The role and significance of alternative electron transport in microalgae

Ph.D. Dissertation

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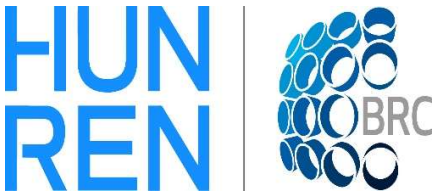

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List of Abbreviations

3-PGA 3-phosphoglycerate

ADP ..... Adenosine diphosphate

AL ..... Actinic light

AOX ..... Alternative Oxidase

A_o ..... Primary electron acceptor in PSI

ATP ..... Adenosine triphosphate

CA ..... Carbonic anhydrase

CBB cycle ..... Calvin-Benson-Bassham cycle

CCM ..... Carbon-concentrating mechanism

CEF ..... Cyclic Electron Flow

CF_1 and CF_0 ..... Coupling factor

Chl ..... Chlorophyll

C_i ..... Inorganic carbon

Cyt b_6f ..... Cytochrome b_6f

Ddx ..... Diadinoxanthin

Dtx ..... Diatoxanthin

EET ..... Extracellular electron transport

ETC ..... Electron transport chain

F_A and F_B ..... Iron–sulfur clusters

Fd ..... Ferredoxin

FNR ..... Ferredoxin-$NADP^+$ reductase

F_X ..... Interpolypeptide iron–sulfur cluster

G3P Glyceraldehyde-3-phosphate

HCO_3^- ..... Bicarbonate

HRFs ..... Host release factors

LHCI and LHCII ..... Light-harvesting complex (I & II)

ML ..... Measuring light

MAMPs ..... Microbe-associated molecular patterns

NADPH ..... Nicotinamide Adenine Dinucleotide Phosphate Hydrogen

NDH ..... NAD(P)H dehydrogenase-like complex

Nda2 ..... Type II NADPH dehydrogenase

NPQ ..... Non-photochemical quenching

Phe ..... Pheophytin

PIF ..... Photosynthesis inhibitory factor

PIFT ..... Post-illumination chlorophyll fluorescence transients

PRRs ..... Pattern recognition receptors

PSI and PSII ..... Photosystem (I & II)

PTOX ..... Plastid terminal oxidase

PQ ..... Plastoquinone

Q_A and Q_B ..... Primary and secondary quinone electron acceptor in PSII

qE ..... Energy-dependent quenching

ROS ..... Reactive oxygen species

RuBP Ribulose-1,5-bisphosphate

RuBisCO ..... Ribulose-1,5-bisphosphate carboxylase oxygenase

SWEET1 ..... Sugars will eventually be exported transporter 1

1. Introduction

Photosynthesis, a central and complex biological process orchestrated by organisms such as plants, algae and certain bacteria, mediates the conversion of solar energy into chemical energy through a series of precisely choreographed biochemical reactions. Photosynthesis sustains life on our planet, providing oxygen and laying the foundation for the trophic pyramid (Whitmarsh & Govindjee, 1999).

1.1 Anoxygenic photosynthesis

Anoxygenic photosynthesis is a distinctive process employed by certain bacteria in which oxygen is not produced as a byproduct. In contrast to oxygenic photosynthesis, which is prevalent in plants, algae, and cyanobacteria and involves the splitting of water to liberate oxygen, anoxygenic photosynthesis utilizes alternative electron donors, including hydrogen sulfide (H_2S), thiosulfate ($S_2O_3^{2-}$), or organic compounds (Blankenship, 2002).

Mechanisms and pathways

In anoxygenic photosynthesis, light energy is captured by bacteriochlorophyll pigments, which differ from the chlorophyll found in oxygenic photosynthesis. This energy drives ATP synthesis and the electron transport chain. Depending on the electron donor, different anoxygenic photosynthetic bacteria use various pathways:

Purple sulfur bacteria use hydrogen sulfide as an electron donor, producing sulfur and sulfate as byproducts. The photosynthetic process of these bacteria can be summarized by the following equation:

$$CO_2+2H_2S \rightarrow CH_2O+2S+H_2O$$ (Overmann & Garcia-Pichel, 2006).

Purple non-sulfur bacteria are more flexible and can use organic compounds or hydrogen as electron donors. They can switch between phototrophic (using light) and chemotrophic (using chemical compounds) modes of metabolism. Similarly, to purple sulfur bacteria, these bacteria utilize hydrogen sulfide but produce sulfur globules outside their cells, which can subsequently be oxidized to sulfate.

Heliobacteria are Gram-positive bacteria that employ a distinctive type of bacteriochlorophyll, designated bacteriochlorophyll g, and possess a more simplified photosynthetic apparatus (Bryant & Frigaard, 2006).

Anoxygenic photosynthetic bacteria flourish in environments where light is available, but oxygen is scarce, such as stratified lakes, hot springs, and sediment layers in water bodies. They play a pivotal role in the biogeochemical cycling of sulfur and carbon in these ecosystems.

1.2 Oxygenic photosynthesis:

The net chemical equation that describes oxygenic photosynthesis is as follows

$6CO_2 + 12H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$

This biochemical reaction not only facilitates the energy conversion required for the growth and development of the photosynthetic organism but also plays a crucial role in the global carbon and oxygen cycles. In the process of photosynthesis, six molecules of carbon dioxide (CO_2) and six molecules of water (H_2O) undergo a transformation driven by the energy from light. This process, which occurs in chloroplasts, involves the harnessing of light energy to convert these reactants into glucose ($C_6H_{12}O_6$), a type of sugar. As a result of this reaction, six molecules of oxygen (O_2) are released into the atmosphere as by-products.

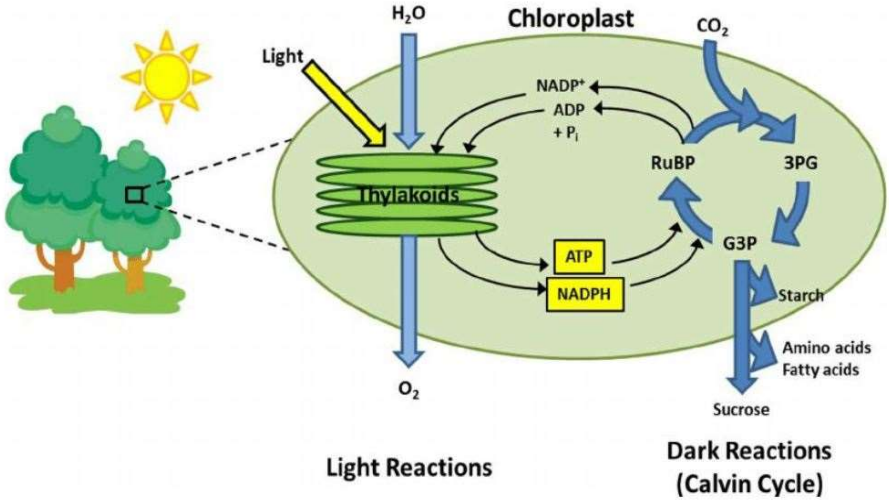

Figure 1.1: Schematic representation of overall process of oxygenic photosynthesis (Rasmussen & Minteer, 2014).

Structure of the chloroplast and thylakoid composition

Photosynthesis takes place in the chloroplast, a specialized organelle found in all green plants and algae. It is a double membrane-bound organelle with two areas of an inner membrane known as the thylakoid, which can be stacked or unstacked, i.e. the grana and the stromal lamellae (Whitmarsh & Govindjee, 1999). The thylakoid membrane integrates pigment-protein complexes as vital elements to capture light energy and carry out light-dependent photosynthetic operations. In algae and higher plants, it consists of six major pigment-protein complexes. These are the photosystem reaction centres (PSI and PSII), the light-harvesting complex of PSI (LHCI), the light-harvesting complex of PSII (LHCII), the cytochrome b_6f (Cyt b_6f) complex, and the chloroplast coupling factor (CF_1 and CF_0), which helps to transfer electrons from water oxidation to the final electron acceptor $NADP^+$ to produce ATP and NADPH.

The stacked region of the thylakoid (grana) is largely composed of PSII and LHCII complexes, whereas the stromal lamellae (non-stacked) contain PSI, LHCI and ATP synthase, and most study suggest Cyt b_6f is uniformly distributed in both types of lamellae but their distribution may change with conditions (Dekker & Boekema, 2005). This form of heterogeneous arrangement increases the packing density of photosynthetic complexes, allowing more effective light-harvesting capacity and energy transfer under a variety of light conditions (Blankenship, 2002; Davidson & Hardison, 1984).

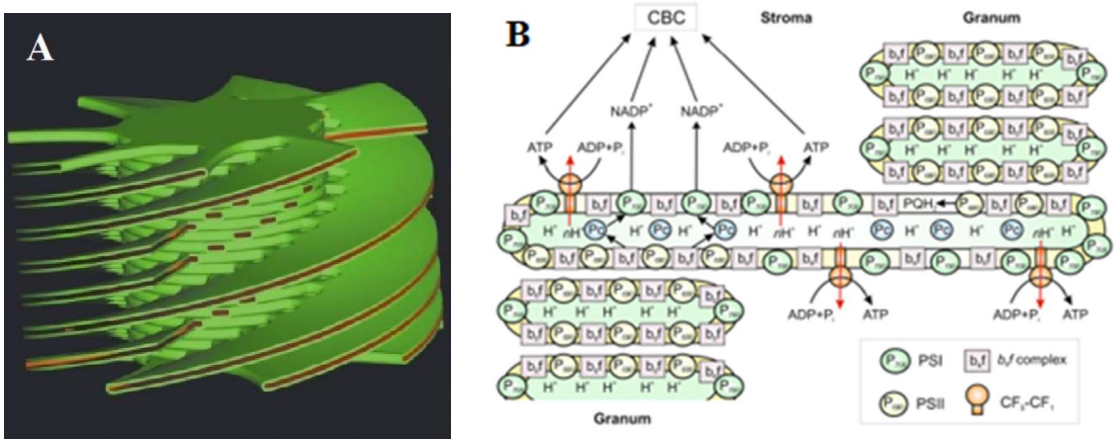

Figure 1.2: Representation of (A) helical arrangement of stromal membranes (Mustardy & Garab, 2003), (B) arrangement of different complexes (Tikhonov, 2017).

Photosynthetic pigments and process

Photosynthetic organisms possess an array of pigments, each characterized by their absorption maximum within distinct spectral regions of the electromagnetic spectrum. These pigments facilitate the capture of solar energy and its subsequent sequestration within the ecosystem through the process of photosynthesis. The pigments are generally divided into three groups: chlorophylls, carotenoids and phycobilins (Whitmarsh & Govindjee, 1999). Chlorophylls contain a porphyrin ring that oxidizes and reduces rapidly to transfer excited electrons to other (Eaton-Rye et al., 2012). Because chlorophylls are the molecules that harvest most of the light, they are called primary pigments. Chlorophylls are divided into several types, including mainly Chl *a*, Chl *b*, Chl c_1, Chl c_2, Chl *d*, Chl *f*. The most common type of chlorophyll is *Chl a*, among plants, algae and cyanobacteria which absorbs strongly in blue and red regions of the visible light spectrum, peaking around 430 nm and 665 nm respectively. Some bacteria contain bacteriochlorophyll, which aids them in utilizing the light to support their growth and development. They are typically adapted for environments with low light intensities, hence absorb light in the longer wavelength ranging from red to infra-red region of 600-900 nm and do not produce molecular oxygen.

Carotenoids and phycobilins are accessory pigments. They do not appear to directly influence the process of photon capture, rather serving in transfer of the absorbed light to chlorophyll molecule. There are more than 600-700 carotenoids in different forms. They are divided into two types: xanthophylls and carotenes. Xanthophylls include violaxanthin, neoxanthin, zeaxanthin and lutein,

while carotenes include α-carotene, β-carotene and lycopene. They absorb light in the 400-550 nm wavelength range and play an important role in protecting chlorophyll molecules from photodamage under intense illumination by enzymatically converting violaxanthin to zeaxanthin via xanthophyll cycle, thereby dissipating excess energy of light. Phycobilins are water-soluble pigments found on the outer stromal surface of the chromophoric membranes of cyanobacteria and red algae. Phycobilins, like chlorophyll, are tetrapyrroles that form phycobilisomes by covalent attachment to proteins and serve as the primary accessory pigments for efficient light harvesting throughout the photosynthetic process (Blankenship, 2002; Demmig-Adams & Adams, 1996).

Photosynthesis can be divided into two main stages:

i) Light-dependent reactions

The first crucial step in photosynthesis is the absorption of light, which occurs in the thylakoid membranes.

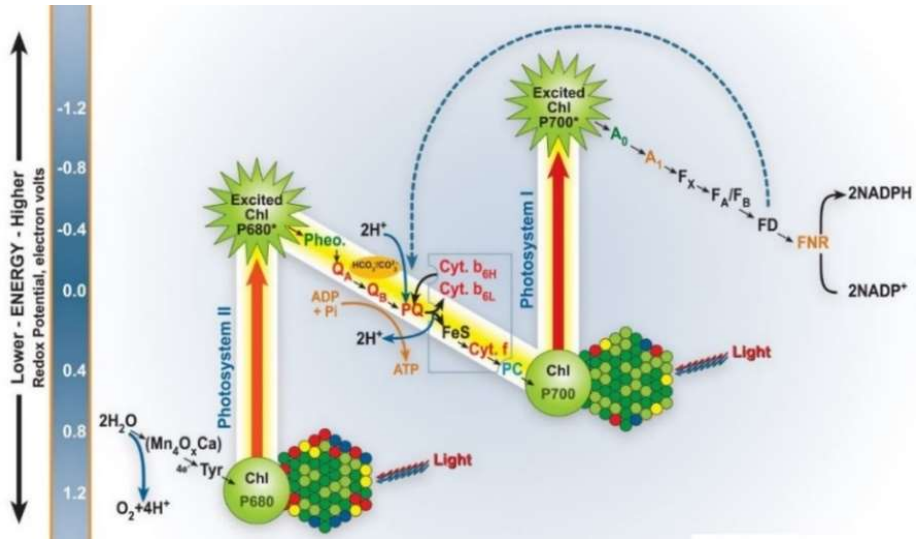

Figure 1.3: Z-scheme of the light reaction of photosynthesis (from http://www.life.illinois.edu/govindjee).

When photons are absorbed by chlorophyll molecules, they create an excited electronic state in the chlorophyll to higher energy levels, a process known as photoexcitation (Blankenship, 2002). The excitation energy is rapidly transferred through a network of light-harvesting complexes, and it is directed to the reaction centres of photosystem II (PSII)(P680) and photosystem I (PSI)(P700).

In the reaction centres of PSII and PSI, special pair of chlorophyll *a* molecules, known as reaction centre chlorophylls are localized. As the excitation energy is trapped by the PSII reaction centre, a charge separation event occurs, leading to the radical formation of $P680^{+}Phe^{-}$. On the acceptor side, the reduced pheophytin transfers the electron to an electron acceptor called the primary quinone

electron acceptor Q_A. From there, the electrons enter the electron transport chain (ETC) located in the thylakoid membrane via a secondary electron acceptor Q_B. The ETC consists of several components, including the PQ (plastoquinone) pool and the cytochrome b_6f complex (Bassham et al., 1950). The PQ acts as an electron carrier, shuttling electrons from PSII to the cytochrome b_6f complex. As the electrons move through the cytochrome b_6f complex, they undergo a series of redox reactions in which energy is used to pump protons (H^+) from the stroma into the thylakoid lumen. This proton pumping creates a proton gradient across the thylakoid membrane.

Water molecules in PSII donor side undergo a process called water oxidation (Lubitz et al., 2019). The oxidation of water is catalyzed by a Mn_4Ca cluster, which undergoes cyclic changes of light-induced oxidation. The complex exhibits five distinct oxidation states, designated as S0, S1, S2, S3 and S4, with the release of oxygen occurring during the transition from S3 to S4 to S0 (Vass, 2012), thereby splitting the water molecules into oxygen (O_2), protons (H^+) and electrons. Utilizing these electrons, Tyr-Z of D1 protein at the donor side reduces back the $P680^+$ formed during the charge separation in PSII, thus maintaining a continuous flow of electrons through the photosystem.

After passing through the cytochrome b_6f complex, these electrons are transferred to photosystem I (PSI) via plastocyanin (PC) a small redox-active protein that is located in the thylakoid lumen, where they reduce the oxidized $P700^+$, which was formed during the previous charge separation event between the P700 and the acceptor A_o in PSI rection centre. The acceptor A_o transfers the electron through a series of transport via iron-sulphur clusters (F_X, F_A, F_B) (Ben-Shem et al., 2003). Ferredoxin, which is a small iron-sulfur protein, acts as the final electron carrier. The electrons from ferredoxin are then transferred to the enzyme ferredoxin-$NADP^+$ reductase (FNR), ultimately leading to the reduction of $NADP^+$ to NADPH, a high-energy molecule that carries the electrons needed for the next stage of photosynthesis i.e. Calvin-Benson-Bassham cycle (CBB cycle).

Meanwhile, the protons generated by the oxidation of water and the redox transfer of electrons by Cyt b_6f lead to the establishment of a proton concentration gradient ($\Delta[H^+]$) and electrochemical gradient across the thylakoid membrane (Kramer et al., 2004), with a higher concentration of protons in the thylakoid lumen than in the stroma. As these protons flow back to the thylakoid lumen via ATP synthase, ADP molecules are converted to ATP, via the process known as photophosphorylation.

The light-dependent reactions culminate in the production of ATP and NADPH, which are essential energy carriers for the light-independent reactions (CBB cycle). This scheme of redox transfer of electrons from H_2O/O_2 system to finally $NADP^+/NADPH+H^+$ system is known as linear electron transport or Z-scheme. In the CBB cycle, carbon dioxide from the atmosphere is fixed and converted into glucose and other essential organic compounds.

ii) Light-independent reactions (CBB cycle)

The light-independent reactions, also known as the CBB cycle, take place in the stroma of chloroplasts. In contrast to the light-dependent reactions, the CBB cycle does not depend directly

on light, but uses the energy carriers ATP and NADPH, which are produced during the light-dependent reactions (Davidson & Hardison, 1984; Whitmarsh & Govindjee, 1999).

The CBB cycle commences with the capture of carbon dioxide (CO_2) from the atmosphere, which subsequently combines with a five-carbon molecule designated as ribulose-1,5-bisphosphate (RuBP). This reaction is facilitated by the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO), which produces a six-carbon compound that rapidly splits into two molecules of 3-phosphoglycerate (3-PGA). The 3-PGA molecules are subsequently transformed into a high-energy molecule known as glyceraldehyde-3-phosphate (G3P). This transformation is dependent on the input of energy in the form of ATP and NADPH, which are generated during light-dependent reactions. In each cycle, three ATP molecules and two NADPH molecules are consumed to produce a single molecule of G3P.

The majority of the G3P produced is employed in the regeneration of the original RuBP, thus enabling the continuation of the CBB cycle and the capture of CO_2. This regeneration process is intricate and involves a series of reactions that rearrange carbon atoms, necessitating the additional input of ATP. Following six cycles, two G3P molecules unite to form a single glucose molecule ($C_6H_{12}O_6$), which serves as the plant's primary source of energy and building material. Additionally, some G3P molecules contribute to the synthesis of other essential compounds, including carbohydrates, amino acids, and lipids, which are necessary for the plant's growth and metabolism. Some G3P molecules can also be used to synthesize other carbohydrates, amino acids, lipids and various organic compounds needed for plant growth and metabolism (Aro & Andersson, 2001).

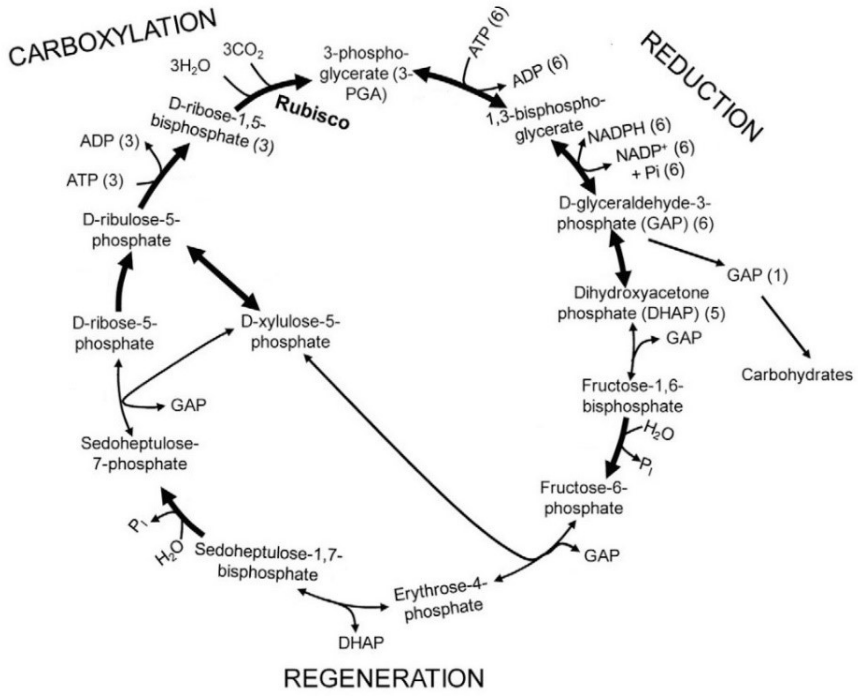

Figure 1.4: Detailed sketch of Calvin-Benson-Bassham cycle in the dark reaction of photosynthesis (Abebe, 2009).

The CBB cycle operates continuously in the presence of light and its rate can be regulated by factors such as light intensity, carbon dioxide concentration and the availability of ATP and NADPH. It is a highly efficient process that allows plants to convert carbon dioxide into glucose and other essential organic compounds required for growth and survival. The glucose produced by the CBB cycle forms the basis of the food chain, supporting the energy needs of animals and other organisms that rely on plants for food.

1.3 Alternative electron transport processes

In the process of photosynthesis, the primary electron transport chain (ETC) is responsible for the efficient conversion of light energy into chemical energy, thereby producing ATP and NADPH, which are essential for the fixation of carbon. However, plants and other photosynthetic organisms have developed alternative electron transport pathways to adapt to varying environmental conditions and metabolic demands. These pathways include Cyclic Electron Flow (CEF), the Alternative Oxidase (AOX) pathway, the Mehler reaction, and Chlororespiration. Each of these processes serves a distinct role in maintaining cellular energy balance, protecting against oxidative stress, and ensuring the flexibility and resilience of the photosynthetic apparatus.

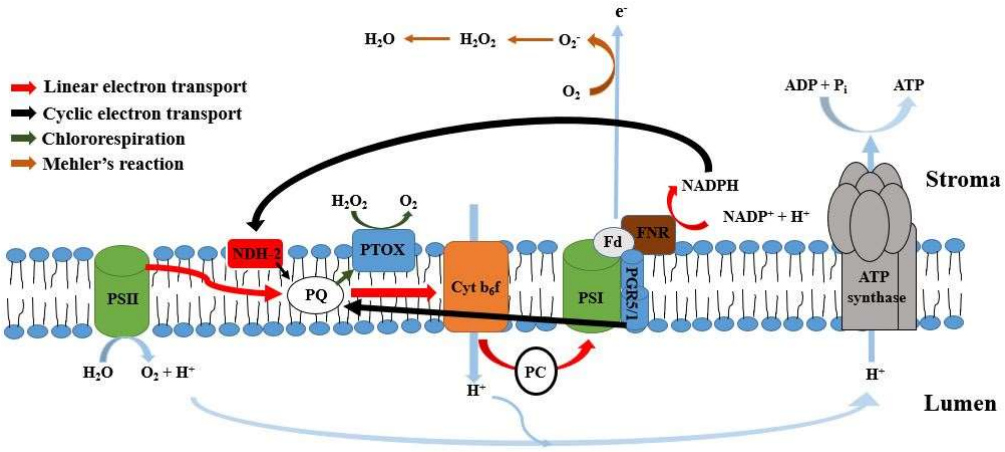

Figure 1.5: Various alternative pathways of electron transport in chloroplast.

Each of the pathways is listed below individually:

Cyclic electron flow (CEF)

Cyclic Electron Flow (CEF) represents an indispensable alternative pathway in photosynthesis, whereby electrons are recycled around photosystem I (PSI) in lieu of the conventional linear trajectory. In contrast to the linear electron flow, CEF does not result in the production of NADPH; rather, it generates additional ATP. This process occurs when electrons from PSI are transferred through a series of carriers, including ferredoxin and the cytochrome b_6f complex, back to the plastoquinone pool. The recycling of electrons thus creates a proton gradient across the thylakoid membrane, which is then used by ATP synthase to produce ATP through photophosphorylation.

This mechanism of ATP generation without NADPH production is essential for maintaining the ATP/NADPH ratio to meet the cell's metabolic demands (Miyake et al., 2005).

There are two discrete cyclic electron flow (CEF) pathways that have been identified in microalgae, which facilitate the efficient production of adenosine triphosphate (ATP) during photosynthesis. The antimycin A-sensitive pathway entails an electron transfer from ferredoxin to plastoquinone, which is facilitated by the involvement of proteins such as protein gradient regulation-5 (PGR5) and PGR5-like (PGRL1) (DalCorso et al., 2008). These proteins form a supercomplex with photosystem I (PSI), LHCI, LHCII, the cytochrome b_6f complex, ferredoxin-NADP reductase (FNR), and PGRL1 (Iwai et al., 2010). The supercomplex is capable of catalyzing cyclic electron flow and responsible for maintaining the proton gradient across the thylakoid membrane, which in turn drives ATP synthesis. In contrast to the antimycin A-sensitive pathway, the antimycin A-insensitive pathway is dependent on NAD(P)H dehydrogenases (NDH) located in the thylakoid membrane (Rumeau et al., 2007). These NDH enzymes reduce the plastoquinone (PQ) pool using reductants derived from the stromal components in a non-photochemical way. The class-I NDH-1 complex found in higher plants (Joët et al., 2002) and cyanobacteria (Battchikova et al., 2011) is absent in *Chlamydomonas*, this organism employs the Class-II NAD(P)H dehydrogenase Nda2 to achieve the same PQ pool reduction (Desplats et al., 2009).

