MARINA TONETTI BOTANA

The role of *Symbiodinium* membrane lipids in response to heat shock: implications for coral bleaching

Thesis presented to the Instituto Oceanográfico of the Universidade de São Paulo, in partial fulfillment of the requirements for obtaining the degree of Master in Sciences, Oceanography Program, concentration area of Biological Oceanography.

Supervisor: Prof. Dr. Paulo Y. G. Sumida

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“Look up at the stars and not down at your feet. Try to make sense of what you see and wonder about what makes the universe exist. Be curious. Science is not only a discipline of reason, but also, one of romance and passion”

Steve Hawking

“Commit yourself to the process. Not the goal. The final score take care of itself”

James Clear

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RESUMO

Recifes de coral do mundo inteiro vêm sendo devastados pelo fenômeno de branqueamento, o qual as evidências indicam que seja causado pelo stress oxidativo promovido pelo aquecimento global e eventos catastróficos de El Niño. A grande variabilidade genética da Família Symbiodiniacea também é sugerida como determinante da susceptibilidade do coral hospedeiro porque cada espécie possui limites fisiológicos específicos, tanto no modo de vida livre como em simbiose. Neste estudo apresentamos pela primeira vez o sucesso da utilização da de técnicas de lipidômica (i.e, caracterização dos lipídeos globais em um determinado organismo) oferecendo suporte para as investigações moleculares de investigação dos mecanismos relacionados ao stress térmico em espécies de endosimbiontes de coral. *Symbiodinium minutum* foi sensível às temperaturas elevadas, enquanto *S. microadriaticum* e *S. goreaui* apresentaram distintos níveis de termo tolerância. Os fenótipos lipídicos das espécies após o stress, incluindo o transportador de elétrons do fotossistema II – plastoquinona – sugerem que cada um apresentou uma estratégia diferente para sobreviver. Além disso, os lipídeos específicos do cloroplasto com ácidos graxos poliisaturados (PUFA) formado, principalmente, por espécies com ômega 3 (n-3) foram essenciais para manter a bioenergética celular à longo prazo (10 dias após stress) em todos os *Symbiodinium* spp. A capacidade de manter altas concentrações de n-3 na membrana dos cloroplastos determinou a sobrevivência dos *S. microadriaticum* e *S. goreaui.* Os dados apresentados nesta dissertação revelam, pela primeira vez, o aumento de ácidos graxos oxidados na membrana do cloroplasto e também na forma livre (FFA) em resposta aos dados de stress oxidativo causados pelo calor. O estudo das membranas lipídicas é fundamental para melhor compreensão da bioenergética dos simbiontes e para determinar a vulnerabilidade da relação de simbiose com o coral aos estressores climáticos em um futuro com temperaturas mais elevadas.

Palavras-chave: *Symbiodinium*, recifes de coral, lipidômica, stress térmico, stress oxidativo.

ABSTRACT

Coral reefs around the world have been largely devastated by the phenomenon of “coral bleaching”, which causes have been reported to be strongly related to oxidative stress promoted by climate change drivers, including mainly global warming and catastrophic El Niño events. Genetic variability in coral endosymbionts from the Family Symbiodiniacea was also suggested as determinant of host susceptibility to stress because they present distinct physiological boundaries when in free living or in symbiosis. Here we present for the first time the successful use of lipidomics (*i.e.*, the global characterization of lipids in a given organism) supporting molecular investigation in the oxidative mechanisms related to thermal stress in coral endosymbionts phylotypes. *Symbiodinium minutum* was thermal sensitive, whereas *S. microadriaticum* and *S. goreaui* presented different levels of thermal tolerance. Their lipid phenotypes after stress, including the photosystem electron transporter - plastoquinone - suggested they had different survival strategies. In addition, chloroplast specific lipids with polyunsaturated fatty acids (PUFAs) mainly formed by omega 3 (n-3) seemed to be essential to sustain *Symbiodinium* cells bioenergetics in the long term (10 days after stress). *S. microadriaticum* and *S. goreaui* capability of keeping high n-3 concentrations in the chloroplast membranes determined their survival. The present thesis reports, for the first-time, upregulation of oxidized lipids derived from precursor chloroplast membranes and free fatty acids (FFA) in response to oxidative stress damage caused by heat. The study of lipid membranes is of paramount importance to better understand the bioenergetics of symbionts and to determine the host/endosymbiont vulnerability to climate change stressors in a warmer future.

