Nutritional ecology of Red Sea soft corals

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Écologie nutritionnelle des octocoralliaires de mer Rouge

Chloé Pupier | Thèse de doctorat, 2020

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A mes grand-parents, dont le cœur est un jardin d'amour

« O tempo dos homens é igual a eternidade dobrada. E você, o que está fazendo do seu tempo ? Preso entre o absurdo e o sublime, é verdade, mas o teu amanhã é seu, é seu o sonho, sua estrada e sua viajem. »

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Sommaire

Avant-Propos

Note sur la traduction française

Ce manuscrit est essentiellement écrit en anglais. Toutefois, un résumé développé en français est donné dans les pages suivantes, en avant-propos au corps du document de thèse.

Contenu

Cette thèse se présente sous la forme de cinq articles, dont trois publiés (Chapitre 2 : article n°1, Chapitre 3 : article n°3, Chapitre 4 : article n°2), un article actuellement soumis (Chapitre 5 : article n°4), ainsi qu'un article en préparation (Chapitre 4, article n°5).

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Liste des publications

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2019

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***Pupier, C. A.**, Bednarz, V. N., Grover, R., Fine, M., Maguer, J. F., & Ferrier-Pagès, C. (2019). Divergent capacity of scleractinian and soft corals to assimilate and transfer diazotrophically-derived nitrogen to the reef environment. *Frontiers in Microbiology*, *10*, 1860.

***Pupier, C. A.**, Fine, M., Bednarz, V. N., Rottier, C., Grover, R., & Ferrier-Pagès, C. (2019). Productivity and carbon fluxes depend on species and symbiont density in soft coral symbioses. *Scientific reports*, *9*(1), 1-10.

Soumis

***Pupier, C.A.**, Mies, M., Fine, M., Francini-Filho, R. B., Brandini, F.P., Zambotti-Villela, L., Colepicolo, P., Ferrier-Pagès, C., Lipid biomarkers reveal the trophic plasticity of octocorals along a depth gradient. *Soumis à Proceedings of the Royal Society B.*

En préparation

***Pupier, C.A.**, Grover, R., Fine, M., Rottier, C., van de Water, J.A.J.M., Ferrier-Pagès, C., Nitrogen acquisition in soft and hard corals: a key to understand their ecological niche? *En préparation.*

Seuls les articles accompagnés d'un astérisque (*) seront détaillés dans le manuscrit de thèse.

Liste des communications orales

Pupier, C. A., Fine, M., Bednarz, V. N., Rottier, C., Grover, R., Ferrier-Pagès, C. (2018). Carbon acquisition of two octocorals from shallow and mesophotic coral reef ecosystems. *5th Young Reef Scientists Meeting*, 5-7 septembre, Munich (Allemagne).

Pupier, C. A., Grover, R., Fine, M., Rottier, C., van de Water, J. A. J. M., Ferrier-Pagès, C. (2021). Assimilation of dissolved nitrogen by soft and hard corals: a multi-species comparison. 14th International Coral Reef Symposium (ICRS), reporté 18-23 juillet, Brême (Allemagne).

Résumé / Abstract

Les octocoralliaires représentent l'un des principaux groupes formant la communauté macrobenthique des récifs coralliens tropicaux. Ils sont notamment abondants au sein des écosystèmes perturbés où les changements environnementaux entraînent le déclin des coraux constructeurs de récifs. Bien que la nutrition joue un rôle fondamental dans la régulation de l'abondance d'une population, l'acquisition de nutriments par les octocoralliaires reste à ce jour peu connue. Dans ce contexte, les objectifs de cette thèse étaient 1) de caractériser l'acquisition et l'assimilation de carbone et d'azote, par différentes espèces d'octocoralliaires de mer Rouge, et 2) d'évaluer les changements de nutrition le long d'un gradient de profondeur, depuis la surface (5 m) jusqu'à la zone récifale mésophotique supérieure (50 m). Les résultats démontrent que les octocoralliaires forment une symbiose nutritionnelle avec leurs dinoflagellés, dont le fonctionnement diffère largement de celui de la symbiose dinoflagellés-scléractiniaire. La symbiose des octocoralliaires se démarque particulièrement par un apport de carbone autotrophe stable le long du gradient de profondeur, alors que les scléractiniaires connaissent un approvisionnement réduit avec l'augmentation de profondeur. De plus, l'assimilation de composés azotés dissous (par les dinoflagellés ou les symbiotes microbiens) par les octocoralliaires est très inférieure à celle des scléractiniaires. Ces résultats suggèrent que les octocoralliaires dépendent largement de sources alimentaires hétérotrophes pour satisfaire leurs besoins nutritionnels. L'importance de l'hétérotrophie est confirmée par de fortes concentrations tissulaires en biomarqueurs lipidiques spécifiques du zooplancton aux deux profondeurs, avec une augmentation en milieu mésophotique chez certaines espèces. Une telle mixotrophie confère aux octocoralliaires une grande plasticité trophique, ce qui pourrait contribuer à une plus grande résistance aux changements environnementaux en cours.

Mots clés : octocoralliaires | symbiose | autotrophie | hétérotrophie | mésophotique

Octocorals are one of the major groups forming the macrobenthic community of tropical coral reefs. They are notably abundant within disturbed ecosystems where environmental changes have led to the decline of reef-building corals. Although nutrition plays a fundamental role in regulating the abundance of a population, the acquisition of nutrients by octocorals has received little attention to date. In this context, the aims of this thesis were to 1) characterize the acquisition and assimilation of carbon and nitrogen by several octocoral species from the Red Sea, and 2) investigate the nutritional changes along a depth gradient, from the shallow (5 m) down to the upper mesophotic (50 m) reef zone. The results show that octocorals form a nutritional symbiosis with dinoflagellates, but the functioning differs significantly as compared to the scleractinian-dinoflagellate symbiosis. Particularly, the octocoral symbiosis is characterized by a stable supply of autotrophic carbon along the depth gradient, whereas scleractinian corals experience a reduced supply with increase in water depth. In addition, octocorals assimilate along the entire depth gradient significantly less dissolved nitrogen compounds (from dinoflagellates or microbial symbionts) as compared to scleractinian corals. These results suggest that octocorals strongly depend on heterotrophic food sources to meet their nutritional requirements. The importance of heterotrophy is confirmed by high concentrations of lipid biomarkers specific to zooplankton in the octocoral tissue at shallow and mesophotic depths, with an increased concentration for some species in the mesophotic environment. Such mixotrophy provides octocorals with a wide trophic plasticity, which may contribute to their higher resistance to cope with already on-going environmental changes.

Key words: octocoral | symbiosis | autotrophy | heterotrophy | mesophotic

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Liste des abréviations

Acronyme	Traduction anglaise	Traduction française
C	Carbon	Carbone
CCA	Crustose coralline algae	Algue coralline encroûtante
N	Nitrogen	Azote
P	Phosphorus	Phosphore
DIC	Dissolved inorganic carbon	Carbone inorganique dissous
DFAA	Dissolved free amino acid	Acide aminé libre dissous
DIN	Dissolved inorganic nitrogen	Azote inorganique dissous
DON	Dissolved organic nitrogen	Azote organique dissous
DIP	Dissolved inorganic phosphorus	Phosphore inorganique dissous
DOP	Dissolved organic phosphorus	Phosphore organique dissous
N_2	Dinitrogen	Diazote
DDN	Diazotrophically-derived nitrogen	Azote issus des diazotrophes
PAR	Photosynthetically active radiation	Radiation photosynthétiquement active
COTS	Crown-of-thorn starfish	Etoile de mer couronne d'épines
OA	Ocean acidification	Acidification des océans
FSW	Filtered seawater	Eau de mer filtrée
DI	Distilled	Distillée
DW	Dry weight	Poids sec
AFDW	Ash-free dry weight	Poids sec sans cendres
DMSP	Dimethylsulfopropionate	Diméthylsulfopropionate
BW	Buoyant weight	Poids flottant
ETS	Electron transport system	Système de transport des électrons
DOM	Dissolved organic matter	Matière organique dissoute
P/A pigments	Photosynthetic and accessory pigments	Pigments photosynthétiques et accessoires
AW	Ash weight	Poids des cendres
P_C	Autotrophic carbon acquisition	Acquisition de carbon autotrophe
PSII	Photosystem II	Photosystème II
ARA	Acetylene reduction assay	Test de réduction de l'acétylène
POC	Particulate organic carbon	Carbone organique particulaire
PN	Particulate nitrogen	Azote particulaire
DOC	Dissolved organic carbon	Carbon organique dissous
OM	Organic matter	Matière organique
NH_4	Ammonium	Ammonium
NH_3	Nitrate	Nitrate
POM	Particulate organic matter	Matière organique particulaire
SDA	Stearidonic acid	Acide stéaridonique
DPA	Docosapentaenoic acid	Acide docosapentaénoique
CGA	Cis-gondoic acid	Acide cis-gondoique
PUFA	Polyunsaturated fatty acid	Acide gras polyinsaturé

Chapter **1** | Introduction

Chapter **1** | Introduction

1.1 Tropical coral reefs

1.1.1 Importance of coral reef ecosystems

Biogenic reefs are hard structures exhibiting topographic relief, which are created through the growth of living benthic organisms (Flügel and Kiessling 2002). The first reef-like carbonate structures were formed by stromatolites and are among the earliest evidences of life on Earth (Allwood et al. 2007). Nowadays, the most common and well-known reefs are those formed by hard corals (Cnidaria, Scleractinia), which are the most emblematic builders.

Coral reefs thus result from the deposition of calcium carbonate by corals over millennia. One of them, the Great Barrier Reef, is one of the world's biggest structures ever built by living organisms. Reef formation depends on the speed of skeletal deposition by hard corals and the extent of skeleton erosion (Rohwer et al. 2010). Reefs are found whenever coral larvae can settle on hard substrate, such as in the deep ocean floor, volcanic islands and continental coastlines. Large reef ecosystems, built by the so-called cold-water corals, exist in the deep zones of all oceans (Roberts et al. 2006). This thesis will however focus on tropical reefs, built by scleractinian corals living in symbiosis with dinoflagellate algae. These corals need optimal living environmental conditions and are thus limited to the photic zone, within latitudes ranging from 30°N to 30°S (Figure 1).

Tropical coral reefs are ecologically important because they constitute complex, diverse, and productive ecosystems. Their extent (260,000-600,000 km²) represents less than 0.2% of the ocean's surface, which equates to a smaller land area than France. Yet, they shelter from one-quarter to one-third of the world's marine species (Knowlton et al. 2010). The epicenter of the planet's marine biodiversity is called the Coral Triangle. Located in Indo-Pacific, this area gathers more species diversity than anywhere else in the world (Figure 1). Secondary centers are identified in the Red Sea and North of Madagascar (Veron et al. 2015). Due to this exceptional biodiversity, coral reefs are often referred to as the 'rainforests of the sea'(Connell et al. 1978). Coral reefs, and their associated mangrove wetlands and seagrass meadows are also among the most productive ecosystems per unit surface area (Costanza et al. 1998). These

three habitats are tightly interlinked, especially for providing nurseries and feeding grounds to reef fishes (Dorenbosch et al. 2005).

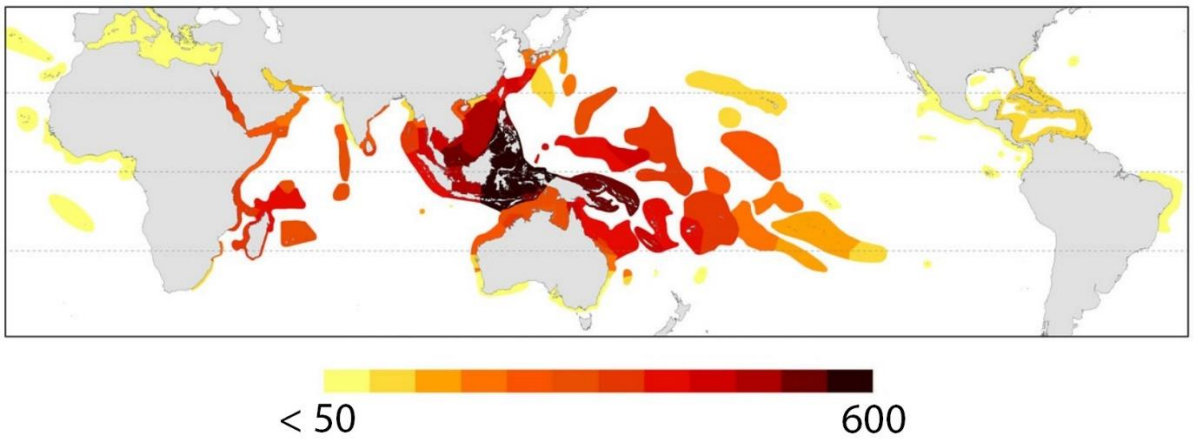


Figure 1. Global diversity of symbiotic hard coral species, indicated by records of occurrences. Modified from Veron et al. (2015).

Besides their ecological role, coral reefs provide humans with multiple essential goods and services. For example, they protect shorelines from erosion, are an important source of food for the population living nearby, generate incomes thanks to fisheries, tourism, and molecules of interest for the pharmaceutical industry (Moberg and Folke 1999; Wells 2006; Laurans et al. 2013). Reefs generate around thirty billion dollars per year (Spalding et al. 2001; Stoeckl et al. 2011) and 500 million people directly benefit from the reefs (Wilkinson 2008).

1.1.2 Major benthic groups: the bricks*, the cement* and the stabilizers

Although hard corals are considered as the bricks of the reef framework, they are not capable of building reefs on their own. The coral reef ecosystem includes a mosaic of ecosystem engineers such as crustose coralline algae (CCA), sponges and octocorals, which contribute to the three-dimensional structure of the reef (Figure 2). All together, they function similarly to terrestrial forests providing architectural complexity, food and shelter to other reef organisms. As trees in the forest, they also contribute significantly to the reef primary productivity and biogeochemical cycles, either directly or through symbiotic associations (Rossi 2013).

* Designation from Dr. Maggie D. Johnson

Crustose coralline algae (CCA) form hard crusts on rocky surfaces and cement the coral bricks together. The CCA crusts provide a settlement substrate for marine invertebrate larvae of corals, abalones and sea urchins among others (Heyward and Negri 1999; Nelson 2009). Fleshy algae, encompassing turfs and macroalgae, are not considered as reef engineers, but are however common components of the reefscape. They are the primary food source of herbivores and the most common competitors with hard corals. Sponges act as reef stabilizers through their impact on benthic substrates. On the one hand, they are one of the primary bioeroders of coral reefs, processing solid carbonate into smaller fragments and fine sediments (Bell 2008) and references therein). On the other hand, they are known to bind coral fragments or rubble together, thereby increasing coral survival by enhancing settlement of carbonate secreting organisms (Wulff and Buss 1979; Wulff 1984).

Octocorals, which are the main subject of this thesis, do contribute to the habitat complexity of the reef but are not considered as reef-builders as such (Fabricius 2011; Schubert et al. 2017 and references therein). Only few exceptions produce a solid skeleton (e.g., *Heliopora coerulea*, *Tubipora musica* and *Corallium* spp.) or massive trunks of consolidated sclerites (some species of the genus *Sinularia*, (Schuhmacher 1997; Jeng et al. 2011), thus contributing to the reef construction and growth. Most of the species contribute to substrate stabilization by releasing sclerites after their death.

Figure 2. Painting representing some of the major benthic groups on reefs (hard coral, octocoral, sponge and algae). Modified from Creartgraphic.

1.2 Ecophysiology of soft corals

1.2.1 Classification and anatomy

Soft corals belong to the phylum Cnidaria, class Anthozoa, subclass Octocorallia and order Alcyonacea (Figure 3). Cnidarian are tentacle-bearing invertebrates, distinguished by stinging structures called nematocysts and a radial symmetry. They are also characterized by an internal cavity that opens through a single orifice (i.e. the mouth, which serves for nutrient uptake and excretion). Members of the class Anthozoa, also named 'flower animals', are represented at the adult stage by small units called polyps. Both hard and soft corals belong to this class and, except few single-polyp species, form a colonial arrangement made of a myriad of polyps. While soft corals belong to the sub-class Octocorallia, reef-building corals belong to the Hexacorallia. The most pronounced characteristic that differentiates these subclasses is that members of Octocorallia have an eight-fold symmetry (i.e. eight tentacles on each polyp) fringed on both sides by one or several rows of pinnules, whereas Hexacorallia tentacules have a six-fold symmetry without pinnules (Fabricius and Alderslade 2001).

At present, Octocorallia consists of three orders, which are Heliporacea (commonly called blue corals), Pennatulacea (commonly called sea pens) and Alcyonacea (Figure 3). The main groups of the order Alcyonacea are sea fans, sea whips, sea rods, sea plumes (all globally termed gorgonians) and soft corals. Unlike 'gorgonians', soft corals do not present a solid internal axis. Throughout this thesis, the term octocoral will qualify any member of Alcyonacea family, without further distinction between taxa.

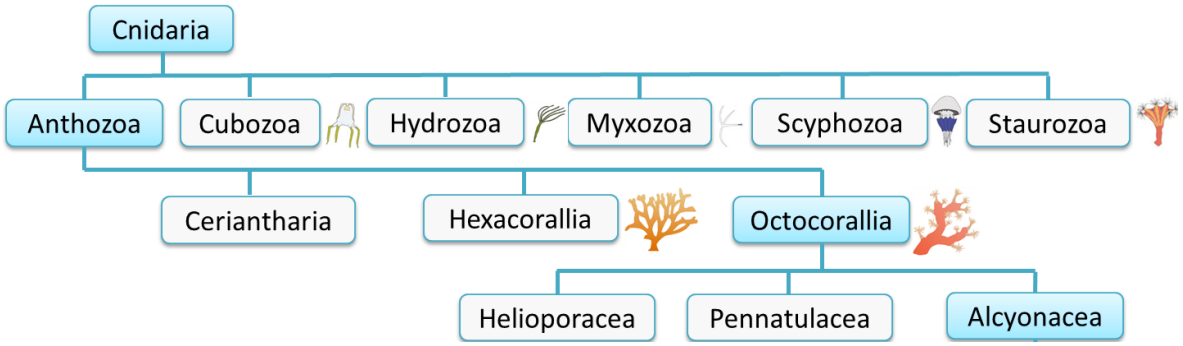


Figure 3. Simplified phylogeny of Cnidaria. Taxonomic position of studied species appears in blue. Adapted from http://www.marinespecies.org/. Picture credits: (top) (Leclère and Röttinger 2017) and (middle) https://ian.umces.edu/.

Soft coral growth forms are divided into categories ranging from encrusting to arborescent (Bayer et al. 1983). The shape and size of soft coral colonies can thus widely vary from one species to another. Even within the same species, these features can considerably vary in response to the environmental conditions. In massive and erect forms, it is often possible to distinguish parts such as stalk, stem, branches, or capitulum (Figure 4).

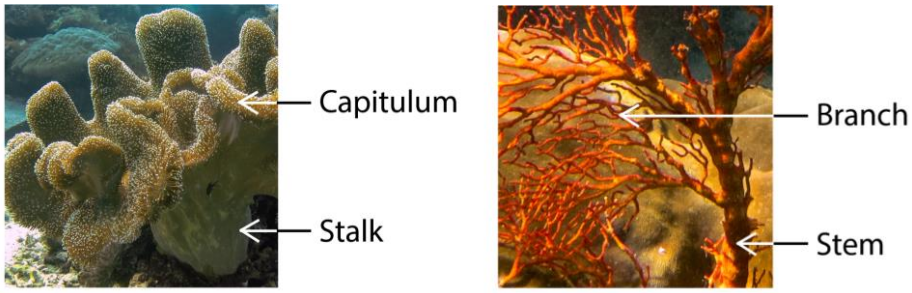


Figure 4. Morphological parts of soft coral (left) and gorgonian (right) colonies. The following terms have been described by Bayer et al. (1983). Capitulum: more or less disk-shaped or hemispherical, polypiferous part of soft coral colony. Stalk: barren basal part of the colony, in Alcyonacea and Pennatulacea; narrow proximal part of a non-retractile polyp in Nephtheidae. Stem: basal part of the colony from which branches may or may not arise, in gorgonians; polypiferous part of the colony usually giving rise to branches in soft corals. Photos credit: C. Pupier.

Soft corals species are called monomorphic or dimorphic depending whether the colony bears one or two polyp categories, respectively. The typical type of polyp is the autozooid, responsible for food capture and reproduction. In addition, some species can have another polyp category called siphonozooid, which ensures seawater irrigation through the colony. Autozooids are cylindrical with a mouth and tentacles at one end whereas siphonozooids are smaller and have no or rudimentary tentacles (Fabricius and Alderslade 2001). Tentacles and their pinnules are mobile, contractile and hollow. An octocoral polyp consists of two main parts. The portion that extends above the colony surface or completely retracts is called anthocodia (cylindrical body with terminal mouth and tentacles). The lower part of the polyp extends from the mouth into the gastrovascular cavity and includes the pharynx (Figure 5). The pharynx is the place where digestion of food particles starts and it encompasses a gutter lined with cilia, which ensures water circulation inside the gastrovascular cavity. From there, can be distinguished eight protruding tissue plates, the mesenteries, which have an important function in the hydraulic system of the polyps. They thicken into mesenterial filaments, including two that create an upward current in the gastrovascular cavity, and six that contain digestive glands

and gonads (they complete the digestion of food particles that has started in the pharynx). At the base of a colony, desmocytes are extensions originating from the ectoderm, which play an active role in anchoring tissue to substrate (Barneah et al. 2002).

Soft corals are diploblastic animals, namely they comprise two epithelial cell layers (Figure 5). The outer tissue layer (i.e. epidermis) is the interface with seawater, which extends over the entire colony. It gathers mucocysts, the cells that produce mucus, along with sensory cells such as nematocysts. The inner tissue layer (i.e. gastrodermis) lines the tentacles, pharynx and gastrovascular cavity. In soft corals living in symbiosis with Symbiodiniaceae dinoflagellates (see 1.2.4), most of the symbionts are localized in the cytoplasm of the gastrodermic coral cells, within a symbiosome (Roth et al. 1988). In between these two cell layers stands an acellular tissue called coenenchyme. It consists of mesoglea usually containing fibrous proteins like collagen, sclerites, along with amoeboid cells that play a role in fighting infections and phagocytosing debris and bacteria. The coenenchyme is thin in the tentacles but makes the main bulk of the colony in most species. It is crossed by a network of canals (solenia), which connect the gastrovascular cavity of each polyp to the others.

Although mostly studied in other Cnidaria taxa, soft corals may have a basic and diffused nervous system (Watanabe et al. 2009), with muscle bundles and nerve fibers located in the coenenchyme and other nerve and muscle cells lying under the epithelial layers (Josephson 2004).

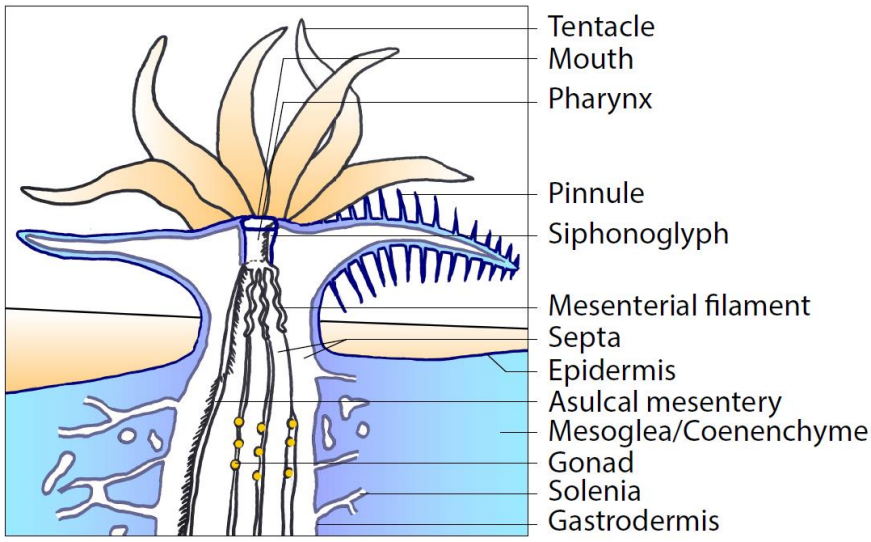


Figure 5. Autozooid polyp of octocoral. The longitudinal section highlights the epithelial layers and internal body organization. For clarity, pinnules are only represented on one tentacle and solenia are limited to a little part of the coenenchyme. This is a simplified scheme, which

should comprise eight mesenteries (i.e. that alternate with the eight tentacles) and eight mesenterial filaments. The gastrovascular cavity ends in a dead-end canal. Retractor septal muscles and transverse fibers of mesenteries, which play a role in polyp retraction and movement of tentacles are not displayed here. Drawing by C. Pupier, based on Fabricius and Alderslade (2001).

Conversely to hard corals, soft corals do not produce a calcium carbonate skeleton. Instead, they rely on hydrostatic pressure for body support. Most soft corals however possess small isolated calcium carbonate structures called sclerites. They are formed by specialized cells (i.e. scleroblasts) in the coenenchyme and are composed of magnesium-enriched calcite. Sclerites are an important feature used for the identification of soft corals since their shape, size and coloration vary widely among species. For example, spindle-shaped sclerites of up to 3 mm long are observed in *Dendronephthya* sp. while tiny oval corpuscules of 0.02 mm long are rather found in *Xenia* sp. (Figure 6).

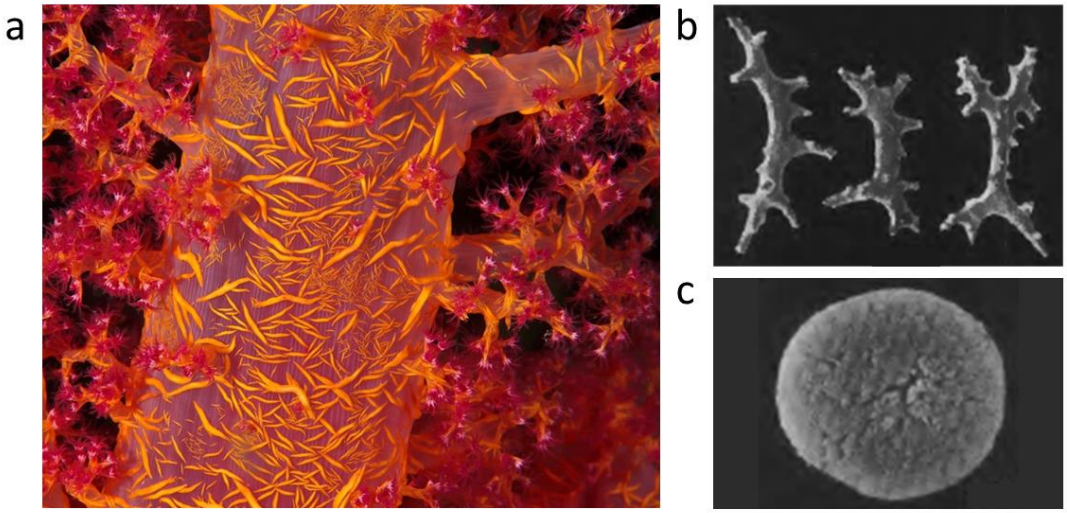


Figure 6. Sclerites of soft corals. (a) Sclerites are visible on the surface of branches of *Dendronephthya* sp. (photo credit: M. Lanini). (b) Sclerites of *Dendronephthya* sp. seen under an electron microscope (× 250, from Bayer et al., 1983). C: Sclerite of *Xenia* sp. seen under an electron microscope (× 1750, from Bayer et al., 1983).

1.2.2 Life history features and habitat requirements

Octocorals are the second most common group of macrobenthic animals on many Indo-Pacific, Caribbean and Red Sea reefs after hard corals (Benayahu and Loya 1981; Fabricius and Alderslade 2001; Benayahu et al. 2019). Some soft coral species of the family Xeniidae and Nephtheidae present high growth rates and asexual reproduction by colony fission (Benayahu

and Loya 1985, 1987; Fabricius et al. 1995a; Karlson et al. 1996). Such features can allow them to be pioneer colonizers of cleared substrates and also increase their chances to successfully compete for space. Species like *Xenia macrospiculata* even have the ability to actively migrate for settling on living colonies of hard corals or natural surfaces (Benayahu and Loya 1981, 1985). Conversely, most Alcyoniidae are characterized by slow growth, low reproduction rates and greater persistence (Fabricius 1995). They are able to perform fission too, though operating slower than for the other families, with asexual recruits making a greater contribution to the population than sexual recruits (Bastidas et al. 2004). These life history features can result in the formation of large aggregations over the long-term (Bastidas et al. 2004). Moreover, some soft coral species, probably introduced through aquarium leak, have been reported as invasive species and overgrew coral communities in Venezuela and Brazil (Lages et al. 2006; Ruiz Allais et al. 2014; Mantelatto et al. 2018).

Most octocorals occur in high abundance in wave-protected areas because they are susceptible to abrasion and dislodgement. They also search for strong currents for maximizing food encounter (Fabricius and Alderslade 2001). For symbiotic soft coral species, the presence of algal endosymbionts limits them to the photic zone, or deeper depending on the slope and water quality (Schubert et al. 2017). Up to 75% of octocoral species however occur in depths greater than 50 m, and are mostly dominated by non-symbiotic species.

1.2.3 Octocorals and reef organisms

Soft corals are well known for their secondary metabolites, which are of particular interest for medical bioprospecting (reviewed in van de Water et al. 2018). In the reef environment, these substances are allelopathic and can act as a barrier to recruitment, growth and survival of hard corals (Sammarco et al. 1983; Fleury et al. 2006). However, octocorals can host many macro organisms such as sponges, hydrozoans and decapods (e.g. (Nakano and Fujii 2014; Maggioni et al. 2019). They provide essential resources for fishes and nudibranchs, including food, shelter and protection (e.g., Avila et al. 1999; Pratchett 2005; FitzPatrick et al. 2012; Epstein and Kingsford 2019). As all multicellular organisms, octocorals are holobiont entities associated with many microorganism assemblages (van de Water et al. 2018). In other words, they are complex meta-organisms, which comprise the coral animal host, its symbiotic

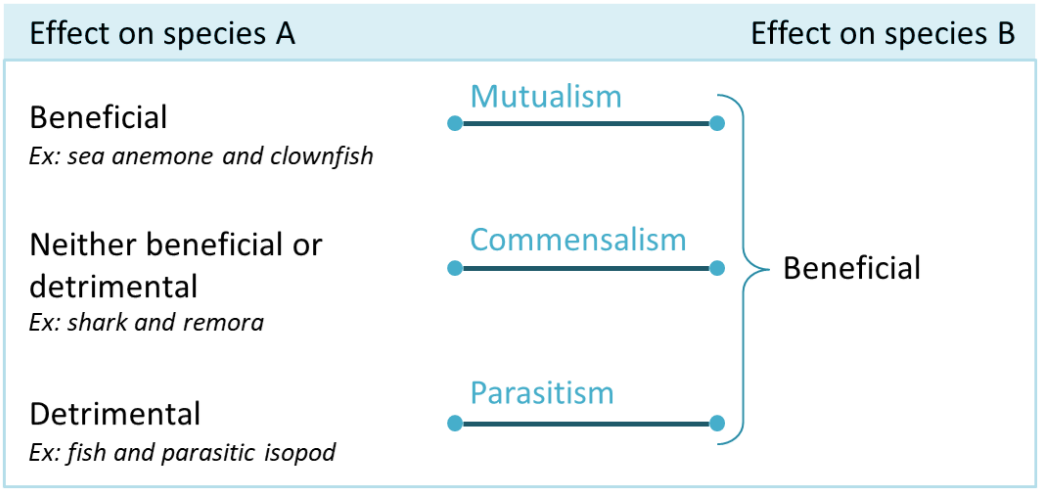
dinoflagellates and other microorganisms, including fungi, bacteria, archaea and viruses (Knowlton and Rohwer 2003).

1.2.4 Symbiosis with Symbiodiniaceae: a nutritional-based partnership

- Symbiosis: definition

A symbiosis is the "living together" of two unlike organisms in a long-term relationship. It is a biological interaction that can span from mutualism to commensalism and parasitism (Table 1). However, the nature of these associations is not completely fixed and can shift from mutualism to commensalism or parasitism depending on environmental conditions. Symbioses can be either obligate, meaning that one or both of the partners entirely depend on each other for survival, or facultative (they can generally live independently). The bigger organism is called host while the smaller is the symbiont. In addition, the symbioses can be designated depending on the location of the symbionts. They can be referred as intra- or extracellular endosymbiosis (symbionts inside host cells or tissue, respectively), or ectosymbiosis (symbionts outside the host tissue).

Table 1. Description of the different symbioses. One example is provided for each type of symbiosis. The species that benefits from the association (B) may obtain nutrients, shelter, support or even locomotion from the host species (A).



Symbiotic associations are ubiquitous and play important roles in ecological and evolutionary processes (Thrall et al. 2007). The symbiotic association between soft or hard

corals and dinoflagellates of the family Symbiodiniaceae (see below) is qualified of mutualistic endosymbiosis. Symbionts are localized inside endodermic host cells (in the tentacles and periphery of the flesh, in the shallow gastrodermis, mostly not deeper than about 100 µm, Y. Benayahu pers. com.) and both partners benefit from this relationship, through nutritional interactions described in detail in the next chapters. In addition, the coral host provides its symbionts with a protected habitat (as compared to free-living Symbiodiniaceae that are more likely grazed). Environmental conditions can create disparity in benefits and costs of the two partners, which rather promotes a hybrid model straddling between mutualism and parasitism (e.g., Baker et al. 2018). In addition to photosynthetic dinoflagellates, corals harbor a diverse array of microorganisms such as bacteria, archaea, fungi and viruses (Knowlton and Rohwer 2003). The metabolic interactions within this symbiotic consortium are fundamental and drive coral's response to environmental changes (e.g., reviews of Ainsworth et al. 2017; Benavides et al. 2017; Bednarz et al. 2019; Pernice et al. 2019).

- Symbiodiniaceae

Dinoflagellates of the family Symbiodiniaceae (kingdom Chromista, infrakingdom Alveolata, class Dinophyceae, order Suessiales) are of particular interest in tropical oligotrophic waters, because they can form mutualistic symbioses with a wide array of benthic metazoan and protist hosts (Figure 7). Dinoflagellates are unicellular microeukaryotes, characterized by two flagella, which allow them to migrate through the water column when they are free living or released in the water by their host.

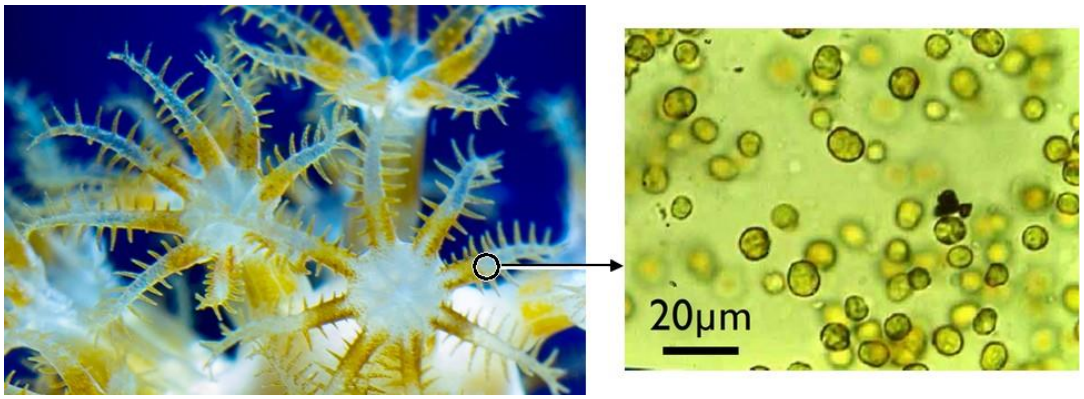


Figure 7. Octocoral polyps and close-up on their Symbiodiniaceae endosymbionts. Photo credits: B. Ping (left) and S. Santos (right).

The taxonomy of the Symbiodiniaceae was recently revised, including a formal description of seven genera (LaJeunesse et al. 2018). Five of them can be associated with octocorals and their distribution and occurrence vary across biogeographic regions (Figure 8).

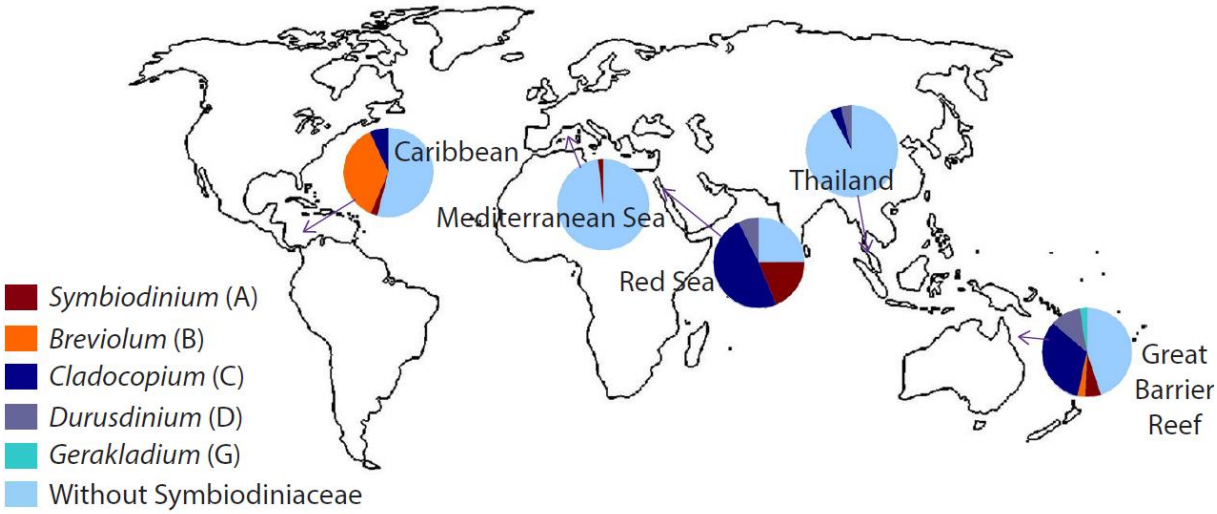


Figure 8. Relative abundance of Symbiodiniaceae genera in octocorals of several biogeographic regions. Genera are indicated in the bottom-left corner, followed by the former clade designation in brackets. A large number of octocoral species does not possess algal symbionts (≤ 50%), similarly to hard corals. Unreported are *Effrenium* sp. (formerly clade E) and *Fugacium* sp. (formerly clade F), which can be found associated with hard corals. Modified from van de Water al. (2018).

The genetic diversity within and between Symbiodiniaceae genera also reflects a physiological diversity, explaining their different response to environmental changes. For example, *Durusdinium* sp. was characterized as tenacious symbiont concerning thermal stress, meaning that it undergoes photo-damage but is not expelled by the host (Silverstein et al. 2017). Due to the physiological diversity of the symbionts, some hard coral species can shuffle or switch their symbionts (*sensu* Baker 2003) to acclimate to changing environmental conditions (but see Stat et al. 2009). Octocorals are however known to present more stable relationships with their dinoflagellates overtime (Goulet and Coffroth 2003; Kirk et al. 2005; Goulet 2006, 2007).

- <u>A nutritional-based partnership</u>

Symbiotic corals are considered as mixotrophic organisms because they can use both inorganic and organic sources of nutrients. This dual mode of nutrition enables them to reap the

maximum benefit in oligotrophic waters, where each of the partner would hardly persist alone due to the low concentration of inorganic nutrients and prey availability (Stoecker et al. 2017).

Most of the knowledge on the nutritional ecology of corals is from hard corals, as described in the next two paragraphs. Corals are animals capable of heterotrophic feeding (i.e., the capture of particulate food in suspension in seawater, (Houlbrèque and Ferrier-Pagès 2009). Heterotrophy is especially important for supplying the host with nitrogen (N), phosphorus (P), amino-acids, or trace elements (e.g., iron and vitamins) for tissue, skeletal growth and reserve stocks (Ferrier-Pagès et al. 2003, 2010, 2018; Seemann et al. 2013; Tremblay et al. 2016). Corals can capture food directly, with their tentacles, or via suspension feeding (Bak et al. 1998; Anthony 1999). The coral host can transfer a fraction of its heterotrophically-acquired nutrients to the symbionts, for their own needs (Tremblay et al. 2015). It also transfers large amounts of dissolved inorganic carbon (DIC) resulting from its respiration, along with metabolic waste products (Muscatine and Porter 1977).

Corals are also autotrophic organisms thanks to the presence of their photosynthetic symbionts, which transform inorganic into organic nutrients. The chloroplasts of Symbiodiniaceae cells possess photosynthetic pigments (chlorophyll *a* and c_2, carotenoids) that enable the symbionts to catch light energy. As a result of cascading chemical reactions, the symbionts produce, from DIC, low molecular weight organic compounds called photosynthates (Muscatine et al. 1981). These organic molecules are rich in carbon and are generally in the form of sugars (e.g., glucose, Burriesci et al. 2012). Besides DIC, symbionts are likely the first site of assimilation of other nutrients dissolved in seawater, such as dissolved inorganic (ammonium, nitrate) and organic (urea, DFAA) nitrogen (DIN and DON, respectively) (Grover et al. 2002, 2003, 2006, 2008; Pernice et al. 2012) or dissolved inorganic and organic phosphorus (DIP and DOP, respectively) (see Ferrier-Pagès et al. 2016) and references therein) for the two major macronutrients. These nutrients can also be transferred to the coral host in the form of amino acids and other phosphorylated nutrients (Wang and Douglas 1999). Since the dinoflagellates are embedded in the animal tissue, dissolved inorganic nutrients first transit through the host tissues, which have developed particular adaptations (Furla et al. 2011). Most of the autotrophically-acquired nutrients, which are not used directly by the symbionts for their own needs and growth, are transferred to the host (Muscatine et al. 1984; Tremblay et al. 2012). A large percentage of the photosynthates is either immediately respired by the host to cover its

metabolic needs, or released as dissolved and particulate organic matter (Tremblay et al. 2012). The remaining photosynthates can be used for growth and energy reserves (Grottoli et al. 2006).

In addition, corals are associated with a large diversity of microorganisms, which can contribute to providing nutrients to the coral host and symbionts. For example, diazotrophs are specialized bacteria able to transform dinitrogen (N_2) gas into a bioavailable form of N. They have been discovered within the surface mucus layer, the coral tissue and skeleton (Lema et al. 2012; Ainsworth et al. 2015). Studies that used the labelled gas $^{15}N_2$ demonstrated that diazotrophically-derived N (DDN) was assimilated by the coral host and symbionts (reviewed by Benavides et al. 2017). Although this form of N contributes only to ca. 1% to the N needs of the coral holobiont compared to the other N forms (inorganic N dissolved in seawater or from the host metabolic wastes), it may help corals recovering faster from bleaching (Bednarz et al. 2017).

→ FOCUS ON OCTOCORALS

In octocorals, the relative contribution of autotrophy and heterotrophy to the holobiont's nutritional requirements is poorly known (reviewed in Schubert et al. 2017). The common belief is that octocorals have low rates of primary productivity and depend on both autotrophy and heterotrophy to meet their metabolic needs (Sorokin 1991; Fabricius and Klumpp 1995). However, the few reports investigating the contribution of photosynthesis to host respiration (P:R ratio) rather support a reliance on autotrophy for the respiratory needs (Mergner and Svoboda 1977; Sorokin 1991; Fabricius and Klumpp 1995; Riegl and Branch 1995; Kremien et al. 2013; Bednarz et al. 2015; Rossi et al. 2018, 2020), except for few species (Ramsby et al. 2014; Baker et al. 2015). The small number of studies, which have addressed a carbon budget observed high interspecies variation, with photosynthate translocation rates ranging from 10% in *Capnella gabonensis* to 75% in *Sinularia flexibilis* (Schlichter et al. 1983; Farrant et al. 1987; Sorokin 1991; Khalesi et al. 2011). In gorgonian octocorals, the symbionts performance is linked to the host morphology because species with thin branches and small polyps are more autotrophic than those with a massive shape and large polyps (Baker et al. 2015; Rossi et al. 2018). The colony morphology may also play an important role in the nutrition of soft corals. The ratio of body surface area to volume, which determines both gas exchange through the epidermal tissue and photosynthetically active radiation (PAR) exposure, is low in soft corals as compared to hard corals (Fabricius and Klumpp 1995). Any increase of this ratio, through the expansion of colony, may enhance primary productivity. More investigations are therefore

needed to better understand the autotrophic capacities of octocorals in general, and soft corals in particular.

Regarding heterotrophy, soft corals have long been considered as mainly suspension feeders, because their nematocysts are small, simply structured and inefficient for paralyzing actively swimming prey (Mariscal and Bigger 1976). However, the recent discovery of a novel type of nematocysts could potentially highlight a more diverse arsenal of stinging structures than previously thought (Yoffe et al. 2012). Moreover, microvilii densely cover the epidermis of soft corals and likely plays a role in the absorption of dissolved matter (Schlichter 1982b, a). The diet of octocorals has been determined via field and feeding experiments, from temperate (Coma et al. 1994, 1998, 2015; Orejas et al. 2003; Rossi et al. 2004) and tropical species (Lewis 1982; Sebens and Koehl 1984; Sorokin 1991; Fabricius and Dommisse 2000; Rossi et al. 2020). It is composed of small zooplankton (Figure 9). In addition, and on the contrary to many hard corals, soft corals can also largely prey on phytoplankton (Figure 9), whose ingestion has been described in both asymbiotic (Fabricius et al. 1995a, b, 1998) and symbiotic species (Ribes et al. 1998; Leal et al. 2014; Piccinetti et al. 2016). A particular behavior called 'deposit feeding' has even been observed in the cold-water soft coral *Gersemia Antarctica*, which bends against the substrate to supplement its capture of prey on sediment (Slattery et al. 1997).

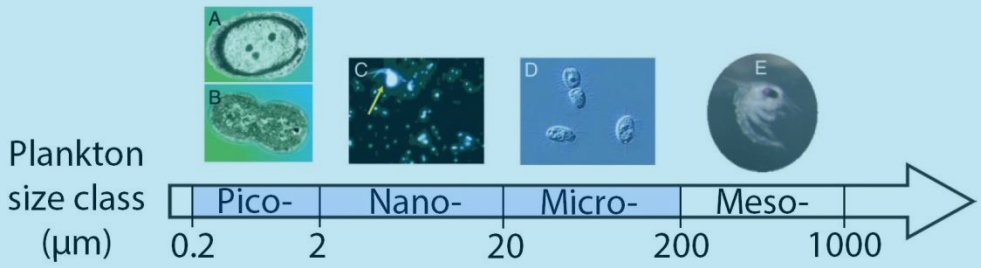


Figure 9. Plankton size classes included in the diet of octocorals. The classes highlighted in blue indicate the microbial fraction. Scanning electron micrographs of A: *Prochlorococcus* sp. (0.6 µm) and B: *Synechococcus* sp. (1 µm). C: epifluorescence microscope image showing one nanoflagellate cell indicated by the arrow. D: ciliates (100-200 µm) taken under phase contrast microscope. E: crab zoea (1000 µm). Modified from Houlbrèque and Ferrier-Pagès (2009).

The relative contribution of auto- and heterotrophy to soft coral nutrition may not be fixed and depend on environmental conditions and/or on the availability of resources (i.e. depth and season; (Rossi et al. 2020). However, this remains to be further investigated.

1.3 Major threats to coral reefs and soft corals

Existing coral reefs are not the result of continuous growth. They have faced both natural and human-induced disturbances that have affected and still affect their integrity periodically or over the long-term. Reefs are generally able to recover from natural disturbances in a few years to decades (e.g., Harmelin-Vivien 1994). However, human activities add up local and global stressors to the natural disturbances, which act together to weaken coral reefs and eventually drive major declines.

1.3.1 Global disturbances

Anthropogenic activities such as fossil fuel combustion, industrialization and deforestation, have raised the concentrations of greenhouse gases in the atmosphere since the beginning of the industrial era (Sabine et al. 2004). Such emissions are the cause of global climate changes including ocean warming and acidification. As a result, marine ecosystems are changing and coral reefs are among the most threatened (Hoegh-Guldberg et al. 2014; Gattuso et al. 2015).

- Ocean warming

All coral live close to their upper thermal maximum and can suffer from thermal stress when sea surface temperatures increase by ≥ 1°C above the average summer maximum (Wooldridge 2013). When corals experience extreme and prolonged heat stress, the symbiosis with their photosynthetic dinoflagellates is disrupted, resulting in the expulsion of the symbionts, a phenomenon called bleaching (Brown 1997). Bleached corals are physiologically damaged, and prolonged bleaching often leads to high levels of coral mortality (Hughes et al. 2018). Although positive thermal anomalies are the main cause for bleaching, it can also be induced by other stress factors such as bacterial infections, burial under sediment or high irradiance (Fabricius and Alderslade 2001). According to the World Meteorological Organization, the last decade was the warmest one on record. Reefs worldwide have therefore encountered unprecedented and recurrent coral mass bleaching events (Hughes et al. 2017). The increased frequency of heat waves has prevented corals to fully recover between two bleaching episodes and continuously reduced coral populations in many places. For example, estimated

losses of hard corals in the Great Barrier Reef (Australia) were respectively due to tropical cyclones (48%), predation by crown-of-thorn starfishes (COTS) (42%) and bleaching (10%) in the 1985-2012 period (De'Ath et al. 2012), whereas marine heatwaves caused the loss of 50% of the shallow corals in the past five years (Hoegh-Guldberg et al. 2019). However, physiological responses to thermal stress and subsequent recovery are highly variable among species, depending on both the symbionts harbored (e.g., different functional traits, Suggett et al. 2017) and the host (e.g., different metabolic and morphological traits, Baird et al. 2009; van Woesik et al. 2012; Wooldridge 2014).

→ FOCUS ON OCTOCORALS

Mass bleaching of tropical octocorals has been reported in many reefs worldwide (Slattery and Mcclintock 1995; Paulay and Benayahu 1999; Marshall and Baird 2000; Arceo et al. 2001; Loya et al. 2001; McClanahan et al. 2001; Celliers and Schleyer 2002; Goulet et al. 2008a; Chavanich et al. 2009; Prada et al. 2010; van Woesik et al. 2011; Dias and Gondim 2015; Slattery et al. 2019). Similarly to other coral groups, their susceptibility to thermal stress is heterogeneous and, some species or individuals exhibit a higher tolerance, depending on the host or symbiont genus or on the general health state, energetic reserves and acclimatization of the colonies (Loya et al. 2001; Lasker 2003; Strychar et al. 2005; Goulet et al. 2008a; van Woesik et al. 2011; Slattery et al. 2019). Records of bleaching may also be biased towards greater numbers of bleached persistent species (e.g. Alcyoniidae), as opposed to the ones that die and disintegrate (e.g. Xeniidae) within several days (Fabricius 1999).

The few experimental studies tackling tropical octocoral responses to thermal stress mostly performed acute heat-stress on a short period of time (e.g. from +2-8°C during 48 h to +5°C during 35 days). Overall, they showed negative responses, including reduced photosynthesis, bleaching, decline in energetic reserves and reproductive output, and necrosis (Michalek-Wagner and Willis 2001; Drohan et al. 2005; Strychar et al. 2005; Imbs and Yakovleva 2012; Sammarco and Strychar 2013; Netherton et al. 2014). The exception is a species of the family Xeniidae (*Bayerxenia* spp.), which did not display changes neither related to the symbionts or host cells, after being exposed to +6°C during one week (Ziegler et al. 2014). Some species also mitigated bleaching through symbionts migration and accumulation in the stolons (Parrin et al. 2012, 2016). Therefore, octocorals seem to be as affected as hard corals by ocean warming. The overall stability of octocoral populations, while hard corals have

steadily declined (e.g., Ruzicka et al. 2013), may involve other processes than resistance (i.e. resilience, acclimatization, and life history features).

- Ocean acidification (OA)

OA is an on-going process, which induces several changes in the seawater carbonate chemistry (e.g. Feely et al. 2004). In particular, it decreases seawater pH and the concentration of carbonate ions. Such modifications therefore negatively affect the biogenic calcification (e.g. of aragonite and calcite) and can also drive the net dissolution of carbonate structures. Thus, OA poses significant problems to marine organisms that form calcium carbonate shells, skeletons, or internal structures (Orr et al. 2005; Gattuso and Hansson 2011). For example, present-day conditions have been shown to dissolve exoskeletons of Dungeness crab larvae (Bednaršek et al. 2020). On reefs, OA can lead to reduced structural complexity and resilience due to decreased coral abundance and calcification rates (Kroeker et al. 2013; Smith et al. 2020). Observations of acidified natural environments such as carbon dioxide seeps have shown a loss of key habitat-forming species and major simplification of the ecosystem (Sunday et al. 2017; Agostini et al. 2018). Overall, reefs acclimatized to acidified waters demonstrate a clear dominance of non-calcareous macroalgae (pH < 7.7, Enochs et al. 2015), followed by monospecific aggregations of hard corals (e.g. *Porites* sp., Fabricius et al. 2011) or soft corals (e.g., *Sarcophyton* sp., Inoue et al. 2013) under moderate conditions (pH > 7.8). Mesocosm experiments have shown that the effects of OA are highly context dependent and will differ greatly between habitats, and depending on species composition (e.g. Comeau et al. 2012, 2014, 2019).

→ FOCUS ON OCTOCORALS

While *in situ* observations suggest shifts from hard coral to octocoral dominance under levels of pH predicted by the end of the century (Inoue et al. 2013), only few experimental studies have addressed octocoral's response to OA. They showed that octocoral tissue descriptors and physiological parameters (i.e. symbiont cell density, pigmentation, sclerite structure, polyp pulsation rate and growth) were not affected by OA (Gabay et al. 2013, 2014; Enochs et al. 2016). Only one study showed a decreased calcification, but the pH tested (7.1) exceeded the values projected by the end of the century (Gómez et al. 2015). OA mitigation by octocorals could be explained by their thick tissue, which can protect sclerites (Gabay et al.

2013, 2014), and the process of calcification itself, which may be more stable than the one of hard corals despite altered carbonate chemistry (Enochs et al. 2016). Although there is a paucity of data on the combined effects of OA and other threats on octocorals, it has been suggested that hard corals, which are already stressed by OA alone, will be more affected than octocorals with the combination of several stressors (e.g., eutrophication, Januar et al. 2017).

1.3.2 Local stressors

Coral reefs can be affected by local anthropogenic pressures such as overfishing, invasive species and various sources of pollution (sediment, nutrients, and pesticides loads) due to coastal and agriculture development (Burke et al. 2011; Anthony et al. 2015; Wear and Thurber 2015). At the ecosystem level, local stressors may be the cause of structural impairment and mortality of most organisms (e.g. destructive fishing). They may also impact reefs indirectly by inducing cascading effects across the ecosystem. For example, overfishing (alteration in top-down controls) depletes stocks of large predators and grazers, and leads to reduced herbivory and subsequent algal overgrowth (Hughes 1994; Jackson et al. 2001; Scheffer et al. 2005). Finally, reduced water quality, due to overloads of nutrients and sediments (alteration of bottom-up controls), can stimulate the growth of algae (McCook et al. 2001), the development of diseases (Pollock et al. 2014) and can favor the emergence of corallivorous *Acanthaster planci* outbreaks (Birkeland 1982; Brodie et al. 2005; Fabricius et al. 2010). At the organism level, the severity of water pollution on hard corals depends on the intensity, duration and frequency of exposure. They contribute to the impairment of coral fitness and to lessen their capacity to withstand global threats. Responses include reduced physiological performance, disruption of the coral-dinoflagellate symbiosis, tissue injury and mortality (e.g., Fabricius 2005; Erftemeijer et al. 2012; Weber et al. 2012; D'Angelo and Wiedenmann 2014; Morris et al. 2019).

→ FOCUS ON OCTOCORALS

Reports on the effects of declining water quality on the abundance of soft corals are not conclusive. They either show a negative impact on the cover of both hard and soft corals (Fabricius and De'Ath 2004), no effect (Fabricius and De'ath 2000), or a relative dominance of soft corals in areas affected by sedimentation (McClanahan and Obura 1997; Schleyer and Celliers 2003), moderate to high concentrations of suspended particulate matter (Fabricius and

Dommisse 2000; Fabricius 2005), or a combination of particulate and dissolved matter (Baum et al. 2016). Data from the Great Barrier Reef, Hong-Kong and Palau suggest that a poor water quality is rather related to a decline in species diversity (Fabricius and De'Ath 2004; Fabricius et al. 2005, 2007; Fabricius and McCorry 2006). Further research is thus needed to explain the confounding effects of water quality on octocorals.

1.3.3 Changes in the reef community structure: phase shifts to octocoral-dominated reefs

Intense competition continuously takes place between benthic reef organisms for the access to light and space. Intra- or interspecific competitive interactions can have major effects on the growth, recruitment and survivorship of benthic competitors (Chadwick and Morrow 2011). Human activities may unbalance the dynamic of these interactions, by favoring the decline of an organism or providing the competitive advantage to another. Phase shifts are community changes in response to a persistent modification in environmental conditions (Done 1992; Dudgeon et al. 2010). On coral reefs, a phase shift results in the transition from a hard coral-dominated reef to a reef dominated by other benthic organisms such as algae, sponges, octocorals, or corallimorphs (McCook 1999; Norström et al. 2009; Barott and Rohwer 2012). This process is often associated with reef degradation, due to the loss of the 'bricks' of the ecosystem and associated functions. Unless few exceptions, a community shift may be difficult to reverse to pre-disturbance conditions (e.g., Diaz-Pulido et al. 2009).

Although shifts to algae-dominated reefs are prominent worldwide, shifts to other major benthic groups, such as octocorals and sponges, have also been reported (Norström et al. 2009). Changes in the community structure ending up towards the dominance of octocorals can occur following COTS outbreaks, anthropogenic pollution, storms, bleaching, diseases and dynamite fishing (Figure 10 and Table 2). These disturbances led to a decreased cover of hard corals whereas soft corals maintained or increased their abundance.

Figure 10. High cover of soft corals. Local dominance of *Sarcophyton* sp. observed in the Seychelles (left, photo credit: M. Boussion) and high abundance of multi-species assemblage of soft corals observed in Indonesia (right, photo credit: C. Pupier).

The colonization of the reef by octocorals occurs when the right conditions regarding their recruitment and growth are met. For example, Wood and Dipper (2008) recorded that, under similar environmental conditions, some fields of coral rubble have provided suitable settlement substrate for soft corals whereas some others did not. The successfully colonized part of the reef underwent a phase shift from hard corals to xeniids-*Clavularia* assemblage and the other part remained unoccupied, likely not stable enough. Moreover, Fabricius (1997) observed that there was no difference in soft coral cover before and after a COTS outbreak that removed a large proportion of hard corals. Even eight years after the event, soft corals showed little tendency to take advantage of increased available space due to the environmental conditions that were not suitable for them (Fabricius and Alderslade 2001).

Table 2. Reported shifts from hard corals to soft corals dominance. COTS: crown-of-thorn starfishes. From van de Water et al. (2018).

Location		Cause	Abundance of octocoral (cover)		References
			From (%)	Up to (%)	
Red Sea	North and Center	Anthropogenic pollution, COTS outbreak	5-10	30-50	(Al-Zibdah et al. 2007; Tilot et al. 2008; Wilkinson 2008)
	South	Anthropogenic pollution, bleaching, storms	4	30	(Reinicke et al. 2003; Bruckner and Dempsey 2015)
Caribbean	Florida Keys	Bleaching	6	14	(Ruzicka et al. 2013)
	US Virgin Islands	Diseases			(Lenz et al. 2015)
Indian Ocean	Madagascar/Seychelles	Bleaching	1.3	2	(Stobart et al. 2005)
	Seychelles	Anthropogenic pollution, bleaching			(Wilkinson 2008)
Pacific Ocean	Malaysia	Bleaching, COTS outbreak, dynamite fishing	1	30	(Wood and Dipper 2008)
	Indonesia	Anthropogenic pollution	7	13	(Baum et al. 2016)
		Dynamite fishing			(Fox et al. 2003)
	Australia	Bleaching, COTS outbreak	10	16	(Wakeford et al. 2008)
	Fiji	Anthropogenic pollution			(Hoffmann 2002)

1.4 Soft corals of the Northern Red Sea

Red Sea coral reefs, like other marine ecosystems, experience global warming and ocean acidification (Fine et al. 2019). However, hard corals in the Gulf of Aqaba have an unusually high thermal tolerance and rarely experience bleaching, despite rapidly rising sea surface temperatures and recurrent positive temperature anomalies. They withstand water temperature anomalies that cause severe bleaching or mortality in most hard corals elsewhere when the water temperature exceeds 1-2°C above the local maximum monthly mean (e.g., Bellworthy and Fine 2017; Grottoli et al. 2017). As a consequence, the Gulf of Aqaba has been identified as a coral refuge from climate change (Fine et al. 2013; Kleinhaus et al. 2020).