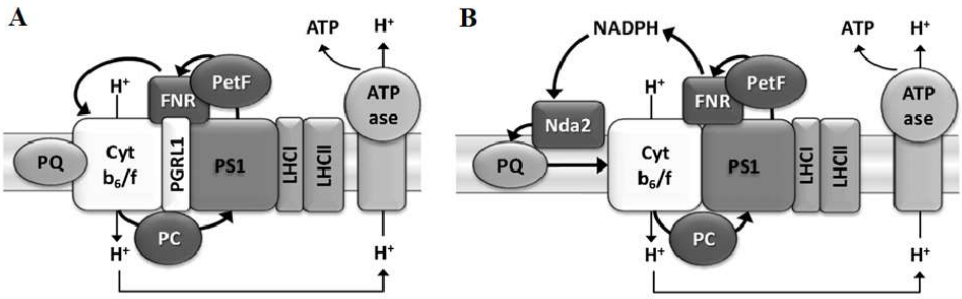

Figure 1.6: Cyclic electron transport in microalgae via (A) Antimycin A-sensitive and (B) Antimycin A-insensitive pathways (Hemschemeier & Happe, 2011).

In circumstances where there is a greater requirement for ATP than NADPH, such as during rapid cell growth, photosynthetic acclimation or in response to stress, CEF assumes particular significance. By modulating the ATP/NADPH production ratio, CEF facilitates the optimization of photosynthetic efficiency and the maintenance of redox balance within the chloroplast. This flexibility is essential for plants to effectively regulate their energy production and utilization, ensuring the uninterrupted functioning of processes requiring ATP even when the demand for NADPH is relatively low (Kramer & Evans, 2010).

Mehler reaction

The Mehler reaction, frequently designated as the water-water cycle, occurs within chloroplasts and encompasses the non-cyclic reduction of oxygen (O_2) to water (H_2O) through the utilization of electrons derived from photosystem I (PSI). The process commences with the reduction of

molecular oxygen by electrons from PSI, facilitated by ferredoxin and the ferredoxin-$NADP^+$ reductase. This results in the formation of superoxide radicals (O_2^-). Subsequently, these superoxide radicals are converted into hydrogen peroxide (H_2O_2) and ultimately into water through the action of enzymes such as superoxide dismutase and catalase (Asada, 1999).

This reaction plays a pivotal role in dissipating surplus excitation energy in PSI, particularly under high light conditions. This prevents the formation of deleterious reactive oxygen species (ROS) and safeguards chloroplasts from oxidative damage. The Mehler reaction plays a pivotal role in maintaining cellular redox balance and ensuring optimal photosynthetic performance by efficiently channeling excess electrons away from PSI. The detoxification of ROS is of paramount importance for plant health, particularly under stress conditions where the risk of oxidative damage is elevated (Foyer & Noctor, 2005).

Chlororespiration

Chlororespiration represents a backup process in chloroplasts that is analogous to mitochondrial respiration. The process is activated when the primary electron pathways are under stress, as occurs during low CO_2 conditions. It involves the transfer of electrons from NAD(P)H to plastoquinone (PQ) and then to oxygen, resulting in the production of water. Key roles are played by PTOX and NDH.

This process helps to maintain redox balance and prevents the formation of harmful reactive oxygen species (ROS), ensuring continued ATP production and protecting the photosynthetic system. This flexibility is vital for plant resilience under fluctuating environmental conditions (Krieger-Liszkay & Feilke, 2016; Li et al., 2016).

Alternative oxidase pathway (AOX)

The alternative oxidase (AOX) pathway represents a further critical electron transport route, which is found in the mitochondria of plants and certain algae. In contrast to the primary electron transport chain, this pathway circumvents complexes III and IV, transferring electrons directly from ubiquinol to oxygen, ultimately leading to the production of water. The AOX pathway is of particular importance in situations where the primary electron transport chain is either inhibited or overwhelmed with reducing equivalents, such as occurs during periods of stress. By dispersing this excess reducing power, AOX helps to prevent the over-reduction of electron carriers, thereby maintaining the cell's redox balance (Vanlerberghe & McIntosh, 1997).

Furthermore, AOX plays a crucial role in regulating ATP synthesis and generating heat (thermogenesis) in response to stressful conditions, including high light intensity, low temperatures, and pathogen attacks. This pathway enables plants to adapt more flexibly to changing environmental conditions. The capacity to utilize AOX for dissipating excess electrons is vital for protecting cells from oxidative damage (Maxwell et al., 1999) and ensuring uninterrupted ATP production. This, in turn, enhances the plant’s overall stress tolerance and resilience, facilitating survival and thriving in the face of environmental challenges. In dinoflagellates, it helps to cope

with adverse condition such as the ROS buildup during high light, temperature, hypoxia and during harmful algal bloom and plays a significant role in maintaining energy balance (Cho et al., 2022; Oakley et al., 2014), particularly in symbiotic relationships with corals and during stressful environmental conditions, contributing to the survival and success of these organisms in diverse marine habitats (Vega de Luna et al., 2020).

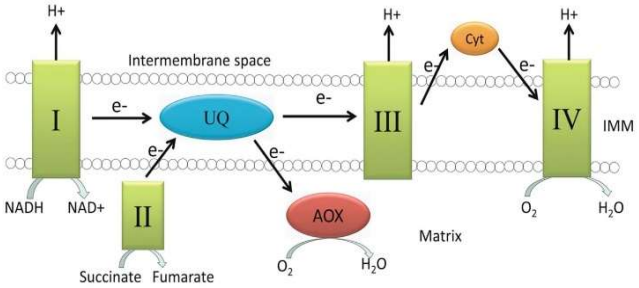

Figure 1.7: Alternate oxidase pathway in Mitochondria (Liu, 2017).

These alternate pathways enable photosynthetic organisms to manage excess energy, maintain redox homeostasis, and survive under stress conditions, underscoring the sophisticated adaptability of photosynthetic systems.

Extracellular electron transport (EET)

Extracellular electron transport (EET) in photosynthetic microorganisms offers an alternative pathway for managing excess electrons generated during photosynthesis. In organisms like *Synechocystis*, the primary electron transport chains of the thylakoid membrane, which drive processes like ATP synthesis and carbon fixation, can become over-reduced under conditions of high light intensity or low carbon availability (Gorby et al., 2006). To alleviate this stress and prevent photodamage, these microorganisms employ EET as a mean to divert surplus electrons to the cell exterior. This alternative pathway involves electron donors such as plastoquinole (PQH_2), which can transfer electrons to cytoplasmic carriers, including ferredoxins and soluble cytochromes, or directly to extracellular components via some unidentified proteins and further electron transport to the exterior could occur via conductive pili in cyanobacteria (Schuergers & Wilde, 2015). In some species membrane bound cytochromes and in other transporters such as Riboflavin in plasma membrane can also mediate electron transfer to extracellular component (Marsili et al., 2008; Lin et al., 2015). This mechanism not only protects photosynthetic machinery but also allows these microorganisms to engage in ecological interactions, such as reducing external compounds or facilitating electron exchange with other microbes in biofilms or mats (McCormick et al., 2011).

EET also provides a critical adaptation for photosynthetic microorganisms in fluctuating or stressful environments. When internal electron sinks, like $NADP^+$ or carbon fixation pathways, are insufficient to balance the electron flow, EET serves as an overflow mechanism, exporting electrons to extracellular acceptors such as iron (Kranzler et al., 2014) or other redox-active substances in the environment. Additionally, EET has been implicated in intercellular signaling

(Michelusi et al., 2014), where exported electrons may convey information about environmental or metabolic states. By linking photosynthetic processes to external electron acceptors, this alternative pathway not only enhances cellular resilience but also enables participation in broader ecological processes, such as nutrient cycling and symbiosis.

1.4 Photosynthesis in extreme environments

Photosynthesis occurs in a wide range of extreme environments. A few examples are described below that highlight the remarkable ability of photosynthetic organisms to thrive in extreme environments, whether on land or in water, demonstrating their resilience and adaptability to different ecological conditions.

Coral reefs are found in shallow, sunlit waters of tropical and subtropical regions (Jones, 2004). Coral polyps, which host symbiotic algae called zooxanthellae, perform photosynthesis to produce energy for themselves and the coral reef ecosystem (Falkowski, 1984). Despite challenges such as high temperatures, intense sunlight and fluctuations in nutrient availability, corals and their symbiotic algae have evolved mechanisms to efficiently capture light and fix carbon dioxide for growth and calcification (Hoegh-Guldberg et al., 2007).

Other examples such as desert plants, including cacti and succulents, have evolved specialized structures to cope with arid conditions. These include waxy coatings and reduced leaves, which minimize water loss while maximizing sunlight absorption for photosynthesis (Jones, 2010). Similarly, in extremely arid deserts, endolithic microorganisms, such as cyanobacteria, reside within rocks to safeguard themselves from the harsh effects of sunlight and desiccation, utilizing solar energy for photosynthesis (Warren-Rhodes et al., 2006). Furthermore, alpine plants in regions such as the Andes or Himalayas also encounter extreme conditions, including intense ultraviolet radiation and low temperatures. Nevertheless, they have adapted to efficiently capture sunlight for photosynthesis and withstand freezing temperatures (Körner, 2003; Pospíšilová, 2003). In polar regions, despite the freezing temperatures and extended periods of darkness, both terrestrial and aquatic photosynthetic organisms, such as algae, mosses, and phytoplankton, flourish. They contribute to photosynthesis during the summer months when sunlight is available (Arrigo et al., 1997).

1.5 Coral reefs

Coral reefs inhabit nutrient-poor marine environments, yet they stand out as some of the most diverse ecosystems on the planet. The diverse underwater ecosystems formed by the accumulation of calcium carbonate secreted by corals. These intricate structures provide a vital habitat for a wide variety of marine organisms, supporting approximately 25% of all marine species despite covering less than 0.1% of the ocean floor. Coral reefs play a crucial role in the global carbon cycle, acting as carbon sinks that sequester carbon dioxide from the atmosphere. Corals and other reef organisms incorporate carbon into their calcium carbonate structures, helping to reduce the levels of greenhouse gases in the atmosphere. This seemingly paradoxical phenomenon stems from the

remarkable ability of coral reef-building species to harness solar energy, functioning as primary producers within the reef (Falkowski et al., 1984). This capability is not unique to coral reefs but is shared by several cnidarian species in various aquatic habitats, facilitated by a specialized mutualistic relationship with unicellular photosynthetic algae (Furla et al., 2000). These algae, known as zooxanthellae, endow their animal hosts with exceptional productivity, driving nutrient recycling throughout the entire coral reef ecosystem (Crossland et al., 1991).

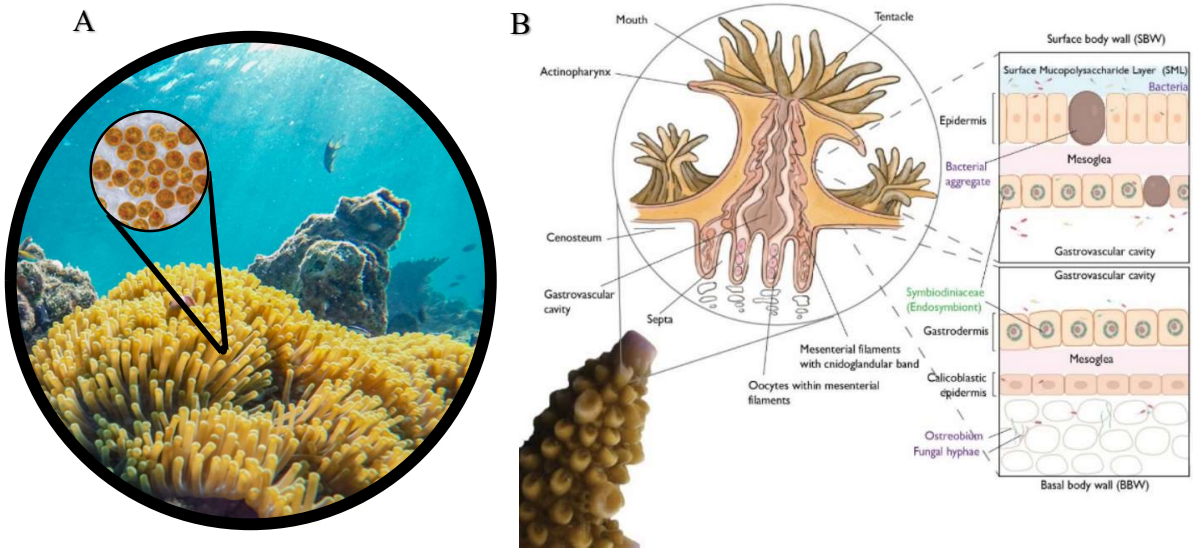

Figure 1.8: Coral reef and its endosymbiont (*Symbiodinium*) (A), and anatomy of the coral polyp showing the exact location of *Symbiodinium* cells in gastrodermis (B) (Page et al., 2024).

Most symbiotic cnidarians host dinoflagellate algae from the Symbiodiniaceae family, previously known as "zooxanthella." Certain species, like *Anthopleura* in Anthozoa, can host both Symbiodiniaceae and Trebouxiophyceae species (zoochlorella). Green Hydra species (*Hydrozoa*) specifically harbor *Chlorella* species as their photosynthetic symbionts (Kawaida et al., 2013).

This mutualistic bond allows corals to flourish in nutrient-deficient waters by utilizing sunlight energy through photosynthesis which interns power their metabolism. This process is based on a complex molecular machinery of pigments and proteins housed in the thylakoid membrane structure located in the chloroplast. Over the course of time, photosynthetic machinery of the dinoflagellate algal symbiont of cnidarians have evolved unique traits to optimize light energy utilization and safeguard against excess energy damage (Dorrell & Howe, 2015) helping them to adapt to varying light intensities and environmental conditions, ensuring efficient energy management within coral reef ecosystems (Roth, 2014).

Additionally, coral reefs contribute to the overall health of marine ecosystems by promoting nutrient cycling and supporting the productivity of fisheries. However, coral reefs are facing unprecedented threats due to climate change, overfishing, pollution, and ocean acidification, leading to widespread coral bleaching events and reef degradation. Understanding the intricate mechanisms of photosynthesis and energy regulation in symbiotic organisms like coral reefs

provides valuable insights into their responses to both natural and human-induced environmental changes. This comprehension, in turn, contributes to the conservation and management efforts aimed at safeguarding these vital ecosystems.

1.6 Symbiosome

Cnidarians exhibit a restricted range of cell types, with specific endoderm cells containing algal symbionts within host-derived vacuoles, designated as symbiosomes (Camaya, 2020; Tivey et al., 2020). These symbionts, belonging to the Symbiodiniaceae family, can occupy over 90% of the cytosol in these cells (Gates et al., 1992). The formation of symbiosis can occur in both the larval and adult stages, although the molecular details remain largely unknown (Byler et al., 2013; Mies et al., 2017).

The process of host-symbiont recognition is facilitated by cell surface components, including lectins and glycoproteins (Fransolet et al., 2012). It is noteworthy that the presence of photosynthetic activity is not a prerequisite for the establishment of symbiosis. The infectivity of Symbiodiniaceae has been observed to correlate with cell size, with larger cells (>10 μm) exhibiting reduced infectivity (Biquand et al., 2017). Following engulfment by gastrodermal cells, the algae are contained within phagosomes and subsequently transformed into symbiosome.

Symbiodinium alternate between a motile gymnodinioid form and a non-motile coccoid form (Fitt & Trench, 1983). Once within the symbiosome, the Symbiodiniaceae cease to be motile and display morphological differences from their free-living counterparts. The structure of the symbiosome comprises additional membrane layers derived from the algae. An acidic environment (pH~4) within the symbiosome, maintained by active proton pumping, facilitates CO_2 enrichment for the algae (Barott et al., 2014; Raven et al., 2020). The existence of this distinctive environment thus requires the precise regulation of metabolite exchange between the host cells and the Symbiodiniaceae, to ensure the continued viability of the mutualistic relationship.

Establishment of in cnidarian-dinoflagellate symbiosis

The establishment of the cnidarian-dinoflagellate symbiosis is initiated by the complex recognition mechanisms that establish the foundation for a fruitful partnership. Molecular signalling pathways play a pivotal role in this inaugural encounter, with parallels drawn from symbioses such as that observed between the green hydra (McAuley & Smith, 1982) and the *Paramecium-Chlorella* (Karakashian & Rudzinska, 1981). The process is centered upon MAMP-PRR signalling, whereby glycan-lectin interactions facilitate communication between the host cnidarian and its symbiotic algae (Jolley & Smith, 1980; Meints & Pardy, 1980). Once contact is established, phagocytosis becomes the primary route for the algae to enter the host, a process that is far from random. Recent studies, particularly with *F. scutaria* larvae, have revealed that selective phagocytosis occurs, with larvae preferentially incorporating specific *Symbiodinium* strains, such as C1f, into their gastrodermal cells, while others, such as C31, are less favored (Rodriguez-Lanetty et al., 2006; V. Weis et al., 2001).

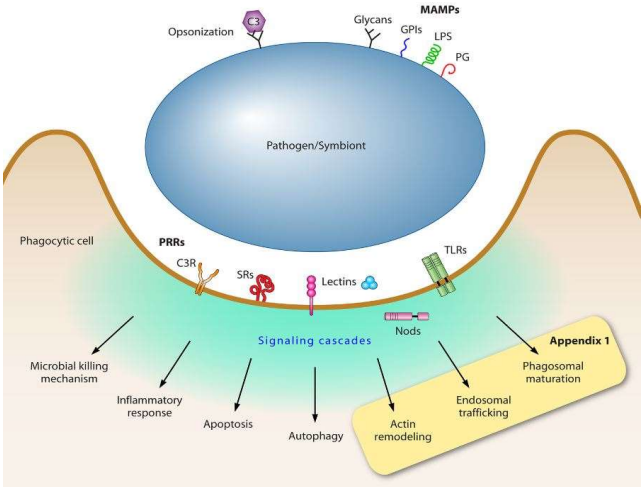

Figure 1.9: MAMP-PPRs signaling and processes afterwards. Recognition and phagocytosis of endosymbiont (Davy et al., 2012).

The process of symbiotic integration is intricate, with symbiotic algae employing strategies to delay phagosomal maturation, thereby evading host intracellular defences and ensuring a stable relationship (Karakashian & Rudzinska, 1981). The role of apoptosis and autophagy further complicates this process, as these cellular mechanisms determine the fate of symbionts post-phagocytosis, often resulting in the elimination of incompatible partners during bleaching responses (Dunn et al., 2007). In this context, the upregulation of genes such as sym32 and calumenin in symbiotic anemones highlights the significance of cell signaling in the processes of recognition and adhesion, thereby providing deeper insights into the dynamics of this symbiotic relationship (Ganot et al., 2011). As research progresses, the molecular intricacies of the cnidarian-dinoflagellate symbiosis continue to be elucidated, revealing a fascinating dance of cellular processes that define this unique biological partnership.

Host-symbiont transport

Symbiodiniaceae algae display remarkable adaptability, exhibiting a range of morphologies, cell cycles, and life histories as they transition from a free-living existence to a symbiotic lifestyle with cnidarians (Trench, 1993). This evolutionary trajectory illustrates the intricate interplay between environmental pressures and biological innovation. As a consequence of their evolutionary transition to a symbiotic lifestyle, these algae developed a series of specialized transporter genes that are essential for the exchange of nutrients with their cnidarian hosts. For example, the SWEET1 transporter, which belongs to a recently identified family of proteins involved in sugar efflux, is upregulated in symbiosis. This suggests that it plays a role in facilitating sugar exchange between the algae and its host. This finding is consistent with prior research on SWEET transporters and their role in glucose trafficking in plants and other organisms (Chen et al., 2010; Feng & Frommer, 2015).

Furthermore, the upregulation of nitrogen transporters, particularly those involved in ammonium recycling, indicates that the algae are assuming an emerging role as an "ammonium sink" (Aranda et al., 2016) within the symbiotic relationship. This adaptation highlights the necessity for both nitrogen and phosphate transporters, as these nutrients are critical for meeting the increased demands of the holobiont. Research has shown that upregulation of phosphate transporter supporting previous findings that suggest the symbiont may act as a phosphate sink (Godinot et al., 2009).

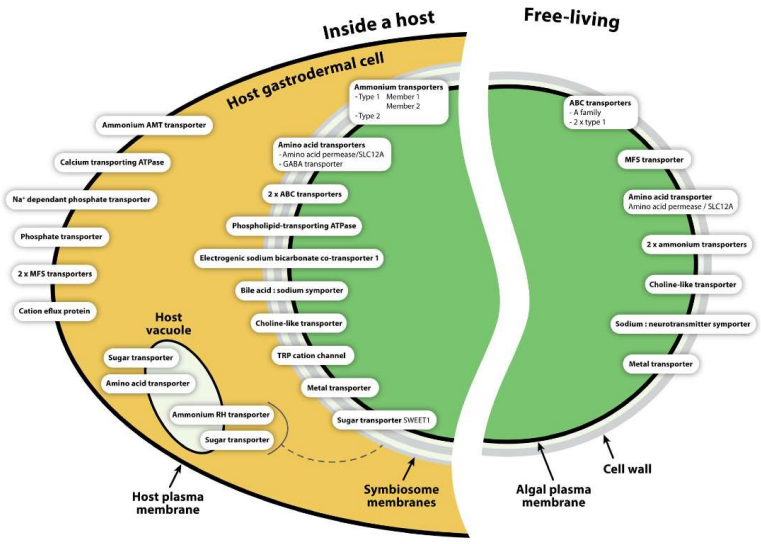

Figure 1.10: Differently expressed transporters in free-living and symbiotic Symbiodinium as well as the holobiont (Maor-Landaw et al., 2020).

In general, compounds exchanged between the symbiont and the host must traverse at least three membranes: the algal plasma membrane, the symbiosome membrane, and the host cell membrane. Therefore, the symbiotic relationship between coral and the algae Symbiodiniaceae relies on the activity of several transporters, which enable the interchange of nutrients and metabolites that are indispensable for the sustained survival of both entities. As an example, the coral host employs bicarbonate transporters to provide the algae with bicarbonate, a vital component of photosynthesis. Furthermore, the provision of nitrogen compounds, including ammonium, nitrite and nitrate, is facilitated by specialized transporters, thereby supporting the assimilation and growth of the algae. In return, Symbiodiniaceae employs glucose transporters to deliver glucose, a key product of photosynthesis, to the coral host, thereby providing it with a vital energy source. Furthermore, Symbiodiniaceae provides the coral host with sugars, lipids, and glycerol via aquaporin and lipid transporters, thereby sustaining the coral's metabolic processes.

Other transporters including riboflavin and folate facilitate the movement of essential cofactors and detoxifying substances, thereby supporting the coral's cellular functions and growth. Riboflavin transporters have also been observed to play a role in EET in bacteria (Marsili et al., 2008), suggesting they could potentially be present in coral systems as well. However, EET in coral systems has not yet been thoroughly documented. The coral host facilitates the detoxification of

toxic arsenate to methylarsenite in collaboration with symbiont via the arsenite transporter. Additionally, phosphate and sulfate transporters facilitate the transfer of these essential ions, which are crucial for ATP production and sulfur metabolism. Metal ion transporters, on the other hand, deliver essential cofactors, including copper, cadmium, zinc, and iron, to symbiont. The collective function of these transporters is to maintain the delicate balance of nutrient exchange, which is fundamental to the health and stability of the coral-Symbiodiniaceae symbiosis.

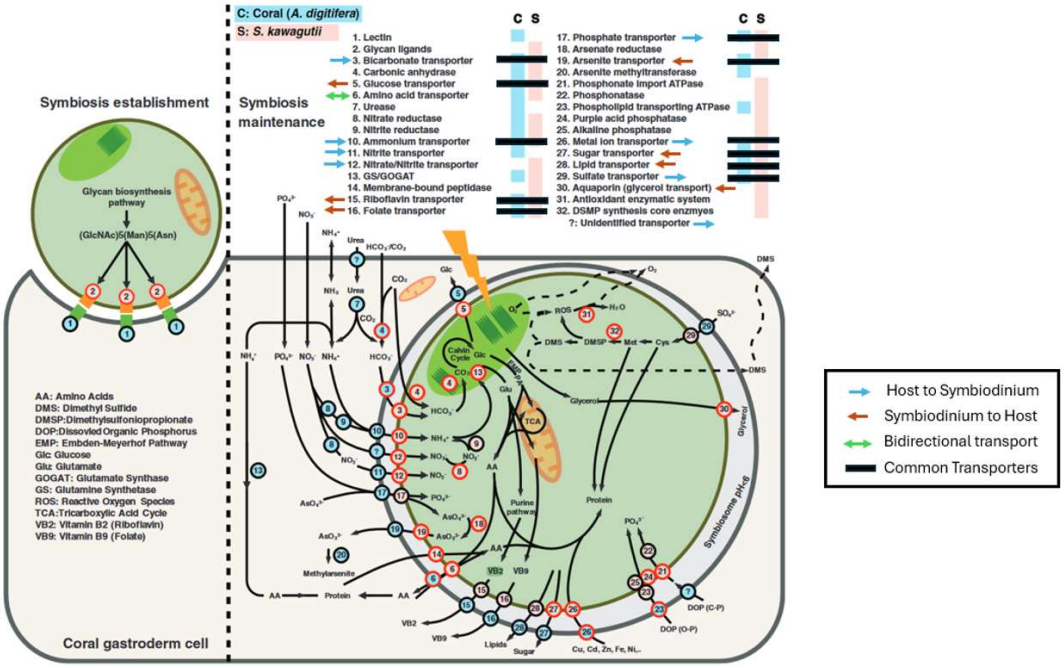

Figure 1.11: Schematic representation of recognition and transport between coral host and Symbiodinium cell (Lin et al., 2015)

Inorganic carbon acquisition and fixation

Photosynthesis in the cnidarian-dinoflagellate symbiosis supports respiration, growth and reproduction, and sometimes releases carbon into the seawater as coral mucus. Inorganic carbon (C_i) for photosynthesis can come from respiration, calcification by-products or seawater (Furla et al., 2000; Harland & Davies, 1995; Ware et al., 1992).

At seawater pH 8.2, most inorganic carbon is bicarbonate (HCO_3^-, ~2.2 mM) rather than CO_2 (~30 µM). Marine phototrophs require either RuBisCO with high CO_2 affinity or a carbon-concentrating mechanism (CCM) to utilise HCO_3^-. The cnidarian host uses a CCM to transport C_i to the dinoflagellate cells, in contrast to nonsymbiotic animals that expel Ci (Allemand et al., 1998; Leggat et al., 2002).

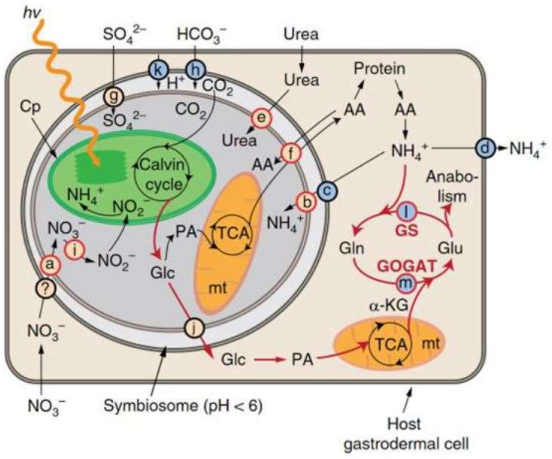

Figure 1.12: Metabolic interactions within the alga-hosting cell and the Symbiodiniaceae (Xiang et al., 2020).

