How do algae endosymbionts mediate for their coral host fitness under heat stress? A comprehensive mechanistic overview

Running head: *Algae endosymbionts response in bleached corals*

Montaser A. M. M. Al-Hammady[1a], Thamara Ferreira Silva[2a], Hussein N. M. Hussein[1], Garima Saxena[3], Luzia Valentina Modolo[3], Hildegard Westphal[4,5*], [*]Mohamed A. Farag[6,7]

[1]National Institute of Oceanography and Fisheries (NIOF), Cairo 11516, Egypt.

[2]Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil

[3]BioDiscovery Institute and Department of Biological Sciences, University of North Texas, 1155 Union Circle#311428, Denton, TX, 76203-5017, United States

[4]Leibniz Centre for Tropical Marine Research, Bremen, Germany

[5]University of Bemen, Department of Geosciences, Bremen, Germany

Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, 11562, Egypt [6]

Chemistry Department, School of Sciences & Engineering, The American University in Cairo, [7] New Cairo 11835, Egypt

Corresponding authors: Mohamed A. Farag, email: Mohamed.farag@pharma.cu.edu.eg (M.A. [*] Farag), hildegard.westphal@zmt-bremen.de

[a] denotes for equal contribution

Abstract

Climate change is considered as one of the biggest threats to coral reefs in the next 100 years. The most significant impact of climate change would be the rise in global seawater temperature. A critical turning point which determines the fate of coral reefs depends on how corals and their associated algae respond to the rise in seawater temperature. Symbiotic plasticity may help corals to survive such environmental threats. The zooxanthella *Symbiodinium* is classified into nine clades (A – I), six of which are known to be coral symbionts. As reaction to thermal stress, symbionts are lost to the host by several mechanisms. Physiological models on the importance of nitrogen fixing bacteria, *i.e.*, diazotrophs, for bleached corals indicate that diazotrophically derived nitrogen either allows corals to better withstand bleaching, or increases bleaching by generating unbalanced nutrient requirements that lead to phosphorous starvation of the

Symbiodinium. Reactive oxygen species (ROS) triggered by symbiosis operation of the damaged photosynthetic machinery causes leaks in the host cell, where they overpower cellular antioxidant mechanisms and potentially damage the host tissue. Both symbiotic partners, however, have significant adaptations for managing ROS to mitigate against cell damage as illustrated herein. Such extensive compile of literature in this review suggest that physiological host plasticity and/or symbiotic components clearly plays a significant role in response to thermal stress that may also vary between different species of corals, as many corals may contain specific ecotypes or clades of zooxanthellae which may vary in their ability to withstand thermal stress.

Keywords: Microalgae; climate change; oxidative stress; algae endosymbionts; zooxanthellae

Introduction

Algae symbiosis and its role in the physiology of the coral and anemone holobiont

The dinoflagellate alga *Symbiodinium* establishes symbiotic relationships with cnidarians (corals and anemones) with mutual benefits allowing for the survival of both symbionts involved in such interaction (Gilbert et al., 2010). Indeed, the relationship (also known as co-development) between the organisms can become very intimate by outsourcing some of the signaling mechanisms to the symbiotic partner. As a result, a new species may arise from the assembly of genetically varied species (Gilbert et al., 2010). Such co-development often imparts improved traits such as thermotolerance, thereby potentially increasing the resilience of organisms adapt to environmental change (Gilbert et al., 2010).

Many cnidarians form symbiotic associations with unicellular photosymbiotic algae, namely zooxanthellae, as well as with cyanobacteria. The genus *Symbiodinium* of photosynthetic dinoflagellate algae is endosymbiotic and encompasses a multitude of species that have originated in the early Eocene period some 50 million years ago (Stambler, 2011). The diversification of the genus started with the formation of Clades A and E around 15 million years ago, with declining ocean temperature (Pochon, Montoya-Burgos, Stadelmann, & Pawlowski, 2006), with significant genetic and phenotypic variations observed nowadays among *Symbiodinium* taxa. A total of nine clades (A – I)

are distinguished, and six of these clades are known to be cnidarian symbionts (Baker, 2003). *Symbiodinium* lives in symbiosis, for example, with tropical shallow-water *Scleractinian* corals (Gilbert et al., 2010), and with sea anemones *Condylactis gigantea* (Loram et al., 2007), *Cassiopeia xamachana*, *Corculum cardissa*, *Aiptasia pulchella*, *Rhodactis lucida*, *Anthopleura elegantissima* and *Montipora verrucose* (Stambler, 2011). Such mutualistic symbiotic relationship allows corals or sea anemones to provide protection and carbon dioxide supply for the algae to perform photosynthesis. In turn, the alga offers nutrients to the symbionts through photosynthesis (Stambler, 2011).

Coral Bleaching

Climate change is considered one of the biggest threats to coral reefs in the next 100 years (Howells et al., 2020). The most significant impact of climate change is thought to be the rise in seawater temperature, which is likely to increase the frequency and prevalence of coral bleaching in many places worldwide (Slattery, Pankey, & Lesser, 2019). While corals are well adapted to seasonal and daily temperature fluctuations, heatwaves with water temperatures above is 2-4°C average cause coral bleaching in days or weeks, while temperature heat waves in range of 1-2°C show effects a bit later (Al-Hammady, 2013; Howells et al., 2020). Bleaching, turning the coral pale, occurs when the coral's relationship with its photosynthetic symbionts breaks down and when the coral displays a strong reduction in the density of *symbiodinium* (Fisher, Malme, & Dove, 2012; Slattery et al., 2019). The past decade has witnessed a steep rise in coral bleaching events, a trend that is expected to continue to rise in the future (Hoegh-Guldberg et al. 2007; Gilbert et al., 2010) with the increased global warming effect, leading to increased mass mortality of corals. Chemical pollutants like heavy metal and herbicides, light and nutrients deprivation are other examples of environmental stress that leads to coral bleaching (Dani et al., 2016; Baumann et al., 2018).

To better understand coral bleaching under controlled conditions, laboratory treatments have been implemented with 48 h of heat stress exposure at 32°C at the Hawaii Coconut Island , found enough to induce disorganization and decomposition of thylakoid structures in the dinoflagellates evidenced by photo-oxidative damages and disturbance of homeostatic processes (Downs et al., 2013). Such reaction outcomes suggest that either the symbiont needs to adapt to environmental stress (particularly heat stress) or that the coral (host) expats the dinoflagellate (Sammarco & Strychar, 2013).

A critical determinant of the fate of coral reefs depends on how corals and their associated zooxanthellae respond to the rise in seawater temperature (AMMAR, Obuid-Allah, & AL-HAMMADY, 2011). In fact, corals and their zooxanthellae are expected to entail different physiological responses to changes in temperature (Lawson, Possell, Seymour, Raina, & Suggett, 2019), as was shown for photosymbiotic larger benthic foraminifers (Stuhr et al. 2018a,b). The current review capitalizes on the role of dinoflagellate symbiosis during bleaching in the cell physiology of corals and also suggests new steps that can support solutions towards higher reef resilience.

This review focuses on the role of algae harbored inside corals in mitigating against heat stress effect and maintaining the symbiosis with regards to its different action mechanisms. Heat stress is known to cause disruptions in cell cycles, by arresting the cell cycle at G1 stage in clade types B, C and D. In addition to this, large triploid nuclei are also produced by members of Clade D (Fujise et al., 2018). When symbionts are exposed to heat stress, metabolic activities often increase and with further increment in temperature to nearly halt respiration and leading to the disruption of endosymbionts physiology. Alga chloroplasts were determined to be the last structures to be affected by heat stress (Villar et al., 2018) suggestive that heat stress affects the host at first prior to symbiont *i.e*., *Symbiodinium*.

Temperature tolerance of Symbiodinium taxa

The different clades of *Symbiodinium* have varying degrees of thermal tolerance and photosynthetic responses (Robison & Warner, 2006). For instance, Clade D can tolerate higher temperatures than Clade C. Many coral species form an association with one defined zooxanthellae strain (Goulet & Coffroth, 2003). Others, in contrast, such as *Acropora millepora*, *Acropora tenuis*, *Stylophora pistillata* and *Turbinaria reniformis*, host several strains of *Symbiodinium* (Berkelmans & Van Oppen, 2006). Clades A and B also associate with the giant sea anemone *Condylactis gigantea* (Loram et al., 2007), Table (1).

The fate of the photosynthetically fixed carbon shows significant differences between clades A and B with respect to their lipids and amino acids metabolism. The two clades also show coral bleaching at varied temperatures with clade A being more resistant to heat stress than clade B (Loram et al., 2007). Whether resistance to thermal stress is linked to *Symbiodinium* metabolism has yet to be concluded, and to date only few studies have addressed this question.

Table (1). List of *Symbiodinium* clades and its associated Symbionts and location

Symbiodinium Clade	*Genus name*	Thermal tolerance degrees	Symbionts	Location	References
Clade D	*Durusdinium*	High	*Coral reef:* *Acropora millepora*, *Acropora tenuis*, *Turbinaria reniformis Acropora millepora*, *Stylophora pistillata*	San Blas Islands, Panama Indo-Pacific Arabian/Persian Gulf Australia	Goulet & Coffroth, (2003) Berkelmans & Van Oppen, (2006) Yorifuji et al., (2017) Núñez-Pons et al., (2017)
Clade C	*Cladocopium*	Low			
Sub Clade C3		Low	*Acropora millepora, Platygyra daedalea, Acropora aspera Acropora formosa*	Heron Island (Australia)	Fisher, et al., (2012)
Sub Clade C15		High			
Subclades C27		--	*Fungia fungites*	South China Sea	Qin et al., (2019)
subclades C40		--	*Echinopora lamellosa, Hydnophora exesa, Goniopora exesa*		
Clade A	*Sensus stricto*	High	sea anemone: *Condylactis gigantea*	Weinland on the Bermuda platform	Loram et al., (2007)
Clade B	*Breviolum*	Low			
Clade G	*Gerakladium*	High	sea anemone: *Anemonia viridis*	Australia Florida Keys (USA)	Berkelmans & Van Oppen, (2006) (Stéphane Roberty,
Clade F	*Kawagutii*	Low			

The thermotolerance of seven coral species from Heron Island (Australia) that harbor the subclades C3 and C15 showed that C3 have less photosynthetic efficiency compared to C15 (Fisher, et al., 2012). Even at normal temperature, rapid light curves indicated that *Symbiodinium* clade D has less capacity than Clade C to absorb light and, therefore, low electron transport rate. Ability of less light absorption in a symbiont may be attributed to heat tolerance abilities, however, the heat stress declines the growth of the organism (Jones & Berkelmans, 2012). Under stressful conditions, *Symbiodinium* clade D

is less prevalent compared to Clade C holobionts. Clade C holobionts assimilate 22% more nitrogen than Clade D at normal conditions, whereas comparable assimilation rates were observed in both clades under stress conditions (Baker et al., 2013). The experiments with untreated and bleached coral *Stylophora* pistillata showed 5–30-fold higher nitrogen assimilation during bleaching events along with alga cells feeding on nitrogen rich phytoplankton *Synechococcus* (Meunier et al., 2019).