1.4.1 Study site

Most of the experiments of this thesis were conducted on corals collected in Eilat, in the reef adjacent to the Interuniversity Institute for Marine Sciences (Figure 11). This site is of particular interest for giving access to shallow and mesophotic reef zones. Mesophotic coral reef ecosystems comprise the light-dependent communities of corals and other organisms found at depths below 30 to 150 m in tropical and subtropical regions (Puglise et al. 2009). Based on measurements of light intensity and spectrum (Tamir et al. 2019), combined with concomitant changes in benthic composition, the mesophotic reefs of Eilat have been classified into two distinctive light habitats: the upper-mesophotic zone (30-80 m), which is characterized by a high diversity of depth-generalist coral species, and the lower mesophotic zone (80-160 m), dominated by a single species of depth-generalist (Eyal et al. 2019). For our work, corals were collected at depths of 8-10 m (shallow) and 40-50 m (upper-mesophotic). Light attenuation at these depths is described in Appendix II. Characteristics of the water are stable along this gradient, including a salinity over 41‰, temperatures ranging from 20.7°C to 30.3°C at 10 m depth and 20.9-27.8°C at 50 m depth over the year, as well as low concentrations of dissolved nutrients (<0.5 µM dissolved nitrogen and <0.2 µM dissolved phosphorus) (data from the Israel National Monitoring Program, Eyal et al. 2019).

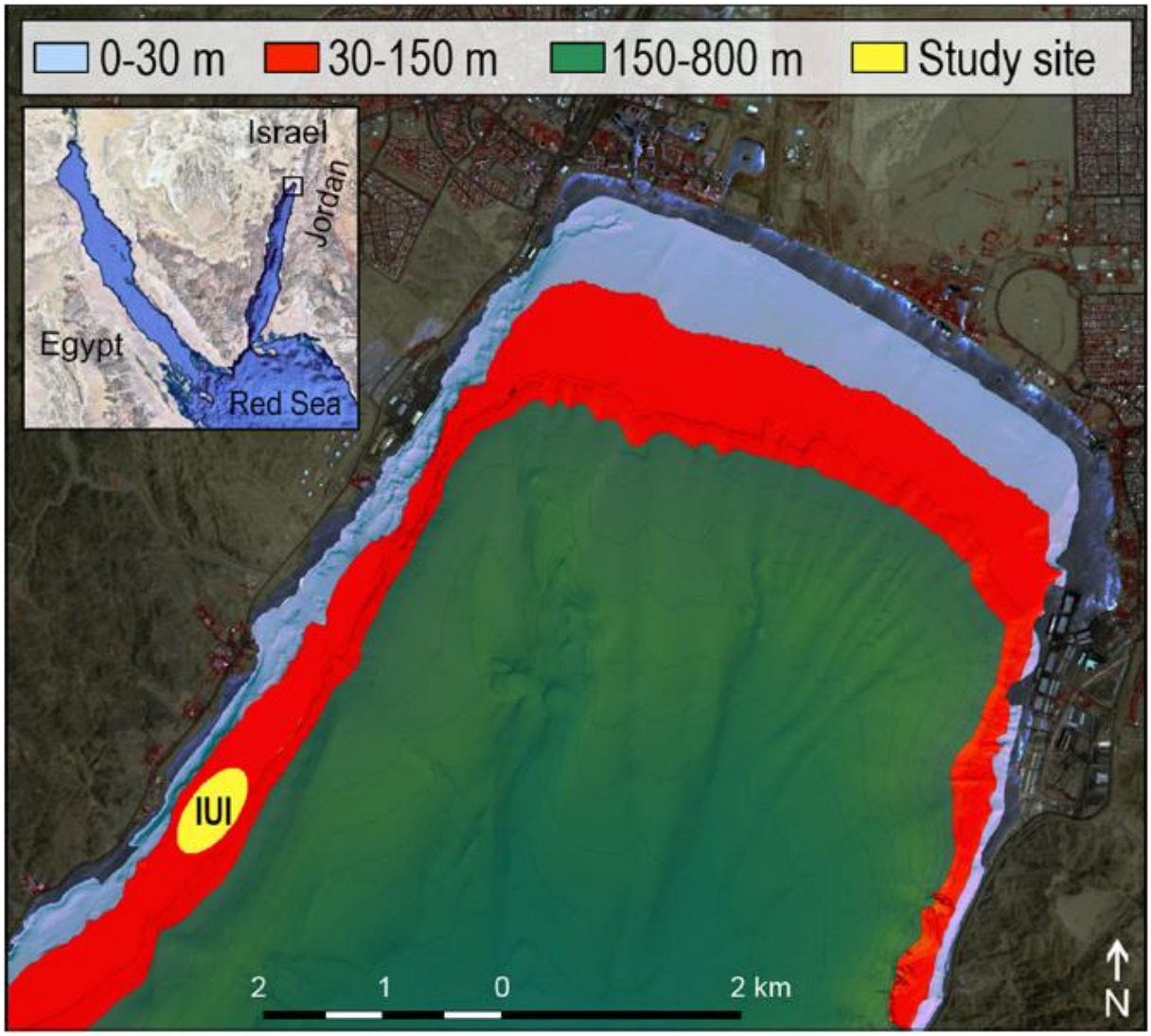


Figure 11. Study site in the Red Sea (Eilat, Israel, Gulf of Eilat/Aqaba). IUI: Interuniversity Institute for Marine Sciences. The three bathymetric zones are represented (shallow water corresponding to 0-30 m depth, in blue; mesophotic zone corresponding to 30-150 m depth, in red; and deep waters corresponding to 150-800 m depth, in green). Modified from Shoham and Benayahu (2016).

Soft corals are the second most abundant faunal group in the shallow and mesophotic reefs of Eilat, after scleractinians. They have a moderate cover of 3-10% along the depth gradient but rarely cover more than 20% of the substrate (Eyal et al. 2019). Two distinct communities of octocorals have been identified at shallow and upper-mesophotic (30-45 m) depths, which only shared 16% of species in common (Shoham and Benayahu 2017). The upper-mesophotic octocoral community features a higher species richness, but a similar diversity, as compared to the shallow one. More particularly, the upper-mesophotic octocoral community is characterized by a predominance of symbiotic species (Shoham and Benayahu 2017).

1.4.2 Biological material

Several species of soft and hard corals were studied in this thesis, chosen depending on their morphology and physiological characteristics (Figure 12 and Figure 13).

Dendronephthya sp. (Kükenthal, 1905) is the only species that does not harbor any Symbiodiniaceae and have ca. 180-200 polyps per g DW, of a diameter of 3-4 mm (Lewis 1982; Sorokin 1995). *Heteroxenia fuscescens* (Kölliker, 1874) have polyps of 12-14 mm (Lewis 1982). Members of the family Xeniidae exhibit a unique, rhythmic pulsation of their tentacles. Such feature induces an upward motion and enables the mixing of the coral boundary layer, which prevents refiltration of water by neighboring polyps and enhances the coral's photosynthesis (Kremien et al. 2013). *Litophyton* sp. (Forskål, 1775) has 550 polyps per g DW of a width and height of 0.5 mm (Ofwegen 2016). *Rhytisma fulvum fulvum* (Forskål, 1775) and *Sarcophyton* sp. (Lesson, 1834) have ca. 180-200 polyps per g DW of a diameter of 2.5-4 mm (Lewis 1982; Sorokin 1995).

Regarding hard corals, the branching species studies here, *Acropora eurystoma* (Klunzinger, 1979), *Pocillopora damicornis* (Linnaeus, 1758), *Seriatopora hystrix* (Dana, 1846) *and Stylophora pistillata* (Esper, 1792), have small polyps (less than 2 mm, Palardy et al. 2005). *Galaxea fascicularis* (Linnaeus, 1758) is particular due to its phaceloid shape. The mounding-shaped corals, *Goniastrea* sp. (Milne Edwards and Haime, 1848) and *Cynarina* sp. (Brüggemann, 1877) have large polyps (> 5mm).

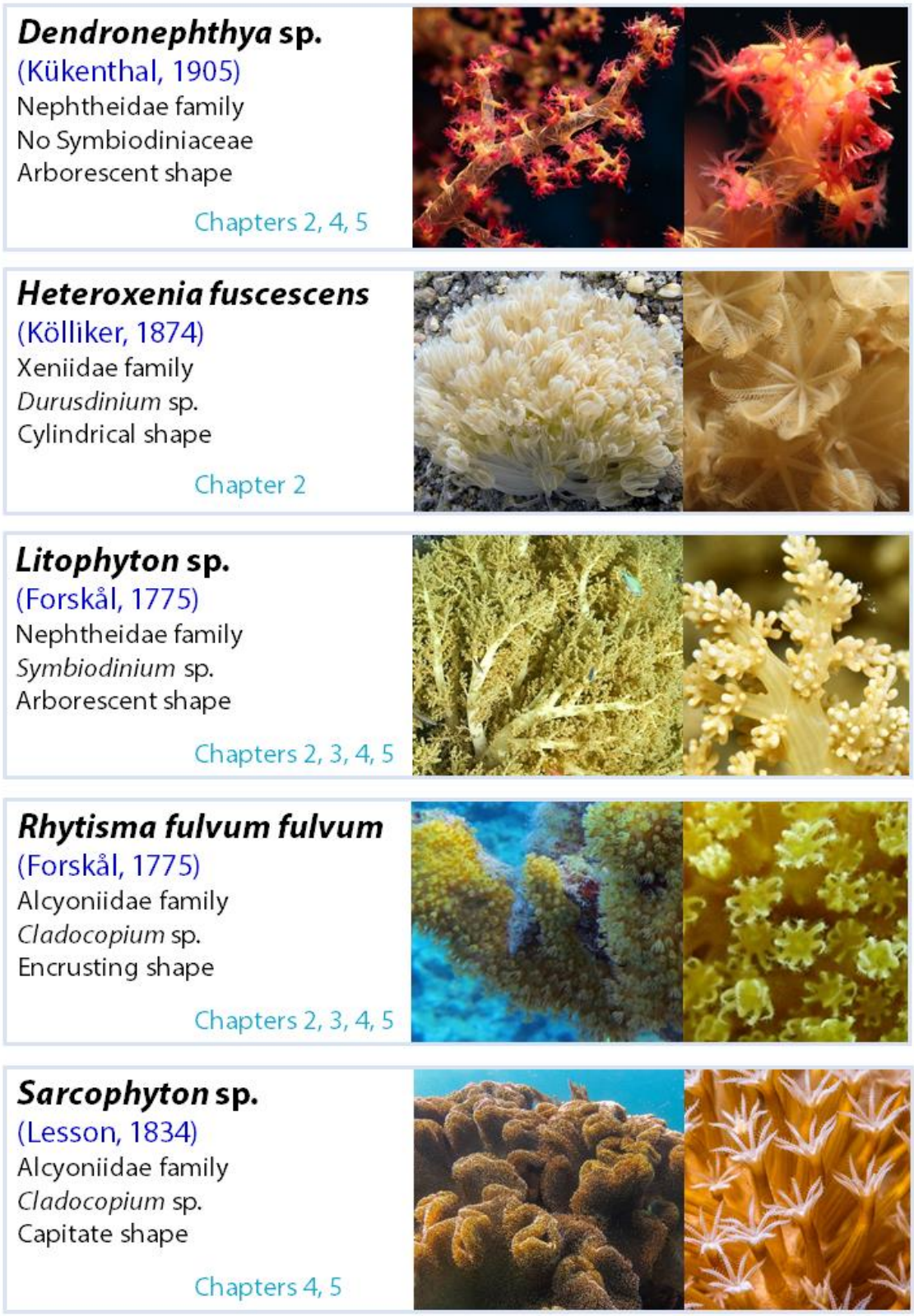


Figure 12. Soft corals used during this thesis. Corresponding family, Symbiodiniaceae associated with the coral and growth form of the colony are mentioned. Photo credits are attributed to M. Boussion for *Dendronephthya* sp. and *H. fuscescens* (right), G. Banc-Prandi for *Litophyton* sp. (left), P. Colla for *Sarcophyton* sp. (right), and C. Pupier for *H. fuscescens* (left), *R. f. fulvum* (left) and *Sarcophyton* sp. (left).

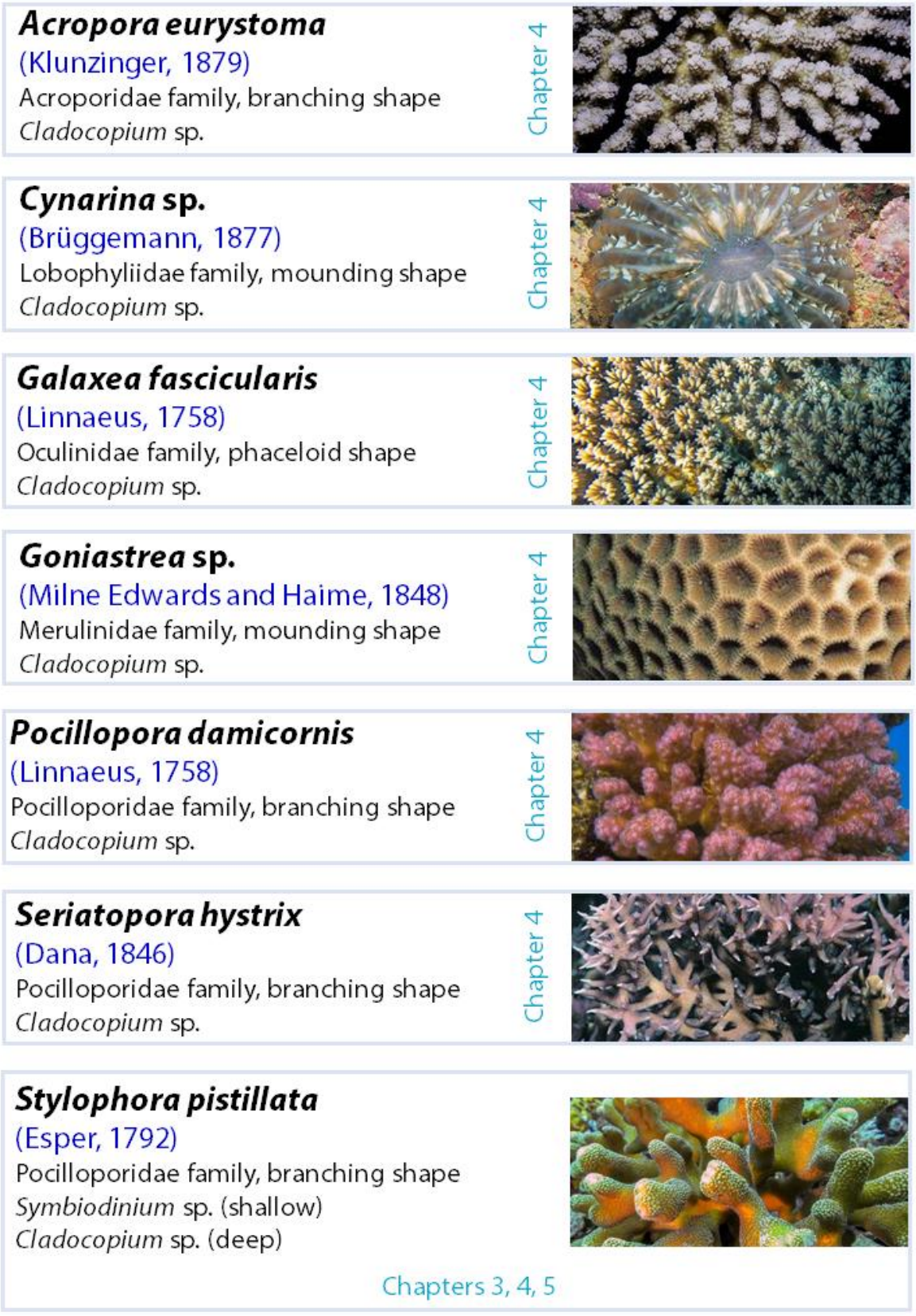


Figure 13. Hard corals used during this thesis. Corresponding family, growth form of the colony and Symbiodiniaceae associated with the coral are mentioned. Photo credits are attributed to E. Turak for *A. eurystoma*, N. Coleman for *Cynarina* sp., G. Paulay for *Goniastrea* sp., C. Veron for *S. hystrix*, and C. Pupier for *G. fascicularis*, *P. damicornis* and *S. pistillata*.

1.5 Aims of the thesis

1.5.1 Why a focus on soft corals?

As described in the above parts, octocorals are important components of reefs:

- They are the second main group of macrobenthic animals on reefs (part 1.2.2).
- They are ecosystem engineers, and thus provide essential habitat and food to reef-associated organisms (parts 1.1.2 and 1.2.3).
- They are often abundant within disturbed reef systems, where they can exhibit a higher survivorship than hard corals in a wide range of environmental conditions (part 1.3).

However, the accumulated data on the physiological characteristics of soft corals, or on their trophic ecology and relationship with endosymbionts is highly fragmented and not exhaustive (Schubert et al. 2017; van de Water et al. 2018). It does not help having a holistic view of the functional role and ecological importance of these organisms. Research on octocorals indeed lags well behind the research on hard corals (Figure 14) and important knowledge gaps related to their physiology, particularly to their nutrition, still need to be addressed.

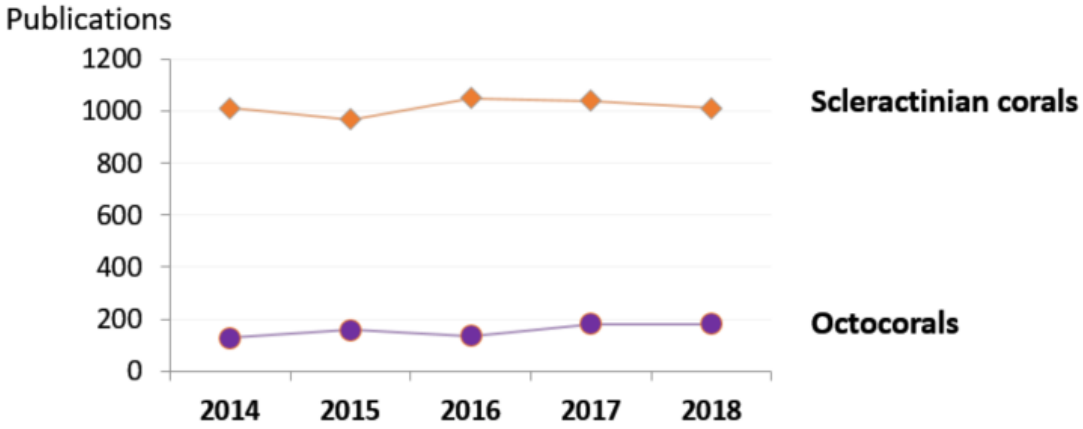


Figure 14. Number of publications obtained in a Google scholar search. Key words used are "Scleractinian corals" and "physiology" versus "Octocorals" and "physiology".

1.5.2 Aims of the thesis

Nutrition is one of the most important factors regulating the abundance and distribution of a community within an ecosystem, along with environmental conditions and interactions with other organisms (e.g., competition and predation). Particularly, nutrient acquisition supplies energy for coral growth, reproduction and can promote coral resistance or resilience to environmental changes.

In the oligotrophic reefs of Eilat (Gulf of Aqaba, northern Red Sea), two distinct communities of octocorals have been identified at shallow and upper-mesophotic depths. Interestingly, the upper-mesophotic octocoral community is characterized by a higher species richness, along with a predominance of symbiotic species. While these characteristics suggest an adequate light regime for coral's photosynthesis to occur there (Shoham and Benayahu 2017), the nutritional strategies of soft corals have received little attention so far.

This thesis therefore focuses on the functioning of the dinoflagellate-soft coral symbiosis and aims to bring insights into the nutritional interactions between different partners of the holobiont. The respective contributions of auto- and heterotrophy in the metabolic needs of soft corals along the depth gradient were of particular interest. For this purpose, the acquisition and allocation of several forms of carbon and nitrogen were assessed. Four objectives corresponding to the following chapters were identified to explore these nutrient fluxes under different environmental conditions and to compare them to processes already known in hard corals.

- Aim 1 - Chapter 2: Normalization metric and processing protocol

Normalization is a fundamental step in data treatment that will deeply affect the outcome of an experiment. Several problems have been identified for soft corals: 1) The gelatinous and leather-like nature of soft coral tissue do not allow an easy extraction of tissue descriptors, such as symbiont density, pigment and protein concentration, 2) the most widely used normalization metric in scleractinian corals, the skeletal surface area, is not relevant for soft corals since they do not possess any skeleton, 3) surface area of a nubbin is difficult to estimate due to their highly variable hydroskeleton, which can change size by several fold depending on environmental conditions, and 4) physiological parameters and processes of soft corals need to be compared with other coral species, such as scleractinians.

An experiment was thus designed to 1) develop a fast protocol for an easy tissue homogenization and 2) identify a normalization metric that can be used to perform inter-studies and inter-species comparisons.

For this purpose, the effect of different tissue sample states and media used for homogenization were tested on the measurement of tissue descriptors in the soft coral *Heteroxenia fuscescens*. Moreover, the suitability of several normalizing metrics was investigated in *Dendronephthya* sp., *Heteroxenia fuscescens*, *Litophyton arboreum* and *Rhytisma fulvum fulvum*.

- Aim 2 - Chapter 3: Acquisition of dissolved inorganic carbon

The autotrophic carbon acquisition contributes largely to the metabolic needs of hard corals, which receive 90% of the photosynthates produced by the symbionts. In octocorals, the contribution of the symbionts to the energetic requirements of their host is still poorly understood. First of all, studies addressing the productivity of soft corals are scarce, limited to a depth of 20 m, and the results obtained present a high variability (see supplementary information in Rossi et al. 2018). There are several identified sources for such variability: 1) soft coral colonies can reach various degrees of expansion depending on the environmental conditions, that confers a high biological variability to the measurements (e.g., Fabricius and Klumpp 1995); 2) the morphology of octocoral colony and polyps, notably their surface area to volume ratio, can be more or less suited for autotrophy (Baker et al. 2015; Rossi et al. 2018); and 3) the use of different methods and normalization metrics (see Chapter 2 for the latter) hardly enables comparisons. In addition, the quantification of photosynthate translocation from the symbionts to the host has been investigated in only one tropical (*Heteroxenia fuscecens*) and one temperate species (*Capnella gaboensis*) using ^{14}C (Schlichter et al. 1983; Farrant et al. 1987).

An experiment was conducted to estimate the importance of autotrophically acquired carbon for the host requirements in soft corals.

For this purpose, bicarbonate labeled with the stable isotope ^{13}C was used and its fate was traced within the host-symbionts association. We tested the effect of light availability

(depth) and Symbiodiniaceae genus on two soft coral species (*Litophyton* sp. and *Rhytisma fulvum fulvum*), sampled in the Red Sea at two depths (8 m and 40 m depth). These species harbor two different Symbiodiniaceae genera and different morphological shapes. The two depths tested enabled the assessment of the effect of light availability on carbon fluxes and the comparison with the carbon acquisition and allocation in the scleractinian coral *Stylophora pistillata* living at the same location and depths.

- Aim 3 - Chapter 4: Acquisition of dissolved inorganic/organic nitrogen

Nitrogen is considered as one of the limiting nutrients in the oceans due to the scarcity of its dissolved forms. As described in this chapter, corals can acquire almost all forms of dissolved nitrogen, the dinitrogen gas, the dissolved inorganic forms (DIN) such as ammonium and nitrate, as well as the dissolved organic forms (DON). In this thesis, we have tackled the assimilation of all these forms by soft corals, and compared the assimilation rates obtained with those measured in scleractinian coral species.

i) Diazotrophy

The most abundant dissolved N form, the dinitrogen, is only bioavailable to corals through the action of diazotrophs. Only two publications have investigated the rates of dinitrogen fixation in the presence of soft corals (e.g., Bednarz et al. 2015; Cardini et al. 2016). However, these studies have used the acetylene reduction assay, which only quantifies gross dinitrogen fixation occurring in the seawater without providing insights into who- among the seawater or coral associated microorganisms, the coral host or the symbionts, is actually assimilating the fixed nitrogen. Only the use of $^{15}N_2$ gas, allows tracing the fate of diazotrophically-acquired nitrogen (DDN) in the different compartments.

An experiment was thus performed to assess the importance of dinitrogen fixation for soft coral nutrition. The point arising from this objective notably questions whether soft corals are associated with active diazotrophs. The influence of the trophic status (i.e. strict heterotroph versus mixotroph) and increased heterotrophic capacity with depth on this process were tested. Moreover, a comparison with hard corals was undertaken.

This experiment was designed to measure net dinitrogen fixation and distinguish between the different compartments (i.e., seawater particles, coral tissue, dinoflagellate

symbionts) for the very first time in soft corals, using labelled $^{15}N_2$ gas. For this purpose, experiments were performed 1) in the laboratory, to assess whether soft and hard corals have the same capacities to assimilate DDN, and 2) to assess dinitrogen fixation in corals collected from the field. For 1), the soft *Sarcophyton* sp. and hard *Stylophora pistillata* corals were used. For 2) soft coral (*Dendronephthya* sp., *Litophyton* sp. and *Rhytisma fulvum fulvum*) and hard coral (*Acropora eurystoma*, *Pocillopora damicornis*, *Goniastrea* sp. and *Cynarina* sp.) species were sampled in the Red Sea at two depths (8-10 m and 40-45 m depth). These species harbor different Symbiodiniaceae genera (or not at all) and different morphological shapes. The depths investigated enable the assessment of the effect of increased heterotrophic capacity on dinitrogen fixation.

ii) Uptake of DIN and DON

A second experiment on nitrogen assimilation investigated the acquisition of dissolved inorganic and organic nitrogen by the soft coral-Symbiodiniaceae symbiosis. It aimed to determine which sources of dissolved nitrogen are acquired by soft corals. The effects of light availability (depth), Symbiodiniaceae genus and increased temperature were tested. As for the previous experiment, a comparison with hard corals was made to evaluate the efficiency of nitrogen assimilation among coral species.

This experiment was designed to measure the assimilation of NH_4^+, NO_3^-, and DFAA in soft and hard corals, using stable isotope labelling. For this purpose, several species of soft corals (*Litophyton arboreum*, *Rhytisma fulvum fulvum* and *Sarcophyton* sp.) and hard corals (*Galaxea fascicularis*, *Stylophora pistillata* and *Seriatopora hystrix*) were sampled in the Red Sea at two depths (8-10 m and 40-50 m depth). These species harbor different Symbiodiniaceae genera and present different morphological shapes.

- Aim 4 - Chapter 5: *In situ* acquisition of autotrophic and heterotrophic nutrients in soft corals

As corals have the ability to capture preys, heterotrophy can represent a significant acquisition of carbon, nitrogen and other nutrients. Literature on the diet of tropical soft corals is however very sparse (e.g., Lewis 1982; Fabricius et al. 1995a; Sorokin 1995) and the heterotrophic capacity of corals has been mostly estimated through laboratory studies, or studies undertaken with incubation chambers. The heterotrophic status of corals under in situ conditions is thus still poorly understood, in all coral species.

An experiment was designed to investigate the autotrophic and heterotrophic status of soft corals *in situ*, using their fatty acid composition. The effect of depth was tested and a comparison with a hard coral species was made.

For this purpose, the concentrations of fatty acids specific to autotrophy (stearidonic and docosapentaenoic acids) and heterotrophy (cis-gondoic acid) were measured in soft and hard corals collected in the field. Species investigated were *Dendronephthya* sp., *Litophyton* sp., *Rhytisma fulvum fulvum*, and *Sarcophyton* sp. for the soft corals and *Stylophora pistillata* for the scleractinian. They were collected in the Red Sea at two depths (8-10 m and 40-50 m depth). These species harbor different Symbiodiniaceae genera and different morphological shapes. The depths investigated enable the assessment of the effect of putative increased heterotrophic capacity.

Chapter **2** | Normalization metric and processing protocol

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Publication n°1

Studies with soft corals - recommendations on sample processing and normalization metrics

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Abstract

Soft corals (Octocorallia) often constitute the second most abundant macrobenthic group on many tropical and temperate reefs. However, the gelatinous and leather-like nature of their tissue and their variable hydroskeleton entails a number of problems for tissue homogenization and data normalization. An easy and fast protocol for tissue homogenization, as well as a normalization metric that can be used to perform inter-studies or inter-species comparisons, are thus needed. In this study, we tested whether the tissue sample state before processing (frozen vs freeze-dried samples) and the media used for tissue homogenization (0.2 µm filtered seawater; FSW vs milliQ water; DI) affects the quantitative measurements of tissue descriptors (chlorophyll, protein and *Symbiodinium* concentrations) in the model species *Heteroxenia fuscescens*. Furthermore, the suitability of dry weight (DW) and ash-free dry weight (AFDW) as size-normalizing metric was investigated across different soft coral species. Our results reveal that freeze-drying the samples and homogenizing them in DI water exhibited several benefits, namely enhancing chlorophyll and protein concentrations up to 50%, saving processing time and providing a more accurate determination of DW and AFDW. Overall, this optimized tissue processing protocol offers a more reliable quantification of tissue descriptors and reduces the chance of underestimating these parameters in soft corals. Finally, since the

contribution of sclerites to the total DW of the colony can highly differ between species, we demonstrate that AFDW is a reliable metric for normalizing soft coral data, particularly when inter-species comparisons are made.

2.1 Introduction

Soft corals (Octocorallia: Alcyonacea) represent, after reef-building scleractinian corals, the second most abundant macrobenthic group on many tropical and temperate reefs (Fabricius and Alderslade 2001). They notably become often dominant within disturbed reef systems (Inoue et al. 2013). High abundances of octocorals (soft corals or gorgonians) have thus been observed in Caribbean and Indo-Pacific eutrophicated reefs, where a reduced water quality does not favour the growth of scleractinian corals (Fabricius et al. 2005; Baum et al. 2016). Despite the fact that soft corals are increasingly recognized as key taxa on reefs, two major issues regarding physiological and ecological studies remain however to be clarified.

First, the coenenchyme (colonial tissue) of soft corals consists of thick gelatinous material containing fibres, amoeboid cells, scleroblast cells and calcareous sclerites (Fabricius and Alderslade 2001). Altogether, these leather-like tissue elements make cell layers difficult to break apart and tissue homogenization challenging. Therefore, measurements of tissue descriptors (*Symbiodinium*, chlorophyll and protein concentrations) are difficult to obtain from frozen or fresh soft coral samples, and a reliable and easy tissue homogenization protocol remains to be implemented.

Second, a common normalization metric to perform inter-species comparisons of physiological and/or ecological processes is required. For example, apprehending functional processes that occur at the reef-ecosystem level (e.g., primary production) implies to understand how soft corals operate over space and time compared to scleractinian corals. Comparative measurements related to these sub-classes are therefore necessary, but are only enabled if they are normalized to the same metric. Normalization of tissue parameters or physiological measurements requires a metric that reflects the size of the organisms and is stable across variable environmental conditions. In scleractinian corals, skeletal surface area is the most commonly used metric for normalizing data to the size of the colony (Edmunds and Gates 2002). However, soft corals lack a calcium carbonate skeleton and rely on hydrostatic pressure for body shape maintenance (Fabricius and Alderslade 2001). Although this hydroskeleton can vary over time depending on environmental fluctuations (Fabricius 1995; Hellström and Benzie

2011), few studies have approximated soft coral surface area based on geometric body measurements (e.g., Bednarz et al. 2012, 2015; Kremien et al. 2013) or linear projections (Fabricius and Dommisse 2000). Such normalization metric is critical when studying processes (e.g., photosynthesis) that strongly depend on a vectorial parameter (e.g., light) as it allows understanding the effectiveness of these processes (Kremien et al. 2013). For other measurements, such as the quantification of structural tissue descriptors or the production of metabolites, the variable size of the hydroskeleton excludes the possibility to accurately normalize to surface area at different time intervals (Haydon et al. 2018). Several other normalization metrics have thus been applied to soft corals and are summarized in Figure 15, Table 3 and Appendix I – Table S1.

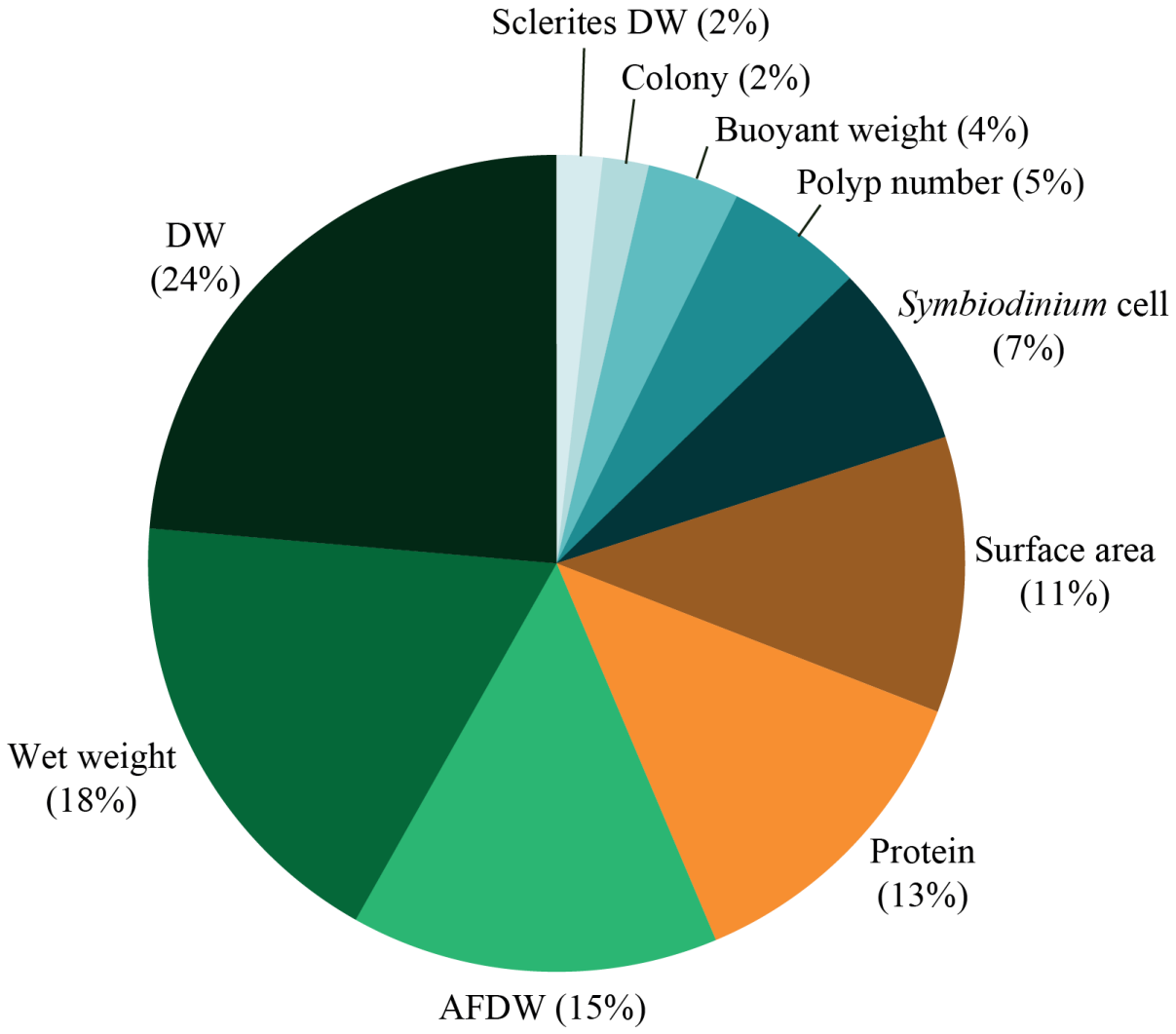

Figure 15. Normalization metrics used in physiological studies on soft corals. Percentages are based on the occurrence of the studies using the metric of interest. DW = dry weight. AFDW = ash-free dry weight.

Table 3. Summary about the range of metrics used in previous studies to normalize physiological processes in soft corals. AFDW = ash-free dry weight. DW = total dry weight. BW = buoyant weight. DMSP = dimethylsulfopropionate. P/A pigments = photosynthetic and accessory pigments. ETS = electron transport system. DOM = dissolved organic matter. The group 'biochemical composition of tissues' gathers measured parameters such as amino acid, protein, carbohydrate and/or lipid content. The asterisk notifies a study for which the normalizing metric (i.e., dry weight of decalcified samples) has been considered as ash-free dry weight by the authors.

Normalization metric	Family	Measured parameter	Reference
AFDW	Alcyoniidae	Biochemical composition of tissues, DMSP concentration, feeding rates, mucus production, nutrient flux, oxygen flux	(Fabricius and Klumpp 1995; Riegl and Branch 1995*; Migné and Davoult 1997a, b, 2002; Van Alstyne et al. 2006; Slattery et al. 2013)
	Briareidae	Oxygen flux	(Fabricius and Klumpp 1995)
	Nephtheidae	Feeding rates, oxygen flux	(Fabricius and Klumpp 1995; Fabricius et al. 1995a, 1998)
	Xeniidae	Oxygen flux	(Fabricius and Klumpp 1995)
DW	Alcyoniidae	Biochemical composition of tissues, caloric content, metabolite concentration, P/A pigments, *Symbiodinium* density	(Slattery and Mcclintock 1995; Ben-David-Zaslow and Benayahu 1998; Khalesi et al. 2007, 2009; Rocha et al. 2013b, b; Leal et al. 2014; Costa et al. 2016)
	Clavulariidae	Biochemical composition of tissues	(Slattery and Mcclintock 1995)
	Nephtheidae	Biochemical composition of tissues, inorganic carbon, oxygen flux, P/A pigments, S*ymbiodinium* density	(Farrant et al. 1987; Slattery and Mcclintock 1995)
	Xeniidae	Biochemical composition of tissues, caloric content, inorganic carbon, P/A pigments	(Schlichter et al. 1983, 1984; Ben-David-Zaslow and Benayahu 1998, 1999; Bednarz et al. 2012)
Sclerites DW	Alcyoniidae	Calcification rate	(Tentori and Allemand 2006)
Wet weight	Alcyoniidae	Biochemical composition of tissues, calcification rate, DMSP concentration, ETS activity, P/A pigments, S*ymbiodinium* density	(Michalek-Wagner and Willis 2001; Tentori and Allemand 2006; Van Alstyne et al. 2006; Khalesi et al. 2007; Imbs et al. 2010; Baum et al. 2016; Haydon et al. 2018)
	Anthothelidae	DMSP concentration	(Haydon et al. 2018)

	Nephtheidae	Biochemical composition of tissues, calcification rate, cell growth, DMSP concentration, ETS activity, inorganic carbon, oxygen flux, P/A pigments, S*ymbiodinium* density	(Farrant et al. 1987; Tentori et al. 2004; Baum et al. 2016; Haydon et al. 2018)
	Xeniidae	Biochemical composition of tissues, DOM flux, inorganic carbon	(Schlichter 1982a)
BW	Alcyoniidae	Budding rate, energy expenditure, oxygen flux	(Khalesi et al. 2009, 2011)
Colony	Xeniidae	Oxygen flux	(Kremien et al. 2013)
Polyp number	Nephtheidae	Feeding rates, oxygen flux, P/A pigments	(Fabricius et al. 1995a, b, 1998)
Protein	Alcyoniidae	Calcification rate, DMSP concentration, P/A pigments, S*ymbiodinium* density	(Tentori and Allemand 2006; Khalesi et al. 2009; Haydon et al. 2018)
	Anthothelidae	DMSP concentration	(Haydon et al. 2018)
	Nephtheidae	Calcification rate, cell growth, DMSP concentration	(Tentori et al. 2004; Haydon et al. 2018)
	Xeniidae	Metabolite concentration, oxygen flux, P/A pigments, symbiodinium density, uptakes of nutrients	(Zeevi Ben-yosef et al. 2006; Gabay et al. 2013; Ezzat et al. 2016)
Surface area	Alcyoniidae	Oxygen flux, S*ymbiodinium* density	(Drew 1972; Bednarz et al. 2015; Cardini et al. 2016)
	Nephtheidae	Dinitrogen fixation, inorganic carbon, oxygen flux, P/A pigments, *Symbiodinium* density	(Farrant et al. 1987; Bednarz et al. 2015; Cardini et al. 2016)
	Xeniidae	Dinitrogen fixation, OM flux, oxygen flux	(Bednarz et al. 2012, 2015; Kremien et al. 2013; Cardini et al. 2016)
***Symbiodinium* cell**	Alcyoniidae	DMSP concentration, P/A pigments	(Van Alstyne et al. 2006; Gabay et al. 2013; Rocha et al. 2013a, b)
	Xeniidae	P/A pigments	(Gabay et al. 2013)

Among these metrics, those involving wet weight and buoyant weight may also be sources of error as soft coral tissue withholds a lot of water and the density of a colony may be similar to that of seawater (Davies 1989). For example, normalization of dimethylsulphoniopropionate (DMSP) production to soft coral fresh weight (Van Alstyne et al. 2006) led to significant lower values compared to the normalization to protein content, underestimating the functional role of soft compared to hard corals (Haydon et al. 2018). Coral biomass may therefore offer a greater potential to accurately reflect the size of a soft coral colony. Protein content has been widely used to normalize physiological data in corals. However it can encounter great variability across environmental conditions, and may thus interfere with the intrinsic pattern of the parameter of interest (Edmunds and Gates 2002). Ash-free dry weight (AFDW) is another common proxy for tissue biomass of many benthic reef organisms such as scleractinian corals, coralline algae, sponges (Kötter and Pernthaler 2002; Schoepf et al. 2013; Comeau et al. 2014) and soft corals (Figure 15). AFDW quantification relies on a completely destructive method, whereas dry weight (DW) allows for potential analysis of tissue parameters before burning the sample. Although tissue energy reserves (i.e., lipid, protein and carbohydrate) of soft corals have been determined from dried material (Ben-David-Zaslow and Benayahu 1999), the possibility to obtain reliable measurements involving *Symbiodinium* (cell density and chlorophyll content) still needs to be tested.

Based on these issues the present study aimed 1) to optimize a protocol for soft coral tissue processing and 2) to find the normalization metric most relevant for physiological experiments involving interspecies comparisons. We tested whether the tissue sample state before processing (frozen vs freeze-dried samples) and the media used for tissue homogenization (0.2 µm filtered seawater; FSW vs milliQ water; DI) affects the quantitative measurements of tissue descriptors in the model species *Heteroxenia fuscescens* (Ehrenberg, 1834). Furthermore, the suitability of DW and AFDW as size-normalizing metric was investigated across different soft coral species (*H. fuscescens*, *Dendronephthya* sp. (Kuekenthal, 1905), *Litophyton arboreum* (Forskål, 1775) and *Rhytisma fulvum* (Forskål, 1775), which are key components of Red Sea reefs. These species particularly exhibit different growth forms ranging from arborescent (*Dendronephthya* sp. and *L. arboreum*) to capitate (*H. fuscescens*) and encrusting (*R. fulvum*).

2.2 Materials and methods

2.2.1 Biological material

Nineteen colonies of *H. fuscescens* with 20 polyps and four colonies of *Dendronephthya* sp., *L. arboreum* and *R. fulvum*, collected in the Red Sea were used for the experiments. They were equally allocated to five 20 L tanks and maintained under the same conditions for eight weeks prior to starting the experiments. Tanks were continuously supplied with seawater, at a renewal rate of 10 L h^{-1}. HQI lamps (Tiger E40, Faeber, Italy) above the tanks provided photosynthetically active radiation (PAR) of 200 µmol photons m^{-2} s^{-1}.

2.2.2 Optimized protocol for *Symbiodinium*, chlorophyll and protein determination

A first experiment, performed with *H. fuscescens*, aimed to compare *Symbiodinium*, chlorophyll and protein concentrations in frozen or freeze-dried samples, homogenized in FSW or DI. Four combinations were thus considered, with 5 replicates per condition: (i) freeze-dried samples homogenized in FSW, (ii) freeze-dried samples homogenized in DI, (iii) frozen samples homogenized in FSW and (iv) frozen samples homogenized in DI. For this purpose, 20 tubes were prepared, each containing one freshly cut polyp of ten colonies of *H. fuscescens* randomly chosen in the tanks. All tubes were flash-frozen in liquid nitrogen. Half of them were processed directly whereas the other half was freeze-dried (Alpha 2-4 LDplus freeze-dryer, Martin Christ, Germany), until obtaining a dried powder. Frozen and freeze-dried samples were ground with a potter tissue grinder and solubilized in 10 mL FSW or DI. This procedure was rapid for the freeze-dried samples (5 minutes) but took more than 30 minutes for the frozen samples. From each homogenate, 500 µL was collected for total protein measurement (see following section). The remaining homogenate was centrifuged for 10 min (at 11,000 g at 4°C) to pellet the *Symbiodinium*. Light microscopy confirmed the total removal of *Symbiodinium* from the supernatant (i.e., host fraction), which was freeze-dried again. This was done to determine the host DW and to subsequently compare the tissue parameters of frozen and freeze-dried samples normalized to the same metric. *Symbiodinium* pellets were rinsed twice to eliminate any remaining host cells (Tremblay et al. 2012), and re-suspended in 10 mL FSW or

DI. After mixing, 100 µL and 5 mL were subsampled for the determination of *Symbiodinium* density and total chlorophyll concentration, respectively (see following section).

2.2.3 Tissue parameter measurements

Proteins were extracted from the 500 µL subsamples in a sodium hydroxide solution (0.5 M) for 5 h at 60°C. Thereafter, protein content was measured using a BC assay kit (Interchim, France) (Smith et al. 1985), and protein standards were prepared using bovine serum albumin (Interchim, France). Absorbance of subsamples was measured at 562 nm by an UVmc² spectrophotometer (SAFAS, Monaco). *Symbiodinium* density was quantified microscopically via eight replicate haemocytometer counts in the 100 µL subsamples (Neubauer haemocytometer, Marienfeld, Germany). For chlorophyll analysis, the 5 mL subsamples were centrifuged for 10 min (at 8,000 g at 4°C). The supernatant was discarded and 5 mL of acetone (100%) amended with magnesium chloride (Sigma Aldrich, Germany) were added to the *Symbiodinium* pellet to extract the chlorophyll over 24h in the dark (at 4°C). After centrifugation at 11,000 g for 15 min at 4°C, absorbances were measured at 750 nm, 663 nm and 630 nm using a Xenius spectrophotometer (SAFAS, Monaco) and chlorophyll concentrations calculated according to (Jeffrey and Humphrey 1975).

2.2.4 Normalization parameters

An experiment was performed with *H. fuscescens* to highlight any correlation between biomass (DW), protein content and colony's body volume. The body volume of 5 colonies with different polyp numbers was estimated using the water displacement technique (Benayahu and Loya 1986), before colonies were freeze-dried and homogenized in 10 mL DI. A subsample of 500 µL was used for protein content determination. We also compared the DW and ash weight (AW) of the four different coral species listed above. For this purpose, colonies of each species were freeze-dried, crushed and split in five powder heaps of different weights (DW). They were combusted at 450°C for 4 h in a muffle furnace (Thermolyne 62700, Thermo Fischer Scientific, the United States). Such combustion process avoids losses of sclerites (Harvell and Fenical, 1989) and allows estimating the DW of sclerites.

2.2.5 Statistical analyses

Analyses were performed using R software (R Foundation for Statistical Computing) and assumptions of normality and homoscedasticity of variance were evaluated through Shapiro's and Bartlett's tests along with graphical analyses of residuals. Protein, *Symbiodinium* and chlorophyll concentrations were analyzed using a two-way ANOVA, in which the sample state (frozen vs freeze-dried) and type of water (FSW vs DI) were fixed effects. Regression equations were established between colony DW and protein content, volume or AW. Pearson's product moment correlation coefficients were calculated to test the strength of the linear associations.

2.3 Results and discussion

This study shows that both the sample state (i.e., frozen and freeze-dried) and the homogenization media (i.e., DI and FSW) significantly influence the quantification of tissue parameters in soft corals, while no effect was observed for *Symbiodinium* counts (Figure 16 and Table 4). Sample state mainly affects chlorophyll determinations with two-fold higher concentrations in freeze-dried as compared to frozen samples (Figure 16b). A previous work on reef sands also demonstrated a 27 to 39 % increased chlorophyll concentration in freeze-dried over frozen samples, likely due to the removal of mucous matrices surrounding the cells and a better exposure of pigments to the solvent in the former (Hannides et al. 2014). Here, the reduced chlorophyll concentration in frozen samples likely results from pigment damage due to the longer homogenization period of frozen fragments (30 min) as compared to the freeze-dried fragments (5 min). Although samples were kept on ice during homogenization, this caution did not prevent tissue slurry from mechanic heating with the Potter grinder. Similarly, (Hannides et al. 2014) highlighted that longer sonication periods caused a significant decline in chlorophyll content, due to pigment degradation (Metaxatos and Ignatiades 2002). Variations in the length of the homogenization procedure can occur for coral samples with different tissue thickness, and may amplify variance in the chlorophyll measurements. Future studies on corals should thus take into account such tissue processing flaws and compare the efficacy of the usual tissue extraction procedure with the freeze-drying one. Care must also be taken when comparing studies that use photosynthetic pigments as normalization metric for other parameters (e.g, coral productivity). As highlighted in this study, a 50% reduced photosynthetic pigment extraction in

frozen as compared to freeze-dried samples can lead to a significant overestimation of the productivity in the former, an issue that can be extended to scleractinian corals too. Finally, saving time is another benefit when processing freeze-dried over frozen samples, which is important in experiments involving numerous samples. Although the homogenization media has an overall low effect on tissue processing, the use of DI compared to FSW significantly increases the protein content measurable in tissue samples (Figure 16c and Table 4). This could have important implications when comparing physiological processes across different coral species and environmental conditions. The use of DI also reduces the amount of salts in homogenates, thereby providing a higher accuracy for the determination of absolute host and *Symbiodinium* DW as well as avoiding salt crystals entering instruments such as mass spectrometers (Baker et al. 2015).

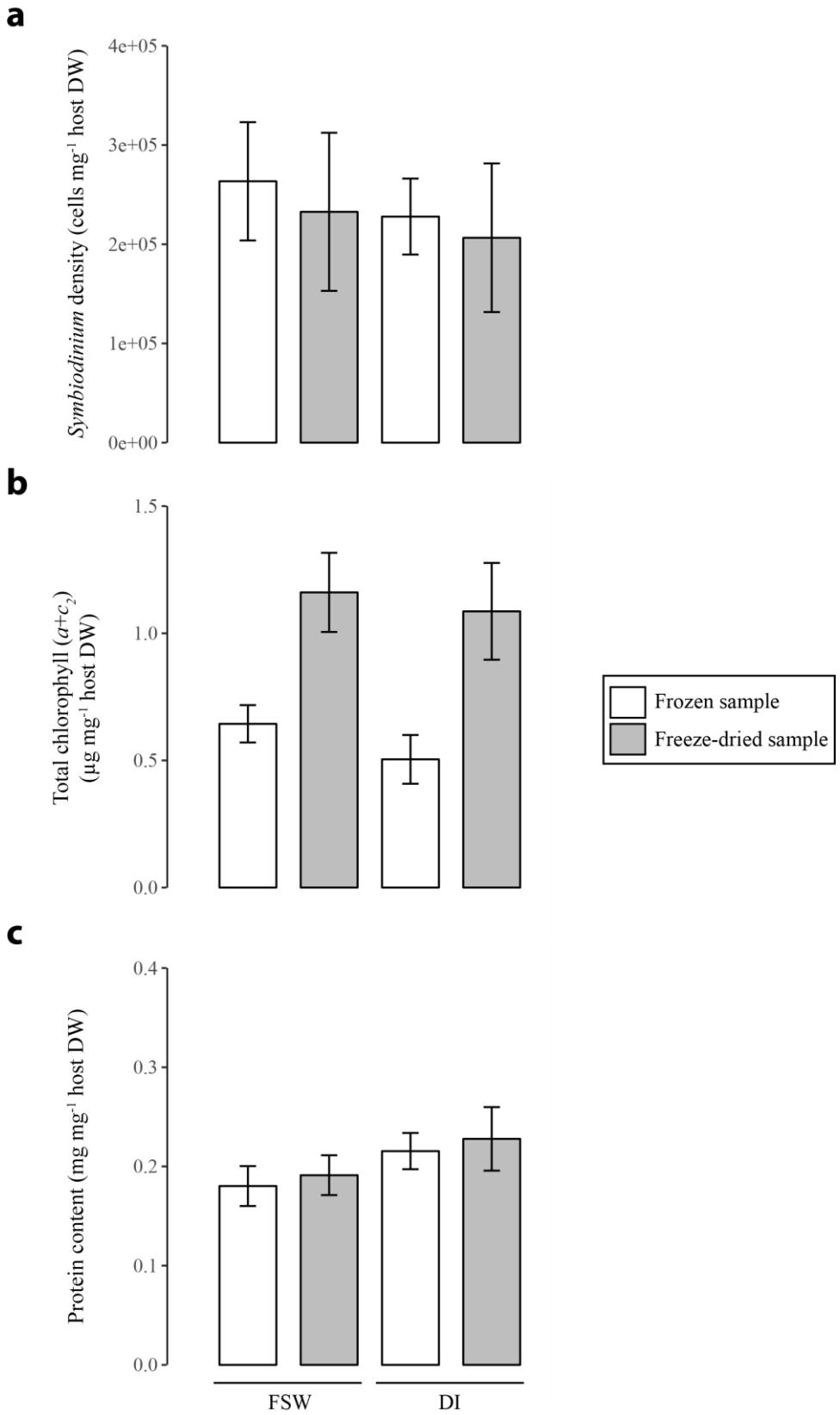

Figure 16. Tissue parameter quantification in soft corals using different sample processing techniques. Samples were either processed frozen or after freeze-drying and homogenized in filtered seawater (FSW) or MilliQ water (DI). All tissue parameters (Symbiodinium density, (a); chlorophyll, (b) and protein content, (c)) are normalized to host dry weight. Error bars represent standard deviation of five samples.

Table 4. Statistical results about the effect of sample state and homogenization media on Symbiodinium density, total chlorophyll and protein concentrations measured in *Heteroxenia fuscescens*.

Factor	df	*F* values	*p* values
Symbiodinium density			
Sample state	1	0.801	0.384
Homogenization media	1	1.121	0.305
Sample state × homogenization media	1	0.026	0.873
Residuals	16		
Total chlorophyll concentration			
Sample state	1	80.478	<0.01
Homogenization media	1	3.058	0.100
Sample state × homogenization media	1	0.284	0.602
Residuals	16		
Protein concentration			
Sample state	1	1.251	0.280
Homogenization media	1	11.925	<0.01
Sample state × homogenization media	1	0.005	0.947
Residuals	16		

As highlighted in the introduction, a literature review identified 36 physiological studies on soft corals, which display a wide variety of normalization metrics (Figure 15, Table 3 and Table S1). Although the choice of the metrics certainly depends on the process under study and the information provided, a consensual normalization metric is important to allow better comparability of results among studies. For a given soft coral species maintained under a constant environmental condition, all metrics can be proxies for biomass and colony size. Results obtained with *H. fuscescens* indeed exhibit significant linear relationships between total DW and body volume or protein concentration (Figure 17a and Table 5). Other studies have also shown significant correlations between AFDW or DW and polyp number, colony height, or carbon and nitrogen content (Migné and Davoult 1993; Fabricius et al. 1995a; Migné et al. 1996). However, the use of several metrics listed above can be unsatisfactory when comparing processes between different species or different environmental conditions. We demonstrate that DW does not provide a reliable normalization metric to compare tissue parameters across coral species (Figure 17b and Table 5), although dried samples enable better tissue processing in soft corals. Linear regression analyses between DW and AW of different soft coral species show species-specific linear relationships; this is due to the presence of sclerites, which can differ in size, shape and abundance within a colony and between species (Van Alstyne et al. 1992).

Sclerites are frequently used as taxonomic indicator, and species-specific differences likely depend on the necessity to accumulate sclerites for structural defence against predators and/or skeletal support against wave action (Van Alstyne et al. 1992). Overall, our results show that the contribution of sclerites to the total DW of the colony can highly differ between species, and thus, we recommend the use of AFDW over DW for normalizing physiological processes in soft corals, particularly when inter-species comparisons are made.

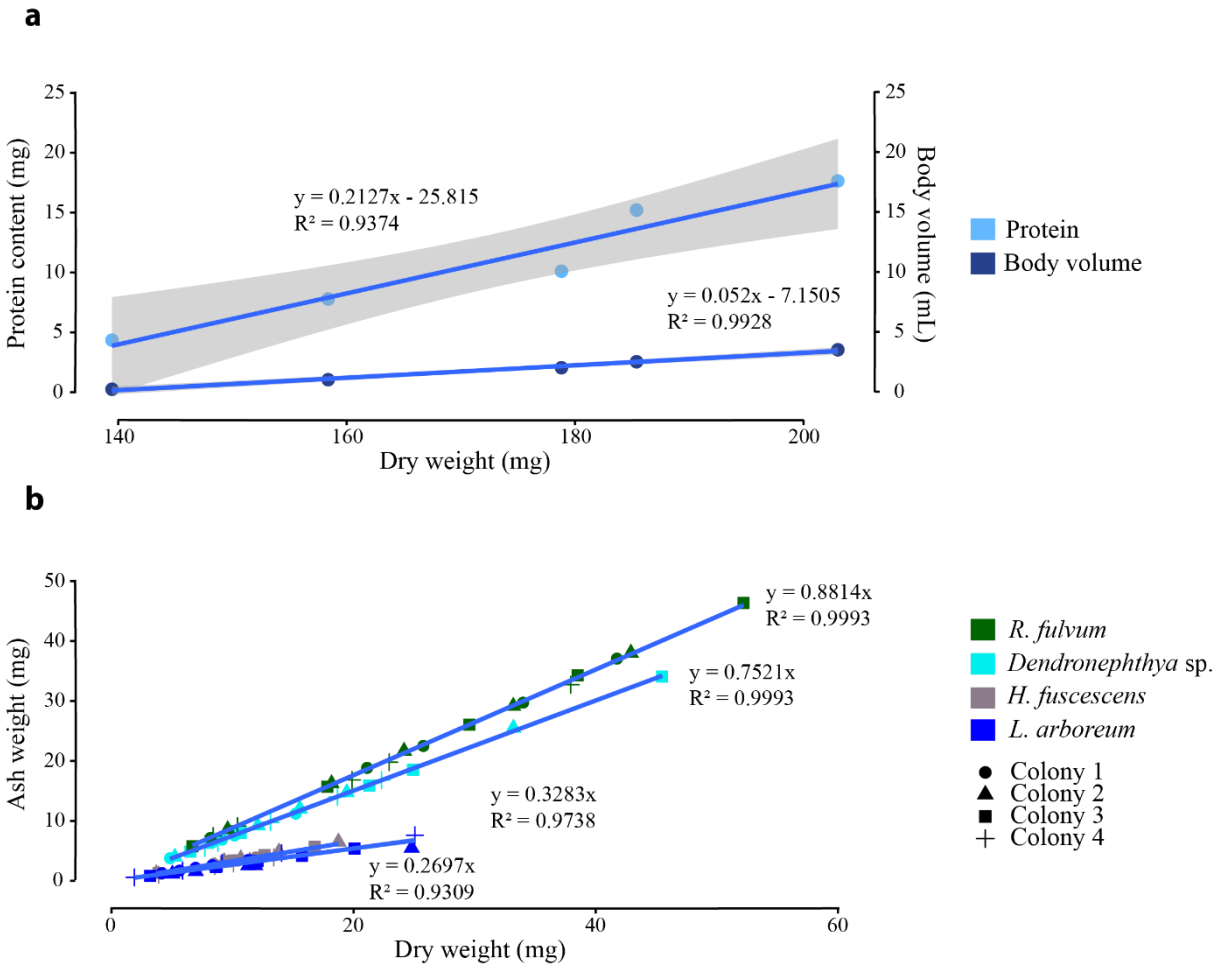

Figure 17. Correlations between the normalizing metrics. (a) Total dry weight with protein content and body volume of five *Heteroxenia fuscescens* colonies. (b) Linear regressions between total dry weight and ash of four colonies of *Rhytisma fulvum*, *Dendronephthya* sp., *H. fuscescens* and *Litophyton arboreum.* Grey areas display confidence interval of 0.95 around the regression lines.

Table 5. Significant correlations between dry weight (DW), protein content, body volume and ash weight (AW) of *Heteroxenia fuscescens*, *Rhytisma fulvum*, *Dendronephthya* sp. and *Litophyton arboreum*.

Species	Tested correlation	R	*t*	df	*p* values
H. fuscescens	DW × protein content	0.9682	6.7005	3	<0.01
	DW × body volume	0.9964	20.341	3	<0.01
R. fulvum	DW × AW	0.9997	161.28	18	<0.01
Dendronephthya sp.	DW × AW	0.9996	157.53	18	<0.01
H. fuscescens	DW × AW	0.9868	25.866	18	<0.01
L. arboreum	DW × AW	0.9648	15.575	18	<0.01

In conclusion, we recommend to dry the biological material for biochemical and tissue parameter analyses and to use a subset of the dried powder for AFDW determination as final normalization metric (Figure 18). AFDW stands for a useful metric to normalize tissue parameters in soft corals, benefiting of the aforementioned advantages owed from its freeze-dried state and allowing best comparability among species. Other non-destructive normalization metrics, such as surface area, can however be used in parallel to provide a different information on the physiological processes studied.

