Defining the bio-energetic limits of *Symbiodinium* sp's host-symbiont relationship under future climate scenarios

Verena Schrameyer

Dipl. Biol. University of Bremen

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student

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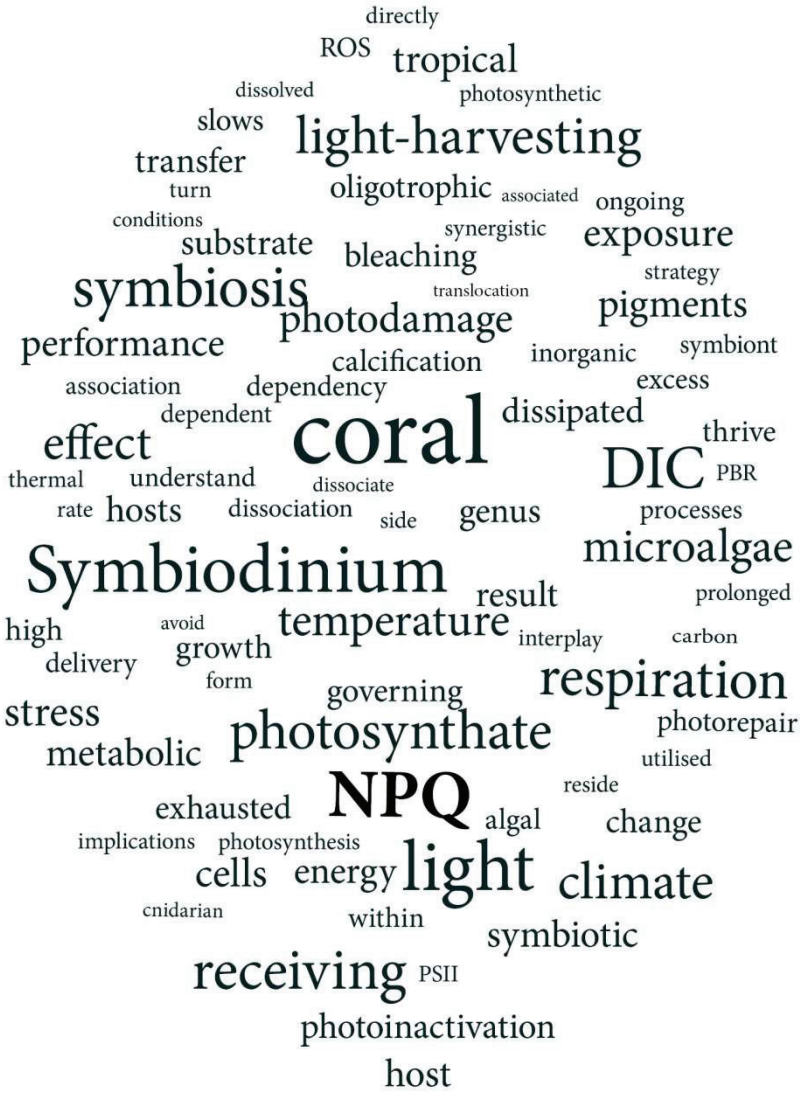
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Abbreviations

acpPC	Chl*a*/c_2 – Peridinin-binding protein complex
AEPS	Alternative electron pathways
ATP	Adenosin triphosphate
CCM	Carbon concentrating mechanism
CET	Cyclic electron transport
Chl	Chlorophyll
CO_2E_c	CO_2 light compensation point
DCMU	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
Dd	Diadinoxanthin
Dt	Diatoxanthin
DIC	Dissolved inorganic carbon
DO	Dissolved oxygen
E_c	Light compensation point
ENSO	El Niño-Southern Oscillation
ETR	Electron transport rate
FSW	Filtered seawater
GFP	Green fluorescent protein