Symbiont shuffling and switching

Symbiont shuffling is a mechanism that occurs when the number of heat-tolerant zooxanthellae algal strains increases over other zooxanthellae strains associated with a coral colony for the sake of protection against coral bleaching events (Gilbert et al., 2010). Thermal tolerance was found to be proportional to the stability of thylakoid and lipid membranes in algal chloroplast. Increased stability is associated with higher levels of unsaturated fatty acids and increased protection against reactive oxygen species (Berkelmans & Van Oppen, 2006). In South China sea, temperature surge resulted in corals beaching, albeit with certain scleractinian corals such as *Galaxea fascicularis* and *Montipora* spp. from three different geographical locations exhibiting plasticity, especially under slow climate change (Tong et al., 2017).

Another plasticity mechanism named 'symbiont switching' involves a complete exchange of the dominant *Symbiodinium* with other *Symbiodinium* species or clades to adapt to environmental changes i.e., increasing temperatures (Jones et al., 2008). A switch to type D1 – 4 under heat stress can aid the survival of the corals (Yorifuji et al., 2017). Experiments performed with *Exaiptasia pallida* anemones associated with *Symbiodinium* clades B, C or D upon gradual thermal stress revealed symbiont switching to clade D (Núñez-Pons et al., 2017). In contrast, coral symbionts in the Arabian/Persian Gulf depict extremely high thermal tolerance while living in symbiosis with *Symbiodinium* clade C with no report of switching or shuffling to clade D. This suggests that this is not a generalized mechanism in coral adaptation to heat stress. Such discrepancy warrants for a large coral taxa or genotypes analysis from different origins to be conclusive about clades thermal tolerance (Hume et al., 2013).

Indeed, the hypothesis that symbiont changes in corals to mitigate heat stress might do not show a general pattern, is supported by further observations e.g., it was observed that *Stylophora pistillata* and *Acropora eurystoma* corals from the Gulf of

Aqaba show thermal resistance during summer heat with no changes in symbiont composition (Bellworthy & Fine, 2017). Several studies suggest that the Gulf of Aqaba (Red Sea) may serve as a reef refugee due to a unique suite of environmental conditions though with no concrete hypothesis behind it (Fine et al., 2013). The Red Sea, as a geologically "young" sea located in one of the warmest regions in the world, has the possibility to provide insight into urgent topics such as speciation processes and the capacity of reef systems to adapt to global climate change (Berumen, et al., 2013)

Aiding survival of corals

With the rise of global sea water temperatures, it is imperative to explore adaptation strategies that potentially can increase the resilience of corals. For instance, short-term (10 days) pre-conditioning of the holobiont *Acropora millepora* to heat stress (3°C below the bleaching event) led to less bleaching events (Bellantuono et al., 2012). Another study suggested a decline of the bleaching effect following acclimatization of *Symbiodinium* spp. to increased temperatures (Takahashi et al., 2013). A study of repeated bleaching and recovery was carried out with the holobiont *Montastraea cavernosa* (Silverstein et al., 2015). Symbiotic switching supported the recovery and prevented bleaching when compared to prior heat exposure (Silverstein et al., 2015). Priming effect in plants involves subjecting the plants with certain stress so that they can have resistance against such stress in the future. A similar effect of thermal priming was tested in *Acropora millepora.* The priming affected photosynthesis but did not alter the bleaching susceptibility of the coral (Middlebrook, Anthony, Hoegh-Guldberg, & Dove, 2012).

During temperature stress, significant amounts of autotrophic carbon is retained in the symbionts and with decrease in carbon partition leading to reduced coral growth and calcification. Supplementation of the symbiont with heterotrophic nutrients (coral polyps feeding on particles in the water column) could assist ambient carbon flow rates although the severity of heat stress or coral bleaching is not attenuated (Tremblay et al., 2016).

South China Sea shows a diversity in Symbiodiniaceae subclades in corals of C15, C27 and C40 that is interpreted to increase resilience against the increasing temperatures (Qin et al., 2019). Consecutive heat stress on coral colonies transplanted in Florida Keys National Marine Sanctuary, showed that larval stages of the corals *Porites astreoides* and *Siderastrea siderea* were infested with seven different clades of *Symbiodinium* and then exposed to heat stress. Colonies of *P. astreoides* showed stronger bleaching compared to S. sidereal (Smith et al., 2019). Consequently, it can be concluded that resilience to bleaching differs not only by coral species but is also influenced by the local abundance

of algal species. Response in dealing with thermal stress was highly variable in experiments performed with the coral *Montipora digitate* from the count of 16 colonies, with 6 colonies tolerated thermal stress, 7 showed sensitivities, while three colonies perished (Kavousi et al., 2020).

Mechanisms in algal endosymbionts that mediate for its natural host fitness or survival under heat stress conditions

Over the next sections, the very proximate cellular events that occur within the Cnidarian–dinoflagellate partnership shall be discussed that ultimately lead to loss of symbionts from host tissue and cause bleaching. We are increasingly gaining a full understanding of the early events that happen in the symbionts but still to lack a deep grasp of the distal events in host tissues that ultimately lead to bleaching. Most previous reviews have focused on coral responses with less emphasis on clearly dissecting the algal response towards heat stress which could be detrimental in the cascade of the coral dysbiosis. This review shall focus in depth on how algal endosymbionts respond to elevated sea water temperatures at the different cellular levels i.e., physiological, molecular, protein and metabolite levels.

Physiological changes

The capacity of corals to cope with the predicted global climate change over the next century is expected to lie in their physiological acclimatization mechanisms. Corals show rapid changes in behavior, morphology and physiology that enable them to survive the ever-changing temperature of the seawater, a scenario that shows considerable physiological flexibility. Recently, several authors reported on the different effects of thermal stresses on coral reef physiology (Bednarz et al., 2019; Dang, Pierangelini, Roberty, & Cardol, 2019; E. M. Gibbin et al., 2018; Lawson et al., 2019; Meunier et al., 2019; Quigley, Randall, van Oppen, & Bay, 2020; Slattery et al., 2019; Weis, 2019).

Symbiotic plasticity as physiological response to thermal stress

Through expanding their niche field, symbiotic plasticity may help corals to survive environmental threats (Sampayo, Ridgway, Bongaerts, & Hoegh-Guldberg, 2008; Silverstein, Cunning, & Baker, 2015; Weis, 2019). Symbiosis symbolizes this dichotomy: the relationship is particularly vulnerable to bleaching, however symbionts that withstand stress may often reduce bleaching (Howells et al., 2020; Silverstein et al., 2015). (Tong et al., 2017) indicated that corals are able to control *Symbiodinium*

238 population mechanisms at various temperatures and to aid them to respond to the rapid
239 change in climate. The 9 clades of *Symbiodinium* (*i.e.*, clades A – I) show varying
240 photosynthetic efficiencies and heat resistance capabilities (Pochon & Gates, 2010; Qin et
241 al., 2019). While clades A–D are the major *Symbiodinium* harbored within scleractinian
242 corals, clades F and G are identified in corals and have been suggested for defined
243 functional roles in the coral reef ecosystem (A. C. Baker, 2003) (**Fig. 1**). Clade A is
244 classified into the genus *Symbiodinium sensus stricto,* while clade B refers to the genus
245 *Breviolum* (Dang et al., 2019). Among the family of *Symbiodiniaceae*, certain
246 *Symbiodinium* or *Durusdinium* species are reported to have higher vulnerability to
247 elevated temperatures being more resistance to acute thermal stress (Silverstein, Cunning,
248 & Baker, 2017). However, coral bleaching reports to conclusively correlate with the
249 absence of thermo-tolerant *Symbiodiniaceae* taxa, and the factors of functional diversity
250 among broadly phylogenetically distributed taxa are still largely unresolved (Suggett,
251 Warner, & Leggat, 2017). With regards to differential thermal responses across
252 *Symbiodinium* clades, (Stéphane Roberty et al., 2016) reported that *Symbiodinium sensus*
253 *stricto* (clade A) was more oxidative stress-tolerant than *S. kawagutii* (clade F) (**Table1**).

254 Photosynthetic electron flux represents another physiological mechanism that may
255 lead to thermal tolerance. (Dang et al., 2019; Stéphane Roberty et al., 2016) suggested
256 that the photosynthetic electron transmission chain plasticity and antioxidant network
257 outlet in some different species of Symbiodiniaceae contribute to the thermal tolerance of
258 the holobiont. Photosynthetic plasticity gadget may embrace greater reactive oxygen
259 species tolerance, higher photoprotective capacities (M. Warner & Berry-Lowe, 2006),
260 and some more efficient electron sinks (M. E. Warner & Suggett, 2016). The potential of
261 cyclic electron flows across photosystem I at temperature stress is greater in clade A than
262 in the clade B (Aihara, Takahashi, & Minagawa, 2016). Moreover, the degree to which
263 the cyclic electron flows and/or the O_2^-dependent electron flow occurs among
264 *Symbiodiniaceae* taxa and the degree of either mechanisms contribution to the species'
265 thermal tolerance is still unknown.

266 Coral photosymbionts within the family of Symbiodiniaceae typically display a high
267 degree of variations in features and local adaptation as reviewed in (Quigley et al., 2020).
268 The change in the symbiont type dominants from *Symbiodinium* clade C to D
269 (*Cladocopium* and *Durusdinium*) was found to improve the resistance of adult colonies to
270 coral bleaching by up to 1.5 ° C (Berkelmans & Van Oppen, 2006), illustrating the role of

Durusdinium (*Symbiodinium* clade D) in bleaching resistance (Mizerek, Baird, & Madin, 2018). Change in Symbiodiniaceae clade during bleaching can also occur in juveniles (Yorifuji, Harii, Nakamura, & Fudo, 2017). Juveniles of the coral species *Acropora tenuis* harboring clade C1 experienced greater mortality compared to those with clade D, during thermal stress (Yuyama, Nakamura, Higuchi, & Hidaka, 2016). In contrast, juvenile corals with mixed populations of types A3, C1, and D1-4 were found more tolerant to thermal stress than juveniles harbouring D1-4 (Quigley et al., 2020; Yorifuji et al., 2017). This demonstrates the significance of the specific symbionts in mediating resistance to the host coral environment. Likewise, coral juveniles harboring *S. microadriaticum* developed better than *S. minutum* (McIlroy & Coffroth, 2017), as well as skeletal growth in juveniles with clade D compared with clade C1 (Yuyama & Higuchi, 2014). Whether such improved growth is associated with improved photosynthetic rates in these *Symbiodinium* has yet to be determined. Host thermal tolerance variations (Cunning, Gillette, Capo, Galvez, & Baker, 2015) are rarely studied in context to the identification of Symbiodiniaceae consortium harbored inside corals, or to be engineered inside corals to determine thermal tolerance impact experimentally on host-symbiotic interaction. (Abrego, Ulstrup, Willis, & van Oppen, 2008) (Manzello et al., 2019).