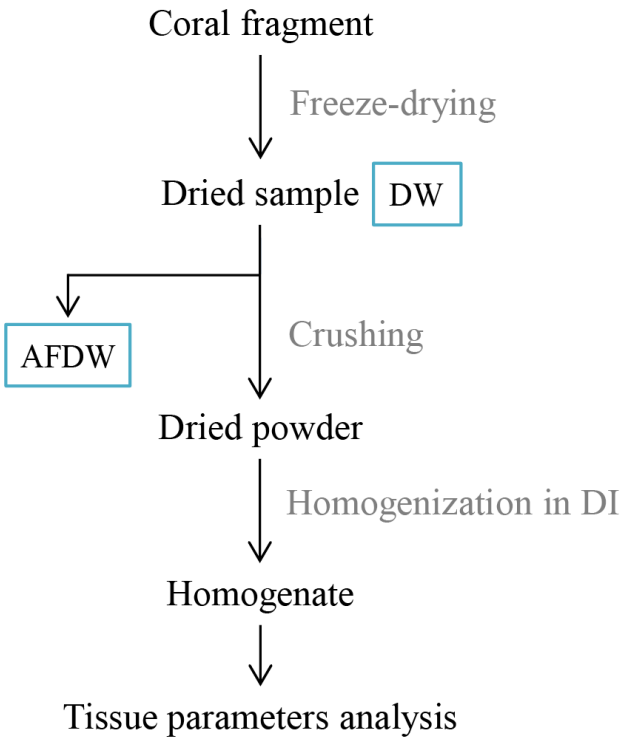

Figure 18. Optimized workflow for processing soft coral tissue samples. DW = dry weight. AFDW = ash-free dry weight. DI = MilliQ water.

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Chapter **3** | Acquisition of dissolved inorganic carbon

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Publication n°3

Productivity and carbon fluxes depend on species and symbiont density in soft coral symbioses

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Abstract

Soft corals often constitute one of the major benthic groups of coral reefs. Although they have been documented to outcompete reef-building corals following environmental disturbances, their physiological performance and thus their functional importance in reefs are still poorly understood. In particular, the acclimatization to depth of soft corals harboring dinoflagellate symbionts and the metabolic interactions between these two partners have received little attention. We performed stable isotope tracer experiments on two soft coral species (*Litophyton* sp. and *Rhytisma fulvum fulvum*) from shallow and upper mesophotic Red Sea coral reefs to quantify the acquisition and allocation of autotrophic carbon within the symbiotic association. Carbon acquisition and respiration measurements distinguish *Litophyton* sp. as mainly autotrophic and *Rhytisma fulvum fulvum* as rather heterotrophic species. In both species, carbon acquisition was constant at the two investigated depths. This is a major difference from scleractinian corals, whose carbon acquisition decreases with depth. In

addition, carbon acquisition and photosynthate translocation to the host decreased with an increase in symbiont density, suggesting that nutrient provision to octocoral symbionts can quickly become a limiting factor of their productivity. These findings improve our understanding of the biology of soft corals at the organism-scale and further highlight the need to investigate how their nutrition will be affected under changing environmental conditions.

3.1 Introduction

Mutualistic symbioses between cnidarians and photosynthetic dinoflagellates of the family Symbiodiniaceae are widespread in marine environments (Venn et al. 2008). In coral reef ecosystems, this association is the backbone for the growth and survival of corals surrounded by oligotrophic waters that hardly provide any exogenous nutrients. Indeed, the dinoflagellate symbionts of scleractinian corals translocate a large proportion of their photosynthetically fixed carbon compounds to the coral host for its own nutrition, growth, reproduction and energetic needs (Muscatine et al. 1984). The percentage of carbon translocation can be as high as 90% in well-lit shallow waters (Falkowski et al. 1984; Tremblay et al. 2012), and can remain high in mesophotic environments (Ezzat et al. 2017). However, the total amount of autotrophically-acquired carbon generally decreases from shallow to deep reef environments through a reduced productivity of dinoflagellate symbionts (Ezzat et al. 2017). Simultaneously, the coral host becomes more dependent on heterotrophic food sources and it is the plasticity between host heterotrophy and symbiont autotrophy that allows the association to thrive in such contrasting reef environments (Houlbrèque and Ferrier-Pagès 2009). So far, our understanding of coral holobiont performance and host-symbiont metabolic interactions under different environmental conditions is limited to studies on scleractinian corals, while there is very little information available for other prominent members of reef systems such as soft corals (Alcyonacea).

Soft corals often constitute the second major benthic group of reef ecosystems (Schubert et al. 2017). They can thrive with relative high abundance and diversity under very different environmental conditions ranging from turbid to clear-water (Fabricius and Dommisse 2000; Fabricius and De'ath 2008) or from shallow to mesophotic reef environments (Shoham and Benayahu 2017). Similarly to scleractinian corals, a large proportion (≥ 50%) of soft coral taxa is associated with Symbiodiniaceae in the Eastern Pacific, Caribbean, Red Sea and Great Barrier Reef (Schubert et al. 2017). There is evidence showing that the abundance of soft coral

populations maintained or increased in most regions worldwide whereas scleractinian coral cover generally declined over the last decades (Stobart et al. 2005; Lenz et al. 2015), due to increased sea surface temperatures, eutrophication and pollution. This is usually explained by a greater nutritional plasticity of soft corals, which are considered as mixotrophic species (Fabricius and Klumpp 1995). They can therefore acquire nutrients through the autotrophic activity of the symbionts, but also through the heterotrophic capacity of the host (Fabricius and Klumpp 1995; Fabricius and Dommisse 2000). Evidence in the literature demonstrates that feeding has a positive effect on coral tissue, enhancing the growth of both partners of the symbiosis (Ferrier-Pagès et al. 2011). In addition, feeding can play a central role in maintaining physiological function when autotrophy is reduced (Anthony et al. 2009). The lower dependency of soft corals on autotrophy, compared to scleractinian corals, has been deduced from few measurements performed in shallow water conditions (0-20 m) (Fabricius and Klumpp 1995; Ramsby et al. 2014; Baker et al. 2015; Schubert et al. 2017; Rossi et al. 2018). Therefore, knowledge gaps exist for the main autotrophic physiological processes in soft corals thriving under different environmental conditions. The few studies which have dealt with their carbon budget observed highly variable contributions of photosynthetically fixed carbon provided by the symbionts to the host, with carbon translocation rates ranging from 10% in *Capnella gabonensis* to 75% in *Sinularia flexibilis* (Schlichter et al. 1983; Farrant et al. 1987; Sorokin 1991; Khalesi et al. 2011). In addition, different normalization metrics for physiological processes in soft and scleractinian corals often hinder comparisons of the trophic characteristics between the two groups.

While tropical soft corals are considered reef engineers which create habitats for other reef species (Benayahu 1985; Schubert et al. 2017), little comprehensive data exist on their trophic ecology, and therefore on their functional role as primary producers and carbon sinks within the reef ecosystem (Fabricius and Klumpp 1995; Fabricius et al. 1995a; Fabricius and Dommisse 2000; Bednarz et al. 2015). Since autotrophic carbon acquisition is a key factor shaping coral productivity, physiology, and ecology, and partly explains coral success or failure under changing environmental conditions, more studies should be dedicated to better understand the autotrophic capacity of soft corals. Therefore, the present study aims to assess the carbon fluxes between dinoflagellate symbionts and host of two common soft coral species (*Litophyton* sp. and *Rhytisma fulvum fulvum*) from shallow and mesophotic reefs of the Gulf of Eilat. For this purpose, we used stable isotope tracers (^{13}C-bicarbonate) to measure the rates of carbon fixed, exchanged and lost by the shallow and mesophotic symbiotic associations under their natural photosynthetically active irradiance (PAR) levels. Importantly, we also compare

our results with those of scleractinian coral species by applying a standardized normalization metric for all parameters (Haydon et al. 2018; Pupier et al. 2018).

3.2 Material and methods

3.2.1 Soft coral collection and experimental setup

The study was conducted in November 2017 at the Inter-University Institute for Marine Science (IUI), Gulf of Eilat. Experiments were performed with two soft coral species harboring dinoflagellate symbionts, *Litophyton* sp. (Forskål, 1775) and *Rhytisma fulvum fulvum* (Forskål, 1775) belonging to the Alcyoniidae family.

Ten colonies per species were sampled on the reef adjacent to the IUI (29°51'N, 34°94'E) at 8 m and 40 m depth, respectively, to generate a total of 40 nubbins per species (two nubbins per colony). Nubbins were allowed to recover for ten days in open-water cages placed at the original collection site before being brought back to the Red Sea Simulator facility (Bellworthy and Fine 2018). There, nubbins were allocated to three different outside aquaria per species and depth. All aquaria were continuously supplied with water directly pumped from the reef and were exposed to the natural diurnal light cycle. Furthermore, aquaria were shaded with several layers of mesh clothes to adjust daily maximum light levels to the maximum irradiance measured at the corresponding depth. Thus, all nubbins were maintained under natural diurnal variation in irradiance and received the depth-corresponding *in situ* maximum irradiance at noon. Levels of photosynthetically active radiation (PAR) were obtained from the Israel National Monitoring program of the Gulf of Eilat (http://www.iui-eilat.ac.il/Research/NMPMeteoData.aspx). During the five days of our experiment, surface water PAR reached 1200 µmoles photon m^{-2} s^{-1} at midday (Appendix II - Figure S1). The PAR received at 10 m and 40 m depth peaked at ca. 550 µmoles photon m^{-2} s^{-1} and 50 µmoles photon m^{-2} s^{-1} respectively, considering attenuation coefficients of 0.072-0.1 m^{-1} at this precise location (Akkaynak et al. 2017; Tamir et al. 2019). These values are in agreement with one-off measurements performed with a data logger during coral collection. The *in situ* seawater temperature (24°C) and nutrient concentrations (< 0.5 µM dissolved nitrogen, and < 0.2 µM dissolved phosphorus) were stable throughout the investigated depth gradient and period of time (http://www.iui-eilat.ac.il/Research/NMPMeteoData.aspx). Coral nubbins were maintained for one day under these conditions before the following measurements were performed in the outside aquaria under exactly the same environmental conditions.

3.2.2 $NaH^{13}CO_3$ incubations

The rates at which carbon was fixed by the shallow and mesophotic coral colonies under their natural PAR levels were estimated using ^{13}C- labelled bicarbonate according to Tremblay et al. (2012). To take into account variation in PAR levels during the day, corals were incubated for 5h, between 10 am and 3 pm, to cover the maximal daily irradiance (Stambler 2006) (Appendix II - Figure S1). For each species and depth condition, 10 coral nubbins, from 10 different colonies were placed in individual beakers filled with 200 mL FSW enriched with 0.6 mM $NaH^{13}CO_3$ (98 atom % ^{13}C, #372382, Sigma-Aldrich, St-Louis, MO, USA). After this "pulse" period of 5h, half of the corals were sampled and stored frozen at -20°C until further analysis (T_0). The other half was transferred into 200 mL of non-enriched FSW for a chase period of 19 h (T_{24}). The determination of $\%^{13}C$ enrichment, and total carbon content in the symbionts and host compartments were performed with a Delta plus Mass spectrometer coupled to a C/N analyser (Thermo Fischer Scientific, Bremen, Germany). The natural isotopic abundance of each species at each depth was determined from the corals used for the respiration measurements. Detailed calculations are described in (Tremblay et al. 2012). Data were normalized to ash-free dry weight of the organisms (Pupier et al. 2018).

3.2.3 Physiological and tissue descriptor measurements

Estimation of the dark respiration rates of the whole colony, as well as of the isolated symbionts, was needed for the establishment of the daily carbon budget. Dark respiration rates were measured on four nubbins per species and depth (from four different colonies), as well as on freshly isolated symbionts from four other nubbins according to Tremblay et al. (2012). Dark-acclimated nubbins/symbionts were individually placed in stirred incubation chambers filled with 0.45 µM filtered seawater and maintained at 24°C. Changes in dissolved oxygen were monitored over 30 minutes using optodes connected to an Oxy-4 (PreSense, Regensburg, Germany). Oxygen fluxes were converted into carbon equivalents (R_C) (Tremblay et al. 2012). The autotrophic carbon acquisition to respiration ratios (P_C/R_C) were estimated considering a maximal autotrophic carbon acquisition rate for 6h, and a half rate for the remaining 6h. At the end of the incubations, nubbins were flash-frozen in liquid nitrogen, freeze-dried, weighed for the total DW determination and processed as described in Pupier et al. (2018) (Appendix II) for the further determination of the symbiont density, total chlorophyll concentration and ash-free

dry weight (AFDW). The genus of the symbionts hosted by the shallow and mesophotic populations of *Litophyton* sp. and *R. f. fulvum* was investigated following the protocol of Santos *et al.* (Santos et al. 2002).

3.2.4 Interspecies comparisons

To compare our data on soft corals, normalized to AFDW, with those found in the literature on scleractinian corals, often normalized to skeletal surface area, it was necessary to use the same normalization metric. As surface area could not be measured with accuracy on soft corals (since they have a highly variable hydroskeleton which expands and shrinks the colony size by several folds depending on the environmental conditions), we normalized all data to AFDW (Pupier et al. 2018). For this purpose, we chose eight nubbins (skeletal surface area ranging from 4 to 29 cm^2) of the scleractinian coral *Stylophora pistillata* originating from the Red Sea and grown at the Monaco Scientific Center, as it is one of the dominant species in the Gulf of Eilat and many data have been acquired on this species. AFDW was determined as described above after separating the tissue from the skeleton and combusting it. Surface area was determined using the wax technique (Veal et al. 2010). A conversion factor (255.65 ± 8.98, Table S5), estimated from the ratio between skeletal surface area (in cm^2) and AFDW (in g) of these nubbins, was used to transform data normalized to surface area (Appendix II - Table S4) into data normalized to AFDW.

3.2.5 Statistical analyses

Analyses were performed using R software (R Foundation for Statistical Computing). All data were expressed as mean ± standard error. Prior to analyses, outlier values were identified using Grubb's test and were excluded when p-values were significant ($p < 0.05$). Assumptions of normality and homoscedasticity of variance were evaluated through Shapiro's and Bartlett's tests along with graphical analyses of residuals. A two-way analysis of variance (ANOVA) was performed to test the effect of species and depth on symbiont density, chlorophyll concentrations and carbon acquisition. The rates at which carbon was assimilated, translocated and lost were analysed separately between the two time points (i.e., pulse and chase periods) using 2-way ANOVAs with species and depth as fixed effects. When the interaction was significant pairwise, Tukey tests were performed as *a posteriori* testing.

3.3 Results

3.3.1 Physiological measurements

The dinoflagellate-host association was species-specific. *Litophyton* sp. harbored *Symbiodinium* sp. (formerly *Symbiodinium* clade A, LaJeunesse et al. 2018) whereas *R. f. fulvum* was associated with *Cladocopium thermophilum* (formerly *Symbiodinium thermophilum* clade C3, Hume et al. 2015; LaJeunesse et al. 2018), regardless of the depth investigated. Symbiodiniaceae density, normalized to AFDW, was two-fold higher in *Litophyton* sp. than in *R. f. fulvum* at both depths and was for both species always higher in shallow than in mesophotic con-specifics (Figure 19a, Appendix II - Table S2). For both species, total chlorophyll concentrations (per AFDW) were significantly higher for mesophotic corals (Figure 19b, Appendix II - Table S2). Also, symbionts contained significantly more chlorophyll per cell in mesophotic corals ($2.8 \cdot 10^{-6} \pm 3.2 \cdot 10^{-7}$ µg cell^{-1} for *Litophyton* sp. and $7.5 \cdot 10^{-6} \pm 4.9 \cdot 10^{-7}$ µg cell^{-1} for *R. f. fulvum*, Appendix II - Table S2) as compared to shallow corals ($1.6 \cdot 10^{-6} \pm 1.5 \cdot 10^{-7}$ µg cell^{-1} for *Litophyton* sp. and $4.2 \cdot 10^{-6} \pm 1.2 \cdot 10^{-6}$ µg cell^{-1} for *R. f. fulvum*, Appendix II - Table S2).

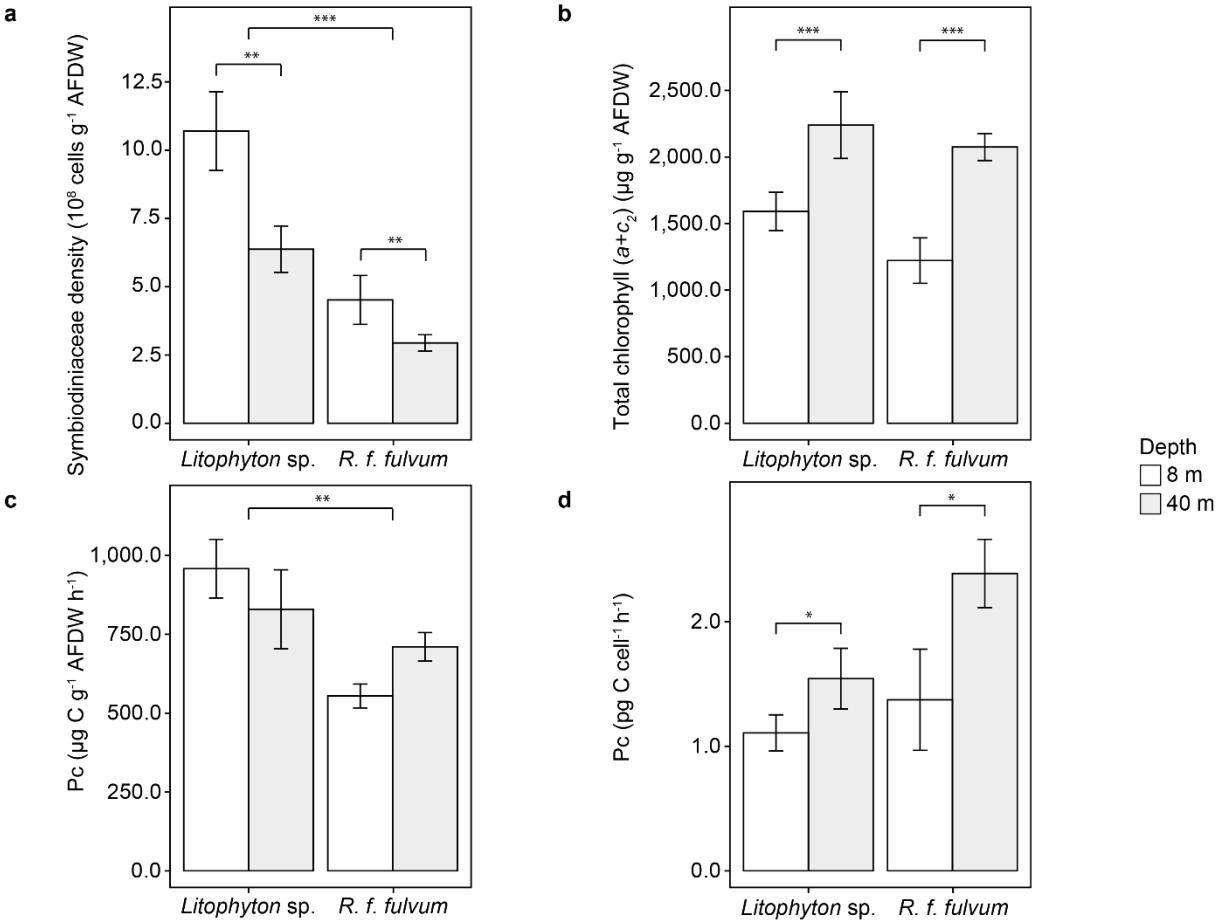

Figure 19. Physiological and tissue descriptors measured in the soft coral species investigated. For panels (a), (b) and (c), data are normalized to ash-free dry weight. (a) Symbiodiniaceae density. (b) Total chlorophyll concentration. (c) Autotrophic carbon acquisition. (d) Autotrophic carbon acquisition per Symbiodiniaceae cell. Error bars represent standard error. There were significant differences between species and depths for (a), between depths for (b) and (d), and between species for (c) (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

Autotrophic carbon acquisition, estimated using ^{13}C-bicarbonate incorporation over 5h under maximal irradiance (P_C, expressed in µg C g^{-1} AFDW h^{-1}), was significantly different between the two coral species, with *Litophyton* sp. exhibiting higher Pc rates than *R. f. fulvum* (Figure 19c, Appendix II - Table S2). When normalized to symbiont cells, Pc rates were significantly different between depths, with higher values obtained in mesophotic conditions (Figure 19d, Appendix II - Table S2). Respiration rates (Rc, expressed in µg C g^{-1} AFDW h^{-1}) were similar between depths for *R. f. fulvum* (347 ± 45 and 489 ± 47 in shallow and mesophotic conditions, respectively), whereas they were two-fold lower for mesophotic (148 ± 47) than shallow (406 ± 18) nubbins of *Litophyton* sp. (Appendix II - Table S2, Tukey HSD $p < 0.01$). While symbiont respiration accounted for less than 1% of the holobiont respiration for

Litophyton sp. in both depths, symbiont respiration accounted ca. 6% and 4% to holobiont respiration for mesophotic and shallow *R. f. fulvum*, respectively.

3.3.2 Carbon budget

After the pulse period (5h), both species exhibited significant differences in their carbon budget between depths (Figure 20a, b, c, d, Appendix II - Tables S2 and S3). Overall, rates of photosynthate translocation were lower in *Litophyton* sp. at both depths (36% to 60% of the total fixed carbon) compared to *R. f. fulvum* (85%), and symbionts of *Litophyton* sp. retained four to five times more carbon than those of *R. f. fulvum* (Figure 20b, d, Appendix II - Table S2). However, no difference was observed in the rate of carbon assimilated by the host between depths and species (134-150 µg C g^{-1} AFDW h^{-1}). For *Litophyton* sp. from mesophotic depth, symbionts translocated less carbon to the host (36% versus 60%) and less carbon was lost by the holobiont (19% versus 44%) as compared to nubbins from shallow depth. On the contrary, photosynthate translocation (85%) and carbon loss (64-70%) were comparable between shallow and mesophotic *R. f. fulvum* corals.

After the chase period (24h), the carbon budget of both species was clearly species-specific but without depth-specific differences (Figure 21a, b, c, d, Appendix II – Table S2). In *Litophyton* sp., symbiont cells assimilated half of the photosynthesized carbon and translocated only 47% to the coral host in both shallow and mesophotic colonies (Figure 21a, b, c, d, Appendix II – Table S2). On the contrary, symbionts in *R. f. fulvum* translocated 86-92% of the photosynthetically fixed carbon to the host and assimilated only 4-11% of this carbon for their own energetic needs. Consequently, significant differences were evident between the two species for the rates of carbon 1) translocated (up to one third lower in *Litophyton* sp. than in *R. f. fulvum*), 2) retained in the symbionts (ten-fold higher in *Litophyton* sp. than in *R. f. fulvum*) and 3) lost from the symbiotic association (two-fold lower in *Litophyton* sp. than in *R. f. fulvum*) (Figure 21a, b, c, d, Appendix II – Table S2).

3.3.3 Relationship between Symbiodiniaceae density and carbon flux

Our results show that in the two coral species at both depths the carbon acquisition per symbiont cell exponentially decreased with increasing symbiont density (Figure 22a). In

addition, we found a positive linear correlation between symbiont cell carbon acquisition and translocation (Figure 22b).

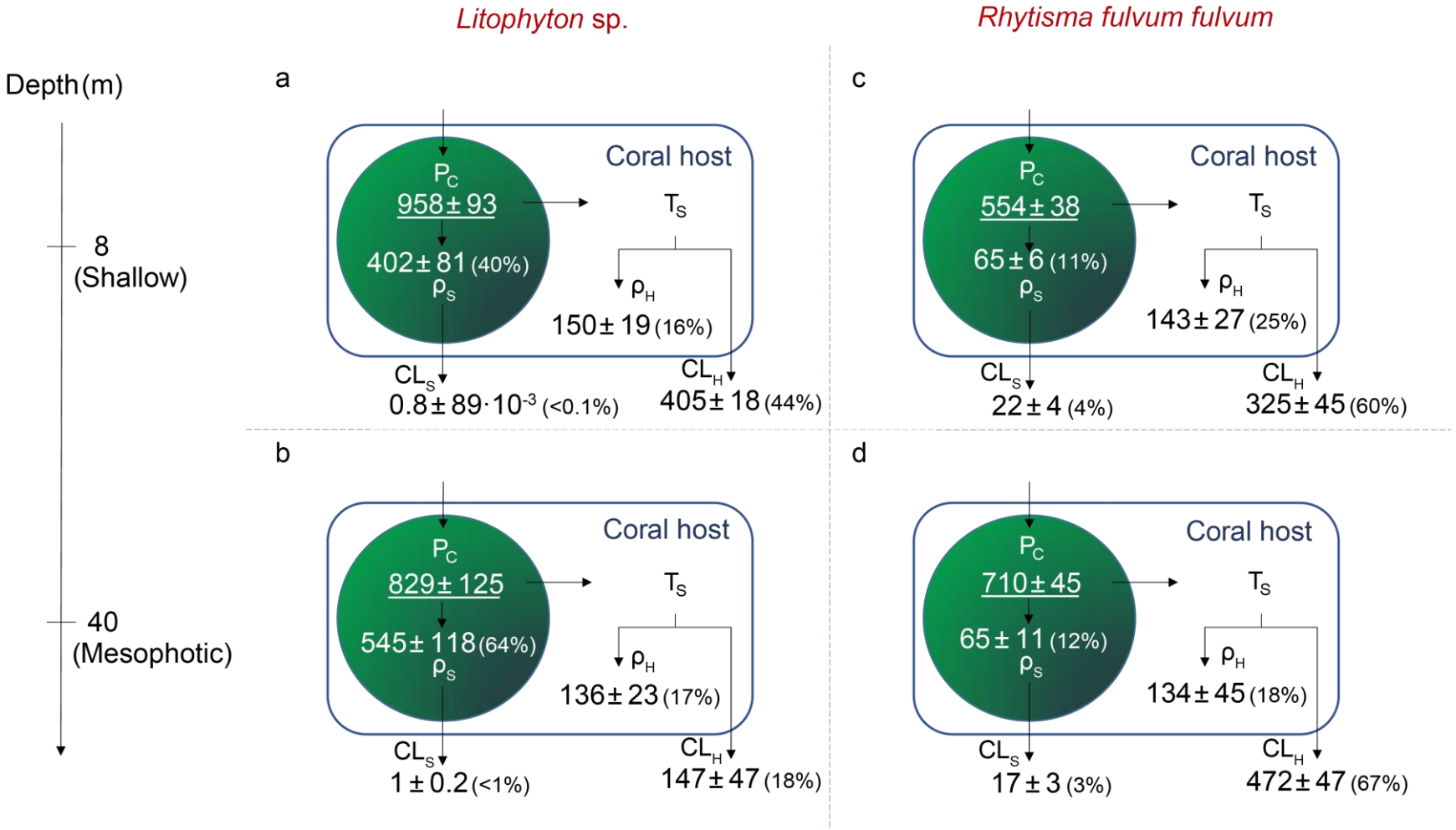

Figure 20. Carbon budgets obtained after the 5 hours pulse period in the soft coral species investigated. (a) Shallow *Litophyton* sp.. (b) Mesophotic *Litophyton* sp.. (c) Shallow *Rhytisma fulvum fulvum*. (d) Mesophotic *Rhytisma fulvum fulvum*. Symbionts are represented by a green circle. P_C = autotrophic carbon acquisition. ρ_S = carbon assimilated in Symbiodiniaceae. ρ_H = carbon assimilated by the coral host. T_S = translocation of photosynthates. C_{LS} = carbon lost by the symbionts through respiration. C_{LH} = carbon lost by the host through respiration. P_C and carbon fluxes are expressed in µg C g^{-1}AFDW h^{-1}.

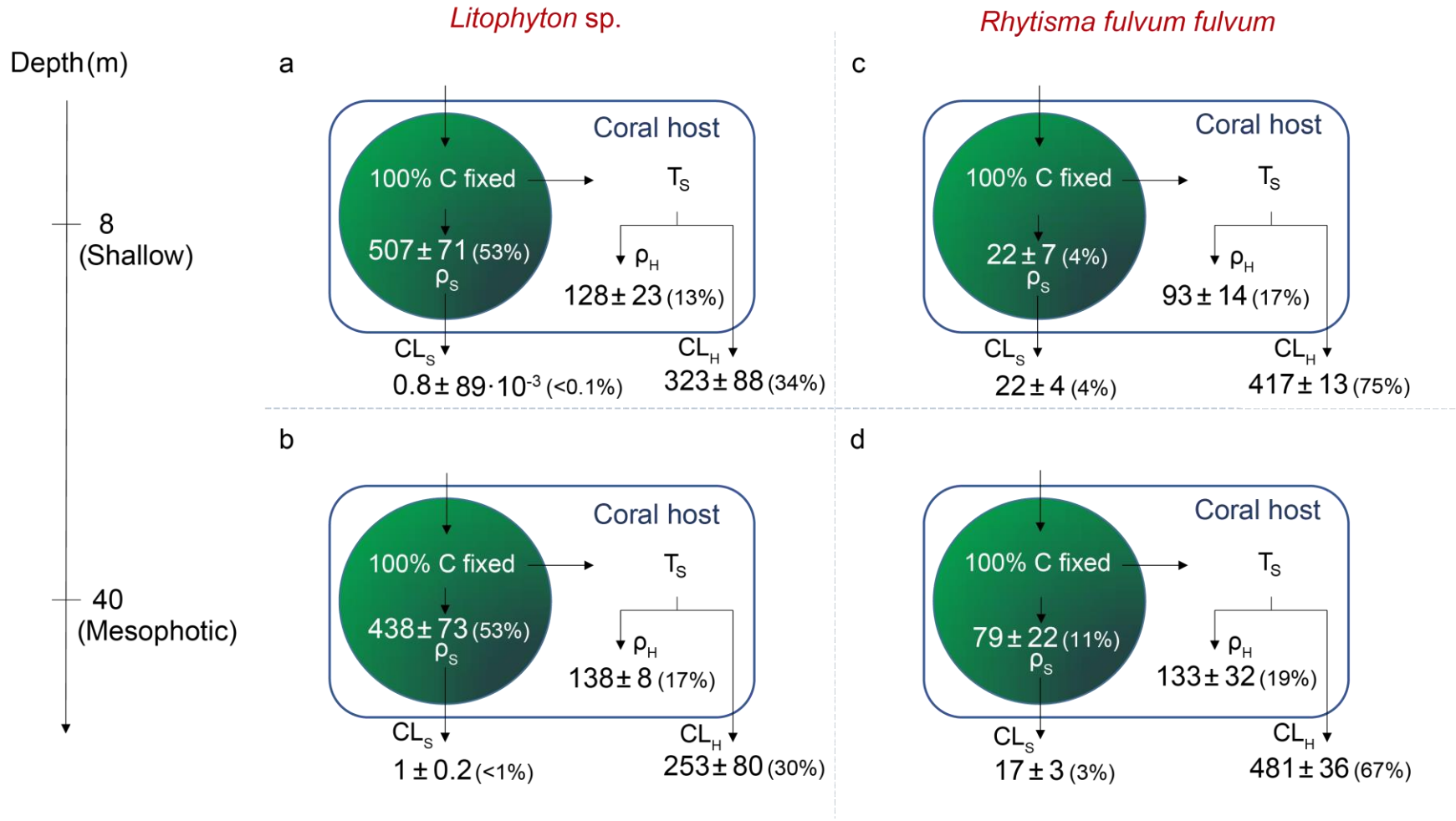

Figure 21. Carbon budgets obtained after the 24 hours chase period in the soft coral species investigated. (a) Shallow *Litophyton* sp.. (b) Mesophotic *Litophyton* sp.. (c) Shallow *Rhytisma fulvum fulvum*. (d) Mesophotic *Rhytisma fulvum fulvum*. Symbionts are represented by a green circle. ρ_S = carbon assimilated in Symbiodiniaceae. ρ_H = carbon assimilated by the coral host. T_S = translocation of photosynthetates. C_{LS} = carbon lost by the symbionts through respiration. C_{LH} = carbon lost by the host through respiration and mucus production. Carbon fluxes are expressed in µg C g^{-1}AFDW h^{-1}.

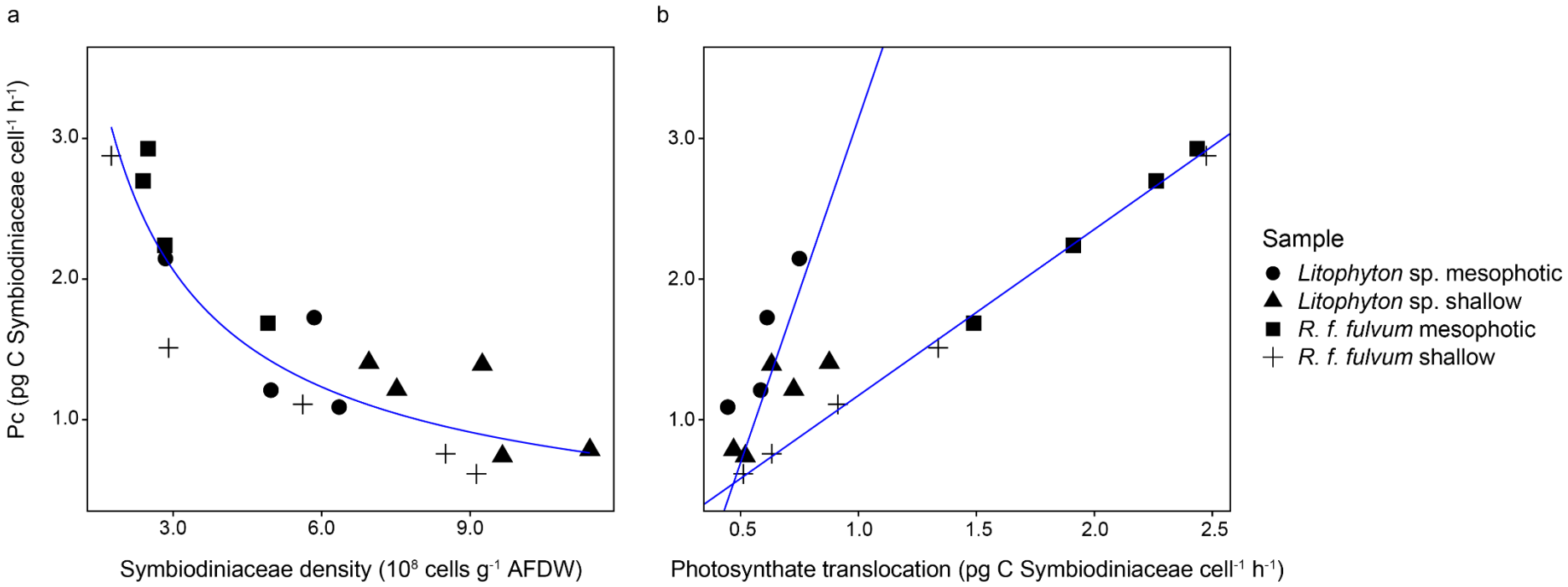

Figure 22. Relationships between Symbiodiniaceae density and carbon fluxes of the soft coral species investigated. (a) Exponential relationship between carbon acquisition per cell and density of Symbiodiniaceae cells of *Litophyton* sp. and *Rhytisma fulvum fulvum* ($R^2 = 0.78$, $y = 4.7087x^{-0.744}$). (b) Orthogonal regressions between carbon acquisition per cell and photosynthates translocation of Symbiodiniaceae cells of Litophyton sp. ($R^2 = 0.60$, $y = 4.8964x - 1.7547$) and *Rhytisma fulvum fulvum* ($R^2 = 0.99$, $y = 1.1818x - 0.0091$).

3.4 Discussion

This study shows that the functioning of the coral-dinoflagellate symbiosis in soft corals holds some different characteristics to that in many scleractinian corals, and that the two groups are differently acclimatized to depth. The two investigated soft coral species maintained an equivalent carbon acquisition from shallow to poorly-lit mesophotic habitats, while carbon acquisition of scleractinian corals generally decreases with depth. This autotrophic efficiency, combined with putative high heterotrophic capacities, may provide soft corals with an ecological advantage in the upper Red Sea mesophotic reefs (Shoham and Benayahu 2017). In addition, the results show that carbon acquisition and translocation rates per symbiont cell were negatively correlated with the symbiont density within the host tissue, with a higher assimilation of carbon in symbiont biomass under high symbiont density. However, the rates at which carbon was translocated to and assimilated by the host were unaffected, indicating that dense symbiont populations can remain mutualistic.

Our findings indicate that the mean autotrophic carbon acquisition in both soft coral species (measured during 5h and using ^{13}C-labelling) remained stable from shallow to mesophotic environments, which suggests that soft corals are either photo-limited in shallow waters or well photo-acclimatized to depth (Figure 23).

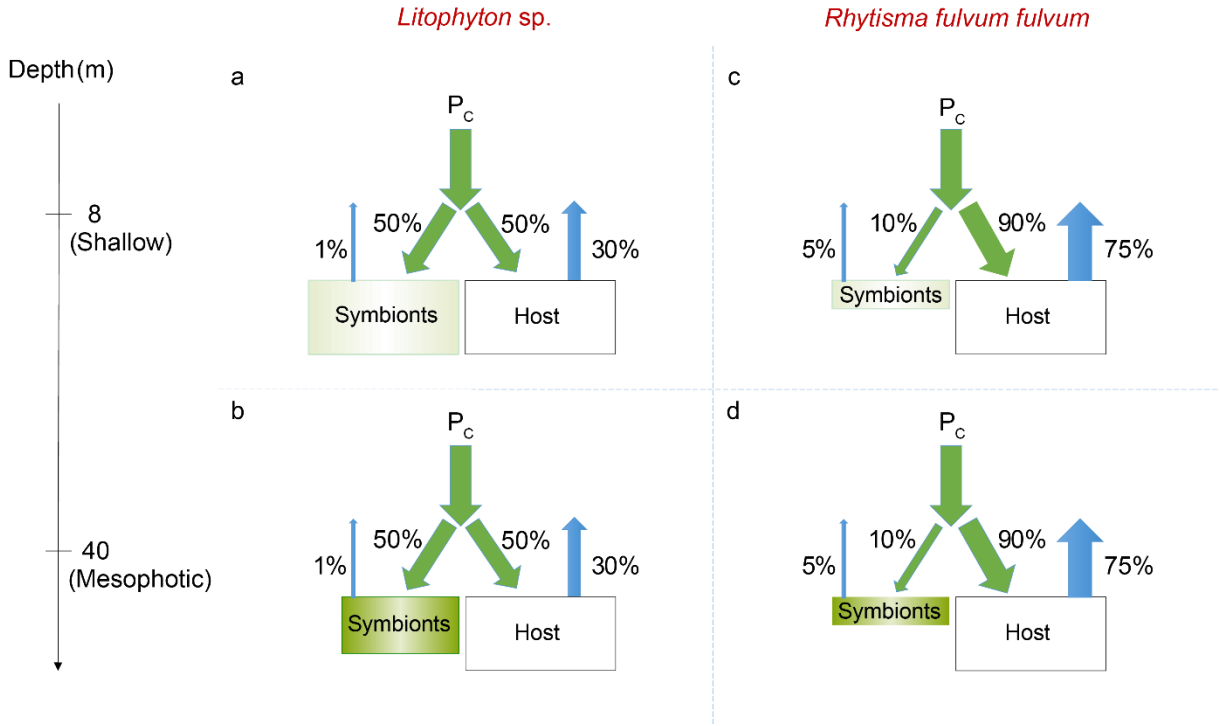

Figure 23. Metabolic interactions between soft corals and their dinoflagellate symbionts. (a) Shallow *Litophyton* sp.. (b) Mesophotic *Litophyton* sp.. (c) Shallow *Rhytisma fulvum fulvum*. (d) Mesophotic *Rhytisma fulvum fulvum*. Boxes and arrows are indicative of differences in density and fluxes. Color intensity of symbionts corresponds to chlorophyll concentration levels.

This is in agreement with previous observations (Fabricius and Klumpp 1995) that maximal photosynthetic rates of several soft coral species from the Great Barrier Reef were measured at 20 m depth rather than in shallower waters (Table 6). Such constant carbon acquisition along the depth gradient is surprising in comparison to the general patterns observed in scleractinian corals. Most scleractinian corals experience a significant decrease in rates of carbon acquisition with depth, although this physiological parameter has been poorly investigated in mesophotic environments (Lesser et al. 2010; Einbinder et al. 2016; Ezzat et al. 2017). However, a recent study, which investigated the photosynthetic performance of the mesophotic coral *Euphyllia paradivisa*, found a high photosynthetic capacity of this coral species under low light conditions (Eyal et al. 2016).

Table 6. Tissues descriptors and autotrophic carbon acquisition reported for different octocorals, normalized to ash-free dry weight (AFDW). P_C = autotrophic carbon acquisition.

OCTOCORALS					
Species	**Depth** (m)	**Symbiont density** (10^8 cell g^{-1} AFDW)	**Total chlorophyll (a+c2)** (µg g^{-1} AFDW)	**P_C** (µg C g^{-1} AFDW h^{-1})	**Reference**
Great Barrier Reef					
Asterospicularia sp.	20			530	Fabricius and Klumpp (1995)
Briareum stechei	5			419-452	
	20			707-838	
Capnella lacertiliensis	5			949-1047	
	20			511	
Efflatounaria sp.	5			975-1375	
	20			772-1191	
Lobophytum spp.	5			367	
	20			419	
Nephthea sp.	5			1145	
	10			1100	
	20			956	
Paralemnalia sp.	5			838-923	
	10			792-1191	
	20			884-956	
Sarcophyton spp.	5			367-445	
	10			1034-3076	
	20			511-707	
Sinularia spp.	5			713-825	
	10			772	
	20			406-903	
Xenia spp.	5			890-1231	
	10			1165-1872	
	20			812	
Caribbean					
Antillogorgia sp.	2-8	1-3	1300-2120	1105-2291	Baker et al. (2015); Rossi et al. (2018)
Briareum asbestinum	3-8			249	Baker et al. (2015)
Eunicea sp.	2-8	1-4	760-2150	51-382	Baker et al. (2015); Rossi et al. (2018)
Gorgonia ventalina	2-8	1	730	457-3284	
Plexaura sp.	2-8	0.5	1280	20-742	
Pterogorgia anceps	2-8	2	3490-4050	184-3338	
Red Sea					
***Litophyton* sp.**	**8**	**9.4**	**1591**	**958**	**This study**
	40	**6.4**	**2240**	**829**	
Rhytisma f. fulvum	**8**	**4.5**	**1222**	**554**	
	40	**2.9**	**2075**	**710**	

Pigment content, symbiont density and symbiont genus are generally involved in shaping the photo-acclimatization of the coral symbiosis from shallow to mesophotic environments. Our results highlight for the two soft coral species a host-specific Symbiodiniaceae genus that remained stable along the depth profile up to 40 m depth. *Litophyton* sp. was associated with *Symbiodinium* sp., as previously reported (formerly *Symbiodinium* clade A, Barneah et al. 2004; LaJeunesse et al. 2009), while *R. f. fulvum* was rather associated with *Cladocopium* sp., in agreement with studies performed in the Red Sea (formerly *Symbiodinium* clade C, Barneah et al. 2004), or the Great Barrier Reef (van Oppen et al. 2005; Goulet et al. 2008b). Such clade specificity is assumed to be persistent both in space and time in octocorals (Goulet and Coffroth 2003; Baker et al. 2013a). Thus, acclimatization of the soft corals to low light levels was independent of the symbiont genus, but potentially driven by higher concentrations of total chlorophyll per AFDW and per symbiont cell in mesophotic corals (Figure 19b), which maximize the light harvesting capacity of the cells under low light levels (Mass et al. 2007; Ezzat et al. 2017). In addition, both species exhibited a decreased symbiont density in mesophotic as compared to shallow colonies which is likely another adaptation to depth by reducing self-shading under reduced PAR (Cohen and Dubinsky 2015; Ziegler et al. 2015). A lower symbiont density also reduces the competition for inorganic nutrient supply (Cunning et al. 2017). It has been demonstrated that mesophotic scleractinian corals can display several strategies to acclimatize to low light levels, such as modifications in the organization of photosynthetic apparatus (shift to a PSII-based system, including additional photosynthetic antenna, Einbinder et al. 2016), modifications in the organization of antenna (growth of specialized paracrystalline light-harvesting antenna domains, Scheuring and Sturgis 2005), or the synthesis of fluorescent pigments by the host (that can perform wavelength transformation to facilitate light penetration, Smith et al. 2017); however, these strategies remain to be further investigated in soft corals.

The autotrophic carbon acquisition to respiration ratios (P_C/R_C) of *Litophyton* sp. ranged from 1 to 2 from shallow to mesophotic depths, the latter value being above the conservative threshold for net autotrophy ($P_C/R_C>1.5$). Considering that the solar radiation level in November is one of the lowest annual levels (Stambler 2006), the data suggest that *Litophyton* sp. is mostly an autotrophic species. On the contrary, P_C/R_C ratios of *R. f. fulvum* in November were always below compensation ($P_C/R_C = 1$) even when considering a maximal autotrophic carbon acquisition rate sustained for 12h. This strongly suggests that this species relies, at least in winter, on heterotrophy to sustain its daily respiratory needs.

The difference in P_C/R_C between the two species may be due to their different growth shapes and different micro-morphological features of the polyps. Two recent studies on gorgonian octocorals, indeed highlighted a correlation between host morphology, polyp size and productivity (Baker et al. 2015; Rossi et al. 2018), thereby corroborating the first observations of Porter (1976).

Symbiont cell carbon acquisition was negatively correlated with symbiont density in both investigated soft coral species and decreased exponentially as soon as the symbiont density increased. Although this pattern is first described on soft corals in this study, similar observations were reported with gorgonian octocorals (Rossi et al. 2018) and scleractinian corals (Scheufen et al. 2017), and might be due to a host-dependent regulation of light and nutrient ressources (Scheufen et al. 2017), or a self-shading of the symbionts (Cunning et al. 2017).

Our results also highlight a positive correlation between carbon acquisition and carbon translocation rates per symbiont cell, similarly to a previous study on scleractinian corals (Leal et al. 2015). In *Litophyton* sp., a low carbon acquisition per cell corresponded to a high symbiont density and consequently, symbiont cells assimilated more carbon for their own metabolism rather than translocating it to the host (50% translocation). In contrast, the symbionts in *R. f. fulvum* translocated significantly more carbon (80 to 90% translocation) to the host, likely due to their lower abundancy and increased rates of carbon acquisition per cell. Carbon translocation rates of >80% have also been reported for other coral species (Falkowski et al. 1984; Muscatine et al. 1984; Tremblay et al. 2012). For example, Scheufen et al. (2017) measured highest productivity rates in corals during the very oligotrophic summer season despite seasonally reduced symbiont density. This may also imply a high carbon translocation to the host that is essential when exogenous food sources are scarce.

Differences in cell-specific carbon assimilation and translocation rates may not only be related to the symbiont density but also to the symbiont genus. Indeed, *Symbiodinium* sp. (associated with *Litophyton* sp. here) is known to translocate less carbon to its host as compared to *Cladocopium* sp. (associated with *R. fulvum fulvum* here) (Starzak et al. 2014; Leal et al. 2015; Baker et al. 2018). However, an important observation is that the lower percentage of carbon translocation in *Litophyton* sp. is not indicative of a shift towards symbiont parasitism (Baker et al. 2018), as the carbon translocation to the host still exceeded the metabolic costs for holobiont respiration. In *R. f. fulvum*, due to the low

symbiont density, an increased translocation rate per cell was needed to transfer the same amount of carbon to the host. In addition, the percentage of carbon lost by the host was lower in *Litophyton* sp. than in *R. f. fulvum*, compensating for the lower percentage of carbon translocated from the symbionts. As a consequence, the rates at which carbon was retained in the host were constant between species and depths (between 13% and 19% of the photosynthesized carbon, or 93 to 138 µg C g^{-1} AFDW h^{-1}). Assimilation rates of 10 to 20% of the photosynthesized carbon by the animal host seems to be a general feature in corals (Tremblay et al. 2012; Ezzat et al. 2017).

Soft and scleractinian corals therefore seem to exhibit a different trend of carbon acquisition along the depth gradient, although more investigations with different scleractinian and soft coral species are still needed. In order to compare carbon fixation per biomass between soft and scleractinian corals, we used data previously obtained on the scleractinian coral *Stylophora pistillata* sampled at the exact same location, depths and month (Ezzat et al. 2017) (Appendix II - Table S4) and normalized the rates to AFDW. The estimates we obtained show that soft corals fix less carbon per biomass as compared to *S. pistillata* at shallow depth in November (estimation of 2426 µg C g^{-1} AFDW h^{-1} for *S. pistillata* versus 554-958 µg C g^{-1} AFDW h^{-1}), although soft corals contain comparable or higher dinoflagellate density (Thornhill et al. 2011) and chlorophyll content per biomass (Appendix II - Table S4) than scleractinian corals. However, in deeper environments, soft corals tend to fix carbon per biomass at similar or even higher rates (estimation of 555 µg C g^{-1} AFDW h^{-1} for *S. pistillata* versus 710-829 µg C g^{-1} AFDW h^{-1} for *R. f. fulvum* and *Litophyton* sp.). This may indicate that in shallow waters, soft corals have particularly less fixed carbon available to maintain or grow their biomass as compared to scleractinian corals. Morphological characteristics of the host, which have an impact on the diffusion and transmission of light to the symbionts, may explain differences in carbon acquisition in shallow water corals. Soft corals lack a calcium carbonate skeleton, contrary to scleractinian corals, and only possess sclerites as calcified structures. However, skeletons present light scattering abilities, which enhance light absorption efficiency of the symbionts (e.g., Enríquez et al. 2017). Although characteristics of sclerites and tissues remain to be investigated to understand the potential light amplification and dispersion in soft corals, we hypothesized that they would affect the light environment of the symbionts to a lesser extent than skeletons do in scleractinian corals. In addition, soft corals have a thick coenenchyme and exhibit a low ratio of colony surface area to volume, which does not

favor light exposure and gas or nutrient exchange through the epidermal tissue (Wangpraseurt et al. 2014). Finally, the variability of their hydroskeleton allows soft corals to contract and expand their tissue. Tissue contraction removes symbionts from the tissue surface, thereby higher irradiance is required to reach the symbionts and to achieve photosynthetic compensation and saturation.

Understanding nutritional ecology of octocorals is still in its infancy. The common belief is that octocorals are more heterotrophic than scleractinian corals, because of their low carbon acquisition in surface waters, and because their morphology is more suited for heterotrophy (Schubert et al. 2017). Two recent studies on Caribbean gorgonian octocorals have observed a possible correlation between host morphology and symbiont performance (Baker et al. 2015; Rossi et al. 2018). Octocorals with thin branches and small polyps can be more autotrophic as compared to those with a massive shape and large polyps. In our work, a different degree of autotrophy was demonstrated for the two investigated soft coral species, which might be linked to morphological traits. The arborescent shape of *Litophyton* sp. may enhance the exposure of symbionts to light and thus favour autotrophy compared to the mat-forming shape of *R. f. fulvum*. In addition, our results strongly support the view that high symbiont density results in lower cell-specific carbon translocation and acquisition. However, soft corals with high symbiont densities (*Litophyton* sp.) reached similar carbon translocation than those with low symbiont density. Further work involving various soft coral species, growth-shapes and symbiont-specificities will be essential to provide more insight into the physiology and trophic ecology of octocorals thriving along environmental gradients.

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Chapter **4** | Acquisition of dissolved inorganic and organic nitrogen

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Publication n°2

Divergent capacity of scleractinian and soft corals to assimilate and transfer diazotrophically-derived nitrogen to the reef environment

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Abstract

Corals are associated with dinitrogen (N_2)-fixing bacteria that potentially represent an additional nitrogen (N) source for the coral holobiont in oligotrophic reef environments. Nevertheless, the few studies investigating the assimilation of diazotrophically-derived nitrogen (DDN) by tropical corals are limited to a single scleractinian species (i.e. *Stylophora pistillata*). The present study quantified DDN assimilation rates in four scleractinian and three soft coral species from the shallow waters of the oligotrophic Northern Red Sea using the $^{15}N_2$ tracer technique. All scleractinian species significantly stimulated N_2 fixation in the coral-surrounding seawater (and mucus) and assimilated DDN

into their tissue. Interestingly, N_2 fixation was not detected in the tissue and surrounding seawater of soft corals, despite the fact that soft corals were able to take up DDN from a culture of free-living diazotrophs. Soft coral mucus likely represents an unfavorable habitat for the colonization and activity of diazotrophs as it contains a low amount of particulate organic matter, with a relatively high N content, compared to the mucus of scleractinian corals. In addition, it is known to present antimicrobial properties. Overall, this study suggests that DDN assimilation into coral tissues depends on the presence of active diazotrophs in the coral's mucus layer and/or surrounding seawater. Since N is often a limiting nutrient for primary productivity in oligotrophic reef waters, the divergent capacity of scleractinian and soft corals to promote N_2 fixation may have implications for N availability and reef biogeochemistry in scleractinian versus soft coral dominated reefs.

4.1 Introduction

Coral reefs are highly productive ecosystems despite thriving in oligotrophic waters, which contain low levels of essential dissolved inorganic nutrients such as nitrogen (N) (de Goeij et al. 2013). Corals, which are the main reef builders, can achieve high productivity thanks to their association with endosymbiotic dinoflagellates, which recycle the animal waste products and are efficient in scavenging inorganic N dissolved in seawater (Wang and Douglas 1999; Grover et al. 2003). Corals also profit from high rates of N recycling in the water column and sediments via microbial processes (Carpenter and Capone, 2008), and from biological dinitrogen (N_2) fixation of diazotrophic symbionts (Bednarz et al. 2017), which are heterotrophic bacteria and cyanobacteria that convert atmospheric N_2 into bioavailable N. In addition to being associated with benthic organisms (reviewed in Benavides et al., 2017), diazotrophs can live pelagic, and colonize reef substrates (e.g., sediments, coral rubble). Many studies using the acetylene reduction assay (ARA) method have recorded high rates of N_2 fixation either in reef waters or in the presence of corals (i.e., Bednarz et al., 2015; Cardini et al., 2016; Rädecker et al., 2015). The ARA technique quantifies gross N_2 fixation without providing insights into DDN (diazotrophically-derived nitrogen) assimilation by the coral-dinoflagellate symbiosis. Instead, the use of labelled $^{15}N_2$ gas measures DDN assimilation (net N_2 fixation) and distinguishes between the different compartments (i.e. seawater particles, tissue,

dinoflagellate symbionts), but it is still poorly known to what extent and under which conditions corals profit from DDN.

Most of the research using the $^{15}N_2$ method to assess DDN assimilation in adult tropical corals has been directed towards only one species, *Stylophora pistillata*, belonging to the Pocilloporidae family. These studies showed that *S. pistillata* can acquire DDN via grazing on a culture of diazotrophic cells (Benavides et al. 2016). However, under natural conditions, this coral species has variable capacities to stimulate diazotrophic activity in seawater as well as variable DDN assimilation rates into the coral holobiont (Grover et al. 2014; Bednarz et al. 2017; Lesser et al. 2018). As DDN can play a major role in supplying N to corals (Cardini et al. 2016; Bednarz et al. 2017), the estimation of the capacity of different coral species to take advantage of this nutrient source can help in understanding how they cope with nutrient limitation. In addition, no study so far investigated DDN assimilation by soft corals, although they represent together with scleractinian corals the two most dominant benthic groups on many reefs worldwide. Soft corals can quickly colonize open space due to their fast growth rates, high fecundity and asexual reproduction and these opportunistic life history features may be facilitated by diazotrophs as additional N source (i.e. DDN). The aim of this study was to perform a multi-species comparison and to investigate the capacity of different scleractinian and soft coral species to either assimilate DDN/diazotrophic cells and/or transfer it to the surrounding seawater.

4.2 Materials and methods

4.2.1 Laboratory experiment: DDN assimilation from external diazotrophs

To assess whether scleractinian and soft corals have the same capacities to assimilate DDN from external active diazotrophs in the seawater, an experiment was performed at the Monaco Scientific Centre. We used the scleractinian coral *S. pistillata* and the soft coral *Sarcophyton* sp., which have been maintained in aquaria for years, and are not associated with active diazotrophs (V. Bednarz, unpublished data; Kooperman et al., 2007). All colonies were maintained under the same light (200 µmoles photons m^{-2} s^{-}

^{1}) and temperature (25°C) conditions to ensure comparability between the species during the following experiment. For this purpose, a culture of diazotrophs (*Crocosphaera watsonii*) was prepared and diluted to obtain two cell concentrations of 100 and 1000 cells mL^{-1}, respectively, matching concentrations of 3-20 µm size class phycoerythrin-containing unicellular cyanobacteria under non-bloom and bloom conditions (Campbell et al. 1997). *C. watsonii* concentrations were determined using a hemocytometer (Z1 particles counter, Beckman Coulter, USA). Four nubbins of each coral species per *C. watsonii* concentration were then individually placed in 320 mL gas tight bottles completely filled with 280 mL of 0.22 µm-filtered seawater amended with the adequate concentration of diazotrophs and 40 mL of $^{15}N_2$ enriched seawater. In addition, three nubbins of each species were incubated under the same above conditions but without *C. watsonii* cells. At the end of the 24h incubation, corals were removed and stored frozen until subsequent analysis. They were then processed as described below.

4.2.2 Field experiment: DDN/diazotrophic cells assimilation and transfer to seawater

- Biological material

The study was conducted during November 2017 at the Interuniversity Institute (IUI) of Marine Sciences, Eilat, Red Sea. Coral fragments were collected by SCUBA diving from different colonies of the reef adjacent to the IUI and were brought back to the Red Sea Simulator facility (see Bellworthy and Fine, 2018 for more details regarding the system) to recover for one day prior to starting the incubations. Scleractinian corals (*Acropora eurystoma*, n = 6; *Pocillopora damicornis*, n = 3; *Goniastrea sp.*, n = 3; and *Cynarina sp.*, n = 2) were sampled in shallow waters (8-10 m depth) and soft corals (n = 5 for all species; *Dendronephthya* sp., *Rhytisma fulvum fulvum* and *Litophyton* sp.) in shallow waters and at upper mesophotic depths (40-45 m). Biological replicates for each species were derived from individual colonies and the different species were chosen in order to cover a broad range of families and morphological traits (Table 7). Three additional nubbins per species per depth were collected to measure natural ^{15}N abundance of the corals. At the collection time, the site was characterized by stable temperature (24-25°C)

and nutrient levels (< 0.5 µM dissolved N and < 0.2 µM dissolved phosphorus) along the depth gradient (data from the Israel National Monitoring program of the Gulf of Eilat, http://www.iui-eilat.ac.il/Research/NMPMeteoData.aspx).

Table 7. Scleractinian and soft coral species investigated in the study.

Coral group	Species	Family	Morphology
Scleractinian	*Acropora eurystoma*	Acroporidae	Branching
	Cynarina sp.	Lobophyliidae	Mounding
	Goniastrea sp.	Merulinidae	Mounding
	Pocillopora damicornis	Pocilloporidae	Branching
Soft	*Dendronephthya* sp.	Nephtheidae	Arborescent
	Litophyton sp.	Nephtheidae	Arborescent
	Rhytisma fulvum fulvum	Alcyoniidae	Mat-forming

- N_2 fixation measurements

The $^{15}N_2$ seawater addition method was used to assess N_2 fixation rates. For this purpose, $^{15}N_2$-enriched seawater was produced prior to the incubation experiment by injection of 10 mL of $^{15}N_2$ gas (98% Eurisotop) into gas-tight 250 mL bottles completely filled with degassed, 0.2 µm filtered seawater, followed by vigorous shaking for 12 h to ensure 100% $^{15}N_2$ equilibration (Mohr et al. 2010). In order to test which coral species can assimilate DDN or can enrich the seawater in DDN, collected nubbins from each coral species were individually placed in gas-tight bottles of 600 mL. Bottles were completely filled with 120 µm-filtered seawater, directly pumped from the reef, with 10% replaced by $^{15}N_2$-enriched seawater (resulting in theoretical enrichment of ~9.8 atom%). A first set of three control bottles was prepared as described above but without corals to measure the baseline of N_2 fixation by planktonic diazotrophs. To evaluate the natural ^{15}N abundance of the corals, a second set of controls consisted in incubating three coral nubbins from each species (only two for *Cynarina* sp.) in 120 µm-filtered seawater incubations without $^{15}N_2$ addition. All bottles were incubated for 24 h in several outdoor aquaria. The seawater temperature was kept constant at *in situ* temperature (~ 24°C) by using continuous supply of seawater in the aquaria. Corals were exposed to the natural diel light cycle and shaded to the corresponding irradiance of their collection site by applying layers of black mesh above the aquaria and considering an attenuation coefficient equal to 0.072-0.1 m^{-1}

(Akkaynak et al. 2017; Tamir et al. 2019). At the end of the incubations, corals were removed from seawater and stored frozen until subsequent analysis. Incubation water of all bottles was filtered onto pre-combusted (450°C for 5 h) GF/F filters which were dried at 60°C for 48h. Corals and filters were processed as described in the sample analysis section.