Key words: *Symbiodinium*, coral reefs, lipidomics, thermal stress, oxidative stress

LIST OF ACRONYMS AND ABBREVIATIONS

ARA - arachidonic acid

AC - *Symbiodinium* phylotype A1 control sample

AT – *Symbiodinium* phylotype A1 temperature stressed sample

ATP – adenosine triphosphate

BC - *Symbiodinium* phylotype B1 control sample

BT - *Symbiodinium* phylotype B1 temperature stressed sample

CC - *Symbiodinium* phylotype C1 control sample

CE – cholesterol ester

Cer - ceramide

Chl-a -chlorophyl-a

CL - cardiolipin

CT - *Symbiodinium* phylotype C1 temperature stressed sample

DAG – diacylglycerol

DGCC - 1,2-diacylglyceryl-3-(O-carboxyhydroxymethylcholine)

DGDG – digalactosyldiacylglycerol

DGTS - diacylglyceroltrimethylhomoserine

DHA - docosahexaenoic acid

EPA - eicosapentaenoic acid

ER - endoplasmic reticulum

ESI-TOFMS – electron spray ionization time of flight mass spectrometer

FFA - free fatty acids

Gluc Acid – glucoronic acid

HUFA – High unsaturated fatty acids

LC - liquid chromatography

MGDG - monogalactosyldiacylglycerol

MUFA - monounsaturated fatty acid

MS – mass spectrometer

PC – phosphatidylcholine

PE - phosphatidylethanolamine

PG – phosphatidylglycerol

PI - phosphatidylinositol

PL – polar lipids

PUFA - polyunsaturated fatty acid

RPLC – reverse phase liquid chromatography

ROS - reactive oxygen species

SFA - saturated fatty acid

SQDG – sulfoquinovosyldiacylglycerol

SM - sphingomyelin

TAG - triacylglycerol

UHPLC - ultra-high-performance liquid chromatography

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CHAPTER 3: Concentration of omega 3 fatty acids in membrane lipids determine *Symbiodinium* phylotypes (A1, B1 & C1) fate after temperature stress

CHAPTER 1: GENERAL INTRODUCTION

1.1 Coral reefs and their symbionts in the context of global warming

Coral reefs are one of the most productive ecosystems on Earth, representing one of the most diverse marine environments (Grigg *et al*., 1984). They hold high biodiversity and ecosystem services important for sustaining higher trophic level organisms and providing goods for large numbers of people (Martinez *et al*., 2007; Alves de Lima *et al*., 2010; Knowlton *et al*., 2010). Corals are defined as ecosystem engineering organisms responsible for controlling the availability of resources to other organisms through physical changes in biotic and abiotic materials (Jones *et al*., 1994). Therefore, studying them is pivotal to understand marine community structure and production of resources to humankind.

Tropical calcium carbonate reefs are built by stony corals (Scleractinia) associated with dinoflagellates of the family *Symbiodinidaceae* perceived as a mutualistic symbiosis. Photosynthetically produced endosymbiont metabolites are exchanged with coral hosts guaranteeing their survival and growth even in nutrient-poor waters (Muscatine & Porter, 1977; Leggat *et al*., 2003). In addition, the family *Symbiodinidaceae* is divided into nine clades (A-I) (Pochon & Gates, 2010) and multiple phylotypes within each clade (Thornhill *et al*., 2014), which shows distinct degrees of host-specificity and different tolerances to environmental conditions (Toller *et al*., 2001; Baker, 2003; Chen *et al*., 2003; Coffroth & Santos, 2005; LaJeunesse, 2005; Berkelmans & van Oppen, 2006; Goulet, 2006). Recent research has focused on the strength of this mutualistic relationship in response to predicted climate alteration (Davy & Cook, 2001; Cervino *et al*., 2004; Barneah *et al*.,2007; Hoegh-Guldberg *et al*.,2007), which is the main culprit causing extensive coral reef degradation (Hoegh-Guldberg & Smith, 1989; Graham *et al.* 2008; Wilkinson, 2008).