The hypothesis of coral algal symbiosis flexibility or plasticity is not fully explained (A. C. Baker, 2003; Hoadley et al., 2019; M. E. Warner & Suggett, 2016), though recognized to play a significant role in response to global change (Bellantuono, Hoegh-Guldberg, & Rodriguez-Lanetty, 2012). (Silverstein et al., 2015) reported that some corals may change their *Symbiodinium* communities to respond to the different environmental impacts, by utilizing heat-tolerant *Symbiodinium* in a higher temperature region, a newly established symbiosis of coral and algae has been proposed to be more effective for coral survival. However, (Thomas, Kendrick, Kennington, Richards, & Stat, 2014) an opposite pattern was observed that some species of coral still maintain a very stable symbiosis of coral-algae during thermal stress.

Under thermal stress, it has been revealed that coral reefs hosting *Symbiodinium* may be put in a continuous chain ranging from mutualist to parasite, that could decrease host health (M. Lesser, Stat, & Gates, 2013; Pettay, Wham, Smith, Iglesias-Prieto, & LaJeunesse, 2015). However, evidence of parasitism and inability in coral food interdependence is dependent on the host fitness associated with certain types of *Symbiodinium* (M. Lesser et al., 2013; Wooldridge, 2013). Thus, we suggest that the

ability to change the symbiosis of coral-algae in response to thermal stress is still debatable considering the discrepancy in thermal stress response in coral reef and its symbiotic zooxanthellae.

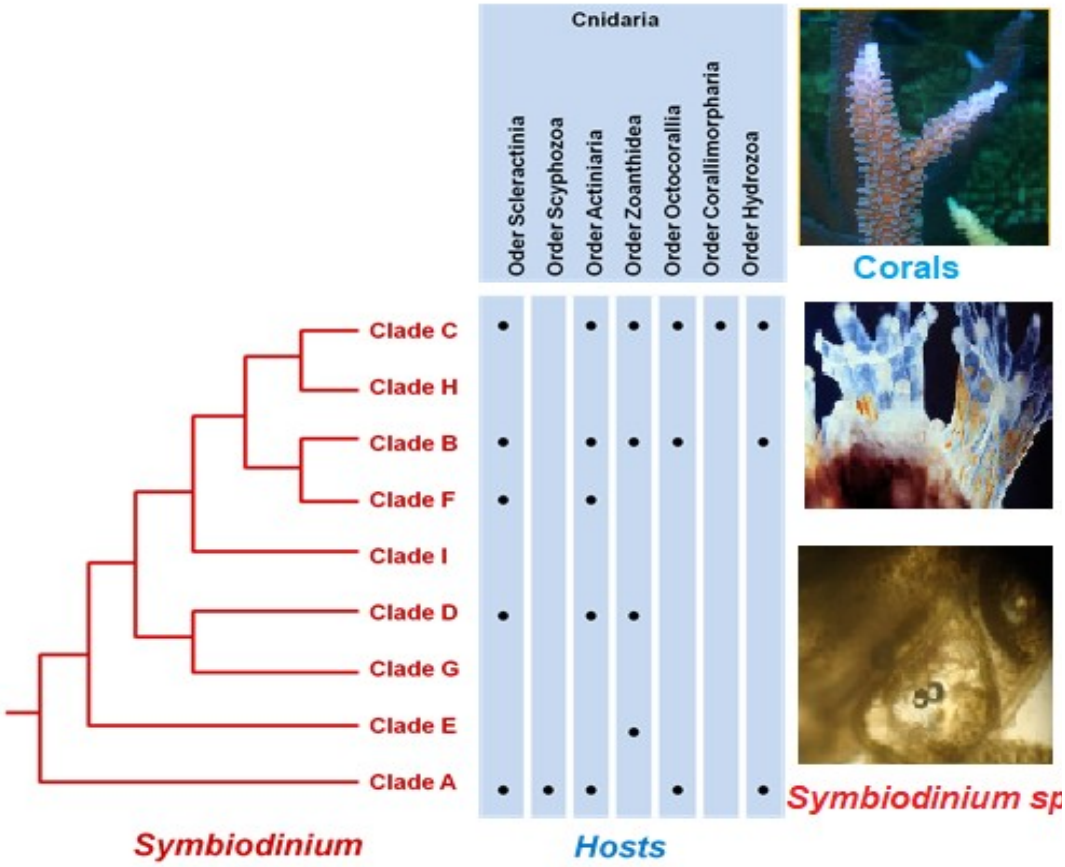

Fig. 1. Phylogenetic relationships among *Symbiodinium* 's major 9 clades; 5 clades (Clades A, D, F, B and C) are associated to coral reef (order: Scleractinia) as well as order: Actiniaria, order: Scyphozoa symbiontant with one clade (Clade A), 4 clades are related to order Zoanthidae (Clades E, D, B and C), 3 clades (Clades A, B and C) for order: Octocorallia as well as order: Hydrozoa, and one Clade (Clade C) associated to order: Corallimorpharia

Physiological mechanisms underlying symbionts exit from host corals in response to thermal stress

Cellular pathways allow the coral host to sacrifice symbionts under thermal stress conditions (El Rahmany, 2019). Nevertheless, the manner in which such processes operate in the wider environmental scenarios of bleaching showed discrepancy. Symbionts are apparently degrading *in situ* in host cells (El Rahmany, 2019; Sammarco &

Strychar, 2013), either due to the death of symbionts themselves and decay from the consequences of thermal stress, or owing to the host deliberately killing the symbionts and ultimately digesting or expelling them (Ashok et al., 2020; Bieri, Onishi, Xiang, Grossman, & Pringle, 2016). There is also some evidence that symbionts are exocytosed alive from stressed corals. The exocytosis profiles and free symbionts have apparently been reported in the host gastric cavity in bleaching animals. Host cell detachment represents another process by which the symbionts are expelled out by the host (Brown, Le Tissier, & Bythell, 1995). Interestingly, (Qin et al., 2019) reported the release of living whole host cells from heat and cold stressed bleaching corals, with symbionts still inside though not yet explained and whether these cells can form another colony under favored conditions. Apoptosis is another action of controlled cell death that is observed in symbionts and their host coral (Weis, 2008), characterized by a group of signaling cascades that leads to cell death. Once it is initiated, morphological changes occur including shrinkage of cells, DNA fragmentation, and chromatin condensation forming apoptotic bodies containing packed cellular debris; it reduces tissue damage from thermal stress and thus to maintain tissue balance by deleting damaged cells (S. Dunn, Thomason, Le Tissier, & Bythell, 2004). In comparison to apoptosis, necrosis is spontaneous cell death, primarily due to external stimuli, which contribute to an empty swelling tissue leading to the break-out of plasma and the release of cellular materials. (Weis, 2008) (**Fig. 2**).

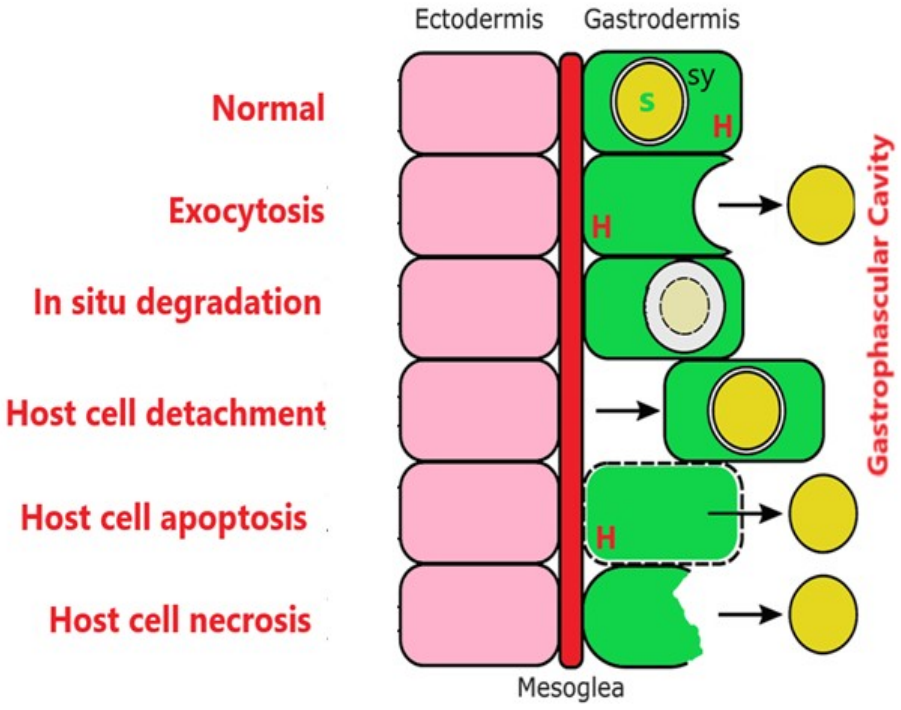

Fig. 2 Physiological mechanisms underlying symbionts exit from host corals in response to thermal stress, the cellular mesoglea (M) is anchored to normal host cells (H). Host vacuoles or symbiosomes (Sy) contain symbionts (S). Five different types of symbiont-loss cell pathways in tissue coral host exist. **1**): symbionts are lost due to either in situ degradation, or are digested or discarded in the host cell (not detected), **2**): within the gastrovascular cavity, symbionts damaged by exocytosis are set free, **3**): during host cell separation, host cells with their symbionts already remain inside, are separated from mesoglyphic and adjacent cells and unchained into the gastrovascular cavity, **4**): coral host cells undergoing apoptosis, 5): host cells which die from necrosis.

