The Photobiology of *Symbiodinium* spp.: Linking Physiological Diversity to the Implications of Stress and Resilience

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Mark E. Warner and David J. Suggett

Abstract

Over the past two decades, our knowledge of *Symbiodinium* genetic diversity and ecological distribution has grown at an incredible pace, while the physiological diversity and how it may be compared to the phylogenetic and evolutionary constraints of these dinoflagellates is still catching up. Our knowledge of the photobiology of *Symbiodinium* has been driven largely by the desire to explain cellular mechanisms of stress and reaction to global events driven by climate change. We tend to focus more on the former in this chapter since this is where the majority of work has been performed to date. However, it is imperative that we return to the first principles of phytoplankton physiological ecology to form a more complete understanding of the photobiology of these algae and the physiological constraints expected in an algal cell capable of living benthic, pelagic and symbiotic life styles. In order to understand not only the pathways of cellular dysfunction, but also resilience, we address specific patterns of light harvesting balance, reaction centre turnover, inorganic carbon acquisition and utilisation, and alternative electron transport across this genus. *Symbiodinium*-focused research, and physiology in particular, poses several challenges that demand a broad perspective gleaned from working with these algae in culture as well as *in hospite* in controlled 'model' and natural cnidarian hosts across many different environments. We outline several key processes related to photosynthesis as well as new directions integrating many of these processes that are required to more fully understand how environmental change shapes the role of photobiology in governing the ecological success of these important algae.

Keywords

Symbiodinium • Photosynthesis • Reaction centre • Light harvesting • Photoacclimation • Photoinactivation • Alternative electron flow

30.1 Introduction

The endosymbiotic dinoflagellates in the genus *Symbiodinium* are a cornerstone of the success of reef building corals and numerous other invertebrate taxa in the tropical marine realm. In particular, these symbioses have played a significant role in supporting the productivity, growth, reproduction and hence evolution of the scleractinian corals over millions of years (Stanley 2003). Over the past 25 years, numerous efforts describing the taxonomy of this genus, largely grounded in molecular phylogenetics, have culminated in a refined description of a highly divergent

M.E. Warner (✉)
School of Marine Science and Policy, University of Delaware,
700 Pilottown Rd., Lewes, DE, USA
e-mail: mwarner@udel.edu

D.J. Suggett
Functional Plant Biology & Climate Change Cluster (C3),
University of Technology Sydney,
PO Box 123 Broadway, Sydney, NSW, Australia
e-mail: David.Suggett@uts.edu.au

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group of microalgae. Phylogenies based on nuclear, chloroplast, and mitochondrial DNA sequences divide the genus into nine primary lineages or "clades," presently designated "A" through "I" and, depending on the resolution of a particular molecular marker used, each of these clades are broken down further into a number of sub-clades or other categories (Coffroth and Santos 2005).

As noted by LaJeunesse (2005) and Thornhill et al. (2014) given the adaptive radiation that has occurred within each clade, it is often difficult if not impossible to map important questions concerning the ecology and physiology of these algae to the "clade-level" taxonomy. For example, while patterns of thermal tolerance are apparent for the many algal samples studied to date in at least one major lineage, clade D, this appears to be the exception rather than the rule (see also Abrego et al. (2008) and LaJeunesse et al. (2014) for even exceptions for some D-*Symbiodinium*), and there is a high range of physiological plasticity to thermal perturbation among several of the algae within other clades (Robison and Warner 2006; Sampayo et al. 2008; Tchernov et al. 2004). Hence, further taxonomic distinctions are necessary before trends concerning the physiological ecology across these taxa may be fully realized. Indeed development of several molecular markers with faster rates of evolution (as compared to nuclear level markers such as the nr18s) provide greater intra-cladal resolution and better detail concerning the ecological distribution of *Symbiodinium*, their particular host associations across spatial and temporal scales, as well as physiological differences that can be related to their photobiology. Such distinctions have further ramifications for how *Symbiodinium* diversity is quantified, as grouping clusters of sequences into operational taxonomic units (OTUs) based on sequence similarity alone when the members of such groups may represent a mixture of intragenomic variants, true species or species clusters (Stat et al. 2013) may not result in accurate categorization of functional diversity (Baird et al. 2009). Further refinement of molecular markers, e.g. detecting allelic variation at microsatellite loci, or the incorporation of several independent markers with different levels of resolution, are now paving the way toward differentiating populations of closely related *Symbiodinium* species as well as clonal variation within a species (LaJeunesse et al. 2014; Pettay and LaJeunesse 2009; Baums et al. 2014; Finney et al. 2010). Hence, we are getting closer to identifying many different species of *Symbiodinium* and relevant strains and are thus closer than ever to being able to map physiological diversity to species diversity and population distributions. Such progress should open a broader understanding of realized niche space for particular symbionts across various host taxa and geographic ranges (LaJeunesse et al. 2010; Finney et al. 2010).