GHG	Greenhouse Gas
GP	Gross photosynthesis
GPP	Gross primary productivity
HPLC	High performance liquid chromatography
IRGA	Infrared gas analyser
LET	Linear electron transport
MAA	Mycosporine-like amino acid
MAP	Mehler-Ascorbate-Peroxidase cycle
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NPQ	Non-photochemical-quenching
OEC	Oxygen evolving complex
PAM	Pulse- amplitude- modulate fluorometry
PBR	Photobioreactor
PCP	Peridinin-Chl*a*-binding protein complex
PPR	Photoprotective pigment ratio
PSP	Photosynthetic pigment pool
PQP	Plastoquinone pool
PSI	Photosystem I
PSII	Photosystem II

PSU	Photosynthetic unit
RC	Reaction center
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SOI	Southern Oscillation Index
XC	Xanthophyll cycling
XP	Xanthophyll pool

Abstract

Hard corals are a living association of a cnidarian and microalgae of the genus *Symbiodinium*. This symbiosis is critical for corals to survive in oligotrophic tropical waters. The algal symbionts reside within the host cells receiving photosynthetic substrate in form of dissolved inorganic carbon (DIC) and in turn transfer photosynthate to their coral host. The photosynthetic performance of the algal symbionts is directly dependent on the DIC substrate delivery, which in turn can have implications on the photosynthate translocation to their coral hosts.

Under thermal and or high light stress, the algal symbiont's photosynthetic substrate can become limited, so that the photosynthetic rate slows down and excess light energy, not utilised for photosynthesis, is dissipated to avoid photodamage. Upon prolonged exposure under these stress conditions, the symbiotic association can dissociate and result in coral bleaching. It was of interest to understand ongoing processes governing the dissociation of a coral symbiosis, focussing on cultured algal symbionts as well as when associated with a coral host.

The photosynthetic apparatus of *Symbiodinium* has different pathways for dissipating excess energy to alleviate the impact of high light stress. Here a novel non-photochemical quenching mechanism was described through the application of picosecond chlorophyll fluorescence measurements on *Symbiodinium* clade C cells suggesting a heterogeneously organized photosystem II (PSII) pool. A model was developed, revealing the re-organization of the alga's photosynthetic apparatus under normal and photoprotective modes, during thermal and high light stress. We propose a new "super-quenching" mechanism, triggered when quenching at the peripheral antennas is insufficient to protect PSII from photodamage. PSII

then transfers its excited state energy to PSI, transforming a non-spillover PSII pool into a spillover pool. The inherently higher stability of PSI and high quenching efficiency of P_{700}^{+} allows dumping excess energy to heat, and resulting in an almost complete cessation of photosynthetic electron transport. A similar breakdown of *Symbiodinium*'s photosynthesis could occur when living *in hospite* associated with corals and this could provide a trigger for coral bleaching.

Symbiodinium is equipped with light-harvesting and reaction centre components in the thylakoid membrane including a water-soluble peridinin-chlorophyll (chl) *a*-protein complex (PCP), and a membrane-bound chl *a*-chl c_2–peridinin- protein complex (acpPC), along with typical photosynthetic electron transport systems such as the PSII reaction centre and the chl *a*-P_{700} reaction centre complex of PSI. Recent findings suggest that structural changes to PSII associated light harvesting pigment-protein antenna complexes (LHC), membrane intrinsic acpPC and peripherally associated PCP, in *Symbiodinium* are a mean of photoprotection, in addition to xanthophyll cycling. How LHC movement and xanthophyll cycling possibly complement each other under thermal and high light conditions, corresponding to coral bleaching conditions (the expulsion of algal symbionts from the coral host) has been addressed in this thesis. Here it could be revealed that thermal stress is the main precursor for movement of light harvesting complexes in order to shunt excess energy away from PSII. The findings presented here demonstrate the substantial non-photochemical quenching capacity of cultured *Symbiodinium*.