Thermal stress causes a heterotrophy of corals on diazotrophs

Biological atmospheric dinitrogen (N_2) fixation of specific prokaryotes (diazotrophs) is a globally important physiological process for marine ecosystems. Scleractinian corals, in addition to their symbiosis with *Symbiodinium*, live in close association with a diverse range of other microorganisms, including diazotrophs located in coral tissue, mucus or skeleton (reviewed in (Bednarz et al., 2019). Corals can obtain nitrogen either from zooxanthellae, or from diazotrophic symbiotic communities (Benavides et al., 2016; Lema, Willis, & Bourne, 2012). (Meunier et al., 2019) reported that bleached corals lose most of their nitrogen supply from *Symbiodinium* and with rising seawater temperature often to limit the capacity to accumulate coral nitrogen. Several studies reported overconsumption of mesoplankton and macroplankton by corals during thermal stress to maintain a sufficient supply of essential nutrients during recovery from bleaching (Goldberg, 2018). Reef feeding capacity was documented on smaller plankton fractions, i.e., picoplankton (0.2–2μm) and nanoplankton (2–20μm), but only one study reported an increase in the ingestion of bacteria and picoflagellates by bleached corals (Tremblay, Naumann, Sikorski, Grover, & Ferrier-Pagès, 2012). The synthesis of nitrogen from planktonic diazotrophs has been shown recently in bleached corals (Benavides et al., 2016), through several mechanisms as revealed using radioactive $15N_2$-label to include: (1) direct feeding of coelenteron- digested planktonic diazotrophs, (2) uptake and extracellular release of ^{15}N-dissolved nitrogen compounds fixed by planktonic diazotrophs, and (3) ingestion of ^{15}N enhanced nondiazotrophic plankton by diazotrophic mediated nitrogen movement. Other studies demonstrated that diazotroph N_2 fixation increased in bleached corals (Bednarz et al., 2019). The production of nitrogen resulting from diazotrophic planktonic behavior in heat induced coral reef has never been examined

(Meunier et al., 2019). Increased diazotrophic N_2 fixation and transfer of diazotrophically derived nitrogen to *Symbiodinium* is likely to occur, especially under limited external nutrient availability or during bleaching (Bednarz, Grover, Maguer, Fine, & Ferrier-Pagès, 2017). Physiological models on the importance of diazotrophs in the functioning of coral holobionts revealed that diazotrophically derived nitrogen either by (i) allowing coral reefs to better withstand and recover from bleaching events by providing the requisite N mechanisms for cell repair to create photosynthetic and photoprotective pigments (Bednarz et al., 2017) or (ii) increase bleaching by generating unbalanced nutrient requirements that lead to phosphorous (P) starvation of the *Symbiodinium* (Pogoreutz et al., 2017). These paradoxical models highlight the need for further studies on determining the diversity and activity of diazotrophs within coral holobionts under the different environmental conditions to be conclusive.

The availability of N overload that depress the threshold of bleaching in corals by moving *Symbiodinium* from N restricted to phosphorus (P) deficient state (Wiedenmann et al., 2013). These stoichiometric changes may lead to phospholipid substitution with sulpholipids in the thylakoid chloroplast membranes, a typical response in photoautotrophs during restricted availability of P (Frentzen, 2004). As the thylakoid membrane 's lipid composition is closely associated with the assembly and efficiency of the photosynthetic machinery, it may determine susceptibility to bleaching in *Symbiodinium* (Tchernov et al., 2004). Therefore, increasing N availability will eventually increase the sensitivity of coral bleaching (Wiedenmann et al., 2013). Recent study on *Orbicella faveolata* reported that extra nitrogen at high temperature drives symbionts towards parasitism and dysregulation of food dynamics

Thermal stress increases ROS levels

Reactive oxygen species (ROS) triggered by symbiosis operation of damaged photosynthetic machinery cause leaks in the host cell, where they overpower cellular antioxidant mechanisms and damage the host tissue (C. Downs, McDougall, Woodley, Fauth, & Richmond, 2013; M. P. Lesser, 2006)[56, 57](Downs et al., 2013; & Lesser, 2006). If ROS production exceeds the mitigation action of antioxidant biological system potential, it leads to macromolecule damage i.e., protein, lipid and DNA (Nielsen, Petrou, & Gates, 2018; Weis, 2008). Furthermore, (McGinty, Pieczonka, & Mydlarz, 2012) revealed for mitochondria and chlorophyll degradation in *Symbiodinium*, and in host

tissue (S. R. Dunn, Pernice, Green, Hoegh-Guldberg, & Dove, 2012), that supports major physiological disorder in coral bleaching. Even though considerable efforts have been made to confirm ROS involvement in bleaching of coral, the degree of the relationship between ROS production coexistence and host physiological pressure is still obscure. **Fig. 3** illustrates action mechanisms that occur in the symbiont as the bleaching process proceeds firstly, with the activation of photosynthesis in the symbiont chloroplasts. Such dynamic photosynthetic events have recently been extensively examined (Weis, 2008). Both symbiotic partners have significant adaptations for managing ROS to mitigate against cell damage (Pey, Zamoum, Christen, Merle, & Furla, 2017), mediated *via* the expression of a wide range of ROS handling enzymes i.e., catalase, ascorbate peroxidase and multiple superoxide dismutase (SOD) (Smith, Suggett, & Baker, 2005; Weis, 2008). SOD serves as the first line of protection in an critical phase of detoxifications (S. R. Dunn et al., 2012) acting *via* transforming superoxide anions (O_2^-), formed by the hydrogen peroxide (H_2O_2) to minimize the accumulation of these cytotoxic products, H_2O_2 being converted to harmless H_2O and O_2 by a significant amount of further enzymatic and/or non-enzymatic antioxidants i.e., glutathione peroxidase (Gaps) in combination with co-factor glutathione, in addition to ascorbate peroxidase (APX) and catalase enzyme. ROS levels show an increase under suboptimal conditions due to the displacement between output and protective mechanism, with once adaptive ROS response to overspread during stress, a subsequent thermal hazard cascades to occur.

The zooxanthaella released from the symbiotic system may be associated with an excessive production of reactive oxygen species (ROS) due to the stress imposed. In case of heat stress, an increase of roughly 70% in ROS levels did not negatively affect the symbionts physiology, which suggested that the overproduction of ROS during the stress is not the reason for expulsion of the algal species (Nielsen et al., 2018). Thermal stress reduces photochemistry efficiency by inflicting damage on numerous photosynthetic proteins, such as; Photosystem II (PSII) (Weis, 2008), light harvesting complex (Takahashi, Whitney, Itoh, Maruyama, & Badger, 2008), Calvin cycle (Weis, 2008), or the thylakoid membrane (Tchernov et al., 2004). As D1 protein is considered one of the core structural proteins of PSII reaction center and responsible for providing the plastoquinone binding pocket, it plays a key role in preserving the intact photosynthetic electron transport chain (McGinley et al., 2012). Particularly important for *Symbiodinium*, high temperature periods interfere with the cellular maintenance of the D1 protein, either

by constraining the overall rate of protein repair or by persuading a faster rate of PSII photo-inactivation (Hill, Brown, DeZeeuw, Campbell, & Ralph, 2011). This unevenness causes a disorder known as photo inhibition, in which a fast accumulation of photo-damaged D1 eventually decreases the production of PSII reaction centers, thus causing photosynthetic dysfunction (McGinley et al., 2012). Such long-lasting photoinhibition episodes are recognized as a factor responsible for a large scale of coral bleaching cases (Takahashi, Nakamura, Sakamizu, Woesik, & Yamasaki, 2004). Although there is less sign of photoinhibition of photosystem I (PSI) and subsequent degeneration of the two main core PSI reaction center heterodimer core proteins encoded with *psaA* and *psaB* chloroplast genes (Smith et al., 2005), photoinhibition and PSI protein loss in some plants may result in chilling stress (Ivanov, Allakhverdiev, Huner, & Murata, 2012).

Thermal stress causes further symbiotic chloroplast and photosynthetic system photoinhibition and damage in at least three interconnected mechanisms to work together and ultimately lead to coral bleaching. (i) The D1 protein is a part of the water division multifaceted in the thylakoid membranes of photosystem II inhabitants (Ohad, 1994). Thermal stress damages the D1 protein in *Symbiodinium*, and causes overriding of the normal repair mechanism (Weis, 2008), and moreover the repair mechanism itself is damaged (Takahashi et al., 2004). The D1 protein damage results in agitation energy backup and malfunction of photosystem II (Hoadley et al., 2019). (ii) Also, the dark reaction of photosynthesis is damaged by heat and light so as to reduce carbon fixation (Jones & Berkelmans, 2012). Ribulose bisphospate carboxylase oxygenase (Rubisco), the enzyme responsible for carboxylation may be the most affected area (M. P. Lesser, 2006). This results in the reduction of ATP and NADPH levels that in turn results in dysfunction of the Photosystem II (S. R. Dunn et al., 2012). (iii) Thermal stress may further damage the thylakoid membranes directly (Tchernov et al., 2004), resulting in a strong electron decoupling in both photosystems. The photosynthetic system therefore continues to produce electrons, albeit without the production of NADPH and ATP. The accumulation of electrons by either of the above mechanisms is believed to finally generate ROS production in symbiosis. As described in the Mehler reaction in photosystem I, overabundant electron production reduces O_2 level, reduction in $NADP^+$ level leading to the production of a highly elevated ROS and (O_2^-) (Tchernov et al., 2004). The most deleterious effect lies in the presence of ferrous iron (Fe_2^+), H_2O_2 that can react to form the most reactive hydroxyl radical (·OH). Furthermore, photochemically overabundant

electrons react with pigments and O_2 to produce a highly reactive singlet oxygen ($^1O_2$) (El Rahmany, 2019; M. P. Lesser, 2006), that additionally aggravates the problem by reacting with other D1 proteins and damaging pigments in the algae thylakoid membrane (Weis, 2008).