We are now on the cusp of discovery for determining much more detail in the taxonomy and specificity of these symbioses and how possible physiological functional diversity relates to such patterns. However, important questions to consider are what are the physiological constraints facing different *Symbiodinium*, and with regard to the physiology of these algae, is there a balance or trade-off between functional diversity and functional redundancy? Likewise, one must consider the context of the specificity of a particular symbiosis, as some host coral species are capable of harboring several different species of *Symbiodinium* at once, while other hosts appear to be relatively fixed with one particular dominant symbiont. Hence, there may be a range of variability in a particular functional trait for these algae, but the specificity of the symbiosis itself can impart a significant constraint such that a particular coral/symbiont combination may be 'locked' into a specific functional redundancy. For a photochemical example, several shallow water symbionts may be able to maximise light energy capture and light energy dissipation but some may have a minimal ability to adjust photosystem II reaction centre turnover (discussed in detail below), which could in turn play a significant role during periods of high water temperature stress.

Trait-based approaches, similar to those investigated in the community ecology of other phytoplankton (Litchman and Klausmeier 2008), are quite germane and applicable to broadening our understanding of *Symbiodinium* photobiology and physiology. The allure of determining functional traits is to form a better understanding of how they may contribute to algal (and symbiotic host) fitness across biological and environmental scales. Importantly, some traits may be driven by genes that have evolved independently across multiple groups, thereby allowing a further investigation of such traits alone with less focus on phylogeny (Barton et al. 2013). However, a key challenge with such an approach is teasing apart which traits define a particular symbiont niche and the associated hurdles with how to measure a particular trait. This last point is especially relevant to this system, as the majority of naturally occurring *Symbiodinium* are not easily cultured, nor should we expect all aspects of cellular physiology to be the same when comparing a symbiont *in* and *ex hospite* (Stochaj and Grossman 1997).

This chapter attempts to summarize some key aspects of the photobiology of the different algae within *Symbiodinium* in the context of specific cellular traits used in the balance of light harvesting and light utilization. Next, we focus on several elements of *Symbiodinium* photosynthesis that have served as primary focal points in examining the response to environmental perturbation, such as elevated water temperature related to coral bleaching, and carbon supply related to ocean acidification. Several functional traits are common across a number of classes of phytoplankton yet also display notable differences that can play a significant role in determining the ecological success and niche space for a particular alga. Similarly, symbiosis research is reliant on a

range of subjects from uni-algal cultures, to 'model' symbiotic cnidarians that are amenable to laboratory manipulations, to reef corals that harbor a dizzying array of morphological and additional layers of complexity. A primary point of our synthesis here is that using photobiological traits to begin to define functional diversity will enable movement away from the reliance of using *Symbiodinium* phylotype as a primary determinant of the response capability and/or pre-determinacy of ecological success.