1.2 Coral bleaching and the oxidative stress theory

Coral bleaching is defined by visible whitening of the coral as a result of decreasing densities of *Symbiodinium* spp. cells and/or declines in their photosynthetic pigments (Fig.1.1). Since corals are extremely dependent on endosymbiont metabolites, their lack leads to coral degradation, eventually followed by coral death (Glynn, 1996; Brown, 1997). A recognized biochemical explanation for the phenomenon was first proposed by Lesser (1997) and coined by Downs *et al.* (2002) as "the oxidative stress theory of coral bleaching". They proposed that excessive light and temperature cause physiological stress in the symbiont in combination with increased production of reactive oxygen species (ROS). ROS (i.e., hydrogen peroxide, superoxide, hydroxyl and singlet oxygen - Valko *et al*. 2007) can trigger the oxidation of essential biomolecules such as proteins and lipids in both coral and *Symbiodinium* spp. cells, thereby leading to disruption of symbiotic association.

Figure 1.1: Bleached corals of the genus *Mussismilia hispida*, endemic from the tropical waters of Brazil, observed in Ubatuba (São Paulo State north shore) in the summer of 2019.

The concept of ROS playing a key role in bleaching events has been an important subject of research in the last decades. The increased ROS production in symbiont cells may be caused by combination of temperature, light and even other stressors (e.g., alterations in

carbonate chemistry) (Tchernov et al., 2004; Smith *et al*., 2005; Ragni *et al*., 2010; Roberty *et al*., 2015; Goyen, 2017). Today, despite the evidences, the specific causes triggering excessive ROS production leading to bleaching and harmful impacts on host physiology and impairment of symbiosis are still a matter of debate and further investigation.

1.3 The effect of high temperature in the viscosity of membranes leading to free radical and ROS formation in the chloroplast

The membranes of both cells and organelles are universally formed by lipids. Lipid composition directly affects membrane motion and fluidity, which are determined by the size and saturation levels of fatty acids chains of polar lipids. Polyunsaturated fatty acids (PUFA) tend to increase membrane's fluidity, whereas saturated fatty acids (SFA) have the opposite effect. Besides, membrane characteristics can also be altered by abiotic factors, such as temperature. Higher temperatures tend to increase spaces between fatty acids chains increasing membrane fluidity (Fig. 1.2). Therefore, organisms must adjust their membrane composition with variations in abiotic factors in order to maintain cellular functions, a process known as homeoviscous stability control (Sinensky *et al*., 1974; Cossins *et al*., 1978; Kellerman *et al.,* 2016).

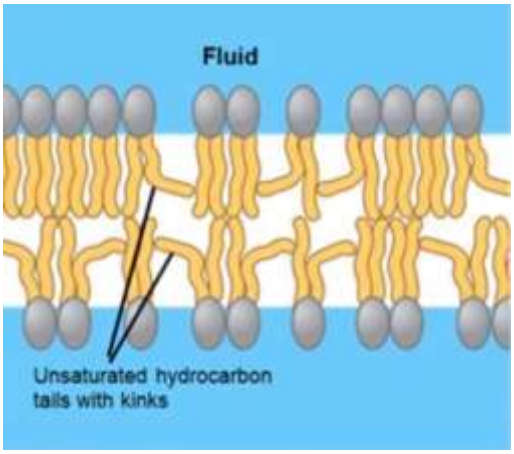

Figure 1.2: **Exemplification of how membrane lipids are affected by fatty acid composition and temperature**. Figure shows how polar lipids with unsaturated fatty acids attribute high motion and low viscosity to the membrane. Plus, elevated temperatures also promote higher membrane's motion and fluidity. Combination of elevated temperatures and high polyunsaturated fatty acids can compromise membrane's electron transport chain viability. Source: adapted from bio.libretexts.org.

Controlling membrane composition is pivotal for survival of all life forms, from bacteria to plants to mammals. The lipid composition of energy transducing membranes (i.e., cytoplasmic membranes of bacteria, thylakoid membranes of chloroplasts and mitochondrial inner membranes) is extremely specialized and must perform two essential functions: 1) control permeability of ions such as protons and sodium; and 2) tighten the electron transport at the membrane level. In chloroplasts (Fig. 1.3), the high-energy electrons coming from the water split at photosystem II (PS II) and must be safely transported within membranes by a plastoquinone to energize protein complexes or proton pumps (cytochrome b6f and PSI), a process known as electron transport chain. These pumps generate a proton gradient (high in the lumen and low in the cytoplasm) needed to promote adenosine triphosphate (ATP) synthesis (Fig.1.3). In retrospect, this ion gradient can only be established by controlling permeability of energy transducing membranes. All living forms must always adapt and keep membrane motion and fluidity stable in order to make ATP or energy, thus, lipids and bioenergetics are inseparable.