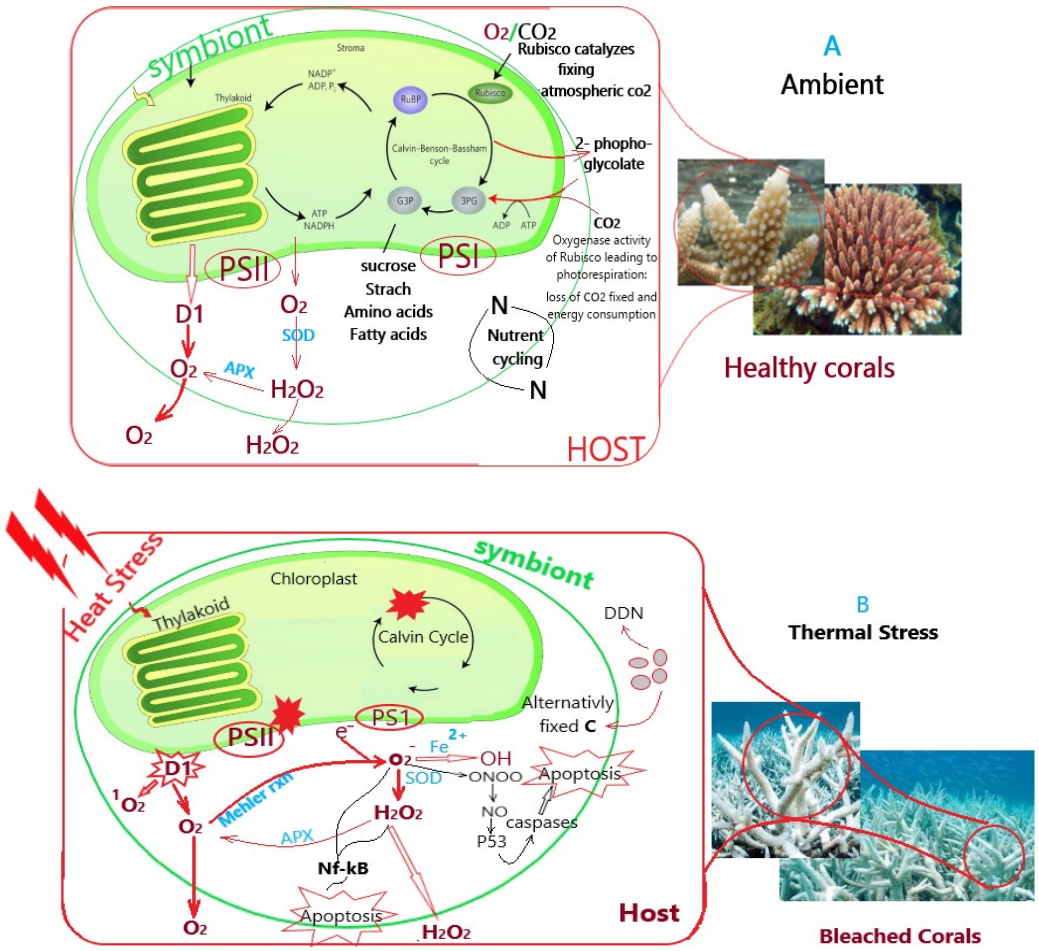

Fig. 3. Schematic overview of the oxygen assimilation pathways in *Symbiodinium* in ambient (A) host cells, and thermal stress conditions (B). The photosynthetic apparatus consisting of Photosystem I (PSI) and Photosystem II (PSII) on the thylakoid, normally operates under ambient conditions and produces large amounts of oxygen dispersed into the host. ROS formed is restored to oxygen with dismutase superoxide and peroxidase ascorbate (APX). In at least three cellular targets under heat stress, damaged photosynthetic apparatus occurs: the protein PSII D1, the process of Calvin, and the thylakoid membranes. Such damage results in a large amount of ROS in form of single oxygen ($^1O_2$) and superoxide (O^-_2) that overwhelm the oxygen- assimilation. O_2 is converted into both the most reactive hydroxyl radical (· OH) and the more stable and highly diffusible hydrogen peroxide (H_2O_2) that can leak into the host. H_2O_2, coming from both the symbiont and the

host, triggers the activation of innate immunity transcription factor NF-£B. In addition, it stimulates apoptosis directly and/or induces the nitric oxide synthase (iNOS) expression that produces nitric oxide (NO). NO may react with O_2^- forming a highly reactive peroxynitrite ($ONOO^-$) that damages the membrane of mitochondria.

Nitric oxide, inter partner signaling and the role of innate immunity for host in bleaching

Nitric oxide (NO) is a membrane-permeable gas molecule that plays an important role in physiological and developmental functions and in the defense of living organisms. In corals, NO can play a vital role in coral bleaching in addition to its role during interactions between coral pathogens (D. M. Baker, Freeman, Wong, Fogel, & Knowlton, 2018; Fang, Huang, & Lin, 1997). It may react with O_2^- to form a potent and highly diffuse peroxynitrite oxidant ($ONOO^-$) (Marangoni et al., 2019). NO host tissue levels increase dramatically in response to increased temperature or symbiont photosynthesis inhibition by photosystem II inhibition (Weis, 2008). (Bouchard & Yamasaki, 2008) similarly reported that NO produced in *Symbiodinium microadriaticum* following exposure to severe thermal stress caused a reduction in PSII (Fv/Fm) photosynthetic capacity.

The original source of the NO is still not clear, whether its source is the host or the algal symbiont, or both. (Detournay, Schnitzler, Poole, & Weis, 2012) suggested that nitric oxide synthase (NOS) is induced from the coral host that catalyzes arginine transformation, NADPH and O_2 to NO. Another hypothesis (Fang et al., 1997) suggests that high level of ROS in the host tissue leads to the induction of an innate immune response by inducing NF-κB. Regardless of the NO source in the host, the exact target of NO and following events that cause symbiont loss are still unknown (Hawkins, Bradley, & Davy, 2013). NO and O_2^- combine to form $ONOO^-$ that damage the mitochondrial membrane, that, in turn results in the release of potent pro-apoptotic molecules that initiate an apoptotic spill (Weis, 2008) (Fig 3).

Thermal stress causes CO_2 fixation dysfunction

The relationship between corals and *Symbiodinium sp.* involves several symbiotic interactions. Chemical interchange involves inorganic nutrients such as CO_2, phosphate and ammonium provided by coral with photosynthetic products such as glycerol and amino acids from *Symbiodinium* (E. Gibbin, Banc-Prandi, Fine, Comment, & Meibom,

2020; Hillyer, Tumanov, Villas-Bôas, & Davy, 2016; Jiang & Lu, 2019; Lilley, Ralph, & Larkum, 2010; Yellowlees, Rees, & Leggat, 2008). Thermal stress appears to be associated with a dysfunction of the CO_2 fixation mechanism in the zooxanthellae. (Wooldridge, 2009) presented a cellular model that indicates that the bleaching response may initially be triggered by the failure of the coral host to maintain a sufficient supply of CO_2 for its endosymbiont partner, particularly during periods of thermal stress when the photosynthetic demand for CO_2 is maximal. Thermal stress that inhibits photosynthesis does not affect though carbon-fixation mechanism in *Symbiodinium*, but Rubisco enzyme activity decreased significantly when subjected to elevated temperatures above 37 ° C (Fisher et al., 2012; Hillyer, Dias, Lutz, Roessner, & Davy, 2017; Lilley et al., 2010). Rubisco, the enzyme which catalyzes the fixation of inorganic carbon dioxide has yet to be cloned from dinoflagellates, though extensively examined from a wide range of bacterial, algal and higher plant sources (Long et al., 2018; Orr et al., 2020). Understanding of the Rubisco enzymatic properties of dinoflagellates may help to better understand the processes in *Symbiodinium* during bleaching events. Isolated Rubisco activities from *Symbiodinium* decreased above 30 °C with almost complete loss of activity above 36 °C (Lilley et al., 2010). On the other hand, the potential for CO_2 limiting conditions to inhibit Rubisco enzyme of the zooxanthellae is reported from other studies confirming that both photosynthesis and calcification in symbiotic reef corals are mostly carbon limited (Herfort, Thake, & Taubner, 2008; Marubini & Davies, 1996; Stephane Roberty, Béraud, Grover, & Ferrier-Pagès, 2020).

Both symbiont partners have developed diverse ways to acquire and concentrate carbon for photosynthesis (Stephane Roberty et al., 2020). In addition to respiration-derived CO_2, corals provide dissolved inorganic carbon (DIC) from the water column (mainly HCO^-_3) to the symbionts through a combination of external and intracellular carbonic anhydrase (Tansik, Fitt, & Hopkinson, 2017). Effective acquisition of (DIC) by symbionts is a key process for the health of the symbiotic association as the amount of DIC fixed and assimilated by the symbionts will also determine the amount of photosynthesis transferred to the host for its own needs (E. Gibbin et al., 2020). Although oceanic DIC levels are high, DIC may be a source of limitation for the symbiont's primary production (Tansik et al., 2017), though not generalized in all coral species or environmental conditions (Buxton, Badger, & Ralph, 2009). The adequacy of DIC 's supply to the symbionts (in

terms of demand saturation) depends on diverse environmental factors, but also on symbiont density or metabolic holobiont needs, and is still not well understood.

Molecular Changes under heat stress

The complementary development of DNA microarrays (cDNA) for non-model organisms has enabled significant advances in coral genomics and bioinformatics studies, allowing large-scale investigations of the expression for thousands of genes at the same time. Microarrays of RNA were used to study the transcriptomic responses for thermal stress in *Stylophora pistillata* (Maor−Landaw et al., 2014), *Acropora palmata* (DeSalvo et al., 2010), *Montastraea faveolata* (DeSalvo et al., 2010), *Porites astreoides* and in the larvae of *Montastraea faveolata* (Polato et al., 2010), *Acropora palmata* (Portune, Voolstra, Medina, & Szmant, 2010), and *Acropora millepora* (RODRIGUEZ−LANETTY, Harii, & Hoegh−Guldberg, 2009).

To support evidence for physiological changes to occur inside bleached corals under thermal stress, several molecular studies were undertaken. Variation in response from one individual to another may be potential due to changes in gene expression (Maor−Landaw et al., 2014; Rose, Seneca, & Palumbi, 2016). Gene expression characterizes cellular processes involved in coral response to heat stress (Maor-Landaw & Levy, 2016; Meron et al., 2019). Under thermal stress, metabolism genes are frequently up-regulated to meet the energy requirements of the cells, but misaligned proteins tend to accumulate in the endoplasmic reticulum during thermal stress and trigger unfolded protein response resulting in cell cycle detention and apoptosis (Hetz, 2012; Maor−Landaw et al., 2014).

Patterns of gene expression characterize the cellular processes engaged in the coral's response to thermal stress (Meron et al., 2019). Large portions of differentially expressed genes are specific to the symbiotic state, suggesting that host coral (or sea anemone) may react to environmental changes in a symbiotic state-dependent manner (Ishii et al., 2019). Investigation of the integral light harvesting complex (LHC) gene families [chlorophyll a-chlorophyll c2-peridinin protein complex (acpPC)] owing to their distinctive gene configuration were monitored as hallmark of the bleaching events in *Symbiodinium* (Ishii et al., 2019; Takahashi et al., 2008; Xiang, Nelson, Rodriguez, Tolleter, & Grossman, 2015). Studies have identified cellular processes involving corals during heat stress,

598 including the reconstruction of cytoskeletons, calcification, Ca^{2+} homeostasis, cell death,
599 metabolism and antioxidant and chaperone synthesis. Molecular changes proved to be
600 generalized regarding the underlying cellular bleaching mechanisms, but though with no
601 specific pathway identified (Bieri et al., 2016; Gierz, Forêt, & Leggat, 2017; Seneca &
602 Palumbi, 2015; Weis, 2008). A total of 62 KEGG-based up-regulated genes were
603 described by (Maor−Landaw et al., 2014) in three cell pathways: ER protein synthesis,
604 ubiquitin-mediated proteolysis and proteasome function. An approach to determine stress
605 response that would identify protein-encoding genes up-regulated following thermal stress
606 and their established functional roles based on concepts in model organisms was
607 proposed.