4.2.3 Sample analysis

For scleractinian corals, the tissue was removed from the skeleton using an air brush and homogenized with a potter tissue grinder. The host tissue and zooxanthellae were separated through a series of centrifugations according to Grover et al. (2003) and each fraction was freeze-dried. Soft corals were treated as described in Pupier et al. (2018). Briefly, they were freeze-dried, the resulting powder homogenized in distilled water and then separated in several centrifugation steps into the host tissue and zooxanthellae fractions as described above. Each fraction was subsequently freeze-dried again. The ^{15}N enrichment, as well as particulate organic carbon (POC) and particulate N content (PN) of each sample and filter were determined using a mass spectrometer (Delta Plus; Thermo Fisher Scientific, Germany) coupled to a C/N analyzer (Flash EA; Thermo Fisher Scientific, Germany). To calculate N_2 fixation in particles of the incubation water or DDN assimilation by corals (host and/or zooxanthellae), the equation of Montoya et al. (1996) was used:

$$N_2 \text{ fixation or DDN assimilation} = \frac{\text{atom\%}^{15}N_{excess} \times PN_{sample}}{t \times 9.8}$$

Where: t is the incubation time, 9.8 the initial ^{15}N enrichment of the incubation water, and PN_{sample} the particulate N content of the samples. For each sample, the atom%$^{15}N_{excess}$ enrichment was calculated by subtracting the natural ^{15}N enrichment of control samples without $^{15}N_2$ exposure (atom% $^{15}N_{control}$) from the ^{15}N enrichment of samples after exposure to $^{15}N_2$ enriched seawater (atom% $^{15}N_{sample}$). The atom% $^{15}N_{sample}$ was considered significant when it was at least three fold higher than the standard deviation of the atom% $^{15}N_{control}$. N_2 fixation and DDN assimilation values were normalized to the volume of water filtered or to the total dry weight of the sample. For comparison with

previous studies, scleractinian corals data were also normalized to the skeletal surface area determined using the wax technique (Veal et al. 2010).

4.2.4 Statistical analyses

Analyses were performed using R software (R Foundation for Statistical Computing). All data were expressed as mean ± standard error. Prior to analyses, outlier values were identified using Grubb's test and were excluded when p-values were significant ($p < 0.05$). Assumptions of normality and homoscedasticity of variance were evaluated through Shapiro's and Bartlett's tests. A non-parametric Kruskal-Wallis test was used to test for differences between groups (hard vs soft corals) and depths (shallow vs deep soft corals) on POC and POC:PN. Analyses of variance (ANOVA) were performed to respectively test the effect of species and morphologies on DDN transfer and assimilation. Tukey tests were performed as *a posteriori* testing.

4.3 Results

In the laboratory experiment, no N_2 fixation (neither in seawater nor in coral tissue) was measured when corals were incubated without the addition of *Crocophaera watsonii* cells to the seawater. Both *S. pistillata* and *Sarcophyton* sp. however demonstrated abilities to assimilate DDN in the presense of active diazotrophs (i.e. *Crocosphaera watsonii*) in seawater (Figure 24). Overall, *Sarcophyton* sp. assimilated 65% more DDN in its tissue than *S. pistillata*. While there was no difference between the DDN assimilations of *S. pistillata* exposed at the two cell concentrations (Tukey HSD: $p = 0.389$), *Sarcophyton* sp. significantly increased its DDN assimilation under higher cell concentration (Tukey HSD: $p = 0.020$).

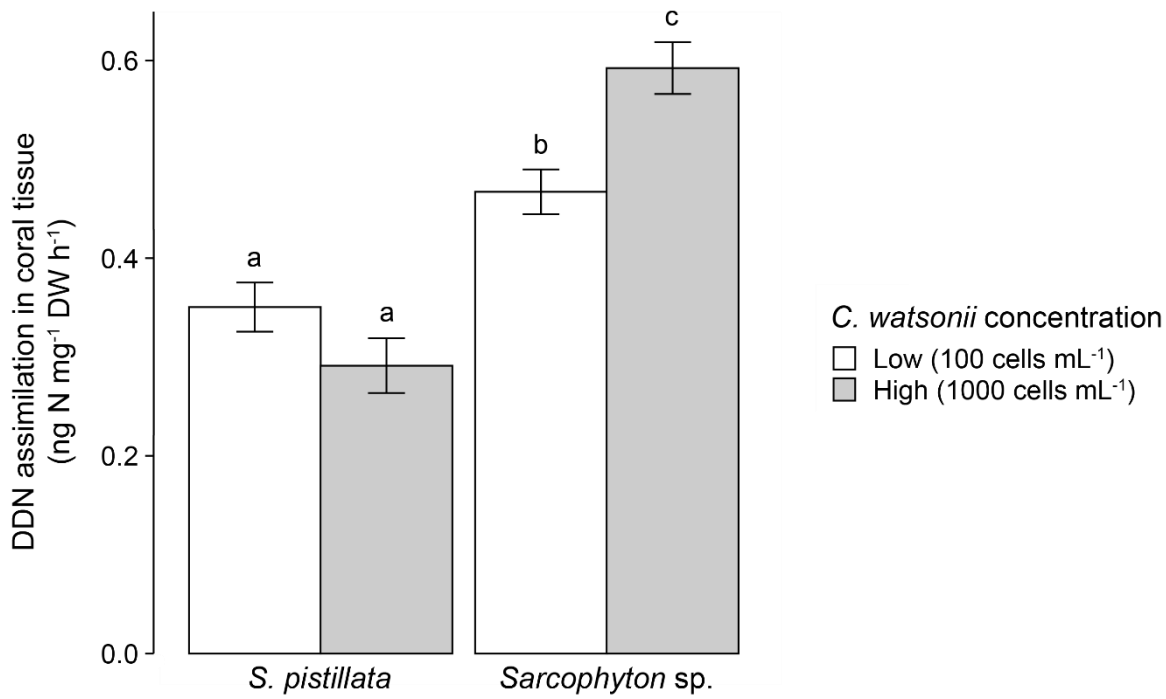

Figure 24. **Scleractinian and soft corals DDN assimilation from two concentrations of active diazotrophs (*Crocosphaera watsonii*) in seawater.** Investigated species are *Stylophora pistillata* (scleractinian coral) and *Sarcophyton* sp. (soft coral). Error bars indicate standard error. Letters refer to Tukey HSD testing. DDN = diazotrophically-derived nitrogen. DW = dry weight.

In the field experiment, a very low level of N_2 fixation was measured in seawater with natural populations of diazotrophs, in the absence of corals (Figure 25a). All coral species enriched the seawater in mucus-containing particles during the 24 h incubation (Figure 25a). Particulate organic carbon, which is a proxy for living and detrital particles present in the water and being released by the corals, was two to seven fold higher in chambers containing scleractinian corals compared to those containing soft corals (Kruskal-Wallis: $p < 0.001$). There was no difference in POC release between shallow and mesophotic soft corals (Kruskal-Wallis: $p = 0.138$). Particles released by scleractinian corals presented a higher POC:PN ratio (between 11.9 and 16.6) compared to those of soft corals (between 4.9 and 7.5) (Figure 25b, Kruskal-Wallis: $p < 0.001$). While there was no N_2 fixation in the incubation water containing all soft coral species sampled in shallow or mesophotic environments, a significant fixation was observed in the incubation water of all four scleractinian species (Figure 26a, b). Fixation rates ranged from 34 to 211 ng N L^{-1} over the 24 h incubation, or between 6 and 135 10^{-3} ng N mg DW^{-1} h^{-1} when expressed per coral biomass. For both normalizations, rates were significantly different between

species (ANOVA: $p = 0.003$ and $p = 0.002$, respectively) and morphologies (branching vs mounding, ANOVA: $p < 0.001$) with higher rates obtained for *A. eurystoma* and *P. damicornis*. Significant assimilation of DDN was observed in the host tissue and zooxanthellae of scleractinian corals, but not for the soft coral species from either shallow or mesophotic environments (Figure 26c). The DDN assimilation in host tissue was equal or higher than the assimilation in the zooxanthellae fraction. Moreover, an inverse trend was observed between N_2 fixation occurring in incubation water and DDN assimilated by the whole symbiotic association (Figure 26d).

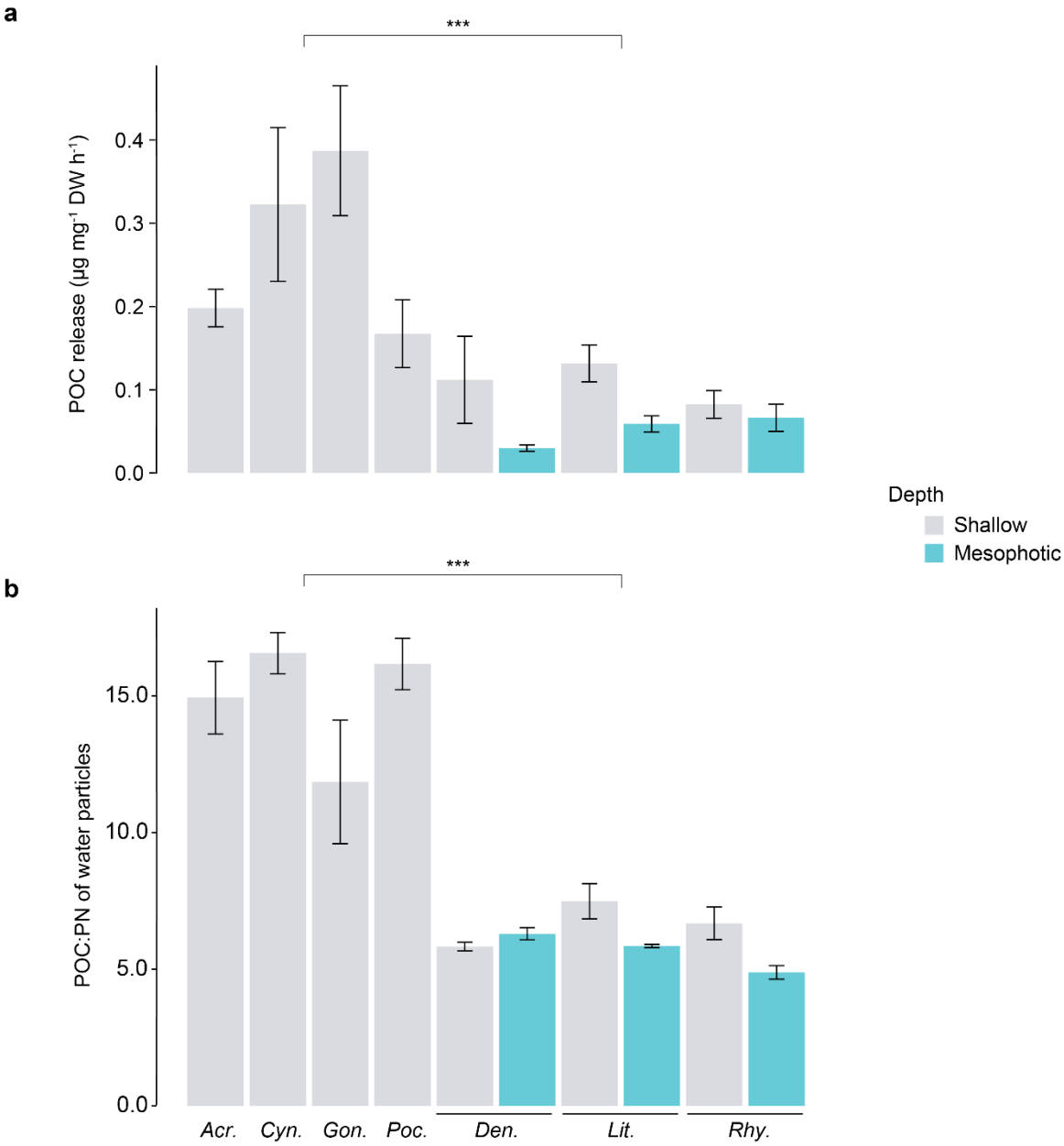

Figure 25. POC (a) and POC:PN ratio (b) measured in the incubation water of corals. POC = Particulate Organic Carbon; PN = Particulate Nitrogen. DW = dry weight. Asterisks indicate a significant difference between the two groups (scleractinian vs soft corals, $p < 0.001$). From left to right: Acr. = *Acropora cervicornis*, Cyn. = *Cynarina* sp., Gon. = *Goniastrea* sp., Poc. = *Pocillopora damicornis*, Den. = *Dendronephthya* sp., Lit. = *Litophyton* sp., Rhy. = *Rhytisma fulvum fulvum*.

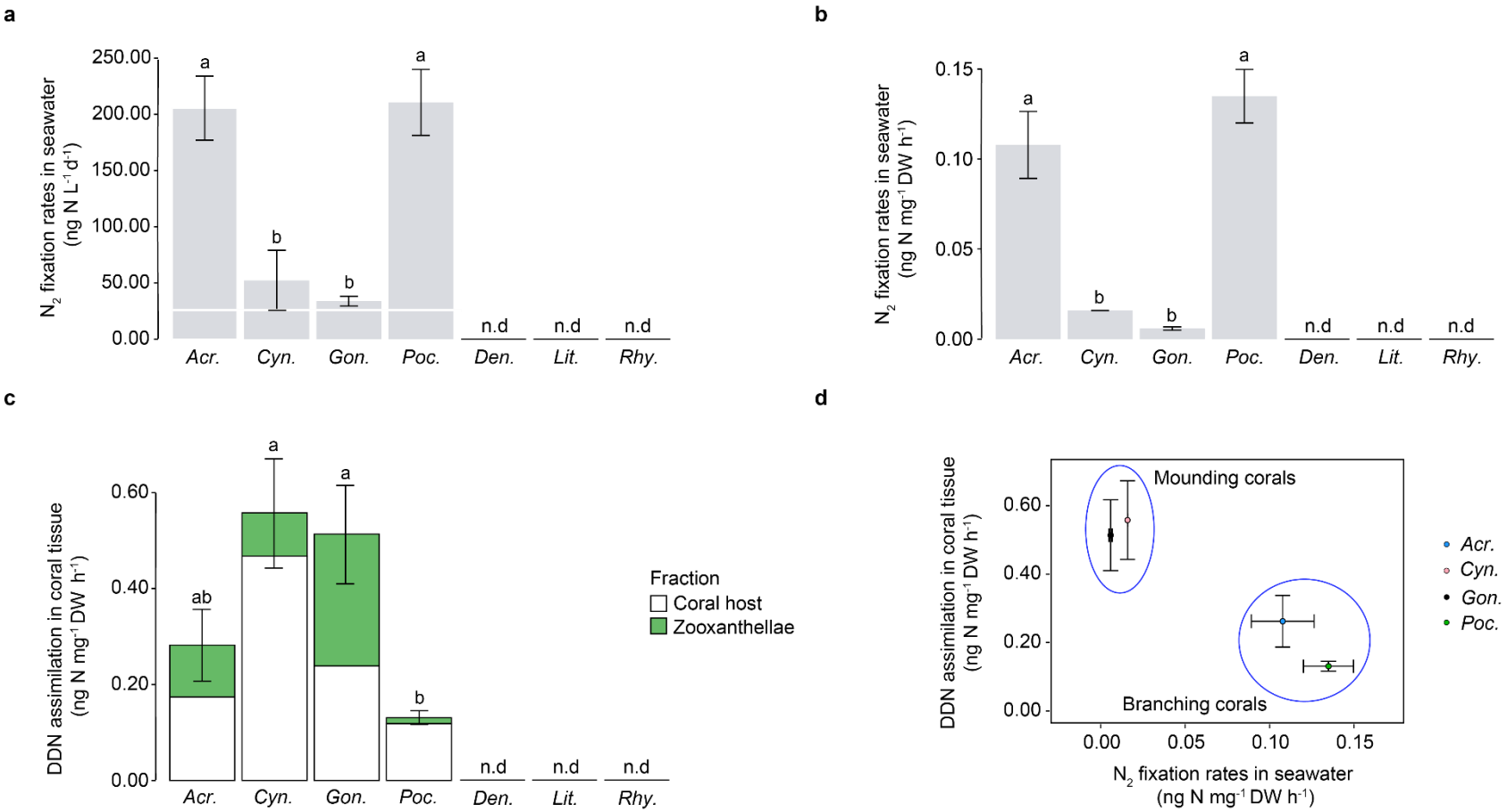

Figure 26. N_2 fixation in seawater (a,b), assimilation in coral tissues (c), and relationship between the two (d). The white line indicates N_2 fixation rate occurring in 120 µm-filtered seawater incubated without corals. Error bars indicate standard error. n.d. = not detected. DDN = diazotrophically-derived nitrogen. DW = dry weight. From left to right: Acr. = *Acropora cervicornis*, Cyn. = *Cynarina* sp., Gon. = *Goniastrea* sp., Poc. = *Pocillopora damicornis*, Den. = *Dendronephthya* sp., Lit. = *Litophyton* sp., Rhy. = *Rhytisma fulvum fulvum*.

4.4 Discussion

4.4.1 Divergent capacity of coral mucus to enrich seawater with DDN

This study first highlights contrasting capacities of scleractinian and soft corals to promote N_2 fixation in seawater. Freshly fixed N_2 was traced in the incubation water containing scleractinian coral species, while no fixation in the seawater occurred in presence of soft corals. A previous study, which has used the ARA method has also recorded much lower rates of gross N_2 fixation in the presence of soft compared to scleractinian corals (Bednarz et al. 2015). A difference in the quality and/or quantity of mucus (particulate and dissolved organic carbon, POC and DOC) released by the two coral groups as observed here, together with a different amount of mucus-associated bacteria most likely explain differences in seawater diazotroph abundance or activity. While the sugar composition of the mucus can be similar between soft and scleractinian corals (Meikle et al. 1988; Hadaidi et al. 2019), both the amount and POC:PN content of mucus is often species-dependent (Naumann et al. 2010; Hadaidi et al. 2019). Pogoreutz et al. (2017) highlighted that DOC enrichment, in the form of sugars, can significantly stimulate diazotrophic N_2 fixation. In our study, scleractinian corals released between 1.9 and 8.9 mg POC m^{-2} h^{-1}, which is in the range of what has been reported for Red Sea scleractinian corals (between 0.3 to 6.5 mg POC m^{-2} h^{-1}) by Naumann et al. (2010). Soft corals however released POC at two- to ten-fold lower rates. This is in agreement with previous measurements performed on another Red Sea soft coral belonging to the Xeniidae family, for which no POC release was observed (Bednarz et al. 2012). As POC is a proxy for detrital and living particles, the lower particle content in the soft coral surrounding seawater can be explained by the well-known antimicrobial properties of soft coral mucus (Kelman et al. 2006; Nakajima et al. 2018). These authors indeed demonstrated that little antimicrobial activity was measured for scleractinian coral mucus whereas soft corals, including those studied in this work, significantly inhibited the growth of co-occurring seawater bacteria through the production of antibiotic compounds. Hence, scleractinian corals release mucus with a large number of bacteria into the surrounding seawater that can influence the activity and diversity of planktonic diazotroph populations. This feature may be greatly reduced in soft corals, as their high antimicrobial activity constitutes a defense strategy against invading pathogens and fouling organisms for the holobiont, thus possibly assisting in competition over space and nutrition (Kelman et al. 2009). Finally, the organic matter (OM) released by soft corals presented a particularly low POC:PN content (of 5 to 7, i.e. particles enriched in N) compared

to the OM released by scleractinian corals (from 12 to 17 in this study; range in agreement with Naumann et al., 2010). This is also in agreement with the findings of two previous studies (Meikle et al. 1988; Nakajima et al. 2018), which observed a higher protein and lower carbohydrate composition of soft coral mucus compared to scleractinian coral mucus. Since N_2 fixation is rather promoted by N deprivation (Knapp 2012), a low POC:PN ratio is not in favour of N_2 fixation. Put together, our results suggest that the lack of N_2 fixation in seawater surrounding soft corals is potentially due to the antimicrobial properties of soft coral mucus and the N repletion of the released particles. On the contrary to soft corals, seawater N_2 fixation was recorded in presence of scleractinian corals. Measured rates (0.01-0.13 ng N mg^{-1} DW h^{-1} or 0.03 nmol N cm^{-2} h^{-1} or 2 to 15 nmol N L^{-1} d^{-1}) are however significantly lower than those recorded for other benthic substrates such as sediment, sands or microbial mats (reviewed in Benavides et al., 2017). Nevertheless, they are in the same range as those previously measured with seawater diazotrophs of the Great Barrier Reef (from 5 to 70 nmol N L^{-1} d^{-1}, Messer et al., 2017).

4.4.2 Divergent capacity of corals to assimilate DDN

Our results demonstrate that all investigated scleractinian species assimilated DDN, since ^{15}N enrichment was observed in both host tissue and symbionts. In contrast, no ^{15}N enrichment of soft coral tissue was detected either in corals collected from shallow or mesophotic reefs. The fact that even mesophotic soft coral colonies did not assimilate DDN stands in contrast to the scleractinian species *S. pistillata*, which shows higher assimilation rates in deep compared to shallow waters (Bednarz et al. 2017). Here, DDN assimilation rates of scleractinian corals (ca. 0.3 to 1 ng cm^{-2} h^{-1} or 0.6 to 1.7 nmol cm^{-2} d^{-1}) are in agreement with those measured for the species *S. pistillata* sampled in shallow waters of the Red Sea or the Great Barrier Reef (Bednarz et al. 2017; Lesser et al. 2018). However, they were 6 to 10 times lower than those measured for corals depending more on heterotrophy, such as bleached corals or those living in mesophotic and temperate environments (Bednarz et al. 2017, 2019). These observations suggest that the contribution of DDN to the N requirements of corals increases during nutrient deprivation, or when the uptake of other inorganic N forms by dinoflagellate symbionts is reduced (Bednarz et al. 2019). The inverse trend observed in this study between seawater N_2 fixation and DDN assimilation rates in corals suggests that a substantial part of the DDN assimilated by corals is obtained from heterotrophic feeding on fixed N compounds

and/or from diazotrophic cells growing in the mucus layer (Bednarz et al. 2017). Therefore, the lack of N_2 fixation in the surrounding seawater of soft corals (highlighting the absence of active diazotrophs in their mucus) may also explain why we did not detect any DDN assimilation by shallow and mesophotic soft corals. Furthermore, coral polyps with a mounding morphology show generally higher heterotrophic feedings rates as compared to those with a branching morphology (Palardy et al. 2005). This corresponds to the higher DDN assimilation rates observed for *Cynarina* sp. and *Goniastrea* sp. (mounding morphology) as compared to *A. cervicornis* and *P. damicornis* (branching morphology). Corals can receive DDN not only from mucus-associated but also from pelagic diazotrophs (Camps et al. 2016). In our experiment on *C. watsonii* cells, both corals assimilated DDN (i.e. in the form of N_2 fixing *C. watsonii* cells or DDN compounds released by *C. watsonii* cells) from the surrounding seawater with rates similar to those observed for our corals from the Red Sea. Moreover, *Sarcophyton* sp. assimilated more DDN in the high versus the low *C. watsonii* concentration suggesting that the assimilation capacity of this species was not saturated even under simulated bloom conditions. This indeed indicates that soft corals also have the capacity to assimilate diazotrophs/DDN from the seawater. Thus, the lack in DDN assimilation by Red Sea soft corals sampled in the field, including the Symbiodiniaceae-free and heterotrophic genus *Dendronephthya* sp., is likely not due to a lower heterotrophy as compared to scleractinian corals, but rather linked to the absence of active diazotrophs in the soft coral mucus. For example, strains of putative diazotrophs (*Vibrio campbellii* and *Vibrio parahaemolyticus*; Chimetto et al., 2008) have been isolated from *Dendronephthya* sp. (Harder et al. 2003). We therefore hypothesize that even if diazotrophs can be present, the mucus of soft corals does not represent a favorable habitat for diazotrophic activity. Moreover, non-negligible DDN assimilation rates have been detected in the skeleton of scleractinian corals due to the activity of endolithic diazotrophs (Sangsawang et al. 2017; Bednarz et al. 2019). Such contribution to the total N budget of the holobiont cannot be observed in soft corals as they lack a calcium carbonate skeleton. *In situ* experiments remain to be investigated to assess whether soft corals benefit from any other external source of N on reefs that could enhance their opportunistic life history features.

4.5 Conclusion

It is widely accepted that N is one of the most limiting nutrients for reef primary productivity (Eyre et al. 2008), and that benthic N_2 fixation plays an important role in supplying bioavailable N within benthic and pelagic reef habitats (Cardini et al. 2016; Messer et al. 2017). The surface structure of benthic organisms and substrates provides an important habitat for the colonization by diazotrophs, but the abundance, composition and activity of the diazotrophic community may depend on the type of organism/substrate. Here, we suggest that soft coral mucus represents likely a less favorable habitat for microbes as compared to scleractinian coral mucus due to its relatively low C but high N content along with antimicrobial properties. The resulting different capacity of scleractinian and soft corals to promote active diazotroph populations and N_2 fixation in reef waters may have several implications for N availability and reef biogeochemistry in the future. Particularly coral reefs that have experienced phase shifts from hard to soft coral dominance (Stobart et al. 2005; Norström et al. 2009; Pratchett 2010; Inoue et al. 2013) may suffer from a significant decrease in N_2 fixation and subsequent N limitation. This may ultimately affect primary production, carbon sequestration and the functioning of coral reef ecosystems and could be an interesting topic for future studies.

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Publication n°5

Dissolved nitrogen acquisition in soft and hard corals: a key to understand their different nutritional strategies?

In preparation

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Abstract

Symbioses between marine organisms and photosynthetic dinoflagellates of the family Symbiodiniaceae are vital for reef ecosystems. Coral holobionts are able to grow and survive in oligotrophic environments through the efficient uptake, conservation and recycling of nutrients. Nitrogen (N) is one essential nutrient for coral holobiont functioning but nitrogen availability is often low in reef waters. Recent studies have advanced our understanding of nitrogen cycling in scleractinian corals but the nutrient metabolism of other major benthic organisms on reefs still needs to be addressed. For example, the capacity of soft corals to assimilate dissolved nitrogen at the organism level remains unexplored. Here, using stable isotope labeling, we performed a multi-species comparison to investigate dissolved nitrogen (i.e. ammonium, nitrate, and free amino acids) assimilation in several species of soft and scleractinian corals sampled in the Red Sea (Eilat, Israel) at two different depths (8-10 m and 40-50 m depth). The main result shows that for all nitrogen sources, scleractinian corals exhibited higher assimilation rates per tissue biomass than soft corals (up to ten fold), despite harboring the same genera of Symbiodiniaceae in similar densities. Soft corals may thus complement their low acquisition of DN by the acquisition of other N forms, thereby rather relying on heterotrophic feeding to meet their N requirements. This finding highlights a very different acquisition of N between the soft and scleractinan-dinoflagellate associations. Such

contrasting capacities to take advantage of this nutrient source can help in understanding how soft and scleractinian corals cope with different nutrient loads.

4.6 Introduction

Nitrogen (N) is essential for the life of all organisms on Earth, as it is required for the biosynthesis of key cellular components. However, N concentrations are low in many marine ecosystems such as coral reefs, with the most abundant N form, dinitrogen gas (N_2), being only available to nitrogen-fixing microorganisms. N is therefore a growth-limiting nutrient for most organisms living in oligotrophic environments. Animals are only able to assimilate particulate and dissolved organic N (DON), such as free amino acids (DFAA) and urea, whereas algae and microorganisms can also assimilate the inorganic forms (DIN) such as nitrate (NO_3^-) and ammonium (NH_4^+) (Muscatine et al. 1979). Consequently, many reef animals have evolved as symbiotic meta-organisms (i.e. holobionts), in which the host is associated with microorganism assemblages, in order to efficiently take up, conserve and recycle the few available nutrients. Consequently, many reef animals have evolved as symbiotic metaorganisms (i.e., holobionts), in which the host is associated with microalgae and/or nitrogen-fixing bacteria to survive in their nutrient-poor environment.

Symbiotic corals are the most iconic reef holobionts. They are indeed associated with a wide range of microorganisms such as diazotrophs and dinoflagellates of the family Symbiodiniaceae (LaJeunesse et al. 2018). As a result, they can use almost all N forms available in their surrounding environment, with the symbionts primarily assimilating DIN (Grover et al. 2002, 2003; Pernice et al. 2012), and the host DON (Grover et al. 2006, 2008). Therefore, when corals bleach (i.e. loss of dinoflagellate symbionts due to environmental stress), they are depleted of a major N source and their chances of recovery are partly determined by their ability to restore the functions of their symbionts (Suggett et al. 2017). Assimilation of dissolved N also depends on the environmental conditions to which the corals are exposed. For example, elevated seawater temperatures, which reduce nutrient availability through water column stratification (Behrenfeld et al. 2006), lessens the ability of the coral holobiont to take up DIN (Godinot et al. 2011; Krueger et al. 2018). Finally, metabolic compatibility between coral hosts and their algal symbionts also determine the nutritional performance of the coral holobiont (Leal et al. 2015; Pernice et al. 2015). Overall, the nutritional status of corals and their capacity

to efficiently exploit nutrients from the surrounding environment are major functional traits influencing their distribution and abundance in a given environment.

However, the nutritional ecology of coral-dinoflagellate symbioses, regarding their N metabolism, has most exclusively concerned few species of hard corals, as they are the main reef builders (Muscatine et al. 1989; Hoegh-Guldberg and Williamson 1999; Mills et al. 2004; Baker et al. 2013b; Ezzat et al. 2017). Other major benthic reef organisms such as soft corals have been overlooked (qualitative assimilation of NH_4^+ in *Lobophyton* sp., Burris 1983); uptake of DFAA by *Heteroxenia fuscescens*, Schlichter 1982b). This lack of data, together with different normalization metrics between the two coral groups prevent any predictions on the nutritional conditions that favour the growth of either hard or soft corals. Soft corals have however been observed in higher abundance in areas with high concentrations of dissolved inorganic nutrients (Baum et al. 2016). This observation suggests that they might have a different nutrient metabolism than hard corals, or a different nutritional relationship with their symbionts, but these hypotheses remain to be investigated. Therefore, this study assessed dissolved N assimilation (i.e. NH_4^+, NO_3^-, and DFAA) in soft corals, addressed which forms present the highest assimilation rates, and compared assimilation rates of soft and hard corals. For this purpose, a multi-species comparison was performed using the ^{15}N stable isotope tracer to follow N incorporation into the tissues of several soft and hard corals sampled in shallow and upper mesophotic reefs of the Red Sea. This study thus allows a better understanding of the functional ecology of both soft and hard corals.

4.7 Material and methods

4.7.1 Biological material

The study was conducted at the Inter-University Institute (IUI) for Marine Sciences (Eilat, Israel) in November 2018. The experiment was performed with 3 species of hard corals (*Galaxea fascicularis*, *Stylophora pistillata* and *Seriatopora hystrix*) and 3 species of soft corals (*Litophyton arboreum*, *Rhytisma fulvum fulvum* and *Sarcophyton* sp.). Coral fragments were collected by SCUBA diving on the shallow (8-10 m depth) and mesophotic (40-50 m depth) reefs adjacent to the IUI. Temperature and nutrient levels at the time of sampling were not different between the shallow and mesophotic reefs. Temperature ranged from 24.7 to 25.4°C during the length of the experiment. Nitrate concentrations ranged from 0.17 to 0.25 µM, whereas dissolved inorganic phosphorus and ammonium were below 0.2 µM (data obtained

from the National Monitoring Program of the Gulf of Eilat (http://www.iui-eilat.ac.il/Research/NMPMeteoData.aspx). The genus of the symbionts hosted by the corals was investigated following the protocol of Santos et al. (2002), using chloroplastic ribosomal deoxyribonucleic acid sequences.

To assess the assimilation rates of N under natural living conditions, twenty nubbins per coral species were collected from different colonies at the shallow and mesophotic depths (except for *L. arboreum* which was only found in shallow waters). Five nubbins were immediately frozen for the determination of Symbiodiniaceae genus and natural isotopic abundance, while the others were brought back to the Red Sea Simulator facility (described in Bellworthy and Fine 2018) to recover for one week prior to starting the incubations with the ^{15}N stable isotope tracers (see below). For this purpose, nubbins were allocated to 12 outdoor aquaria (one per species and depth), which were continuously supplied with seawater directly pumped from the reef. Corals were maintained under the natural light cycle at levels corresponding to their *in situ* depth (ca. 450 and 30 µmole photons m^{-2} s^{-1} at midday, for shallow and mesophotic environments respectively). These levels were adjusted using mesh clothes.

In addition to the conditions described above, 3 additional aquaria were prepared, each containing 5 nubbins per species of soft corals collected from the shallow reef (5 m). Aquaria were maintained under the exact same conditions as above, except that temperature was gradually increased (+ 0.5°C per day) up to 30°C (+5°C than the control condition). Corals were kept at this higher temperature for one week prior to the incubations with ^{15}N tracers, run at the same time as the other incubations.

4.7.2 Incubations

^{15}N stable isotope tracers were used to assess the assimilation rates of dissolved inorganic and organic N sources by hard and soft corals. To this end, 15 L of 0.45 µm-filtered seawater were enriched with either $^{15}NH_4Cl$, $^{15}NO_3Cl$ and ^{15}N-DFAA (all at 98 atom %^{15}N, Sigma-Aldrich, St-Louis, USA) to reach a final N concentration of 3 µM. Five 200 mL beakers were prepared per species, depth and N compound and entirely filled with the ^{15}N-enriched solution. Incubations were performed in the Red Sea simulator under the natural irradiance of shallow and mesophotic depths. Corals were incubated at 25°C or at 30°C for five hours, between 11:00 am and 04:00 pm to cover the maximal daily irradiance. At the end of the

incubations, coral nubbins were rinsed in 0.2 µm-filtered seawater (FSW) and frozen at -80°C until further analysis.

4.7.3 Samples processing

Soft coral samples were processed as described in Pupier et al. (2018). Briefly, they were freeze-dried, weighed and crushed into powder. For hard corals, the tissue was first removed from the skeleton using an air-brush and homogenized with a Potter-Elvehjem tissue grinder, before being freeze-dried, weighed and crushed into powder. The skeleton was kept for the determination of the skeletal surface area using the wax dipping technique (Veal et al. 2010). A fraction of the powder (i.e. ca. 30 mg) was used for the determination of the ash-free dry weight (AFDW) as described below and the remaining tissue was homogenized in 10 mL distilled water with a Potter-Elvehjem tissue grinder. The host and symbiont fractions of each sample were separated through a series of centrifugations (Grover et al. 2003). The symbiont pellet was re-dissolved in 10 mL distilled water. A 500 µL subsample was used for the determination of algal symbiont density via haemocytometer counts (Neubauer-improved haemocytometer, Marienfeld, Germany) using a light microscope. In addition, a 2.5 mL subsample from the symbiont fraction was used for the determination of the chlorophyll concentration according to Jeffrey and Humphrey (1975), using a spectrophotometer (SAFAS, Monaco). The remaining fractions of the host and symbionts were subsequently freeze-dried and weighed. Approximately 500 µg of host and symbiont powder were transferred in tin caps for analysis of the ^{15}N enrichment, N content and the natural $\delta^{15}N$ signatures using a mass spectrometer (Delta Plus, Thermo Fisher Scientific, Germany) coupled to a C/N analyzer (Flash EA, Thermo Fisher Scientific, Germany). The total amount of N assimilated in each compartment was then determined using the modified equations of Dugdale and Wilkerson (1986) according to Grover et al. (2002, 2003, 2008). These equations compare the N enrichment obtained in the incubations with ^{15}N with the natural isotopic values of control corals, sampled at the beginning of the experiment.

All data were normalized to the AFDW of the nubbins, which was used as a proxy for biomass. The subsample used for the determination of the AFDW was first weighed, and then combusted at 450°C for 4 h in a muffle furnace (Thermolyne 62700, Thermo Fischer Scientific, the United States). AFDW was determined as the difference between the dry weight and ash weight of the subsample and extrapolated to the total weight of the nubbin. AFDW was chosen

for normalization because the surface area of soft corals cannot be accurately determined, as besides polyp expansion, their fleshy body can extend or retract extensively depending on environmental conditions (e.g., Fabricius and Klumpp 1995). Moreover, since N is assimilated into coral biomass, it is therefore more relevant to estimate the assimilation rates per biomass. This is in agreement with the observations of Edmunds and Gates (2002), who also concluded that normalizing data to biomass is most effective in accounting for coral size in comparative studies.

4.7.4 Statistical analyses

Analyses were performed using the R environment for statistical computing and graphics (R Core Team, 2017 Generalized linear models were used to test: 1) the effect of species, depth and N source on the assimilation rates of N by the host or the dinoflagellate fractions at 25°C, 2) the effect of species, temperature and N source on the assimilation rates of N by the host or dinoflagellate fractions in shallow soft corals, and 3) the effect of species and depth on tissue descriptors. We did not differentiate between the three N sources tissue descriptor data, as we assumed that the incubation time and N concentration used here would not affect symbiont density and chlorophyll concentration (Hoegh-Guldberg 1994), and the N assimilated represented less than 0.5% of the total N content. Generalized linear models (family = Gaussian) were fitted on data transformed using the Box-Cox transformation procedure ($(x^{\lambda}-1)/\lambda$, Appendix III - Table S6) as implemented in the R-package *MASS* (Venables and Ripley, 2002). Compliance with the assumptions of a normal distribution of the model residuals and homoscedasticity was verified using, respectively, the Shapiro-Wilk test and Levene's test as implemented in the R-package *car* (Fox and Weisberg, 2019). The *emmeans* package (Lenth et al. 2020) was used to calculate estimated marginal means and compute biologically relevant contrasts (i.e. between depths for each species and between species for each depth). The Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) was used for multiplicity adjustments of p-values.

4.8 Results

For clarity, the results of the statistical treatments are reported in Appendix III (Table S7) only. Shallow nubbins of *L. arboreum* and *S. pistillata* harbored *Symbiodinium* sp.

(formerly clade A), while nubbins from all other species and depths were associated with *Cladocopium* sp. (formerly clade C). Symbiodiniaceae densities per tissue biomass (Figure 27) were overall similar between hard and soft corals. They were significantly different between depths only for *G. fascicularis*, *S. hystrix* (lower at mesophotic depth) and *R. f. fulvum* (higher at mesophotic depth). Total chlorophyll concentration per tissue biomass was significantly higher in *R. f. fulvum* at shallow depth, compared to the other species (Appendix III -Figure S2a). Mesophotic colonies presented a higher chlorophyll concentration per biomass than shallow colonies in *S. hystrix* and *S. pistillata* (Appendix III -Figure S2a). At shallow depth, *R. f. fulvum* and *Sarcophyton* sp. contained higher chlorophyll concentrations per symbiont cell than hard corals (Appendix III - Figure S2b). All hard coral species, as well as *Sarcophyton* sp, showed significant higher concentrations in chlorophyll at the mesophotic depth as compared to shallow (Appendix III - Figure S2b).

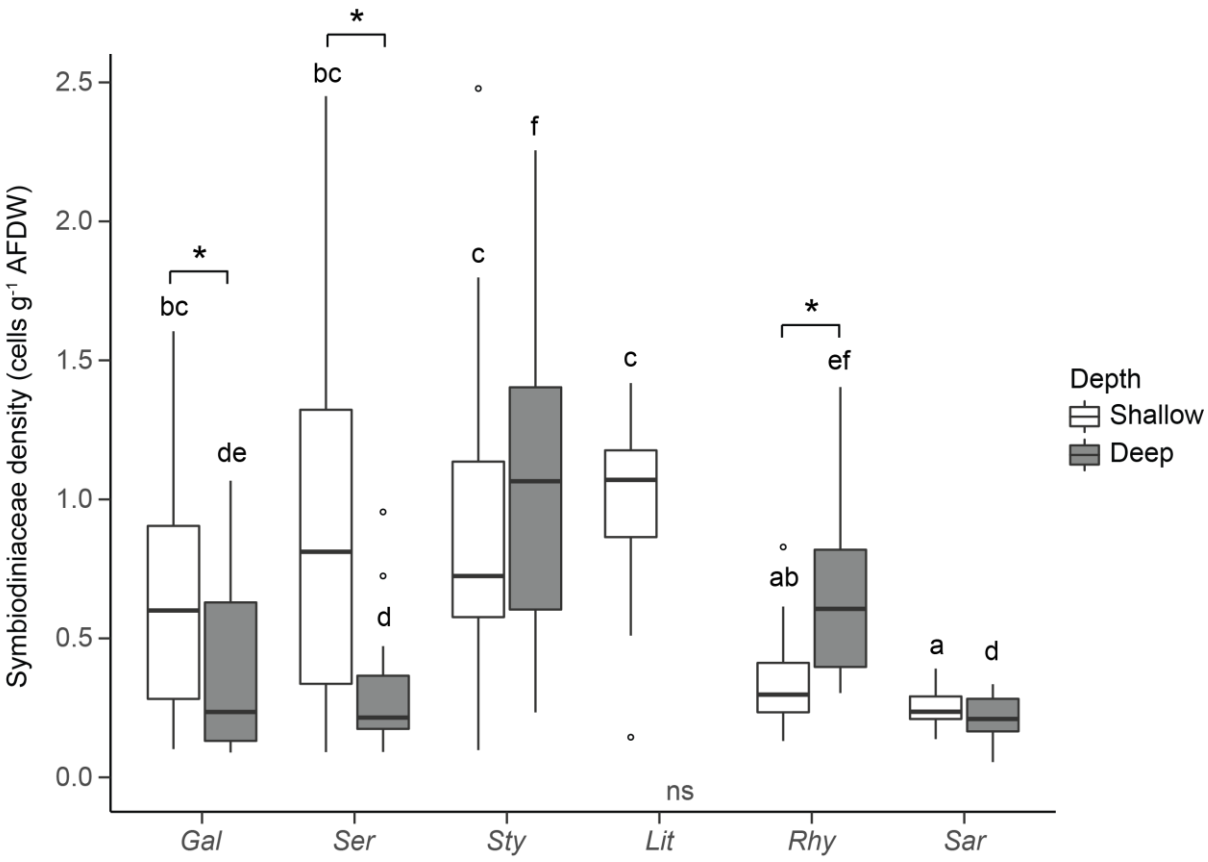

Figure 27. Symbiodiniaceae density in the species investigated. The first three species (*Galaxea fascicularis*, *Seriatopora hystrix*, *Stylophora pistillata*) are hard corals and the last three (*Litophyton arboreum*, *Rhytisma fulvum fulvum*, *Sarcophyton* sp.) are soft corals. Significant differences between depths are displayed with an asterisk. Significant differences between species are distinguished with letters by depth. AFDW = ash-free dry weight. ns = not sampled.

Concerning the elemental composition of the host tissue, the C:N ratios increased from ca. 5 in scleractinian corals and *L. arboreum* from shallow and mesophotic depths to ca. 8 in shallow and mesophotic *Sarcophyton* sp, and to more than 15 in *R. fulvum fulvum* (Figure 28a). Such C:N increase in the last two species was explained by a higher C content rather than a lower N content, which was not different between species (Figure 28b). The C:N ratio decreased with depth in *G. fascicularis* and *S. hystrix*. Again, these changes were not entirely due to changes in the N content of the coral tissue, which increased in *S. hystrix* only. The Symbiodiniaceae fraction presented variable C:N ratios (Figure 29a), which were however higher in shallow colonies of *R. f. fulvum*, due to a lower N content compared to the other corals (Figure 29b). Overall, the C:N ratios increased with depth except in *R. f. fulvum*, due to a decrease in N content.

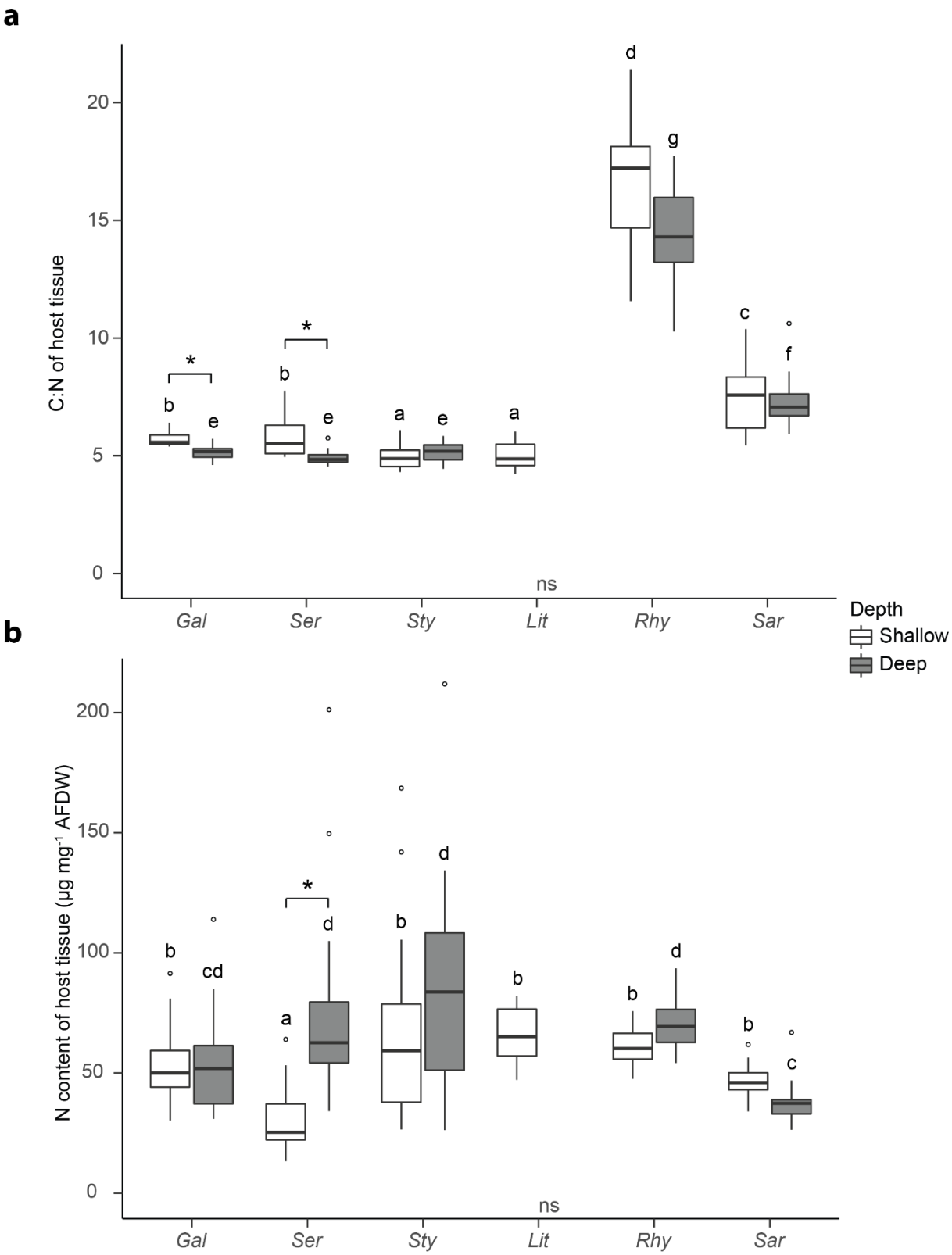

Figure 28. Elemental composition of the host tissue in the species investigated. (a) C:N ratio. (b) N content. The first three species (*Galaxea fascicularis*, *Seriatopora hystrix*, *Stylophora pistillata*) are hard corals and the last three (*Litophyton arboreum*, *Rhytisma fulvum fulvum*, *Sarcophyton* sp.) are soft corals. Significant differences between depths are displayed with an asterisk. Significant differences between species are distinguished with letters by depth. AFDW = ash-free dry weight. ns = not sampled.

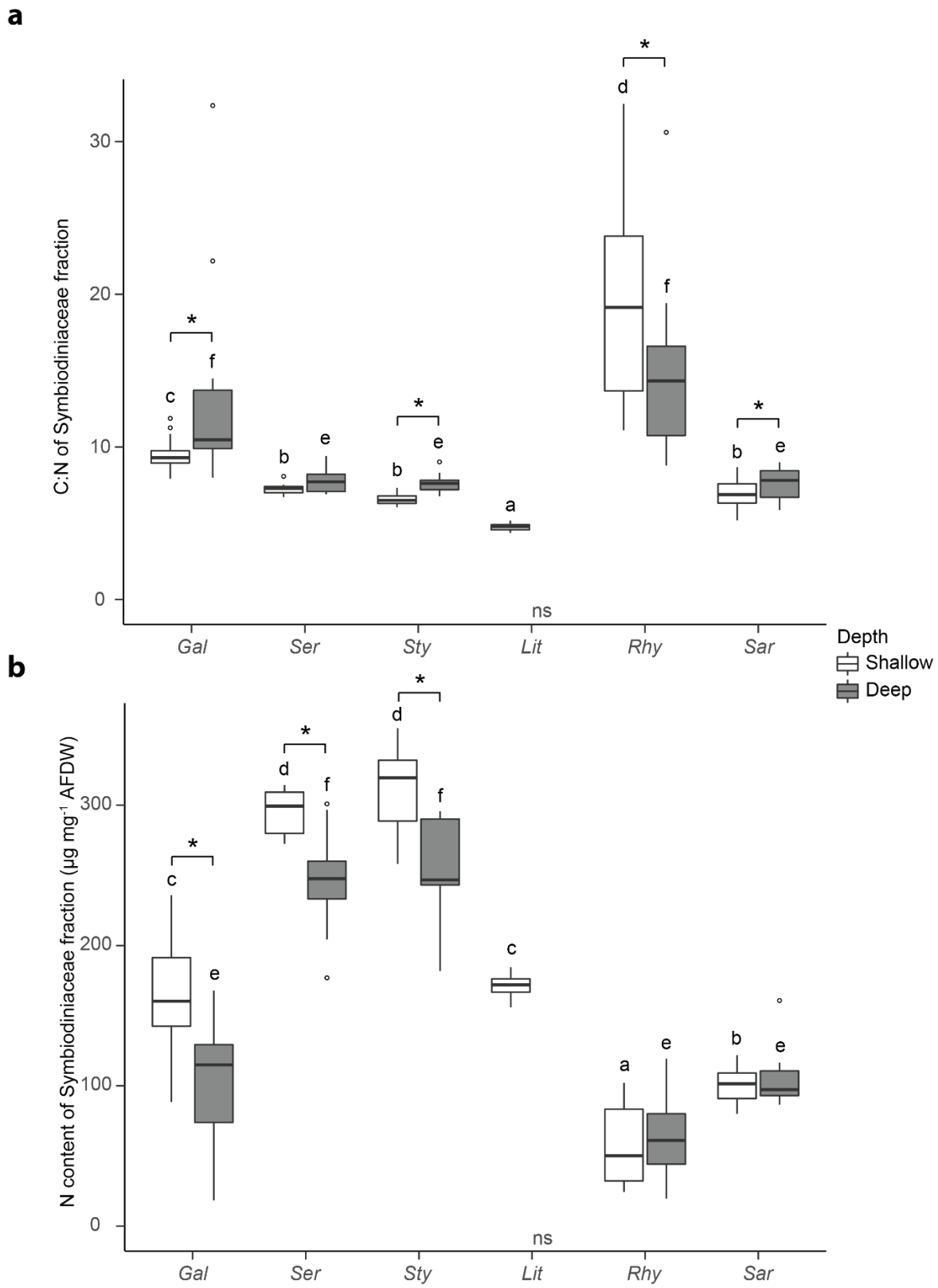

Figure 29. Elemental composition of the Symbiodiniaceae fraction in the species investigated. (a) C:N ratio. (b) N content. The first three species (*Galaxea fascicularis*, *Seriatopora hystrix*, *Stylophora pistillata*) are hard corals and the last three (*Litophyton arboreum*, *Rhytisma fulvum fulvum*, *Sarcophyton* sp.) are soft corals. Significant differences between depths are displayed with an asterisk. Significant differences between species are distinguished with letters by depth. AFDW = ash-free dry weight. ns = not sampled.

The assimilation of N per tissue biomass in the host is ca. ten-fold higher in hard compared to soft corals for all investigated N sources (Figure 30). At each depth, scleractinian species had similar assimilation rates of NH_4, NO_3 and DFAA into host tissue, except *G. fasicularis,* which had higher NO_3 assimilation compared to the other species at shallow depth (Table S2, Figure 30a). Rates of DFAA assimilation into host tissue were consistently higher at mesophotic compared to shallow depth (Figure 30a and Appendix III - Table S7). The same depth effect was observed on the rates of NO_3 assimilation into the host tissue of *S. hystrix* and *S. pistillata*. No effect of depth was observed for NH_4 assimilation rates. Finally, NH_4 was assimilated at higher rates than the other N sources in shallow hard corals, whereas there was no difference in assimilation rates between sources in mesophotic colonies (Appendix III - Table S7). Concerning the soft coral species, and at each depth, NH_4 and DFAA assimilation rates were significantly lower in *Sarcophyton* sp. than in the two other species, while NO_3 assimilation rates were higher in *R. fulvum fulvum* (Figure 30b and Appendix III - Table S7). There was no difference in the assimilation rates of NH_4, NO_3 and DFAA between shallow and mesophotic soft corals, except for NO_3 assimilation rate in *R. fulvum fulvum*, which was higher at mesophotic depth (Appendix III - Table S7). Soft corals assimilated NH_4 at higher rates at both depths, as compared to NO_3 and DFAA (Figure 30b).

Assimilation rates in algal symbionts were also overall higher in hard compared to soft corals, with the following exceptions (Figure 31): symbionts of shallow colonies of *L. arboreum* and mesophotic colonies of *R. f. fulvum* had similar NH_4 assimilation rates than symbionts of *G. fascicularis* at the corresponding depths. DFAA assimilation rates were not different between the symbionts of shallow colonies of *L. arboreum*, *S. pistillata* and *G. fascicularis*. Mesophotic depth decreased the assimilation rates of NH_4 and NO_3 in *Sarcophyton* sp, as well as NO_3 in *R. fulvum fulvum*. However, it increased DFAA assimilation in *R. fulvum fulvum*. There was no preferential assimilation of a nitrogen source by the symbionts of soft corals.

After exposing shallow soft corals to +5°C during one week (Figure 32), we observed, at 30°C, higher assimilation rates of NH_4 in the host tissue and symbionts of *R. f. fulvum* as well as in the host tissue of *L. arboreum*. Assimilation rates of NO_3 were enhanced in the host tissue and symbionts of *R. f. fulvum* while assimilation rates of DFAA were higher in both the host tissue and symbionts of *R. f. fulvum* and *Sarcophyton* sp. at 30°C. At both 25°C and 30°C, *Sarcophyton* sp. assimilated NH_4 (both host and symbiont fractions) and DFAA (host fraction) at lower rates than the other species. Assimilation rates of NO_3 were significantly higher in *R. f. fulvum* at the two temperatures (both fractions) and the assimilation of DFAA was higher in

L. arboreum (symbiont fraction). NH_4 remained the source assimilated at the highest rate at 30°C in all host fractions (Appendix III - Table S7).

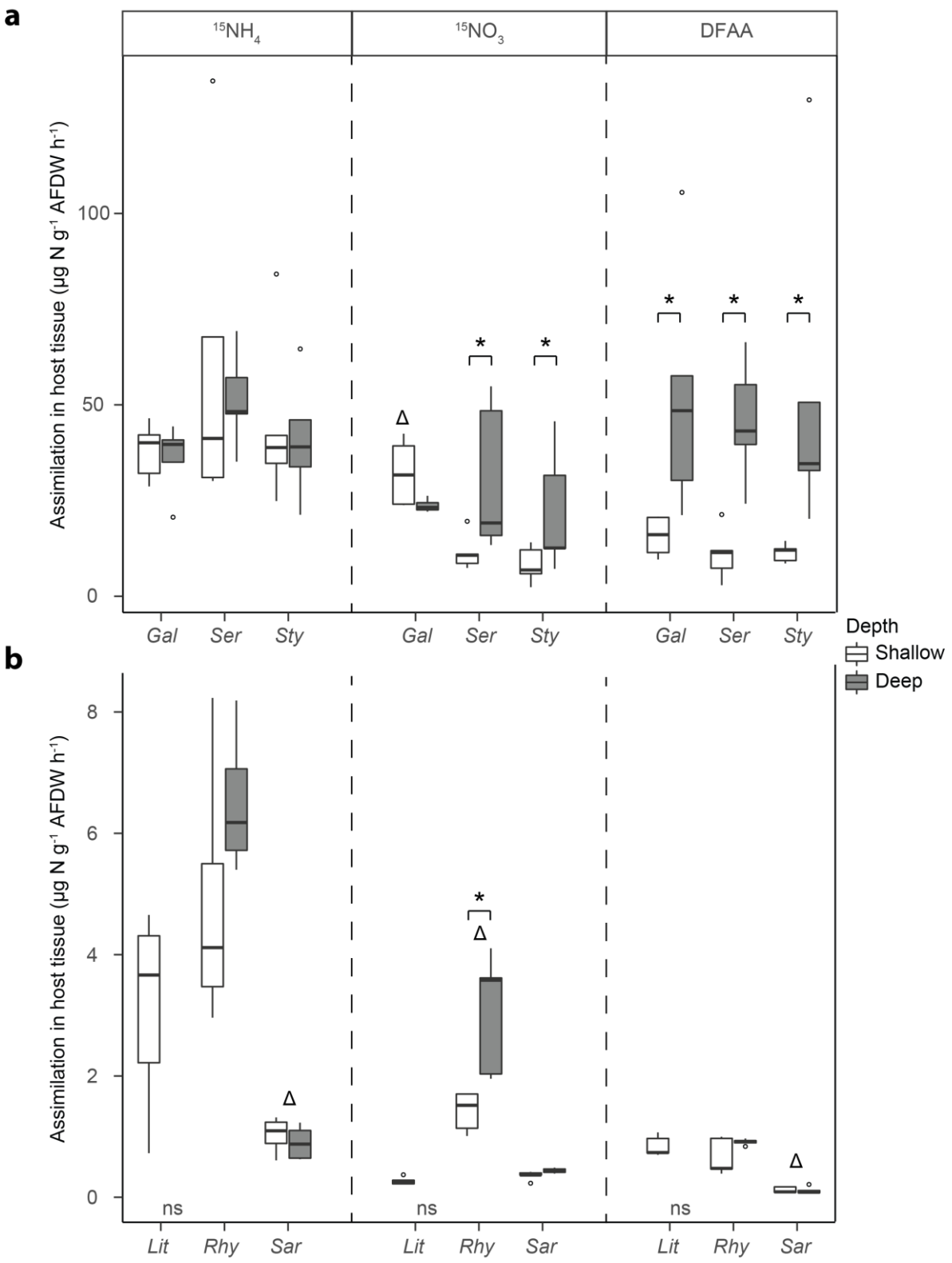

Figure 30. Assimilation rates of dissolved N in the host tissue in (a) hard corals (*Galaxea fascicularis, Seriatopora hystrix, Stylophora pistillata*) and (b) soft corals (*Litophyton arboreum, Rhytisma fulvum fulvum, Sarcophyton* sp.). Significant differences between depths are displayed with an asterisk. Since there was no similar assimilation rates between hard and soft corals, Δ shows the species that differentiate from the other two within its group (i.e. either hard or soft corals, for a single N source). AFDW = ash-free dry weight. DFAA = dissolved free amino acids. ns = not sampled.

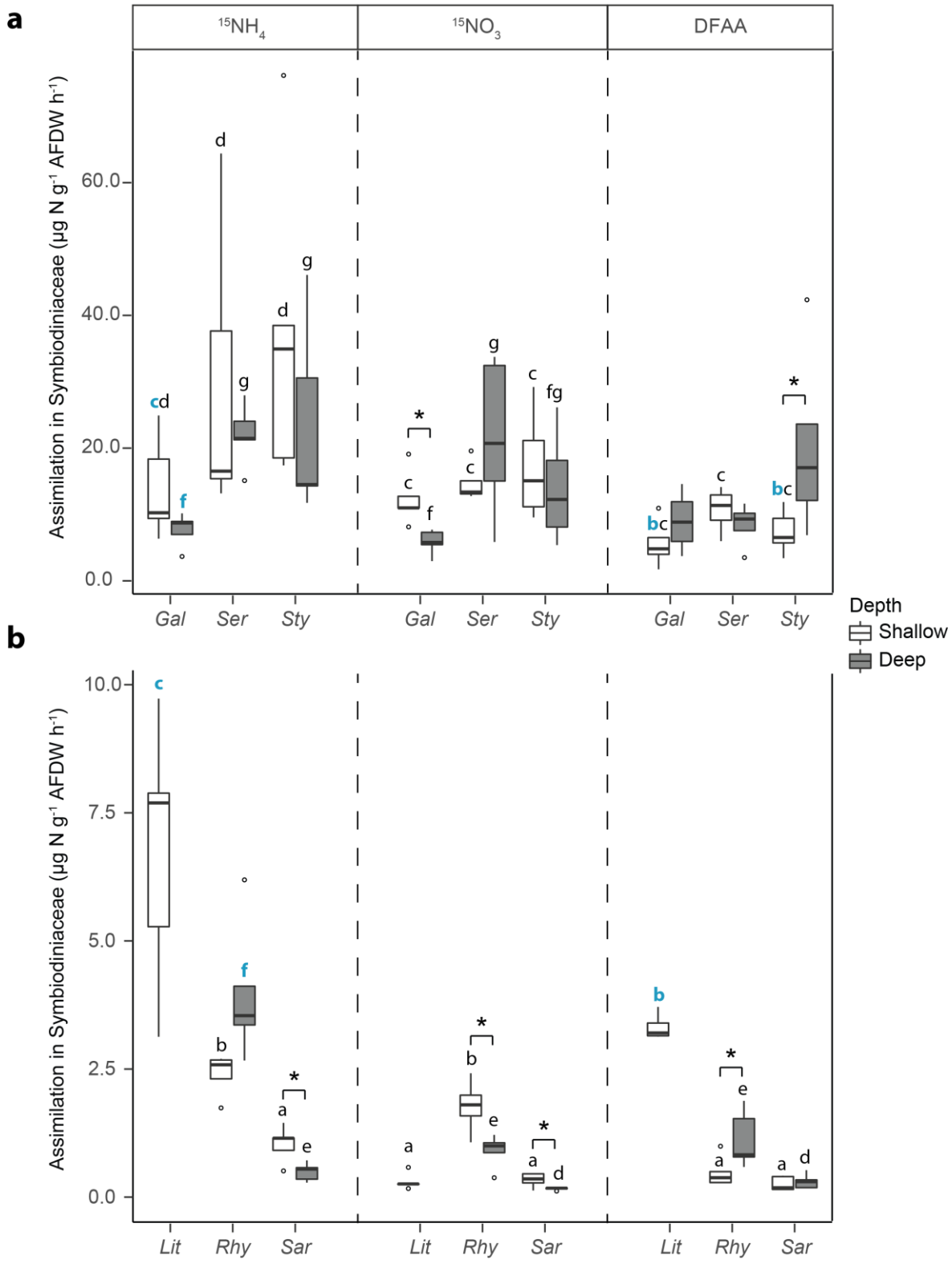

Figure 31. Assimilation rates of dissolved N in the Symbiodiniaceae fraction in (a) hard corals (*Galaxea fascicularis*, *Seriatopora hystrix*, *Stylophora pistillata*) and (b) soft corals (*Litophyton arboreum*, *Rhytisma fulvum fulvum*, *Sarcophyton* sp.). Significant differences between depths are displayed with an asterisk. Significant differences between species are distinguished with letters by depth. Blue bold letters highlight a similar rate between hard and soft coral species. AFDW = ash-free dry weight. ns = not sampled. DFAA = dissolved free amino acids. ns = not sampled.