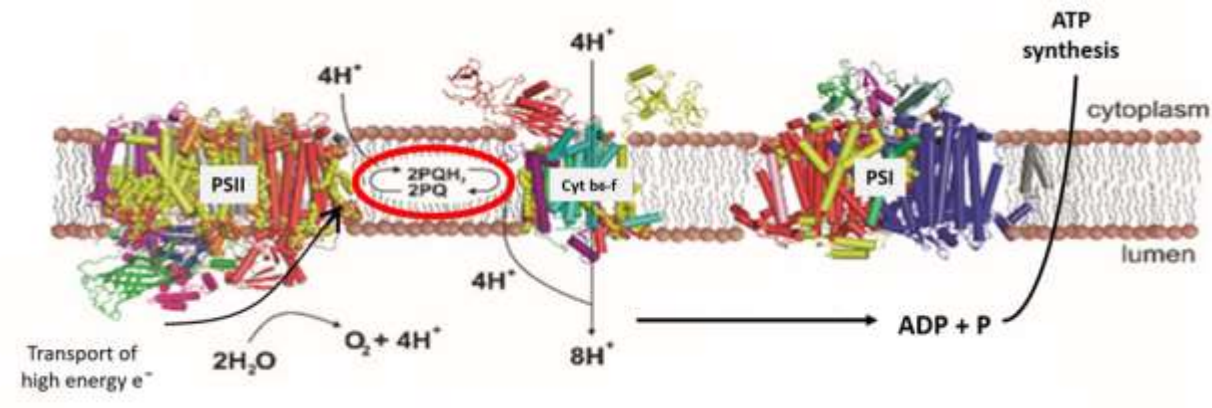

Figure 1.3: Electron transport chain resulting in ATP synthesis in the thylakoid membranes of chloroplasts. Protein complexes photosystem II (PSII), cytochrome b_6-f (cyt b_6-f) and photosystem I (PSI) are highlighted. Plastoquinone pool responsible for electron transport from PSII to cyt b_6-f is circled in red. Source: Adapted from Wada & Murata, 2009.

Thylakoid membranes of chloroplasts are very unique in that they are composed of glycolipids and specially at protein complexes such as the PS II. They are also decorated with several pigments including carotenoids and chlorophylls (Wada & Murata *et al*., 2009). The arrangement or conformation of lipids and pigments has been honed by Darwinian evolution to prevent the leakage of both protons and electrons at the membrane level. Any leakage of protons through the membrane may prevent the generation of proton gradients needed for ATP synthesis (e.g., Kellerman *et al*., 2016; Yoshinaga *et al*., 2016). On the other hand, leakage of high-energy electrons may lead to free radical generation, which combined with oxygen leads to the formation of ROS (Polle, 1996). Both free radicals and ROS, if not contained by the cell's antioxidant machinery (including carotenoids and antioxidant enzymes such as catalases), can cause oxidation of biomolecules: proteins, pigments and lipids (Augusto & Miyamoto, 2011; Yin *et al*. 2011). Lipid peroxidation is the substitution of a bis-allylic hydrogen in the lipid structure by a free radical, yielding the formation of a lipid radical (L•). This lipid radical can easily react with oxygen, generating a lipid peroxyl radical (LOO•) and undergo complex cyclic reactions that might propagate through the membrane through a process best known as chain reaction (Niki, 2009; Yin *et al*., 2011). In this context, therefore, fatty acids containing high unsaturation levels, such as polyunsaturated fatty acids (PUFAs),

are the main targets of radical and ROS attack in their double bounds and propagate lipid oxidation even further unless stopped by an antioxidant agent.

The combination of high temperatures and high concentration of PUFAs in the chloroplasts of *Symbiodinium* is likely a strong trigger for coral bleaching, and the associated "oxidative theory of coral bleaching" (Lesser, 1997; Downs *et al,* 2002). Drastic changes in membrane permeability and fluidity are expected to occur as a consequence of high temperatures. Altering the thylakoid membrane conformation most likely promotes leakage of protons and electrons, thereby leading to decreased energy production and increased oxidative stress, respectively. It is, however, unknown whether the disruption of symbiosis occurs by a decreased supply of metabolites to the coral host, death of symbionts or simply symbionts themselves representing a potential threat to the host due to high ROS production.