30.2 Balancing Light Harvesting and Light Utilisation

Microalgal cells within both benthic and pelagic environments are continually subjected to highly variable light fields. Light availability is largely stochastic and thus cells have evolved a variety of mechanisms, which operate over a continuum of time scales from microseconds to hours (Falkowski and LaRoche 1991; MacIntyre et al. 2002) that ensure physiological and biochemical plasticity to continually optimise photosynthetic rates, i.e. photoacclimation. Upper and lower thresholds of photosynthetic optimization to changes in light availability, as either light intensity or spectral quality (Falkowski and LaRoche 1991; Hickman et al. 2009; Moore et al. 2006), are set according to genotypic constraints in cellular functioning, i.e. photoadapation; as such, photoacclimation potential can be highly variable between microalgal species (Kulk et al. 2011; Lavaud and Lepetit 2013; Six et al. 2008) and even between different genetic variants of the same species (Hennige et al. 2009; Suggett et al. 2007). Photoadaptation thus ensures dynamic changes to microalgal community and population structure over space and time according to the light niche to which species are best optimized. *Symbiodinium* as a genus indeed appears highly plastic in both photoacclimation and photoadaption potential (Frade et al. 2008; Hennige et al. 2009; Iglesias-Prieto and Trench 1994), which no doubt is a major contributor towards its broad ecological success of inhabiting a dynamic range of spatial and even temporal light environments, e.g. shallow reef flats to mesophotic reefs, but also of life history, i.e. free living benthic and pelagic as well as *in hospite* within invertebrate hosts.

Light-niche optimization imposed through photoacclimation is almost universally characterized through use of the so-called photosynthesis-light response curve (PE curve). The shape and magnitude of the PE-curve reflect the underlying cellular processes that interact to regulate photosynthesis as a function of light availability (MacIntyre et al. 2002); as such, both shape and magnitude are inherently dependent upon availability of environmental factors that regulate microalgal growth, in particular temperature and inorganic nutrient availability (including CO_2), as well as the "currency" used to describe photosynthesis (sensu Suggett et al. 2009), i.e. O_2 evolution, CO_2 uptake, or linear electron transport. Applying models describing the light-dependency of photosynthesis (Sakshaug et al. 1997; Silsbe and Kromkamp 2012) to PE curves thus provide an objective means to quantify how environmental history influences: (i) extent of light absorption under "sub-saturating" light intensities, and so the light-dependent photosynthesis rate (termed α); (ii) the rate of maximum photosynthetic turnover under "saturating" light intensities, and so the light-independent maximum photosynthesis rate (termed P^{max}); and (iii) extent of photoinhibition under "supersaturating" light intensities. These various parameters are not static but continually undergo "fine tuning", or dynamic balancing (Geider et al. 1998), based on the scales of operation of cellular processes relative to those of environmental variability. The saturation light intensity of photosynthesis (termed E_K) is particularly useful in understanding the mechanism behind dynamic balancing. In a classic experiment by Escoubas et al. (1995), inhibitors were used to mimic the process of changes in light availability as either $E > E_K$ (high light) or $E < E_K$ (low light) by either reducing or oxidising the plastoquinone (PQ) pool. As such, cells underwent photoacclimation via "signaling" according to the redox state of the PQ pool where $E \neq E_K$ (i.e. E/E_K was greater or less than a value of 1). Analysing PE curves where E is normalised to the corresponding value of E_K thus provides a powerful means to differentiate patterns of photoacclimation from adaptation (MacIntyre et al. 2002; Hennige et al. 2008, 2011; Suggett et al. 2012b), in particular where inter-comparing data from across different light fields using easily collected active-fluorescence based light curves (Hennige et al. 2008). In this context of the PE curve, the extent and timescale that E_K is effectively exceeded will ultimately govern the nature by which photoacclimation potential can maintain cellular fitness according to (i) relatively long term changes in light availability (e.g. hours to seasons) that predominantly influence the rate of macromolecular synthesis, notably of key proteins associated with light harvesting and CO_2 uptake; and (ii) relatively rapid light variability (e.g. seconds to hours) that predominantly influences physiological fine tuning to light utilization pathways and minimisation of photodamage.