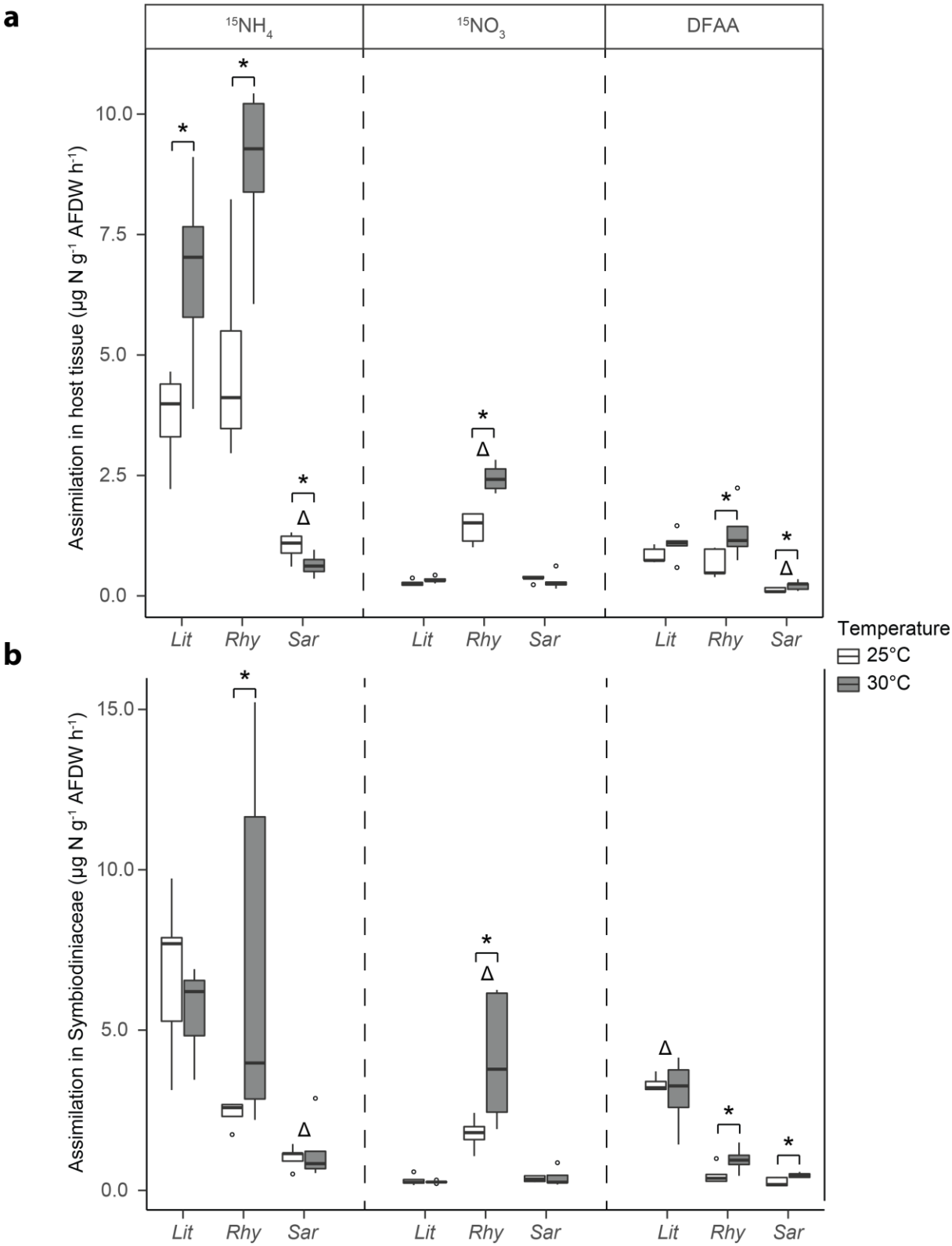

Figure 32. Assimilation rates of dissolved N in shallow soft corals exposed at two temperatures. (a) gathers rates related to the host tissue and (b) to the Symbiodiniaceae fraction. (*Litophyton arboreum*, *Rhytisma fulvum fulvum*, *Sarcophyton* sp.). Significant differences between temperatures are displayed with an asterisk. Δ shows the species that differentiate from the other two within its group (i.e. either host or Symbiodiniaceae fraction, for a single N source). AFDW = ash-free dry weight. DFAA = dissolved free amino acids. ns = not sampled.

4.9 Discussion

N availability is a major factor determining coral growth and productivity, and its acquisition is thus among one of the major processes affecting coral fitness (Rädecker et al. 2015). Therefore, the capacity of different coral species to efficiently exploit nutrients from the surrounding environment, or compete for nutrients with other species, can explain coral distribution and response to environmental stress. We thus compared the assimilation rates of different forms of N in soft and hard corals, sampled at shallow and mesophotic depths, in order to better understand their ecological strategies and the conditions favouring their growth. This study first highlights a different capacity of soft and hard corals to assimilate dissolved N forms. The lower assimilation rates per unit biomass observed in soft corals are likely due to divergent nutritional strategies, along with different morphological traits. In addition, we observed, for most but not all species, an increased assimilation of N with increasing depth or seawater temperature.

4.9.1 Differences in N assimilation between soft and hard corals

This study first highlights ten-fold lower rates of DIN and DON assimilation per unit biomass in soft compared to hard corals, whose uptake rates are comparable to previous studies (Figure 30, Figure 31, Figure 33, Appendix III – Figure S2; Grover et al. 2002; Pernice et al. 2012; Ezzat et al. 2017). These lower assimilation rates of DIN/DON in soft corals suggest that they have lower needs in these N sources for tissue growth or alternatively, lower uptake capacities than hard corals, due to differences in Symbiodiniaceae characteristics. Uptake rates of dissolved nutrients have been linked to the Symbiodiniaceae densities in host tissue, or Symbiodiniaceae species, some being less efficient than others in nutrient acquisition (Baker et al. 2013a; Ezzat et al. 2017). In our study, however, the genus and density of Symbiodiniaceae were similar between the soft and hard corals investigated and can thus not explain the differences observed in N assimilation rates.

The similar N contents per unit biomass in soft and hard corals (Figure 28) do not support lower N needs in soft corals. Therefore, a different nutritional strategy of the soft and hard corals is the most likely explanation for the lower assimilation of DN by soft corals. Since N_2 fixation does not occur in many soft corals (Pupier et al. 2019a), they should likely rely on the heterotrophic feeding on particulate organic matter (POM) to meet their N requirements

(Fabricius and Dommisse 2000). Soft corals, and octocorals in general, are known to capture high amounts of phyto- and zooplankton, as well as other forms of POM suspended in the surrounding seawater (Sebens and Koehl 1984; Fabricius et al. 1995a, b, 1998; Fabricius and Dommisse 2000; Migné and Davoult 2002). Ingestion of diatoms has also been observed in soft coral species such as *Sinularia flexibilis* (Piccinetti et al. 2016).

Several morphological and metabolic characteristics can contribute to higher N assimilation rates in hard corals compared to soft corals. For example, photosynthesis of hard corals living in shallow waters is higher than the one of soft corals (e.g., Fabricius and Klumpp 1995). As N uptake is proportional to symbiont's photosynthetic activity (e.g., Grover et al. 2002), the higher photosynthesis of hard corals can explain their higher rates of N assimilation. In addition, soft corals are primarily characterized by the absence of a calcium carbonate skeleton and only possess sclerites as calcified structures (Fabricius and Alderslade 2001). In hard corals, the deposition of a hard skeleton (calcification) generates protons that have to be neutralized to avoid tissue acidification (Comeau et al. 2012). Crossland and Barnes (1974) but also Biscéré et al. (2018), suggested that ammonia may be involved in proton neutralization, through the ornithine cycle and urea production, therefore possibly promoting the uptake of DIN by the holobiont. Moreover, coral skeletons harbor endolithic communities, which can assimilate nitrogenous compounds and translocate them back to the coral host (see review by Pernice et al. 2019). Finally, soft corals comprise a large coenenchyme mostly constituted of mesoglea, which includes collagen fibers, sclerites and free amoeboid cells (Helman et al. 2008; Orgel et al. 2017). Although they have a large gastrovascular system allowing efficient transport of metabolites within the colony (Gateño et al. 1998), several experiments on sea anemones suggested that the mesoglea can represent a significant barrier to transepithelial diffusion of molecules (oxygen in Bradfield and Chapman 1983); bicarbonate in Furla et al. 1998). The expansion state, together with the low surface area to body volume ratio, usually found for soft corals exhibiting fleshy and massive morphologies, are also not in favor of exchanges through the epidermal tissue (Kirschner 1991; Shick 1991; Fabricius and Klumpp 1995).

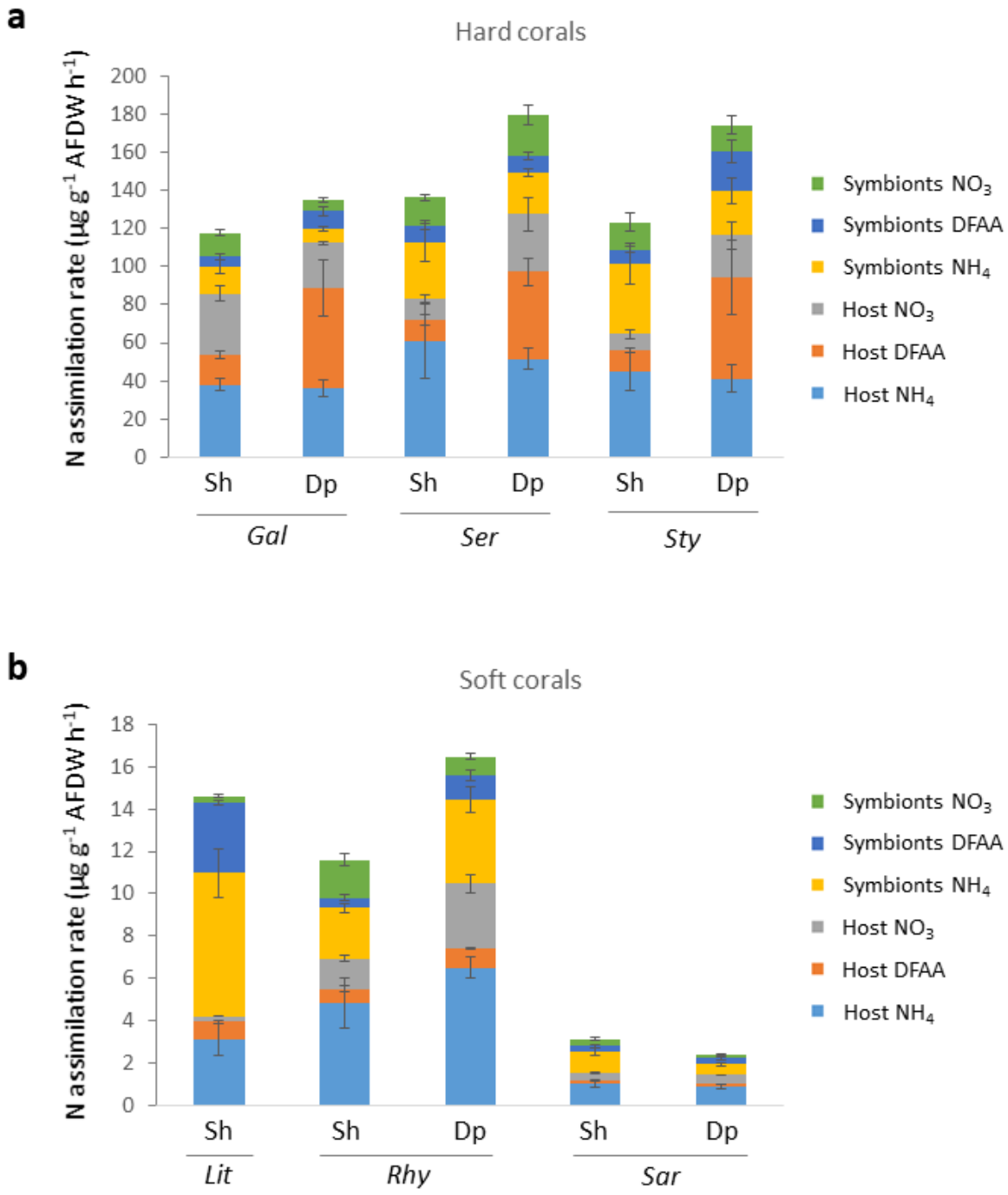

Figure 33. Total N assimilation rates in the species investigated. Values displayed are means ± standard error. (a) gathers hard corals (Gal = *Galaxea fascicularis*, Ser = *Seriatopora hystrix*, Sty = *Stylophora pistillata*) and (b) soft corals (Lit = *Litophyton arboreum*, Rhy = *Rhytisma fulvum fulvum*, Sar = *Sarcophyton* sp.). Sh = shallow. Dp = Deep. AFDW = ash-free dry weight. DFAA = Dissolved free amino acids.

4.9.2 Effect of the N form, depth and seawater temperature on N assimilation rates

The higher assimilation rates of NH_4, over the other N forms, in both hard and soft corals sampled in shallow waters is in agreement with previous studies (Grover et al. 2002, 2003, 2008). As NH_4 assimilation does not involve a redox reaction, it requires less energy than the assimilation of NO_3. As NH_4 is also a smaller molecule compared to DFAA, and can be absorbed more easily, it is likely the preferred dissolved N source. At mesophotic depths, however, the increased needs in N with depth (see below) caused corals to assimilate all available forms of N in seawater equally.

Both soft and hard corals increased either their chlorophyll content per unit biomass or per symbiont cell, which is a likely a strategy to increase the capture of light energy (Kahng et al. 2019) for acclimation to the lower light levels at mesophotic depth. Depth had however a different effect on the assimilation rates of DN and the tissue N content of hard and soft corals. In hard corals, the overall assimilation rates of N in host tissue (especially DFAA, and NO_3), were enhanced at mesophotic depth. This finding is in contrast with previous observations that light stimulates N uptake and assimilation (Grover et al. 2002). Such discrepancy may be due to the fact that previous measurements of assimilation rates were normalized to surface area whereas our data is normalized to tissue biomass, which decreases with depth. The higher assimilation of organic N (DFAA) corresponds with a reduced reliance on autotrophy due to reducing light levels with increasing depth in mesophotic hard corals. Consequently, they may rely more on heterotrophy (Williams et al. 2018), as well as obtain more N from diazotrophic bacteria (Bednarz et al. 2018). Overall, the enhanced assimilation rates of N at mesophotic depth suggest higher N needs in mesophotic colonies to sustain their metabolism, but this hypothesis remains to be further investigated. In contrast to hard corals, there were consistent assimilation rates of N throughout the depth gradient in soft corals, suggesting that low rates of N supply may be sufficient for all basal requirements of soft corals. Increased temperatures, however, tended to enhance the assimilation of N in both the host tissue and symbiont fractions of soft corals, which is in agreement with a previous study performed on N assimilation rates of *S. pistillata* at high temperature (29°C) (Godinot et al. 2011). Such increase in N assimilation can be of a mechanistic nature, for example a thermal optimum reached for the enzymes

involved in N assimilation. It could also be linked to an increased metabolism at high temperature (Gillooly et al. 2001), but this hypothesis remains to be tested.

4.9.3 Conclusion

This study corroborates the dependency of soft corals on another N source than dissolved compounds to meet their N requirements for tissue growth. This observation suggests that soft corals rely primarily on heterotrophy, especially for species bearing polyps with low surface area to volume ratios, which were shown to have lower photosynthetic rates and rely less on autotrophy than polyps with high surface area to volume ratios (Baker et al. 2015; Rossi et al. 2018). A higher reliance on heterotrophy can help soft corals to cope with higher turbidity or sedimentation regimes, two conditions, which decrease the amount of light received by corals (decreased autotrophy), but increase the amount of particles in suspension in seawater (McClanahan and Obura 1997). Further, soft corals may benefit from lower DN assimilation rates in areas with low water quality. Indeed, under excess N availability, symbionts can shift from an N limited to a phosphorus‑starved state (Wiedenmann et al. 2012), which then causes the substitution of phospholipids with sulpholipids in the chloroplast thylakoid membranes. Since phospholipids are essential to the stability of the membranes under heat-stress (Tchernov et al. 2004), increased N availability may increase the bleaching susceptibility of corals (Wiedenmann et al. 2012). In addition, it was shown that excess N can reduce photosynthate translocation rates by Symbiodiniaceae (Ezzat et al. 2015), leading to the starvation of the coral host. Since soft corals have low assimilation rates of N, it is unlikely that excess N enters the tissue and disrupts the symbiosis. Soft corals have thus been shown to be more abundant than hard corals in reefs, which suffer from high concentrations of dissolved inorganic nutrients (Baum et al. 2016). Further studies investigating the N and phosphorus budget of soft versus hard corals along a eutrophication gradient are needed to investigate how nutrients may explain the ecological niches of these two coral groups.

Chapter **5** | *In situ* acquisition of autotrophic and heterotrophic nutrients

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Publication n°4

Lipid biomarkers reveal the trophic plasticity of octocorals along a depth gradient

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Abstract

Octocorals are important reef inhabitants, from the shallow to mesophotic depths and beyond. However information regarding their nutritional ecology along the depth gradient is limited, despite the fact that nutrient acquisition is a fundamental process explaining the health condition and distribution of reef organisms. In particular, the contribution of the autotrophic versus heterotrophic acquisition of nutrients in symbiotic soft corals is poorly known. Here, the abundance of three lipid biomarkers, specific for autotrophy and heterotrophy, was investigated in the tissue of four octocoral species and one scleractinian sampled in shallow and upper mesophotic reefs of the oligotrophic Northern Red Sea. Our findings show functional

mixotrophy for all dinoflagellate-bearing species, with a significant input of heterotrophic feeding on crustacean zooplankton at both depths. Although octocorals maintained similar concentrations of autotrophic markers with increasing depth, the scleractinian species exhibited a decrease in autotrophy with depth, in agreement with previous measurements of photosynthetically-fixed carbon acquisition. The increase in heterotrophic capacity with depth was species-specific, likely related to physiological and morphological characteristics. The relatively high level of the heterotrophic marker in all symbiotic species in shallow conditions does not corroborate the common idea that corals rely mostly on autotrophy in shallow waters. This study thus highlights the importance of heterotrophy across the euphotic-upper mesophotic depth gradient and brings major advances on our understanding of the ecological significance of feeding for reef corals. Furthermore, it allows for a better understanding of the capacity of different octocoral species to exploit nutrient resources and help improving predictions on the impacts of climate and environmental changes on this important reef-dwelling taxon.

5.1 Introduction

Mixotrophs are some of the most widespread organisms on Earth (Selosse et al. 2016; Stoecker et al. 2017) because their dietary flexibility often allows them to use both inorganic and organic nutrients (Berge et al. 2017). This dual mode of feeding is particularly advantageous in oligotrophic ecosystems with low concentrations of inorganic nutrients and prey availability (Stoecker et al. 2017). Mixotrophic species can span from predominantly autotrophic to predominantly heterotrophic (Rottberger et al. 2013), although it is often difficult to disentangle the relative contributions of each feeding modes to the diet. Therefore, the trophic ecology of mixotrophic species is still not fully understood.

Symbiotic corals are some of the most well-known mixotrophic organisms, because they build the framework and form the basis of coral reefs, which are among the most productive and biologically diverse ecosystems in the planet (Knowlton et al. 2010; Sheppard et al. 2017). Corals display a tripartite trophic feeding strategy: (i) photoautotrophy performed by their dinoflagellate endosymbionts (Symbiodiniaceae – see LaJeunesse et al. (LaJeunesse et al. 2018)) which translocate photosynthates to the coral host, (ii) heterotrophy by actively feeding on plankton and suspended particles, and (iii) osmotrophy, which is a form of heterotrophy that is based on the absorption of dissolved organic matter (Muscatine 1990; Sorokin 1991; Al-Moghrabi et al. 1993; Ferrier-Pagès et al. 2003; Houlbrèque and Ferrier-Pagès 2009). Most

studies on coral nutritional ecology have focused on the scleractinian (reef-building) coral group. Symbiotic scleractinian corals living in shallow waters largely rely on the symbiont-derived autotrophic supply of nutrients for most of their immediate metabolic needs (Muscatine et al. 1984; Tremblay et al. 2014). These corals can however switch to a dominant heterotrophic feeding mode whenever their autotrophic nutrition is impacted. For example, heterotrophy is enhanced during bleaching (loss of the dinoflagellate symbionts – see Glynn 1993), or in deep waters where zooplankton availability is higher and light levels are too low for effective photosynthesis to occur (Muscatine et al. 1989; Hughes and Grottoli 2013; Tremblay et al. 2016). Therefore, heterotrophy is a key factor that contributes to the resistance and resilience of corals to environmental stress. Independently of a stressful situation, a few studies have also suggested that coral heterotrophy can be significant even when corals receive a large nutritional input from their autotrophic symbionts (Radice et al. 2019), but this aspect remains to be further investigated. Since nutrition is an important driver of coral population dynamics, further studies are needed to quantify variation in coral nutrition in the field, both for scleractinians and other important but understudied taxa, such as octocorals (soft corals).

Octocorals are important members of shallow and mesophotic coral reef communities (Schubert et al. 2017; Benayahu et al. 2019) and create complex three-dimensional habitats for other reef species (*i.e.* marine forests – see Rossi et al. 2017). Although some symbiotic octocoral species have been reported as mixotrophic in shallow waters (Fabricius and Klumpp 1995), two recent studies performed on gorgonian octocorals have concluded that the extent of autotrophy and heterotrophy depends on host morphology (e.g., colony shape and polyp size) and symbiont specificity (Baker et al. 2015; Rossi et al. 2018). Therefore, octocorals may differ from scleractinians in the extent to which they depend on heterotrophy for nutrition. These differences remain however poorly defined, with a small number of studies dedicated to their trophic ecology (but see Rossi et al. 2020). With natural and anthropogenic impacts on coral reefs leading to the overall decline in scleractinian coral cover (Hoegh-Guldberg et al. 2007; De'Ath et al. 2012; Hughes et al. 2018), octocorals may become one of the dominant reef taxa in the next decades (Lasker et al. 2020). It is therefore urgent to understand the conditions that favor octocoral growth and survivorship. In particular, their nutritional ecology may explain their diversity and abundance in locations where those of scleractinian corals are lower (Schubert et al. 2017).

Lipids such as fatty acids (FA) have been widely applied in marine food webs as markers of predator-prey interactions. Since FA composition is often specific to particular groups of organisms (Volkman 1986; Volkman et al. 1989) and animals are generally unable to produce

all necessary FA (Dalsgaard et al. 2003; Ruess et al. 2005; Budge et al. 2006), their content in coral tissue can give useful information on their autotrophic or heterotrophic origin. While specific FA have been validated as tracers of the photosynthetic contribution of the symbionts, such as stearidonic acid (SDA, 18:4ω3) and docosapentaenoic acid (DPA, 22:5ω3) (Papina et al. 2003; Treignier et al. 2008; Imbs et al. 2014; Mies et al. 2017, 2018), other FA may be specific to crustacean zooplankton and therefore heterotrophy, such as the cis-gondoic acid (CGA, 20:1ω9) (Dalsgaard et al. 2003; Dodds et al. 2009; Naumann et al. 2015; Mies et al. 2018).

In this study, we quantified autotrophy- (SDA, DPA) and heterotrophy-specific (CGA) markers to investigate whether symbiotic octocorals of the upper mesophotic reefs (40-50 m) of the Northern Red Sea rely more on heterotrophy than those living at shallow depths. For this purpose, four octocoral species (*Dendronephthya* sp., *Litophyton arboreum*, *Rhytisma fulvum fulvum*, and *Sarcophyton* sp.) and one scleractinian coral species (*Stylophora pistillata*) were sampled. Our results shed light on the nutritional ecology of octocorals, which are one of the less studied coral groups. In addition, because nutrition is an important factor involved in coral resistance and resilience, studies on this subject may contribute to understanding coral reef response to environmental changes.

5.2 Materials and methods

5.2.1 Collection and sampling procedures

Colonies of *Dendronephthya* sp. (a non-symbiotic species), *L. arboreum*, *R. f. fulvum*, *Sarcophyton* sp. and *S. pistillata* (Figure 34) were sampled by scuba diving on the reef adjacent to the Inter-University Institute for Marine Science (IUI) (29°51'N, 34°94'E), Gulf of Eilat, Israel, in November 2018. Five nubbins of approximately 3 cm were collected from different colonies for each of the five species. Samples were directly frozen at -80°C and freeze-dried after one week. These procedures were performed at both shallow (8-10 m) and upper mesophotic (40-50 m) sections of the reef.

Figure 34. The five Red Sea coral species investigated for their nutritional ecology. From left to right: the asymbiotic octocoral *Dendronephthya* sp. (photo credit: M. Boussion) and symbiotic octocorals *Litophyton arboreum* (photo credit: G. Banc-Prandi), *Rhytisma fulvum fulvum* (photo credit: C. Pupier) and *Sarcophyton* sp. (photo credit: C. Pupier), along with the symbiotic scleractinian *Stylophora pistillata* (photo credit: E. Tambutté).

5.2.2 Laboratory experimental controls

Although SDA, DPA and CGA have been validated as precise and specific trophic markers for corals and other marine taxa (Dalsgaard et al. 2003; Mies et al. 2017, 2018), four controls were performed to further confirm their specificity, especially for octocorals: (i) symbiont cells (isolated from *S. pistillata* grown in the laboratory), to confirm the presence of SDA and DPA and the absence of CGA; (ii) *S. pistillata* and *Sarcophyton* sp. colonies, originating from the Red Sea and grown in the laboratory, maintained in strict autotrophy to confirm presence of SDA and DPA and, most importantly, absence of CGA; (iii) *S. pistillata* and *Sarcophyton* sp. colonies fed with zooplankton (to confirm presence of CGA); and (iv) a mixture of zooplankton to confirm the absence of SDA and DPA and the presence of CGA. For this purpose, ten nubbins per species (*S. pistillata and Sarcophyton* sp.) were kept in 20-L tanks for one month prior to starting the experiment. The tanks received a continuous supply of oligotrophic seawater (< 0.5 µM dissolved inorganic nitrogen and < 0.2 µM inorganic phosphate) at a flow rate of 20 L h^{-1}. Water was mixed using mini-pumps (404 L h^{-1}, Newa, Loreggia, Italy). Seawater temperature was kept at 25ºC with the use of submersible resistance heaters (300 W Titanium, Schego, Offenbach, Germany) and light level was maintained at 200 µmol photons m^{-2} s^{-1} (12:12) using metal halide lamps (Philips, HPIT 400 W, Distrilamp, Bossée, France). Nubbins were then separated in two groups, one unfed (i.e. strict autotrophy) and the other fed (i.e. mixotrophy). *Sarcophyton* sp. was fed for two months with a mixture of rotifers and copepods (Ocean Nutrition, San Diego, USA) three days a week, and *Artemia salina* nauplii (Ocean Nutrition, San Diego, USA) twice a week. The scleractinian *S. pistillata* was only fed twice a week with *A. salina* nauplii. At the end of the experiment, nubbins were

frozen at -80°C and freeze-dried for subsequent analyses. Each sample was crushed into powder using a mortar and pestle and then divided in two subsamples. A subsample was kept for lipid extraction and gas chromatography analysis, while the other was weighed and combusted at 450°C for 4 h in a muffle furnace (Thermolyne 62700, Thermo Fischer Scientific, USA). Ash-free dry weight (AFDW) was determined as the difference between the dry weight (DW) and ash weight, and extrapolated to the total weight of powder used for lipid extraction (Appendix IV - Table S8).

5.2.3 Symbiont identification

The predominant symbiont phylotype had been previously identified (C. Pupier, unpublished data) for all sampled coral species through gDNA extraction and standard PCR and sequencing protocols (Sambrook et al. 1989). The primer set (taken from (Santos et al. 2002) used for PCR amplification targeted amplicons for the chloroplast large subunit (cp23S-rDNA), a marker that is widely used for assessments of Symbiodiniaceae diversity (Santos et al. 2002; LaJeunesse et al. 2018).

5.2.4 Lipid extraction and gas chromatography analysis

Lipid extraction was performed in similar fashion to (Bligh and Dyer 1959), with minor modifications. One milliliter of a solution of methanol and dichloromethane (1:1) was added to each sample tube together with 125 µL of the internal standard (C13:0 triacylglyceride, T3882, Sigma) solution (5 mg mL^{-1} in hexane). Thereafter, the tubes were agitated for 1 min and centrifuged at 10,000 X g (-4ºC). The solvent was dried in pure N_2 gas, then the samples were methylated by dissolving in 500 µL of 5% HCl in methanol, before heating to 100ºC for 2 h. Fatty acid methyl esters were then extracted with hexane.

Gas chromatography procedures were nearly identical to Martins et al. (2018). Fatty acid methyl esters were analysed by gas chromatography coupled with mass spectrometry (QP2010, Shimadzu, Japan). A 30-m fused silica capillary column (VF-Max, 0.25 µm film, Agilent) was utilized. One microliter of each sample was injected at 220ºC in split mode. The carrier gas was helium, at a flow rate of 1 mL min^{-1}. Initial temperature was 60ºC with an increase of 5ºC per minute until it reached 260ºC, which was then maintained for 10 min. The fatty acid methyl esters of the three polyunsaturated fatty acids, including two autotrophy

markers (SDA and DPA) and one heterotrophy marker (CGA), were identified by comparison of their retention times against those in the certified reference standard 47033 PUFA No. 1 Marine Source (Sigma).

5.2.5 Statistical analyses

The following analysis was performed using the R environment for statistical computing and graphics (R Core Team 2017). Generalized linear models (GLM) were fitted to test for differences in SDA, DPA and CGA concentrations between species, depths, and the interaction of these factors. Data were transformed using the Box-Cox transformation procedure ($(x^{\lambda-1})/\lambda$, with λ = -0.060606 for SDA, λ = -0.343434 for DPA, and λ = 0.101010 for CGA), as implemented in Venables and Ripley (2002) to comply with assumptions of normality of residuals (tested for with the Shapiro-Wilk test) and homoscedasticity (tested for with Levene's test; Fox and Weisberg 2018). Biologically relevant pairwise comparisons (i.e., between depths for each species and between species for each depth) were made for species with detectable fatty acid concentrations based on estimated marginal means (Lenth et al. 2020) and simultaneous general linear hypothesis testing (glht() function of the multcomp package; Hothorn et al. 2008). P-values were adjusted using the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995).

To address multivariate differences in markers' concentration between the five species and the two depth strata we used a non-metric multidimensional scaling (nMDS) ordination to summarize similarities (Bray-Curtis) on SDA, DPA and CGA content. The Spearman non-parametric correlation coefficient, based on ranks, was used to investigate the relationship between variables and the MDS axes, with results illustrated as superimposed vectors in the MDS diagram. In addition, a 2-way crossed analysis of similarities (ANOSIM) was used to evaluate significant differences according to species and depth levels. The MDS and ANOSIM analyses were performed using the PRIMER 6 software (Clarke and Gorley 2006).

5.3 Results

The identities of Symbiodiniaceae phylotypes associated with the four symbiotic species are summarized in Table 8. While the three octocorals were associated with the same symbiont phylotype in both shallow and mesophotic depths (*Symbiodinium* sp. A10 or *Cladocopium*

goreaui), *S. pistillata* displayed a different association between depths (*Symbiodinium microadriaticum* in the shallow and *Cladocopium goreaui* at mesophotic depths).

Table 8. Predominant symbiont species associated with the four symbiotic Red Sea coral species sampled at shallow and mesophotic depths of the reef adjacent to the Inter-University Institute for Marine Sciences in Eilat, Israel.

Host coral species	Shallow (8-10 m)	Mesophotic (40-50 m)
Litophyton arboreum	*Symbiodinium* sp. A10	*Symbiodinium* sp. A10
Rhytisma fulvum fulvum	*Cladocopium goreaui* C1	*Cladocopium goreaui* C1
Sarcophyton sp.	*Cladocopium goreaui* C1	*Cladocopium goreaui* C1
Stylophora pistillata	*Symbiodinium microadriaticum* A1	*Cladocopium goreaui* C1

The first laboratory experimental control confirmed the presence of both SDA and DPA and the absence of CGA in the symbiont cells (Figure 35). The exact opposite was detected for the plankton mixture, with presence of CGA and absence of SDA and DPA. Colonies of *Sarcophyton* sp. kept in strict autotrophy also displayed higher concentration of the autotrophic markers and absence of the heterotrophy marker. The same was observed for *S. pistillata*, although with trace amounts of CGA. The last controls, *S. pistillata* and *Sarcophyton* sp. colonies fed with the plankton mix, displayed similar SDA and DPA content to those unfed, but also displayed up to 20 times more CGA. In addition, in situ nubbins of the non-symbiotic octocoral *Dendronephthya* sp. contained CGA, while SDA was found in trace concentrations.

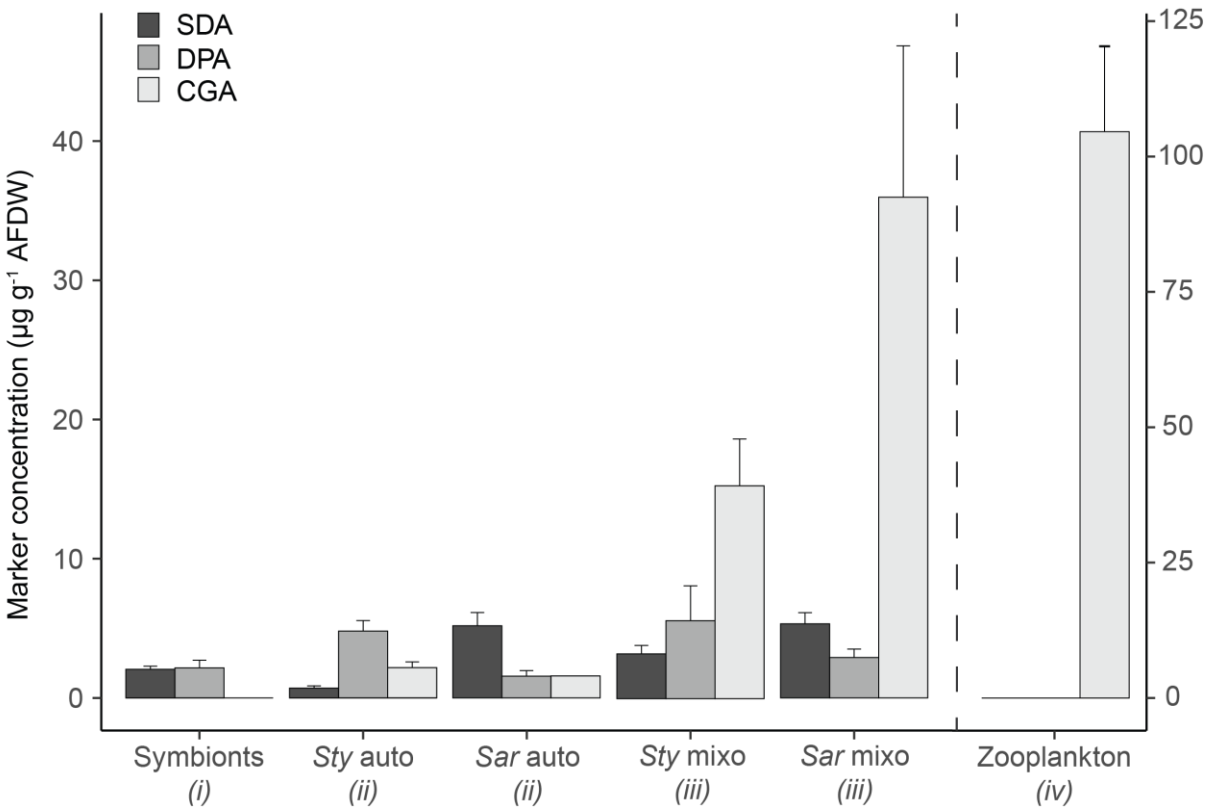

Figure 35. Controls used for validation of the specificity of the fatty acid trophic markers SDA, DPA (stearidonic acid and docosapentaenoic acid, autotrophy markers) and CGA (cis-gondoic acid, heterotrophy marker). Sty and Sar respectively refer to *Stylophora pistillata* and *Sarcophyton* sp. grown at the laboratory. auto = autotrophy. mixo = mixotrophy. (i) Isolated symbiont cells from *Stylophora pistillata*, (ii) *Stylophora pistillata* and *Sarcophyton* sp. tissue from nubbins maintained under strict autotrophy, (iii) *Stylophora pistillata* and *Sarcophyton* sp. tissue from nubbins fed with zooplankton, and (iv) zooplankton mix, comprising rotifers, copepods and *Artemia salina* nauplii, used to feed the coral nubbins. Fatty acid concentration (µg g^{-1} ash-free dry weight) is expressed as mean ± standard error.

The ANOSIM detected significant differences between both species (R = 0.488; P = 0.001) and depths (R = 0.197; P = 0.011). The two-dimensional MDS ordination diagram (with a stress value equal to 0.08) presented a clear gradient of species showing, on one side, high CGA and SDA content and low DPA content (*e.g. R. f. fulvum*), intermediate values (e.*g. L. arboreum*) and, in the other side, species showing lower CGA and SDA content and higher DPA content (*e.g. Sarcophyton* sp.) (Figure 36). Figure 37 shows that all corals sampled *in situ* contained the three markers at different concentrations. Pairwise comparisons highlighted significant differences between depths in SDA for *L. arboreum* (decrease with depth), in DPA for *S. pistillata* (decrease with depth) and in CGA for *Dendronephthya* sp. and *R. f. fulvum* (increase with depth) (Appendix IV - Table S9).

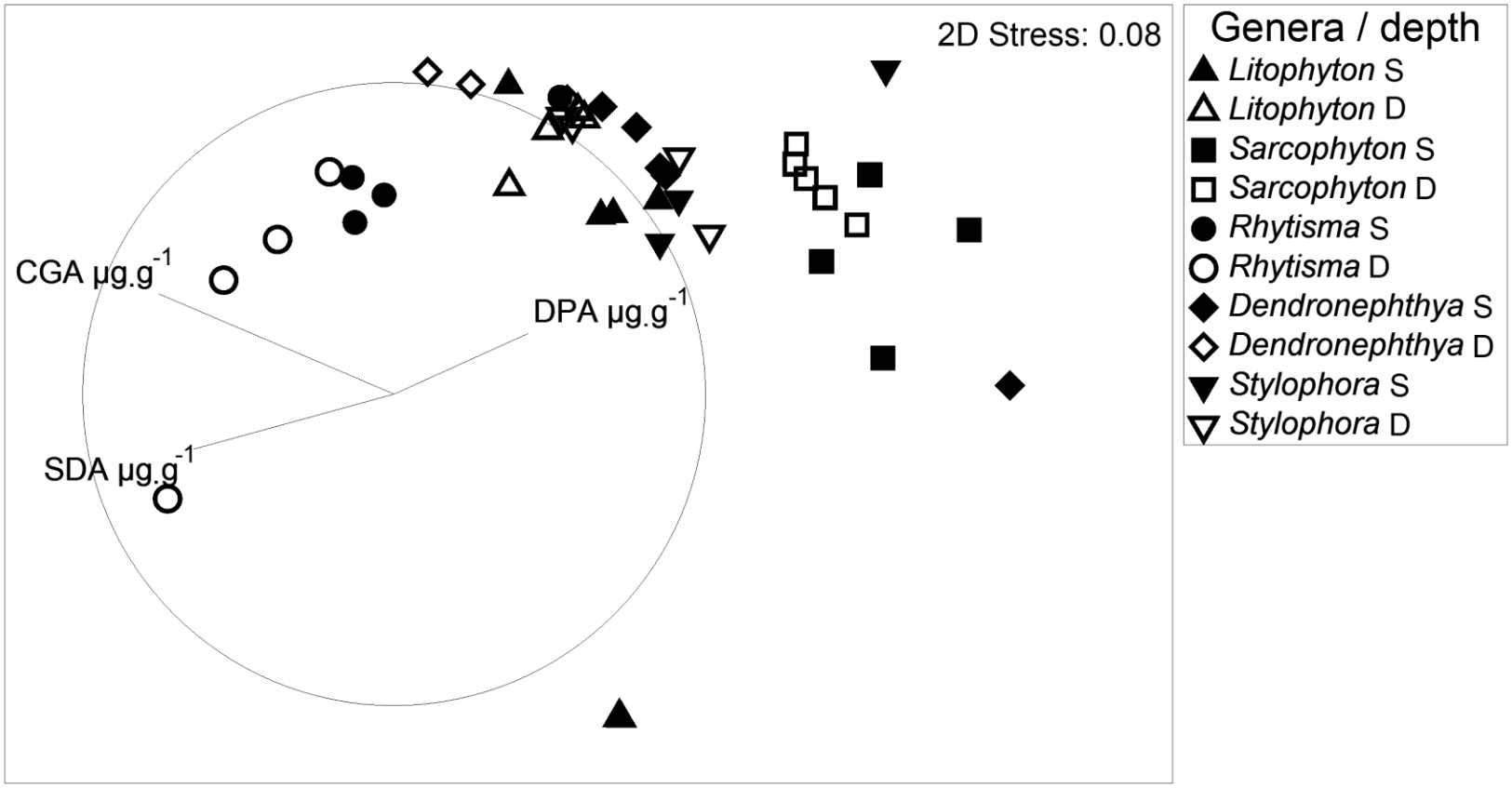

Figure 36. Non-metric multidimensional scaling (MDS) ordination used to assess similarities in the concentration (µg g^{-1} ash-free dry weight) of the fatty acids SDA, DPA (autotrophy markers) and CGA (heterotrophy marker) in the tissues of *Dendronephthya* sp., *Litophyton arboreum, Rhytisma fulvum fulvum, Sarcophyton* sp., and *Stylophora pistillata,* sampled in both shallow (8-10 m) and mesophotic (40-50 m) sections of the reef adjacent to the Inter-University Institute for Marine Sciences, in Eilat, Israel.

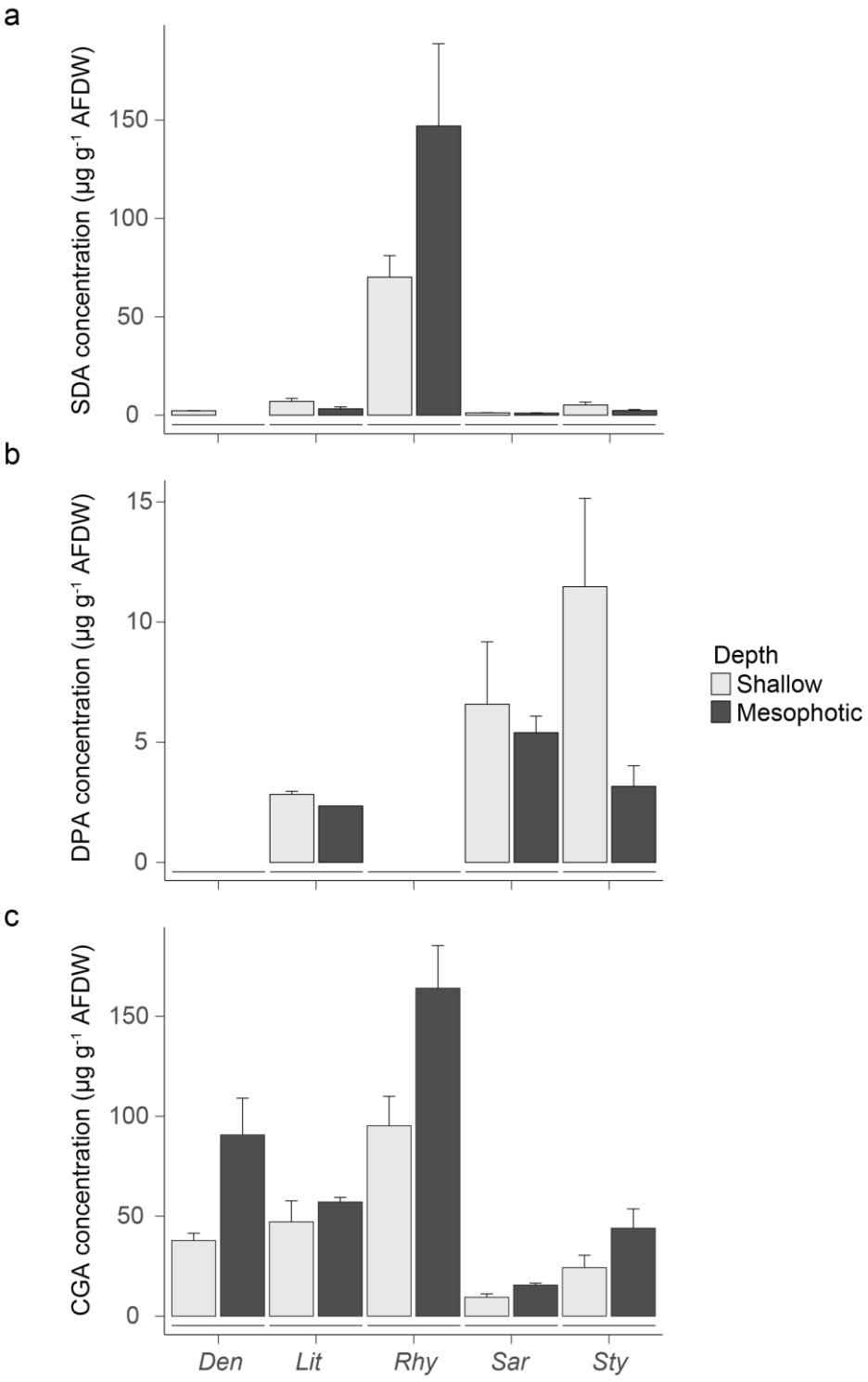

Figure 37. Concentration (µg g^{-1} ash-free dry weight) of the fatty acid trophic markers (a) SDA, (b) DPA (stearidonic acid and docosapentaenoic acid, autotrophy markers) and (c) CGA (cis-gondoic acid, heterotrophy marker) from the tissues of *Dendronephthya* sp. (asymbiotic octocoral, Den), *Litophyton arboreum* (symbiotic octocoral, Lit), *Rhytisma fulvum fulvum* (symbiotic octocoral, Rhy), *Sarcophyton* sp. (symbiotic octocoral, Sar), and *Stylophora pistillata* (symbiotic scleractinian coral, Sty), sampled in both shallow (8-10 m) and mesophotic (40-50 m) sections of the reef adjacent to the Inter-University Institute for Marine Sciences, in Eilat, Israel. Data are expressed as mean ± standard error.

5.4 Discussion

This study is one of the first to evaluate the shift in autotrophic/heterotrophic nutrition along the depth gradient for several species of octocorals sampled in the Northern Red Sea and to compare their nutritional ecology with the most abundant scleractinian coral species (*Stylophora pistillata*) of this oligotrophic area. Octocorals are indeed one of the most abundant groups of organisms in the upper mesophotic zone of the Red Sea reefs, often displaying higher species richness than in shallow reefs (Shoham and Benayahu 2017; Eyal et al. 2019). A better knowledge of their trophic ecology along the depth gradient is therefore of prime importance to understand their distribution and growth in nutrient-poor conditions. Our findings highlight several species-specific aspects of the nutritional ecology of octocorals. The analysis of specific fatty acid markers shows that soft corals did not reduce their autotrophic capacity in mesophotic reefs, and that both symbiotic and asymbiotic species can display an increased heterotrophy with increasing depth. The symbiotic reef-building coral, on the contrary, showed significant decrease in autotrophic activity with depth. However, it displayed a high heterotrophic capacity throughout the depth gradient, and even at very shallow depths.

The fatty acids SDA, DPA and CGA had been previously validated as specific markers of feeding strategies for reef corals (Mies et al. 2017, 2018; Marangoni et al. 2019). The additional experimental controls produced here all confirmed their specificity. Although nearly undetectable amounts of CGA were found in *S. pistillata* maintained in strict autotrophy, such residual trace concentrations are not unusual for corals that have been kept under starvation for short periods (Mies et al. 2018). Therefore, our controls agree with previous experiments and validations. It is important to note that CGA concentration reflects predation on crustacean zooplankton only; therefore, although copepods may comprise the most abundant and ecologically relevant group of zooplankton in coral reef systems (Hamner and Carleton 1979), uptake of other dietary items such as salps is not detected. Thus, CGA is a valid proxy, but likely an underestimation of the total heterotrophic capacity (Mies et al. 2018).

Field analyses show that *R. f. fulvum* is the species with the highest concentration of autotrophy markers at both shallow and deep sites, likely being the most autotrophic of all octocorals investigated. Although there may be some correlation between the concentration of autotrophic markers and symbiont density in host tissue, it is often weak because of variations in fatty acid production, storage and translocation by Symbiodiniaceae (Zhukova and Titlyanov 2003; Imbs et al. 2014; Mies et al. 2017, 2018), as well as environmental influence on these

processes (Kneeland et al. 2013). Further evidence for this is that the highest symbiont density for the five coral species investigated was observed in *L. arboreum* (Pupier et al. 2019b), which did not have the highest concentration of autotrophy markers. Symbiont genotypic diversity can also affect the quantity of translocated material to the host (Loram et al. 2007; Starzak et al. 2014), although no clear relationship between CGA levels and symbiont identity could be observed in the present study. The concentration of autotrophy markers did not significantly decrease with depth for any of the octocoral species investigated, in agreement with a recent finding that carbon acquisition in octocorals is constant from shallow to at least 40 m depth (Pupier et al. 2019b). For *S. pistillata*, however, autotrophic markers were found in higher concentration in the shallow colonies, which is also in agreement with findings that photosynthesis and carbon acquisition in this species decrease along the depth gradient (Mass et al. 2007; Einbinder et al. 2016; Ezzat et al. 2017). Altogether, these results suggest that octocorals may have a greater ability to transform light in energy at deep reefs, which might explain their relatively high abundance/diversity at mesophotic depths.

The bathymetric stability of the symbiont-host association might explain the constant level of autotrophic markers in octocoral species. Symbiodiniaceae is a functionally-diverse group, with species displaying differences in adaptation to light intensity, thermal stress, and many other environmental conditions (Baker 2003; Robison and Warner 2006; Stat et al. 2008; Swain et al. 2017). All octocoral species investigated here were associated with the same symbiont phylotype at shallow and mesophotic depths, as often observed for octocorals (van Oppen et al. 2005; Goulet 2007). However, our findings show that *S. pistillata* shifts from *Symbiodinium microadriaticum* to *Cladocopium goreaui* at mesophotic depths. *Symbiodinium microadriaticum* has been reported to assimilate less autotrophic carbon and have a lower capacity of photosynthate translocation than C. *goreaui* (Stat et al. 2008; Ezzat et al. 2017), indicating that plasticity in symbiont association may explain between-species differences in bathymetric distribution.

Photosynthetic products are carbon-rich but may be deficient in other critical elements for growth including nitrogen and phosphorus (Battey and Patton 1987; Titlyanov et al. 2001; Palardy et al. 2006). Therefore, heterotrophic feeding is critical for fulfilling such deficiency (Al-Moghrabi et al. 1993; Anthony and Fabricius 2000; Ferrier-Pagès et al. 2003; Houlbrèque and Ferrier-Pagès 2009). Most octocorals are known to ingest organic matter, bacteria, phytoplankton and zooplankton (Fabricius et al. 1995a, 1998; Widdig and Schlichter 2001; Ribes et al. 2003; Coma et al. 2015; Piccinetti et al. 2016). Our findings show that the heterotrophic input in Red Sea octocorals is species- and depth-specific. *Rhytisma f. fulvum*

presented the highest CGA content at both shallow and mesophotic depths, suggesting that it is also the most effective heterotrophic species, out of the four ones evaluated here. CGA values for *R. f. fulvum* were even higher than for *Dendronephthya* sp., an asymbiotic and obligate heterotroph. Combined with its high autotrophic capacity, this might explain why *R. f. fulvum* has high resilience, efficiently colonizing reefs and forming large carpets after major environmental disturbances (Benayahu and Loya 1977; Buckley et al. 2004, 2008; Stobart et al. 2005).

The scleractinian *S. pistillata* showed a relatively high degree of heterotrophy (i.e high CGA content) even at shallow depth. This is contrary to the common belief that shallow scleractinian corals rely almost exclusively on autotrophy (Muscatine et al. 1989; Muscatine and Kaplan 1994; Lesser et al. 2010). In fact, our findings suggest that heterotrophy may not compensate for a lack of autotrophy, but rather complement it. A more profound investigation of CGA content in other scleractinian coral species should confirm whether this heterotrophic capacity is restricted to *S. pistillata*, to Eilat reefs, or a general feature of shallow scleractinian corals. It is important to note that there is a strong diel pattern of increased zooplankton biomass at dusk and dawn in the Gulf of Aqaba/Eilat, often ten-fold higher during the night than during the day (Yahel et al. 2005). In addition, the region is characterized by convective mixing of approximately 300 m in autumn and winter (when our collections were performed) (Labiosa et al. 2003), which may have enhanced coral heterotrophy in shallow waters (Gove et al. 2006; Roder et al. 2010; Sevadjian et al. 2012; Williams et al. 2018). If most coral species are as heterotrophic as *S. pistillata* in shallow areas, zooplankton abundance should then be considered as a major driver of scleractinian abundance and health and further explorations on this topic are warranted.

A bathymetric trophic zonation was evident for *R. f. fulvum* and *Dendronephthya* sp., which displayed CGA increase at mesophotic depths. This pattern may be explained by an increase in predation pressure and/or increased zooplankton availability (reviewed in Houlbrèque and Ferrier-Pagès 2009). However, CGA content for *L. arboreum* and *Sarcophyton sp.* did not differ between shallow and deep sites. This, together with the lower CGA content in these two species, suggest that they either have a limited prey capture ability or rely on other resources such as bacteria and/or dissolved organic matter. This agrees with previous reports that species within *Litophyton* and *Sarcophyton* genera are poor zooplankton-feeders and rely more on bacterioplankton (Sorokin 1991). Nonetheless, our results reinforce that depth is a critical factor in shaping Red Sea octocoral communities also because species diversity increases significantly at 20-60 m (Shoham and Benayahu 2017; Eyal et al. 2019).

The species-specificity in heterotrophic capacity for the five coral species studied here can be related to the identity and/or functioning of the symbionts hosted by each species, as it has been demonstrated that heterotrophy was positively correlated with the amount of translocated carbon (Leal et al. 2015). This finding matches with the observation that the most autotrophic species (*Rhytisma f. fulvum*) is also the most heterotrophic one. Alternatively, heterotrophy can be associated with the morphophysiological characteristics of the species. Increased heterotrophy and predation capacities have been linked to polyp and/or corallite size (Anthony and Fabricius 2000; Marangoni et al. 2019). *Rhytisma f. fulvum*, a species that presented high CGA content, has a polyp diameter similar to *Sarcophyton* sp. (2.4-4 mm; Lewis 1982; Sorokin 1991), while *L. arboreum*, a low-CGA species, features polyps almost ten times smaller (Ofwegen 2016). Species-specificity is also evident for the autotrophic markers. *Rhytisma f. fulvum* presented unusually high SDA and absence of DPA, which, as of now, is unique for all symbiotic octocorals/scleractinians investigated (Mies et al. 2018; Marangoni et al. 2019). It is unclear whether the host, symbiont or the environment, controls the concentration of specific autotrophy markers, but *R. f. fulvum* has been reported as a species with unusual biochemical composition and processes (Trifman et al. 2016). Therefore, the cause for variations in trophic behavior is likely a multifactorial interaction between holobiont physiology and environmental conditions.

This study shows that Red Sea octocorals are functional mixotrophs in both shallow and mesophotic reefs. In addition, our findings show that heterotrophy becomes even more relevant at mesophotic reefs, and without a reduction in autotrophic input. This later finding was unexpected, as soft corals display a different pattern than the one known for scleractinians. This consistent autotrophic capacity may have major implications for explaining differences in bathymetric distribution/dominance of species across depth gradients. In addition, it suggests that soft corals may find more efficient thermal-stress refugia in deep reefs than scleractinians, and may become the dominant group in some coral reefs in the future. This study helps understanding coral functional and trophic ecology, especially in mesophotic reefs, which are conservation-dependent areas (Rocha et al. 2018; Eyal et al. 2019).

Acknowledgements

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Chapter **6** | General discussion and perspectives

Chapter **6** | General discussion and perspectives

6.1 General discussion

The main objective of this thesis was to provide insights into the nutritional ecology of several symbiotic octocoral species from the Northern Red Sea, down to mesophotic depths. The dinoflagellate-octocoral symbiosis is known to be different from the one of scleractinian corals, in terms of diversity and stability. While octocorals exhibit low symbiont diversity, scleractinian corals generally host multiple symbiont types and are more flexible depending on the prevalent environmental conditions (Baker and Romanski 2007). This difference has been attributed to octocorals having a greater dependence on heterotrophy and therefore having less to gain from retaining flexibility as a symbiotic trait (van Oppen et al. 2005). However, the importance of autotrophy and heterotrophy and their respective contribution to the host's energy budget has been scarcely studied in octocorals, and mainly tested in gorgonians (Rossi et al. 2020). Advancing knowledge on the nutritional ecology of soft coral symbioses is though of increasing importance, considering that soft corals are one of the main components of reef ecosystems and that climate change and local disturbances are predicted to increasingly induce transitions from hard coral- to octocoral-dominated reefscapes (e.g., Tsounis and Edmunds 2017).

Therefore, in this thesis, we investigated the acquisition and fluxes of organic and inorganic sources of nutrients, relative to carbon and nitrogen, in several soft coral hosts, their dinoflagellates symbionts, and other possibly associated microorganisms such as diazotrophs. Comparisons with scleractinian-dinoflagellate symbioses highlighted major functional differences between the two groups and allowed the discussion of our results in a broader context.

6.1.1 Soft and scleractinian corals form different functional symbioses from the shallow down to the upper mesophotic reef zone

- Autotrophy remains stable along the depth gradient in soft corals whereas it decreases in scleractinian corals

The autotrophic acquisition of carbon by the coral symbionts was assessed in this thesis in three different soft coral species via two different but complementary approaches. The first one (Chapter 3) consisted in measuring the acquisition and assimilation of dissolved inorganic carbon into the coral host tissue and symbionts, using a ^{13}C-bicarbonate tracer. The second technique consisted in measuring the concentration of two fatty acids (stearidonic and docosapentaenoic acids) specific to autotrophy (Chapter 5) in the coral tissue. A comparison was made with the scleractinian coral *Stylophora pistillata*, using the same techniques.

The two approaches showed, and for all soft coral species investigated, that the short and long-term assimilation of carbon in the coral host tissue was stable along the depth gradient or even increased at mesophotic depths. The stability in autotrophic nutrient supply along the depth gradient suggests that soft corals are either photo-limited in shallow waters and/or well photo-acclimatized to depth. This is in agreement with previous observations that maximal photosynthetic rates of several soft coral species from the Great Barrier Reef were measured at 20 m depth rather than in shallower waters (Fabricius and Klumpp 1995). Strategies of acclimatization to low light levels were noticed in the symbionts (i.e., reduced self-shading and increased light harvesting capacity of the symbiont cells; Cohen and Dubinsky 2015; Ziegler et al. 2015; Einbinder et al. 2016). However, the autotrophic stability was independent of the Symbiodiniaceae genus harbored by the soft coral host, since the associations were species-specific and are prone to be stable over space and time (Goulet and Coffroth 2003). On the contrary, the hard coral *S. pistillata* experienced a two-fold decrease in autotrophic carbon acquisition with depth and shifted from *Symbiodinium* (previously clade A) to *Cladocopium* (previously clade C) from shallow to deep reefs (Mass et al. 2007; Ezzat et al. 2017).