1.4 Lipid membrane profiles of symbionts and their fate after thermal stress

Strong evidence suggests that *Symbiodinium* spp. can modulate the lipid composition of cells and organelles membranes in order to keep homeoviscous stability and adapt to environmental alterations (D'amico *et al*., 2006). Higher abundance of SFAs relative to PUFAs has been reported to enhance physical stability of thylakoid membranes of *Symbiodinium* sp. in response to thermal stress (Tchernov *et al*., 2004; Bachok *et al*., 2006; Tolosa *et al*., 2011). The rationale is that *Symbiodinium* spp. thylakoid membranes are enriched in PUFA, which are highly susceptible to oxidative damage by free radicals and ROS (Wada, 1994; Lesser, 2006; Catalá, 2009). However, *Symbiodinium* spp. may protect the photosynthetic membranes against ROS and thus acquire thermal tolerance altering the ratio saturated/unsaturated fatty acids. Tchernov *et al.* (2004) have even suggested that thermal tolerance is not associated with a single monophyletic phylotype, but rather with the level of saturation of their membrane lipids.

The above-mentioned studies marked the initial investigations of the role of lipids in coral bleaching. They were essential to establish that bulk fatty acid composition is crucial for survival and supports the "oxidative theory of coral bleaching" (Lesser, 1997; Downs *et*

al, 2002). In this dissertation, we generated data based on the global lipidome of some *Symbiodinium* phylotypes (e.g., glycolipids, phospholipids, aminolipids and storage lipids), including their pigments, in response to thermal stress. Thus, we not only report data on fatty acids, but also the lipid molecular species containing these fatty acids. That is, we are able to pinpoint whether thermal stress affects thylakoid membranes by characterizing their specific glycolipids rather than bulk fatty acids derived from other pool of lipids such as the triglycerides or phospholipids. Such detailed and comprehensive lipid analysis could only be achieved by recent analytical developments in mass spectrometry and the advance of lipidomics (Jones *et al*., 2012; Yao *et al*., 2015; Nygren *et al*., 2017).

1.4.1 Lipidomics as tool to better characterize microalgae lipids

Lipidomics is a fairly recent technique that evolved from metabolomics in its own research field (Tomita & Nishioka, 2006; German *et al*., 2007). Previous lipid analytical techniques allowed qualitative information about polar lipids such as acquired by thin-layer chromatography or quantitative analysis of bulk fatty acids by gas chromatography. Contrasting with past lipid analytical techniques, lipid characterization by liquid chromatography coupled to mass spectrometry (LC-MS) enabled precise characterization and quantification of every lipid molecular species present in a given sample (German *et al*., 2007; Oresic *et al*., 2008), including molecules that are specific markers of chloroplast, such as glycolipids and plastoquinones. For example, a great diversity of glyco and amino membrane lipids in *Symbiodinium* spp. and other dinoflagellates has been described by LC-MS analysis, including few alterations when growing in distinct temperatures (Leblond *et al.,* 2000, 2006, 2010, 2015; Gray *et al.,* 2009; Dahmen *et al.,* 2013; Flaim *et al.,* 2014; Anesi *et al*., 2015, 2016). These studies were mostly focused on a specific class of polar lipid such as glycolipids or aminolipids, and not aimed at characterizing the global lipidome together with specialized lipids, such as plastoquinone and pigments using LC-MS. Besides the culture-based investigations, important data have been generated in environmental studies reported by Van Mooy *et al.* (2006, 2009 and 2010), Moutin *et al.* (2007), Schubotz *et al.* (2009), Xie *et al.* (2014) and Becker *et al.* (2018). These include not only data from phospho, glyco and

aminolipids, but also storage lipids and quinone molecules (e.g., ubiquinone in Becker *et al*., 2018). These studies describe the lipidome of the water column of the oceans, where a diverse variety of phytoplankton occurs, in response to diel temperature oscillations and distinct nutrient conditions. The present study is, to the best of our knowledge, the first attempt to characterize the global lipidome of a microalga, describing not only membrane lipids, but also storage lipids, pigments and plastoquinone from *Symbiodinium* phylotypes. This information will be linked to cell physiology data to further knowledge on their thermal sensitivity.

2. GOALS AND SCOPE OF THIS THESIS

The overall goal of this thesis is to investigate how the lipidomes of *S. microadriaticum* (phylotype A1), *S. minutum* (phylotype B1) and *S. goreaui* (phylotype C1) most prevalently associated with scleractinian corals are related to their thermal sensitivity. Description of *Symbiodinium* spp. lipids and pigments is very scarcely found in the literature, let alone their lipid alterations with stress events. For best comprehension all studied *Symbiodinium* spp. will be referred as their phylotypes A1, B1 and C1. Therefore, this thesis is divided into two further chapters:

Chapter 2:

Description and quantification of global lipidome and pigment profiles of *Symbiodinium* phylotypes A1, B1 and C1 growing under optimum conditions of temperature, light and nutrients. Here, the goals are to examine whether differences in lipidome and pigments profiles between *Symbiodinium* phylotypes can predict their thermal tolerance to heat stress events based on previous studies (e.g., Tchernov *et al*., 2004).