608 Heat shock proteins (HSPs) are molecular chaperones that play a key role in the
609 maintenance of regular cellular functions in protein transport, aggregation, degradation
610 folding and unfolding, (Sørensen, Kristensen, & Loeschcke, 2003). Based on their
611 molecular mass (kDa), HSPs are organized into two main families, namely HSP 70 and
612 HSP 90. Hsp70 isoforms originate from the gene duplication process and exhibiting
613 differential gene expression profiles under various stress conditions (Lin et al., 2001).
614 HSP 90 representatives are among the most abundant proteins and amount to 1–2 % of all
615 cell proteins under non-stressed conditions (Csermely, Schnaider, So, Prohászka, &
616 Nardai, 1998). Thermal stress is considered the major factor influencing the degree of
617 HSP expression, so far HSPs may be used as stress markers for many species (N. N.
618 Rosic, Pernice, Dove, Dunn, & Hoegh-Guldberg, 2011). HSPs were up-regulated after
619 exposure to thermal stress in reef building coral *Acropora millepora* (N. N. Rosic et al.,
620 2011), *Montastraea faveolata* (C. A. Downs, Mueller, Phillips, Fauth, & Woodley, 2000),
621 *Goniopora djiboutiensis* (Sharp, Brown, & Miller, 1997) and others (Van Oppen & Gates,
622 2006). Also, host HSP 70 protein level increased in two coral species *Stylophora*
623 *pistillata* and *Porites cylindrica* post exposure to term thermal stress (Fitt et al., 2009).
624 Microarray data confirmed that thermal stress stimulate up-regulation of Hsp90 gene in
625 the host coral *M. faveolata* (Voolstra et al., 2009), as well as of Hsp70 and Hsp90 in
626 *Acropora millepora* larvae (RODRIGUEZ−LANETTY et al., 2009).
627 In a study on *S. Cladocopium* (clade C) colonized coral *Acropora millepora* reported
628 that HSP90 and HSP70 showed differential expression in response to heat stress and
629 suggestive that multiple factors, including environmental and phylogenetic constraints to
630 regulate symbiont HSP expression.

Symbiodinium from genotype clade D increases significantly the coral host's bleaching thr esholds relative to clade C (Barfield, Aglyamova, Bay, & Matz, 2018; Ladner, Barshis, & Palumbi, 2012). Another HR-DEG symbiont, FKB62, encoded a peptidyl prolyl isomerase FKBP62, which interacts with HSP90 and stabilizes small HSPs at high temperature tolerance (Meiri & Breiman, 2009). Contrary to the up-regulation of host HSPs, FKB62 and many HSPs were down-regulated in the symbionts in response to heat, (Ishii et al., 2019) suggested that symbiont HSPs could adversely regulate the expression of heat-responding genes as reported in another study (N. N. Rosic et al., 2011).

(N. Rosic et al., 2015) reported on differential 1053 genes in four *Symbiodinium* clades (A, B, C and D), conserved genes such as heat shock proteins (Hsp70 and Hsp90), and antioxidant genes, which are important in stress responses. However, differential gene expression responses to heat stress have not yet been studied in apo-symbiotic vs. symbiotic corals. (Meron et al., 2019) found that the presence of endosymbionts has a more negative impact on the host than the environmental temperature of the holobiont. That the coral species, *Euphyllia paradivisa* to thrive as apo-symbionts, may have a greater chance of meeting the upcoming global climate change challenge. The higher tolerance of asymbiotic coral may be attributed to the lack of photosymbionts and thus to the suppression of host physiological processes by thermal stressed algal cells (Caroselli et al., 2015). In contrast, (Ishii et al., 2019) found that gene expression patterns revealed that large portions of differentially expressed genes in response to thermal stress are specific to the symbiotic state, suggesting that sea anemone host may react to environmental changes in a symbiotic state-dependent manner. Coral hosting of alternative types *Symbiodinium* is related to the different local adaptation pathways (Barfield et al., 2018).

A recent study in *Symbiodinium* of the coral *Turbinaria reniformis* revealed a significant reduction in PsaC chloroplast genes, a stromal PSI protein when exposed to 32 ^{o}C. Yet, this decrease was ascribed to the potentially defensive decrease in PSI content, since electron flow of PSI was not impeded while PSII electrons were being transported (Hoogenboom, Campbell, Beraud, DeZeeuw, & Ferrier-Pages, 2012). Several detailed genome-level analyses (e.g. genomics, metabolomics, and transcriptomics) were undertaken to explain molecular symbiosis processes between coral hosts and its *Symbiodinium* symbionts (Ishii et al., 2019; Manzello et al., 2019; Meron et al., 2019; Rivest, Kelly, DeBiasse, & Hofmann, 2018; N. Rosic et al., 2015; Weizman & Levy,

2019; Yu, Huang, Zhou, Tang, & Yu, 2017). Thermal stress may influence, in particular, the degradation of lysosomes and transportation of substrates such as carbohydrates *via* the symbiosome membrane (phagosome-deriving organic symbiont containing), causing symbiotic stability to decrease and to ultimately lead to a symbiotic transition associated with bleaching (Ishii et al., 2019). Transcriptomic studies are currently carried out to discover the possible acclimatization and adaptation among of corals during thermal stress (Kenkel, Meyer, & Matz, 2013; Maor−Landaw et al., 2014). Such research focused on the partial or almost transcriptome of symbiotic cnidarians including anemones (Moya et al. 2012), corals (DeSalvo et al., 2010) (Bellantuono et al., 2012) (Barshis, Ladner, Oliver, & Palumbi, 2014) and coral larvae (Meyer, Aglyamova, & Matz, 2011). (Seneca & Palumbi, 2015) investigated transcriptome response of 20 *Acropora hyacinthus* colonies 5 and 20 h post exposure to control (29 °C) or heated (35 °C) conditions. A wide dynamic transcriptome response was revealed exemplified in 27% of the coral transcriptome found significantly regulated 1 h after heat exposure. Yet 15 h later, when coral holly bleached, only 12% of the transcriptome was differentially regulated.

(Weizman & Levy, 2019) identified 1309 genomic sites that can change their accessibility in response to thermal changes. Moreover, accessible sites of apo-symbiotic *Aiptasia* were enriched with NFAT, ATF4, GATA3, SOX14, and PAX3 motifs and expressed genes related to immunological pathways. Symbiotic *Aiptasia* accessible sites were enriched with NKx3-1, HNF4A, IRF4 motifs and expressed genes related to oxidative stress pathways. While changes in apoptosis, antioxidant and heat-shock protein genes were observed frequently post treatment with high temperatures, several other gene families are identified in only one or a few of studies (Seneca & Palumbi, 2015). (Petrou, Nielsen, & Heraud, 2018) found that, thermal stress resulted in biomolecular changes indicating cellular stress, relatively increased free amino acids pool and molecular phosphorylation, and consequently a decrease in protein content that indicates protein transition and degradation.

A direct comparison of metagenomic datasets from normal to bleached corals revealed substantial changes in microbial associates during heat stress, including bacteria, viruses , fungi, and microalgae, and suggestive that the microbial community's metabolism shifted from autotrophy to heterotrophy, which include increases in genes associated with fatty acid metabolism, proteins, simple carbohydrates, phosphorus and sulfur (Littman, Willis, & Bourne, 2011). (Thurber et al., 2008) investigated taxa shifts and microbiota function associated with *Porites compressa* under thermal stress, and

highlighted increases in the relative abundance of stress resistance and genes in stressed corals. Coral microbiota may play an important role in nutrients cycling and coral holobiont antimicrobial defense. It is therefore important to study the possible effects of thermal and bleaching on the molecular genes of coral associates of microbial communities.

Biochemical changes in Symbiodiniaceae under heat stress

A wide range of chemicals are produced and exchanged during the establishment of a symbiosis between corals and Symbiodiniaceae to guarantee its maintenance (Muscatine & Hand, 1958; Burriesci et al., 2012; Imbs et al., 2014), several of which show changes either as a consequence to the bleaching event or as a tolerance induction response to thermal stress. The extent of the effects of heat on the symbiosis between corals and Symbiodiniaceae has been investigated in recent studies using "omics" approaches, to address differences in thermotolerance among symbiodiniaceae species and to help identify metabolite biomarkers useful for the diagnosis of heat stress (Ladner et al., 2012; Barchis et al., 2014; Seneca & Palumbi, 2015; Hillyer et al., 2016; Farag et al., 2018). Variations in various metabolic products i.e., lipids, carbohydrates, proteins and secondary metabolism were observed due to the interaction between corals and Symbiodiniaceae species, as described below though with no conclusion on the origin of these chemicals in all studies.

Fig. 4 depicts major biochemical changes that take place in *Symbiodinium* in response to heat stress and that might contribute to its thermotolerance.

Lipid metabolism

High temperature exerts a great influence on biological membranes by triggering changes in their composition and consequently membranes fluidity (Los & Murata, 2004). In addition, Symbiodiniaceae are able to change their cell and organelle membrane composition to adjust to environmental variations (D'amico et al., 2006). As higher temperatures tend to increase the fluidity of the membrane, an increase in the composition of saturated fatty acids (SFA) should be expected, which tend to make the membrane more rigid. In contrast, polyunsaturated fatty acids (PUFA) have the opposite effect (Wada, 1994).

In *Cladocopium* C1 (*Symbiodinium* C1) (LaJeunesse et al., 2018), acclimatization at high temperature resulted in increased saturated lipids (Diaz-Almeyda et al., 2011). The *Symbiodinium* ITS2 type C8 also accumulated large amounts of saturated lipids when expelled from its host following thermal stress when compared to unexpelled *Symbiodinium* (Petrou et al., 2018). In contrast, *Cladocopium* sp. (Clade C) exposed to 34 °C did not show such changes in saturated lipids levels (Farag et al., 2018).

It has been proposed that the combination of high temperature and high concentrations of PUFAs in the chloroplasts of Symbiodiniaceae is probably a strong trigger for coral bleaching (Lesser, 1997; Downs et al., 2002). This is because the double bonds of PUFAs are the main targets of free radical and ROS attack, spreading lipid oxidation (Wada, 1994). Thus, a high ratio of SFAs to PUFAs has been reported to increase the physical stability of the thylakoid membranes of Symbiodiniaceae under heat stress (Papina et al., 2003; Tchernov et al., 2004; Bachok et al., 2006; Tolosa et al., 2011), and consequently protection of the photosynthetic apparatus. Despite this, some amount of PUFA is needed to regulate the fluidity of the membrane and consequently the efficiency of the PSII. *Synechocystis* cells unable to produce PUFAs were more sensitive to heat stress than the wild type (Gombos et al. 1994). Consequently, it seems that the ability to adjust the proportion of membrane lipids enables repair damage to PSII structures can also determine tolerance to thermal stress in Symbiodiniaceae (Takahashi et al., 2009).