Carbonic anhydrase (CA), which converts HCO_3^- to CO_2, was first identified in symbiotic cnidarians by Isa Y, Yamazato K (Isa, 1984). Weis et al. found CA activity in 22 symbiotic cnidarian species, with higher CA activity in host tissues, which are crucial for photosynthesis (Weis et al., 1989). CA inhibitors reduced photosynthetic carbon assimilation by 56-85%. CA is found around dinoflagellate cells and on ectodermal and gastrodermal cell membranes (Al-Moghrabi et al., 1996; Bertucci et al., 2011). H^+-ATPase in ectodermal cells secretes H^+ into seawater to form carbonic acid, which is converted to CO_2 by CA for passive diffusion into cells (Furla et al., 2000).

Bertucci et al. showed an H^+-ATPase in dinoflagellates that maintains an acidic symbiosome vacuole, which aids HCO_3^- dehydration. *Galaxea fascicularis* dinoflagellates uptake CO_2 and HCO_3^- via a Na^+-dependent carrier, suggesting active C_i uptake and CCM (Goiran et al., 1996). Higher C_i concentrations than allowed by passive diffusion are found in symbiotic dinoflagellates from *Tridacna gigas* (Leggat et al., 1999) and *Stylophora pistillata* (Furla et al., 2000). CA is associated with thylakoids and the large chloroplast pyrenoid in *Symbiodinium* spp., with RuBisCO localized in the pyrenoid.

Structure of chloroplast and thylakoid composition

Comparative genomic studies have revealed significant sequence and structural divergence among the different genera within the Symbiodiniaceae family. This diversity is also evident within the Symbiodinium genus, particularly between species with disparate lifestyles, such as the free-living *Symbiodinium natans* and the symbiotic *Symbiodinium tridacnidorum* (González-Pech et al., 2021).

The chloroplasts of dinoflagellates, including those belonging to the Symbiodiniaceae family, provide an intriguing insight into their evolutionary history. These chloroplasts, which contain the pigment peridinin, were acquired through secondary endosymbiosis with red algae (Ishida &

Green, 2002). They are surrounded by three membranes (Yoon et al., 2002) and have a complex, reticulated structure that is thought to optimize light absorption (LaJeunesse et al., 2010). The chloroplasts contain a peripheral pyrenoid encased by a cytosolic starch plate, which is invaginated by the chloroplast envelope but not penetrated by the thylakoids (Camaya, 2020; Yamashita et al., 2009). Furthermore, these chloroplasts contain small plasmid-like DNA minicircles that encode essential photosynthetic proteins (Barbrook et al., 2014). The gene content of Symbiodiniaceae chloroplasts is notably reduced in comparison to other microalgae, primarily due to extensive gene transfer from the chloroplast to the nucleus (Mungpakdee et al., 2014).

The primary pigments present in these dinoflagellates include chlorophyll *a* (Chl *a*), chlorophyll c_2 (Chl c_2), peridinin (Per), and diadinoxanthin (Ddx) (Niedzwiedzki et al., 2014). Furthermore, *Symbiodinium* and *Durusdinium* species have evolved the capacity to synthesize mycosporine-like amino acids (MAAs), which provide protection by absorbing ultraviolet (UV) radiation. However, this ability is not universal within the Symbiodiniaceae family. For instance, *Breviolum* and *Cladocopium* species have lost the gene cluster responsible for MAA biosynthesis (Shoguchi et al., 2020).

The primary light-harvesting complex (LHC) in these organisms is the membrane-embedded Chl *a*-Chl c_2-Per protein complex (acpPC), which exhibits a distinctive pigment ratio of 4/6/6/1 (Chl *a*/Chl c_2/Per/Ddx) (Niedzwiedzki et al., 2014). A phylogenetic analysis has identified three distinct lineages of acpPC genes. The LHCR complex is associated with PSI, while the LHCF complex is related to fucoxanthin-Chl *a*-Chl *c* binding proteins in diatoms(Boldt et al., 2012; Maruyama et al., 2015). Another significant light-harvesting complex is the soluble Per-Chl *a* Protein (PCP) Complex, which may be situated within the thylakoid lumen (Norris & Miller, 1994). This complex, which exhibits considerable variation across different species, is indispensable for the protection of cells from photodamage. It accomplishes this by rapidly quenching the triplet state of Chl *a*, thereby preventing the formation of deleterious singlet oxygen species (Niedzwiedzki et al., 2013). The regulatory mechanisms governing the abundance of these light-harvesting complexes in symbiotic Symbiodiniaceae remain largely unexplored, despite the critical role they play in photo-acclimation (Supasri et al., 2021).

1.7 Photosynthesis within cnidarians

The process of harvesting light energy into ATP and NADPH molecules remains conserved in photosynthesis, as in all other eukaryotic algae. Regarding carbon fixation, Symbiodiniaceae employs a C3 pathway. The incorporation of [^{14}C]-bicarbonate into freshly isolated samples of *Acropora formosa* revealed that glycerate-3-phosphate is the primary product (Streamer et al., 1993). In contrast to other higher organisms, Symbiodiniaceae employs a type II RuBisCO enzyme for CO_2 fixation. This form of RuBisCO is more heat sensitive (Lilley et al., 2010) and lower CO_2:O_2 specificity (Oakley et al. 2014). The enzyme is nuclear-encoded and expressed as a polyprotein (Rowan et al., 1996). It is probable that this gene originated from a process of horizontal gene transfer, exhibiting a high degree of similarity with the type II RuBisCO enzyme found in *Rhodospirillum rubrum*. It has been postulated that RuBisCO from Symbiodiniaceae may

form trimers of homodimers (Rydzy et al., 2021). It is noteworthy that the expression of the RuBisCO gene exhibits a circadian rhythm in free-living conditions, but not in symbiotic states (Mayfield et al., 2014). Furthermore, the discrepancy in RuBisCO molecular mass between algal cultures and symbiotic states suggests the potential involvement of post-translational modifications in regulatory mechanisms (Stochaj & Grossman, 1997). In general, RuBisCO content is higher in cultured cells than in symbiotic ones. Furthermore, two cultured Symbiodiniaceae from coral reefs exhibited comparable RuBisCO levels but disparate chlorophyll contents. Their photosynthetic activity and growth rates exhibited a stronger correlation with chlorophyll content than with RuBisCO content (Brading et al., 2013).

Translocation of photosynthetic products

Muscatine and Hand first demonstrated photosynthate translocation in a symbiotic cnidarian by incubating the temperate sea anemone *A. elegantissima* with $Na_2{}^{14}CO_3$ in the light for 1 to 5 weeks (Muscatine & Hand, 1958). They used autoradiography to visualize radiolabeled organic carbon in the anemone's tissues. The most common method for studying translocation is to label with [^{14}C]-bicarbonate in the light and measure the label in the host's tissues. Translocation has been measured as between 5% and 60% of photosynthetically fixed carbon in various coral and cnidarian-dinoflagellate symbioses (Hofmann & Kremer, 1981; Muscatine & Cernichiari, 1969; Sutton & Hoegh-Guldberg, 1990; von Holt & von Holt, 1968). For example, autoradiograms from the temperate sea anemone *Anemonia sulcata (Anemonia viridis)* suggested that about 60% of photosynthate was translocated (Taylor, 1969).

Given the limitations of ^{14}C labelling, an alternative method of quantifying translocation was formed based on the assumption that all photosynthetic carbon not used for respiration or growth by the dinoflagellate symbionts is transferred to the host (Muscatine et al., 1983). This method requires knowledge of net photosynthetic production (gross photosynthetic carbon fixation minus carbon used for dinoflagellate respiration) and the amount of carbon used for dinoflagellate growth, which is discussed in detail in the references (Davy et al., 1996; Steen & Muscatine, 1984). This "growth rate method" typically estimates that about 90%, and sometimes up to 99%, of the photosynthetically fixed carbon is translocated to the host under well-lit conditions (Davies, 1991; Davy et al., 1996; Steen & Muscatine, 1984).

Forms of translocated photosynthetic carbon

In the cnidarian-dinoflagellate symbiosis, over 90% of the photosynthetically fixed carbon is translocated primarily as glycerol, with glucose and a ninhydrin-positive compound also significant in the green hydra symbiosis. Glycerol accounts for 24.8% to 95% of translocated carbon, with glucose, alanine and organic acids such as fumarate, succinate, malate, citrate and glycolate also present (Markell & Trench, 1993; Sutton & Hoegh-Guldberg, 1990). Fat droplets indicate lipid translocation (Kellogg & Patton, 1983; Patton & Burris, 1983).

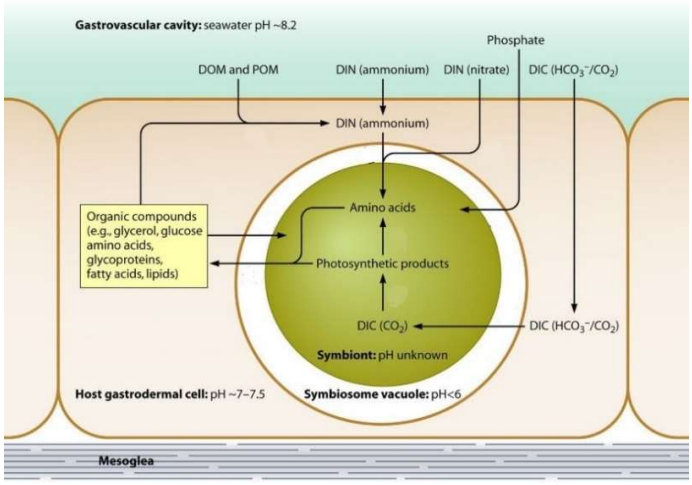

Figure 1.13: Summary of nutritional interactions in the cnidarian-dinoflagellate symbiosis (Davy et al., 2012).

Glycoproteins exuded by *Symbiodinium* cells are part of the translocated material (Davy et al., 2012) and their transfer from symbiont to host has been confirmed in hospite. Symbiotic dinoflagellates take up ^{35}S from food, suggesting reverse translocation of organic compounds, although inorganic sulphate transfer cannot be excluded (Cook, 1971).

Stimulation and control of photosynthetically fixed carbon translocation

One hypothesis is that the host limits symbiont growth by restricting nutrients or blocking mitosis, thereby creating a surplus of photosynthetic carbon for release (Muscatine et al., 1983). However, nutrient limitation may result in carbon storage rather than release in symbiotic dinoflagellates, similar to free-living microalgae (Davy & Cook , 2001; Turpin, 1991). Another hypothesis is that isolated symbiotic dinoflagellates release a significant proportion of their photosynthetically fixed carbon (over 50%) when incubated in homogenized host tissue, compared to less than 5% in seawater. This is often accompanied by an increased rate of photosynthesis, but the chemical signals responsible, known as host release factors (HRFs), have not been identified. Initially thought to be proteinaceous and below 10 kDa (Muscatine et al., 1972; Sutton & Hoegh-Guldberg, 1990), HRFs vary in stability, specificity and molecular weight between different hosts (Gates et al., 1995; Grant et al., 1998; Hinde, 1988).

Gates and colleagues found that HRFs in *Pocillopora damicornis* and *A. pulchella* consist of free amino acids (FAAs) that induce carbon release and enhance photosynthesis by optimizing the intracellular environment of the symbiont (Biel et al., 2007). Research by Hinde and co-workers on *Plesiastrea versipora* identified two signaling molecules below 1000 kDa: one that stimulates photosynthate release (HRF) and one that inhibits photosynthesis (PIF). However, their identification is hampered by thermal lability and loss of activity when salt is removed during analysis (Grant et al., 2003).

In nutrient-poor tropical seas, coral-dinoflagellate symbioses have also evolved sophisticated mechanisms for acquiring, storing, and recycling essential nutrients such as nitrogen (N) and phosphorus (P), which are vital for the health of coral reefs. Nitrogen is sourced from both the host's metabolic processes and the surrounding seawater. The dinoflagellates primarily handle ammonium assimilation and convert nitrate to ammonium through enzymatic processes (Grover et al., 2002, 2003; Lipschultz & Cook, 2002). Phosphorus, primarily in the form of phosphate, is absorbed by both the coral host and the symbionts via active transport mechanisms involving specific phosphate transporters. The dinoflagellates utilise acid phosphatases for phosphate mobilization (Jackson et al., 1989; Jackson & Yellowlees, 1990). Recent evidence indicates that, rather than passive diffusion, nutrient fluxes are actively regulated by the host, which controls the availability of nutrients to maintain a balanced symbiosis and regulate symbiont dynamics within the reef ecosystem (Davy et al., 2012).

1.8 Mechanisms of photoprotection

In the event of elevated temperatures, corals and their algal symbionts, such as Symbiodiniaceae, employ a range of alternative electron transport mechanisms to protect against oxidative damage. One crucial strategy is the process of cyclic electron flow around PSI, whereby electrons are redirected from the plastoquinone pool back to PSI, thus circumventing PSII. This process generates additional ATP without producing NADPH, which helps to reduce the formation of reactive oxygen species (ROS) and alleviate oxidative stress caused by excessive light energy (Aihara et al., 2016; Dang et al., 2019; Shikanai & Yamamoto, 2017). Another protective mechanism is the Mehler reaction, whereby electrons are diverted to molecular oxygen rather than being used to reduce $NADP^+$ to NADPH. This reaction results in the production of ROS, such as superoxide anions, which assist in the management of excess energy and the control of ROS levels, thereby reducing oxidative damage to the photosynthetic apparatus (Roberty et al., 2014).

Concurrently, when photosynthetic organisms encounter CO_2 limitation or fluctuating light intensity, the regeneration of essential molecules such as ADP and $NADP^+$ is diminished, which affects ATP synthase activity and results in the acidification of the thylakoid lumen in chloroplasts (Kanazawa et al., 2017). A reduction in pH levels affects the oxidation of plastoquinol (PQH_2), increasing the reduced state of the plastoquinone (PQ) pool and disrupting electron transfer, particularly at the PSI acceptor side. This disruption impedes the de-excitation rate of chlorophyll *a* (Chl *a*), thereby facilitating the generation of deleterious ROS, including singlet oxygen ($^1O_2$) and superoxide (O_2^-) (Khorobrykh et al., 2020; Tikhonov, 2014).

To mitigate these deleterious effects, photosynthetic organisms deploy non-photochemical quenching (NPQ) mechanisms to safely dissipate excess light energy. One of the principal NPQ processes, energy-dependent quenching (qE), is dependent on the xanthophyll cycle. This involves the conversion of diadinoxanthin (Ddx) to diatoxanthin (Dtx) via the enzyme diadinoxanthin de-epoxidase under conditions of high light intensity, with the dissipation of excess energy occurring as heat (Goss & Lepetit, 2015). Corals such as *Goniastrea aspera* have been observed to exhibit increased Dtx levels in response to increased light intensity, thereby underscoring the role of this process in photoprotection (Brown, 1997). On the other hand, when the light is low diatoxanthin

epoxidase converts diatoxanthin back to diadinoxanthin. This xanthophyll cycle is known as diadinoxanthin cycle, helping the symbiont to photosynthesize efficiently under fluctuating light conditions (Zigman et al., 2012). Furthermore, the mechanisms of energy spillover from PSII to PSI and efficient NPQ activation enhance photochemical efficiency and energy distribution, thereby providing additional protection against light stress. Furthermore, plants and algae undergo state transitions involving kinases (STN7 in plants, STT7 in algae) that phosphorylate LHCII proteins to optimise photosynthesis (Minagawa, 2011; Slavov et al., 2016). Previously, similar mechanisms were proposed that high light intensity caused the PCP or acpPC antenna complexes in Symbiodiniaceae to detach from photosystems. However, later studies revealed that there is no detachment of PCP under high light, instead a strong NPQ by xanthophyll-associated quenching mechanisms that protect against oxidative damage, while PCP helps in an efficient energy transfer to acpPC and PSI (Kanazawa et al., 2014).

1.9 Photosynthesis and coral bleaching

The phenomenon of climate change is responsible for an increase in the frequency and severity of coral bleaching events, which are caused by rising temperatures. Coral bleaching is a phenomenon whereby corals lose their algal partners, which are responsible for providing them with their color. It tends to occur when temperatures rise significantly and sunlight becomes too intense (Brown, 1997).

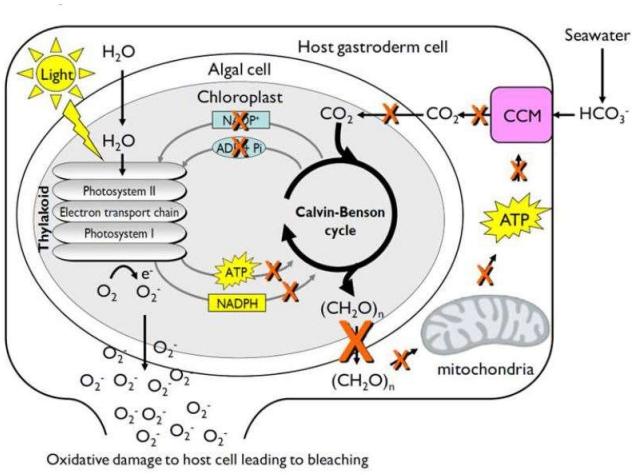

Figure 1.14: Disturbances occurring during a bleaching event. (Wooldridge, 2013)

This phenomenon has been observed to affect not only various coral species but also other marine communities, including jellyfish that inhabit tropical lakes (Maas et al., 2020) The consequences are significant and include reduced local productivity and loss of biodiversity.

The loss of the coral's colorful algal partners during bleaching results in the exposure of the white skeleton beneath. This can occur through a variety of processes, including the breakdown of algae or their expulsion from coral tissues (Bieri et al., 2016). The bleaching phenomenon is thought to be caused by an imbalance between the coral's ability to manage oxidative stress and the production

of reactive oxygen species (ROS). ROS, which is typically produced during photosynthesis in algae, can become overwhelming and damage the symbiotic relationship (Smith et al., 2004).

A reduction in photosynthetic efficiency is a common consequence of bleaching events, as evidenced by a decline in the quantum yield of PSII (Hill et al., 2004; Kemp et al., 2014). Additionally, elevated temperatures and intense light exposure have been demonstrated to inflict damage upon the Symbiodiniaceae, the algae that reside within coral ecosystems. This damage manifests as pigment loss and impaired photosynthesis (Lesser, 1996; Suggett et al., 2008). Recent studies have demonstrated that, while antioxidants could avoid anemone bleaching, they do not halt the production of reactive oxygen species (ROS). This challenges previous assumptions about the role of ROS in the process, that it is not the net ROS change, but the bleaching phenotype arise due to an excess ROS which can be successfully reduced by the application of exogenous antioxidants such as Ascorbate + catalase and mannitol (Dungan et al., 2022).

Furthermore, disturbances in the algae's carbon fixation process may also contribute to bleaching. While elevated temperatures impair the algae's capacity to concentrate carbon (Oakley et al., 2014), alternative factors such as the inhibition of the CBB cycle may also be involved (Bhagooli, 2013). The resilience of different coral species to thermal stress varies depending on the type of algae they host. Some species are better equipped to handle high temperatures than others like *Durusdinium* species provide more tolerance to thermal stress than *Cladocopium* species when hosted in same coral species (Rowan, 2004).

2. Research objectives

This study examines the impact of heat stress on the photosynthetic processes within the coral-Symbiodiniaceae symbiosis, with a particular focus on understanding the electron transport mechanisms and their role in heat tolerance. The research aims to identify how these stress-induced changes affect coral resilience and adaptability to environmental challenges. The objectives of the present study are listed below:

i) Understand the impacts of acute heat stress on the photosynthetic efficiency of coral endosymbiont algae Symbiodiniaceae, using chlorophyll fluorescence techniques like OJIP curves and flash-induced fluorescence relaxation.

ii) Determine how electron transport is affected under heat stress, focusing on fluorescence changes and their reversibility when normal conditions are restored. This will help uncover how the photosynthetic machinery adapts to heat stress.

iii) Explore alternative electron transport pathways by utilizing the fluorescence "wave phenomenon", and $P700^{+}$ reduction kinetics measurements to probe the roles of specific pathways like PGR5/PGRL1 and NDH-2 in heat tolerance.

iv) Study the fluorescence relaxation wave in intact corals, specifically the slow 1-400 second fluorescence wave, to see how it links to electron transport and the overall stress response in the coral host and the algae.

v) Assess the role of extracellular electron transport (EET) in the coral-Symbiodiniaceae system, investigating its potential contribution to stress adaptation and coral resilience in a changing environment.

3. Materials and methods

3.1 Conditions for Symbiodiniaceae cultures and the coral tank

Symbiodiniaceae cultures, including CCMP2465 (*Symbiodinium tridacnidorum*, formerly clade A3), CCMP2467 (*Symbiodinium microadriaticum*, formerly clade A1), and CS156 (*Symbiodinium kawagutii*, formerly clade F1), were cultivated at 24°C under 50 µmol photons m^{-2} s^{-1} white light in F/2 media for a week, reaching the mid-log growth phase.

For the coral tanks, small coral fragments were glued to rocks and cultivated within a controlled tank environment with water parameters maintained as: Calcium (425-440 mg/L), Magnesium (1350-1380 mg/L), Alkalinity (7.8-8 KH), Phosphate (0.03-0.05 mg/L), Nitrate (3-5 mg/L), and Specific Gravity (1.025-1.026), which were monitored every second day. The pH exhibited fluctuations, ranging from 8.1 at night to 8.35 during the day. Each week, 10% of the water was refreshed with Red Sea salt water with a water flow set to 33 times turnover per hour, and lighting maintained at a PAR level of 250-350 µmol photons m^{-2} s^{-1}.

Estimation of chlorophyll content of Symbiodinium cells

Chlorophyll content was estimated by centrifuging the cells at 17000 g for 5 minutes, then pelleted cells were resuspended in 100% methanol at 4 ºC for 10 minutes. After that, cells were again pelleted and the absorbance of the supernatant were measured at 750, 665 and 632 nm.

Total chlorophyll concentration was calculated as the sum of *Chl a* and *Chl c.,* according to the following equations (Ritchie, 2006).

$$Chl\ a\ = (13.6849 * (A_{665} - A_{750}) - 3.4551 * (A_{632} - A_{750})) * Dilution) \quad \text{(eq. 1)}$$

$$Chl\ c\ = (32.9371 * (A_{632} - A_{750}) - 7.014 * (A_{665} - A_{750})) * Dilution) \quad \text{(eq. 2)}$$

Cells were then centrifuged at 5000 g. Following centrifugation, the cells were re-suspended in fresh F/2 media, and the final Chl concentration was adjusted to 5 µg/mL. Specifically, for $P700^{+}$ reduction kinetics measurements, *Symbiodinium tridacnidorum* (CCMP2465) was adjusted to a final Chl concentration of 20 µg/mL.

3.2 Measurement of flash-induced chlorophyll fluorescence relaxation kinetics

The measurement of flash-induced chlorophyll fluorescence relaxation kinetics (FF) was conducted using a double-modulation fluorimeter (FL-3000, Photon Systems Instruments, Brno, Czech Republic) (Trtilek, 1997). 2 mL sample was positioned in a cuvette with a 1 cm path length and continuously stirred in the dark using a small magnetic stirrer bar.

To initiate the measurement, four measuring flashes with a duration of 8 µs, separated by intervals of 200 µs (wavelength of 620 nm), were applied to determine the minimum fluorescence in the dark (F_0). Subsequently, a single turnover saturating actinic flash (30 µs, wavelength of 639 nm) was administered to induce the formation of Q_A^-, resulting in a rise in fluorescence intensity. This

maximal fluorescence recorded after the single turnover actinic flash was specifically denoted as $F_{m(ST)}$. This flash-induced increase in fluorescence yield typically exhibits a monotonous decrease when applying measuring flashes in the time range from 150 µs to 100 s on a logarithmic time scale. It can be assigned into three distinct phases: (i) a fast decay phase with a duration of approximately 300–500 µs due to Q_A^- reoxidation by Q_B (or Q_B^-), (ii) a middle phase due to reduction of plastoquinone (PQ), which binds to the Q_B site after the flash, lasting about 5–15 ms, and (iii) a slow phase as a result of recombination of the electron on $Q_AQ_B^-$ with the oxidized S_2 (or S_3) state of the water-oxidizing complex, which lasts approximately 10–20 s (Deák et al., 2014).

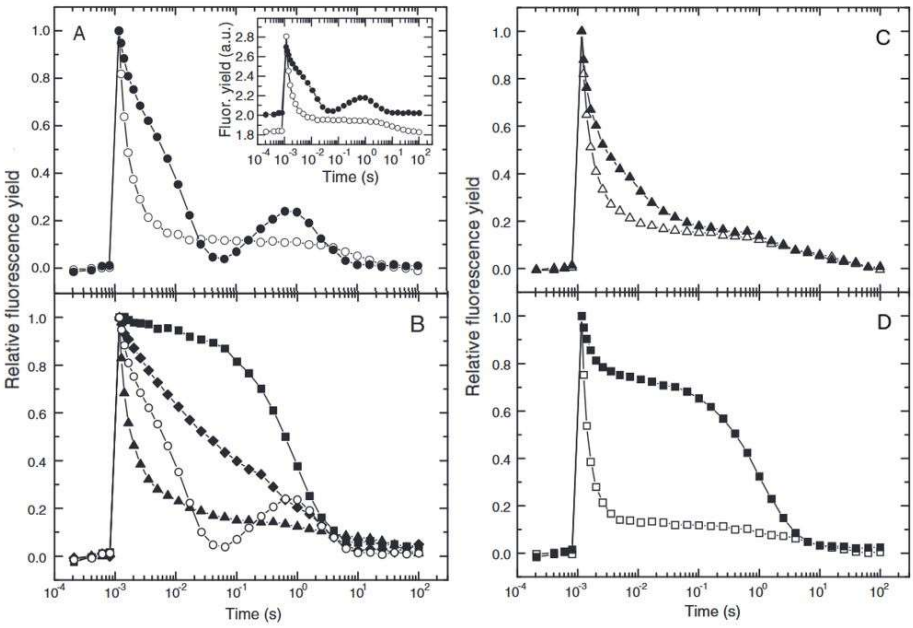

Figure 3.1: Flash-induced fluorescence yield in (A) aerobic (blank) and anaerobic (dark) conditions, (B) presence of electron transport inhibitors 10 µM DCMU (squares), 5 µM DBMIB (diamonds), 0.5 mM DMBQ (triangles), no addition anaerobic (circles), (C) anaerobic the NDH1-less M55 (dark) and control aerobic (blank)and (D) anaerobic PSI-less mutant cells (dark) and control aerobic cells (blank) (Deák et al. 2014).