The comparison of the carbon budget in soft and scleractinian corals indicates that the rates of autotrophic carbon acquisition is at least two-fold higher in *S. pistillata* at shallow depth, as compared to the soft coral species investigated. However, the carbon acquired was also lost (as respiration and mucus production) twice faster in scleractinian corals, suggesting a different functioning of the symbioses and a different metabolic demand between soft and hard coral species. The higher metabolic demand in scleractinian corals is likely due to calcification, since most of the carbon respired is used for calcium carbonate deposition. Indeed, almost 70-75% of the carbon used for calcification in hard corals comes from respiration, and the remaining 25-30% comes from external seawater carbon (Erez 1978; Furla et al. 2000; Hughes et al. 2010). Moreover, the presence of skeleton enhances coral productivity through internal light scattering, which may also contribute to explain the differences in carbon acquisition between soft and hard corals.

- Heterotrophy is important at all depths and increases in the mesophotic reef for several species

Another main finding of this thesis points out that symbiotic soft corals from both shallow and mesophotic depths are most likely to depend more on heterotrophy than autotrophy for nutrient acquisition, as compared to hard corals. This finding is supported by the large amount of lipid biomarkers, specific to crustacean zooplankton, measured in the soft coral tissue from shallow and mesophotic reefs (Chapter 5). For some species, the amount of biomarker significantly increased with depth, suggesting that this type of nutrition is favored in the mesophotic reef. In addition, we found in Chapter 4 that soft corals have a low assimilation of dissolved inorganic or organic nitrogen (DIN and DON) and therefore need to acquire nitrogen from particulate feeding.

6.1.2 Soft and scleractinian corals form different functional symbioses with their dinoflagellates, which might explain the greater resistance of soft corals to eutrophication

We observed that soft corals have a lower capacity than scleractinian corals to assimilate inorganic and organic nitrogen dissolved in seawater. As low amounts of inorganic nutrients enter the soft coral-dinoflagellate symbiosis, it is unlikely that changes in water quality related to nutrients (e.g., eutrophication) disrupt the symbiosis.

First of all, we showed that soft coral mucus likely represents an unfavorable habitat for the colonization and activity of diazotrophs, as it is known to present antimicrobial properties and contains particles with a relatively high N content (Kelman et al. 2006; Nakajima et al. 2018). As a result, soft corals neither promoted N_2 fixation in their surrounding seawater nor assimilated DDN. Moreover, up to ten-fold lower assimilation rates of dissolved N (NH_4, NO_3, DFAA) were observed in soft corals as compared to hard corals, with no clear difference between depths. This could not be explained by i) a different capacity for nutrient acquisition related to the dinoflagellate symbionts (Baker et al. 2013a; Ezzat et al. 2017), since both groups shared the same symbiont genotypes and/or density or ii) a different need in structural N, since soft and hard corals exhibited similar N tissue content. Soft corals therefore likely rely on heterotrophic feeding to meet their N requirements.

Overall, due to the poor capacity of soft coral symbionts to acquire dissolved nutrients, it is unlikely that changes in water quality related to nutrients (e.g., eutrophication) disrupt the soft coral-dinoflagellate symbiosis. Although this finding has not been experimentally tested in this thesis and remains hypothetical, it could be mechanistically supported by the conceptual model on unfavorable ratios of dissolved inorganic nutrients, introduced by Wiedenmann et al. (2012). In a nutshell, in hard corals, increased dissolved inorganic nitrogen concentrations promote a fast population growth of dinoflagellates. A proliferating symbiont population can induce an enhanced cellular demand, thereby leading to an undersupply in phosphorus or other essential nutrients, and ultimately to the starvation of symbiont cells. Such condition alters the lipid composition of their membranes, which renders corals more susceptible to bleaching through the production of reactive oxygen species (D'Angelo and Wiedenmann 2014). Therefore, since less dissolved nitrogen enters the tissue of soft corals, the scenario described above may be restricted to hard corals.

As a matter of fact, the relative dominance of soft corals has been reported in areas affected by high concentrations of dissolved and particulate matter (McClanahan and Obura 1997; Fabricius and Dommisse 2000; Schleyer and Celliers 2003; Fabricius 2005; Baum et al. 2016). In addition, the capacity of soft corals to thrive under environmental conditions ranging from turbid to clear-water (Fabricius and Dommisse 2000; Fabricius and De'ath 2008) may also be attributed to their nutritional flexibility. For example, under high turbidity or sedimentation regimes, a lower reliance on the symbiosis could help soft corals to cope with reduced light availability, while suspended particles can represent a potential source of nutrients for heterotrophic feeding.

6.1.3 Graphical summary

The main results of this thesis are summarized in Figure 38. The stability of the octocoral symbiosis along the depth gradient is ensured by a stable association (one symbiont genus), and the maximization of nutrient acquisition and conservation at the two depths by both autotrophy and heterotrophy. This is supported by a low assimilation of DIN and DON, a stable acquisition of DIC (per AFDW and per µg chlorophyll) a large amount of POM assimilated at both depths and no DDN assimilation nor promotion of N_2 fixation in the surrounding seawater. Moreover, the same amounts of carbon acquired through photosynthesis were translocated from the symbionts to the host at the two depths. In comparison to soft corals, scleractinian corals

exhibited a higher assimilation of DIN and DON (with increased rates of DON assimilated with depth), an acquisition of DIC higher in the shallow but similar (per µg chlorophyll), perhaps lower (per AFDW), one at mesophotic depths, significant assimilation of DDN and promotion of N_2 fixation in the surrounding seawater (although not investigated in the deeper corals). They also had a higher carbon loss in the shallow but lower carbon loss than soft corals at the mesophotic depth and symbionts transferred much lower amounts of carbon with depth. The presence of a calcium carbonate skeleton in hard corals likely plays an important role in the differences of metabolism (i.e., energy requirements) and productivity highlighted here. Finally, species-specific differences in nutrient acquisition and allocation may be due to i) the ratio of colony surface area to volume (a low ratio do not favor light exposure and gas or nutrient exchange through epidermal tissue), ii) micro-morphological features of the polyps (thin branches, small polyps, and high polyp surface area to volume ratio being more suited for autotrophy; big polyps for heterotrophy), iii) the symbiont species hosted (influencing cell-specific carbon assimilation and translocation), iv) the symbiont density (influencing cell-specific carbon assimilation and translocation), and v) the nutritional status (higher heterotrophic efficiency being correlated with higher rates of translocated carbon).

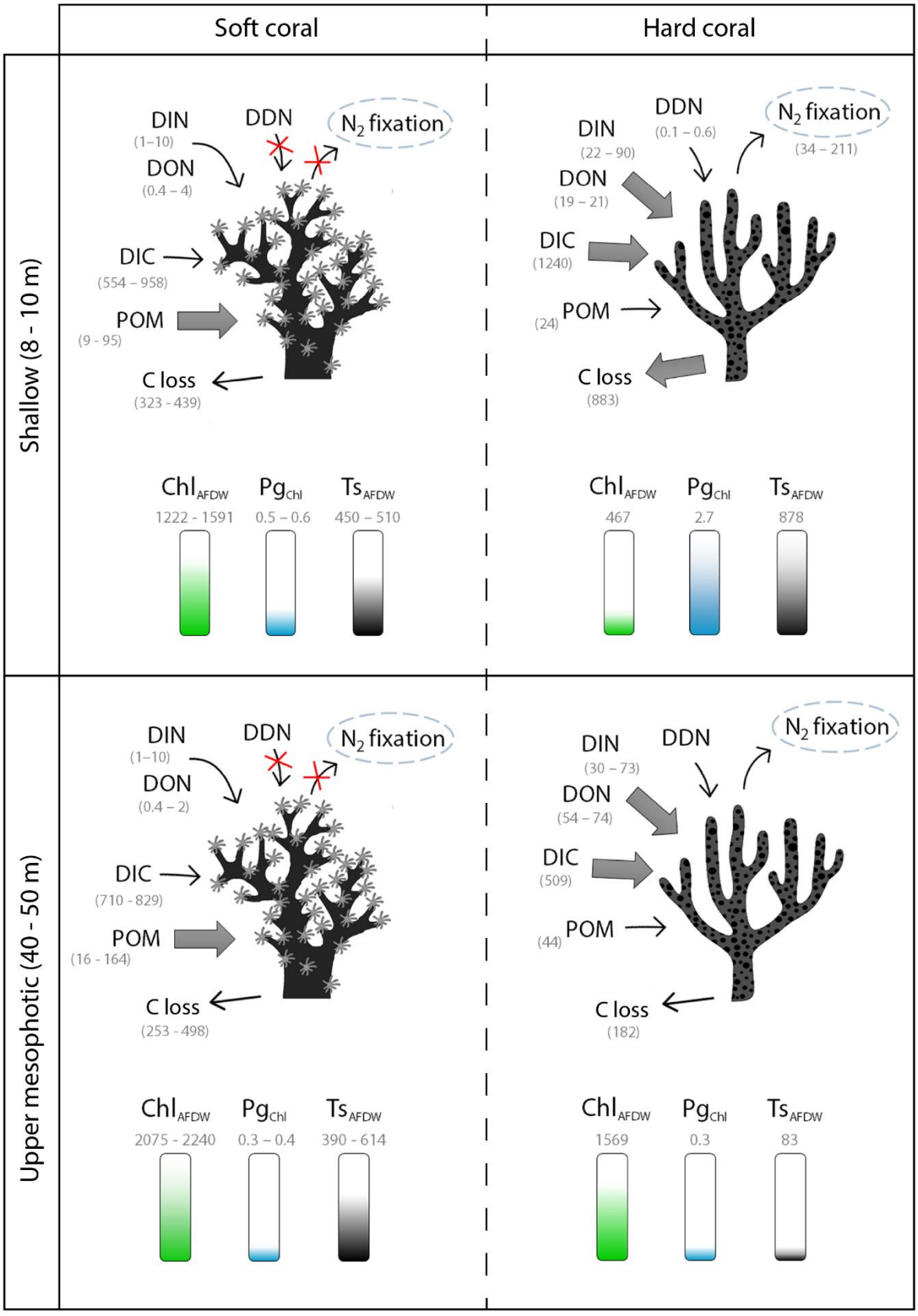


Figure 38. Contrast between the functioning of soft coral versus hard coral nutritional symbioses from shallow to mesophotic reef zones in Eilat (Red Sea). The range of rates corresponding to nutrient acquisition and fluxes is indicated below the nutrient acronym. These

rates are not comparable between each other but between groups (soft vs hard corals) and depths (shallow vs mesophotic) only. Chl = Chlorophyll concentration (µg Chl (a + c_2) g^{-1} AFDW). Pg = Autotrophic carbon acquisition (µg C $µg^{-1}$ Chl). Ts = Carbon translocated from the symbionts to the host (µg C g^{-1} AFDW h^{-1}). AFDW = ash-free dry weight. DIN = Dissolved inorganic nitrogen (including NH_4 and NO_3, in µg N g^{-1} AFDW h^{-1}). DON = Dissolved organic nitrogen (including DFAA, in µg N g^{-1} AFDW h^{-1}). DFAA = Dissolved free amino acids. DDN = Diazotrophically-derived nitrogen (ng N mg^{-1} DW h^{-1}). DW = Dry weight. Rate of N_2 fixation in seawater are in ng N L^{-1} d^{-1}. DIC = Dissolved inorganic carbon (µg C g^{-1} AFDW h^{-1}). POM = Particulate organic matter. The assimilation of POM has been investigated with a lipid biomarker specific to crustacean zooplankton (cis-gondoic acid (CGA, 20:1ω9), in µg g^{-1} AFDW). Lipid biomarkers specific to autotrophy, stearidonic acid (SDA, 18:4ω3) and docosapentaenoic acid (DPA, 22:5ω3), do not appear on the figure. Concentrations of SDA were 1-70 and 1-146 µg g^{-1} AFDW in shallow and mesophotic corals, respectively, and decreased from 11 to 3 µg g^{-1} AFDW with depth in the hard coral. Concentrations of DPA were 3-7 and 3-5 µg g^{-1} AFDW in shallow and mesophotic corals, respectively, and decreased from 5 to 2 µg g^{-1} AFDW with depth in the hard coral.

6.2 Conclusion

A functional mixotrophy was observed throughout the depth gradient for all symbiotic soft coral species, with a significant input of heterotrophic feeding on crustacean zooplankton at all depth. Mixotrophy was also observed for the only scleractinian species investigated in this thesis (*Stylophora pistillata*), suggesting that all coral species need to fulfill the deficiency of photosynthetic products in nitrogen and phosphorus through heterotrophy (see Houlbrèque and Ferrier-Pagès 2009). Moreover, heterotrophic feeding on zooplankton significantly increased with depth for several species, thereby emphasizing an increase in predation pressure and/or zooplankton availability with depth.

This thesis therefore highlights the importance of both autotrophic and heterotrophic inputs in soft coral nutrition, which provides them with a trophic plasticity along the depth gradient. Such dual mode of nutrition surely represents an asset for the successful settlement in different habitats and may provide them with an adaptive mechanism to sustain growth under stressful conditions.

6.3 Perspectives

The findings obtained in each chapter paved the way for future experiments, allowing a deeper understanding of the processes observed here at the organism scale. Some examples are given below.

6.3.1 Investigate the internal light environment of soft corals: the role of tissue and sclerites

Scleractinian corals have the ability to regulate the light environment perceived by their symbionts in hospite. For example, they can filter out the excess of light that can be harmful to the algae or enhance their internal light diffusion and scattering due to unique optical properties of their tissue and skeleton. Such amplification in the local light field of the symbionts notably improves photosynthesis. While soft corals do not have a skeleton, which might explain their lower rates of carbon acquisition and assimilation compared to scleractinian corals, they possess a large amount of sclerites. Sclerites could act as light diffusers, and their species-specific size, shape and color could help explaining differences in photosynthetic efficiencies. It would thus be interesting to investigate the internal light environment of soft corals as well as the possible role of sclerites in light scattering, using light microsensors. The hydroskeleton of soft corals may however entail some technical problems as it is likely to shrink on microsensors.

6.3.2 Importance of phosphorus acquisition for the stability of the soft coral symbiosis

In scleractinian corals, it has been shown that the nitrogen to phosphorus ratio (N:P) plays a major role in the stability of the symbiosis. Mainly, an increase in nitrogen availability (mostly in the form of nitrate) without a similar increase in phosphorus, can enhance the population growth of the dinoflagellates and subsequently enhance coral bleaching. Since soft corals are less prone to take up dissolved inorganic nitrogen, it would be interesting to test i) the levels of nitrate inducing a disruption of the symbiosis, 2) the role of phosphorus availability for the maintenance of such symbiosis under diverse nitrogen and/or environmental conditions. The results could bring information on how soft corals cope with eutrophication and thermal stress.

6.3.3 Investigate the oxidative stress in soft corals

The presence of symbionts in corals is often beneficial due to the energetic input supplied from photosynthesis. However, it can also impose restrictions (e.g., bathymetric distribution) and risks (e.g., higher increase in reactive oxygen species released under stressful conditions through photosynthesis). At the cellular level, the production of reactive oxygen and nitrogen species represents a pivotal factor responsible for coral bleaching. Since the results of this thesis show different nutritional strategies displayed by soft and hard corals along the depth gradient (especially regarding nitrogen), a different reliance on autotrophy, along with an overall flexibility towards the symbiosis, it could be interesting to investigate mechanisms possibly leading to a breakdown of the symbiosis in soft corals, in a context of thermal stress.

6.3.4 Heterotrophy

As heterotrophic nutrition was only assessed through a lipid biomarker specific to zooplankton, experiments involving incubations with a wide size range of labelled prey (pico- and nano-plankton) would help to further characterize the diet of soft corals. Particularly, the use of stable isotope labelling would confirm the effective ingestion of the prey, since the mucus of soft corals is known to have antimicrobial properties, which could eventually affect the concentrations of plankton measured in the chambers.

6.3.5 Contribution of autotrophy versus heterotrophy in the nutritional budget of soft corals

Finally, this thesis could not quantitatively provide any relative contribution of auto- and heterotrophic nutrient supply in the metabolic needs of the holobiont. Another experiment using lipid biomarkers specific to auto- and heterotrophy could be designed for this purpose and must involve different nutritional situations encountered by the same colonies (e.g., seasonal follow-up). Another possibility would be to use a multi-biomarkers approach, by combining stable isotope ($\delta^{13}C$ and $\delta^{15}N$) signatures, along with total lipids (discriminate between neutral and polar lipid's fatty acids).

Résumé du contenu de la thèse

Résumé développé

1. Introduction et objectifs de la thèse

1.1 Récifs coralliens tropicaux

Les récifs coralliens tropicaux sont considérés comme l'un des écosystèmes les plus productifs de la planète, du fait de la forte biomasse qu'ils renferment. Leur étendue (260000-600000 km²) représente moins de 0,2 % de l'océan, ce qui équivaut à une surface terrestre plus petite que la France. Pourtant, les récifs abritent entre un quart et un tiers des espèces marines du monde (Knowlton et al., 2010). Outre leur rôle écologique, les récifs coralliens fournissent à l'humanité de multiples services essentiels à son développement et à sa survie. Par exemple, ils protègent les côtes de l'érosion, constituent une source importante de nourriture pour la population vivant à proximité, génèrent des revenus grâce à la pêche, au tourisme et à des molécules d'intérêt pour l'industrie pharmaceutique (Moberg et Folke, 1999 ; Wells, 2006 ; Laurans et al., 2013). Les récifs génèrent environ trente milliards de dollars par an (Spalding et al., 2001 ; Stoeckl et al., 2011) et 500 millions de personnes bénéficient directement des récifs (Wilkinson, 2008).

La structure tri-dimentionnelle des récifs est formée, à la base, par le dépôt d'un squelette de carbonate de calcium par les polypes des coraux scléractiniaires, encore appelés coraux durs ou constructeurs de récifs. Cependant, les coraux scléractiniaires ne sont pas les seuls bâtisseurs de ces immenses structures. L'écosystème corallien comprend une mosaïque d'espèces « ingénieurs d'écosystème » tels que les algues corallines calcifiantes, les éponges et les octocoralliaires qui contribuent à la structure tridimensionnelle du récif. Ensemble, ils constituent une « forêt animale marine », qui fonctionne de la même manière que les forêts terrestres en fournissant une complexité architecturale, de la nourriture et un abri aux autres organismes du récif. En tant qu'arbres de la forêt, ils contribuent également de manière significative à la productivité primaire et aux cycles biogéochimiques du récif, soit directement, soit par le biais d'associations symbiotiques (Rossi, 2013).

1.2 La symbiose, à la base de la productivité des récifs coralliens

Les coraux constructeurs de récifs (scléractiniaires), ainsi que de nombreux octocoralliaires, sont des méta-organismes complexes, constitués par le corail animal hôte, des

dinoflagellés photosynthétiques et un large éventail d'autres microorganismes tels que des bactéries, des archées, des champignons et des virus (Knowlton et Rohwer, 2003 ; van de Water et al., 2018). La taxonomie des Symbiodiniaceae a été récemment révisée, avec une description formelle de sept genres (LaJeunesse et al., 2018). L'association entre les coraux et les dinoflagellés est appelée symbiose mutualiste et se présente comme un partenariat basé sur la nutrition. Les coraux symbiotiques sont ainsi considérés comme des organismes mixotrophes car ils peuvent utiliser des sources de nutriments à la fois inorganiques et organiques. Alors que l'animal est capable de capturer des proies en suspension dans le milieu, les dinoflagellés fixent le carbone inorganique via la photosynthèse et sont également capables d'absorber d'autres nutriments dissous, comme l'ammonium, les nitrates et les phosphates. Certaines bactéries telles que les diazotrophes fixent quant à elles le diazote. Chaque partenaire échange des nutriments avec les autres partenaires pour soutenir sa propre nutrition et subvenir à ses propres besoins métaboliques. Ainsi, les dinoflagellés transfèrent plus de 90% de leurs photosynthétats à l'animal (dans le cas des coraux scléractiniaires) dans les récifs de surface. Ce mode de nutrition multiple permet aux coraux de tirer le meilleur parti des eaux oligotrophes, où chacun des partenaires ne persisterait guère seul en raison de la faible concentration de nutriments inorganiques et de la faible disponibilité des proies (Stoecker et al. 2017). Les interactions métaboliques au sein de ce consortium symbiotique, qui sont nombreuses et diverses, conditionnent également la réponse du corail aux changements environnementaux (par exemple, voir Ainsworth et al. 2017 ; Benavides et al. 2017 ; van de Water et al. 2018 ; Pernice et al. 2019).

1.3 Zoom sur la symbiose et la nutrition des octocoralliaires

Les octocoralliaires, qui sont le sujet principal de cette thèse, appartiennent au phylum Cnidaria et à la classe Anthozoa. Actuellement, les octocoralliaires se composent de trois ordres : Helioporacea (communément appelés « coraux bleus »), Pennatulacea (communément appelées « plumes de mer ») et Alcyonacea. Les principaux groupes de l'ordre Alcyonacea incluent les gorgones, le corail rouge et les coraux mous. Contrairement aux gorgones et au corail rouge, les coraux mous ne présentent pas d'axe interne solide. Tout au long de ce résumé, le terme « octocoralliaire » qualifie donc tout membre de cette sous-classe sans distinction entre taxons, alors le terme « coraux mous » se réfère uniquement aux octocoralliaires ne disposant pas d'un axe interne solide.

Le principal trait qui distingue les octocoralliaires des scléractiniaires (coraux durs), outre une symétrie d'ordre huit, est l'absence d'un squelette de carbonate de calcium. De ce fait, les coraux mous dépendent, pour le soutien de leur corps, à la fois de la pression hydrostatique et d'une « micro-ossature » constituée d'une multitude de petites structures isolées de carbonate de calcium appelées sclérites. Les octocoralliaires constituent le deuxième groupe d'animaux macrobenthiques le plus abondant sur de nombreux récifs de l'Indo-Pacifique, des Caraïbes et de la mer Rouge, après les coraux durs (Benayahu et Loya, 1981 ; Fabricius et Alderslade, 2001 ; Benayahu et al., 2019 et références dans ce document). Les octocoralliaires peuvent être associés à cinq genres de Symbiodiniaceae selon les régions biogéographiques. La diversité génétique au sein des genres de la famille Symbiodiniaceae reflète également une diversité physiologique, expliquant différentes réponses aux changements environnementaux.

Chez les octocoralliaires, la contribution relative de l'autotrophie et de l'hétérotrophie aux besoins nutritionnels de l'holobionte est mal connue (revue dans Schubert et al., 2017). On pense généralement que les octocoralliaires ont un faible taux de productivité primaire et qu'ils dépendent à la fois de l'autotrophie et de l'hétérotrophie pour satisfaire leurs besoins métaboliques (Sorokin, 1991 ; Fabricius et Klumpp, 1995). Cependant, les quelques études portant sur la contribution de la photosynthèse à la respiration de l'hôte (rapport P:R) soutiennent plutôt une dépendance à l'autotrophie pour satisfaire les besoins respiratoires (Mergner et Svoboda, 1977 ; Sorokin, 1991 ; Riegl et Branch, 1995 ; Fabricius et Klumpp, 1995 ; Kremien et al., 2013 ; Bednarz et al., 2015 ; Rossi et al., 2017, 2020), excepté pour quelques espèces (Ramsby et al., 2014 ; Baker et al., 2015). Le peu d'études qui ont porté sur un bilan de carbone montrent une forte variation inter-espèces, avec des taux de transfert de photosynthétats allant de 10 % chez *Capnella gabonensis* à 75 % chez *Sinularia flexibilis* (Schlichter et al., 1983 ; Farrant et al., 1987 ; Sorokin, 1991 ; Khalesi et al., 2011). La performance des symbiotes semble liée à la morphologie de l'hôte, les espèces ayant des branches fines et de petits polypes étant plus autotrophes que celles ayant une forme massive et de gros polypes (Baker et al., 2015 ; Rossi et al., 2018). La morphologie de la colonie entière peut également jouer un rôle important dans la nutrition des coraux mous : le rapport entre la surface corporelle et le volume, qui détermine à la fois l'échange gazeux à travers le tissu épidermique et l'exposition au rayonnement photosynthétiquement actif, est faible chez les coraux mous par rapport aux coraux durs (Fabricius et Klumpp, 1995). Toute augmentation de ce rapport, induit par l'expansion de la colonie, peut améliorer la productivité primaire. Des recherches

supplémentaires sont donc nécessaires pour mieux comprendre les capacités autotrophes des octocoralliaires en général, et des coraux mous en particulier.

En ce qui concerne l'hétérotrophie, les coraux mous ont longtemps été considérés comme étant principalement des suspensivores, car leurs nématocystes sont petits, simplement structurés et inefficaces pour paralyser des proies nageant activement (Mariscal et Bigger, 1977). Toutefois, la découverte récente d'un nouveau type de nématocystes pourrait potentiellement mettre en évidence un arsenal de structures urticantes plus diversifiées qu'on ne le pensait auparavant (Yoffe et al., 2012). De plus, des microvillosités recouvrent l'épiderme des coraux mous de manière dense et jouent probablement un rôle dans l'absorption de la matière dissoute (Schlichter, 1982a, 1982b). Le régime alimentaire des octocoralliaires a été déterminé par des expériences impliquant des espèces tempérées (Coma et al., 1994 ; 1998 ; 2015 ; Orejas et al., 2003 ; Rossi et al., 2004) et tropicales (Lewis, 1982 ; Sebens et Koehl, 1984 ; Sorokin, 1991 ; Fabricius et Domisse 2000 ; Rossi et al. 2020), et comprend du zooplancton de petite taille, ainsi que phytoplancton (Fabricius et al., 1995a, 1995b ; 1998 ; Ribes et al. 1998 ; Leal et al. 2013 ; Piccinetti et al., 2016). La contribution relative de l'autotrophie et de l'hétérotrophie à la nutrition des coraux mous n’est pas fixe et dépend des conditions environnementales et/ou de la disponibilité des ressources (par exemple de la profondeur et de la saison ; Rossi et al. 2020). Cependant, cette notion reste à étudier plus en détails.

1.4 Les principales menaces pesant sur les récifs coralliens et les coraux mous

Les récifs coralliens sont généralement capables de se remettre des perturbations naturelles en quelques années ou décennies s’ils ne subissent pas d’autres dommages entre temps. Cependant, les activités humaines ajoutent aux perturbations naturelles des facteurs de stress locaux et globaux, qui agissent ensemble pour affaiblir les récifs coralliens et finissent par entraîner des déclins importants. En conséquence, les écosystèmes marins se modifient et les récifs coralliens sont parmi les plus menacés.

Les activités anthropiques telles que la combustion d’énergies fossiles, l'industrialisation et la déforestation, ont augmenté les concentrations de gaz à effet de serre dans l'atmosphère depuis le début de l'ère industrielle (Sabine et al., 2004). Ces émissions sont la cause de changements climatiques à l’échelle mondiale, notamment le réchauffement et l'acidification des océans.

- Le réchauffement de l'océan

Les coraux vivent dans des conditions environnementales proches de leur maximum thermique et peuvent souffrir de stress thermique lorsque la température de la surface de la mer augmente de ≥ 1°C au-dessus du maximum estival moyen (Wooldridge, 2013). Lorsque les coraux subissent un stress thermique extrême et prolongé, la symbiose avec leurs dinoflagellés photosynthétiques est perturbée, ce qui entraîne généralement l'expulsion des symbiotes, un phénomène appelé blanchissement (Brown, 1997). Les coraux blanchis sont physiologiquement endommagés, et un blanchissement prolongé entraîne souvent des niveaux élevés de mortalité chez les coraux (Hughes et al., 2018).

Ce phénomène aujourd'hui généralisé a été signalé dans de nombreux récifs du monde entier et n'épargne pas les octocoralliaires (Paulay et Benayahu, 1999, Marshall et Baird 2000, Arceo et al. 2001, Loya et al. 2001, McClahanan et al. 2001, Cellier et Schleyer 2002, Goulet et al. 2008, Chavanich et al. 2009, Prada et al. 2009, van Woesik et al. 2011, Dias et Gondim 2015, Slattery et al. 2019). Tout comme pour les coraux durs, il a été montré que la sensibilité des octocoralliaires au stress thermique n'est pas uniforme et qu'il existe une tolérance plus élevée chez certaines espèces, voire entre individus d'une même espèce (Loya et al., 2001 ; Lasker 2003, Strychar et al. 2005 ; Goulet et al. 2008 ; van Woesik et al. 2011 ; Slattery et al. 2019). Les quelques études expérimentales qui portent sur les réponses des octocoralliaires tropicaux au stress thermique ont montré une majorité de réponses négatives, impliquant notamment une réduction de la photosynthèse, un blanchissement, une diminution des réserves énergétiques et de la production reproductive, ainsi que de la nécrose (Michalek-Wagner et Willis 2001, Drohan et al. 2005, Strychar et al. 2005 ; Imbs et Yakatova, 2012 ; Sammarco et Strychar, 2013 ; Netherton et al. 2014). Par conséquent, les octocoralliaires semblent être aussi affectés que les coraux durs par le réchauffement de l'océan.

- Acidification des océans

L'acidification des océans est un processus se produisant actuellement de manière continue, qui induit plusieurs changements dans la chimie des carbonates de l'eau de mer (e.g., Feely et al., 2004). En particulier, il diminue le pH de l'eau de mer et la concentration des ions carbonate. Ces modifications ont donc un effet négatif sur la calcification biogénique (e.g., de l'aragonite et de la calcite) et peuvent également entraîner la dissolution nette des structures

carbonatées. Ainsi, l'acidification des océans pose d'importants problèmes aux organismes marins qui forment des coquilles, des squelettes ou des structures internes en carbonate de calcium (Gattuso et Hansson, 2011 ; Orr et al., 2005).

Alors que les observations *in situ* suggèrent qu'une baisse du pH de l'eau de mer dans les années à venir devrait induire une transition de la dominance des coraux scléractiniaires vers une prépondérance des coraux mous et autres espèces non-calcifiantes (Inoue et al., 2013), seules quelques études expérimentales ont abordé la réponse des octocoralliaires à l'acidification des océans. Elles montrent que les descripteurs tissulaires et les paramètres physiologiques (c'est-à-dire la densité des cellules des symbiotes, la pigmentation, la structure des sclérites, le taux de pulsation des polypes et la croissance) ne sont pas affectés par l'acidification (Gabay et al., 2013, 2014, Enochs et al. 2015b). Une seule étude a montré une diminution de la calcification, mais le pH testé (7,1) dépasse les valeurs prévues pour la fin du siècle (Gomez et al., 2014). L'atténuation de l'acidification par les octocoralliaires pourrait s'expliquer par leur tissu épais, qui peut protéger les sclérites (Gabay et al., 2013 ; 2014), et par le processus de calcification lui-même, qui pourrait être moins affecté que celui des coraux durs malgré l'altération de la chimie des carbonates (Enochs et al., 2015b). Bien qu'il existe peu de données sur les effets combinés de l'acidification et d'autres menaces sur les octocoralliaires, il a été suggéré que les coraux durs, qui sont déjà stressés par l'acidification seule, seront plus touchés que les octocoralliaires s'ils sont déjà confrontés à d'autres facteurs de stress tels que l'eutrophisation (Januar et al., 2017).

- Eutrophisation et autres facteurs de stress locaux

L'eutrophisation de l'eau est un phénomène complexe qui implique une augmentation de la concentration en sels nutritifs dissous (nitrates, phosphates), une augmentation des particules en suspension et une réduction de la luminosité. Les récifs côtiers sont les plus impactés par l'eutrophisation, qui résulte d'une agriculture et/ou urbanisation intensive, ainsi que du rejet de polluants inorganiques dans l'eau. Les effets de l'eutrophisation sur l'abondance des coraux mous ne sont pas concluants. En effet, les études portant sur le taux de couverture des coraux durs et mous montrent un impact négatif (Fabricius et De'ath 2004), un effet nul (Fabricius et De'ath, 2001), ou bien une dominance relative des coraux mous dans des zones affectées par la sédimentation (McClanahan et Obura, 1997, Schleyer et Celliers, 2003) ou soumises à de fortes concentrations de particules en suspension et matières dissoutes (Fabricius,

2005 ; Fabricius et Dommisse, 2000 ; Baum et al., 2016). Des données provenant de la Grande Barrière de Corail, de Hong-Kong et de Palau suggèrent qu'une pauvre qualité d'eau (incluant tous les paramètres cités ci-dessus) serait plutôt liée à une diminution de la diversité des espèces (Fabricius et De'ath, 2004 ; Fabricius et McCorry, 2006 ; Fabricius et al., 2005 ; 2007). Des recherches supplémentaires sont donc nécessaires pour expliquer les effets de la qualité de l'eau sur les octocoralliaires.

En conséquence, les octocoralliaires sont souvent abondants dans les systèmes récifaux perturbés où ils peuvent présenter un taux de survie plus élevé que les coraux durs sous un large éventail de conditions environnementales. Des changements dans la structure de la communauté aboutissant à la dominance des octocoralliaires ont été observés suite à des épidémies d'étoiles de mer corallivores (*Acanthaster planci*), à la pollution anthropique, à des tempêtes, au blanchissement, à des maladies et à la pêche à la dynamite. Ces perturbations ont conduit à une diminution de la couverture des coraux durs alors que les coraux mous ont maintenu ou augmenté leur abondance.

1.5 Objectifs de la thèse

La communauté scientifique n'a encore que très peu de données sur les caractéristiques physiologiques des coraux mous, ou sur leur écologie trophique et leur relation avec leurs symbiotes (Schubert et al., 2017, van de Water et al., 2018). La recherche sur les octocoralliaires est en effet très en retard par rapport à la recherche sur les coraux durs et d'importantes lacunes dans les connaissances relatives à leur physiologie, en particulier à leur nutrition, doivent encore être comblées.

La nutrition est l'un des facteurs les plus importants impliqués dans la régulation de l'abondance et la distribution d'une communauté au sein d'un écosystème, au même titre que les conditions environnementales et les interactions avec d'autres organismes (par exemple la compétition et la prédation). En particulier, l'acquisition de nutriments fournit l'énergie nécessaire à la croissance et à la reproduction des coraux et peut favoriser la résistance ou la résilience des coraux aux changements environnementaux. Dans les récifs oligotrophes d'Eilat (golfe d'Aqaba, nord de la mer Rouge), deux communautés distinctes d'octocoralliaires ont été identifiées, l'une en surface et l'autre dans la zone mésophotique supérieure (30-45 m). Il est intéressant de noter que la communauté d'octocoralliaires qui se développe en profondeur est

caractérisée par une plus grande richesse en espèces, ainsi que par une prédominance d'espèces symbiotiques. Bien que ces caractéristiques suggèrent un régime lumineux adéquat pour que la photosynthèse du corail s'y déroule (Shoham et Benayahu, 2017), les stratégies nutritionnelles de ces coraux mous ont fait l'objet de peu d'attention jusqu'à présent.

Cette thèse se concentre donc sur le fonctionnement de la symbiose dinoflagellé-corail mou et vise à apporter des connaissances sur l'écologie nutritionnelle de plusieurs espèces d'octocoralliaires symbiotiques du nord de la mer Rouge, jusqu'à des profondeurs mésophotiques. La symbiose dinoflagellé-octocoralliaire est connue pour être différente de celle des coraux scléractiniaires, en termes de diversité et de stabilité. Alors que les octocoralliaires présentent une faible diversité de symbiotes, les coraux scléractiniaires hébergent généralement plusieurs types de symbiotes et sont plus flexibles en fonction des conditions environnementales dominantes (Baker et Romanski, 2006). Cette différence a été attribuée au fait que les octocoralliaires dépendent davantage de l'hétérotrophie et ont donc moins à gagner d'une telle flexibilité symbiotique (van Oppen et al., 2005). Cependant, l'importance de l'autotrophie et de l'hétérotrophie, ainsi que leur contribution respective aux besoins métaboliques de l'hôte ont été peu étudiées chez les octocoralliaires et principalement testées chez les gorgones (Rossi et al., 2020). Les coraux mous étant l'une des principales composantes des écosystèmes récifaux et le changement climatique et les perturbations locales étant susceptibles d'induire des transitions de plus en plus importantes vers des récifs dominés par les octocoralliaires (par exemple Tsounis et Edmunds, 2017), il devient primordial de faire progresser les connaissances sur l'écologie nutritionnelle de leur symbiose.

Par conséquent, dans cette thèse, nous avons étudié l'acquisition et les flux de nutriments organiques et inorganiques, chez plusieurs hôtes de corail mou, leurs symbiotes dinoflagellés, et d'autres micro-organismes avec lesquels ils seraient éventuellement associés tels que les diazotrophes. Les comparaisons avec les symbioses scléractiniaires-dinoflagellés ont mis en évidence des différences fonctionnelles majeures entre les deux groupes et ont permis de discuter nos résultats dans un contexte plus large.

2. Résultats

2.1 Métrique de normalisation et protocole de traitement des échantillons (Chapitre 2)

La normalisation est une étape fondamentale dans le traitement des données, dont la pertinence influencera profondément le résultat d'une expérience et son interprétation. Chez les coraux durs, ce choix se porte généralement sur la surface du squelette, ainsi que la quantité de chlorophylles ou de protéines après extraction des tissus. Cependant, la structure des coraux mous entraîne un certain nombre de problèmes pour l'homogénéisation des tissus et la normalisation des données : 1) La nature gélatineuse, ou semblable au cuir, des tissus des coraux mous ne permet pas une extraction facile des descripteurs tissulaires, tels que la densité des symbiotes, la concentration de pigments et de protéines, 2) la métrique de normalisation la plus utilisée chez les coraux scléractiniaires, la surface du squelette, n'est pas pertinente pour les coraux mous puisqu'ils ne possèdent pas de squelette, 3) la surface d'un fragment de corail mou est difficile à estimer en raison de leur hydrosquelette très variable, qui peut rapidement changer de taille en fonction des conditions environnementales et 4) ils ne permettent pas la comparaison de paramètres et processus physiologiques des coraux mous à ceux d'autres espèces de coraux comme les scléractiniaires.

Un protocole simple et rapide pour l'homogénéisation des tissus, ainsi qu'une métrique de normalisation pouvant être utilisée pour effectuer des études ou des comparaisons inter-espèces, sont donc nécessaires. Dans cette première étude, nous avons vérifié si l'état de l'échantillon de tissu avant traitement (échantillons congelés vs lyophilisés) et le milieu utilisé pour l'homogénéisation des tissus (eau de mer filtrée à 0,2 μm vs eau distillée) affectent les mesures des descripteurs de tissu (concentrations en chlorophylles, protéines et dinoflagellés) chez l'espèce modèle *Heteroxenia fuscescens*. En outre, la pertinence du poids sec (après lyophilisation) et du poids sec sans cendres (après calcination à 450°C pendant plusieurs heures) comme métrique de normalisation a été étudiée chez différentes espèces de coraux mous. Nos résultats ont révélé que la lyophilisation des échantillons et leur homogénéisation dans de l'eau distillée présentent plusieurs avantages, à savoir la minimalisation des pertes de chlorophylles et protéines (jusqu'à 50%), un gain de temps, ainsi qu’une détermination plus précise du poids sec et du poids sec sans cendres. Dans l'ensemble, ce protocole optimisé offre une quantification plus fiable des descripteurs tissulaires et réduit le risque de sous-estimation de ces paramètres

chez les coraux mous. Enfin, étant donné que la contribution des sclérites au poids sec total de la colonie peut être très différente d'une espèce à l'autre, nous avons démontré que la différence entre le poids sec et le poids sec sans cendre, qui correspond à la quantité de matière organique de l'échantillon, est une mesure fiable pour normaliser les données relatives aux coraux mous, notamment lorsque des comparaisons entre espèces sont effectuées.

2.2 Acquisition du carbone inorganique dissous (Chapitre 3)

L'acquisition autotrophe de carbone contribue largement aux besoins métaboliques des coraux durs, qui reçoivent 90% des photosynthétats produits par les symbiotes. Chez les octocoralliaires, la contribution des symbiotes aux besoins énergétiques de leur hôte est encore mal comprise. Tout d'abord, les études portant sur la productivité (primaire) des coraux mous sont rares, limitées à une profondeur de 20 m et les résultats obtenus présentent une forte variabilité (voir les informations supplémentaires dans Rossi et al., 2018). Il existe plusieurs sources identifiées pour cette variabilité : 1) les colonies de coraux mous peuvent atteindre différents degrés d'expansion en fonction des conditions environnementales, ce qui confère une grande variabilité biologique aux mesures (voir par exemple Fabricius et Klumpp, 1995) ; 2) la morphologie de la colonie et des polypes, notamment le rapport surface/volume, peut être plus ou moins adaptée à l'autotrophie (Baker et al., 2015 ; Rossi et al., 2018) ; et 3) l'utilisation de différentes méthodes et mesures de normalisation ne permet guère de faire des comparaisons. En outre, la quantification du transfert de photosynthétats des symbiotes vers l'hôte a été étudiée chez une seule espèce tropicale (*Heteroxenia fuscecens)* et une seule espèce tempérée (*Capnella gaboensis)* en utilisant le ^{14}C (Schlichter et al., 1983 ; Farrant et al., 1987).

Nous avons donc réalisé des expériences de traçage du carbone inorganique acquis par voie autotrophe au moyen de bicarbonate enrichi en ^{13}C, qui est un isotope stable, sur deux espèces de coraux mous (*Litophyton* sp. et *Rhytisma fulvum fulvum*), provenant des récifs coralliens de la mer Rouge de surface et de la zone mésophotique supérieure (40-50 m), afin de quantifier l'acquisition et l'allocation de carbone autotrophe au sein de l'association symbiotique. Les mesures de l'acquisition et de la respiration du carbone distinguent *Litophyton* sp. comme une espèce principalement autotrophe et *Rhytisma fulvum fulvum* comme une espèce plutôt hétérotrophe. Chez les deux espèces, l'acquisition du carbone était constante aux deux profondeurs étudiées. Cela représente une différence majeure avec les coraux scléractiniaires, dont l'acquisition de carbone diminue avec la profondeur. De plus, l'acquisition du carbone et

le transfert de photosynthétats vers l'hôte diminuent avec l'augmentation de la densité des symbiotes, ce qui suggère qu'à forte densité, les symbiotes conservent les nutriments pour leurs propres besoins au lieu de les transférer à leur hôte.

2.3 Acquisition de l'azote inorganique/organique dissous (Chapitre 4)

La présence des dinoflagellés symbiotiques procure de nombreux avantages aux organismes qui les accueillent au sein de leurs cellules. Parmi ces avantages figurent la capacité de survivre dans des environnements oligotrophes grâce à une absorption, une conservation et un recyclage efficaces des nutriments, réalisés essentiellement par les symbiotes. L'azote est un élément nutritif essentiel au fonctionnement des holobiontes coralliens, mais sa disponibilité est souvent faible dans les eaux récifales du fait de la rareté de ses formes dissoutes. Des études récentes ont fait progresser notre compréhension du cycle de l'azote chez les coraux scléractiniaires. Il a été montré que ces coraux peuvent acquérir presque toutes les formes d'azote dissous, dont le diazote gazeux, les formes inorganiques telles que l'ammonium et le nitrate, ainsi que des composés organiques dissous tels que des acides aminés. Malgré ces connaissances obtenues sur les coraux scléractiniaires, la capacité des autres groupes coralliens, tels que les octocoralliaires, à assimiler les différentes formes d'azote n'a pratiquement jamais été évaluée. Dans cette thèse, nous avons donc abordé l'assimilation de toutes ces formes d'azote par les coraux mous, et comparé les taux d'assimilation obtenus avec ceux mesurés chez les espèces de coraux scléractiniaires.

- Diazotrophie

La forme la plus abondante d'azote dissous, le diazote atmosphérique, ne peut être rendue biologiquement disponible que par l'action de bactéries fixatrices de diazote appelées diazotrophes (qui réduisent le diazote en ammonium). En milieu marin, ces bactéries diazotrophes existent sous forme libre ou forment une association symbiotique avec de nombreux invertébrés qui vont tirer profit de leur activité fixatrice. Les quelques études portant sur l'assimilation de l'azote dérivé des diazotrophes (DDN) par les coraux tropicaux se limitent à quelques espèces de scléractiniaires (e.g. *Stylophora pistillata*) et seules deux publications ont étudié les taux de fixation du diazote en présence de coraux mous (Bednarz et al., 2015, Cardini et al., 2016). Cependant, ces études ont utilisé la technique de réduction de l'acétylène, qui ne quantifie que la fixation de diazote se produisant dans l'eau de mer brute, sans fournir

d'indications sur les bénéficiaires de l'azote fixé parmi les constituants de l'holobionte. Seule l'utilisation du gaz $^{15}N_2$, permet de suivre le devenir de l'azote diazotrophe (DDN) dans les différents compartiments de l'holobionte corallien.

Une première étude a donc été réalisée pour évaluer l'importance de la fixation d'azote pour la nutrition des coraux mous. L'objectif de cette étude était notamment de savoir si les coraux mous sont associés à des diazotrophes actifs. Nous avons quantifié les taux d'assimilation de DDN chez quatre espèces de scléractiniaires et trois espèces de coraux mous des eaux peu profondes de la mer Rouge en utilisant la technique du traceur $^{15}N_2$. Toutes les espèces de scléractiniaires ont stimulé de manière significative la fixation de N_2 dans l'eau de mer (et le mucus) entourant le corail et ont assimilé le DDN dans leurs tissus. Au contraire, la fixation de N_2 n'a pas été détectée dans l'eau d'incubation des coraux mous, et aucune assimilation de DDN n'a été mesurée dans les tissus de ces coraux. Cependant, des incubations au laboratoire de coraux mous avec des cultures de diazotrophes ont montré que les coraux mous étaient capables d'absorber le DDN provenant de diazotrophes libres. Le mucus des coraux mous représente probablement un habitat défavorable pour la colonisation et l'activité des diazotrophes car il contient une faible quantité de matière organique particulaire, avec une teneur en azote relativement élevée, par rapport au mucus des coraux scléractiniaires. De plus, il est connu pour présenter des propriétés antimicrobiennes. Dans l'ensemble, cette étude suggère que l'assimilation du DDN dans les tissus du corail dépend de la présence de diazotrophes actifs dans la couche de mucus du corail et/ou dans l'eau de mer environnante. Étant donné que l'azote est souvent un nutriment limitant la productivité primaire dans les eaux oligotrophes des récifs, la capacité divergente des coraux scléractiniaires et des coraux mous à favoriser la fixation de l'azote peut avoir des implications sur la disponibilité de l'azote et la biogéochimie des récifs dominés par des scléractiniaires par rapport aux récifs dominés par les coraux mous.

- Assimilation du DIN et du DON

La capacité des coraux mous à assimiler l'azote dissous à l'échelle de l'organisme restant inexplorée, une deuxième expérience sur l'assimilation d'azote a eu pour objet l'étude de l'acquisition de l'azote inorganique et organique dissous par la symbiose coraux mous-Symbiodiniaceae. En utilisant le marquage avec des isotopes stables, nous avons effectué une comparaison multi-espèces pour étudier l'assimilation d'ammonium, de nitrate et d'acides

aminés libres chez plusieurs espèces de coraux mous et scléractiniaires échantillonnés dans la mer Rouge (Eilat, Israël) à deux profondeurs différentes (8-10 m et 40-50 m). Le principal résultat montre que pour toutes les sources d'azote, les coraux scléractiniaires présentent des taux d'assimilation par biomasse plus élevés que les coraux mous (jusqu'à dix fois), bien qu'ils abritent les mêmes genres de Symbiodiniaceae en densités similaires. Cela suggère que les coraux mous doivent compléter leur faible acquisition d'azote dissous par l'acquisition d'autres formes d'azote, s'appuyant ainsi plutôt sur une alimentation hétérotrophe pour satisfaire leurs besoins en azote structurel. Cette découverte met en évidence une acquisition d'azote très différente entre les symbioses de coraux mous et scléractiniaires avec leurs dinoflagellés. Un tel contraste dans la capacité à tirer parti de cette source de nutriments peut aider à comprendre comment les coraux mous et scléractiniaires répondent à différents degré d'enrichissement d'eau de mer en nutriments.

2.4 Acquisition *in situ* de nutriments autotrophes et hétérotrophes chez les coraux mous (Chapitre 5)

Les octocoralliaires sont d'importants habitants des récifs, de la surface aux profondeurs mésophotiques et au-delà. Cependant, l'acquisition autotrophe ou hétérotrophe de nutriments est mal connue chez les coraux mous symbiotiques. Comme les coraux ont la capacité de capturer des proies, l'hétérotrophie peut représenter une acquisition importante de carbone, d'azote et d'autres nutriments. La littérature sur le régime alimentaire des coraux mous tropicaux est cependant très rare (par exemple Lewis, 1982 ; Sorokin, 1991 ; Fabricius et al., 1995) et la capacité hétérotrophe des coraux a été principalement estimée par des études en laboratoire. Le statut hétérotrophe des coraux en conditions *in situ* est donc encore mal compris, et ce pour toutes les espèces de coraux.

Une expérience a été conçue pour étudier le statut autotrophe et hétérotrophe des coraux mous *in situ*. À cette fin, l'abondance de trois biomarqueurs lipidiques, spécifiques de l'autotrophie et de l'hétérotrophie, a été étudiée dans les tissus de quatre espèces d'octocoralliaires, et une de scléractiniaire, prélevées au sein de récifs peu profond et mésophotique supérieur de mer Rouge. Nos résultats montrent une mixotrophie fonctionnelle pour toutes les espèces symbiotiques, avec un apport significatif de zooplancton aux deux profondeurs. Bien que les octocoralliaires aient maintenu des concentrations similaires de marqueurs autotrophes avec l'augmentation de la profondeur, l'espèce scléractiniaire a montré

une diminution de l'autotrophie avec la profondeur, en accord avec les mesures précédentes d'acquisition de carbone par la photosynthèse. L'augmentation de la capacité hétérotrophe avec la profondeur était spécifique à l'espèce, probablement liée aux caractéristiques physiologiques et morphologiques. Le niveau relativement élevé du marqueur hétérotrophe chez toutes les espèces symbiotiques en eaux peu profondes ne corrobore pas l'idée reçue selon laquelle les coraux dépendent principalement de l'autotrophie dans les eaux peu profondes. Cette étude souligne donc l'importance de l'hétérotrophie à travers le gradient de profondeur euphotique-mésophotique supérieur et apporte des avancées majeures dans notre compréhension de l'importance écologique de l'alimentation chez les coraux. En outre, elle permet de mieux comprendre la capacité des différentes espèces d'octocoralliaires à exploiter les ressources en nutriments et contribue à améliorer les prévisions concernant les effets des changements climatiques et environnementaux sur cet important taxon vivant dans les récifs.

3. Discussion générale et perspectives

3.1 Les coraux mous et scléractiniaires forment des symbioses fonctionnelles différentes avec leurs dinoflagellés tant dans les récifs de surface que dans les récifs de la zone mésophotique supérieure

- L'autotrophie reste stable le long du gradient de profondeur chez les coraux mous alors qu'elle diminue chez les coraux scléractiniaires

L'acquisition autotrophe de carbone par les symbiotes du corail a été évaluée dans cette thèse par deux approches complémentaires (traceur bicarbonate ^{13}C et acides gras spécifiques à l'autotrophie, Chapitres 3 et 5). Les deux approches ont montré, pour toutes les espèces de coraux mous étudiées, que l'assimilation à court et à long terme du carbone dans le tissu hôte du corail était stable le long du gradient de profondeur ou même augmentée en profondeur mésophotique. La stabilité de l'apport en nutriments autotrophes le long du gradient de profondeur suggère que les coraux mous sont soit photo-limités dans les eaux peu profondes et/ou photo-acclimatés en profondeur. Ceci est en accord avec de précédentes observations indiquant des taux de photosynthèse maximaux mesurés à 20 m de profondeur (en comparaison avec des eaux de surface) chez plusieurs espèces de coraux mous de la Grande Barrière de Corail (Fabricius et Klumpp, 1995). Des stratégies d'acclimatation à de faibles niveaux de lumière ont été observées chez les symbiotes (c'est-à-dire une réduction de l'auto-ombrage et

une augmentation de la capacité de captage de la lumière des cellules des symbiotes ; Cohen et Dubinsky, 2015 ; Ziegler et al., 2015 ; Einbinder et al., 2016). Cependant, la stabilité de l'autotrophie le long du gradient de profondeur était indépendante du genre de Symbiodiniaceae hébergé par le corail hôte, puisque les associations étaient spécifiques à l'espèce et sont susceptibles d'être stables dans l'espace et le temps (Goulet et Coffroth, 2003). Au contraire, chez le corail dur *S. pistillata*, on observe une diminution de l'acquisition de carbone autotrophe avec la profondeur et le remplacement du genre de symbiote *Symbiodinium* (anciennement clade A) au genre *Cladocopium* (anciennement clade C) avec la profondeur (Mass et al., 2007 ; Ezzat et al., 2017).

La comparaison du bilan carbone chez les coraux mous et scléractiniaires indique que le taux d'acquisition du carbone autotrophe est au moins deux fois plus élevé chez *S. pistillata* à faible profondeur par rapport aux espèces de coraux mous étudiées. Cependant, le carbone acquis est également perdu (sous forme de respiration et de production de mucus) deux fois plus vite chez les coraux scléractiniaires, ce qui suggère un fonctionnement différent des symbioses, ainsi qu'une demande métabolique différente entre les espèces de coraux mous et durs. La demande métabolique plus élevée chez les coraux scléractiniaires est probablement due à la calcification, puisque la majeure partie du carbone respiré est utilisée pour le dépôt de carbonate de calcium. En effet, près de 70-75% du carbone utilisé pour la calcification chez les coraux durs provient de la respiration, et les 25-30% restants du carbone externe de l'eau de mer (Erez, 1978 ; Furla et al., 2000 ; Hughes et al., 2010). En outre, la présence du squelette améliore la productivité du corail grâce à la diffusion interne de la lumière, ce qui peut également contribuer à expliquer les différences d'acquisition du carbone entre les coraux mous et les coraux durs.

- L'hétérotrophie est importante à toutes les profondeurs et augmente en zone mésophotique pour plusieurs espèces

Une autre conclusion principale de cette thèse est que les coraux mous symbiotiques de surface et de profondeur mésophotique sont plus susceptibles de dépendre de l'hétérotrophie que de l'autotrophie pour l'acquisition de nutriments, par rapport aux coraux durs. Cette conclusion est étayée par la grande quantité de biomarqueurs lipidiques, spécifiques au zooplancton, mesurée dans les tissus des coraux mous aux deux profondeurs (Chapitre 5). Pour certaines espèces, la quantité de biomarqueurs augmente significativement avec la profondeur,

ce qui suggère que ce type de nutrition est favorisé dans le récif mésophotique. En outre, nous avons constaté au Chapitre 4 que les coraux mous ont une faible assimilation d'azote inorganique ou organique dissous et ont donc besoin d'acquérir de l'azote à partir d'une autre source telle que l'alimentation particulaire.

3.2 Les coraux mous et scléractiniaires forment des symbioses fonctionnelles différentes avec leurs dinoflagellés, ce qui pourrait expliquer la plus grande résistance des coraux mous à l'eutrophisation

Tout d'abord, nous avons montré que le mucus des coraux mous représente un habitat probablement défavorable pour la colonisation et l'activité des diazotrophes, car il est connu pour présenter des propriétés antimicrobiennes et contient des particules à teneur relativement élevée en azote (Kelman et al., 2006 ; Nakajima et al., 2018). En conséquence, les coraux mous ne favorisent pas la fixation de diazote dans l'eau de mer qui les entoure et n'assimilent pas le DDN. De plus, des taux d'assimilation de l'azote dissous jusqu'à dix fois inférieurs ont été observés chez les coraux mous par rapport aux coraux durs, sans différence nette entre les profondeurs. Cela ne peut pas s'expliquer par 1) une capacité différente d'acquisition des nutriments liée aux symbiotes dinoflagellés (Baker et al., 2013 ; Ezzat et al., 2017), puisque les deux groupes partagent les mêmes génotypes et/ou densité de symbiotes ou 2) un besoin différent en azote structurel, puisque les coraux mous et durs présentent une teneur en azote similaire. Les coraux mous dépendent donc probablement d'une alimentation hétérotrophe pour satisfaire leurs besoins en azote.

Du fait de la faible capacité des coraux mous à assimiler l'azote dissous dans l'eau de mer, de faibles quantités de nutriments inorganiques pénètrent dans leurs tissus. Il est donc peu probable que les changements de la qualité de l'eau liés aux nutriments (e.g., eutrophisation) perturbent la symbiose des coraux mous, contrairement aux coraux durs. Bien que cette conclusion n'ait pas été testée expérimentalement dans le cadre de cette thèse et reste hypothétique, elle pourrait être illustrée par le modèle conceptuel des rapports défavorables des nutriments inorganiques dissous, introduit par Wiedenmann et al. (2012). En résumé, les coraux durs ont de fortes capacités d'assimilation d'azote dissous, ainsi de grandes quantités d'azote pénètrent au sein de leurs tissus lorsque l'azote est disponible en fortes concentrations dans l'eau de mer. Cela favorise une croissance rapide de la population de dinoflagellés, donc une demande cellulaire accrue, à laquelle ne peuvent subvenir de faibles concentrations en

phosphore ou autres nutriments essentiels. Un apport insuffisant en phosphore entraîne une modification de la composition lipidique des membranes des symbiotes (qui passent des phospholipides aux sulpholipides), ce qui les rend plus sensibles au blanchissement (D'Angelo et Wiedenmann, 2014). La théorie selon laquelle ce modèle ne serait applicable qu'aux coraux durs, et donc que la symbiose des coraux mous ne serait perturbée qu'en moindre mesure par une augmentation de nutriments, est soutenue par des observations de terrain : une dominance relative des coraux mous a été signalée dans les zones impactées par de fortes concentrations de matières dissoutes et particulaires (McClanahan et Obura, 1997, Fabricius et Dommisse, 2000 ; Schleyer et Celliers, 2003 ; Fabricius, 2005 ; Baum et al., 2016). De plus, d'autres propriétés physiologiques pourraient s'ajouter à leur faible capacité à assimiler l'azote dissous pour expliquer ce phénomène : 1) l'eutrophisation entraînant une plus forte turbidité de l'eau, une moindre dépendance des coraux mous sur leur symbiose avec leurs dinoflagellés pourrait les aider à faire face à une diminution de la quantité de lumière disponible pour la photosynthèse et 2) les particules en suspensions pourraient représenter une source potentielle de nutriments pour l'alimentation hétérotrophe.

3.3 Synthèse graphique

Les principaux résultats de cette thèse sont résumés dans la figure ci-après. La stabilité de la symbiose des octocoralliaires le long du gradient de profondeur est assurée par la maximisation de l'acquisition et de la conservation des nutriments aux deux profondeurs par autotrophie et hétérotrophie. Ceci est soutenu par une faible assimilation d'azote dissous, une acquisition stable de carbone inorganique dissous, une grande quantité de matière organique particulaire assimilée aux deux profondeurs et aucune assimilation de DDN. De plus, les mêmes quantités de carbone acquises par photosynthèse ont été transférées des symbiotes à l'hôte aux deux profondeurs. Par rapport aux coraux mous, les coraux scléractiniaires ont une assimilation plus élevée d'azote dissous (avec des taux d'assimilation d'azote organique dissous plus élevés en profondeur), une acquisition de carbone inorganique dissous plus élevée dans les eaux peu profondes mais similaire (voire plus faible) en profondeur, une assimilation significative de DDN et une stimulation de la fixation de diazote dans l'eau de mer environnante. Ils subissent également une perte de carbone plus importante en eaux peu profondes, mais plus faible que les coraux mous à la profondeur mésophotique. Les symbiotes des coraux scléractiniaires ont transféré des quantités de carbone beaucoup plus faibles avec la profondeur que ceux des coraux mous. La présence d'un squelette de carbonate de calcium chez les coraux durs joue

probablement un rôle important dans les différences de métabolisme (c'est-à-dire de besoins énergétiques) et de productivité mises en évidence ici. Enfin, les différences spécifiques aux espèces en matière d'acquisition et de répartition des nutriments peuvent être dues 1) au rapport entre la surface et le volume de la colonie (un faible rapport ne favorise pas l'exposition à la lumière et l'échange de gaz ou de nutriments à travers le tissu épidermique), 2) aux caractéristiques micro-morphologiques des polypes (les branches fines, les petits polypes et le rapport élevé entre la surface et le volume des polypes étant plus adaptés à l'autotrophie ; grands polypes pour l'hétérotrophie), 3) l'espèce de symbiote hébergée (influence l'assimilation et le transfert de carbone), 4) la densité des symbiotes (influence l'assimilation et le transfert du carbone), et 5) l'état nutritionnel (une efficacité hétérotrophe plus élevée étant corrélée à des taux plus élevés de carbone transféré).