Based on what has been exposed, several studies have also attempted to correlate the initial membrane constitution and responses to thermal stress in Symbiodiniaceae. Zhukova & Titlyanov, (2003) and Tchernov et al., (2004) suggested that the increase in saturated fatty acids composition in *Durusdinium trenchii* could account for their thermal tolerance. Similar results were also reported in other *Symbiodinium* including *Cladocopium C1* and *Durusdinium trenchii* (Kneeland et al., 2013) or *Cladocopium C1* and *Symbiodinium microadriaticum* (Type A1) (Dias-Almeydaet al., 2011). At different cellular level, transcriptome analysis of *Durusdinium trenchii* suggested that the desaturation of its fatty acids might be involved in the thermotolerance (Ladner et al., 2012).

Notably, several studies suggested that changes in fatty acids saturation may be, instead, a result of stress-triggered ROS overproduction and rather not necessarily acclimatization effect to heat stress (Tchernov et al., 2004; Papina et al., 2007).

764 Several attempts have been made to understand the role of structural lipids
765 specific to thylakoids for the thermotolerance in *Symbiodiniaceae*. Under heat stress,
766 *Cladocopium C3* (a thermosensitive species) presented great lipidomic changes upon heat,
767 with relatively lower content of sulfoquinovosyldiacylglycerol (SQDG) in comparison to
768 the lipidome of *Durusdinium trenchii* (a thermotolerant species) (Rosset et al., 2019). The
769 lack or lower content of glycolipid SQDG in plants is known to cause structural disorders
770 in the protein D1 within the PSII complex. Thus association of SQDG within the PSII
771 complex is indispensable for its normal conformation and consequently heat stress
772 tolerance, mainly in *symbiodiniaceae* which damage in PSII leads to photo inhibition and
773 is a major factor for coral bleaching (Frentzen, 2004; Warner & Suggett, 2016). Under
774 ambient conditions, *D. trenchii* showed higher lyso-lipids than *Cladocopium C3*. This
775 large amount of lyso-lipids may constitute a mechanism involved in thermal stress
776 tolerance of *D. trenchii,* whereas lyso-lipids are correlated to molecular chaperones,
777 facilitating the renaturation of proteins (Kern et al., 2001). High temperature further led
778 to a decrease in the pool of lyso-lipids in *D. trenchii*, but with levels though still higher
779 than those reported in *Cladocopium C3*, failing to change lyso-lipid levels under the same
780 experimental conditions.
781 Additionally, higher ratio of DGDG/MGDG was measured in *D. trenchii* as
782 compared to *Cladocopium sp.* symbionts. It was proposed that an increased
783 digalactosyldiacyl glycerol (DGDG), a bilayer lipid (to monogalactosyl diacyl glycerol
784 (MGDG), a non-bilayer lipid, ratio could function as an indicator of thermotolerance. It
785 confers more stability to the thylakoids, playing a direct role in assisting the adequate
786 folding or renaturation of integral membrane proteins (Bogdanov et al., 2010).
787 *Arabidopsis* mutants unable to increase this proportion have failed to acquire
788 thermotolerance (Chen et al., 2006). Similarly, Leblond et al. 2015 demonstrated an
789 increase in the DGDG/MGDG ratio in *S. microadriaticum* after exposure to elevated
790 temperatures.
791 Another report suggested for the use of the ratio between free fatty acids and
792 sterols as sensitive indicators of thermal stress in *Symbiodiniaceae* (Kneeland et al.,
793 2013). In this work, a threshold response was observed in the ratio of fatty acids to sterols.
794 In the more sensitive, type C1, the response occurred earlier and was more severe at
795 intermediate levels of thermal stress than for type D1. Exposure to high temperature (32
796 °C) in type C1, high fatty acid to sterol ratios showed a decrease slightly in the first week
797 and much more after 2 weeks. While in type D1, this ratio increased slightly after 1 week

at 32 ºC and dropped dramatically in subsequent weeks. Changes in this ratios would not be related to the increase in sterols, but rather reduction in PUFAs. Thus, decrease in PUFA pool would be related to its degradation by ROS generated in stressed cells, or as a protective mechanism, since PUFAs can cause a cascade of lipid peroxidation events (Downs et al., 2002; Kneeland et al., 2013).

A decrease in lipids biosynthesis is documented in *Symbiodiniaceae* at high temperatures, which may reflect ATP deprivation due to photosynthesis limitation (Hillier et al., 2016; Prout et al., 2018). Accumulation of acetate, a molecule essential for energy exchange between the symbiont and the host, was observed in *Cladocopium* C8 (Wang & Douglas, 1997). Acetate is supplied by the coral host and consumed in lipids biosynthesis by the symbiont (Wang & Douglas, 1997), and suggestive for a limiting lipid biosynthesis in algae. Such hypothesis is confirmed from accumulation of intermediates of (PUFAs) biosynthesis in *Breviolum minutum* (Hillyer et al., 2016), and an increase of lipogenesis intermediates (*e.g.* monoglycerides and glycolipids) in *Cladocopium* C3 (Hillyer et al., 2018). Besides the accumulation of lipogenesis intermediates, the increase in temperature prompts larger accumulation of lipids in the symbiont (Leggat et al., 2011; Hilley et al., 2016; Petrou et al., 2018). Concomitantly, lipid deprivation in the host appears to take place during the response to heat stress (Bachok et al., 2006; Imbs & Yakovleva, 2012; Revel et al., 2016; Hillyer et al., 2016; Farag et al., 2018). Lower lipid and fatty acid content concurrent with lower PUFA levels were found in completely bleached *Pavona frondifera* corals compared to control (Bachok et al., 2006). Such accumulation of carboxylates, lipids and fatty acids in the symbiont could be explained by the reduction of cellular energy, due to photosynthetic deprivation, causing a decline in both biosynthesis and translocation of lipids back to the host (Petrou et al., 2018).

In summary, thermal stress is capable of modifying reserve, structural lipids and even lipogenesis. It is evident that lipids key role in the constitution of the membrane, as well as its adjustment, aid to tolerate or acclimate to thermal stress. The guarantee of balance between SFAs and PUFAs is of great importance to assurance the fluidity of the membrane, at the same time, reducing oxidative damage. Recent work has focused on certain chloroplast lipids revealing for multiple mechanisms of heat tolerance in *Symbiodiniaceae*, such as the presence of lyso-lipids. Reserve lipid metabolism should also be better explored, in phylotypes with different thermosensitivity, in an attempt to elucidate the increased accumulation of lipids in the symbiont and its possible correlation with vulnerability of the symbiosis relationship.

Carbohydrates metabolism

A decrease in glucose levels in zooxanthella would be expected when the Calvin–Benson cycle is impaired by heat stress, although different responses were reported. For instance, decreased levels of glucose and fructose were detected in *Cladocopium* sp. submitted to a gradual increase (1 °C a day) of temperature from 30 °C to 36 °C (Farag et al., 2018). However, a rapid temperature increase (1 °C every 3 h) from 25 °C to 32 °C did not affect glucose levels in *Breviolum minutum* (*Symbiodinoium* Type B1) along with 7 days of experiment. No significant variation in glucose level was observed in *Cladocopium* C3, extracted from *Acropora aspera,* under temperature increase from 25 °C to 32 °C (1 °C a day) (Hillyer et al., 2017a). Whether such discrepancy is due to different application of heat stress or due to differences in harbored *Symbiodiniaceae* or other symbiont is not clear. In contrast, investigation using *Cladocopium* C3 extracted from the same coral at similar conditions (Hillyer et al., 2017a) detected large accumulation of glucose after 6 and 9 days (Hillyer et al, 2018). The contrasting responses observed for the same organism are likely due to specimen-specific or intra-species variations of different *Symbiodiniaceae* genotypes (Lohr et al., 2019). Differences in the experimental design, sampling methods, sampling time, acclimatization time, water quality, light exposure and the natural variation among colonies can all account for differential responses to the same stimulus (Louis et al., 2016).

With regards to thermal stress effect on corals themselves, high temperature affected the metabolism and composition of carbohydrates inside corals (Sogin et al., 2016). Large amounts of sugar alcohols (*e.g.* xylitol, galactinol and chirositol) were reported to accumulate in *Acropora aspera* after temperature increase (Hillyer et al., 2017a; Hillyer et al., 2018). Likewise, the cyclic polyol sugar myo-inositol showed induction in *Pocillopora damicornis* (Sogin et al., 2016), whereas xylitol was observed in *Sarcohpyton ehrenbergi* upon heat stress (Farag et al., 2018). These carbohydrates are mostly associated with the regulation of cell osmotic pressure (Mayfield & Gates, 2007; DeSalvo et al., 2010), an event that usually occurs in response to stress.

Protein metabolism

The pool of free amino acids was found to increase in both Symbiodiniaceae and in its host coral under elevated temperature. Temperature increase promoted the accumulation of isoleucine and valine in *Breviolum minutum* (Hillyer et al., 2016), whereas expelled isolated symbiont showed increase in free amino acid pool that led to a

decrease in protein pool in its host (Petrou et al., 2018). This increase in free amino acids has been related to an increment in catabolism for energy supply, biosynthesis of signaling molecules and compatible solutes in response to heat stress (Richier et al., 2005). The increment in sulfur-containing amino acids (L-cysteine and L-methionine) exhibits also secondary functions acting as redox sensors and/or ROS scavengers as in the case of L-cysteine (Mayer et al., 1990; Hillyer et al., 2016).

Elevated temperature also led to induction of histidine and β-alanine metabolic pathways in *Cladocopium* C3, whereas increase in phenylalanine and tryptophan metabolism was observed in the host *Acropora aspera* under heat stress (Hillyer et al., 2017a), suggestive for a qualitative difference in amino acids induced with heat stress within the symbiont. The accumulation of non-protein amino acids (such as γ-aminobutyric acid) and polyamines (such as putrescine) was observed in coral hosts *Sarcophyton glaucum* and *Sarcophyton ehrenbergi* (Farag et al, 2018), though not observed in *Symbiodinium* (Sogin et al., 2016).