Under aerobic conditions in *Synechocystis* a monotonous decay was observed, but in anaerobic conditions, a wave like fluorescence decay occurred. This wave phenomenon is composed of a dip of a fluorescence (at around 30-50 ms after the flash) related to the transient oxidation, followed by a transient rise (at around 1 s) related to the re-reduction of PQ pool (Fig. 3.1). The wave phenomenon was found to be eliminated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), 2,6-dimethoxybenzoquinone (DMBQ). It was also absent in PSI and NDH-1 mutants, indicating that it is related to a transient oxidation and reduction of the PQ pool by the donor side of PSI and cycling back of the electron from the acceptor side to PQ pool via NDH-1, respectively (Deák et al., 2014).

The same technique was also used further with the modification of measuring flashes in the time range from 150 µs till 400 s on a logarithmic time scale, with and without actinic flash for both isolated *Symbiodinium* cells and intact coral, in order to study the fluorescence relaxation at a much longer time scale.

3.3 Measurement of chlorophyll *a* fluorescence induction curves

Chlorophyll *a* fluorescence induction curves were measured using an FL-3000 fluorimeter (Photon Systems Instruments, Brno, Czech Republic). This method, known as the Kautsky induction curve, or the polyphasic rise of chlorophyll fluorescence OJIP curve, is designed to monitor the kinetics of the chlorophyll fluorescence induction during the transition from a dark to a light-adapted state (Kalaji et al., 2016). The process entails tracking the transition between the state where all PSII reaction centers are open, referred to as the O step or F_0 at 20 μs, to the state where all reaction centers are closed and the fluorescence intensity reaches its maximum, nominally called the peak or P step (F_m ~ 300 ms) (Strasser & Govindjee, 1992). Additionally, two intermediate steps are typically observed: the J step at around 2 ms and the I step at around 20 ms. PSII functionality is measured using the JIP-test (Strasser & Strasser, 1995), and is used to translate the original measurements of fluorescence transients into a variety of phenomenological and biophysical expressions (Tóth et al., 2007). Because the usual fluorescence transient is susceptible to stress brought on by changes in a variety of environmental variables, this test can be used for in vivo examinations of the photosynthetic process. As such, it functions as an extremely sensitive biophysical biomarker of the electron transport chain.

To conduct these measurements, an automated protocol was applied. This protocol involved the application of red actinic light with a wavelength at the peak of 650 nm and a spectral line half-width of 22 nm. The intensity of the actinic light was set at 3500 μmol m^{-2} s^{-1} to induce fluorescence, and OJIP transients with a duration of 3 seconds were recorded.

3.4 Measurement of post-illumination chlorophyll fluorescence transient

The measurement of post-illumination chlorophyll fluorescence transients (PIFT) was conducted using a Dual-PAM-100 instrument (Heinz-Walz GmbH, Effeltrich, Germany). The procedure involved dark-adapting a 2 mL sample for 5 min, after which chlorophyll fluorescence was recorded over a 6-min period.

The sample was exposed to a weak measuring light (ML) with an intensity of approximately 0.1 μmol photons m^{-2} s^{-1} during the initial 30 seconds. Subsequently, an actinic light (AL) with an intensity of 50 μmol photons m^{-2} s^{-1} was applied for 3 minutes. Following the exposure to the actinic light, the chlorophyll fluorescence rise during the subsequent dark period was recorded.

3.5 Measurement of the activity of photosystems

The activity of Photosystem II (Y(II)) and Photosystem I (Y(I)) was assessed using a Dual-PAM-100 instrument (Heinz-Walz GmbH, Effeltrich, Germany). 8 mL of the sample with a chlorophyll content of 40 μg/mL was filtered evenly onto Whatman GF/C filter paper and dark-adapted for 5 min. Y(II) was calculated as $(F_m'-F_t)/F_m'$, where F_m' represents the maximum fluorescence upon the saturation pulse, and F_t is the steady-state value of fluorescence immediately before the flash (Maxwell & Johnson, 2000). In the dark-adapted state, activity of PSII (F_v/F_m) is calculated as $(F_m-F_0)/F_m$, where F_m is the maximal fluorescence, and F_0 is the minimal fluorescence (Sipka et al.,

2021). Analogously, P_m represents the maximal level of P700 signal obtained by a saturation pulse under far-red illumination when P700 is fully oxidized ($P700^+$). Y(I) is calculated as $(P_m'-P)/P_m$, where P_m' is the maximal level of P700 signal obtained by a saturation pulse at a defined actinic light (AL) illumination, and P is the P700 oxidation level at a defined AL illumination (Klughammer & Schreiber, 1994, 2008).

The length of the saturation pulse used to determine Y(II) and Y(I) was 300 ms, with an intensity of approximately ~10,000 µmol photons m^{-2} s^{-1}. The measuring light intensity for F_0 determination was kept below 0.5 µmol photons m^{-2} s^{-1}. This experimental approach allowed for the quantification of the activity of Photosystem II and Photosystem I in the studied samples, providing valuable insights into the photosynthetic performance under different heat treatments.

3.6 $P700^+$ reduction kinetics

The $P700^+$ reduction kinetic traces were measured using a Dual-PAM-100 instrument (Heinz-Walz GmbH, Effeltrich, Germany). In this experimental setup, a 2 mL sample was placed in a cuvette with a 1 cm path length, positioned between the emitter and detector, which were placed at 180° from each other. The sample was initially dark-adapted for 3 minutes.

The experimental protocol involved exposing the sample to a strong actinic illumination with an intensity of 2000 µmol photons m^{-2} s^{-1} for 1500 ms. This illumination induced the formation of photo-oxidized $P700^+$. Subsequently, a dark phase of 1000 ms followed, during which the kinetics of $P700^+$ re-reduction was measured.

The use of $P700^+$ reduction kinetics provides valuable insights into the efficiency and dynamics of electron transfer processes within the photosynthetic apparatus, particularly when performed in the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), which blocks electrons from PSII specifically at the Q_B binding site hence offering a specific focus on cyclic electron flow (Aihara et al., 2016) in the studied samples under the specified conditions.

3.7 Partial cell wall digestion of *Symbiodinium tridacnidorum*

In the process of preparing cells with partially digested cell wall from *Symbiodinium tridacnidorum*, a modified protocol was employed based on the guidelines outlined in reference (Bashir et al., 2022). Notably, certain adjustments were made to the standard procedure: sorbitol was excluded, and the concentration of cellulase (from Sigma-Aldrich) was reduced to 2%, while macerozyme and kanamycin remained the same i.e. 1% and 100 µg/mL respectively in sterile filtered F/2 media. Additionally, the *Symbiodinium* cells were incubated in the solution for a shortened period of 2 hours in dark and on continuous shaking.

This controlled digestion process was intended to preserve the key properties of fluorescence relaxation in Symbiodiniaceae while facilitating the isolation of protoplasts. By avoiding the use of sorbitol and adjusting the cellulase concentration and incubation time, we aimed to achieve a balance between breaking down the cell wall to obtain protoplasts and retaining the essential

characteristics of fluorescence relaxation specific to Symbiodiniaceae. This tailored approach allowed for the investigation of fluorescence properties in a manner that better reflected the native conditions of these organisms.

3.8 Ferricyanide (FeCN) reduction assay

For investigation of extracellular electron transfer, FeCN reduction assay was performed with 1 mM concentration. *Symbiodinium* cells and coral fragments were kept in FeCN solution respectively on a shaker under growth light. Absorption spectrum of the filtered supernatant FeCN solution for *Symbiodinium* cells and the supernatant of coral fragments was recorded between 380 and 650 nm. The change at 420 nm over a period of 120 minutes was observed. The rate of reduction was calculated with the formula (Gonzalez-Aravena et al., 2018):

$$Rate = \frac{Absorbance\ change\ at\ 420nm}{(Molar\ ext\ coefficient * Chl\ concentration * pat\quad length * time)} \quad \text{(eq. 3)}$$

3.9 Inhibitors

In our study, we employed various electron transport inhibitors to characterize distinct pathways of photosynthetic electron transport in *Symbiodiniaceae*. The inhibitors were used at the concentrations indicated below, to selectively target different components of the photosynthetic machinery. The concentrations utilized for each inhibitor were as follows:

a) 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU): 20 µM
b) 2,6-Dimethoxy-1,4-benzoquinone (DMBQ): 500 µM
c) Methyl viologen (MV): 40 mM
d) Hydroxylamine: 8 mM
e) Glycolaldehyde: 50 mM
f) Polymyxin B (PolyB): 800 µM
g) Antimycin A (AntiA): 50 µM

Each inhibitor plays a specific role in perturbing or blocking certain steps of the electron transport chain, allowing for the investigation and understanding of the distinct pathways involved in photosynthesis. These concentrations were carefully chosen from a range of previously tested concentrations, to ensure effective inhibition while maintaining the integrity of the biological system under study.

Experimental procedure and statistical analysis

The algae samples were adjusted first to the desired chlorophyll concentration and pre-illuminated under 50 µmol photons m^{-2} s^{-1} (growth light condition) for an hour to acclimatize, prior to any measurement. Variations in the response to acute heat stress among different species of Symbiodiniaceae were observed through the recording of changes in photosynthetic parameters, including FF, OJIP, PIFT, and photosystem activity. This observation was conducted under three

conditions: normal growth conditions (24 °C), heat-stress conditions (34 °C for 2 hours), and recovery conditions (for 2 hours at 24 °C). The heat treatment was conducted at a gradual temperature increase in an incubator to simulate natural conditions and a slow recovery of 2 hours also allows us for a more comprehensive examination of the recovery process. Additionally, a series of heat stress experiments were carried out at different temperatures (24 °C, 36 °C, 38 °C, and 40 °C for 1 hour in incubator shaker) to monitor temperature-dependent changes in flash-induced chlorophyll fluorescence relaxation.

To create a microaerobic condition, the sample was subjected to dark incubation with 7 U mL^{-1} glucose oxidase, 60 U mL^{-1} catalase, and 10 mM glucose for 15 minutes. The microaerobic condition was coupled with acute heat stress for a period of 10 minutes, which was conducted using a cuvette heating system (ThermoRegulator TR2000, Photon Systems Instruments, Brno, Czech Republic) with the objective of reducing PSII activity relative to PSI. The resulting changes in flash-induced chlorophyll fluorescence relaxation were recorded across all Symbiodiniaceae strains.

Further studies were done on strain CCMP2465 (*Symbiodinium tridacnidorum*). Inhibitors were employed either in whole cells or in cells with partially digested cell wall, to observe the differences in the decay pattern of FF done in microaerobic condition coupled with the acute heat stress. $P700^+$ reduction kinetics were also performed at normal growth temperature (control) and after acute heat stress, with a 10-minute incubation of DCMU. Furthermore, these $P700^+$ reduction rates were compared to those in presence of glycolaldehyde, antimycin A and polymyxin B, in order to investigate the involvement of specific pathways of electron transport in the photosynthetic apparatus of *Symbiodinium*.

Flash-induced chlorophyll fluorescence relaxation kinetics (FF) was applied on intact corals as well. The decay phase was extended to 400 seconds in order to study any changes in fluorescence that are related to the slow electron transport processes leading to the reduction of PQ pool. Changes in the fluorescence relaxation pattern around 1-400 seconds were measured in presence of 20 μM DCMU and 1 mM Potassium ferricyanide (FeCN) respectively. Experiments were also performed with and without actinic flash, and with different oxygen conditions on intact coral. Lastly FeCN reduction assay were performed and compared between isolated Symbiodiniacea cultures, Symbiodiniacea cultures with partially digested cell wall, and intact coral fragments.

Origin Pro (Origin-Lab Corporation, Northampton, MA, USA) was utilized for statistical analysis. One-way analysis of variance (ANOVA) was conducted on independent samples to identify statistically significant differences among treatments. Tukey's post hoc multiple comparison tests ($\alpha = 0.05$) were then employed to further analyze and compare the treatments. Normality tests were carried out using the Kolmogorov–Smirnov method, and the homogeneity of variance test was conducted using Levene's method.

4. Results

4.1 The response of the photosynthetic activity to acute heat stress in Symbiodiniaceae

To investigate the impact of acute heat stress on flash-induced chlorophyll fluorescence relaxation, various strains of Symbiodiniaceae were analyzed (see Figure 4.1).

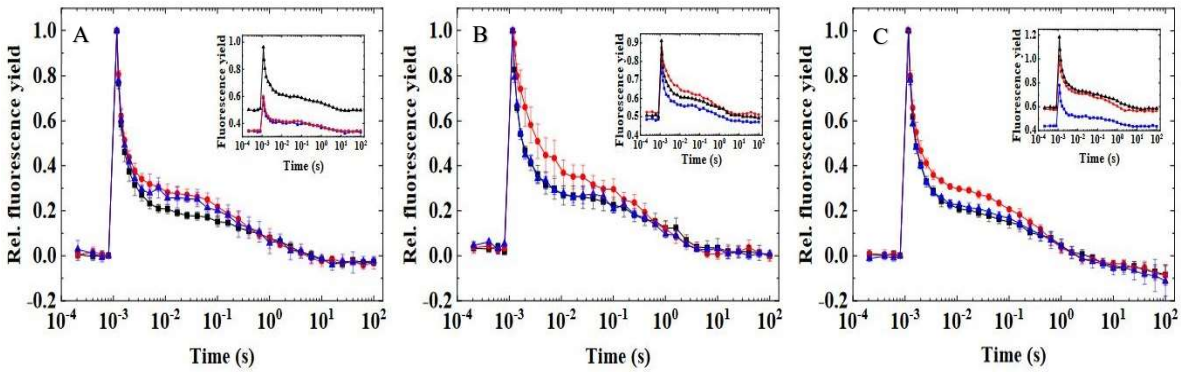

Figure 4.1: Flash-induced fluorescence relaxation in three different strains of Symbiodiniaceae under elevated temperatures. (A) *Fugacium kawagutii* (CS156), (B) *Symbiodinium tridacnidorum* (2465), and (C) *Symbiodinium microadriaticum* (2467), including control (black), 34°C 2 h (red), and recovery (blue). Data averaged from 3 biological replicates.

Exposure to acute heat stress at 34°C resulted in a notable elevation in the middle phase (0.003-0.1 s) of the fluorescence relaxation pattern across all strains examined, with the most pronounced effect observed in *S. tridacnidorum* (strain 2465). This augmentation in the middle phase correlates with increased reduction of the plastoquinone (PQ) pool. This phenomenon was also validated with fast fluorescence induction (OJIP) measurements.

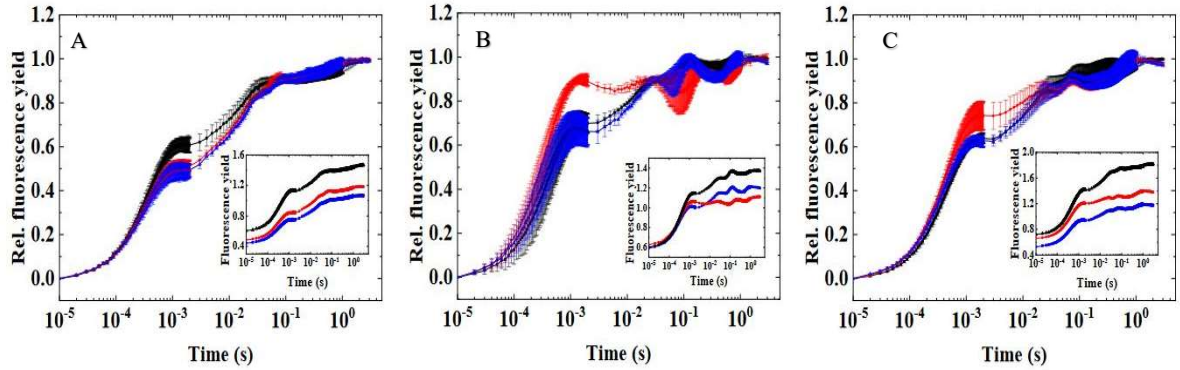

Figure 4.2: OJIP induction curves in three different strains of Symbiodiniaceae under elevated temperatures. (A) *Fugacium kawagutii* (CS156), (B) *Symbiodinium tridacnidorum* (2465), and (C) *Symbiodinium microadriaticum* (2467), including control (black), 34°C 2 h (red), and recovery (blue). Data averaged from 3 biological replicates.

Specifically, the OJIP traces demonstrated that the most substantial increase in the J phase (around ~1 ms) occurred in *S. tridacnidorum* as compared to the other strains (Figure 4.2 (B)), which is indicative of the PQ pool's reduction state (Hill et al., 2014; Tóth et al., 2007) , aligning with middle phase in the flash-induced fluorescence transient (Figure 4.2).

Conversely, *F. kawagutii* (strain CS156) exhibited the smallest elevation in the middle phase of the flash-induced fluorescence transient, corresponding to minimal alterations in the J phase of the OJIP curve. Intriguingly, in *F. kawagutii*, the J phase even exhibited a decrease at elevated temperatures (Fig. 4.2 (A)). Remarkably, the heat-induced modifications in the flash-induced fluorescence relaxation traces were largely reversible upon incubation of samples at the growth temperature (24°C), consistent with the decline observed in the J phase of the OJIP curves. This suggests that the PQ pool gets reduced under elevated temperature and tends towards a more oxidized state upon alleviation of heat.

The impact of elevated heat also manifested in significant alterations in the post-illumination chlorophyll (Chl) fluorescence rise. Following the cessation of light, a transient Chl fluorescence rise ensues due to the reduction of the PQ pool by stromal reductants, thereby serving as an indicator of the redox kinetics of the PQ pool in Symbiodiniaceae (Claquin et al., 2020; Gotoh, Matsumoto, et al., 2010; Reynolds et al., 2008). These dynamics exhibited strain-specific characteristics (Figure 4.3).

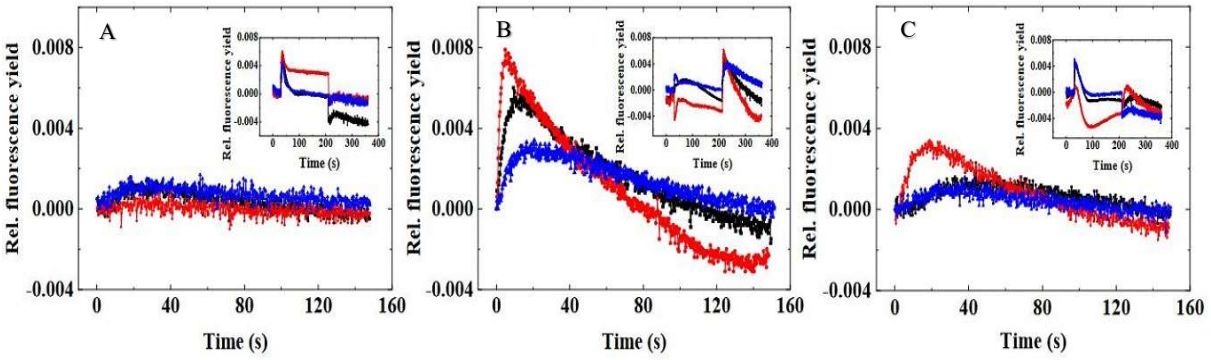

Figure 4.3: Chl fluorescence rise in *Fugacium kawagutii* (CS156) (A), *Symbiodinium tridacnidorum* (2465) (B), and *Symbiodinium microadriaticum* (2467) (C) under control conditions, including control (black), 34°C 2 h (red), and recovery (blue). Fluorescence was normalized to zero at the beginning of the dark phase, with insets showing original traces adjusted for initial fluorescence.

The least pronounced response was observed in *F. kawagutii* (strain CS156), where acute heat stress did not significantly alter the post-illumination Chl fluorescence characteristics (see Fig. 4.3 (A)). In *S. microadriaticum* (strain 2467), heat stress notably accelerated and increased the post-illumination Chl fluorescence rise (Fig. 4.3 (C)), while *S. tridacnidorum* (strain 2465) displayed the most substantial response (Fig. 4.3 (B)). Notably, in strain 2465, the post-illumination fluorescence rise was already considerable even at 24°C in darkness, indicative of a robust dark-

induced reduction of the PQ pool (Hill & Ralph, 2008). At 34°C, the post-illumination fluorescence rise occurred more rapidly compared to 24°C, and subsequently decelerated upon re-incubation of cells at 24°C.

To unveil the alterations in the activity of both photosystems under acute heat stress, measurements of PSII and PSI activities based on variable fluorescence were conducted (refer to Figure 4.4).

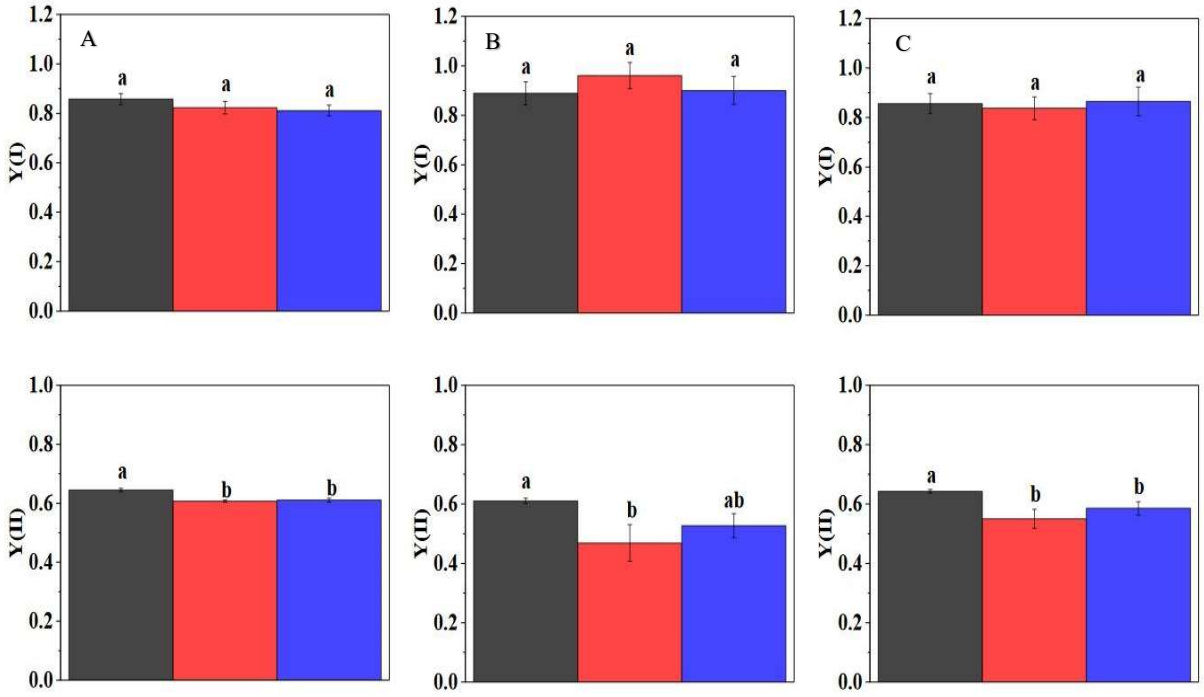

Figure 4.4: Activity of PSI (Y(I)), upper panel) and PSII ((Y(II), lower panel) in three different strains of Symbiodiniaceae under elevated temperatures. (A) *Fugacium kawagutii* (CS156), (B*) Symbiodinium tridacnidorum* (2465), and (C) *Symbiodinium microadriaticum* (2467), including control (black), 34°C 2 h (red), and recovery (blue). Data averaged from 3 biological replicates.

Acute heat stress yielded no discernible change in the activity of Photosystem I (Y(I)) in strains CS156 (see Fig. 4.4 (A)) and 2467 (see Fig. 4.4 (C)). However, in strain 2465, there was a slight elevation in Y(I) following acute heat stress, albeit not statistically significant (see Fig. 4.4 (B)). Conversely, the activity of Photosystem II (Y(II)) exhibited a decline in all strains, with the most pronounced decrease observed in strain 2465. Notably, Y(II) only partially recovered under growth temperature conditions in all three strains. These observations underscore the differential responses of PSI and PSII to acute heat stress across the examined strains of Symbiodiniaceae.

4.2 Temperature dependence of the flash-induced chlorophyll fluorescence relaxation kinetics in Symbiodiniaceae

The flash-induced fluorescence relaxation phenomenon is renowned for its characteristic wave pattern observed in cyanobacteria, signifying the reoxidation of the reduced PQ (plastoquinone) pool through electron transfer towards PSI, followed by the re-reduction of the PQ pool via alternative electron transport pathways such as cyclic electron transport (Deák et al., 2014). This distinctive wave pattern has also been documented in *Chlamydomonas reinhardtii* under H_2-producing conditions (Krishna et al., 2019) and in instances where PSII activity was partially impaired in combination with microaerobic conditions (Patil et al., 2022).

To investigate the potential inducibility of this wave phenomenon in fluorescence relaxation within Symbiodiniaceae, cells were subjected to acute heat stress treatment. As illustrated in Fig. 4.1, acute heat stress at 34°C resulted in an observable increase in the middle phase of flash-induced fluorescence relaxation due to a reduction in the PQ pool. However, intriguingly, this condition did not prompt the formation of the fluorescence wave, contrary to expectations based on observations in other organisms. Therefore, investigations were undertaken to explore whether temperatures exceeding 34°C induced additional changes in flash-induced fluorescence relaxation dynamics (see Figure 4.5).

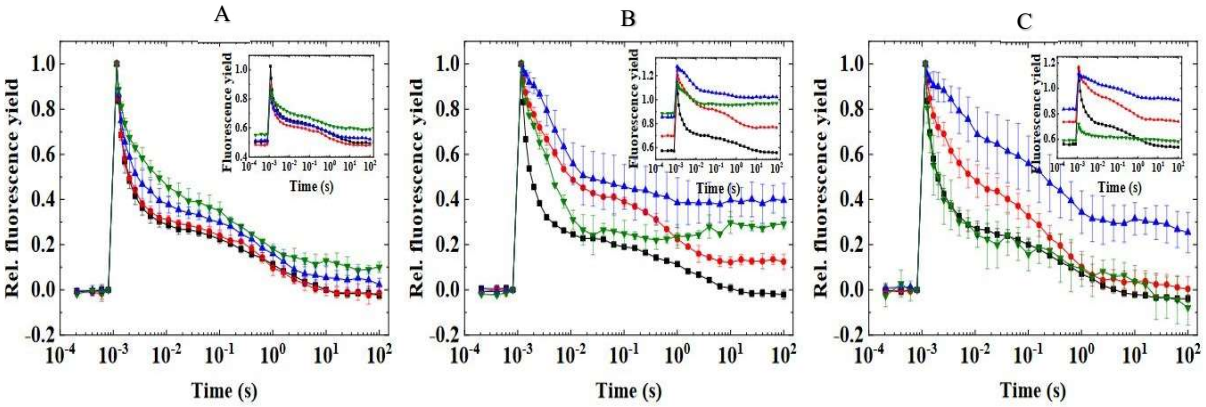

Figure 4.5: Flash-induced fluorescence relaxation in three different species of Symbiodiniaceae under elevated temperatures. (A) *Fugacium kawagutii* (CS156), (B) *Symbiodinium tridacnidorum* (2465), and (C) *Symbiodinium microadriaticum* (2467), including control (black), 36°C (red), and 38°C (blue), and 40°C (green). Data averaged from 3 biological replicates.