Photosystem II (PSII) and photosystem I (PSI) harvest light in concert to provide the electrochemical energy required for linear electron transport, and so evolve O_2 and generate the energy (ATP) and reductant (NADPH) required for cellular growth and maintenance (Fig. 30.1). As such, the product of absorption by PSII and PSI determine the total light harvested for photosynthesis according to: (i) the extent of photon absorption by the type and arrangement of chromophoric pigment-protein complexes, (ii) the efficiency with which energy is funneled to the photosynthetic reaction centres, and the number of reaction centres present to then

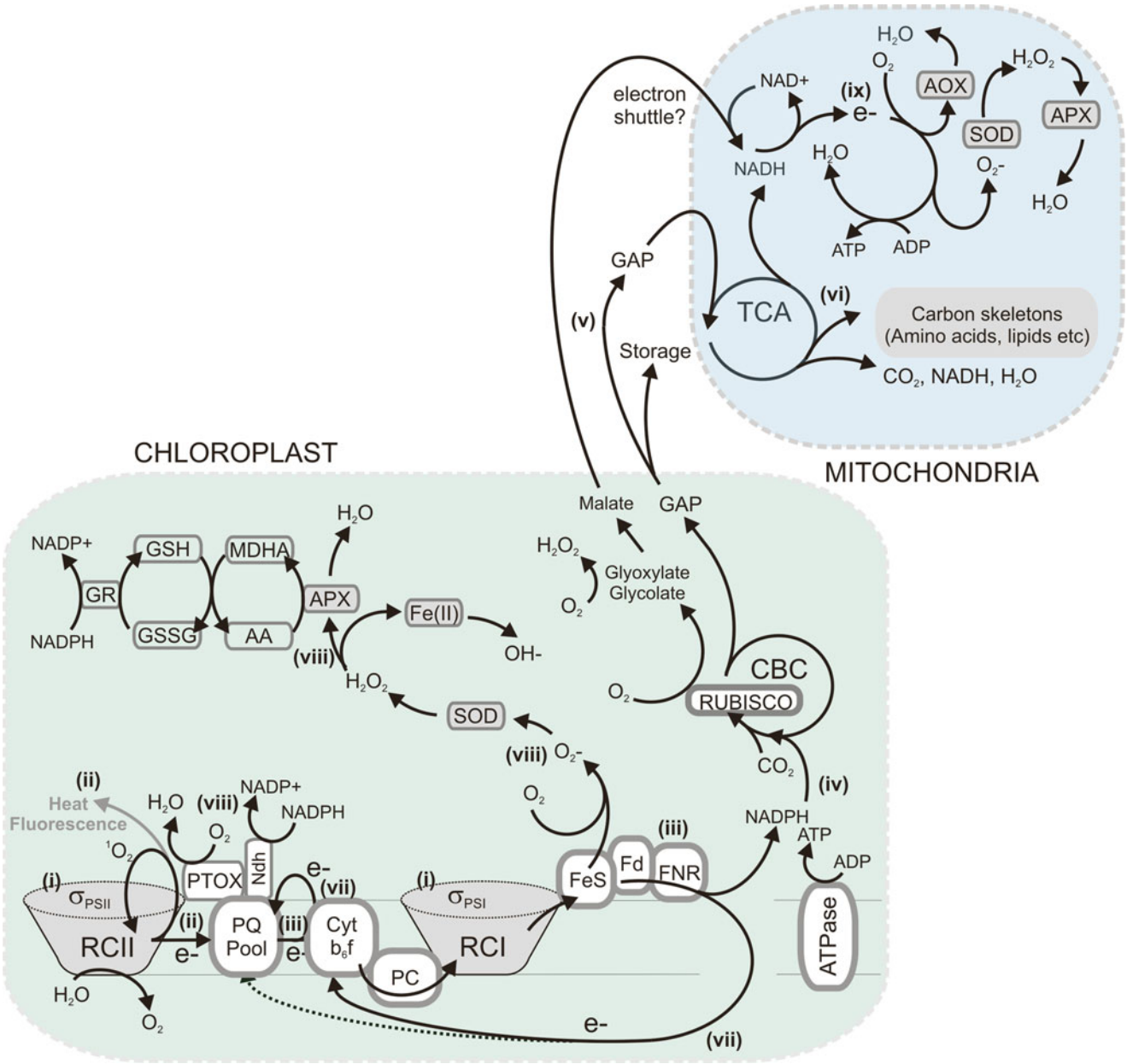

Fig. 30.1 Photochemistry and associated pathways within the chloroplast and mitochondria acting as intracellular electron sinks and/or sources of Reactive Oxygen Species (ROS) (Modified from Behrenfeld et al. (2008) and Suggett et al. (2011); see also main text): (i) light harvesting by the light harvesting complexes (the coupled antennae, σ_{PSII} and σ_{PSI}, and reaction centres, RCII and RCI); (ii) competing pathways for absorbed photons, photochemistry (splitting water to generate an electron) within, and fluorescence and heat emission from, the PSII complex; (iii) electron flow via the linear electron transport chain from RCII via the plastoquinone pool (PQ pool), Cytochrome b_6f (Cyt b_6f) and plastocyanin (PC), to generate the transthylakoid proton motive force required for ATP formation via ATPase. Also, linear electron flow from PSI from RCI via iron-sulphur complex (FeS), Ferrodoxin (Fd) and Ferrodoxin-NADP+ reductase (FNR) to generate reductant (NADPH); (iv) consumption of ATP and NADPH from competing pathways of RuBisCO within the Calvin-Benson-Cycle (CBC) of carboxylation (CO_2) through to glyceraldehyde 3-phosphate (GAP) – CO_2 assimilation – versus oxygenation through to malate – photorespiration; (v) substrate