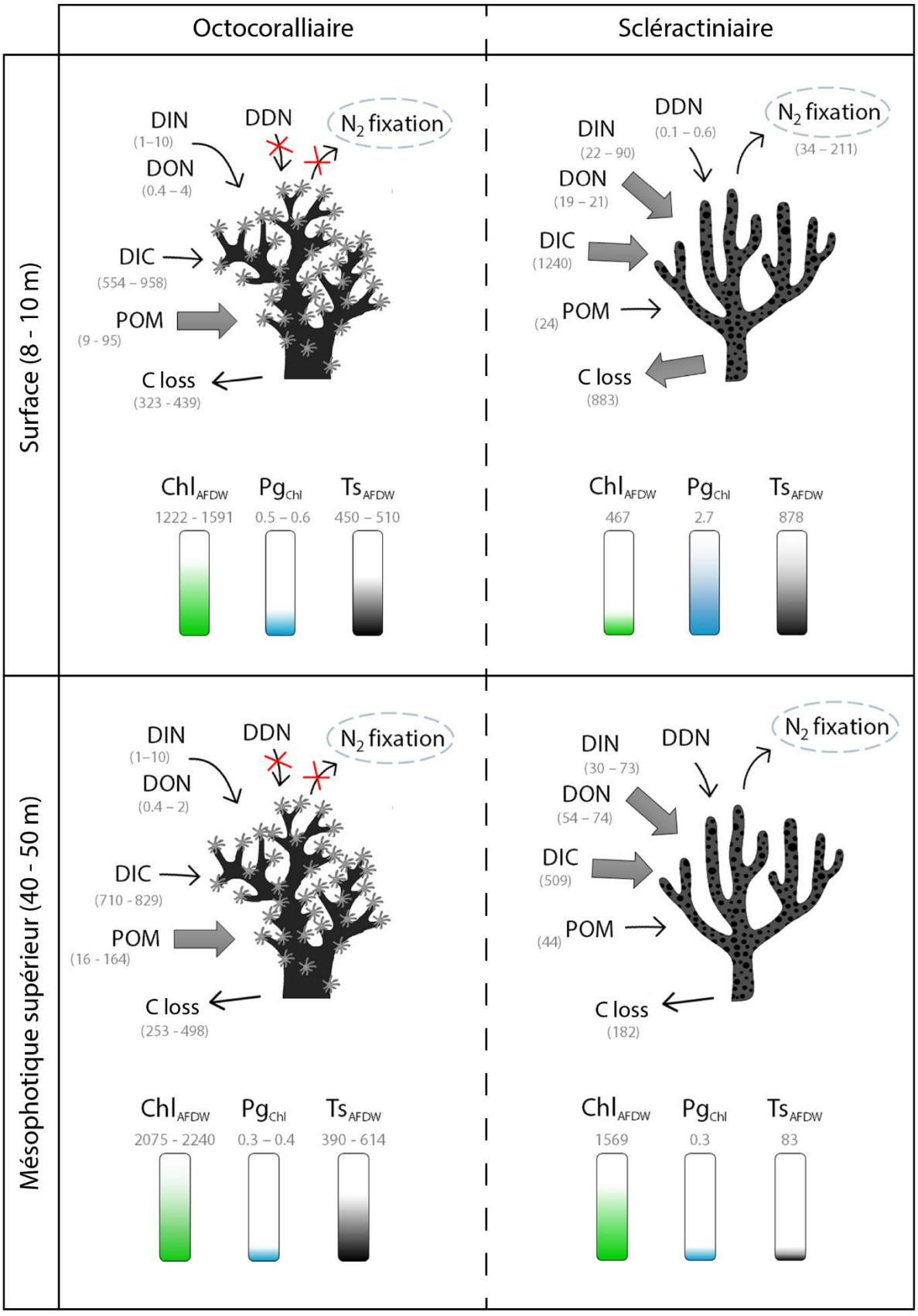


Figure récapitulative. Différence de fonctionnement des symbioses nutritionnelles du corail mou et du corail dur des zones récifales peu profondes à mésophotiques en mer Rouge. La gamme des taux correspondant à l'acquisition et aux flux de nutriments est indiquée sous l'acronyme des nutriments. Ces taux ne sont pas comparables entre eux mais uniquement entre les groupes

(coraux mous contre coraux durs) et les profondeurs (peu profond contre mésophotique). Chl = concentration en chlorophylles (µg Chl (*a* + *c2*) g^{-1} AFDW). Pg = Acquisition de carbone autotrophe (µg C $µg^{-1}$ Chl). Ts = Carbone transféré des symbiotes vers l'hôte (µg C g^{-1} AFDW h^{-1}). AFDW = poids sec sans cendres. DIN = Azote inorganique dissous (comprenant NH_4 et NO3, en µg N g^{-1} AFDW h^{-1}). DON = Azote organique dissous (comprenant les acides aminés libres dissous, en µg N g^{-1} AFDW h^{-1}). DFAA = Acides aminés libres dissous. DDN = Azote provenant des diazotrophes (ng N mg^{-1} DW h^{-1}). DW = Poids sec. Les taux de fixation de N_2 dans l'eau de mer sont exprimés en ng N L^{-1} d^{-1}. DIC = Carbone inorganique dissous (µg C g^{-1} AFDW h^{-1}). POM = Matière organique particulaire. L'assimilation de POM a été étudiée avec un biomarqueur lipidique spécifique du zooplancton (acide cis-gondoïque (CGA, 20:1ω9), en µg g^{-1} AFDW). Les biomarqueurs lipidiques spécifiques de l'autotrophie, l'acide stéaridonique (SDA, 18:4 ω3) et l'acide docosapentaénoïque (DPA, 22:5 ω3), n'apparaissent pas sur la figure. Les concentrations de SDA étaient de 1-70 et de 1-146 µg g^{-1}AFDW dans les coraux peu profonds et mésophotiques, respectivement, et ont diminué de 11 à 3 µg g^{-1} AFDW avec la profondeur dans le corail dur. Les concentrations de DPA étaient de 3-7 et 3-5 µg g^{-1} AFDW dans les coraux peu profonds et les coraux mésophotiques, respectivement, et diminuaient de 5 à 2 µg g^{-1} d'AFDW avec la profondeur dans le corail dur.

3.4 Conclusion et perspectives

Cette thèse souligne l'importance des apports autotrophes et hétérotrophes dans la nutrition des coraux mous, ce qui leur confère une plasticité trophique le long du gradient de profondeur. Ce double mode de nutrition représente certainement un atout pour la colonisation de différents habitats et peut leur fournir un mécanisme d'adaptation pour soutenir leur croissance en conditions stressantes. Les résultats obtenus dans chaque chapitre ont ouvert la voie à de futures expériences, qui permettraient une compréhension plus approfondie des processus observés ici à l'échelle de l'organisme. Quelques exemples sont donnés ci-dessous.

- Étudier l'environnement lumineux interne des coraux mous : le rôle des tissus et des sclérites

Les coraux scléractiniaires ont la capacité de réguler l'environnement lumineux perçu par leurs symbiotes à l'intérieur de leurs tissus. Par exemple, ils peuvent filtrer l'excès de lumière qui peut être nocif pour les algues ou améliorer la diffusion et la dispersion interne de la lumière grâce aux propriétés optiques uniques de leurs tissus et de leur squelette. Une telle amplification dans le champ lumineux local des symbiotes améliore notablement la photosynthèse. Bien que les coraux mous n'aient pas de squelette, ce qui pourrait expliquer leur taux d'acquisition et d'assimilation du carbone plus faible par rapport à ceux des coraux scléractiniaires, ils possèdent une grande quantité de sclérites. Les sclérites pourraient agir

comme diffuseurs de lumière. Leur taille, leur forme et leur couleur spécifiques à l'espèce pourraient notamment contribuer à expliquer les différences d'efficacité de la photosynthèse. Il serait donc intéressant d'étudier l'environnement lumineux interne des coraux mous, ainsi que le rôle possible des sclérites dans la diffusion de la lumière, en utilisant des microcapteurs de lumière. L'hydrosquelette des coraux mous peut cependant poser quelques problèmes techniques car il est susceptible de se contracter sur les microcapteurs.

- Importance de l'acquisition du phosphore pour la stabilité de la symbiose des coraux mous

Chez les coraux scléractiniaires, il a été démontré que le rapport azote/phosphore joue un rôle majeur dans la stabilité de la symbiose. Principalement, une augmentation de la disponibilité en azote (majoritairement sous forme de nitrate) sans une augmentation similaire de phosphore, peut augmenter la croissance de la population de dinoflagellés et par conséquent augmenter le blanchissement des coraux. Comme les coraux mous sont moins enclins à absorber l'azote inorganique dissous, il serait intéressant de tester 1) les niveaux de nitrate induisant une rupture de la symbiose et 2) le rôle de la disponibilité en phosphore pour le maintien de cette symbiose dans diverses conditions d'azote et/ou environnementales. Les résultats pourraient apporter des informations sur la manière dont les coraux mous font face à l'eutrophisation et au stress thermique.

- Étudier le stress oxydant chez les coraux mous

La présence de symbiotes chez les coraux est souvent bénéfique en raison de l'apport énergétique fourni par la photosynthèse. Toutefois, elle peut également imposer des restrictions (e.g., la distribution bathymétrique) et des risques (e.g., une augmentation plus importante des espèces réactives à l'oxygène libérées dans des conditions de stress par la photosynthèse). Au niveau cellulaire, la production d'espèces réactives d'oxygène et d'azote représente un facteur responsable du blanchissement des coraux. Puisque les résultats de cette thèse montrent différentes stratégies nutritionnelles affichées par les coraux mous et durs le long du gradient de profondeur (en particulier en ce qui concerne l'azote), une dépendance différente à l'autotrophie, ainsi qu'une flexibilité générale envers la symbiose, il pourrait être intéressant d'étudier les mécanismes pouvant conduire à une rupture de la symbiose chez les coraux mous, dans un contexte de stress thermique.

- Hétérotrophie

La nutrition hétérotrophe n'ayant été évaluée qu'à l'aide d'un biomarqueur lipidique spécifique au zooplancton dans cette thèse, des expériences impliquant des incubations avec une large gamme de proies marquées (picoplancton et nanoplancton) permettraient de mieux caractériser le régime alimentaire des coraux mous. En particulier, l'utilisation d'un marquage à l'aide d'isotopes stables confirmerait l'efficacité de l'ingestion des proies, puisque le mucus des coraux mous est connu pour avoir des propriétés antimicrobiennes et pourrait éventuellement affecter les concentrations de plancton mesurées dans les chambres.

- Contribution de l'autotrophie par rapport à l'hétérotrophie dans le budget nutritionnel des coraux mous

Enfin, cette thèse n'a pu fournir d'information sur la contribution relative de l'apport en nutriments autotrophes et hétérotrophes dans les besoins métaboliques de l'holobionte. Une autre expérience utilisant des biomarqueurs lipidiques spécifiques de l'autotrophie et de l'hétérotrophie pourrait être conçue à cette fin et devrait impliquer différentes situations nutritionnelles rencontrées par les mêmes colonies (e.g., un suivi saisonnier). Une autre possibilité serait d'utiliser une approche multi-biomarqueurs, en combinant les signatures d'isotopes stables avec les lipides totaux.

Appendices

Appendices

Appendix I – Supplementary material of publication n°1 (Chapter 2)

Table S1 Review of the literature: metrics used for normalizing soft coral data in physiological studies. AFDW = ash-free dry weight. DW = dry weight. DMSP = dimethylsulfopropionate. DOM = dissolved organic matter. The asterisk notifies a study for which the normalizing metric (i.e., dry weight of decalcified samples) has been considered as ash-free dry weight by the authors.

Normalizing metric	Family	Taxa	Measured parameter	Reference
AFDW	Xeniidae	*Asterospicularia* sp.	Oxygen flux	(Fabricius and Klumpp 1995)
	Briareidae	*Briareum stechei*	Oxygen flux	
	Nephtheidae	*Capnella lacertiliensis*	Oxygen flux	
	Nephtheidae	*Dendronephthya* spp.	Oxygen flux	
	Xeniidae	*Efflatounaria* sp.	Oxygen flux	
	Alcyoniidae	*Lobophytum* spp.	Oxygen flux	
	Nephtheidae	*Nephthea* sp.	Oxygen flux	
	Nephtheidae	*Paralemnalia clavata*	Oxygen flux	
	Nephtheidae	*Paralemnalia digitiformis*	Oxygen flux	
	Alcyoniidae	*Sarcophyton* spp.	Oxygen flux	
	Nephtheidae	*Scleronephthya* sp.	Oxygen flux	
	Alcyoniidae	*Sinularia* spp.	Oxygen flux	
	Xeniidae	*Xenia* spp.	Oxygen flux	
	Nephtheidae	*Scleronephthya corymbosa*	Oxygen flux	Fabricius et al. (1995b)
	Alcyoniidae	*Alcyonium digitatum*	Nutrient flux (ammonium)	Migné and Davoult (1997a)

	Alcyoniidae	*Alcyonium digitatum*	Oxygen flux	Migné and Davoult (1997b)
	Nephtheidae	*Dendronephthya hemprichi*	Feeding rates (phytoplankton)	Fabricius et al. (1998)
	Alcyoniidae	*Alcyonium digitatum*	Feeding rates (phyto- and zooplankton)	Migné and Davoult (2002)
	Alcyoniidae	*Sarcophyton trochellophorum*	DMSP concentration	Van Alstyne et al. (2006)
	Alcyoniidae	*Sinularia macropodia*	DMSP concentration	
	Alcyoniidae	*Sinularia maxima*	DMSP concentration	
	Alcyoniidae	*Sinularia pauli*	DMSP concentration	
	Alcyoniidae	*Sinularia polydactyla*	DMSP concentration	
	Alcyoniidae	*Sinularia maxima*	Biochemical composition of tissues (protein, carbohydrate and lipid content)	Slattery et al. (2013)
	Alcyoniidae	*Sinularia polydactyla*	Biochemical composition of tissues (protein, carbohydrate and lipid content)	
	Alcyoniidae	*Sinularia polydactyla* x *maxima* hybrid	Biochemical composition of tissues (protein, carbohydrate and lipid content)	
	Alcyoniidae	*Lobophytum patulum*	Oxygen flux and mucus production	Riegl and Branch (1995)*
	Alcyoniidae	*Lobophytum depressum*	Oxygen flux and mucus production	
	Alcyoniidae	*Sinularia dura*	Oxygen flux and mucus production	
	Alcyoniidae	*Sinularia leptoclados*	Oxygen flux and mucus production	
	Alcyoniidae	*Sarcophyton glaucum*	Oxygen flux and mucus production	
DW	Xeniidae	*Heteroxenia fuscescens*	Biochemical composition of tissues (protein content)	Schlichter et al (1983)
	Xeniidae	*Heteroxenia fuscescens*	Inorganic carbon	
	Xeniidae	*Heteroxenia fuscescens*	Photosynthetic and accessory pigments	
	Xeniidae	*Heteroxenia fuscescens*	Inorganic carbon	Schlichter et al (1984)
	Nephtheidae	*Capnella gaboensis*	Inorganic carbon	Farrant et al. (1987)
	Nephtheidae	*Capnella gaboensis*	Oxygen flux	

	Nephtheidae	*Capnella gaboensis*	Photosynthetic and accessory pigments (chlorophyll content)	
	Nephtheidae	*Capnella gaboensis*	*Symbiodinium* density	
	Alcyoniidae	*Alcyonium paessleri*	Biochemical composition of tissues (carbohydrates, lipids, protein, refractory, ash)	Slattery and McClintock (1995)
	Clavulariidae	*Clavularia frankliniana*	Biochemical composition of tissues (carbohydrates, lipids, protein, refractory, ash)	
	Nephtheidae	*Gersemia antartica*	Biochemical composition of tissues (carbohydrates, lipids, protein, refractory, ash)	
	Xeniidae	*Xenia umbellata*	Caloric content	Ben-David-Zaslow and Benayahu (1998)
	Alcyoniidae	*Rhytisma fulvum*	Caloric content	
	Xeniidae	*Heteroxenia fuscescens*	Biochemical composition of tissues (carbohydrates, lipids, protein, ash)	Ben-David-Zaslow and Benayahu (1999)
	Alcyoniidae	*Sinularia flexibilis*	*Symbiodinium* density (DW)	Khalesi et al. (2007)
	Alcyoniidae	*Sinularia flexibilis*	Metabolite concentration (flexibilide)	Khalesi et al. (2009)
	Xeniidae	*Xenia*	Photosynthetic and accessory pigments (chlorophyll *a* content)	Bednarz et al. (2012)
	Alcyoniidae	*Sinularia flexibilis*	Photosynthetic and accessory pigments	Rocha et al. (2013a)
	Alcyoniidae	*Sinularia flexibilis*	*Symbiodinium* density	
	Alcyoniidae	*Sarcophyton* cf. *glaucum*	*Symbiodinium* density	Rocha et al. (2013b)
	Alcyoniidae	*Sinularia flexibilis*	Photosynthetic and accessory pigments (chlorophyll *a* content)	Leal et al. (2014)
	Alcyoniidae	*Sarcophyton* cf. *glaucum*	Photosynthetic and accessory pigments (chlorophyll *a* content)	
	Alcyoniidae	*Sarcophyton* cf. *glaucum*	Photosynthetic and accessory pigments	Costa et al. (2016)

	Alcyoniidae	*Sarcophyton* cf. *glaucum*	*Symbiodinium* density	
Sclerites DW	Alcyoniidae	*Cladellia* sp.	Calcification rate	Tentori and Allemand (2006)
Wet weight	Xeniidae	*Heteroxenia fuscescens*	DOM flux	Schlichter (1982)
	Xeniidae	*Heteroxenia fuscescens*	Inorganic carbon	
	Xeniidae	*Heteroxenia fuscescens*	Amino acids and glucose	
	Nephtheidae	*Capnella gaboensis*	Inorganic carbon	Farrant et al. (1987)
	Nephtheidae	*Capnella gaboensis*	Oxygen flux	
	Nephtheidae	*Capnella gaboensis*	Photosynthetic and accessory pigments (chlorophyll content)	
	Nephtheidae	*Capnella gaboensis*	*Symbiodinium* density	
	Alcyoniidae	*Lobophytum compactum*	*Symbiodinium* density	Michalek-Wagner and Willis (2001)
	Nephtheidae	*Litophyton arboreum*	Biochemical composition of tissues (protein content)	Tentori et al (2004)
	Nephtheidae	*Litophyton arboreum*	Calcification rate	
	Nephtheidae	*Litophyton arboreum*	Cell growth	
	Nephtheidae	*Litophyton arboreum*	Biochemical composition of tissues (protein content)	
	Alcyoniidae	*Cladellia* sp.	Calcification rate	Tentori and Allemand (2006)
	Alcyoniidae	*Sarcophyton trocheliophorum*	DMSP concentration	Van Alstyne et al. (2006)
	Alcyoniidae	*Sinularia maxima*	DMSP concentration	
	Alcyoniidae	*Sinularia pauli*	DMSP concentration	
	Alcyoniidae	*Sinualria polydactyla*	DMSP concentration	
	Alcyoniidae	*Sinularia macropodia*	DMSP concentration	
	Alcyoniidae	*Sinularia flexibilis*	Biochemical composition of tissues (protein content)	Khalesi et al. (2007)

	Alcyoniidae	*Sinularia flexibilis*	Photosynthetic and accessory pigments (chlorophyll *a* content)	
	Alcyoniidae	*Sinularia flexibilis*	*Symbiodinium* density	
	Alcyoniidae	*Sinularia* sp.	Biochemical composition of tissues (lipids and fatty acids)	Imbs et al. (2010)
	Nephtheidae	*Nephthea* spp.	Electron transport system activity	Baum et al. (2016)
	Alcyoniidae	*Sarcophyton* spp.	Electron transport system activity	
	Alcyoniidae	*Sinularia* sp.	DMSP concentration	Haydon et al. (2018)
	Alcyoniidae	*Sarcophyton* sp.	DMSP concentration	
	Nephtheidae	*Capnella gaboensis*	DMSP concentration	
	Anthothelidae	*Erythropodium hicksoni*	DMSP concentration	
Buoyant weight	Alcyoniidae	*Sinularia flexibilis*	Budding rate	Khalesi et al. (2009)
	Alcyoniidae	*Sinularia flexibilis*	Energy expenditure	Khalesi et al. (2011)
	Alcyoniidae	*Sinularia flexibilis*	Oxygen flux	
Colony	Xeniidae	*Heteroxenia fuscescens*	Oxygen flux	Kremien et al. (2013)
Polyp number	Nephtheidae	*Dendronephthya hemprichi*	Photosynthetic and accessory pigments (chlorophyll *a*, phaeopigments and total photopigments)	Fabricius et al. (1995a)
	Nephtheidae	*Dendronephthya sinaiensis*	Photosynthetic and accessory pigments (chlorophyll *a*, phaeopigments and total photopigments)	Fabricius et al. (1995b)
	Nephtheidae	*Dendronephthya hemprichi*	Feeding rates (phytoplankton)	
	Nephtheidae	*Dendronephthya sinaiensis*	Feeding rates (phytoplankton)	
	Nephtheidae	*Scleronephthya corymbosa*	Feeding rates (phytoplankton)	
	Nephtheidae	*Dendronephthya hemprichi*	Feeding rates (phytoplankton)	
	Nephtheidae	*Dendronephthya hemprichi*	Feeding rates (phytoplankton)	Fabricius et al. (1998)

	Nephtheidae	*Dendronephthya hemprichi*	Oxygen flux	
Protein	Nephtheidae	*Litophyton arboreum*	Calcification rate	Tentori et al (2004)
	Nephtheidae	*Litophyton arboreum*	Cell growth	
	Alcyoniidae	*Cladellia* sp.	Calcification rate	Tentori and Allemand (2006)
	Xeniidae	*Heteroxenia fuscescens*	Metabolite concentration (palythine)	Zeevi Ben-Yosef et al. (2006)
	Alcyoniidae	*Sinularia flexibilis*	Photosynthetic and accessory pigments (chlorophyll *a* content)	Khalesi et al. (2009)
	Alcyoniidae	*Sinularia flexibilis*	*Symbiodinium* density	
	Xeniidae	*Ovabunda macrospiculata*	*Symbiodinium* density	Gabay et al. (2013)
	Xeniidae	*Heteroxenia fuscescens*	Oxygen flux	Ezzat et al. (2016)
	Xeniidae	*Heteroxenia fuscescens*	Photosynthetic and accessory pigments (total chlorophyll content)	
	Xeniidae	*Heteroxenia fuscescens*	*Symbiodinium* density	
	Xeniidae	*Heteroxenia fuscescens*	Uptakes of nutrients (ammonium, nitrate and phosphorus)	
	Alcyoniidae	*Sinularia* sp.	DMSP concentration	Haydon et al. (2018)
	Alcyoniidae	*Sarcophyton* sp.	DMSP concentration	
	Nephtheidae	*Capnella gaboensis*	DMSP concentration	
	Anthothelidae	*Erythropodium hicksoni*	DMSP concentration	
Surface area	Alcyoniidae	*Alcyonium fulvum*	*Symbiodinium* density	Drew (1972)
	Alcyoniidae	*Sarcophyton*	*Symbiodinium* density	
	Nephtheidae	*Capnella gaboensis*	Inorganic carbon	Farrant et al. (1987)
	Nephtheidae	*Capnella gaboensis*	Oxygen flux	
	Nephtheidae	*Capnella gaboensis*	Photosynthetic and accessory pigments (chlorophyll content)	
	Nephtheidae	*Capnella gaboensis*	*Symbiodinium* density	
	Xeniidae	*Xenia*	OM flux	Bednarz et al. (2012)

	Xeniidae	*Xenia*	Oxygen flux	
	Xeniidae	*Heteroxenia fuscescens*	Oxygen flux	Kremien et al. (2013)
	Nephtheidae	*Sarcophyton*	Dinitrogen fixation	Bednarz et al. (2015)
	Xeniidae	*Xeniidae*	Dinitrogen fixation	
	Alcyoniidae	*Sarcophyton*	Oxygen flux	
	Xeniidae	*Xeniidae*	Oxygen flux	
	Nephtheidae	*Sarcophyton*	Dinitrogen fixation	Cardini et al. (2016)
	Xeniidae	*Xeniidae*	Dinitrogen fixation	
	Alcyoniidae	*Sarcophyton*	Oxygen flux	
	Xeniidae	*Xeniidae*	Oxygen flux	
Symbiodinium cell	Alcyoniidae	*Sarcophyton trochellophorum*	DMSP concentration	Van Alstyne et al. (2006)
	Alcyoniidae	*Sinularia maxima*	DMSP concentration	
	Alcyoniidae	*Sinularia maxima* x *polydactyla* hybrid	DMSP concentration	
	Alcyoniidae	*Sinularia pauli*	DMSP concentration	
	Alcyoniidae	*Sinualria polydactyla*	DMSP concentration	
	Alcyoniidae	*Sinularia macropodia*	DMSP concentration	
	Alcyoniidae	*Sarcophyton* cf. *glaucum*	Photosynthetic and accessory pigments	Rocha et al. (2013b)
	Alcyoniidae	*Sinularia flexibilis*	Photosynthetic and accessory pigments	Rocha et al. (2013a)
	Xeniidae	*Heteroxenia*	Photosynthetic and accessory pigments (chlorophyll *a* content)	Gabay et al. (2013)
	Alcyoniidae	*Sarcophyton* sp.	Photosynthetic and accessory pigments (chlorophyll *a* content)	

Appendix II – Supplementary material of publication n°3 (Chapter 3)

Material and Methods

Physiological and tissue descriptor measurements

For the determination of the symbiont density, total chlorophyll concentration and ash-free dry weight (AFDW) from the experimental nubbins, we followed the protocol of Pupier et al.(Pupier et al. 2018). Briefly, freeze-dried samples were homogenized in a Potter tissue grinder. A subsample was weighed, and then combusted at 450°C for 4 h in a muffle furnace (Thermolyne 62700, Thermo Fischer Scientific, the United States). AFDW was determined as the difference between the DW and ash weight (AW) of the subsample and extrapolated to the total weight of the nubbin. The remaining sample was also weighed, and then homogenized with 10 mL distilled water (DI). The homogenate was centrifuged for 10 min (at 11,000 g at 4°C) to separate the animal host (supernatant) from the symbionts (pellet). Light microscopy confirmed the total removal of symbionts from the supernatant. The symbiont pellet was rinsed twice to eliminate any remaining host cells(Tremblay et al. 2012), and re-suspended in 10 mL DI. After mixing, 500 µL and 2.5 mL were subsampled for the determination of symbiont density and total chlorophyll concentration, respectively. Symbiont density was quantified microscopically via eight replicate haemocytometer counts (Neubauer-improved haemocytometer, Marienfeld, Germany). For chlorophyll analysis, the 2.5 mL subsamples were centrifuged for 10 min (at 8,000 g at 4°C) and the supernatant was discarded. Pellets were re-suspended into 4 mL of acetone (100%) amended with magnesium chloride (Sigma Aldrich, Germany) in order to extract the chlorophyll during 24h in the dark at 4°C. Chlorophyll a and c_2 concentrations were determined following the method of Jeffrey & Humphrey(Jeffrey and Humphrey 1975) by the use of a spectrophotometer (SAFAS, Monaco). Data were normalized to the total AFDW of the nubbin.

$NaH^{13}CO_3$ incubations

Carbon incorporation rates in the symbionts (ρ_S) and coral host tissue (ρ_H) were calculated as follow:

$$\rho = \frac{(C_{meas} - C_{nat}) * M_{sample} * M_c}{(C_{inc} - C_{meas}) * (t_{pulse} + t_{chase}) * AFDW}$$

where C_{meas} and C_{nat} are the percentages of ^{13}C measured on the samples (symbionts or host tissues) from ^{13}C-enriched experiments and controls, respectively. C_{inc} is the percent ^{13}C enrichment of the incubation medium; M_{sample} is the mass of the freeze-dried sample (mg); M_c is the mass of carbon per milligram of symbiont or host tissue (µg mg^{-1}); AFDW is the ash-free dry weight (g); and t_{pulse} and t_{chase} are incubation times (h) of the nubbins in the enriched and non-enriched incubation media, respectively. C_{inc} varied during the pulse chase and was calculated as:

$$C_{inc} = \frac{(C_{pulse} * t_{pulse}) + (C_{chase} * t_{chase})}{(t_{pulse} + t_{chase})}$$

where C_{pulse} and C_{chase} are the percent ^{13}C enrichment of the enriched and non-enriched incubation media, respectively (C_{chase}=1.1%).

The amount of carbon acquired through photosynthesis, translocated from the symbionts to the host (T_S) was calculated as:

$$T_S = P_C - \rho_S - R_S$$

where P_C is the total amount of autotrophic carbon produced by the symbionts; ρ_s is the carbon assimilation rate and R_S the respiration rate of the symbionts.

Finally, the amount of carbon lost (C_L) through respiration (R_C) and through particulate and dissolved carbon release ($\rho_{POC/DOC}$) is:

$$C_L = P_C - \rho_S - \rho_H$$

Table S2: Statistical results (two-way ANOVA) about the effect of species and depth on tissue descriptors (Symbiodiniaceae density and total chlorophyll (a+c_2) concentrations, *n* = 9 to 10 per condition) and carbon budgets in *Litophyton* sp. and *Rhystima fulvum fulvum* from 8 m and 40 m depth (*n* = 3 to 5 per condition). AFDW = ash-free dry weight. P_C = autotrophic carbon acquisition. Rc = respired carbon. Pulse and chase refer to the periods of time to trace the fate of inorganic carbon assimilated by host (ρ_H) and symbionts (ρ_S). T_S = photosynthates translocation. C_L = carbon lost as respiration of the holobiont and organic matter release.

		Df	F-value	p-value
TISSUE DESCRIPTORS				
Symbiodiniaceae density				
	Species	1	31.230	< **0.01**
	Depth	1	9.783	< **0.01**
	Species:Depth	1	0.996	0.32518
	Residuals	35		
Chlorophyll concentration (µg g^{-1} AFDW)				
	Species	1	2.326	0.135965
	Depth	1	18.395	< **0.01**
	Species:Depth	1	0.341	0.563096
	Residuals	36		
Chlorophyll concentration (µg symbiont $cell^{-1}$)				
	Species	1	27.996	< **0.01**
	Depth	1	10.260	< **0.01**
	Species:Depth	1	2.188	0.14806
	Residuals	35		
Pc (µg C g^{-1} AFDW h^{-1})				
	Species	1	9.619	< **0.01**
	Depth	1	0.004	0.9524
	Species:Depth	1	2.654	0.1241
	Residuals	15		
Pc (µg C symbiont $cell^{-1}$)				
	Species	1	3.231	0.0938
	Depth	1	6.124	**0.0267**
	Species:Depth	1	0.978	0.3395
	Residuals	14		
Rc (µg C g^{-1} AFDW h^{-1})				
	Species	1	13.504	< **0.01**
	Depth	1	3.151	0.10352
	Species:Depth	1	23.537	< **0.01**
	Residuals	11		
PULSE				
ρ_H (µg C g^{-1} AFDW h^{-1})				
	Species	1	0.020	0.888
	Depth	1	0.177	0.680
	Depth:Species	1	0.008	0.931

	Residuals	15		
ρ_S (µg C g^{-1} AFDW h^{-1})				
	Species	1	27.360	< **0.01**
	Depth	1	1.285	0.274752
	Depth:Species	1	0.623	0.442087
	Residuals	15		
T_S (µg C g^{-1} AFDW h^{-1})				
	Species	1	15.25	< **0.01**
	Depth	1	7.95	**0.01294**
	Depth:Species	1	52.82	< **0.01**
	Residuals	15		
C_L (µg C g^{-1} AFDW h^{-1})				
	Species	1	78.426	< **0.01**
	Depth	1	6.758	**0.0201**
	Depth:Species	1	14.945	< **0.01**
	Residuals	15		
CHASE				
ρ_H (µg C g^{-1} AFDW h^{-1})				
	Species	1	0.908	0.357
	Depth	1	1.309	0.272
	Depth:Species	1	0.515	0.485
	Residuals	14		
ρ_S (µg C g^{-1} AFDW h^{-1})				
	Species	1	51.692	< **0.01**
	Depth	1	0.052	0.823
	Depth:Species	1	1.147	0.302
	Residuals	14		
T_S (µg C g^{-1} AFDW h^{-1})				
	Species	1	5.850	**0.0298**
	Depth	1	0.049	0.8277
	Depth:Species	1	1.935	0.1859
	Residuals	14		
C_L (µg C g^{-1} AFDW h^{-1})				
	Species	1	6.785	**0.0208**
	Depth	1	0.033	0.8584
	Depth:Species	1	0.866	0.3678
	Residuals	14		

Table S3 Atom% ^{13}C of studied corals (Host) and their Symbiodiniaceae (Symbionts) after a 5h pulse of $H^{13}CO_3^-$ and a 19h chase in filtered seawater. Data represent the mean ± standard error of five replicates collected from the shallow (8 m depth) and mesophotic (40 m depth) reef in Eilat, Israel. Nat. ab. = Natural abundance; reflects the natural abundance isotope composition prior to tracer incubation.

		Host			Symbionts		
		Nat. ab. (%)	Pulse (%)	Chase (%)	Nat. ab (%)	Pulse (%)	Chase (%)
Litophyton sp.	Shallow	1.0816 ± 0.0003	1.2272 ± 0.0205	1.2216 ± 0.0198	1.0844 ± 0.0002	1.2645 ± 0.0346	1.2986 ± 0.0325
	Mesophotic	1.0803 ± 0.0003	1.2449 ± 0.0175	1.2348 ± 0.0094	1.0823 ± 0.0005	1.3123 ± 0.0287	1.3021 ± 0.0279
Rhytisma fulvum fulvum	Shallow	1.0835 ± 0.0003	1.1730 ± 0.0065	1.1734 ± 0.0132	1.0904 ± 0.0008	1.1245 ± 0.0055	1.1026 ± 0.0029
	Mesophotic	1.0830 ± 0.0001	1.1617 ± 0.0142	1.1532 ± 0.0092	1.0957 ± 0.0018	1.1324 ± 0.0025	1.1242 ± 0.0066

Table S4: Total chlorophyll and autotrophic carbon acquisition of *Stylophora pistillata* along the depth gradient. (a) Parameters normalized to surface area of the skeleton(Ezzat et al. 2017). (b) Parameters normalized to ash-free dry weight. The values of this table represent estimates calculated from A using a conversion coefficient (255.65 ± 8.98) determined in this study. P_C = autotrophic carbon acquisition.

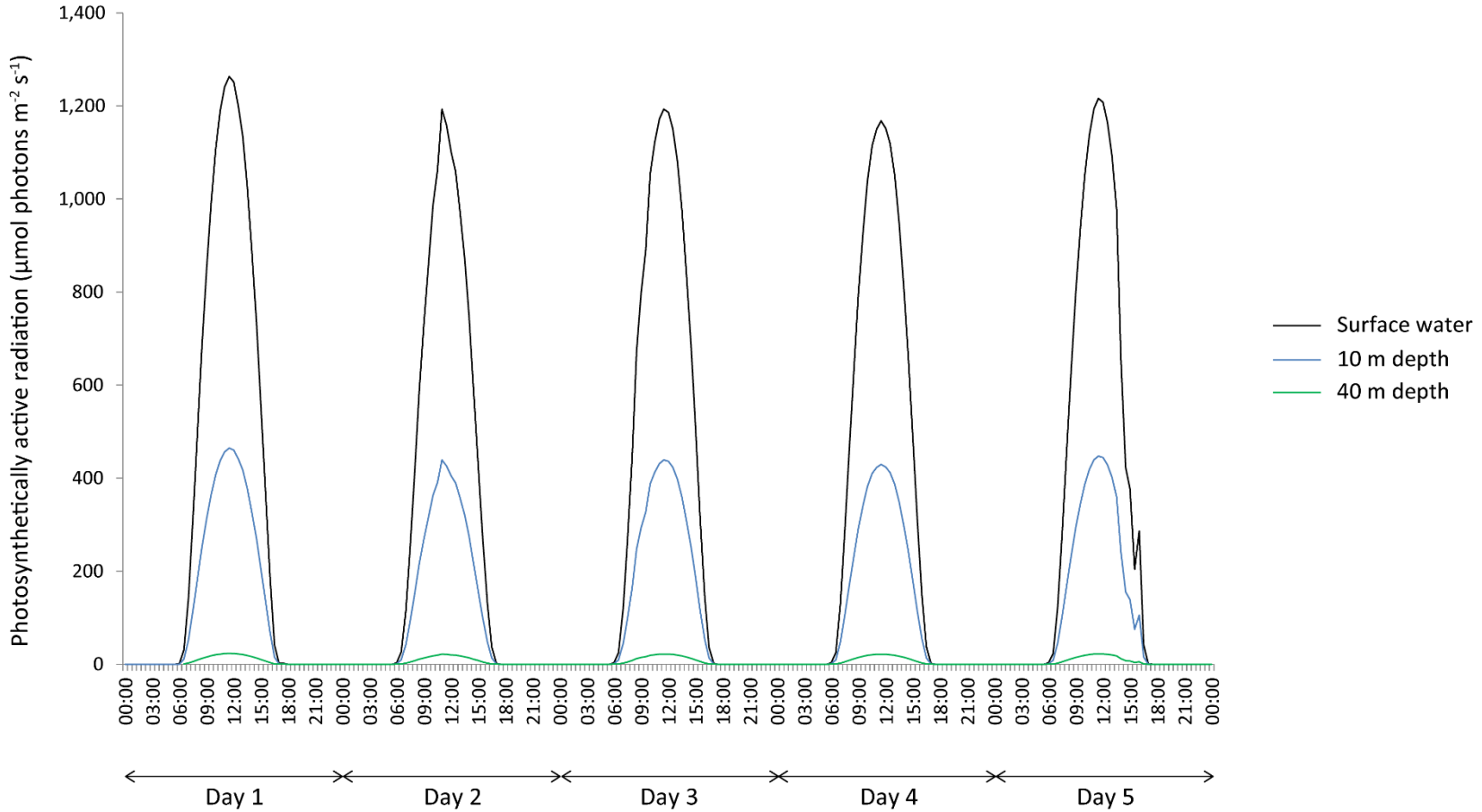
a	Total chlorophyll ($\mu g\ cm^{-2}$)	P_C ($\mu g\ C\ cm^{-2}\ h^{-1}$)
Depth (m)	November	November
5	3.61	9.49
50	6.87	2.17

b	Total chlorophyll ($\mu g\ g^{-1}$ AFDW)	P_C ($\mu g\ C\ g^{-1}$ AFDW h^{-1})
Depth (m)	November	November
5	923	2,426
50	1,756	555

Table S5: Coefficients estimated to convert data normalized to surface area of the skeleton into data normalized to ash-free dry weight (mean ± standard error = 255.65 ± 8.98). The nubbins (n = 8) were fragmented from *Stylophora pistillata* colonies grown at the Monaco Scientific Center. DW = dry weight. AFDW = ash-free dry weight. Calculated coefficients are the ratio between surface area of the skeleton and ash-free dry weight.

Nubbin	DW (g)	AFDW (g)	Surface area (cm^2)	**Coefficient**
1	0.0609	0.0488	11.921	**244.416**
2	0.0610	0.0460	10.461	**227.373**
3	0.0992	0.0836	19.183	**229.524**
4	0.0855	0.0688	16.154	**234.830**
5	0.1341	0.0984	28.983	**294.543**
6	0.1071	0.0892	24.614	**276.018**
7	0.1112	0.0991	25.748	**259.860**
8	0.0158	0.0146	4.065	**278.638**

Figure S1: Levels of photosynthetically active radiation (PAR) received at the seasurface, 10 m depth and 40 m depth in Eilat during the five days of our experiment. Surface data was provided by the Israel National Monitoring program of the Gulf of Eilat and levels along the depth gradient were calculated according to Beer-Lambert law, with an attenuation coefficient of 0.1 m^{-1} (Akkaynak et al. 2017; Tamir et al. 2019).



Appendix III - Supplementary material of publication n°5 (Chapter 4)

Table S6. Lambda of Box-Cox transformation ($x^{\lambda-1}/\lambda$) used for statistical tests.

Variable	Figure	Lambda
Symbiodiniaceae density	1	0.101010
C:N of the host	2A	-0.666666
N content of the host	2B	-0.181818
C:N of the symbionts	3A	-1.474747
N content of the symbionts	3B	-1.030303
DN assimilation rate in the host (per AFDW)	4	-0.060606
DN assimilation rate in the symbionts (per AFDW)	5	-0.060606
DN assimilation rate in the host (per AFDW)	6A	0.060606
DN assimilation rate in the symbionts (per AFDW)	6B	0.020202
Chlorophyll concentration (per AFDW)	S1A	0.262626
Chlorophyll concentration (per symbiont cell)	S1B	0.262626
DN assimilation rate in the host (per surface)	S2A	0.343434
DN assimilation rate in the symbionts (per surface)	S2B	0.141414

Table S7. Statistical differences estimated between sources in the host and Symbiodiniaceae fractions.

DN assimilation in the host fraction (per AFDW) – Figure 4						
Depth = Deep, Species = Galaxea:						
Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	2.8788	0.167	Inf	2.5517	3.206	a
Ammonium	3.1948	0.167	Inf	2.86767	3.522	a
DFAA	3.3961	0.167	Inf	3.06897	3.723	a
Depth = Deep, Species = Rhytisma:						
Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-0.0938	0.167	Inf	-0.42085	0.233	a
Nitrate	1.0336	0.167	Inf	0.70652	1.361	b
Ammonium	1.76	0.167	Inf	1.43296	2.087	c
Depth = Deep, Species = Sarcophyton:						

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-2.4887	0.167	Inf	-2.81578	-2.162	a
Nitrate	-0.8421	0.167	Inf	-1.16916	-0.515	b
Ammonium	-0.1498	0.167	Inf	-0.47686	0.177	c

Depth = Deep, Species = Seriatopora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	2.9323	0.167	Inf	2.60519	3.259	a
DFAA	3.3656	0.167	Inf	3.03852	3.693	a
Ammonium	3.4852	0.167	Inf	3.15813	3.812	a

Depth = Deep, Species = Stylophora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	2.6151	0.167	Inf	2.28801	2.942	a
Ammonium	3.2699	0.167	Inf	2.9428	3.597	b
DFAA	3.3586	0.167	Inf	3.03148	3.686	b

Depth = Shallow, Species = Galaxea:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	2.4936	0.167	Inf	2.16654	2.821	a
Nitrate	3.1077	0.167	Inf	2.78057	3.435	b
Ammonium	3.2491	0.167	Inf	2.92197	3.576	b

Depth = Shallow, Species = Litophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	-1.388	0.167	Inf	-1.7151	-1.061	a
DFAA	-0.1899	0.167	Inf	-0.51702	0.137	b
Ammonium	1.2232	0.187	Inf	0.85749	1.589	c

Depth = Shallow, Species = Rhytisma:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-0.5066	0.167	Inf	-0.83364	-0.179	a
Nitrate	0.3197	0.167	Inf	-0.00742	0.647	b
Ammonium	1.4321	0.187	Inf	1.06641	1.798	c

Depth = Shallow, Species = Sarcophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-2.3626	0.167	Inf	-2.68966	-2.035	a
Nitrate	-1.0863	0.167	Inf	-1.41334	-0.759	b
Ammonium	-0.015	0.187	Inf	-0.38067	0.351	c

Depth = Shallow, Species = Seriatopora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	2.0527	0.167	Inf	1.72558	2.38	a
Nitrate	2.2123	0.167	Inf	1.88516	2.539	a
Ammonium	3.4938	0.167	Inf	3.1667	3.821	b

Depth = Shallow, Species = Stylophora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	1.8189	0.167	Inf	1.49184	2.146	a
DFAA	2.2423	0.167	Inf	1.91523	2.569	a
Ammonium	3.3253	0.167	Inf	2.99819	3.652	b

DN assimilation in the Symbionidinaceae fraction – Figure 5

Depth = Deep, Species = Galaxea:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	1.6264	0.199	Inf	1.2367	2.016	a
Ammonium	1.8664	0.199	Inf	1.4766	2.256	a
DFAA	1.9423	0.222	Inf	1.5066	2.378	a

Depth = Deep, Species = Rhytisma:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	-0.1784	0.199	Inf	-0.5682	0.211	a
DFAA	0.0133	0.199	Inf	-0.3764	0.403	a
Ammonium	1.284	0.199	Inf	0.8943	1.674	b

Depth = Deep, Species = Sarcophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	-1.9264	0.199	Inf	-2.3161	-1.537	a
DFAA	-1.3339	0.199	Inf	-1.7236	-0.944	ab
Ammonium	-0.781	0.199	Inf	-1.1707	-0.391	b

Depth = Deep, Species = Seriatopora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	1.915	0.222	Inf	1.4793	2.351	a
Nitrate	2.6506	0.199	Inf	2.2609	3.04	b
Ammonium	2.8001	0.199	Inf	2.4104	3.19	b

Depth = Deep, Species = Stylophora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	2.2872	0.222	Inf	1.8515	2.723	a
DFAA	2.5943	0.199	Inf	2.2046	2.984	a
Ammonium	2.7448	0.199	Inf	2.3551	3.135	a

Depth = Shallow, Species = Galaxea:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	1.477	0.199	Inf	1.0873	1.867	a
Nitrate	2.2978	0.199	Inf	1.9081	2.688	b
Ammonium	2.3215	0.199	Inf	1.9318	2.711	b

Depth = Shallow, Species = Litophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	-1.3415	0.199	Inf	-1.7312	-0.952	a
DFAA	1.1549	0.199	Inf	0.7652	1.545	b
Ammonium	1.734	0.199	Inf	1.3443	2.124	b

Depth = Shallow, Species = Rhytisma:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-0.8722	0.199	Inf	-1.2619	-0.483	a
Nitrate	0.521	0.222	Inf	0.0853	0.957	b
Ammonium	0.8382	0.222	Inf	0.4025	1.274	b

Depth = Shallow, Species = Sarcophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-1.5362	0.199	Inf	-1.9259	-1.147	a
Nitrate	-1.2347	0.199	Inf	-1.6245	-0.845	a
Ammonium	-0.0254	0.199	Inf	-0.4151	0.364	b

Depth = Shallow, Species = Seriatopora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	2.1631	0.222	Inf	1.7274	2.599	a
Nitrate	2.4711	0.222	Inf	2.0353	2.907	ab
Ammonium	2.8849	0.199	Inf	2.4952	3.275	b

Depth = Shallow, Species = Stylophora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	1.8023	0.199	Inf	1.4126	2.192	a
Nitrate	2.5301	0.222	Inf	2.0944	2.966	b
Ammonium	3.1161	0.199	Inf	2.7264	3.506	b

DN assimilation in the host fraction (per AFDW) – Figure 6 Temperature

Temperature = 25, Species = Litophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	-1.35	0.157	Inf	-1.6575	-1.043	a
DFAA	-0.1886	0.157	Inf	-0.4961	0.119	b
Ammonium	1.2564	0.175	Inf	0.9125	1.6	c

Temperature = 25, Species = Rhytisma:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-0.4983	0.157	Inf	-0.8058	-0.191	a
Nitrate	0.3227	0.157	Inf	0.0152	0.63	b
Ammonium	1.4785	0.175	Inf	1.1347	1.822	c

Temperature = 25, Species = Sarcophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-2.2549	0.157	Inf	-2.5624	-1.947	a
Nitrate	-1.0623	0.157	Inf	-1.3698	-0.755	b
Ammonium	-0.0132	0.175	Inf	-0.357	0.331	c

Temperature = 30, Species = Litophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	-1.1274	0.157	Inf	-1.4349	-0.82	a

DFAA	0.0221	0.157	Inf	-0.2854	0.33	b
Ammonium	1.826	0.157	Inf	1.5185	2.134	c

Temperature = 30, Species = Rhytisma:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	0.1935	0.175	Inf	-0.1503	0.537	a
Nitrate	0.8811	0.175	Inf	0.5372	1.225	b
Ammonium	2.1178	0.157	Inf	1.8103	2.425	c

Temperature = 30, Species = Sarcophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-1.6453	0.157	Inf	-1.9528	-1.338	a
Nitrate	-1.3083	0.157	Inf	-1.6158	-1.001	a
Ammonium	-0.512	0.175	Inf	-0.8558	-0.168	b

DN assimilation in the Symbiodiniaceae fraction (per AFDW) – Figure 6 Temperature

Temperature = 25, Species = Litophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	-1.2491	0.236	Inf	-1.712	-0.786	a
DFAA	1.212	0.211	Inf	0.798	1.626	b
Ammonium	1.8732	0.211	Inf	1.459	2.287	b

Temperature = 25, Species = Rhytisma:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-0.834	0.211	Inf	-1.248	-0.42	a
Nitrate	0.5358	0.236	Inf	0.073	0.998	b
Ammonium	0.8691	0.236	Inf	0.406	1.332	b

Temperature = 25, Species = Rhytisma:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-1.4385	0.211	Inf	-1.852	-1.025	a
Nitrate	-1.0351	0.211	Inf	-1.449	-0.621	a
Ammonium	-0.0202	0.211	Inf	-0.434	0.394	b

Temperature = 30, Species = Litophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	-1.3204	0.211	Inf	-1.734	-0.907	a
DFAA	1.0603	0.211	Inf	0.646	1.474	b
Ammonium	1.6943	0.273	Inf	1.16	2.229	b

Temperature = 30, Species = Rhytisma:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	-0.1224	0.236	Inf	-0.585	0.34	a
Nitrate	1.324	0.211	Inf	0.91	1.738	b
Ammonium	1.7141	0.211	Inf	1.3	2.128	b

Temperature = 30, Species = Sarcophyton:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	-1.0584	0.211	Inf	-1.472	-0.645	a
DFAA	-0.7463	0.211	Inf	-1.16	-0.332	a
Ammonium	0.0184	0.211	Inf	-0.395	0.432	b

DN assimilation in the host fraction (per surface) – Figure S2

Depth = Shallow, Species = Galaxea:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	11.52	0.829	Inf	9.89	13.14	a
Nitrate	14.54	0.742	Inf	13.09	15.99	b
Ammonium	15.09	0.829	Inf	13.46	16.71	b

Depth = Shallow, Species = Seriatopora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	7.81	0.742	Inf	6.36	9.27	a
DFAA	9.61	0.829	Inf	7.99	11.24	a
Ammonium	19.77	0.742	Inf	18.32	21.23	b

Depth = Shallow, Species = Stylophora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	4.05	0.742	Inf	2.6	5.5	a
DFAA	5.82	0.742	Inf	4.37	7.28	a
Ammonium	11.23	0.742	Inf	9.78	12.68	b

Depth = Deep, Species = Galaxea:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	9.41	0.742	Inf	7.95	10.86	a
DFAA	9.84	0.742	Inf	8.39	11.29	a
Ammonium	12.82	0.742	Inf	11.37	14.28	b

Depth = Deep, Species = Seriatopora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	7.37	0.742	Inf	5.91	8.82	a
DFAA	13.75	0.742	Inf	12.3	15.21	b
Ammonium	16.3	0.829	Inf	14.67	17.92	b

Depth = Deep, Species = Stylophora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	5.57	0.829	Inf	3.94	7.2	a
DFAA	9.67	0.742	Inf	8.21	11.12	b
Ammonium	12.67	0.829	Inf	11.04	14.3	c

DN assimilation in the Symbiodiniaceae fraction (per surface) – Figure S2

Depth = Shallow, Species = Galaxea:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	3.2	0.292	Inf	2.63	3.77	a
Ammonium	3.37	0.292	Inf	2.8	3.94	a
Nitrate	3.8	0.292	Inf	3.23	4.38	a

Depth = Shallow, Species = Seriatopora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	3.96	0.292	Inf	3.39	4.53	a
Nitrate	4.21	0.326	Inf	3.57	4.85	ab
Ammonium	4.97	0.292	Inf	4.4	5.54	b

Depth = Shallow, Species = Stylophora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	2.48	0.292	Inf	1.91	3.05	a
Nitrate	3.07	0.292	Inf	2.5	3.64	a
Ammonium	4.27	0.292	Inf	3.7	4.84	b

Depth = Deep, Species = Galaxea:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	1.99	0.326	Inf	1.35	2.63	a
Nitrate	2.15	0.292	Inf	1.58	2.73	a
Ammonium	2.62	0.292	Inf	2.05	3.19	a

Depth = Deep, Species = Seriatopora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
DFAA	2.43	0.326	Inf	1.79	3.07	a
Nitrate	3.2	0.292	Inf	2.63	3.77	a
Ammonium	4.6	0.292	Inf	4.03	5.17	b

Depth = Deep, Species = Stylophora:

Source	emmean	SE	df	asymp.LCL	asymp.UCL	.group
Nitrate	2.78	0.326	Inf	2.14	3.42	a
DFAA	2.88	0.292	Inf	2.31	3.45	a
Ammonium	3.37	0.292	Inf	2.8	3.94	a

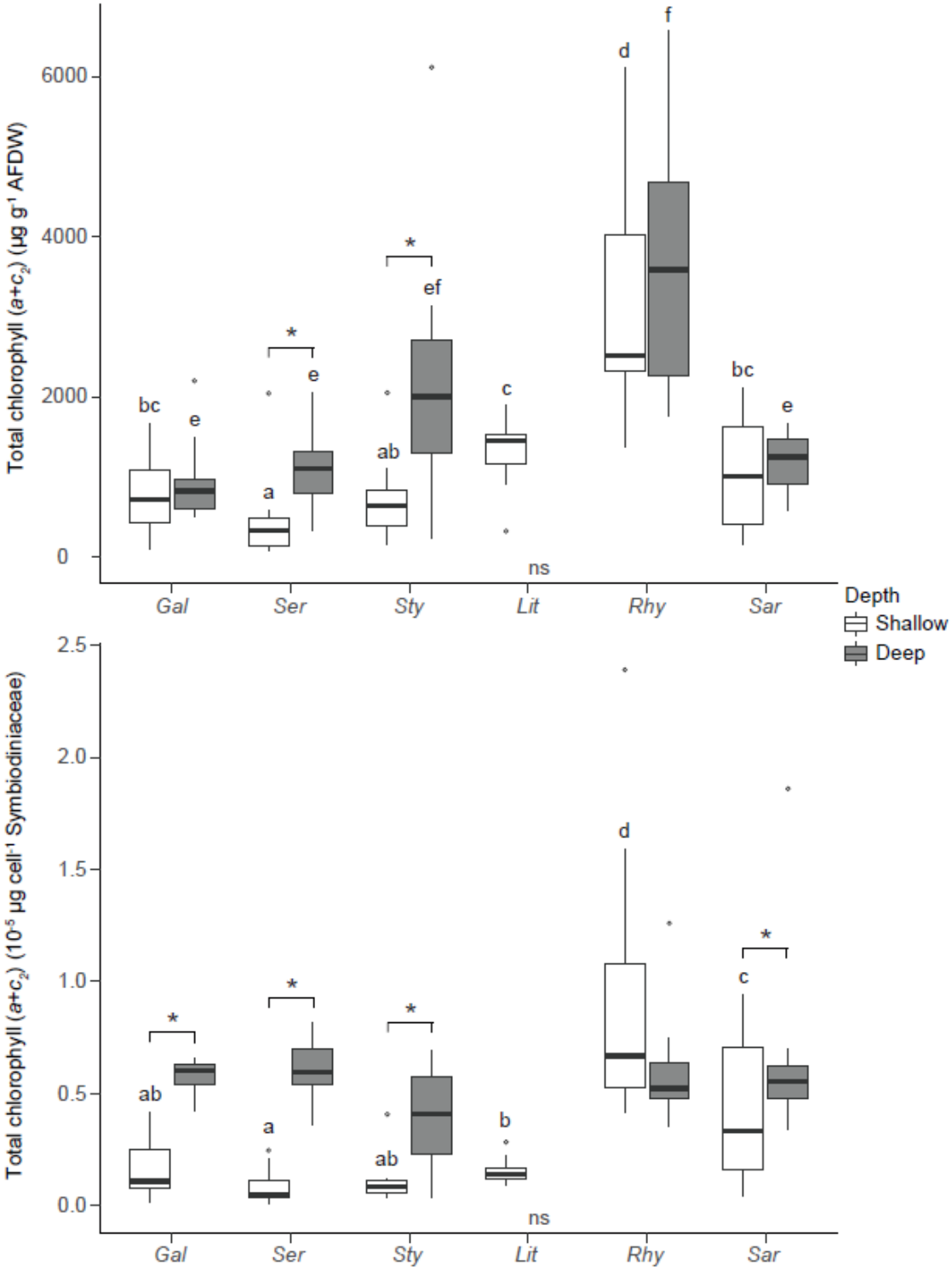


Figure S2. Total chlorophyll concentration in the species investigated. Normalized to biomass in the top panel (AFDW = ash-free dry weight) and normalized to Symbiodiniaceae cell in the bottom panel. The first three species (*Galaxea fascicularis*, *Seriatopora hystrix*, *Stylophora pistillata*) are hard corals and the last three (*Litophyton arboreum*, *Rhytisma fulvum fulvum*, *Sarcophyton* sp.) are soft corals. Significant differences between depths are displayed with an asterisk. Significant differences between species are distinguished with letters by depth. ns = not sampled.

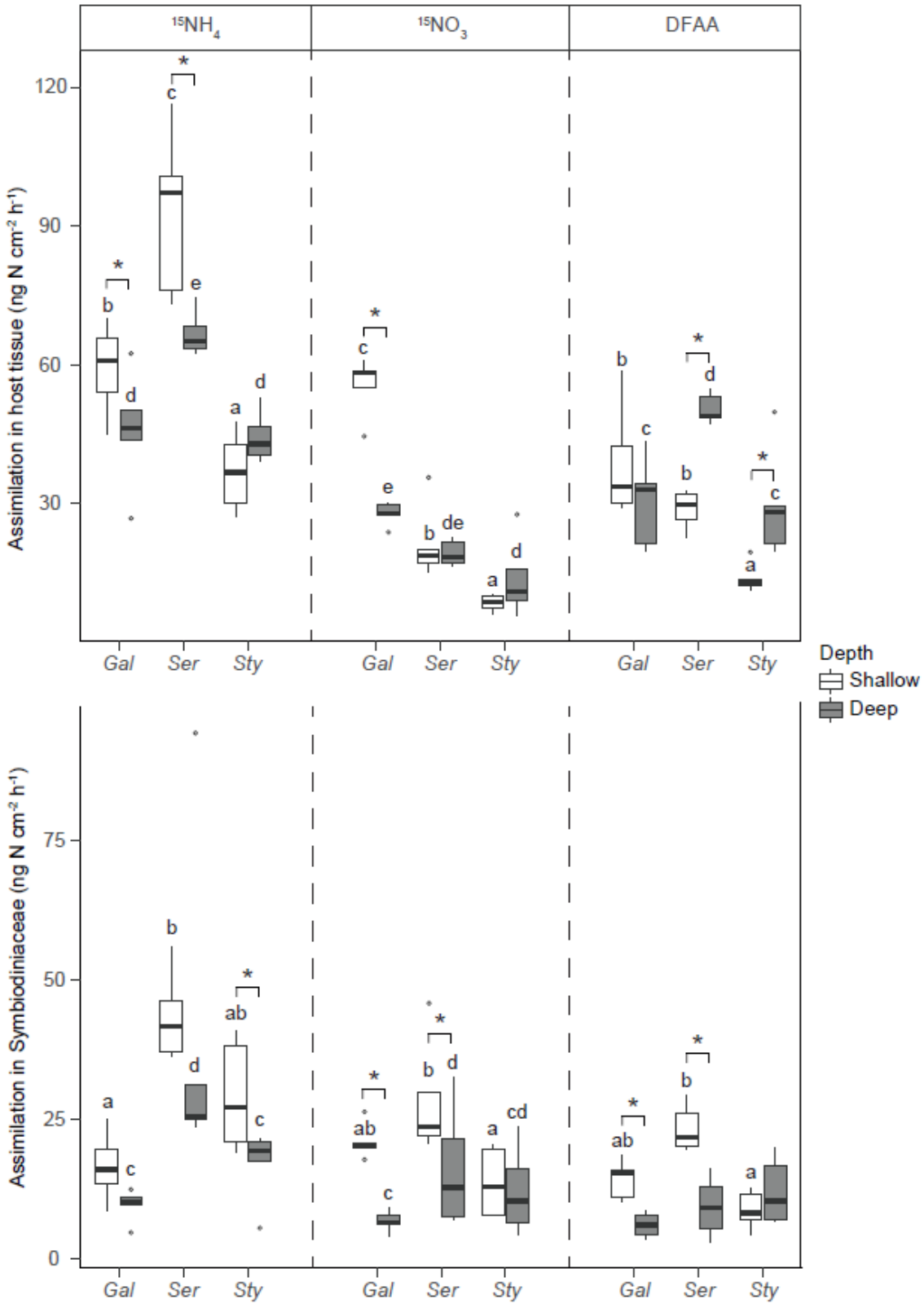


Figure S3. Assimilation rates of the three N sources in hard corals, normalized to surface area of the skeleton, in the host tissue (top panel) and in the Symbiodiniaceae fraction (bottom panel). Significant differences between depths are displayed with an asterisk. Significant differences between species are distinguished with letters by depth. Gal = *Galaxea fascicularis*. Ser = *Seriatopora hystrix*. Sty = *Stylophora pistillata*.

Appendix IV – Supplementary material of publication n°4 (Chapter 5)

Table S8. Coefficient used to convert dry weight (DW) of the nubbins into their ash-free dry weight (AFDW), used to normalize marker concentrations (AFDW = coefficient × DW). Asterisks denote samples containing seawater before being freeze-dried.

Samples collected in Eilat		
Species	Shallow (8-10 m)	Mesophotic (40-50 m)
Dendronephthya sp.	0.2479	0.2479
Litophyton arboreum	0.6145	0.6545
Rhytisma fulvum fulvum	0.2094	0.1254
Sarcophyton sp.	0.7029	0.7029
*Stylophora pistillata**	0.3068	0.3068
Samples grown in laboratory		
Stylophora pistillata isolated symbionts	0.8375	
Stylophora pistillata	0.4805	
Sarcophyton sp.	0.2836	
Mix of zooplankton*	0.2703	

Table S9. Biologically relevant pairwise comparisons based on estimated marginal means and simultaneous general linear hypothesis testing. Data are p-values adjusted using Benjamini-Hochberg procedure. Significant values ($p < 0.05$) are displayed in bold. Species are *Dendronephthya* sp., *Litophyton arboreum*, *Rhystima fulvum fulvum* and *Sarcophyton* sp. for the octocorals, and *Stylophora pistillata* for the scleractinian.