Heat treatment at higher temperatures instigated notable alterations in the profiles of flash-induced fluorescence relaxation, and these changes exhibited strain-specific characteristics. In *F. kawagutii* (strain CS156), the flash fluorescence profiles remained relatively stable until reaching 38°C; however, at 40°C, there was a discernible increase in the middle phase, indicating a reduced PQ pool (see Fig. 4.5 (A)).

Conversely, in *S. microadriaticum* (strain 2467), the PQ pool became substantially reduced at 36°C, with further increases in reduction observed at 38°C. However, at 40°C, the fluorescence

profile reverted to a pattern like that observed at the growth temperature (see Fig. 4.5 (C)). This phenomenon might be attributed to the diminishment of the signal at 40°C, which consequently hindered accurate measurement. In *S. tridacnidorum* (strain 2465), a reduced PQ pool was evident at 36°C due to the heightened middle phase of fluorescence relaxation. However, as the temperature increased further, there was a dip followed by a slight rise in fluorescence, reminiscent of a wave phenomenon (see Fig. 4.5 (B)). These findings underscore the notably different heat sensitivities among the various strains, with CS156 exhibiting the least sensitivity to elevated temperatures, even up to 40°C. These observations provide insights into the intricate responses of Symbiodiniaceae to thermal stress and highlight the diverse adaptive strategies employed by different strains in response to heat-induced challenges.

4.3 The induction of the flash-induced wave phenomenon is species specific in Symbiodiniaceae

In the realm of microalgal physiology, the role of microaerobic conditions in inducing the wave phenomenon within the chlorophyll fluorescence dynamics of microalgae has garnered considerable interest. Previous research has elucidated that microaerobic environments alone are not sufficient to provoke the wave phenomenon in *Chlamydomonas*, despite their ability to induce a substantially reduced PQ pool (Krishna et al., 2019; Patil et al., 2022). Hence, an inquiry was undertaken to ascertain whether microaerobic conditions alone, which result in a markedly reduced PQ pool owing to O_2 depletion that inhibits the terminal oxidases responsible for maintaining PQ pool oxidation (Deák et al., 2014; Ermakova et al., 2016), could induce the wave phenomenon in Symbiodiniaceae.

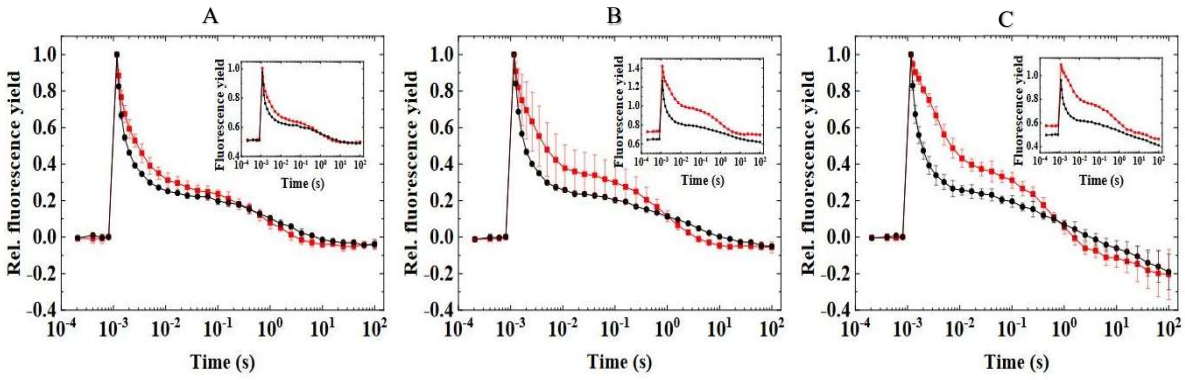

Figure 4.6: Flash-induced fluorescence relaxation in three different species of Symbiodiniaceae under aerobic and microaerobic conditions, recorded at growth temperature. (A) *Fugacium kawagutii* (CS156), (B) *Symbiodinium tridacnidorum* (2465), and (C) *Symbiodinium microadriaticum* (2467), including control (black) and microaerobic condition (red). Data averaged from 3 biological replicates.

Under the controlled conditions of reduced oxygen availability, a noticeable reduction in the PQ pool was indeed observed, as evidenced by the elevated middle phase of fluorescence relaxation

and the increased F_0 levels across various strains (see Fig. 4.6 and inset). Remarkably, microaerobic conditions elicited similar changes across different strains; however, none exhibited the induction of the wave phenomenon. This observation aligns with findings in other microalgae, where microaerobic conditions failed to induce the wave phenomenon.

Upon subjecting heat-treated Symbiodinium samples to microaerobic conditions, distinct fluorescence patterns emerged, exhibiting notable characteristics. Figures 4.7 and Figure 4.8 further underscored the significance of these patterns across different heat treatment conditions (34°C, 36°C, 38°C and 40°C).

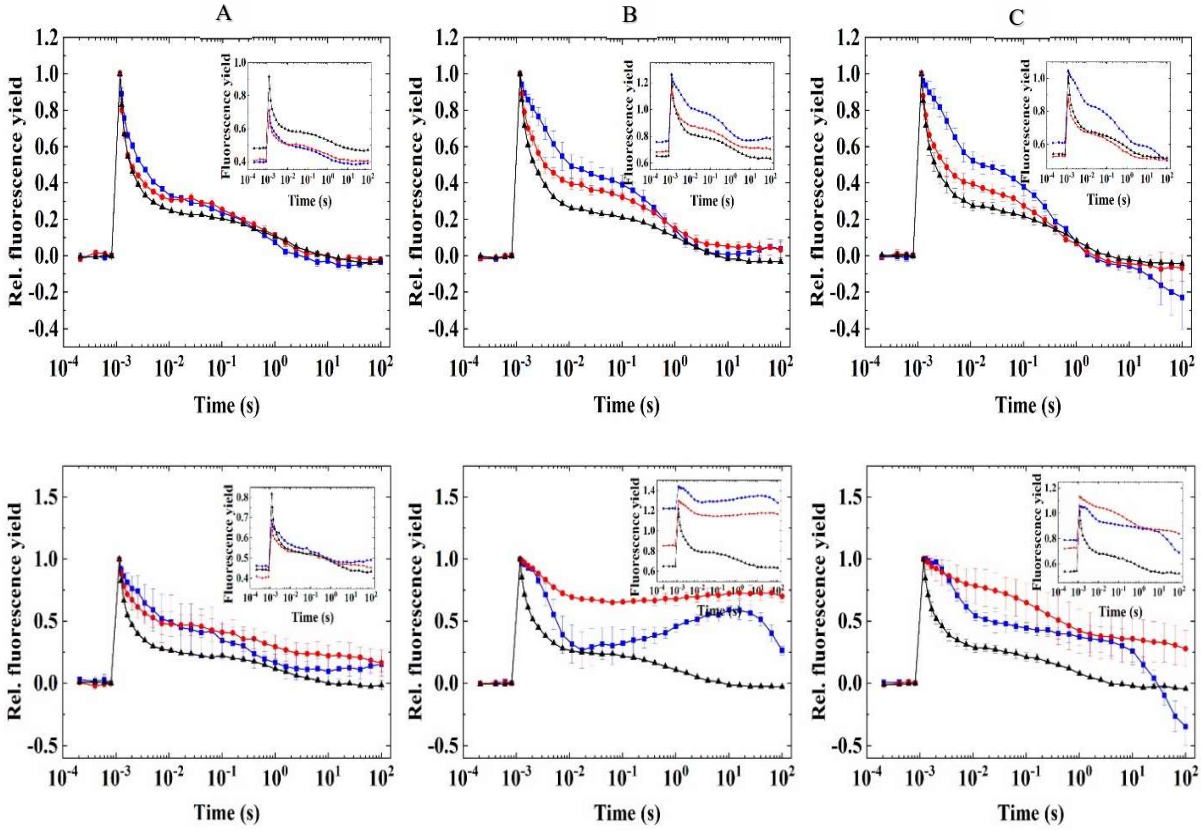

Figure 4.7: Flash-induced fluorescence relaxation in three different species of Symbiodiniaceae under aerobic and microaerobic conditions, recorded at growth temperature. (A) *Fugacium kawagutii* (CS156), (B) *Symbiodinium tridacnidorum* (2465), and (C) *Symbiodinium microadriaticum* (2467), including control (black), acute heat (34°C top) (36°C bottom) for 10 minutes (red) and heat+microaerobic condition (blue). Data averaged from 3 biological replicates.

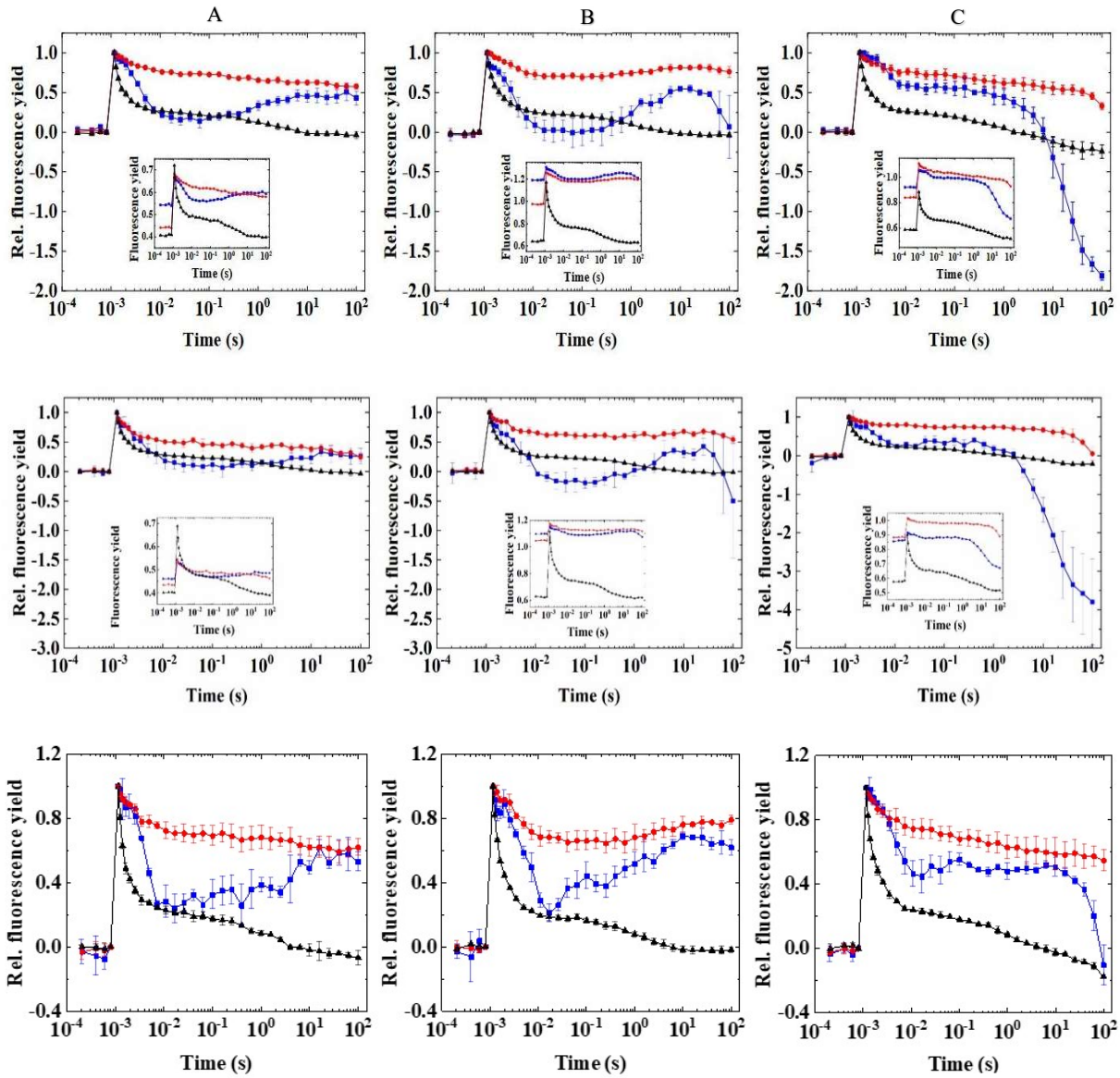

Figure 4.8: Flash-induced fluorescence relaxation in three different species of Symbiodiniaceae under aerobic and microaerobic conditions, recorded at growth temperature. (A) *Fugacium kawagutii* (CS156), (B) *Symbiodinium tridacnidorum* (2465), and (C) *Symbiodinium microadriaticum* (2467), including control (black), acute heat (38°C top) (40°C middle) and (38°C +10% Ficoll, bottom) for 10 minutes (red) and heat+microaerobic condition (blue). Data averaged from 3 biological replicates.

Strain *S. tridacnidorum* exhibited the most pronounced wave phenomenon, particularly at 38°C, under acute heat combined with microaerobic treatment. This temperature revealed a pronounced manifestation of the wave phenomenon, characterized by a transient dip followed by a subsequent rise in fluorescence intensity. Strain CS156 also exhibited the wave phenomenon at this temperature, although with a smaller amplitude compared to strain 2465. In contrast, strain 2467

displayed less evident indications of the wave phenomenon, with a noticeable decrease observed in longer timescales (>10 s), possibly attributed to cell clumping.

While the addition of 10% ficoll to the cell suspension partially mitigated this effect (Figure 4.8), challenges with cell clumping and sedimentation persisted in heat-treated samples under microaerobic conditions. However, amidst these challenges, it becomes evident that the transient dip followed by an increase in fluorescence, characteristic of the wave phenomenon, is most pronounced in strain 2465. These nuanced observations shed light on the intricate interplay between heat stress, microaerobic conditions, and strain-specific responses in *Symbiodinium*.

4.4 Effect of blocking linear electron flow in *S. tridacnidorum* (strain 2465)

Since *S. tridacnidorum* (strain 2465) showed the most pronounced wave expression under acute heat combined with microaerobic treatment, this strain was investigated further to explore its electron transfer pathways and mechanisms associated with this wave phenomenon from a few milliseconds to seconds. The influence of DCMU, an inhibitor of linear electron transport, on the wave phenomenon induced by acute heat combined with microaerobic treatment was investigated.

Under ambient temperature conditions, DCMU effectively blocked the fast and middle phases of fluorescence relaxation following the flash, leaving only the slow phase unaffected (refer to Figure 4.9).

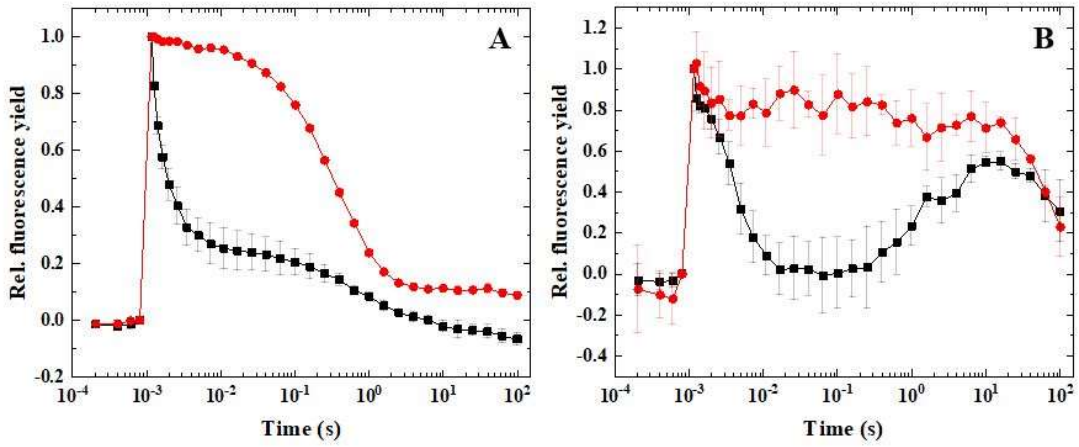

Figure 4.9: Effect of DCMU on the wave phenomenon of flash-induced chlorophyll (Chl) fluorescence relaxation. Treatments were conducted under two conditions: (A) at 24°C (black) and 24°C with DCMU (red), and (B) at 38°C under microaerobic conditions (black) and 38°C under microaerobic conditions with DCMU (red). Data averaged from 3 biological replicates.

However, under conditions conducive to inducing the wave phenomenon (i.e., heat combined with microaerobic treatment), the dip in fluorescence relaxation i.e. a decline in fluorescence yields over a couple of hundred milliseconds after the flash, was absent in the presence of DCMU. This absence can be attributed to the blockade of electron transfer between Q_A and Q_B by DCMU, thereby

disrupting one of the essential conditions for the formation of the wave. Specifically, the uninterrupted operation of sequential electron transfers between PSII and the PQ pool, as outlined by previous studies (Deák et al., 2014), was not fulfilled in the presence of DCMU, thus precluding the manifestation of the wave phenomenon.

DMBQ, a soluble electron acceptor to PSII known to maintain the PQ pool largely oxidized, elicited minor changes in fluorescence relaxation under control conditions (refer to Figure 4.10). However, under conditions conducive to inducing the wave phenomenon (38°C combined with microaerobic conditions), DMBQ treatment hindered the dip below the original F_0 level.

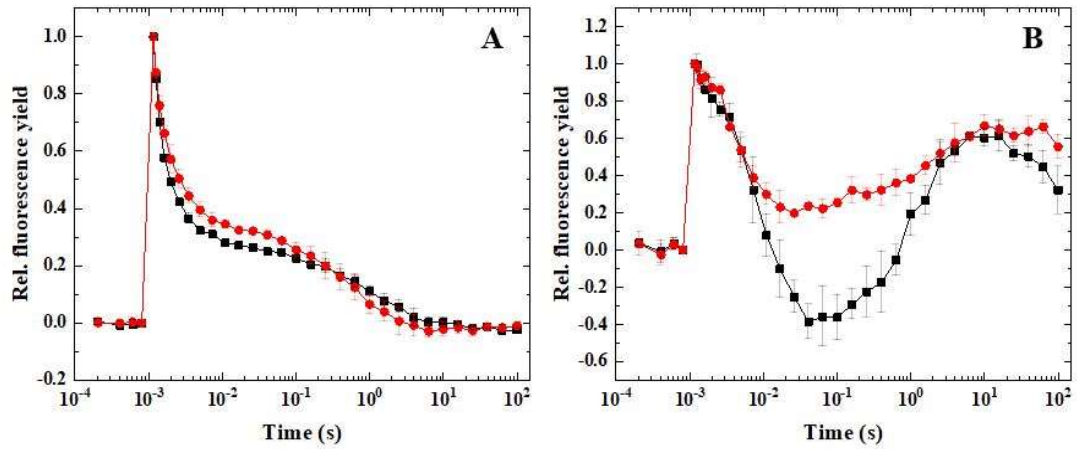

Figure 4.10: Effect of DMBQ on the wave phenomenon of flash-induced Chl fluorescence relaxation. Measurements were conducted under two conditions: (A) at 24°C (black) with the addition of DMBQ (red), and (B) at 38°C under microaerobic conditions (black) with the addition of DMBQ (red). Data averaged from 3 biological replicates.

Nonetheless, unlike observations in *Synechocystis*, where DMBQ treatment resulted in the complete elimination of the wave phenomenon, the wave persisted to some extent under wave-inducing conditions in the case of *Symbiodinium*.

Methyl-viologen, an electron acceptor known to compete with ferredoxin for electrons from the FeS clusters at the acceptor side of PSI, effectively suppresses cyclic electron flow (CEF) (Schansker et al., 2005). Interestingly, under normal conditions, methyl-viologen did not elicit any discernible effect on fluorescence relaxation dynamics. However, under conditions conducive to wave induction, methyl-viologen significantly impeded the wave phenomenon. Only a minor dip in fluorescence was observed, with the fluorescence level remaining above the F_0 baseline (refer to Figure 4.11). This observation underscores the role of methyl-viologen in disrupting the wave phenomenon, highlighting its impact on the electron flow dynamics and chlorophyll fluorescence behavior under specific environmental conditions.

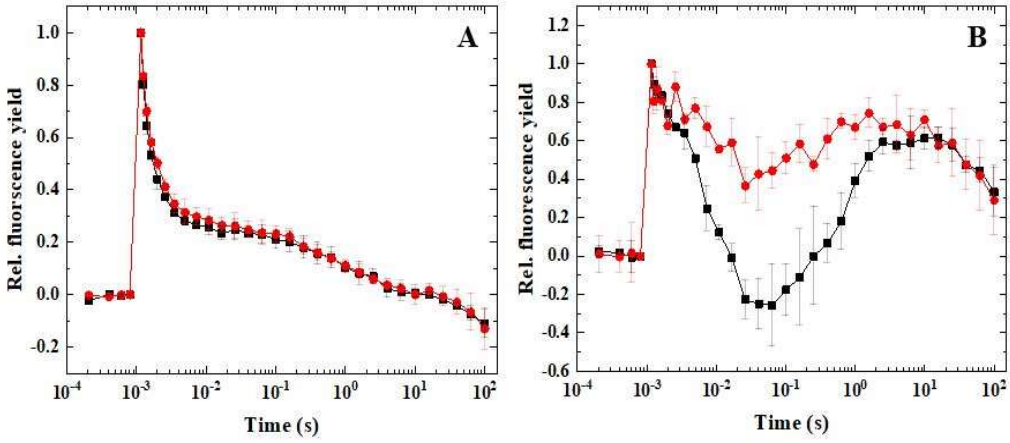

Figure 4.11: Effect of MV on the wave phenomenon of flash-induced Chl fluorescence relaxation. Measurements were conducted under two conditions: (A) at 24°C (black) with the addition of MV (red), and (B) at 38°C under microaerobic conditions (black) with the addition of MV (red). Data averaged from 3 biological replicates.

4.5 Effect of inhibiting the primary electron donor of photosystem II and the terminal electron acceptor in CO_2 fixation in *S. tridacnidorum*

In order to test the condition when the PSII activity relative to the PSI activity was decreased, a hydroxylamine (HA) treatment was applied, either under aerobic or under microaerobic condition. Hydroxylamine did not have a pronounced effect on the fluorescence relaxation after the flash as compared to the non-treated control (Figure 4.12). Under microaerobic conditions and HA treatment a strongly reduced PQ pool was observed (increased middle phase). However, unlike in *Chlamydomonas* (Patil, et al., 2022), the large dip below F_0 could not be observed, and only a small increase in the Chl fluorescence (in the 1-100 s timescale) occurred.

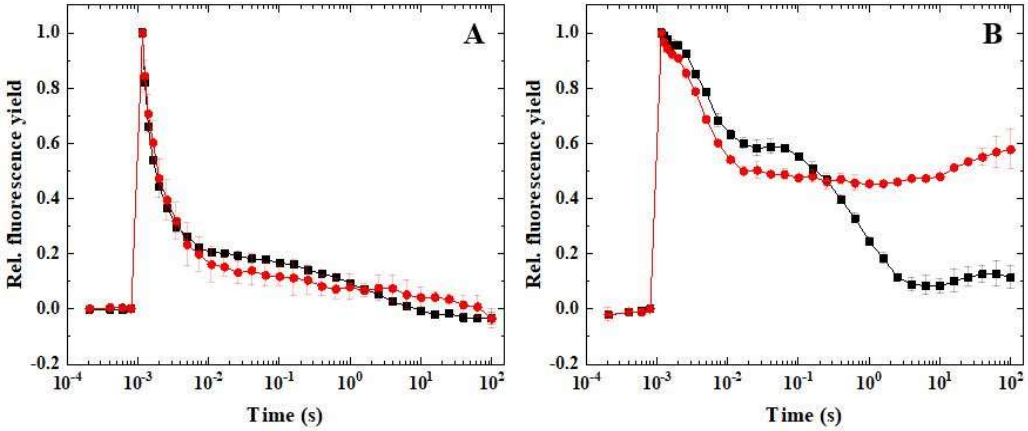

Figure 4.12: Effect of hydroxylamine (HA) on the wave phenomenon of flash-induced Chl fluorescence relaxation. Measurements were conducted under two conditions: (A) at 24°C (black) with the addition of hydroxylamine (HA) (red), and (B) at 38°C under microaerobic conditions (black) with the addition of hydroxylamine (HA) (red). Data averaged from 3 biological replicates.

In samples treated with HA, the characteristic wave phenomenon observed in the heat+microaerobic treatment was notably absent. The absence of the slow component in the relaxation on the timescale of seconds can be attributed to the compromised functionality of the donor side of PSII after HA treatment. Consequently, the fluorescence relaxation profile under the HA+microaerobic treatment closely resembled that of the heat treatment, both of which exhibited damage to the donor side of PSII (Mohammad Aslam et al., 2022).

Under conditions where the terminal electron acceptor in other words, CO_2 fixation is obstructed, thereby inhibiting linear electron flow from PSI towards the CBB cycle, alternative electron flow processes such as cyclic electron flow (CEF) may operate unhindered or even accelerate.

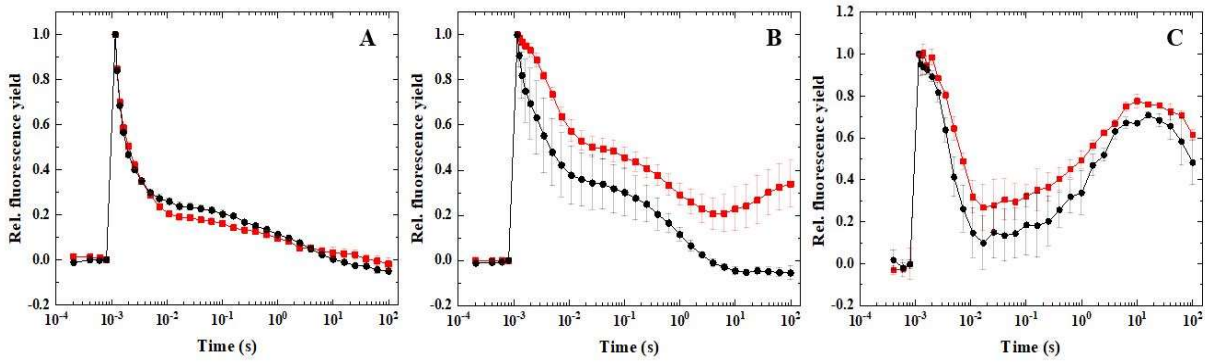

Figure 4.13: Effect of glycolaldehyde (GA) on flash-induced chlorophyll fluorescence relaxation profile. Treatments were conducted at (A) 24°C (black) with glycolaldehyde (red), (B) 24°C under microaerobic conditions (black) with glycolaldehyde (red), and (C) 38°C under microaerobic conditions (black) with glycolaldehyde (red). Data averaged from 3 biological replicates.