connectivity of the chloroplast to the mitochondria – GAP to fuel the tricarboxylic acid cycle (TCA) and export/storage to the cytosol, and/or possible energetic shuttling via malate- oxaloacetate inter-conversion; (vi) fates for an 'incomplete' TCA cycle: drawing off reductant (NADH) prior to mitochondrial electron transport or drawing off carbon skeletons (where the cycle is 'complete' ATP is generated by mitochondrial electron transport); (vii) cyclic electron flow around PSII (via Cyt b_6f to the PQ pool) and/or around PSI (via the PSI electron carriers back to either Cyt b_6f or the PQ pool); (viii) non-linear (alternative) electron flow to convert O_2 back to H_2O: plastoquinone terminal oxidase activity (PTOX) (as well as associated "chlororespiration" from a plastid NAD(P)H dehydrogenase (Ndh) complex via the PQ pool). and Mehler-Ascorbate-Peroxidase (MAP) activity. MAP involves a cascade of electron flow via superoxide dismutase (SOD) and ascorbate peroxidase (APX) that result in intermediate ROS superoxide (O_2^-) and hydrogen peroxide (H_2O_2), with possible catalysis of H_2O_2 to hydroxyl radicals (OH^-) by iron (Fenton's Reaction). Activity of APX is inherently coupled to glutathione cycling (Foyer-Halliwell-Asada cycle) via glutathione reductase (GR) and inter-conversion of glutathione disulphide (GSSG), reduced glutathione (GSH), monodehyroascorbate (MDHA) and ascorbate (AA). Other potential sources of ROS within the chloroplast are from inter-conversion of glycolate to glyoxylate (H_2O_2) via photorespiration and generation of singlet oxygen ($^1O_2$) from energy transfer within the antennae and reaction centers; and (ix) mitochondrial respiration via cytochrome-c or alternative oxidase (AOX) leading to non-linear electron flow to convert O_2 back to H_2O via SOD and APX. To date, how these various pathways and processes interact as a connected network remains unexplored for *Symbiodinium*