SDA	
Differences between depths (shallow – mesophotic)	
Litophyton	**0.0278**
Rhytisma	0.2454
Sarcophyton	0.4656
Stylophora	0.0536
Shallow corals: differences between species	
Litophyton - Rhytisma	**3.82E-09**
Litophyton - Sarcophyton	**3.87E-07**
Litophyton - Stylophora	0.491963
Rhytisma - Sarcophyton	**< 2.00E-16**
Rhytisma - Stylophora	**2.38E-08**
Sarcophyton - Stylophora	**0.000351**
Mesophotic corals: differences between species	
Litophyton - Rhytisma	**< 2.00E-16**
Litophyton - Sarcophyton	**0.000725**
Litophyton - Stylophora	0.428486
Rhytisma - Sarcophyton	**< 2.00E-16**
Rhytisma - Stylophora	**< 2.00E-16**
Sarcophyton - Stylophora	**0.009185**
DPA	
Differences between depths (shallow – mesophotic)	
Litophyton	0.79007
Sarcophyton	0.79007

Stylophora	**0.00132**
Shallow corals: differences between species	
Litophyton - Sarcophyton	0.1368
Litophyton - Stylophora	**0.0249**
Sarcophyton - Stylophora	0.3715
Mesophotic corals: differences between species	
Litophyton - Sarcophyton	0.1368
Litophyton - Stylophora	0.6981
Sarcophyton - Stylophora	0.0928
CGA	
Differences between depths (shallow – mesophotic)	
Dendronephthya	**0.000697**
Litophyton	0.30513
Rhytisma	**0.001709**
Sarcophyton	0.222839
Stylophora	0.069579
Shallow corals: differences between species	
Dendronephthya - Litophyton	0.424288
Dendronephthya - Rhytisma	**2.21E-05**
Dendronephthya - Sarcophyton	**5.12E-05**
Dendronephthya - Stylophora	0.120509
Litophyton - Rhytisma	**0.000536**
Litophyton - Sarcophyton	**1.37E-06**
Litophyton - Stylophora	**0.025377**
Rhytisma - Sarcophyton	**< 2.00E-16**
Rhytisma - Stylophora	**4.08E-08**
Sarcophyton - Stylophora	**0.027373**
Mesophotic corals: differences between species	
Dendronephthya - Litophyton	**0.040002**
Dendronephthya - Rhytisma	**0.000894**

Dendronephthya - Sarcophyton	**4.45E-11**
Dendronephthya - Stylophora	**0.001042**
Litophyton - Rhytisma	**2.69E-08**
Litophyton - Sarcophyton	**1.37E-06**
Litophyton - Stylophora	0.183275
Rhytisma - Sarcophyton	**< 2.00E-16**
Rhytisma - Stylophora	**1.14E-11**
Sarcophyton - Stylophora	**0.000569**

Appendix V – Other publication

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Flow-driven micro-scale pH variability affects the physiology of corals and coralline algae under ocean acidification

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Natural variability in pH in the diffusive boundary layer (DBL), the discrete layer of seawater between bulk seawater and the outer surface of organisms, could be an important factor determining the response of corals and coralline algae to ocean acidification (OA). Here, two corals with different morphologies and one coralline alga were maintained under two different regimes of flow velocities, pH, and light intensities in a 12 flumes experimental system for a period of 27 weeks. We used a combination of geochemical proxies, physiological and micro-probe measurements to assess how these treatments affected the conditions in the DBL and the response of organisms to OA. Overall, low flow velocity did not ameliorate the negative effect of low pH and therefore did not provide a refugia from OA. Flow velocity had species-specific effects with positive effects on calcification for two species. pH in the calcifying fluid (pH_{cf}) was reduced by low flow in both corals at low light only. pH_{cf} was significantly impacted by pH in the DBL for the two species capable of significantly modifying pH in the DBL. The dissolved inorganic carbon in the calcifying fluid (DIC_{cf}) was highest under low pH for the corals and low flow for the coralline, while the saturation state in the calcifying fluid and its proxy (FWHM) were generally not affected by the treatments. This study therefore demonstrates that the effects of OA will manifest most severely in a combination of lower light and lower flow habitats for sub-tropical coralline algae. These effects will also be greatest in lower flow habitats for some corals. Together with existing literature, these findings reinforce that the effects of OA are highly context dependent, and will differ greatly between habitats, and depending on species composition.

Ocean acidification (OA) is a major threat to many marine calcifying species, acting to reduce the calcification of ecologically important species such as reef-forming corals and coralline algae[1]. However, there is evidence that responses to OA are context-dependent, with interactions across species and the environment mediating the strength of responses[2,3]. One potentially important and still poorly studied controller of the direction and magnitude of responses is the seawater velocity that resident organisms are exposed to[4,5]. This is expected to be most important at the discrete boundary layer between marine organisms and ambient seawater, where flow is reduced to the point such that the movement of dissolved substances between the bulk seawater and the surface of the organism is dominated by molecular diffusion[6]. This layer, known as the diffusion/diffusive boundary layer (DBL), increases in thickness as seawater velocity decreases[7,8].

There are three main reasons via which mass transfer limitation in the DBL could influence the response of calcifying organisms to OA: (1) by changing the pH environment at their surface[9,10], (2) by limiting the transfer of essential nutrients and dissolved inorganic carbon[11,12], (3) or by causing the build-up of waste substances, such

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as protons during calcification[13]. The concentration gradient within the DBL is directly related to its thickness and the metabolic activity of the organism[5,14]. Therefore, when water velocity decreases, it increases the thickness of the DBL which favours the increase of pH at the surface of photosynthetic organisms during the day, and decreases in pH at night due to respiration[10,15]. There has been significant discussion of whether such diurnal changes in DBL pH may ameliorate the impacts of OA, whereby pH during the day and mean pH encountered by the organism are increased relative to the overlying seawater[16].

Conversely, reduced dissolved inorganic carbon or nutrients within thicker DBLs could have negative ramifications under OA. In many temperate systems, reduced nutrient concentrations at the surface of the organism might not have great impacts, but in already nutrient-poor systems such as coral reefs, these effects could have large impacts on organism metabolism. These would act additively with the impacts of OA. Reduced export of protons from the calcifying fluid to the external seawater is another possible negative ramification of thicker DBLs that could alter responses to OA, following the proton flux hypothesis[13]. This would manifest by reducing pH in the calcifying fluid (pH_{cf}), with the effects expected to be most pronounced under both OA and slow flow.

Only two studies have examined the interactive effects of water velocity and OA, finding contrasting effects. Cornwall *et al.*[5] found that the effects of OA were ameliorated for a temperate articulate coralline alga under slow flow (interactive effects). Daily increases in pH within the DBL were greater under slow flow and OA than nightly decreases in pH, thereby increasing mean pH at the surface, corresponding to faster calcification rates. Conversely, Comeau *et al.*[4] found that a coral reef community comprised of corals, crustose coralline algae (CCA) and carbonate sediments had lower calcification under both reduced pH and under slower flow (additive effects). Three logical explanations exist for the contrasting results due to: (1) differences between organism physiology; (2) environmental conditions; or (3) the inclusion of sediment in Comeau *et al.*'s study. Coral reef communities are more likely to display mass-transfer limitation than temperate macroalgae[17], therefore any positive effects of pH modification in the DBL may be overwhelmed by the negative impacts of nutrient limitation during Comeau *et al.*[4]. Tropical corals (and even CCA) might therefore be expected to respond differently from temperate articulate coralline algae. This could be due to the fact that the capacity of corals to modify pH at their surface might be much less than that of the coralline algae, or because their export of protons from their calcifying site might be differentially impacted by flow. Unfortunately, neither Cornwall *et al.*[5] nor Comeau *et al.*[4] measured pH_{cf}, nor did Comeau *et al.*[4] measure pH in the DBL of the different species in their communities.

Here we attempt to gain a greater understanding of how seawater velocity will interact with OA for key calcifying species, corals and CCA, by testing two competing questions, based on the results of seemingly contradictory studies[4,5]. Will slow flow provide a refugia from OA for calcifying species[16], or will faster flow reduce the impacts of OA? We tease out the physiological mechanisms responsible for both past results by growing corals and CCA under an interactive combination of slow/fast flow and low/ambient pH over a longer duration than used previously (27 weeks), and measure pH both at the surface of all organisms within the DBL, and pH within their calcifying fluids. We do this by using both taxa (corals and CCA) and by also crossing the already complex design with two irradiance regimes, with both high and low light for each taxon. While light is known to influence calcification and photosynthesis rates of both taxa[18,19], and can be particularly important in mediating the response of macroalgae to OA[20], these regimes also allow us to have one condition where pH modification in the DBL is potentially high and another where it is low (i.e. high and low light respectively). We conduct these experiments under conditions where nutrient concentrations are similar to those in coral reef systems (low concentrations), to represent the natural conditions where the organisms were collected and to test whether mass transfer limitation could be responsible for past results[21]. We hypothesize (1) that if slow flow acts ubiquitously as a refugia, then day time pH in the DBL will be directly correlated to calcification rates and favourable calcifying fluid chemistry across all treatments, (2) that if the export of protons limits calcification under slow flow, then pH_{cf} will be lower under slow flow for all taxa, particularly when combined with OA, and (3) if neither hypothesis is correct then mass-transfer limitation is responsible for the interactive effects.

Materials and Methods

Organism collection and preparation. The experiment was carried out from February 15th until August 25th 2017 in the University of Western Australia's Indian Ocean Marine Research Centre at Watermans Bay. Two weeks prior to the start of the experiment organisms were collected from Salmon Bay, Rottnest Island, WA, Australia (32°01′16.44″S, 115°31′16.60″E), which is located ~15 km offshore the marine laboratory (see Ross *et al.*[22] for the site description). A total of 48 branches (~5 cm tall) of the coral *Acropora yongei* were hand-collected and 48 whole colonies (~5 cm in diameter) of the coral *Plesiastrea versipora* were chiselled out from the reef at ~1–2 m depth. *Acropora* branches were collected ~5 m apart to maximise genetic diversity. Additionally, 48 whole rhodoliths of the coralline alga (CCA) *Sporolithon durum* (~5–8 cm) were hand picked at ~1–2 m depth. Only *S. durum* with limited or no exposed skeleton were selected. Back at the laboratory, the corals were glued to plastic supports using epoxy glue and tags were attached to *S. durum* using nylon lines. Organisms were acclimated to the laboratory lighting, temperature, and flow conditions (similar to the one experienced *in situ* and then used during the experiment) in a flow trough system during two weeks under ambient pH (pH_T ~ 8.1). At the end of the acclimation period, skeletons of the organisms were stained by placing the samples for 24 hours in a bath of seawater enriched with calcein at 50 mg l^{-1} with pH adjusted to 8.1 by addition of NaOH. The stain line was used during the geochemical analyses as a visual indicator to select portion of the skeleton grown under the experimental conditions (i.e. section above the stain line).

Experimental set-up. Twelve custom-made flumes were used to maintain the organisms under controlled conditions of flow, temperature, light and pH. The 90 L flumes consisted of a 1.5 m × 0.2 m × 0.2 m working section. Seawater was circulated at the upstream side of the flume through a 0.3 m long transition chamber mounted with flow straighteners made of stacked PVC pipes (diameter of 1.5 cm) covered by a shade cloth to obtain a

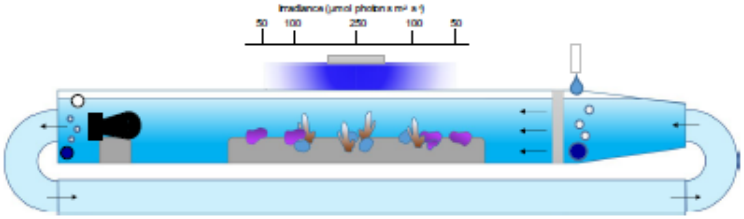

Figure 1. Diagram representing the experimental set-up used to maintain the corals and coralline algae for 27 weeks. The organisms were maintained under two flow velocities created by pumps placed at the end (left of the diagram) of the flumes. pH was regulated independently in each flume using a pH controller. Positions of the organisms within the flumes were used to control light levels. Organisms directly under the light received ~250 μmol quanta m^{-2} s^{-1} at midday (High light treatment for corals), while organisms on the side received either ~100 μmol quanta m^{-2} s^{-1} (Low light for corals, High light for CCA) at midday or ~50 μmol quanta m^{-2} s^{-1} at midday (Low light for CCA).

unidirectional semi-laminar flow. The return section was made of 0.1 m diameter PVC pipes. The organisms were maintained at two flow velocities of 0.025 m s^{-1} (Slow Flow) and 0.08 m s^{-1} (Fast Flow) that were obtained using two types of underwater pumps (D1 and D3 – DC wave maker pumps, Macro Aqua, China). Flow velocities were chosen to remain within ecologically relevant conditions. Flow velocity was determined at ~15 cm depth, where the organisms were placed, and adjusted for each flume using an Acoustic Doppler Velocimeter (Nortek Vectrino, Norway).

The flume experiments were designed to test the interactive effects of two regimes of flow velocities (0.025 and 0.08 m s^{-1}), pH (ambient pH: pH_T = 8.05 and low pH: pH_T = 7.65) between flumes, and light levels (Fig. 1). For clarity, in the following sections the ambient pH treatment is described as 'pH 8.0' and the low pH treatment as 'pH 7.6'. Treatments of flow and pH were randomly assigned to obtain three flumes per combination of flow and pH. Light levels were controlled by the relative position of organisms within the flumes. Organisms directly under the light received ~250 μmol quanta m^{-2} s^{-1} at midday (High light treatment for corals only), while organisms on the side received either ~100 μmol quanta m^{-2} s^{-1} (Low light for corals, High light for CCA) at midday or ~50 μmol quanta m^{-2} s^{-1} at midday (Low light for CCA only). The light levels corresponded to light intensities regularly experienced by organisms in Salmon Bay[22]. Prior experimentation determined that these light conditions were optimal for growing these species in the laboratory[23,24]. Four corals per species were randomly placed under 250 or 100 μmol quanta m^{-2} s^{-1} (n = 2 per light treatments for each flume). Four coralline algae were randomly placed under 100 or 50 μmol quanta m^{-2} s^{-1} (n = 2 per light treatments for each flume). With this design, 6 organisms of each species were exposed to each combination of treatments.

Temperature was maintained constant at ~20.5 °C, which is the mean *in situ* temperature at the collection site[25], during the experiment. Light was provided by 150 W LED (Maltbu LED, Ledzeal) that followed a diel cycle. Light was gradually ramped-up in the morning, commencing from 6:00 h until 10:00 am to reach maximum intensity, remained at maximum intensity for four hours, and then ramped down until total darkness at 18:00 h. pH was manipulated in the flumes using pH-controllers (AquaController, Neptune systems, USA) that control the bubbling of pure CO_2. Ambient air was continuously bubbled in each flume to maintain the O_2 and ambient pH constant. The flumes worked as a flow through system with sand-filtered seawater (porosity ~25 μm) delivered continuously at ~0.5 L min^{-1}.

Carbonate chemistry measurement and calculations. Seawater pH and temperature were measured every ~1–2 d in each flume using a pH meter calibrated every 2 d on the total scale using Tris/HCl buffers made following[26]. Total alkalinity (A_T) was measured weekly in all the flumes using an open cell potentiometric method (Mettler Toledo, T50). A_T was calculated using a modified Gran function and titrations of certified reference materials (CRM, batch 161) provided by A.G. Dickson lab yielded A_T values within 5 μmol kg^{-1} of the certified value. A_T, pH_T, temperature, and salinity were used to calculate the carbonate chemistry parameters using the seacarb package running in R.

Physiological measurements. Calcification was measured over the 27-week experimental duration using the buoyant weight method[27]. Net calcification was determined on each organism by converting the difference in weight between the beginning and the end of the incubation period to dry weight using an aragonite density of 2.93 g cm^{-3} (for the corals) and a calcite density (for the CCA) of 2.73 g cm^{-3}. Net calcification was normalized to the surface area determined using the foil method[28] for *P. verstipora* and *S. durum*, and the relationship between skeleton weight and surface area for *A. yonget* determined by CT scanning.

Light short-term incubations were carried out after 2 months of exposure to the treatments to assess the response of photosynthesis and respiration. Incubations were conducted at this time to ensure adequate acclimation to the growing conditions. Each individual was placed into an incubation chamber filled with seawater originating from its respective flume. Flow and light were manipulated to approximate the respective conditions in the flumes. Organisms were chosen randomly for a total of 4 replicates of each species per treatment combination (96 individuals total) and controls with only flume seawater were run during each incubation. Incubations lasted

~1.5–2 h and changes in dissolved oxygen (using an A323 dissolved oxygen portable meter, Orion Star, Thermo Scientific, USA) and temperature between the beginning and end of each incubation were determined. All rates were normalized to surface-area of the organisms.

Calcifying fluid pH_{cf} and DIC_{cf}. Calcifying fluid pH (pH_{cf}) for all organisms and DIC (DIC_{cf}) for corals was calculated using the $\delta^{11}B$ proxy method for pH_{cf} and the $\delta^{11}B$ and B/Ca method for DIC_{cf}[29]. Geochemical measurements were done on the material representing the average calcium carbonate deposited during the 27 weeks of incubation (as confirmed by calcein staining)[23,24]. The portions of the skeleton grown under the experimental conditions were sampled using cutting pliers (for *A. yongei*) or a dental drill (for *S. durum* and *P. verstpora*).

All powders of selected material were processed in the clean laboratory of the Advanced Geochemical Facility for Indian Ocean Research [AGFIOR, University of Western Australia (UWA)] for dissolution and dilution to 10-ppm Ca solutions. Ten mg of each sample was placed in 6.25% NaClO for 15 mins, rinsed in MilQ water 3 times and then dried for 24 h. Samples were then dissolved in 0.51 N HNO_3, and the boron was quantitatively separated on ion exchange columns. $\delta^{11}B$ was measured on a multicollector inductively coupled plasma mass spectrometry (NU II). Measurements of the international carbonate standard JCp-1 yielded a mean value of 24.35 ± 0.13‰ (mean ± SE, n = 5), which was similar to the nominal value of 24.33 ± 0.11‰ (SE) reported previously[30]. Calculations of pH_{cf} based on $\delta^{11}B$ were made using the calculations of[31]:

$$pH_{cf} = pK_B - \log\left[\frac{(\delta^{11}B_{SW} - \delta^{11}B_{carb})}{(\alpha_{(B3-B4)}\delta^{11}B_{carb} - \delta^{11}B_{SW} + 1000\,(\alpha_{(B3-B4)} - 1))}\right] \quad (1)$$

where pK_B is the dissociation constant dependent on temperature and salinity, $\delta^{11}B_{sw} = 39.61$[32], and $\alpha_{B3\text{-}B4}$ is the boron isotopic fractionation factor for the pH dependent equilibrium of the borate ($B(OH)_4^-$) relative to the boric acid ($B(OH)_3$) species in the calcifying fluid, with a value of 1.0272[33].

B/Ca ratios and $\delta^{11}B$ measured on the same material was utilized to determine $[CO_3^{2-}]$ and then [DIC] at the site of calcification $[DIC]_{cf}$ following[29,34]. B/Ca ratios were determined on the same aliquot of the solution used for pH_{cf} estimates, and DIC_{cf} was calculated from estimates of CO_3^{2-} using the following equations described in[29]:

$$[CO_3^{2-}]_{cf} = K_D[B(OH)_4^-]_{cf}/(B/Ca)_{CaCO_3} \quad (2)$$

Where $K_D = K_{D,0}\exp(-k_{KD}[H^+]_T)$ with $= 2.97 \pm 0.17 \times 10^{-3}$ (±95% CI), $k_{K_D} = 0.0202 \pm 0.042$. The concentration of DIC_{cf} was then calculated from estimates of pH_{cf} and $[CO_3^{2-}]_{cf}$ using the R package seacarb.

Raman spectroscopy. We utilized confocal Raman spectroscopy to determine sample mineralogy (aragonite versus calcite) and as a proxy of calcifying fluid saturation state (Ω). Measurements were conducted on a WITec Alpha300RA + using a 785 nm laser, 1200 mm^{-1} (~1.3 cm^{-1} spectral resolution), and a 20x objective with 0.5 numerical aperture following[35]. The wavenumber was routinely calibrated with a silicon chip (nominal peak at 520.5 cm^{-1}). Topography maps were made with the TrueSurface module for skeleton samples placed on glass slides (powders for corals, and cut sections for CCA). The topography maps were then followed with an automated stage while conducting Raman measurements to ensure the optics remained in focus. For each sample, 100 spectra were collected in a square grid, 300 µm by 300 µm using 1 s integrations for corals and 1 mm by 1 mm using 2 s integrations for CCA. Spectra with poor signal (< 100 arbitrary intensity units or signal/noise ratio of ~10) or contaminated by cosmic rays were excluded.

Sample mineralogy was evaluated by first confirming each sample is $CaCO_3$ based on the ν_1 peak at ~1085–1090 cm^{-1}. Next, each sample was distinguished between aragonite and calcite based on the shape and position of the ν_4 peak between 700–720 cm^{-1}, where a double peak < 710 cm^{-1} is indicative of aragonite and a single peak >710 cm^{-1} is indicative of calcite. We found only aragonite in our coral samples and only high-Mg calcite in our CCA samples, confirming the mineralogy expected for each species.

The widths of the ν_1 peaks were used as proxy measures of calcifying fluid Ω[19]. We used the abiogenic aragonite calibration equation of[35] to calculate Ω_a for the two coral species from the ν_1 full width at half maximum intensity (FWHM). Although there is no published abiogenic high-Mg calcite Ω calibration, we used the Mg concentration-normalized peak widths as relative indicators of Ω for CCA[36,37]. The effect of Mg on ν_1 FWHM was accounted for using the equations of[38], where [Mg] is determined from ν_1 wavenumber, and the residual ν_1 FWHM is determined for qualitative calcite Ω interpretations

Calcifying fluid Ca_2 + *cf*. $[Ca^{2+}]_{cf}$ was calculated as:

$$[Ca^{2+}]_{cf} = \Omega_{Ara}K_{sp}/[CO_3^{2-}]_{cf} \quad (3)$$

where $[CO_3^{2-}]_{cf}$ and $\Omega_{Arag\text{-}cf}$ are derived from boron systematics and Raman spectroscopy, respectively.

Micro-sensor measurements. The DBL pH was determined after 16–27 weeks of incubations using a Unisense microprofiling system (Unisense A/S, Denmark). Measurements were made directly in the flumes on the organisms maintained under their respective conditions of light, pH, and flow. pH_T of the flumes bulk seawater was measured following the method described above before each profile determinations. pH in the DBL was measured with a pH-50 microelectrode with a 40–60 µm tip diameter and an external reference electrode (Radiometer analytical). The pH microelectrode was calibrated using NBS buffers every day, and standardised to the total scale based on Tris mV prior to any further measurements.

Flume	Flow	pH_T	A_T (μmol kg^{-1})	pCO_2 (μatm)	Temperature (°C)
1	Slow	7.61 ± 0.01	2358 ± 3	1309 ± 29	20.5 ± 0.1
2	Slow	7.61 ± 0.01	2361 ± 2	1278 ± 24	20.5 ± 0.1
3	Fast	8.03 ± 0.01	2357 ± 2	427 ± 13	20.8 ± 0.1
4	Slow	7.61 ± 0.02	2360 ± 2	1350 ± 57	20.6 ± 0.2
5	Slow	8.04 ± 0.00	2361 ± 2	416 ± 5	20.6 ± 0.1
6	Slow	8.04 ± 0.00	2357 ± 3	412 ± 6	20.7 ± 0.2
7	Fast	7.63 ± 0.01	2360 ± 2	1222 ± 27	20.9 ± 0.1
8	Fast	7.61 ± 0.01	2359 ± 2	1311 ± 34	20.8 ± 0.2
9	Fast	7.63 ± 0.01	2359 ± 2	1221 ± 23	20.7 ± 0.1
10	Fast	8.03 ± 0.01	2358 ± 2	439 ± 20	20.6 ± 0.2
11	Fast	8.04 ± 0.00	2357 ± 2	410 ± 5	20.6 ± 0.2
12	Slow	8.03 ± 0.00	2356 ± 2	422 ± 6	20.5 ± 0.2

Table 1. Mean carbonate chemistry in the flumes during the 27 weeks experiment. pCO_2 was calculated using measured pH_T, total alkalinity (A_T), temperature and a salinity of 36.3 (SE < 0.1). All values are mean ± SE (n = 64).

A Unisense manual micro-manipulator and a hand-held magnifying glass were used to position the sensors. For *A. yongei*, micro pH electrodes were positioned between polyps as close as possible to the surface of the organisms, while for *P. verstpora* the probes were positioned inside the gap made by their large polyps. For *S. durum*, the sensors were placed as close as possible to the surface in the gap between protuberances. After a 45 min acclimation period, measurements of pH were made every 100 μm above the organisms (50 μm for the first 4 steps) surface up to 1800 μm, with a final measurement at 2800 μm. At each step, measurements were collected for 2 min. The difference in pH between the surrounding seawater and the diffusion boundary layer (DBL) were determined on at least three *P. verstpora* and *S. durum* for each experimental treatment. Measurements on *A. yongei* were only done on three individuals because it was not possible to detect a consistent change in pH in the DBL. pH in the DBL in the dark was also determined on two *P. verstpora*., two *S. durum* and one *A. yongei*. A 2-month period was necessary to perform all the measurements so they could be conducted roughly at the same time of day.

Statistical analyses. Factorial ANOVA models were used to detect differences in calcification, photosynthesis, pH_{cf}, DIC_{cf}, $Ca^{2+}{}_{cf}$, Ω_{cf}, and DBL pH for the corals, and calcification, photosynthesis, pH_{cf}, B/Ca, Raman-derived FWHM and DBL pH for *S. durum*. pH, flow and light were fixed factors in the models, where flume of origin was also included as a random factor. The random factor was dropped from the analyses when it was not significant ($p < 0.25$). All data conformed to normality and homogeneity of variance. All analyses were done in R. All data will be archived in the Pangaea database.

Results

During the 27 weeks of the experiment, carbonate chemistry was successfully maintained constant across the treatments (Table 1). pH was maintained on average at 8.04 ± 0.02 (mean ± SE, n = 384) in the pH 8.0 treatment and 7.62 ± 0.03 (mean ± SE, n = 384) in the pH 7.6 treatments, corresponding to respective pCO_2 of 421 ± 26 and 1282 ± 83 μatm. Over the course of the experiment, the nutrient concentrations were: NH_4^+ = 3.07 ± 2.6 $\mu g l^{-1}$ (n = 8), NOx = 0.83 ± 1.42 $\mu g l^{-1}$, and PO_4 = 6 ± 1.42 $\mu g l^{-1}$. No bleaching or mortality of corals as a function of the treatment was found, though 7 CCA experienced mortality (not linked to the treatment) and were subsequently excluded from further analysis.

Calcification. For *P. verstpora*, the highest calcification was found in the pH 8.0 - Fast Flow conditions under High and Low Light, while calcification was the slowest in the pH 7.6, High Light, Slow Flow treatment (Fig. 2A). Calcification was significantly affected by pH ($p = 0.011$, Table S1), but there was no significant effect of Light ($p = 0.675$). Flow significantly affected calcification ($p = 0.034$) that was on average higher at Fast Flow than Slow Flow. There was no significant interactive effect between the tested parameters.

For *A. yongei*, the highest mean net calcification was measured in the pH 8.0 – High Light – Slow Flow treatment and the lowest in the pH 7.6 – Low Light –Fast Flow (Fig. 2B). Net calcification was affected by pH ($p < 0.001$, Table S1), Light ($p = 0.003$), and their interaction ($p = 0.007$) with calcification on average higher at pH 8.0 and High Light compare to pH 7.6 and Low Light. Flow did not impact calcification ($p = 0.293$), but there was a significant interaction between pH, Light and Flow because the negative effects of flow were larger at Low Light and pH 7.6 ($p < 0.016$).

Calcification of the CCA *S. durum* was fastest in the High Light treatment at pH 8.0 (for the Fast and Slow Flow) and negative in the two pH 7.6 - Slow Flow treatments (Fig. 2C). CCA calcification was significantly affected by pH ($p < 0.001$), Light ($p < 0.001$), and Flow ($p = 0.019$). However, there were no statistically significant interactive effects.

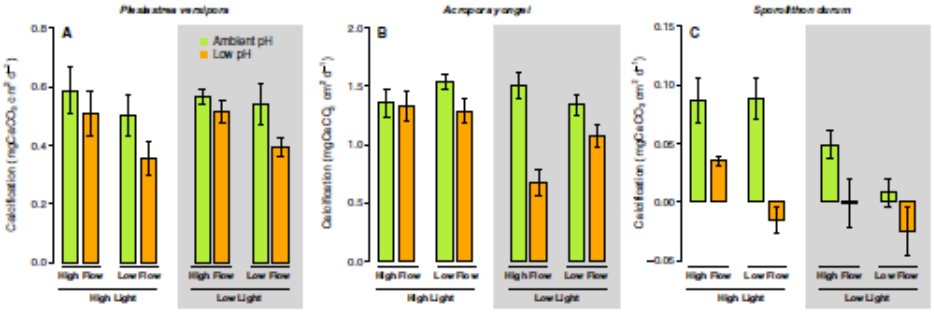

Figure 2. Effects of pH, flow and light on the surface-area normalized net calcification rates of the corals *Plestastrea verstpora* and *Acropora yonget* and the coralline alga *Sporolithon durum*. Calcification was measured on organisms grown in flumes for 27 weeks. pH was maintained at ambient pH (green bars, pH = 8.1) and low pH (orange bars, pH = 7.65). Seawater velocity was adjusted to high flow (8 cm s^{-1}) and low flow (2.5 cm s^{-1}). Light levels were manipulated within the flumes to high light (250 μmol photon m^{-2} s^{-1} for corals and 100 μmol photon m^{-2} s^{-1} for the CCA) and low light (100 μmol photon m^{-2} s^{-1} for corals and 50 μmol photon m^{-2} s^{-1} for the CCA) levels. Values displayed are mean ± SE (n = 6).

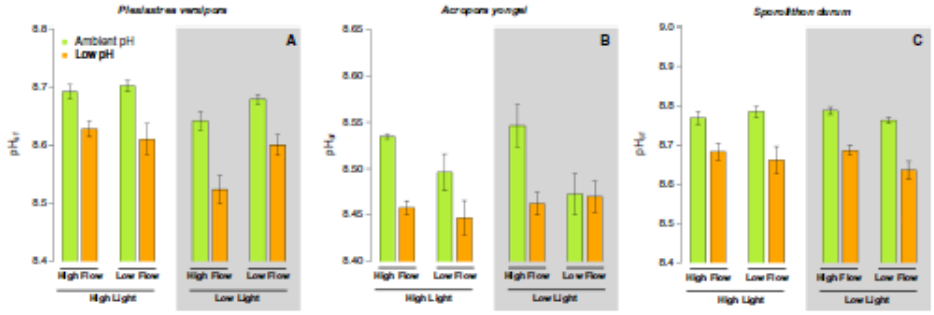

Figure 3. Estimates of pH in the calcifying fluid (pH_{cf}) derived from $\delta^{11}B$ for the corals *Plestastrea verstpora* and *Acropora yonget* and the coralline alga *Sporolithon durum*. pH_{cf} was determined at the end of the 27 week incubation period on organisms maintained at ambient pH (green bars, pH = 8.1) and low pH (orange bars, pH = 7.65). Seawater velocity was adjusted to high flow (8 cm s^{-1}) and low flow (2.5 cm s^{-1}). Light levels were manipulated within the flumes to high light (250 μmol photon m^{-2} s^{-1} for corals and 100 μmol photon m^{-2} s^{-1} for the CCA) and low light (100 μmol photon m^{-2} s^{-1} for corals and 50 μmol photon m^{-2} s^{-1} for the CCA) levels. Values displayed are mean ± SE (n = 6).

Calcifying fluid pH. For *Plestastrea*, the lowest pH_{cf} was found in the pH 7.6 – Low Light – Fast Flow treatment and the highest in the pH 8.0 under High Light at both flows. As a result, there was an effect of pH ($p < 0.001$, Table S2), Light ($p = 0.001$), and the interaction between Light and Flow ($p \sim 0.048$) (Fig. 3A).

For *A. yonget*, estimates of pH_{cf} were the highest in the pH 8.0 - Fast Flow treatments under both Light conditions (Fig. 3B). There was a significant effect of pH ($p < 0.001$, Table S2) and Flow ($p = 0.034$) as well as an interactive effect between Flow and pH ($p = 0.017$) with pH_{cf} being reduced by slow flow rates under seawater pH 8.0.

pH_{cf} of the coralline *S. durum* ranged from 8.64 (pH 7.6 – Low Light – Slow Flow) to 8.78 (pH 8.0 – High Light – Slow Flow and pH 8.0 – Low Light- Fast Flow). pH_{cf} was only affected by pH ($p < 0.001$, Table S2) with the lowest values recorded in all the pH 7.6 treatments (Fig. 3C).

Calcifying fluid DIC. For *P. verstpora*, DIC_{cf} was affected by pH ($p = 0.001$, Table S3) and Light ($p = 0.044$). The interaction between pH, Light and Flow ($p = 0.044$) was also significant because the effects of Flow and pH were reversed at Low Light (Fig. 4A). Estimates of DIC_{cf} for *A. yonget* were only affected by pH ($p = 0.003$, Table S3) with DIC_{cf} more elevated on average in the pH 7.6 treatments (Fig. 4B).

B/Ca of *S. durum*, which is expected to be indicative of DIC_{cf}, was the highest under the pH 7.6 – Slow Flow conditions and was similar in all the pH 8.0 treatments (Fig. 4C). Flow significantly affected B/Ca ($p = 0.039$, Table S3) and there was a trend towards a significant interactive effect of Flow and pH ($p = 0.063$) because B/Ca was more elevated at pH 7.6 only under Slow Flow.

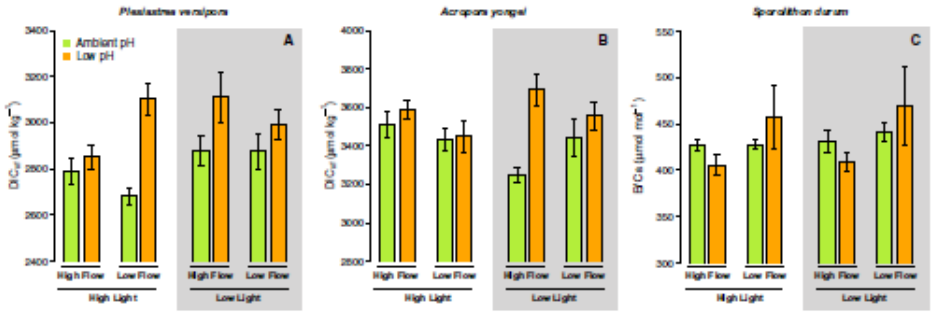

Figure 4. Estimates of DIC in the calcifying fluid (DIC_{cf}) derived from $\delta^{11}B$ and B/Ca for the corals *Plestastrea verstpora* and *Acropora yonget*. B/Ca is used here as a proxy of DIC_{cf} for the coralline alga *Sporolithon durum*. Note that an increase in B/Ca indicates a decrease in DIC_{cf}. Values displayed are mean ± SE (n = 6).

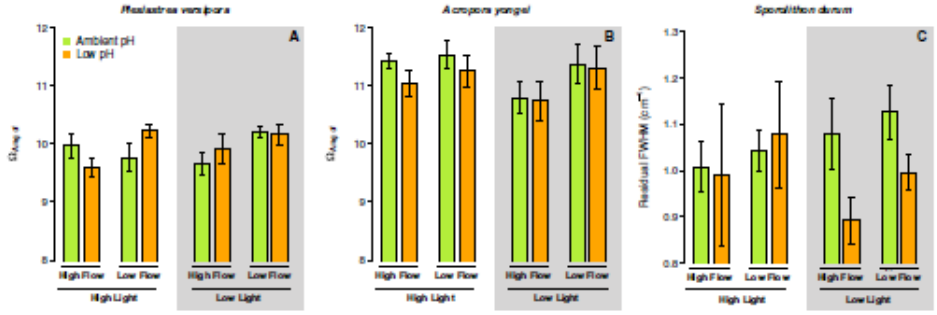

Figure 5. Estimates of the aragonite saturation state in the calcifying fluid ($\Omega_{arag\,cf}$) for the corals *Plestastrea verstpora* and *Acropora yonget*. $\Omega_{arag\,cf}$ was estimated using Raman microscopy. Residual FWHM is directly used as a proxy of $\Omega_{calc\,cf}$ for the coralline alga *Sporolithon durum* because no empirical relationship between residual FWHM and Ω_{calc} exist. Values displayed are mean ± SE (n = 6).

Ω_{cf} and residual FWHM. For *P. verstpora*, Ω_{cf} was affected by Flow ($p = 0.034$, Table S4) because Ω_{cf} was on average more elevated under low flow. However, there were no effects of pH and Light on *P. verstpora* Ω_{cf} (Fig. 5A, Table S4). The Ω_{cf} of *A. yonget* was not significantly affected by any treatment (Fig. 5B).

The treatments did not significantly affect the residual FWHM (indicator of the high-Mg calcite saturation state in the calcifying fluid) of *S. durum* (Fig. 5C, Table S4).

Calcifying fluid Ca^{2+}. Ca^{2+}_{cf} in *P. verstpora* was significantly affected by pH ($p < 0.001$, Table S5) and Light ($p = 0.014$). Ca^{2+}_{cf} was more elevated under low pH and low light (Fig. 6A). For *A. younget*, Ca^{2+}_{cf} was only affected by flow, with the highest values measured under low flow (Fig. 6B).

Photosynthetic rates. Net photosynthetic rates of *P. verstpora* were only affected by Light ($p = 0.043$), with lower rates recorded in the Low Light treatment. For *A. yonget*, net photosynthetic rates were only significantly affected by Flow ($p = 0.042$) because of lower rates under Low Flow (Fig. 7B, Table S6). Net photosynthetic rates of *S. durum* were not affected by the treatments (Fig. 7C, Table S6).

Metabolic alteration of pH in the DBL. Delta pH (pH values recorded near the organism surface with micro-sensors minus mainstream seawater pH) in the DBL in the light was not successfully measured on *A. yonget* because the changes in pH were too small. The only profile completed in the dark showed a strong decrease of ΔpH in the DBL of 0.7 unit. For, *P. verstpora*, there was a significant effect of pH ($p = 0.050$, Table S6), Flow ($p = 0.002$), and their interaction ($p = 0.002$) on ΔpH. This interactive effect was due to greater ΔpH in the low pH treatments under low flow (Fig. 8A). As a consequence pH in the DBL was similar between pH treatments under low flow (Fig. S1). Light ($p < 0.001$) and the interaction between Light and pH ($p = 0.021$) also affected ΔpH. This was caused by higher ΔpH under high light, particularly in the low pH treatment. The two measurements of ΔpH in the dark in the pH 8.0 – Fast Flow treatments showed a decrease in pH in the DBL of ~0.6 unit.

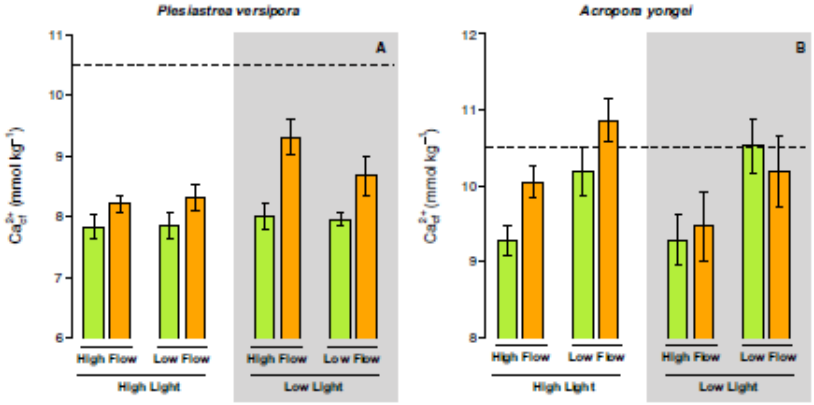

Figure 6. Estimates of the calcium concentration in the calcifying fluid (Ca^{2+}_{cf}) for the corals *Plesiastrea versipora* and *Acropora yongei*. Ca^{2+}_{cf} was calculated from estimates of $\Omega_{arag\,cf}$ and estimates of DIC_{cf}. Values displayed are mean ± SE (n = 6). The horizontal dashed line shows the approximate [Ca] in seawater during the experiment. Values displayed are mean ± SE (n = 6).

For *S. durum*, only Light had a significant effect on ΔpH in the DBL ($p = 0.006$), with higher ΔpH in the high light treatments. There was also a trend toward an interaction between pH and Flow ($p = 0.061$) because ΔpH was higher at pH 8.0 in the Slow Flow conditions. In the dark, ΔpH in the DBL was 0.07 and 0.05 for the two test measurements made on *S. durum* from the pH 8.0 – Fast Flow treatments.

Discussion

Understanding the role played by different physical parameters is necessary to explain the large range of responses to OA measured in past studies, and to accurately predict how OA will manifest across habitats with differing environmental conditions. We demonstrate here that flow, light, and pH and their interactions are critical factors impacting organisms' physiology, internal chemistry, and conditions in the DBL. As a result of these complex interactions, calcification responded differently to the treatments in all the tested organisms because of morphological and physiological differences. In contrast to our initial hypotheses (1) low flow conditions did not alleviate the negative effects of OA on calcification and (2) the export of protons is likely not the main driver of calcification in all species, because there was not a consistent effect of flow on pH_{cf}. In contrast, our results suggest that mass-transfer limitation of nutrients or night-time dissolution play an important role in the control of net calcification rates.

Low flow does not provide a general refugia from OA. In the present experiment, low flow did not alleviate the effect of OA for the three species. These results are in contrast to Cornwall *et al.*[5], who showed that low flow conditions ameliorate the negative effect of OA on calcification of the temperate articulate coralline alga *Arthrocardia corymbosa*. Here, the coralline alga exhibited negative rates of calcification under both irradiances under low flow and low pH conditions. The discrepancy between the two studies can likely be explained by two non-exclusive hypotheses. First, the present study was performed on sub-tropical algae that were grown in low nutrient concentrations similar to undisturbed coral reefs[39], while Cornwall *et al.*[5] used temperate algae grown under higher nutrient concentrations. It is therefore likely that under low flow *S. durum* were nutrient limited, which in turn limited their calcification rates. Nutrient uptake in oligotrophic waters is indeed dependent on flow[23] and can have large impacts on calcification rates. Second, it is possible that the increase of pH in the DBL measured here was not sufficient to limit the effect of OA on calcification. Despite a linear relationship between pH in the DBL and calcification in *S. durum* (Fig. S2), the increase in pH in the DBL was not sufficient to ameliorate the effect of OA. Here, the relatively low photosynthetic activity of coralline algae in all the treatments and the flow velocities used (minimum of 2.5 cm s^{-1}) can explain the limited increase of pH in the DBL under OA. Furthermore, it is possible that the increase in pH during the day was not sufficient to counteract the lower pH at night in the DBL, and therefore that mean DBL pH was not elevated on average over a 24 hours cycle under slow flow. Unfortunately, pH in the DBL at night was not measured because of logistical and experimental constraints.

Our results are partially in agreement with Comeau *et al.*[4] who found that calcification of coral communities is enhanced by faster flow under ambient and OA conditions. Here, this is true for one of the two coral species, as the effects of OA on calcification of *P. verstpora* were ameliorated under high flow. However, this was not true for *A. yongei*. These species-specific responses were likely driven by different morphologies (branching vs mounding) and different physiologies. The mounding physiology of *P. verstpora*, with deep polyps, likely created areas with alternatively low and thick DBL. However, enhanced pH in the DBL during the day was not associated with increased calcification in *P. verstpora*. Calcification was the lowest in the low flow and low pH treatments where the largest DBL ΔpH was measured. This result indicates a control of calcification by either bulk seawater pH

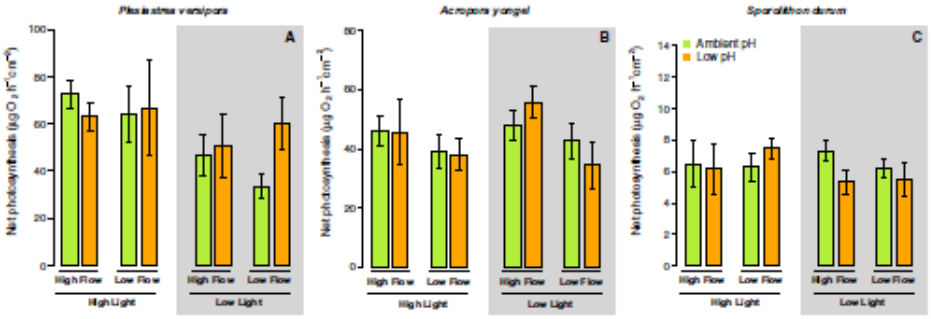

Figure 7. Net Photosynthesis rates determined on the corals *P. verstpora* and *A. yongei* and the coralline alga *S. durum*. Net photosynthesis was determined during incubations performed after 2 months in the experimental treatments. Values displayed are mean ± SE (n = 6).

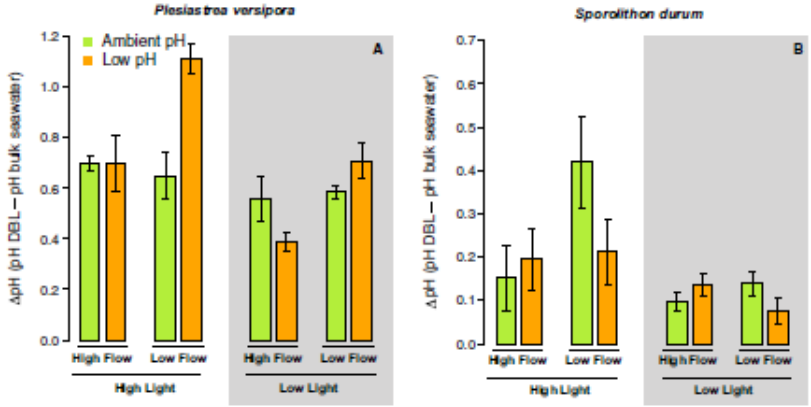

Figure 8. Maximal differences in pH between the diffusive boundary layer (DBL) and the bulk seawater in the light for the corals *Plestastrea verstpora* and the coralline alga *Sporolithon durum*. Values displayed are mean ± SE (n = 3).

(lower calcification recorded in the low pH treatment), nutrient concentrations (lower nutrient available at low flow) and/or low pH in the DBL at night. The few measurements of pH in the DBL in the dark for *P. verstpora* showed a negative delta pH of similar magnitude to the light ΔpH, which could indicate that the positive effect of higher pH during the day is balanced by the negative effects of lower pH at night under slow flow. However, further studies will be necessary to confirm this observation.

The export of protons is not the main driver of calcification in all species. Our results demonstrate that the chemistry at the site of calcification is strongly impacted by the physical and chemical conditions in which the organisms are living. The relationship between pH_{cf} and seawater pH is species-specific in corals and CCA[24,40,41]. This trend was repeated here with the three tested taxa exhibiting different pH_{cf}. For example, mean pH_{cf} in the ambient seawater pH treatments was 8.68, 8.51, and 8.78 for *P. verstpora*, *A. yongei*, and *S. durum* respectively. These values are within the range of what has been reported previously using a variety of techniques such as micro-electrodes[42], pH-sensitive dye[43], and boron isotope proxies[44]. In addition, the present study also demonstrates that pH_{cf}, and the general chemistry at the site of calcification (DIC_{cf}, Ca^{2+}_{cf}, Ω_{cf}), is also modulated by flow and/or light depending on the species. This has important repercussions, as it demonstrates that using pH_{cf} or DIC_{cf} as indicators of the seawater chemistry[45] can be confounded by other physical parameters. This result also suggests that seasonal variation in pH_{cf} and DIC_{cf}[29] could be partly driven by seasonal variations in both light and flow, which should now be measured in the future *in situ* proxy research to improve accuracy of any reconstructions of the physical or chemical environment.

Generally, seawater pH was the main driver of pH_{cf} in the three tested species, while the effects of flow and light were species-specific and subtler. Light had the strongest effects on *P. verstpora*, with pH_{cf} being lower within low light treatments. This could have been driven by generally lower DBL ΔpH under low light for *P. verstpora*. The linear relationship between DBL ΔpH and pH_{cf} for *P. verstpora* and *S. durum* (Fig. S3) suggests that the elevation of pH at the surface of the organisms can slightly modify pH_{cf} and favour the export of protons from the site of calcification by reducing the proton gradient between the calcifying fluid and the mainstream seawater. This indicates that pH near the surface of the organism could be more important than bulk seawater pH in influencing pH_{cf}, particularly for mounding species. However, this is not found in all the corals, as it was not possible to link pH_{cf} and DBL ΔpH in *A. yongei*, because DBL ΔpH was nearly impossible to measure (i.e. close to 0) here, something which has been recently repeated with another *Acropora* species[46]. We also did not find any direct relationships between calcification and pH_{cf} for the two corals (Fig. S4). This is in agreement with our previous work that showed that pH_{cf} does not always drive calcification[23,37,47]. For *S. durum*, there was also no linear relationship between pH_{cf} and calcification but calcification and pH_{cf} were the lowest in the low pH treatments.

Chemistry in the calcifying fluid. $[Ca^{2+}]_{cf}$ of *P. verstpora* increased at lower seawater pH, though it remained lower than seawater $[Ca^{2+}]$, as found previously in *Pocillopora damicornis*[48]. This could be one of the mechanisms used by this species to maintain elevated Ω_{cf} and calcification under low pH. *A. yongei* $[Ca^{2+}]_{cf}$ also showed similar patterns to that observed previously in this species, as $[Ca^{2+}]_{cf}$ was not affected by seawater pH[48]. In contrast, the higher $[Ca^{2+}]_{cf}$ of *A. yongei* under low flow conditions were not correlated with higher calcification rates. Together with previous observations[42], this collectively demonstrates that $[Ca^{2+}]_{cf}$ is both driven by environmental parameters and is highly species-specific. Nevertheless, $[Ca^{2+}]_{cf}$ and $[CO_3^{2-}]_{cf}$ were linearly correlated in both species (Fig. S5), demonstrating that increasing $[Ca^{2+}]_{cf}$ can be used by both species as a compensatory mechanism in response to declining $[CO_3^{2-}]_{cf}$.

There were strong species-specific effects of the experimental conditions on DIC_{cf}. The few studies that have investigated DIC_{cf} have assumed that DIC_{cf} was driven by photosynthesis, seawater pH, or seawater DIC[29,47,49,50]. Here we also show that flow and light can have complex effects on DIC_{cf} and that these effects are pH dependent. The relationship between B/Ca and the employed treatments for the coralline alga was also complex. Under high flow, B/Ca was lower (DIC_{cf} higher) at low seawater pH, which is similar to the effects of seawater pH on corals[51]. However, here the opposite was found for *S. durum* at low flow, demonstrating that seawater carbonate chemistry is not the only driver of CCA DIC_{cf}. This lower B/Ca could be the result of the very low consumption of DIC by calcification in the low flow, low pH treatments where the lowest rates of calcification were measured.

The overall lack of an effect of the treatment on $\Omega_{arag\,cf}$ or FWHM while calcification varied between treatments is indicative of three processes. First, $\Omega_{arag\,cf}$ and FWHM represents the chemical condition in the calcifying fluid when the precipitation of calcium carbonate occurred. Here, our results show that corals and CCA need to reach a certain species-specific threshold $\Omega_{arag\,cf}$ (or FWHM) to initiate the precipitation process (i.e., $\Omega_{arag\,cf} \sim 11$ for *A. yongei* and ~10 for *P. verstpora*, FWHM ~ 1.0 for *S. durum*), regardless of the external conditions of flow, light and pH. Second, $\Omega_{arag\,cf}$ (or FWHM) does not provide information on the bulk rate of calcification (i.e. the instantaneous precipitation rate integrated over time and surface area). Finally, the discrepancy between $\Omega_{arag\,cf}$ (or FWHM) and calcification could be the sign that dissolution in some treatments played a role, as this would decrease calcification without affecting the chemistry at the site of calcification during calcification.

Conclusion

The present study shows that light, flow, and pH have complex species-specific effects on corals and coralline algae. Slow flow conditions did not provide refugia from ocean acidification, and in contrast it had no effect or negative effects on calcification. Nor did elevated seawater velocity show clear evidence of increased proton export. Here we clearly demonstrate the role of flow is context and species-specific. This highlights the necessity of assessing hypotheses regarding how climate change will manifest across multiple species under a variety of environmental conditions, while at the same time evaluating the physiological effects of these parameters. Further, the strong effects of irradiance and flow on the carbonate chemistry within the calcifying fluid confirm the difficulties associated with using skeletal proxies to estimate environmental and physiological conditions[23,45,51]. Therefore, caution must be applied to assuming constant offsets of these parameters from seawater carbonate chemistry across different sites, even within the same species. Most importantly however, we demonstrate here that the effects of ocean acidification will manifest differently between habitats with differences in light and seawater velocity. These differences are complex, difficult to predict based on existing hypotheses regarding the impacts of seawater velocity, and are species-specific. This indicates the need for more targeted research that further assesses the impacts of seawater velocity; based on our findings further work should couple similar research with assessment of dissolution and/or manipulations of nutrient concentrations.

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Author Contributions

S.C., C.E.C. and M.T.M. designed the research. S.C. and C.E.C. wrote the paper. All co-authors edited the paper. S.C., C.E.C., C.P., A.C. and R.T. ran the experiment. S.C., C.E.C. and T.M.D. performed geochemical and statistical analysis.

Additional Information

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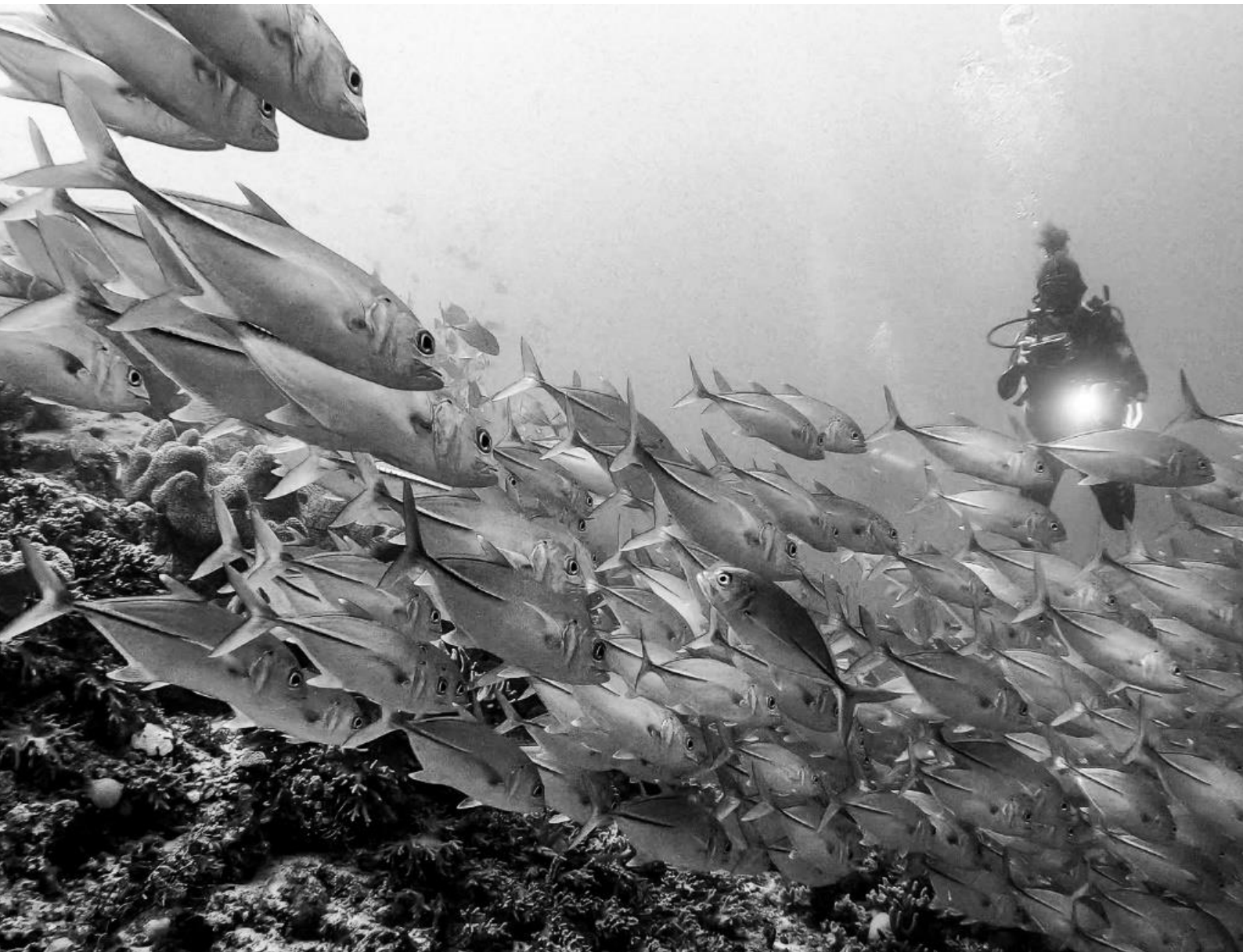
Références



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Résumé / Abstract

Les octocoralliaires représentent l'un des principaux groupes formant la communauté macrobenthique des récifs coralliens tropicaux. Ils sont notamment abondants au sein des écosystèmes perturbés où les changements environnementaux entraînent le déclin des coraux constructeurs de récifs. Bien que la nutrition joue un rôle fondamental dans la régulation de l'abondance d'une population, l'acquisition de nutriments par les octocoralliaires reste à ce jour peu connue. Dans ce contexte, les objectifs de cette thèse étaient 1) de caractériser l'acquisition et l'assimilation de carbone et d'azote, par différentes espèces d'octocoralliaires de mer Rouge, et 2) d'évaluer les changements de nutrition le long d'un gradient de profondeur, depuis la surface (5 m) jusqu'à la zone récifale mésophotique supérieure (50 m). Les résultats démontrent que les octocoralliaires forment une symbiose nutritionnelle avec leurs dinoflagellés, dont le fonctionnement diffère largement de celui de la symbiose dinoflagellés-scléractiniaire. La symbiose des octocoralliaires se démarque particulièrement par un apport de carbone autotrophe stable le long du gradient de profondeur, alors que les scléractiniaires connaissent un approvisionnement réduit avec l'augmentation de profondeur. De plus, l'assimilation de composés azotés dissous (par les dinoflagellés ou les symbiotes microbiens) par les octocoralliaires est très inférieure à celle des scléractiniaires. Ces résultats suggèrent que les octocoralliaires dépendent largement de sources alimentaires hétérotrophes pour satisfaire leurs besoins nutritionnels. L'importance de l'hétérotrophie est confirmée par de fortes concentrations tissulaires en biomarqueurs lipidiques spécifiques du zooplancton aux deux profondeurs, avec une augmentation en milieu mésophotique chez certaines espèces. Une telle mixotrophie confère aux octocoralliaires une grande plasticité trophique, ce qui pourrait contribuer à une plus grande résistance aux changements environnementaux en cours.

Mots clés : octocoralliaires | symbiose | autotrophie | hétérotrophie | mésophotique

Octocorals are one of the major groups forming the macrobenthic community of tropical coral reefs. They are notably abundant within disturbed ecosystems where environmental changes have led to the decline of reef-building corals. Although nutrition plays a fundamental role in regulating the abundance of a population, the acquisition of nutrients by octocorals has received little attention to date. In this context, the aims of this thesis were to 1) characterize the acquisition and assimilation of carbon and nitrogen by several octocoral species from the Red Sea, and 2) investigate the nutritional changes along a depth gradient, from the shallow (5 m) down to the upper mesophotic (50 m) reef zone. The results show that octocorals form a nutritional symbiosis with dinoflagellates, but the functioning differs significantly as compared to the scleractinian-dinoflagellate symbiosis. Particularly, the octocoral symbiosis is characterized by a stable supply of autotrophic carbon along the depth gradient, whereas scleractinian corals experience a reduced supply with increase in water depth. In addition, octocorals assimilate along the entire depth gradient significantly less dissolved nitrogen compounds (from dinoflagellates or microbial symbionts) as compared to scleractinian corals. These results suggest that octocorals strongly depend on heterotrophic food sources to meet their nutritional requirements. The importance of heterotrophy is confirmed by high concentrations of lipid biomarkers specific to zooplankton in the octocoral tissue at shallow and mesophotic depths, with an increased concentration for some species in the mesophotic environment. Such mixotrophy provides octocorals with a wide trophic plasticity, which may contribute to their higher resistance to cope with already on-going environmental changes.

Key words: octocoral | symbiosis | autotrophy | heterotrophy | mesophotic