In such scenarios, it is anticipated that the wave phenomenon would manifest faster phases. To examine this hypothesis, the CBB cycle was inhibited using glycolaldehyde (GA). Interestingly, GA did not induce significant changes in the fluorescence relaxation profile under aerobic conditions at 24°C (refer to Figure 4.13 (A)). Moreover, GA did not elicit significant alterations in the fluorescence wave induced under heat+microaerobic conditions (refer to Figure 4.13 (C)). This observation suggests that under these conditions, the wave phenomenon, characterized by transient reduction-oxidation and re-reduction of the PQ pool, operates at its maximal rate. Thus, blocking the CBB cycle could not further accelerate it, especially considering that the CBB cycle might have been inhibited by the heat treatment itself.

However, when GA was introduced to microaerobic samples at ambient temperature (24°C), a wave-like phenomenon emerged, characterized by a dip at around 4 seconds followed by a subsequent increase in fluorescence (refer to Figure 4.13 (B)). Notably, this dip was absent in microaerobic, non-GA treated cells at 24°C, despite the strong reduction of the PQ pool induced by microaerobic treatment.

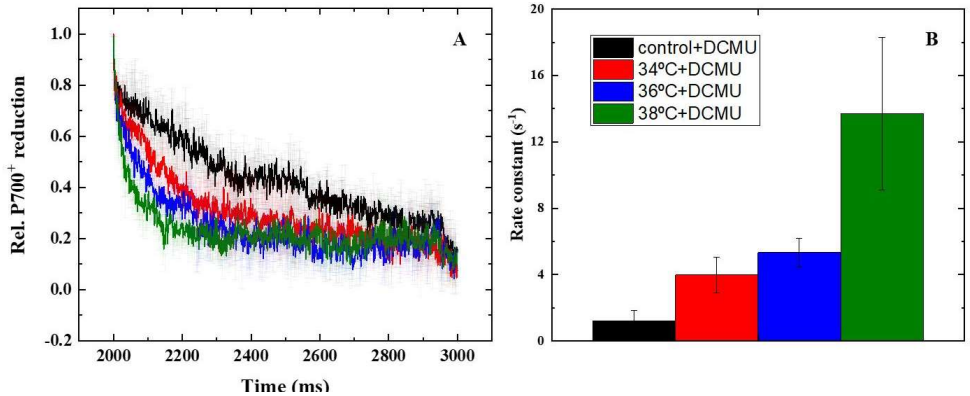

Figure 4.14: $P700^+$ re-reduction original traces (mean±S.D) (A) and the rates of re-reduction (B) as a function of temperature, including 24°C+DCMU (black), 34°C+DCMU (red), 36°C+DCMU (blue) and 38°C+DCMU (green). Data averaged from 3 biological replicates.

We observed that $P700^+$ re-reduction kinetics exhibited temperature dependence in this species, which aligns with our previous observations of fluorescence relaxation under increasing temperatures (se Figure 4.14).

To explore the impact of glycolaldehyde on the kinetics of $P700^+$ re-reduction, P700 absorption measurements were conducted in the presence of DCMU, which blocks electron flow from PSII to PSI, leading to a dominance of PSI cyclic electron flow (PSI-CEF).

Compared to non-treated control cells, glycolaldehyde notably accelerated the re-reduction of $P700^+$ in DCMU-poisoned cells at ambient temperature. Interestingly, this acceleration was comparable in magnitude to that observed with acute heat treatment (see Figure 4.15). These findings suggest that glycolaldehyde treatment, akin to acute heat stress, influences the electron transport processes, potentially modulating the balance of electron flow between PSI and CBB cycle (Mohammad Aslam et al., 2023), and thereby impacting the re-reduction kinetics of $P700^+$.

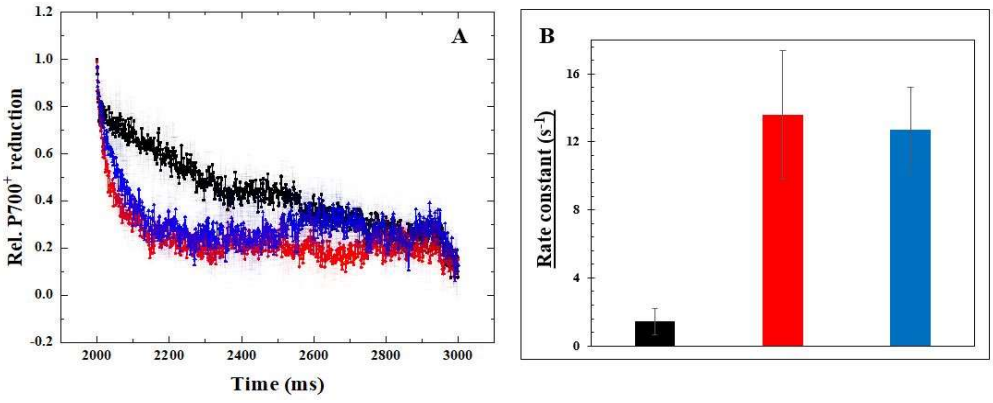

Figure 4.15: Effect of glycolaldehyde or heat treatment on $P700^+$ re-reduction kinetics with PSII activity blocked by DCMU, highlighting cyclic electron flow around PSI. (A) Original traces (mean±S.D): 24°C+DCMU (black), 38°C+DCMU (red), and control+glycolaldehyde (blue). (B) Respective rate constants of $P700^+$ re-reduction from exponential fitting (red). Data averaged from 3 biological replicates.

4.6 Effect of cyclic electron flow inhibitors

Antimycin A, an inhibitor of the PGR5/PGRL1 pathway, and polymyxin B, an inhibitor of the NDH-2 pathway, did not exert a significant effect on the wave phenomenon in Symbiodiniaceae (as shown in figure 4.16 (A)).

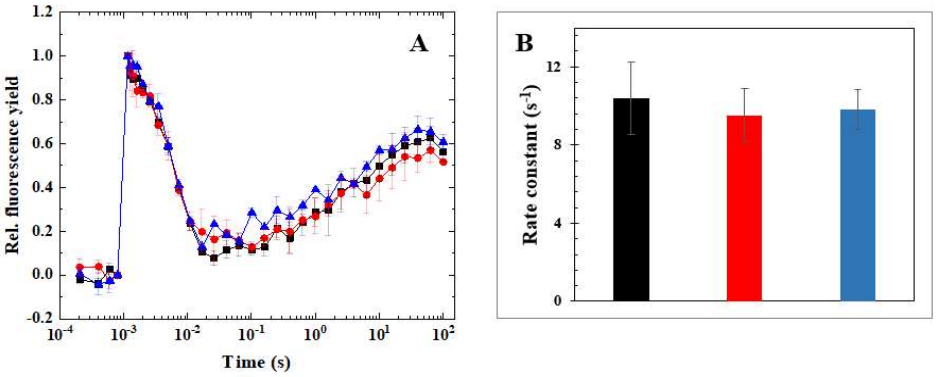

Figure 4.16: Effect of antimycin A and polymyxin B on flash-induced chlorophyll fluorescence relaxation profile (A) and $P700^+$ re-reduction kinetics (B) of undigested cells. Treatment groups include control (38°C + microaerobic, black), polymyxin B treatment (red), and antimycin A treatment (blue). Data averaged from 3 biological replicates.

Furthermore, these inhibitors did not markedly alter the $P700^+$ re-reduction kinetics (as shown in figure 4.16 (B)). However, it is plausible that these inhibitors fail to penetrate intact *Symbiodinium* cells due to the presence of a thick cell wall, as previously suggested for antimycin A (Aihara et al., 2016).

To address this limitation, protoplasts were prepared by digesting the cell wall using cellulase, following a modified protocol (Bashir et al., 2022). Notably, the protoplast preparation procedure imposed considerable stress on the cells, significantly impacting fluorescence relaxation and causing the wave phenomenon to disappear under these conditions (as depicted in Figure 4.17). Consequently, it became imperative to optimize the cell wall digestion procedure to preserve photosynthetic activity and retain the characteristic fluorescence wave feature of *Symbiodinium*.

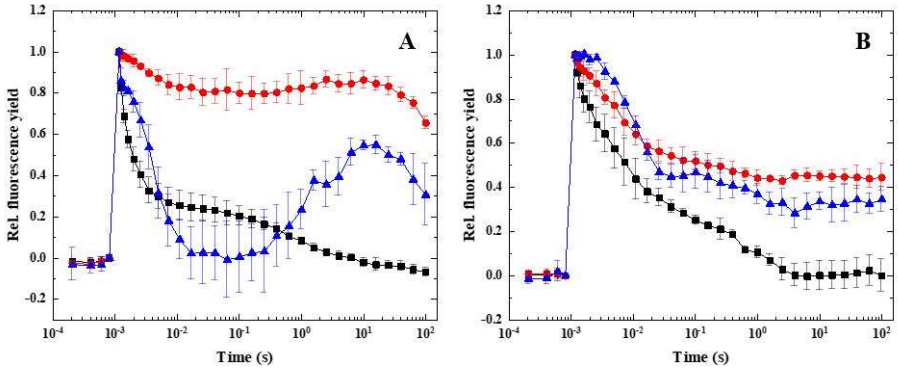

Figure 4.17: Flash-induced fluorescence relaxation in intact cells (A) and protoplasts (B) prepared according to Bashir et al. 2022. Treatments were conducted under three conditions: 24°C (black) and 38°C for 10 minutes (red), and 38°C under microaerobic conditions (blue). Data averaged from 3 biological replicates.

Symbiodinium cells with partially digested cell wall retained the distinctive fluorescence wave phenomenon, induced through heat+microaerobic treatment in a manner analogous to intact cells (see Figure 4.18, black trace).

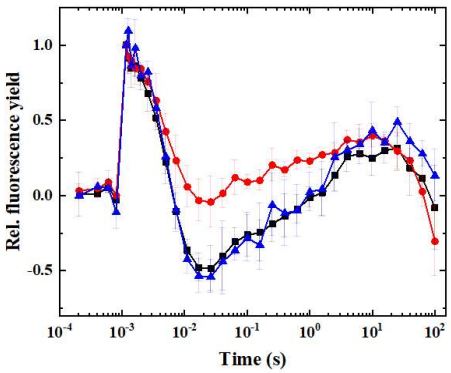

Figure 4.18: Effect of antimycin A and polymyxin B on flash-induced chlorophyll fluorescence relaxation profile in cells with partially digested cell walls. Treatments: control (38°C+microaerobic, black), polymyxin B (red), antimycin A (blue). Data averaged from 3 biological replicates.

Subsequent inhibitor treatments on these cells with partially digested cell wall revealed that antimycin A exhibited no discernible inhibitory effect on the wave phenomenon. In contrast, polymyxin B demonstrated a significant inhibitory impact on the wave, albeit without complete elimination of the fluorescence dip phase post-flash (refer to Figure 4.18).

This heightened inhibitory effect of polymyxin B was further evident in the $P700^+$ re-reduction kinetics (see Figure 4.19), suggesting a potential predominant role of the NDH-2 pathway in electron transport between the acceptor side of PSI and the PQ pool through cyclic electron flow (Mohammad Aslam et al., 2023).

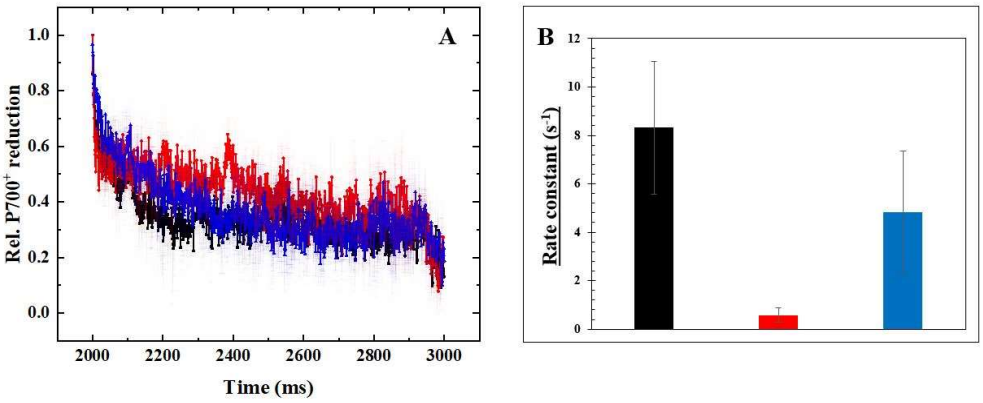

Figure 4.19: Effect of antimycin A and polymyxin B on $P700^+$ re-reduction kinetics in cells with partially digested cell walls. Treatments were conducted at 38°C in the presence of DCMU. (A) Original traces: 38°C+DCMU (black), control+polymyxin B (red), and control+antimycin A (blue). (B) Respective rate constants of $P700^+$ re-reduction from exponential fitting. Data averaged from 3 biological replicates.

4.7 Flash-induced chlorophyll fluorescence relaxation kinetics in longer time scale

In contrast to the rapid rise in fluorescence decay from a few milliseconds observed in *Symbiodinium* under heat and anaerobic conditions, anaerobic conditions alone result in a gradual, slow rise in fluorescence, forming a wave-like pattern over a period of 1-400 seconds. Furthermore, it is evident that this pattern of fluorescence rise was also found in intact coral fragments, where it was also observed to be faster than in isolated *Symbiodinium* cells. The application of DCMU resulted in the complete elimination of the fast and intermediate phases of fluorescence relaxation, with only the slow phase remaining, which originates from the charge recombination of Q_A^- and S2 state. Furthermore, the DCMU treatment also led to the disappearance of the slow fluorescence wave, which suggests that the linear electron transfer from Q_A^- to Q_B is a crucial factor in the formation of this wave (Figure 4.20).

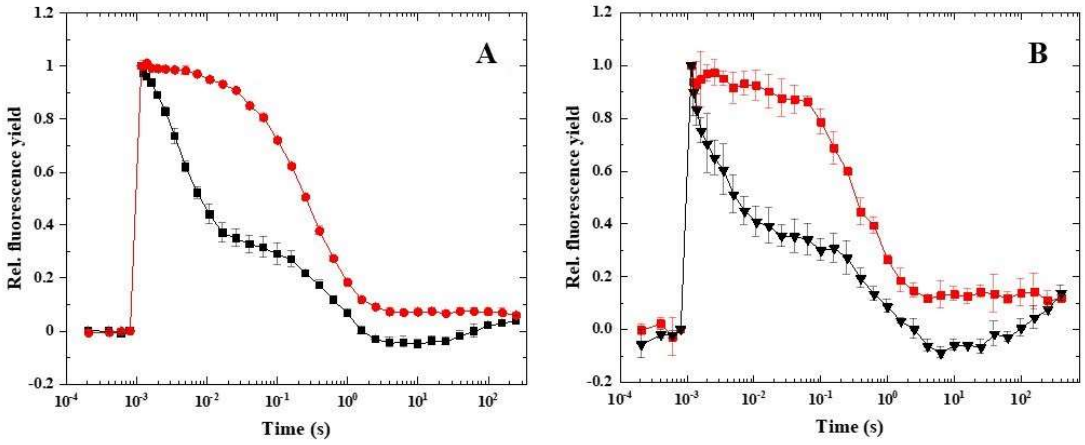

Figure 4.20: Flash-induced fluorescence relaxation in (A) *Fugacium kawagutii* (CS156), (B) *Pocillopora damicornis* including control anaerobic (black) and DCMU (red). Data averaged from 3 biological replicates.

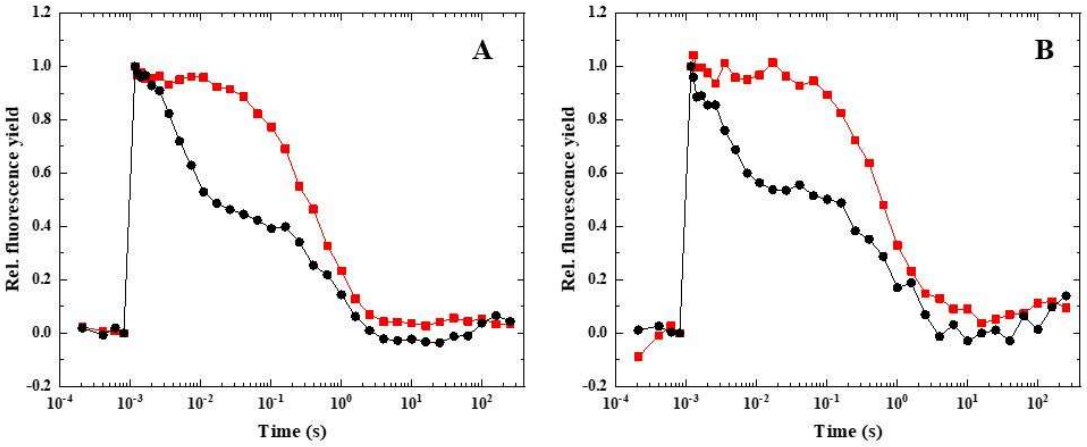

Figure 4.21: Flash-induced fluorescence relaxation in (A) *Acropora*, (B) *Seriatopora* including control anaerobic (black) and DCMU (red). Data averaged from 3 biological replicates.

This fluorescence wave pattern was also observed in other coral species, such as *Acropora* and *Seriatopora*, thus indicating its uniformity across species (Figure 4.21).

To determine if the observed slow fluorescence wave is caused by the actinic flash in coral, and if the slow wave phenomenon is associated with the redox reactions of formed Q_A^-, measurements were conducted with and without the application of actinic flash.

In the absence of the actinic flash, a fluorescence increase could be observed after approximately 100 seconds, indicating a reduction in the PQ pool through the back flow of electrons from the host. However, in this instance, the fluorescence dip was not observed. Upon application of the actinic flash, the fluorescence dip was observed to occur within seconds, followed by an increase after approximately 10 seconds (Figure 4.22). The fluorescence dip and subsequent fluorescence rise were more pronounced following the actinic flash, indicating a surplus of electrons from the symbiont, in addition to those originating from the coral host, which were sufficient to reduce the PQ pool to a greater extent.

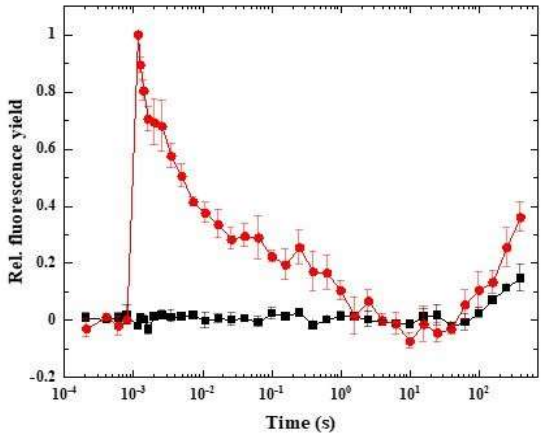

Figure 4.22: Flash-induced fluorescence relaxation in *Pocillopora damicornis* including without actinic flash (black) and with actinic flash (red). Data averaged from 3 biological replicates.

4.8 Oxygen dependent change in fluorescence relaxation kinetics of corals

The fluorescence wave at the timescale of 0-400 seconds was not observed in aerobic conditions in *Symbiodinium*, indicating that reduction of PQ pool was required for the slow rise. However, in coral fragments, it can be observed even in aerobic treatments. To gain further insight, the changes in dissolved oxygen were monitored throughout the measurement of flash-induced chlorophyll fluorescence relaxation kinetics.

The longer the corals were kept in the medium, the more rapidly the dissolved oxygen decreased. This was due to the respiratory activity of the algae and host cells in the 10 mm cuvette.

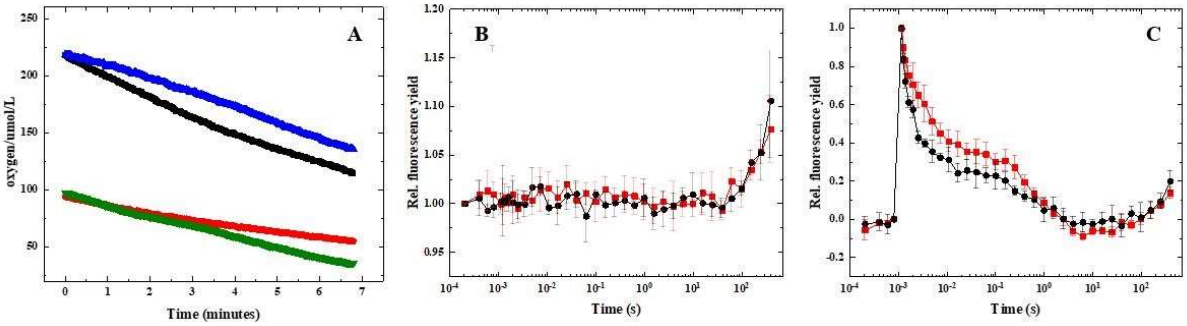

Figure 4.23: Dissolved oxygen changes in *Pocillopora damicornis* without actinic flash (black & red) and with actinic flash (blue & green) (A). Flash-induced fluorescence relaxation including without actinic flash (B) and with actinic flash (C) with black being first measurement above 200 µmol/L and red being second under 100 µmol/L. Data averaged from 3 biological replicates.

Following the initial measurement above 200 µmol/L, a notable enhancement in the PQ pool redox state was apparent, which subsequently exhibited a further intensification of the dip and rise of the fluorescence as the dissolved oxygen concentration declined below 100 µmol/L (second measurement). In the absence of the actinic flash, the gradual rise in fluorescence following 100 seconds persisted, but with a slight decline in intensity (Figure 4.23).

4.9 Potassium ferricyanide (FeCN) reduction assay

It is hypothesized that the slow wave phenomenon is associated with electron transport activity between the algal symbiont and the coral host. Extracellular electron transport from the symbiont to the host may serve as an important mediator of the symbiosis, which may occur via several potential transporters. The application of an artificial acceptor, potassium ferricyanide (FeCN), was employed to elucidate whether electron transport modulates the slow wave phenomenon in Symbiodiniaceae.

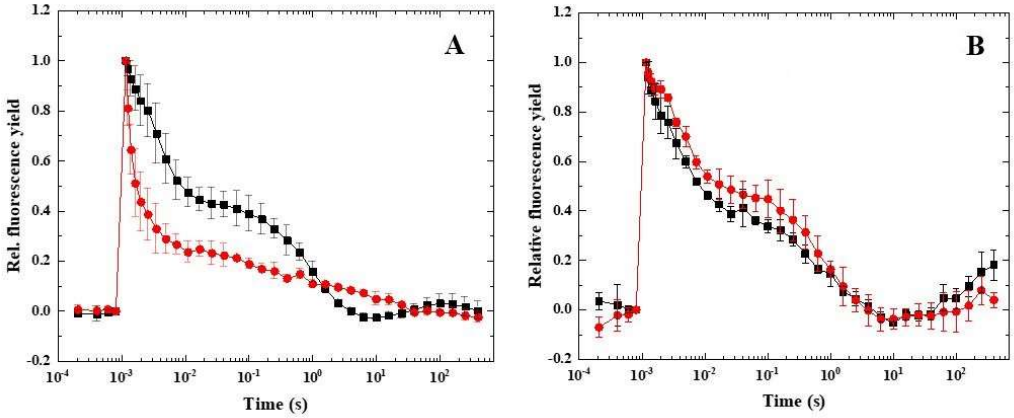

Figure 4.24: Flash-induced fluorescence relaxation in (A) *Fugacium kawagutii* (CS156), (B) *Pocillopora damicornis* including control anaerobic (black) and FeCN (red). Data averaged from 3 biological replicates.

FeCN functions as an electron acceptor for the entire photosynthetic electron transport chain, specifically for PSII and PSI (Izawa, 1980). Moreover, it has been employed as an efficacious electron transport mediator for the transfer of electrons from a range of plasma membrane redox proteins and enzymes (Barr & Crane, 1981; Izawa, 1980; Khorobrykh, 2023). Given that FeCN does not penetrate intact cells, it can be assumed that the reduction of FeCN in *Symbiodinium* suspensions can only occur via electron transport directed to the extracellular space, with a partial origin in photosynthetic electron transport. The elimination of the wave phenomenon and the alleviation of the reduction of PQ pool (which was induced by the anaerobic treatment) indicate the drainage of electrons from the photosynthetic electron transport chain that were transported to the extracellular space (Figure 4.24 (A)). Additionally, FeCN did not affect the middle phase but did reduce fluorescence increases in intact corals over extended time periods (10-400 seconds), suggesting a reduction in the electron flow from the host to the symbiont (Figure 4.24 (B)).

To verify the electron transport directed outwards of the cells, a FeCN reduction assay was conducted. The change in absorbance was recorded at 420 nm over a period of two hours. The assay was performed under a variety of conditions, including those involving isolated *Symbiodinium* cultures, *Symbiodinium* cells whose cell wall was partially digested (mimicking a better transport as in symbiotic lifestyle), and intact coral fragments.

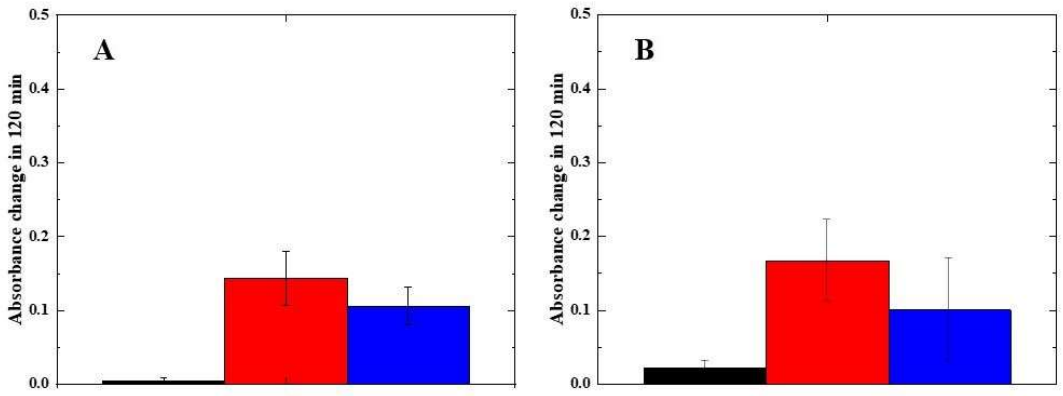

Figure 4.25: Change in FeCN reduction in (A) *Fugacium kawagutii* (CS156), (B) *Fugacium kawagutii* (CS156) cells with partially digested cell walls including control (black), FeCN (red) and in presence of DCMU (blue). Data averaged from 3 biological replicates.