actively "trap" energy for photochemistry. A particularly useful parameter in describing this process is the "effective absorption cross section" (termed σ, see Mauzerall 1972), which parameterizes the effective antennae size (i.e. the target area) of the light harvested for photosynthesis, and in the case of PSII is easily measured using active chlorophyll *a* fluorometry (σ_{PSII}, Kolber et al. 1998; Gorbunov et al. 2001; Szabó et al. 2014). Absorption-specific to PSII (a_{PSII}) can then be calculated from the product of σ_{PSII} and the number of PSII reaction centers, [RCII] (Suggett et al. 2007, 2011). Whilst the same principle applies to PSI, i.e. $a_{PSI} = \sigma_{PSI} \cdot [RCI]$, measuring σ_{PSI} is extremely challenging (e.g. Ryan-Keogh et al. 2012). Even so, this biophysical-based consideration of light harvesting capacity has provided a primary framework for improving our understanding as to how microalgal cells (Strzepek and Harrison 2004; Six et al. 2008; Suggett et al. 2007), including some *Symbiodinium* genotypes (Suggett et al. 2015; Hennige et al. 2009; Iglesias-Prieto and Trench 1997; Falkowski and Dubinsky 1981), have adapted different strategies for allocating resources and light niche optimization.

Photoacclimation reflects a trade-off between modifying the pigment bed (σ), "photosynthetic unit size", versus reaction centre content (n), "photosynthetic unit number" (Falkowski and Dubinsky 1981; Falkowski and Owens 1980). Cellular resources required for production of reaction centers far outweigh those for pigment-protein antenna complexes; consequently, σ_{PSII} often appears to be larger, and preferentially modified over [RCII], in resource limited environments (Moore et al. 2006; Strzepek and Harrison 2004; Suggett et al. 2009). In contrast, dynamic environments where light is highly variable but nutrients in greater supply, appears to favour photoacclimation through modification of [RCII] over σ_{PSII} (Six et al. 2008). Interestingly, *Symbiodinium* predominantly appears to photoacclimate according to this "n-type" strategy more associated with dynamic environments (Hennige et al. 2009; Iglesias-Prieto and Trench 1994), including the highly variable light fields of many reefs (Roth 2014). Whilst such a strategy is potentially at odds with the very static environmental conditions with which *Symbiodinium* cultures have been used to derive these photoacclimation properties, this response is in fact not universal with some genotypes preferentially modifying σ_{PSII} (Falkowski and Dubinsky 1981; Hennige et al. 2009). As such, persistent life in laboratory cultures does appear to conserve adaptive diversity in photoacclimation strategy amongst isolates, although whether inherent strategy after years in culture actually reflects the strategy at the time originally isolated is untested. However, we now know that this variation in strategy amongst *Symbiodinium* genotypes is not consistent with phylogenetic variation but best explained by differences in cell size (Suggett et al. 2015). These authors demonstrated that changes of a_{PSII} amongst 18 genotypes (covering five clades) of *Symbiodinium* could be closely related to changes in cell size; this finding would appear highly consistent with the notion that *Symbiodinium* chloroplasts appear to be arranged as reticulated plastids encircling the cell, and thus light harvesting will be under selective pressure form cell size/volume (LaJeunesse et al. 2010). Interestingly their study also confirmed that co-variability of a_{PSII} with cell size appears largely (but not exclusively) under control from [RCII] (n-type) rather than from σ_{PSII} (σ-type) patterns. Such biophysical explorations of light harvesting have thus begun to establish a broad operational basis to explain adaptive differences in light niche partitioning ("ecotypes") observed for this alga (Falkowski and Dubinsky 1981; Frade et al. 2008; Iglesias-Prieto et al. 2004).