Chapter 3:

Monitor *Symbiodinium* phylotypes (A1, B1 and C1) growth rates and lipidome/pigment alterations after a heat shock event (4h under 34˚C) describing short-term (4 and 24 h) and long-term phenotypical alterations (10 days).

Main assumption: “High temperatures known to change thylakoid membrane stability and enabling scape of high energy electrons generate higher concentration of ROS leading to lipid peroxidation accompanied by reduction of ATP production. Both facts affect *Symbiodinium* spp. growth rates and reflect in drastic changes in their lipidomes and pigments profiles”.

Specific hypotheses:

1) *Symbiodinium* spp. growth rates are negatively affected by heat shock;

2) Changes in lipids and pigments are different between *Symbiodinium* phylotypes after heat shock;

3) Lipidome and pigments profiles of *Symbiodinium* phylotypes after stress are good indicators of oxidative stress caused by heat shock experiment;

4) Lipid peroxidation preferably occurs in polyunsaturated fatty acids (PUFA) from chloroplast membranes.

FINAL REMARKS

In Chapter 2 we verified the use of lipidomics as a precise tool for description, quantification and monitoring of total lipids and pigments of *Symbiodinium* phylotypes A1, B1 and C1 growing under optimum conditions of temperature, light and nutrients. We also showed that statistically significant differences among phylotypes were mainly determined by their membrane lipids. Although A1 and B1 were more similar based in the heatmap analysis, A1 was more alike to C1 considering their higher concentrations of omega-3 polyunsaturated fatty acids and plastoquinone.

In Chapter 3 we analyzed variations in *Symbiodinium* growth rates and both short and long terms lipidomes after a heat shock event summarized in Figure F1. Phylotype B1 was not heat shock resistant and its high decrease in biomass and cell death occurred after a downregulation of essential membrane omega-3 fatty acids and all identified pigments from chloroplasts. Thus, lipidome and pigments changes of B1 could not guarantee its cell's energetic requirements. However, phylotypes A1 and C1 both resisted mainly because they were capable of maintaining higher concentrations of essential omega-3 fatty acids in the thylakoid membranes and supply cells energetic requirements in the long term.

Combined information from both chapters demonstrated lipidomics as a functional tool to comprehend cell physiological alterations caused by thermal stress by a unified concept. We noticed that functional thylakoid membrane lipid structure cannot vary much in order to feasibly supply the cell bioenergetic demands. Therefore, the fate of organisms is likely to be determined by the cell antioxidant machinery, which were not analyzed in the present study, but that might protect membranes against ROS and keep efficient energy output even under stress conditions.