The results demonstrated a notable reduction of FeCN in both intact cells and cells with partially digested cell wall when the samples were kept in growth light (50 µmol photons m^{-2} s^{-1}) for two hours. DCMU did not entirely inhibit the reduction process, but rather resulted in a decrease in FeCN reduction, suggesting that not all electrons are derived from the light reaction of photosynthesis (Figure 4.25).

The reduction of FeCN by the intact coral was significantly higher than that observed in isolated *Symbiodinium* cultures (Figure 4.26 (A)). To account for variations, the reduction rates were normalized relative to the weight of the coral fragments, showing comparable results across

samples (Figure 4.26(B)). However, the amount of photosynthetically active *Symbiodinium* cells within the coral fragments is not straightforward to compare, even when the fragments are grown under identical conditions in the same tank. To address this, FeCN reduction rates were also normalized to the total oxygen-evolving capacity of each coral fragment (Figure 4.26 (C)).

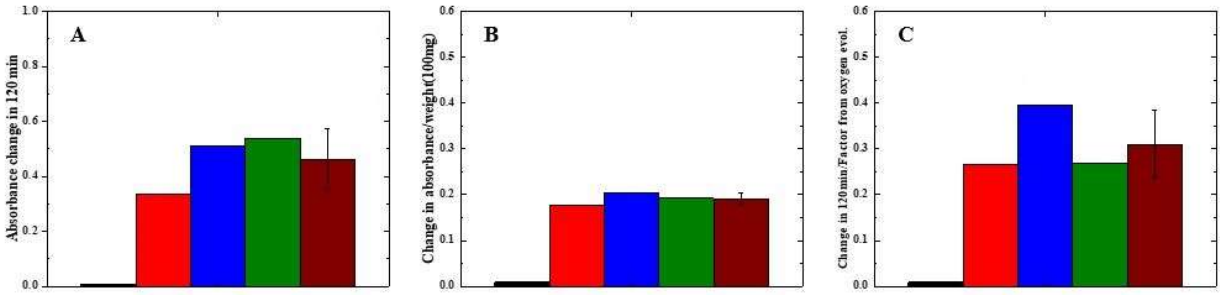

Figure 4.26: Change in FeCN reduction in *Pocillopora damicornis* (A) total absorbance change, (B) change relative to fragment weight and (C) change relative to oxygen evolution, including control with no fragment (black), fragments (red, blue & green) and average (avg) of the fragments (brown). Data averaged from 3 biological replicates.

A comparison of FeCN reductions with respect to chlorophyll concentration per hour revealed that intact *Fugacium* (CS156) cells exhibited an FeCN reduction rate of approximately 13.5 ± 2.4 µmol mg Chl^{-1} h^{-1}. The rate was somewhat higher in CS156 cells with partially digested cell wall, at 15.8 ± 5.3 µmol mg Chl^{-1} h^{-1}. The FeCN reduction rate of intact *P. damicornis* coral fragments was approximately 29.5 ± 7 µmol mg Chl^{-1} h^{-1} (Figure 4.27). The elevated rates observed in intact corals may be attributed to the contribution of the host to electron transfer processes or to somewhat distinct optical conditions that reach the symbiont cells within the coral tissue, as compared to those encountered by *Fugacium* cells in culture. These findings confirmed extracellular electron transport, as evidenced by the reduction of FeCN by algae and coral host cells.

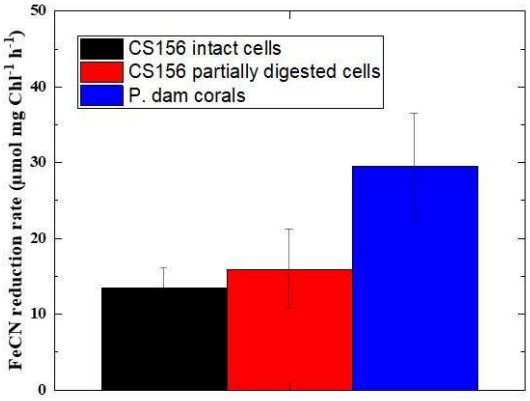

Figure 4.27: FeCN reduction rates in *Fugacium kawagutii* (CS156) (black), CS156 cells with partially digested cell walls (red), and *Pocillopora damicornis* (blue) normalized with the photosynthetic oxygen evolution, which was determined in both cultured *Fugacium* and intact corals. Data averaged from 3 biological replicates.

5. Discussion

5.1 Impact of acute heat stress on photosynthetic properties in Symbiodiniaceae

The characterization of flash-induced chlorophyll fluorescence relaxation in coral endosymbiont algae, specifically Symbiodiniaceae, remains a crucial yet unexplored area of research. Given the severe threat posed by heat stress to coral reefs, there is a pressing need to develop non-intrusive techniques for monitoring physiological changes in coral reef organisms. Symbiodiniaceae species play a pivotal role in providing photosynthetic products to coral hosts, making it imperative to understand the regulation of photosynthetic electron transport processes within these algae.

In our investigation, various strains of Symbiodiniaceae were subjected to acute heat stress, a condition previously shown to damage PSII (Hill & Ralph, 2008b), induce cyclic electron flow (Aihara et al., 2016; Dang et al., 2019), and impact PSI activity and electron donation between PSII and PSI in intact corals (Szabó et al., 2017). The acute heat stress led to alterations in the fluorescence relaxation profile, notably manifesting as an elevated middle phase indicative of enhanced reduction of the plastoquinone (PQ) pool (Figure 4.1). This observation was corroborated by fast fluorescence induction and post-illumination chlorophyll fluorescence rise measurements. Remarkably, strain 2465 exhibited the most significant and reversible increase in PQ pool reduction in response to acute heat stress. PIFT results (Figure 4.3), which originate from electron transfer from DHAP with reverse reactions in the CBB cycle and to PQ via the NDH complex (Gotoh et al., 2010), also indicate a potential role for cyclic electron transport in mitigating the effects of heat stress. The variation in heat sensitivity among different Symbiodiniaceae species has been documented in previous studies (Dang et al., 2019; Fisher et al., 2011; Hennige et al., 2008; Suggett et al., 2015), highlighting the existence of different functional groups within Symbiodiniaceae. Our research reveals species-specific changes in flash-induced fluorescence relaxation during acute heat stress, notably in the PQ pool reduction level. Understanding the responses of Symbiodiniaceae to heat stress informs coral reef resilience mechanisms which is crucial for conservation strategies amid escalating climate threats.

5.2 Exploring temperature dependency in flash-induced chlorophyll fluorescence relaxation and the wave phenomenon in Symbiodiniaceae

The temperature dependency of the flash-induced chlorophyll fluorescence relaxation and the wave phenomenon in Symbiodiniaceae is a critical aspect of understanding their response to environmental stressors. The inducibility of the wave phenomenon, a key indicator of stress response, is closely linked to the ratio of PSI:PSII activities. Therefore, investigating how different strains respond to temperature variations and whether the decrease in PSII activity relative to PSI triggers wave formation is essential.

Raising the temperature up to 40°C revealed that while heat treatment alone did not induce wave formation in any investigated strains (Figure 4.5), strain 2465 exhibited the most prominent dip

and subsequent increase in fluorescence. Interestingly, previous studies in microalgae, such as *C. reinhardtii*, demonstrated that both a strong reduction of the PQ pool and a partial decrease in PSII activity are necessary for wave formation (Krishna et al., 2019; Patil et al., 2022). Our findings suggest a similar pattern in Symbiodiniaceae; heat-treated cells, which experienced a loss of PSII activity relative to PSI, displayed wave formation, particularly pronounced in strain 2465 (Mohammad Aslam et al., 2022).

The heat-induced changes in flash-induced fluorescence relaxation were evident through the relative decrease in variable fluorescence, an increase in F_0, and alterations in the fluorescence relaxation profile (Hoogenboom et al., 2012). Elevated temperatures primarily impact oxygen evolving capacity and PSII activity in corals, with PSII being more sensitive to heat than PSI. This progressive imbalance in electron flow from PSII to the PQ pool and PQ pool oxidation via forward electron transfer to PSI favors wave formation. However, heat treatment alone up to 40°C was insufficient to induce the wave phenomenon. Microaerobic conditions were also crucial, generating a strong redox poise for the PQ pool. The wave phenomenon in Symbiodiniaceae could serve as a valuable environmental indicator of heat stress, particularly when combined with hypoxic conditions in coral reef ecosystems (Hughes et al., 2020; Kühl et al., 1995; Ulstrup et al., 2005). Understanding the temperature-dependent features of wave phenomenon formation could provide insights into strain-specific tolerance to heat stress in Symbiodiniaceae. The tolerance of reef-building corals to various stressors ultimately determines coral reef ecosystems' ability to cope with acute and chronic changes in abiotic environmental conditions.

5.3 The wave phenomenon observed in flash-induced chlorophyll fluorescence relaxation

Flash-induced chlorophyll fluorescence relaxation kinetics is a powerful tool utilized to scrutinize the processes include the dynamic interplay of intersystem PSII-PSI electron flow, charge recombination phenomena, and alternative electron transfer pathways like cyclic electron flow (Deák et al., 2014; Havurinne & Tyystjärvi, 2020; Krishna et al., 2019; Vass et al., 1999; Volgusheva et al., 2016).

Under standard conditions, fluorescence relaxation manifests distinct and well-separated phases, providing an insight into the various sections of photosynthetic electron transport. However, under specific environmental stressors or experimental conditions, characteristic waves in fluorescence relaxation emerge, signifying the nuanced reduction-reoxidation sequences of the plastoquinone (PQ) pool. In cyanobacteria, this intriguing wave phenomenon is attributed to the operation of the NDH-1 complex, facilitating electron transfer from ferredoxin at the acceptor side of PSI to the PQ pool (Deák et al., 2014). Conversely, in microalgae like *Chlamydomonas reinhardtii*, the wave phenomenon correlates with the activity of NDH-2 (Krishna et al., 2019; Patil et al., 2022).

Importantly, the manifestation and nature of the wave phenomenon exhibit considerable diversity across taxonomic groups. While microaerobic conditions are sufficient to evoke the wave in cyanobacteria like *Synechocystis*, microalgae typically necessitate more than microaerobic treatment for its induction. This discrepancy stems from differences in the stoichiometry of

photosystems. In cyanobacteria, characterized by a high PSI:PSII ratio, potent reduction of the PQ pool is ample to induce the wave. Conversely, in eukaryotic microalgae with a lower PSI:PSII ratio, a balanced electron supply and withdrawal from the PQ pool impede wave induction. As exemplified in *C. reinhardtii*, partial inactivation of PSII, coupled with microaerobic treatment, proves requisite to induce a significant dip and subsequent rise of fluorescence following the flash (Patil, et al., 2022). Thus, the photosystem ratio significantly influences the wave phenomenon, acting as a discerning indicator of intersystem electron flow balance. However, this wave phenomenon was less understood particularly in Symbiodiniaceae (Mohammad Aslam et al., 2022).

5.4 Exploring the correlation between the wave phenomenon and cyclic electron flow in Symbiodiniaceae

The wave phenomenon observed in flash-induced chlorophyll fluorescence relaxation has been linked to cyclic electron flow (CEF) in various photosynthetic organisms, including cyanobacteria and certain microalgae. In Symbiodiniaceae, our observations under conditions known to induce CEF, such as acute heat stress, suggest a potential association between the wave phenomenon and CEF activation. Although the *Symbiodinium* genome contains genes for both the PGR5/PGRL1 and NDH-2 pathways, indicating their potential involvement, concrete evidence regarding specific components or CEF pathways in wave induction is lacking at present (Aihara et al., 2016; Deák et al., 2014; Krishna et al., 2019; Mohammad Aslam et al., 2022; Patil et al., 2022).

Alternative electron transfer pathways within *Symbiodinium*, like the Mehler reaction, PTOX, or Flavodiiron proteins, may also influence PQ pool reduction and fluorescence relaxation kinetics. However, the most pronounced wave occurrence was observed under microaerobic conditions in heat-treated cells, where oxygen photoreduction by these pathways is unlikely. This suggests a role for CEF, which accelerates under microaerobic conditions in *Symbiodinium*. Nonetheless, the rise in fluorescence relaxation after the dip phase argues against direct electron cycling from PSI to the PQ pool, as seen in *C. reinhardtii* (Alric, 2010).

In conclusion, flash-induced fluorescence decay emerges as a sensitive indicator of heat stress in Symbiodiniaceae, with the wave phenomenon indicative of partial PSII activity reduction and strong PQ pool reduction under microaerobic conditions. The species-specific characteristics of wave appearance and heat sensitivity in flash-induced fluorescence relaxation highlight its potential as a marker for stress-induced changes in photosynthetic electron transport. While the wave phenomenon may be associated with CEF processes, further research is needed to elucidate its precise mechanism in Symbiodiniaceae.

5.5 Linear electron flow inhibitors largely blocked the fluorescence wave phenomenon in Symbiodiniaceae

While a single report exists regarding the induction of the wave phenomenon in Symbiodiniaceae (Mohammad Aslam et al., 2022), it remains unclear whether this phenomenon is associated with linear or alternative (cyclic) electron flow.

To address these questions, selective inhibitors were employed in this study to probe the underlying mechanisms. As previously demonstrated, functional linear electron transport from PSII to stromal electron carriers is a critical condition for inducing the wave phenomenon (Deák et al., 2014). The application of DCMU disrupts electron transfer from Q_A^- to Q_B, resulting in the disappearance of the fast and middle phases of relaxation. In Symbiodiniaceae, akin to *Synechocystis*, DCMU abolishes the fast and middle phases, leaving only the slow phase related to charge recombination between Q_A^- and the S2 state of the water oxidizing complex observable (Vass et al., 1999) (Figure 4.9). Additionally, DCMU blocks the fast and middle phases even under wave-inducing conditions (i.e., acute heat+microaerobic treatment), preventing the fluorescence dip and subsequent rise, albeit a minor decrease in the fast phase in 38°C+microaerobic treatment persists.

DMBQ, another inhibitor employed, intercepts electrons at PSII, hindering their delivery to PSI via the Cyt b_6f complex, thereby inhibiting linear electron flow and maintaining the PQ pool largely oxidized. DMBQ substantially eliminates the dip in the fluorescence wave. However, unlike *Synechocystis*, DMBQ incompletely halts the fluorescence decrease in *Symbiodinium* (Figure 4.10). This discrepancy suggests that while DCMU and DMBQ largely impede the wave phenomenon by inhibiting linear electron flow, complete inhibition is not achieved in Symbiodiniaceae, possibly due to acute heat-induced damage to the PSII quinone-binding site.

The abolition of the wave by MV underscores the significance of the intact electron transport chain up to the CO_2 fixation step (Figure 4.11). MV, intercepting electrons at the PSI acceptor side, primarily inhibits cyclic electron flow (CEF) rather than linear electron flow (LEF). The withdrawal of electrons at the PSI acceptor side hampers the cycling of electrons from Fd (or NADH) back to the PQ pool.

5.6 Impact of donor side inhibition in PSII and Calvin Benson cycle on the wave phenomenon

When the donor side of PSII was inhibited by using hydroxylamine in conjunction with microaerobic treatment, the anticipated wave phenomenon did not manifest in Symbiodiniaceae. This observation contrasts with findings from studies involving *Chlamydomonas reinhardtii* and other green algae, where such conditions induced a remarkable wave response (Patil et al., 2022). The inhibition of the donor side of PSII by hydroxylamine results in a reduction of PSII activity relative to PSI activity. This, combined with the presence of a strongly reduced PQ pool under microaerobic conditions, creates a notable imbalance between the electron flow from PSII to the PQ pool and the electron withdrawal from the PQ pool towards PSI. Despite these conditions, the expected wave phenomenon, observed in certain microalgae under similar circumstances, was not elicited in Symbiodiniaceae (Patil et al., 2022) (Figure 4.12). Previous studies have demonstrated

that the elimination of the oxygen-evolving complex of PSII through sulfur deprivation (which induced H_2-producing conditions) induced a characteristic wave phenomenon in *C. reinhardtii* (Krishna et al., 2019), highlighting the significance of reduced PSII activity relative to PSI activity in inducing the wave in this species. This effect was replicated with HA treatment in *C. reinhardtii*, resulting in the appearance of the wave (Patil et al., 2022). However, in Symbiodiniaceae, the reduction of PSII activity relative to PSI activity was apparently insufficient to induce a similar wave phenomenon even under micro-aerobic conditions, possibly due to inadequate electron flow back to the PQ pool after the flash. This raises the question: could the wave phenomenon be enhanced by expediting the rerouting of electrons from the acceptor side of PSI to the PQ pool by inhibiting the CBB cycle? Previous research has demonstrated that glycolaldehyde, a chemical inhibitor of the CBB cycle, functional in corals as well (Bhagooli, 2013; Hill et al., 2014), slows down the linear electron flow and causes elevated levels of NADPH and ferredoxin (due to the inhibited NADPH uptake by the CB cycle), leading to an over-reduced PQ pool via cyclic electron flow and blockage of the PSI acceptor side (Szabó et al., 2017). Under ambient conditions, glycolaldehyde (GA) did not affect fluorescence relaxation in our study, likely because GA alone is not sufficient to increase to PQ pool reduction level needed to see the wave. However, under microaerobic conditions at ambient temperature, GA treatment induced a wave-like phenomenon, highlighting the importance of a highly reduced PQ pool (Figure 4.13). Additionally, significant acceleration of $P700^+$ re-reduction in the presence of GA, even at ambient temperature (with DCMU), suggests that the fluorescence wave induction correlates with cyclic electron flow activation, consistent with prior findings, although some studies noted only a moderate effect on $P700^+$ re-reduction with GA (Aihara et al., 2016; Dang et al., 2019).

Through various experimental approaches aimed at inducing a wave or wave-like phenomenon, we gained deeper insights into the fluorescence wave in Symbiodiniaceae. Our findings suggest that the appearance of the wave phenomenon is not primarily determined by the elimination of donor side activity but rather by the blockage of the CBB cycle and its associated impacts on PSI activity. This conclusion is supported by the similarity between the effects of glycolaldehyde (GA) and acute heat, both leading to enhanced $P700^+$ re-reduction (Figure 4.15). However, it is important to note that none of these experimental treatments fully induced the wave to its maximum extent, as seen with the application of acute heat stress+microaerobic treatment. Therefore, in Symbiodiniaceae, the comprehensive effects of acute heat on both PSII donor and PSI acceptor and the CBB cycle are necessary for the complete manifestation of the wave phenomenon. These conditions hold ecological significance as they mimic the extreme heat events experienced by corals, with temperatures reaching up to 38°C, as extensively discussed in recent literature (Camp et al., 2018). High heat stress can lead to oxygen-depleted environments due to increased respiratory activity in both the host and algal symbiont, compounded by various other contributing factors (Hughes et al., 2020). Consequently, employing non-invasive monitoring the phenomenon as flash-induced Chl fluorescence relaxation and its associated wave phenomenon, emerges as a valuable approach for uncovering specific alterations in photosynthetic electron transport within coral endosymbiont algae under such challenging circumstances.

5.7 The role of NDH-2-mediated electron flow in the wave phenomenon

To decipher the specific components of cyclic electron flow contributing to the induction of the wave phenomenon, inhibitors targeting the antimycin-sensitive (PGR5) and insensitive (NDH-2) pathways, namely antimycin A and polymyxin B, were administered. Despite the presence of the coding genes for both pathways in Symbiodiniaceae, antimycin A demonstrated inefficacy in blocking cyclic electron flow or non-photochemical quenching (NPQ) (Aihara et al., 2016). Our findings align with the observation that antimycin A was ineffective in impeding the wave and $P700^+$ re-reduction kinetics, a pattern similarly observed with the NDH-2 inhibitor polymyxin B (Figure 4.16). However, it is important to consider that the cell wall might render these inhibitors impermeable, making it challenging to assess their effects in intact cells. To overcome this limitation and facilitate the entry of antimycin A and polymyxin B, the cell wall was eliminated through enzymatic digestion using cellulase (Bashir et al., 2022; Levin et al., 2017).

In cells with partially digested cell walls, antimycin A remained ineffective, while polymyxin B successfully obstructed the wave phenomenon (Figure 4.18) and decelerated $P700^+$ re-reduction kinetics (Figure 4.19) (Mohammad Aslam et al., 2023). Hence, in the case of Symbiodiniaceae, NDH-2 likely contributes to the genesis of the wave phenomenon by facilitating electron transfer between NADPH and the PQ pool, akin to observations in *C. reinhardtii* and certain other green algae (Krishna et al., 2019; Patil et al., 2022). As previously discussed (Mohammad Aslam et al., 2022), the relatively slow rise in fluorescence following the dip, occurring over seconds, does not align with the rate of cyclic electron flow (CEF), suggesting that the wave phenomenon is not directly attributable to CEF operation. Moreover, studies have shown that the rate of PGR5 and NDH-2 is too slow to link their activity to CEF (Nawrocki et al., 2019; Nawrocki et al., 2019b). Nevertheless, the extent and timescale of CEF can vary widely, implying the existence of different scales of CEF, possibly modulated by the NDH-2 pathway. Moreover, it is probable that electron donation from Cyt b_6f and PSI operate at a rate typical for cyclic electron flow (CEF), as evidenced by the $P700^+$ re-reduction occurring within the 50-100 ms timescale under heat+microaerobic conditions. However, the electrons involved in the re-reduction of the PQ pool, manifesting over a much longer timescale, indicated by the increase in fluorescence after the dip, in seconds likely originate from alternative sources from the stroma rather than being mediated only by CEF. This slow PQ pool re-reduction, however, could be readily mediated by the NDH-2 pathway, as indicated by the inhibitory effect of polymyxin B.

Based on the characteristics of the fluorescence wave phenomenon investigated in the current study (and supported by the $P700^+$ re-reduction kinetics results), it appears that different electron sources with vastly different kinetics play a role in the overall formation of the wave. NDH-2 seems to have a more direct regulatory function on the slow alternative electron transport than the PGR5/PGRL1 pathway in Symbiodiniaceae. However, it cannot be excluded that both systems have important functions under certain conditions. For a comprehensive understanding of the regulation of CEF and other alternative electron transport processes in Symbiodiniaceae, the role of NDH-2 and the PGR5/PGRL1 system must be systematically investigated in the future, particularly under environmentally relevant, complex stress scenarios.

5.8 Flash-induced chlorophyll fluorescence relaxation as stress marker in corals

The Chl fluorescence patterns of corals are influenced by a range of environmental conditions, with a particular focus on the relationship between the coral and its symbionts. In the presence of low oxygen levels (anaerobic conditions), the relaxation of flash-induced chlorophyll fluorescence exhibits a gradual increase in a wave-like pattern, spanning a duration of up to 400 seconds. This phenomenon occurs fast in intact coral fragments as compared to isolated *Symbiodinium* cells (Figure 4.20). This fluorescence behavior is observed consistently across multiple coral species, such as *Acropora* and *Seriatopora* (Figure 4.21). This suggests that coral's internal structure facilitates more efficient electron transfer processes. In the absence of the flash, the fluorescence of intact corals increases gradually from 1 to 400 seconds. However, in the presence of the flash, there is a distinct dip followed by a sharp rise (Figure 4.22). This wave-like fluorescence pattern depends on a highly reduced PQ pool and is not observed in Symbiodinium under aerobic conditions. However, intact corals remarkably demonstrate this phenomenon, underscoring the complexity of their metabolic processes. As oxygen levels decline over time (due to respiration in the host tissue), intensity of the dip and slow rise in fluorescence decay increases due to enhanced reduction of the PQ pool since the terminal oxidase becoming less operable, demonstrating how corals adapt to changing oxygen conditions through complex energy transfer processes (Figure 4.23). This indicates that, as the availability of dissolved oxygen in a symbiosome is less than in the ambient seawater, even minor alterations due to stressors, such as a reduction in tidal current, heat stress, the buildup of reactive oxygen species (ROS) caused by photodamage, or an elevated respiration rate in the host (Anthony & Kerswell, 2007; Rädecker et al., 2021), can have significant impacts on the symbiont. Hence transfer of metabolites from host to the symbiont and vice-versa might become very crucial to survive in adverse conditions. Therefore, the presence of this wave in both isolated symbionts and intact corals under stress conditions may permit further utilization as a tool to study any stress condition in intact corals in the field.

5.9 Extracellular electron transport

The last part of the thesis demonstrates for the first time that *Symbiodinium* is capable of transferring electrons out of the cells to external acceptors. In the EET activity *Symbiodinium* is more effective than cyanobacteria such as *Synechocystis*, but they are similar to other photosynthetic microorganisms such as *C. vulgaris* and *Synechococcus elongatus*, exhibiting characteristics of a "poor" exoelectrogens (Gonzalez-Aravena et al., 2018). The majority of electrons generated during light-driven reactions are consumed in CO_2 fixation and other intracellular processes, leaving only a limited number of electrons available from photosynthesis to be directed to extracellular electron transport (EET). In contrast to this, "good" exoelectrogenic bacteria, such as *Shewanella oneidensis* and *Geobacter sulfurreducens*, have specialised adaptations that enable them to couple respiration with extracellular electron acceptors in an efficient manner. These bacteria employ outer membrane c-type cytochromes that facilitate direct electron transfer to external surfaces, in addition to redox-active molecules that facilitate electron flow outside the cell (Bouhenni et al., 2010; Lovley, 2012).

In the case of poor exoelectrogens, such as photosynthetic microorganisms, soluble artificial redox mediators that are impermeable to the plasma membrane, typically ferricyanide, are employed to facilitate exoelectrogenesis. The ferricyanide assay, which consists of measuring the electrochemical conversion of ferricyanide ($Fe(CN)_6^{3-}$) to ferrocyanide ($Fe(CN)_6^{4-}$), provides a quantitative measurement of exoelectrogenesis. In *Symbiodinium*, FeCN assays demonstrated a significant level of EET, as evidenced by a reduction in middle phase of the fluorescence decay and the rise over a period of 1-400 seconds following the addition of FeCN (Figure 4.24A). The absorption change over a period of 2 hours at 420 nm also indicated the reduction of ferricyanide, suggesting that electrons from the photosynthetic electron transport chain reaching the extracellular environment (Figure 4.25). However, its reduction in intact coral was found to be significantly higher than that observed in isolated Symbiodinium, indicating that the coral host may play a role in enhancing EET (Figure 4.27). This may be attributed to the presence of additional redox-active compounds or structural factors within coral tissues that facilitate electron flow. Moreover, both Symbiodinium and coral host cells have been observed to possess riboflavin transporters in symbiotic lifestyle (Lin et al., 2015), which may facilitate the transfer of electrons from the symbiont to the coral via riboflavin as a natural redox mediator (Canstein et al., 2008). This indicates that while Symbiodinium may not be an effective electrogen on its own, its association with the coral host facilitates a more robust EET pathway. This partnership is likely to play an important role in maintaining coral health, particularly under conditions of stress where efficient redox balancing is crucial, demonstrating how mutualistic relationships can facilitate enhanced metabolic resilience.