Secondary metabolism

Biotic and abiotic stresses are well-known for their up regulation of secondary metabolites. These chemicals are fundamental for ecological, cellular signaling processes besides controlling the pool of oxidant molecules inside cells. Such events are essential for the maintenance of symbiosis under stressing conditions (Lesser, 1996; Jones & King, 2015; Murray et al., 2016). The functional diversity of the dinoflagellate is also a determinant of the coral resilience (Suggett et al., 2017). The elevation of temperature has led to the production of betalains and phenylpropanoids in *Cladocopium* C3 (Hillyer et al., 2017a). Likewise, the biosynthesis of antioxidant mycosporine-like amino acids (MAAs) was also described in dinoflagellates following thermal stress (Rosic & Dove, 2011). MAAs are photoprotective molecules that functions in marine organisms (Oren & Gunde-Cimerman, 2007), such as to reduce reactive oxygen species and scavenge free radicals involved in anti-oxidative thermal stress response (Singh et al., 2008; Rosic et al., 2015), alleviating photo-oxidative damage in coral bleaching (Gao & Garcia-Pichel, 2011; wada et al., 2015). Yakovleva et al., (2004) suggested that a high abundance of mycosporine-glycine (Myc-Gly) may make corals more tolerant to oxidative toxicity and increase their ability to survive in habitats with harmful temperature disturbances. Among the wide range of MAAs found in nature, some are commonly found in *Symbiodiniaceae*, such as Porphyra, mycosporine-2-glycine, mycosporine-glycine, shinorine, palythine (Rosic, 2019). Contrastingly, in other genera, including *Brevolium*, *Cladocopium* and

Fugacium are unable to produce MAAs (Banaszak et al., 2000), due to no functional pathway (Liu et al., 2018; Shoguchi et al., 2018). In contrast, adaptation to stressful environmental conditions of Clade a *Symbiodinium* appears to be related to the production of substantial amounts of mycosporin-like amino acids (MAAs) (Banaszak et al., 2000.)

Symbiodiniaceae produces carotenoids, xanthins, peridinins and phenolic compounds as antioxidants and the acclimatization to heat fluctuations results from photoprotective mechanisms, such as increased production of phenolic compounds (Louis et al., 2016). Other compound such as terpenes appeared to be related to abiotic stresses i.e. UV light, signaling molecules (Newberger et al., 2006) though their role in heat stress has yet to be explored.

Changes in the production of biogenic volatile organic compounds (BVOCs) were also investigated in *Symbiodiniaceae* (Deschaseaux et al., 2014; Lawson et al., 2019). Heat stress (32 °C) provoked an increase by 98.5% in the levels of dimethyl-disulphide in *Cladocopium goreaui* and 99.9% in the levels of nonanoic acid in *Durusdinium trenchii* (Lawson et al., 2019). The levels of dimethyl disulfide were not affected by heat in *Durusdinium trenchii* (Deschaseaux et al., 2014). How specialized volatiles in *Symbiodiniaceae* respond to chronic, and short-term heat stress has yet to be explored and whether these function as signaling molecules similar to jasmonic acid well reported *in planta* (Thaler et al., 2002; Farag et al., 2002) is an area that is totally unexplored.

Signaling molecules named elicitors have also been used to induce alterations in Symbiodiniaceae secondary metabolism. For example, *Symbiodinium* sp. can respond to elicitors commonly used in plants, such as methyl jasmonate, salicylic acid and gibberellic acid. Such elicitors led to increased biosynthesis of the non-volatile diterpenes pseudopterosin and fuscol in *Symbiodinium* sp. (Newberg et al., 2006). The induction of volatiles terpenoids in *Symbiodinium* in response to thermal stress is difficult considering that volatiles analysis in surrounding water is more complicated than within corals or isolated *Symbiodinium* culture. High temperature can induce the production of massive ROS in terrestrial algae, which benefits to the oxidation of fatty acids and carotenoids, leading to the formation of volatiles and carotenoid degradants (Zuo., 2019), and has yet to be confirmed in the case of marine symbiont counterpart.

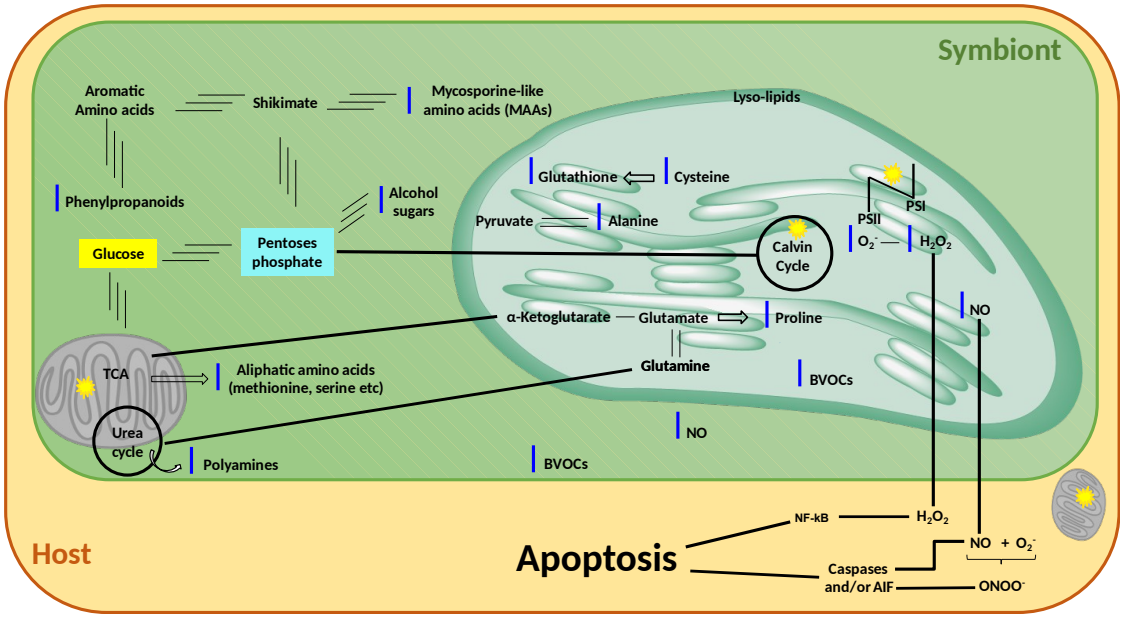

Fig. 4 Summarizes some biochemical changes that take place in *Symbiodinium* in response to heat stress and that might contribute to its thermotolerance or acclimatization. The schematic representation was based on the findings revealing the following changes. (**a**) Lyso-lipids from thylakoid membrane possess chaperone-like properties and increased digalactosyldiacylglycerol (DGDG) to monogalactosyldiacylglycerol (MGDG) ratio to confer higher stability to thylakoid membranes; (**b**) The amounts of cysteine, glutathione, methionine and biogenic volatile organic compounds (BVOCs) increase upon heat stress, strengthening the non-enzymatic antioxidant system in response to the oxidative triggered by stress; (**c**) Compatible solutes, such as alanine, alcohol sugars, proline, serine etc.. act as osmoregulators; (**d**) Augment of superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) results from an increased leakage of electrons from the photochemical reaction of photosynthesis. H_2O_2 can be diverted to the host and stimulate factor nuclear kappa B (NF-kB) and ultimately apoptosis, depending on the severity of the stress; (**e**) Nitric oxide (NO) originated from the activity of nitrite reductase in chloroplasts or nitrate reductase in cytosol is a gaseous transmitter that can reach the host cells to induce apoptosis directly *via* the stimulation of caspases or indirectly by its conversion to peroxinitrite ($ONOO^-$), a powerful oxidant that stimulates the apoptosis inducing factor (AIF) and caspases as well, culminating in apoptosis events; (**f**) Pentoses phosphate derived from the Calvin cycle are directed to the cytosol for the formation of alcohol sugars (including myo-inositol), glucose, and shikimate; (**g**) Shikimate is the precursor of mycosporine-like amino acids (MAAs), antioxidant molecules known to accumulate in response to heat; (**h**) Aromatic amino acids (phenylalanine and tyrosine) originated from the shikimic acid pathway lead to the accumulation of phenylpropanoids; (**i**) the individual survival during the stress is guaranteed by slowing down the energy production from the tricarboxylic acid (TCA) cycle. The carbon skeletons of TCA cycle intermediates are directed to the production of aliphatic (non-aromatic) amino acids; (**i**)

Polyamines, known to improve heat stress tolerance, accumulate due to the intense activity of urea cycle. Triple arrows indicate multiple reaction steps. The size of mitochondria and chloroplast is merely illustrative and does not represent the actual relative size in the symbiont cells.

Conclusion & future directions

In summary, considerable progress has been made towards understanding coral-dinoflagellate symbiosis associated biological processes over the last decade. Nevertheless, the rapid decline in coral reef ecosystems due to climate change is outstripping our pace of discovery. Despite some efforts made, such as protection of marine areas and efforts to reduce the emission of greenhouse gases, the rapid decline of coral reefs continues. Therefore, understanding how the increase in temperature affects the establishment of symbiosis and what factors are related to different thermal sensitivity is crucial to plan future management. Despite an intensive study of symbiont and host responses to heat stress in recent years, a complete understanding of thermotolerance mechanisms remains elusive.

With this in mind, we present up recent discoveries made towards understanding such phenomena and helping to find solutions to the coral reef crisis. Such extensive compile of literature suggest that physiological host plasticity and/or symbiotic components clearly plays a significant role in response to global, and that response to thermal stress may also vary between different species of corals, as many corals may contain specific ecotypes or clades of zooxanthellae which may vary in their ability to withstand thermal stress(Pochon & Gates, 2010; Qin et al., 2019)[1, 2].

Innate immunity, as a physiological function, can play a key role in helping corals to survive such thermal stress. In that regards, symbionts can emerge through several physiological pathways to mitigate against thermal stress, of which to include host cells *in situ* degradation, host cell detachment, exocytosis apoptosis and necrosis. According to coral bleaching associated reactive oxygen species (ROS) theory, ROS is triggered by symbiosis operation of damaged photosynthetic machines cause leaks in the host cell, and to lead to cellular damage, including macromolecules. Another negative impact of thermal stress includes reduced photochemistry efficiency by inflicting damage on numerous photosynthetic proteins, such as photosystem II (PSII), light harvesting complex, Calvin cycle or the thylakoid membrane.

At the metabolic level, several chemical and biochemical changes occur in both partners in the face of thermal stress. These responses are also species - specific and may also vary according to the experimental conditions and to account for discrepancy in results among researchers. Therefore, as a short-term perspective, it would be interesting to standardize experimental stress conditions, in an attempt to make them consistent with environmental changes. Understanding what biochemical changes or chemicals produced may reflect trade-offs associated with thermotolerance. For this, metabolic and molecular approaches, expanding in recent years, can be very useful, to understand which metabolic responses and signaling pathways are related to thermotolerance. In addition, field experiments to investigate real coral reef responses are also needed, and will be important to identify chemicals or pathways be engineered inside corals for management practices that can mitigate coral bleaching likewise already applied in terrestrial *planta*.

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