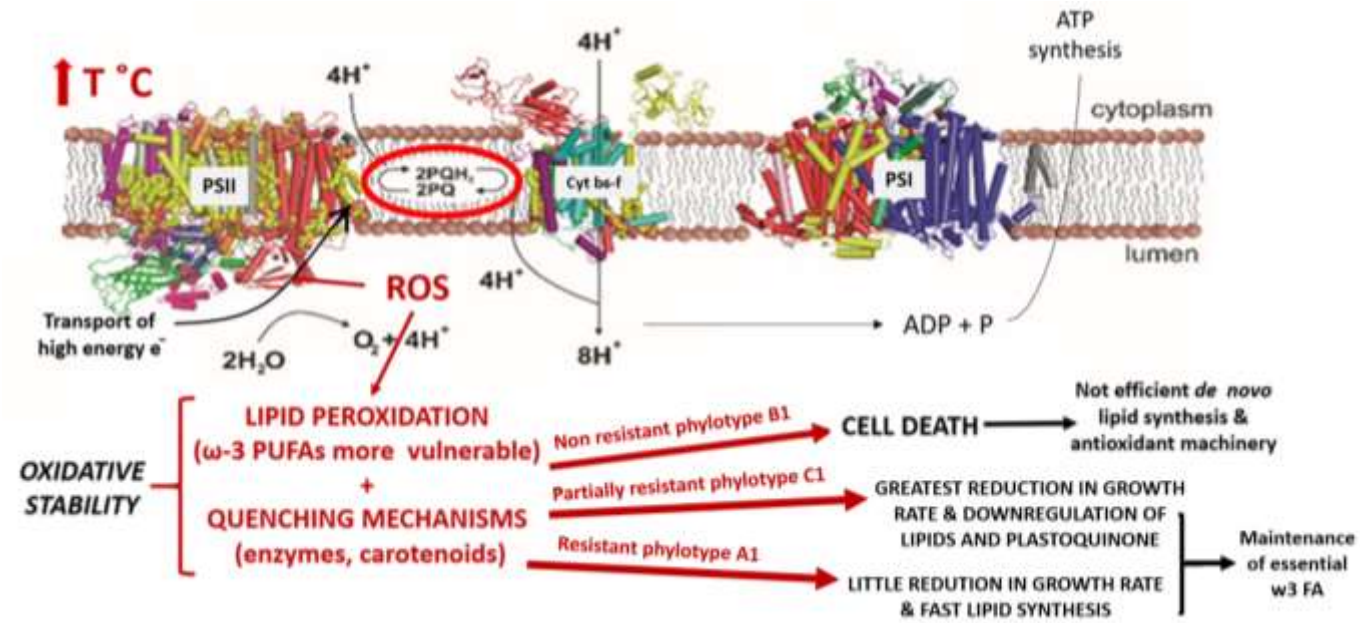

Figure F1: Summary of *Symbiodinium* phylotypes cell's proposed mechanisms caused by upregulation of ROS. Temperature alters membrane viscosity and enable scape of high energy electrons from electron transport chain. This might be responsible for upregulation of ROS that will concomitantly lead to the peroxidation of most vulnerable omega-3 polyunsaturated fatty acids and activate quenching mechanisms. We propose that phylotype B1 did not survive because it could not avoid lipid peroxidation of essential ω-3 in the membranes, neither synthesize *de novo* epoxidized lipids. On the other hand, it was not true for phylotypes A1 and C1. Both kept efficient energy output, although we suggest that they followed distinct strategies already discussed in chapter 3. Protein complexes photosystem II (PSII), cytochrome b_6-f (cyt b_6-f) and photosystem I (PSI) were highlighted. Plastoquinone pool responsible for electron transport from PSII to cyt b_6-f was also highlighted in red. Scape of high energy electron leading to ROS was highlighted in dark red. Source: Adapted from Wada & Murata, 2009.

Finally, we attempted to elucidate specific hypothesis delineated in this dissertation/thesis:

1) "*Symbiodinium spp. growth rates are negatively affected by heat shock"*

Growth rates were all reduced after heat shock. Besides, response was different between phylotypes. *S. minutum* (B1) did not survive stress, whereas *S. microadriaticum* (A1) and *S. goreau* (C1) were resistant to heat shock but decreased their growth rates.

2) "*Changes in lipids and pigments are different between Symbiodinium phylotypes after heat shock*"

Symbiodinium spp. lipidomes and pigments profiles were differently altered by heat shock. However, downregulation of n-3 fatty acids 4 hours after stress was common in all phylotypes. After 24 hours and in the long term considered as 10 days, each phylotype had a specific survival strategy and fate summarized in figure 4. This demonstrates the essential role of omega-3 fatty acids for cellular energy as suggested by Valentine and Valentine (2004).

3) "Lipidome and pigments profiles of Symbiodinium spp. after stress are good indicators of oxidative stress caused by heat shock experiment"

We considered variations in the pigments ratios as indicators of chloroplast "health status" and chlorophyll decrease strongly indicated damage in the chloroplasts caused by heat shock. This data together with variations in epoxy/de-epoxy xanthophylls suggest that damage was likely caused by oxidative stress but could not prove it. However, oxidized fatty acids in the free form and connected with polar lipids analytically proved damage provoked by oxidative stress.

4) "*Lipid peroxidation preferably occurs in polyunsaturated fatty acids (PUFAs) from chloroplast membranes*"

Lipid peroxidation was evidenced by the presence of oxidized fatty acids mostly derived from omega 3 polyunsaturated fatty acids in free (FFA) and membrane associated (PL) forms of MGDG, DGDG, DGCC and PC. Glycolipids (MGDG and DGDG) are well known structural thylakoid membrane lipids, plus, evidences discussed in chapter 3 strongly indicated DGCC presence also in the chloroplast. Therefore, chloroplast membranes were main targets of lipid peroxidation. Plus, significant changes in DGCC and DGDG compounds suggest that they might be specifically located closer to chloroplast ROS

formation sources when compared to MGDG because they did not change significantly after heat shock.