6. Thesis points

Coral bleaching has become a major ecological threat in recent decades, driven primarily by rising global temperatures. Environmental stresses cause the expulsion of zooxanthellae from coral hosts, leading to mass bleaching events in the Great Barrier Reef and many more destructions across the globe. Since corals rely on carbohydrates produced by these photosynthetic algae, understanding how stress affects zooxanthellae is critical. This thesis investigates the impact of stress on the photosynthetic efficiency of the algal endosymbiont, *Symbiodinium,* and explores potential alternative electron transport pathways in both free-living *Symbiodinium* as well as intact coral fragments under stress. The key findings are summarized below:

- Heat susceptibility varies from species to species in Symbiodiniaceae, hence the intact corals that host the specific species, may also respond differently.

- Acute heat stress makes the PQ pool more reduced, decreases Photosystem II efficiency, and in some species accelerates Photosystem I activity slightly.

- Flash-induced Chl fluorescence decay exhibited a wave phenomenon under heat treatment and microaerobic conditions, which is related to the transient oxidation and re-reduction of the PQ pool.

- These fluorescence phenomena are not only potentially associated with decreased relative activity of PSII:PSI, but also with a decreased Calvin-Benson-Bassham cycle activity and increased alternative electron flow, which can be correlated well with $P700^{+}$ reduction kinetics.

- The NDH-2 complex was observed as a potential mediator in channeling the electrons from stromal components back to the PQ pool under stress conditions in Symbiodiniaceae.

- Stress conditions usually intensify from intact coral to the symbiosomes, as high respiration of the host might lead to hypoxia in the symbiosomes much before the ambient oxygen reaches zero.

- FeCN reduction shows extracellular electron transport from the symbiont and the host to its environment, while the rise of the F_0 fluorescence in the dark indicates electron inflow from the coral host to the symbiont. These phenomena indicate a novel, two-way electron transport between the symbiont and the host.

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9. Summary

The study of flash-induced chlorophyll fluorescence relaxation in *Symbiodiniaceae*, the algal symbionts of corals, is of particular importance for the monitoring of physiological responses to acute heat stress, which represents a significant threat to coral reefs. These algae play a vital role in providing essential photosynthetic products to their coral hosts, making it imperative to assess their electron transport processes under stressful conditions. During our investigation, various strains of *Symbiodiniaceae* subjected to acute heat stress exhibited altered fluorescence relaxation profiles, notably an increase in the middle phase, which indicates a pronounced reduction of the PQ pool. Strain 2465 exhibited the most substantial reversible reduction, which aligns with previous studies that highlight the variability in heat sensitivity among different species of *Symbiodiniaceae*. This species-specific response to heat stress is crucial for understanding the resilience of coral reefs and for developing effective conservation strategies in the context of climate change. Furthermore, investigating the temperature dependency of fluorescence relaxation and wave phenomena in these algae offers crucial insights into their response to environmental stressors. It is noteworthy that our findings indicated that merely elevating the temperature to 40°C without additional stress did not result in wave formation across strains. However, strain 2465 exhibited a pronounced decline and subsequent increase in fluorescence as compared to other strains, which lacked this behavior. This pattern indicates that both a substantial reduction in the PQ pool and a partial loss of PSII activity are prerequisites for wave formation. The considerable alterations in fluorescence relaxation observed in heat-treated cells emphasize the deleterious effects of elevated temperatures on oxygen-evolving capacity and PSII activity. Ultimately, understanding the temperature-dependent aspects of wave formation will be invaluable in assessing strain-specific tolerance to heat stress, which is critical for the resilience of coral reef ecosystems in the context of a rapidly changing environment.

The wave-like phenomenon observed in flash-induced chlorophyll fluorescence relaxation has attracted attention due to its potential connection to cyclic electron flow (CEF) in a range of photosynthetic organisms, including cyanobacteria and certain microalgae. In our observations of *Symbiodiniaceae* under conditions that stimulate CEF, we identified a potential relationship between the wave phenomenon and CEF activation. Although Symbiodinium contains genes for both the PGR5/PGRL1 and NDH-2 pathways, further evidence was required to establish a definitive link between specific components of these pathways and wave induction. It is also possible that alternative electron transfer pathways, including the Mehler reaction, PTOX, and flavodiiron proteins, may affect the reduction of the plastoquinone (PQ) pool and the kinetics of fluorescence relaxation. It is noteworthy that the most pronounced wave activity was observed under microaerobic conditions in heat-treated cells, which suggests that CEF, which tends to accelerate in these environments, may contribute to the observed phenomenon. However, the rapid increase in fluorescence following the dip phase indicates reduction of the PQ pool via alternate electron transport pathway, as observed in *C. reinhardtii*, does not occur in the same manner in *Symbiodiniaceae*. The flash-induced fluorescence decay serves as a sensitive indicator of heat stress in *Symbiodiniaceae*, with the wave phenomenon reflecting both a reduction in partial PSII activity and a significant reduction in the PQ pool under microaerobic conditions. The distinctive

features of the wave's appearance and heat sensitivity across species indicate its potential as a marker for stress-related alterations in photosynthetic electron transport.

To gain further insight into the relationship between fluorescence relaxation components and the photosynthetic electron transport chain, we employed inhibitors of linear electron flow. The application of these inhibitors resulted in a notable disruption of the fluorescence wave phenomenon observed in *Symbiodiniaceae*. It became evident that functional linear electron transport from PSII to stromal electron carriers is a prerequisite for wave induction. For example, DCMU, which disrupts electron transfer from Q_A^- to Q_B, results in the disappearance of the rapid and intermediate phases of fluorescence, leaving only the slow phase associated with charge recombination. Similarly, DMBQ and MV, which interrupt electron flow from PSII to PSI and electron flow back from PSI to the PQ pool respectively, also affect the fluorescence dynamics, thereby underscoring the interconnected nature of various electron transport within these organisms. Inhibition of the donor side of photosystem II with hydroxylamine under microaerobic conditions did not result in the anticipated fluorescence wave phenomenon, indicating that a reduction in PSII activity alone is insufficient to induce this phenomenon in *Symbiodiniaceae*. This may be attributed to an insufficient supply of electrons to the PQ pool. Further investigation into the potential for enhancing the wave phenomenon through the inhibition of the CBB cycle using glycolaldehyde revealed that, while ambient conditions did not affect fluorescence relaxation, glycolaldehyde treatment under microaerobic conditions did induce wave-like activity, underscoring the significance of a reduced PQ pool. In conclusion, these findings indicate that the wave phenomenon is influenced by more than just the elimination of donor-side activity; it is also affected by the blockage of the CBB cycle, which correlates with CEF activation. Nevertheless, the complete manifestation of the wave phenomenon remains contingent upon a combination of acute heat stress and microaerobic conditions. In order to identify the specific components of CEF involved in wave induction, a series of tests were conducted using inhibitors targeting the antimycin-sensitive and the antimycin-insensitive (NDH-2) pathways. Despite the existence of both pathways in *Symbiodiniaceae* genome, antimycin A did not inhibit CEF or non-photochemical quenching. Although NDH-2 was demonstrated to facilitate electron transfer between NADPH and the PQ pool, its involvement alone was insufficient to fully account for the observed wave phenomenon. These findings of our present study indicate that different electron sources with varying kinetic characteristics perform discrete functions in wave formation, thereby emphasizing the regulatory role of NDH-2 in slower alternative electron transport processes. Further research is required to investigate the combined effects of both NDH-2 and the PGR5/PGRL1 systems under environmentally relevant stress scenarios.

In the broader context of coral health, the flash-induced chlorophyll fluorescence patterns provide insight into the complex interplay between environmental conditions and the relationships between corals and their symbiotic algae. In the context of low oxygen levels or anaerobic conditions, the flash-induced chlorophyll fluorescence displays a gradual, wave-like increase over a longer timescale, spanning 1 to 400 seconds. This phenomenon occurs at a more rapid rate in intact coral fragments in comparison to isolated Symbiodinium cells. The uniformity of this fluorescence behaviour across diverse coral species, including *Acropora* and *Seriatopora*, indicates that the

coral's internal structure facilitates electron transfer processes. The application of DCMU, an inhibitor, results in the cessation of the rapid and intermediate phases of fluorescence, with only the slow phase, associated with specific charge recombination in the photosynthetic system, remaining. In the absence of a flash, intact corals still exhibit a gradual increase in fluorescence over time. However, the introduction of a flash results in a distinct dip, followed by a rapid rise in fluorescence. This suggests that the symbiotic algae provide additional electrons to the coral system. This wave-like fluorescence pattern is dependent on a highly reduced PQ pool and is absent in *Symbiodinium* under aerobic conditions, which highlights the intricate metabolic processes occurring within intact corals. As oxygen levels decline, the fluorescence intensity increases, demonstrating how corals are able to adapt to fluctuating oxygen conditions through the utilization of sophisticated energy transfer processes. This adaptability indicates that minor stressors, such as reduced tidal currents, heat stress, reactive oxygen species (ROS) accumulation from photodamage, or increased respiration rates in the host, can have a significant impact on the symbionts, with a critical role of charge particles being transferred between the host and symbiont.

Finally, the utilization of potassium ferricyanide (FeCN) as an artificially introduced electron acceptor emphasized the crucial significance of extracellular electron transfer. Since FeCN is unable to penetrate cells, a reduction in fluorescence observed in both isolated *Symbiodinium* cells and intact coral fragments suggests that electrons are transported to the extracellular space. The disappearance of the fluorescence wave over a period of 1 to 400 seconds under anaerobic conditions with FeCN suggests that electron flow from the algae's photosynthetic chain is a crucial factor in this wave phenomenon. The addition of FeCN also led to a diminution in the fluorescence rise observed in intact coral samples, which may indicate a reduction in electron transfer from the algae to the coral host. Interestingly, while the middle fluorescence phase is eliminated in algae exposed to FeCN, it remains unaffected in intact corals, indicating a surplus of electron availability that can still reduce the PQ pool of the symbiont. It is noteworthy that intact corals exhibited a significantly higher reduction rate of FeCN in comparison to isolated *Symbiodinium* cells. This implies that the coral host may enhance electron transfer processes. The higher reduction rate could be attributed to contributions from the host or the unique microenvironment within coral tissues. Overall, these findings underscore the significance of extracellular electron transport in maintaining the vital symbiotic relationship between algae and coral hosts, particularly in adverse conditions.

10. A dolgozat összefoglalása

A flash-indukálta klorofill-fluoreszcencia lecsengés vizsgálata különösen fontos a korallokkal szimbiózisban élő páncélos ostoros algákon (Symbiodiniaceae), a korallzátonyokra jelentős veszélyt jelentő akut hőstresszre adott fiziológiai válaszok nyomonkövetése szempontjából. Ezek az algák létfontosságú szerepet játszanak a korallok fotoszintetikus produktivitásának biztosításában, ami elengedhetetlenné teszi elektrontranszport folyamatok tanulmányozását stressz-indukálta körülmények között. Vizsgálataink során a Symbiodiniaceae különböző törzseit akut hőstressznek vetettük alá, amely során az algák fluoreszcencia lecsengés profiljai jelentős változásokat mutattak. Különösen a középső lecsengési fázis növekedése volt megfigyelhető, ami a plasztokinon (PQ)-pool redukálódását jelzi. A PQ-pool legjelentősebb, reverzibilis redukálódását a 2465-ös törzsben figyeltük meg, összhangban korábbi tanulmányokkal, amelyek rávilágítanak a Symbiodiniaceae különböző fajainak hőérzékenységében megnyilvánuló változatosságára. A hőstresszre adott fajspecifikus válaszok kulcsfontosságúak a korallzátonyok ellenálló képességének megértésében és az éghajlatváltozással kapcsolatos hatékony természetvédelmi stratégiák kidolgozásában. A fluoreszcencia lecsengésben megfigyelhető hullámjelenségek hőmérsékletfüggésének vizsgálata betekintést nyújt a környezeti stresszorokra adott válaszreakciókba is. Eredményeink azt mutatták, hogy a hőmérséklet 40 °C-ra történő emelése további stresszhatások nélkül nem okozta a hullámjelenség megjelenését a különböző fajokban. A 2465-ös törzsben megfigyelhető volt azonban a fluoreszcencia jellegzetes csökkenése, majd növekedése, míg a többi vizsgált törzsben ez a jelenség nem volt kifejezett. Ez a mintázat azt jelzi, hogy a hullámjelenség előfeltétele a PQ-pool jelentős redukálódása, továbbá a PSII-aktivitás részleges elvesztése. A hőkezelt sejtekben a fluoreszcencia lecsengésében megfigyelt jelentős változások a megemelkedett hőmérséklet oxigénfejlesztő kapacitásra és a PSII aktivitásra gyakorolt káros hatásait jelzik. Elmondható tehát, hogy a hullámjelenség hőmérsékletfüggő viselkedésének megértése igen fontos a hőstresszel szembeni fajspecifikus tűrőképesség jellemzésében, ami kritikus fontosságú a korallzátony-ökoszisztémák ellenálló képessége szempontjából a gyorsan változó környezetben.

A flash-indukálta klorofill fluoreszcencia lecsengésében megfigyelt hullámszerű jelenség a ciklikus elektrontranszporttal (CEF) való lehetséges kapcsolata miatt kiemelt figyelmet érdemel számos fotoszintetikus szervezetben, beleértve a cianobaktériumokat és bizonyos mikroalgákat. A Symbiodiniaceae-n végzett megfigyeléseink során azonosítottuk a hullámjelenség és a CEF aktiválása közötti lehetséges kapcsolatot, a CEF-et előidéző körülmények között. Bár a *Symbiodinium* mind a PGR5/PGRL1, mind az NDH-2 útvonalak génjeit tartalmazza, további bizonyítékokra volt szükség ahhoz, hogy egyértelmű kapcsolatot állapítsunk meg ezen útvonalak specifikus komponensei és a hullámjelenség indukciója között. Lehetséges, hogy alternatív elektronátviteli útvonalak, beleértve a Mehler-reakciót, a PTOX-ot és a Flavodiiron fehérjéket, befolyásolhatják a PQ-pool redukcióját és a fluoreszcencia relaxáció kinetikáját. Fontos megjegyezni, hogy a hőkezelt sejtekben mikroaerob körülmények között volt a legkifejezettebb a hullámjelenség, ami arra utal, hogy a CEF, amely ilyen környezetben fokozott aktivitással működik, hozzájárulhat a megfigyelt jelenséghez. A fluoreszcencia gyors növekedése az átmeneti csökkenési fázist követően, amely megfigyelhető pl. a *C reinhardii* fajban, másként viselkedik a

Symbiodiniaceae fajokban, amely azt jelzi, hogy az elektronok ciklikus áramlása a PSI-ből a PQ-pool-ba más módon történik. A flash-indukált fluoreszcencia lecsengés a Symbiodiniaceae-ben a hőstressz érzékeny indikátoraként szolgál, a hullámjelenség a részleges PSII-aktivitás csökkenését és a PQ-pool jelentős redukálódását tükrözi mikroaerob körülmények között. A hullám megjelenésének és hőérzékenységének fajonként eltérő jellemzői arra utalnak, hogy a hullámjelenség a fotoszintetikus elektrontranszport stresszválaszokkal összefüggő változásainak markereként szolgálhat.

A fluoreszcencia relaxáció komponensei és a fotoszintetikus elektrontranszportlánc közötti kapcsolat további megértéséhez lineáris elektrontranszport gátlószereket alkalmaztuk. Ezen inhibitorok alkalmazása a Symbiodiniaceae-ben megfigyelt fluoreszcencia hullámjelenség jelentős megváltozását eredményezte. Megállapítottuk, hogy a hullámjelenség indukciójának előfeltétele a PSII-től a sztróma elektron-hordozók felé irányuló működőképes lineáris elektrontranszport. A DCMU, amely gátolja a Q_A-ból a Q_B-be történő elektronátvitelt, a fluoreszcencia lecsengés gyors és középső fázisának eltűnését eredményezi, és csak a töltésrekombinációhoz kapcsolódó lassú fázis figyelhető meg. Hasonlóképpen, a DMBQ és a MV, amelyek a PSII-ből a PSI-be történő elektronáramlást, illetve PSI-ből a PQ-poolba történő elektronáramlást gátolják, szintén befolyásolták a fluoreszcencia lecsengés dinamikáját, ezáltal megerősítettük a különböző elektrontranszport folyamatok összekapcsolódásának a fontosságát ezekben a fajokban. A PSII donor oldalának hidroxilaminnal történő gátlása mikroaerob körülmények között nem eredményezte fluoreszcencia hullámjelenség kialakulását, ami azt jelzi, hogy a PSII aktivitásának csökkenése önmagában nem elegendő a hullámjelenség kiváltásához a Symbiodiniaceae fajokban. Ez a PQ-poolba történő elégtelen elektronáramlásnak tudható be. A hullámjelenség a Calvin-Benson-ciklus glikolaldehiddel történő gátlásával történő vizsgálata kimutatta, hogy míg a glikolaldehid nem befolyásolta a fluoreszcencia lecsengést kontroll körülmények között, mikroaerob körülmények között végzett glikolaldehid-kezelés hullámszerű jelenséget váltott ki, ami kiemeli a redukált PQ-pool jelentőségét. Összefoglalva, ezek az eredmények azt mutatják, hogy a hullámjelenséget nem csak a donor oldali aktivitás csökkenése befolyásolja; a Calvin-Benson-ciklus gátlása is jelentős hatást gyakorol erre a folyamatra, ami összefügg a CEF aktiválásával. Mindazonáltal a hullámjelenség teljes mértékű megnyilvánulása az akut hőstressz és a mikroaerob körülmények kombinációjától függ. A CEF hullámindukcióban részt vevő specifikus komponenseinek azonosítása érdekében vizsgálatokat végeztünk az antimycinre érzékeny és az antimycinre nem érzékeny (NDH-2) útvonalakat célzó inhibitorokkal. Annak ellenére, hogy mindkét útvonal létezik a Symbiodiniaceae genomban, az antimycin A nem gátolta a CEF-et és a nem-fotokémiai kioltást. Bár ismert, hogy az NDH-2 elősegíti az elektrontranszfert a NADPH és a PQ-pool között, ennek aktivitása önmagában nem volt elegendő a megfigyelt hullámjelenség teljes megnyilvánulásához. Munkánk eredményei arra utalnak, hogy a különböző kinetikai jellemzőkkel rendelkező különféle elektronforrások egyedi funkciókat töltenek be a hullámjelenség előidézésében, ezzel kihangsúlyozva az NDH-2 szabályozó szerepét a lassabb alternatív elektrontranszport folyamatokban. További kutatásokra van szükség az NDH-2 és a PGR5/PGRL1 rendszerek együttes hatásainak vizsgálatához környezeti szempontból releváns stresszkörülmények között.

A korallok élettanának tágabb értelmezésében a flash-indukálta klorofill-fluoreszcencia lecsengés mintázatok betekintést nyújthatnak a korallok és a velük szimbiózisban élő algák összetett kölcsönhatásába. Csökkent oxigénszint vagy anaerob körülmények között a flash-indukálta klorofill-fluoreszcencia fokozatos, hullámszerű növekedést mutatott hosszabb időskálán, 1 és 400 másodperc között. Ez a jelenség gyorsabb ütemben jelentkezik az ép, teljes koralldarabokban, mint az izolált *Symbiodinium* sejtekben. A fluoreszcencia lecsengés viselkedésének egységessége a különböző korallfajok, köztük az *Acropora* és a *Seriatopora* esetében azt jelzi, hogy a korallok belső szerkezete elősegíti az elektronátviteli folyamatokat. A DCMU inhibitor alkalmazása a fluoreszcencia gyors és középső fázisának megszűnését eredményezi, és csak a lassú fázis marad meg, amely a fotoszintetikus rendszerekben a töltésrekombináció folyamataihoz kapcsolódik. Egész korallokban az aktinikus villanófény hiányában a fluoreszcencia fokozatosan növekszik az idő függvényében. Az aktinikus villanófény alkalmazása azonban egy jellegzetes fluoreszcencia csökkenést eredményez, amelyet a fluoreszcencia gyors emelkedése követ. Ez arra utal, hogy a szimbiózisban élő algák többlet elektronokat biztosítanak a korallok számára. Ez a hullámszerű fluoreszcencia-mintázat erősen függ a PQ-pool redukáltságától, és aerob körülmények között nem figyelhető meg *Symbiodinium* sejtekben, ami rávilágít az ép korallokban zajló bonyolult anyagcsere folyamatokra. Az oxigénszint csökkenésével a fluoreszcencia intenzitása növekszik, ami azt mutatja, hogy a korallok kifinomult energiaátviteli folyamatok felhasználásával képesek alkalmazkodni az ingadozó oldott oxigén tartalomhoz. Ez az alkalmazkodóképesség azt jelzi, hogy egyes stresszorok, mint például a csökkent árapály-áramlat, a hőstressz, a fotokémiai rendszerek károsodásával kapcsolatba hozható reaktív oxigénformák (ROS) felhalmozódása vagy a gazdaszervezet megnövekedett légzési rátája jelentős hatással lehet a szimbiontákra, és ebben döntő szerepet játszhatnak a gazdaszervezet és a szimbionta között átadott töltéssel rendelkező molekulák.

Végül a kálium-ferricianid (FeCN), mint mesterséges elektronakceptor alkalmazásával kimutattuk az extracelluláris elektronátvitel fontos szerepét korallokban. Mivel a FeCN nem képes behatolni a sejtekbe, az izolált *Symbiodinium* sejtekben és az ép koralldarabokban megfigyelt fluoreszcencia csökkenés arra utal, hogy az elektronok az extracelluláris térbe kerülnek. A fluoreszcencia hullám eltűnése az 1 és 400 másodperc közötti időintervallumban FeCN jelenlétében, anaerob körülmények között azt sugallja, hogy az alga fotoszintetikus rendszereiből származó elektronáramlás döntő tényező ebben a hullámjelenségben. A FeCN hozzáadása az ép korallmintákban megfigyelt fluoreszcencia-emelkedés eltűnéséhez is vezetett, ami az algából a korall gazdaszervezet felé történő elektronátvitel csökkenésére utalhat. Érdekes módon, míg a FeCN-dal kezelt algákban megszűnik a középső fluoreszcencia lecsengési fázis, addig az ép korallokban ez nem változik, ami arra utal, hogy az szimbiotikus algák PQ-pool-ját redukálni képes többlet elektronok állnak rendelkezésre. Figyelemre méltó, hogy az ép korallokban a FeCN-redukciós ráta jóval magasabb volt az izolált *Symbiodinium* sejtekhez képest. Ez arra utal, hogy a korall gazdaszervezet képes fokozni az elektronátviteli folyamatok hatékonyságát, ugyanis a magasabb redukciós ráta a gazdaszervezet vagy a korallszövetek egyedi mikrokörnyezetének hozzájárulásával magyarázható. Összességében ezek az eredmények kihangsúlyozzák az extracelluláris elektrontranszport jelentőségét az algák és a korallok közötti létfontosságú szimbiózis fenntartásában, különösen kedvezőtlen körülmények között.

Appendices

List of relevant publications (MTMT: 10075181)

- Mohammad Aslam, S., Vass, I., & Szabó, M. (2023). Characterization of the Flash-Induced Fluorescence Wave Phenomenon in the Coral Endosymbiont Algae, Symbiodiniaceae. International Journal of Molecular Sciences, 24(10), 8712. (Impact Factor: 4.9).

- Mohammad Aslam, S., Patil, P. P., Vass, I., & Szabó, M. (2022). Heat-induced photosynthetic responses of Symbiodiniaceae revealed by flash-induced fluorescence relaxation kinetics. Frontiers in Marine Science, 9, 932355. (Impact Factor 2.8).

- Patil, P. P., Mohammad Aslam, S., Vass, I., & Szabó, M. (2022). Characterization of the wave phenomenon of flash-induced chlorophyll fluorescence in Chlamydomonas reinhardtii. Photosynthesis Research, 152(2), 235-244. (Impact Factor 2.9).

Prize and Award

- **Straub Young Scientist Prize**, HUN-REN Biological Research Center, Szeged, Hungary. April 2024

- **Young Researcher Award**, 11th International Conference Photosynthesis and Hydrogen Energy Research for Sustainability, Turkey. July 2023

List of conferences attended

- Exploring the dynamic responses of Symbiodiniaceae to acute heat stress: insights into coral symbiosis and regulatory electron transport processes. Mohammad Aslam, Sabit; Vass, Imre; Szabó, Milán. European Coral Reef Symposium – ECRS 2024, Naples, Italy. (Oral-Presentation)

- Deciphering heat stress response in coral endosymbiont algae Symbiodiniaceae: unraveling photosynthetic electron transport dynamics and regulatory pathways. Mohammad Aslam Sabit, Milán Szabó and Imre Vass. FIANÖVELŐ 2024, Debrecen, Hungary. (Oral-Presentation)

- Differences in heat susceptibility and involvement of NDH-2 in alternative electron transport in coral endosymbiont algae, Symbiodiniaceae. Mohammad Aslam, Sabit; Szabó, Milán; Vass, Imre. 11th International Conference Photosynthesis and Hydrogen Energy Research for Sustainability-ICPRS 2023, Istanbul, Turkey. (Poster)

- Photosynthetic responses of the coral endosymbiont algae Symbiodiniaceae under acute heat stress, revealed by flash-induced fluorescence relaxation kinetics. Sabit Mohammad Aslam, Priyanka P Patil, Imre Vass, Milán Szabó. 18th International Congress on Photosynthesis Research – ICPR 2022, Aotearoa, New Zealand. (Virtual Poster)

- Heat induced responses in different clades of the coral endosymbiont algae, Symbiodiniaceae. Sabit Mohammad Aslam, Milán Szabó and Imre Vass. Straub Napok HUN-REN Biological Research Center, 2022, Szeged, Hungary. (Poster)

- The role and significance of cyclic electron transport in the coral endosymbiont algae, Symbiodiniaceae. Sabit Mohammad Aslam, Milán Szabó and Imre Vass. XIII. Hungarian Plant Biology Congress, HUN-REN Biological Research Center, (2021), Szeged, Hungary. (Poster)