Coral bleaching resilience has been found to be species-specific, with differential impairment of *Symbiodinium*'s photobiology. Whilst much is known about the importance of considering *Symbiodinium* clades and resulting differential photophysiological characteristics affecting the overall coral physiology, the influence of the host upon autotrophic/photophysiological performance of their symbionts is largely unstudied. With the application of an inhibitor

preventing the *de novo* synthesis of the PSII core protein D1, coral species were incubated in natural high light stress conditions for 4 days. Gross photoinhibitory conditions of *Symbiodinium* clade C1 were examined when harboured in two distinct coral host organisms. Algal symbionts harboured in *Pavona decussata*, a bleaching resilient coral species displayed lower photodamage, compared to algal symbionts harboured in *Pocillopora damicornis*, a bleaching sensitive coral species, which was found to exhibit different photoprotective strategies. Despite differences in photodamage and resulting photorepair requirements (re-synthesis of D1 and incorporation in the PSII to create a functional reaction centre), both species displayed constant maximum quantum yields throughout exposure to high light conditions. Results clearly suggest that the photophysiological viability of *Symbiodinium* can be influenced depending on the harbouring coral host species.

In order to understand intricate physiological processes occurring in a coral holobiont and to further assess interdependencies of a *Symbiodinium*-coral host symbiosis, a closed metabolic chamber system (photobioreactor; PBR) was developed for the simultaneous assessment of three key integrated parameters of aquatic oxygenic phototrophs; chlorophyll fluorometry, oxygen and dissolved inorganic carbon (DIC) exchange. The performance of the PBR was evaluated for *in hospite Symbiodinium* associated with the scleractinian coral *Pocillopora damicornis*. The 'two-phase' PBR utilised circulation of a gas-phase through a liquid-phase (seawater) along with continuous stirring to reach equilibrium and allowed for determination of CO_2 as an indirect measure of changes in DIC concentration within the liquid-phase. Simultaneous measures of photosynthetic efficiency (using pulse amplitude modulated fluorometry) and metabolic gas exchange (using state-of-the art dissolved oxygen sensor technology and an infra-red gas analyser for CO_2 detection) were performed. The developed instrumental setup can be used to examine any aquatic phototroph, where the preliminary results presented, show the great capacity of this application.

Further, the novel PBR and additional O_2 microsensors were used to examine the photophysiology and metabolic gas exchange of the symbiont subclade (C1) harboured in two morphologically different coral species; *Pocillopora damicornis* and *Pavona decussata*. Here light respiratory dynamics were described through the application of O_2 microsensors under photosynthesis – irradiance (P – E) curve measurements. Comparable light respiratory dynamics but differing gross primary production, as well as light utilisation were found between the two species examined. *P. decussata*, displayed a much lower CO_2 light compensation point at only half the photon flux density compared to *P. damicornis*, indicating differing DIC supply to its algal symbionts. It was therefore concluded that *P. damicornis* has a comparable respiratory activity per symbiont to *P. decussata*, but as *P. damicornis* harbours less than half the symbionts per unit area compared to *P. decussata*, the holobiont exhibits a higher CO_2 compensation point (CO_2E_c) irradiance. Dissipative energy pathways also differed during photosynthesis-irradiance (P – E) curve measurements, where *P. decussata* displayed an increase of non-light induced energy quenching (Y(NO)) and *P. damicornis* increased active energy quenching (Y(NPQ)). O_2 microsensor derived light respiration rates were demonstrated for the first time from P – E curve measurements for coral holobionts. This is a significant contribution to the field as respiration increased with irradiance ~ 20 times compared to steady-state dark respiration for both species. Light respiration rate results demonstrated here clearly highlight that enhanced post-illumination rates, which have commonly been used to infer about light respiratory activity, are not reflecting the much greater actual light respiratory activity.

In concert, this thesis has revealed that *Symbiodinium* is equipped with a broad capacity of non-photochemical quenching pathways, where the species-specific pairing of *Symbiodinium* and its coral host can mediate photoprotective capacity. The importance of sufficient DIC as

photosynthetic substrate available to the algal symbionts has been identified as a possible key role governing bleaching resilience in hard coral species.