Importance of phytoplankton lipids in the global carbon budget and bottom-up effects in the marine food chains

Symbiodinium sp. takes part of the Dinophyceae class and many lipid compounds characterized and monitored in our work have been previously reported in the phenotypes of relatives from the same class and other microalgal classes that inhabit oligotrophic ocean gyres (e.g., Crytophyceae, Haptophyceae) (Van Mooy *et al*., 2009, 2010; Shemi *et al*., 2016). We suggest our temperature-related alterations in the lipidome of *Symbiodinium* spp. are similar to those that may occur in phytoplankton communities considering a universal biochemical principle of lipid composition in bioactive membranes.

Becker *et al.* (2018) showed that in the North Pacific subtropical gyre (NPSG) nearly half of the relative abundance of organisms was composed of Dinophyceae and Haptophyceae (ca. 25.3% each). This region comprises 40% of Earth's total surface area, representing the world's largest biome (Emmerson *et al*., 1997; Sarmiento *et al*., 2004). Local and global alterations in phytoplankton communities were noticed with elevated temperatures and other climate change stressors in the past years leading to alterations in global primary production and carbon sinking (Sarmiento *et al*., 2004; Behrenfeld *et al*., 2006; Schmittner *et al*., 2008; Nagelkerken & Connell, 2010), but the physiological mechanisms responsible for such alterations were poorly discussed. We suggest that changes are likely to happen because of phytoplankton photosynthetic structure vulnerability to oxidative stress considering that they are the major DHA producers in the biosphere (Valentine, 2009). High concentrations of DHA were reported by Becker *et al*. (2018) in the NPSG community associated with DGCC betaine polar head. Their most abundant DGCC had C38:6 – 800.6035 m/z - acyl chains of palmitic acid (16:0) and docosahexaenoic acid (DHA), which was also the most abundant feature in our samples. Photosynthesis and phytoplankton growth in phenotypes with high DHA concentration can be impaired through mechanisms already discussed in our work.

Furthermore, the high abundance of DHA in *Symbiodinium* phylotypes and in other flagellate microalgae (Leblond *et al.,* 2000, 2006, 2015; Gray *et al*., 2009; Awai *et al*., 2012; Armada *et al*., 2013; Dahmen *et al*., 2013; Anesi & Guella, 2015; Anesi *et al.,* 2016) sustains the idea proposed by Valentine (2009): phytoplankton represents the global stock of DHA production in the marine ecosystem. Life cycle of zooplankton species depend on the nutritional quality of phytoplankton, which is defined by lipid content (Søreide *et al*., 2010). Ingestion of DHA and other omega 3 enables development of their sensorial mechanisms essential for survival, growth and reproduction (Müller-Navarra *et al*., 2000; Falkowski & Oliver, 2007). Higher in the food chains, other organisms also evolved with the same dependence in n-3 and their populations may either decrease or present individuals with neurological and sensorial deficiencies if n-3 consumption is low (Davis *et al.,* 1992; Budge *et al.,* 2001; Jonasdottir *et al.,* 2002). Consequently, we highlight the importance of DHA ingestion in the marine food chains and how they are also likely to be impacted by global warming. Behrenfeld *et al.* (2006) showed that from 1999 to 2004 the average global primary productivity dropped by about 200 tons a year. Local changes had a decrease as high as 50% (see also Bopp *et al*., 2013). If ocean temperatures keep increasing progressively as predicted by climate models (Hansen *et al*., 2010; Rogelj *et al*., 2012; Cabré *et al*., 2015), omega 3 producing phytoplankton might follow an opposite way and promote a bottom-up effect in all marine food chains that could potentially lead to ecological collapse of the whole ecosystem

Omega-3 presence in microalgae membranes mediate cell death cascades with temperature changes in the environment (Valentine, 2009). It was highly evidenced in our experiments with *Symbiodinium* spp. and generated valuable data for coral reef symbiosis and bleaching research. Besides, the concomitant omega-3 presence in phytoplankton populations with large distribution patterns enhances our insights of large detrimental effects in marine food chains following ocean warming. Therefore, we highlight the importance of the present study in mechanistically explaining a universal biochemical principle for all living creatures, from algae cells to more complex organisms. A principle useful to understanding cell physiology and also how slight modifications can impact the whole environment.

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