Understanding how absorbed light is subsequently utilised (dissipated) is especially important when considering photobiological responses to short term environmental variability. Cells must minimise transient excessive exposure to conditions that induce photoinhibition via "photoprotective pathways", and thus represent an important component of overall photoacclimation strategy and associated resource investment. The overall photosynthetic yield that is generated from the total quanta absorbed is referred to as the quantum yield (identified by the symbol ϕ); again, as with the PE curve, a quantum yield can therefore be generated for different photosynthetic currencies (O_2 evolved, CO_2 fixed or extent of photochemistry). In the case of PSII photochemistry, the quantum yield (termed ϕ_{PSII}) is easily measured using active chlorophyll *a* fluorometry and predominantly describes the balance of absorbed light that is dissipated as photochemistry versus heat, relative to the quantum yield of fluorescence itself. The balance of photochemical versus heat dissipation is therefore regulated by (i) longer-term acclimation processes that determine "σ-type" versus "n-type" strategies, and so govern the maximum achievable PSII quantum yield, and (ii) transient short term photoprotective process that occur under light exposure to increase heat dissipation (decrease photochemical dissipation), to decrease the maximum PSII quantum yield in the light. As such, the extent with which changes in the PSII quantum yield under transient light exposure reflect alterations to heat versus photochemical dissipation provides a secondary framework to evaluate photoacclimation strategies (Lavaud et al. 2007; Suggett et al. 2007). For example, diatom species evolved to more stable oceanic environments appear to have invested in cellular machinery that drives a lower heat-to-photochemistry ratio than for diatom species evolved to more variable coastal waters (Lavaud et al. 2007; Lavaud and Lepetit 2013). *Symbiodinium* genotypes appear to similarly express differences in their inherent heat-to-photochemistry ratio (Suggett et al. 2015). Whilst these differences appear to show some relationship with phylogenetic designation (Suggett et al. 2015), they also appear to relate to niche

separation in terms of shallow versus deeper corals (Iglesias-Prieto et al. 2004) as well as tolerance to transient heat stress (Hennige et al. 2011; Suggett et al. 2012b); however, such relationships are still largely unexplored. Similarly, how these differences in light utilization strategy may ultimately reflect a trade off against corresponding light absorption strategy is still unresolved.

Whilst the balance of heat versus photochemical dissipation of absorbed excitation energy provides a useful means to identify patterns of photoacclimation strategy, it is in reality difficult to interpret since cells can have a number of "safety valves" to maintain photochemical electron turnover that may or may not feedback to whether/how energy is transiently dissipated as heat, i.e. "non-photochemical quenching". Linear electron transport through the photosynthetic apparatus is required to generate a proton gradient across the thylakoid membrane, which is used to drive energy (ATP) formation but also acts as the trigger for heat dissipation. However, up-regulation of alternative (non-linear) electron flow (AEF) pathways can sustain linear electron flow and in turn maintain the accumulation of the proton gradient across the thylakoid membrane/extent of heat dissipation (Fig. 30.1). An important basis for these pathways is not only to photoprotect the electron transport chain against excessive excitation but also balance ATP and reductant (NADPH) supply needed for optimum cellular functioning (e.g. Cardol et al. 2011); we return to the importance of AEF later in this chapter. An additional shortcoming to RUBISCO is especially important in the context of AEF and photoprotection (see Sect. 30.3.4).

In spite of the numerous pathways that exist to "photoprotect", it is inevitable that many microalgae are still periodically subjected to light stress-induced photoinhibition given the dynamic nature of light availability. Energy transfer from the triplet state of excited chlorophyll molecules within both the antennae and reaction centers (Telfer 2014) results in formation of the Reactive Oxygen Species (ROS) singlet oxygen ($^1O_2$). Ordinarily, carotenoid molecules quench $^1O_2$; however, when this quenching capacity is exceeded the excess $^1O_2$ causes "acceptor side" inhibition by directly damaging the reaction centre complex integrity (Yadav and Pospíšil 2012), although evidence suggests that in some cases $^1O_2$ may actually be beneficial by modifying the redox state of the PQ-pool to signal down-regulation (Kruk and Szymańska 2012; Triantaphylidès and Havaux 2009). Such loss of reaction center integrity (notably the PSII reaction centre) has been frequently observed for *Symbiodinium* under stress (Warner et al. 1999, see Sect. 30.3.2) and appears linked with $^1O_2$ targeting of the light harvesting complexes (Takahashi et al. 2008). However, the very nature of photoprotection via AEF makes cells additionally vulnerable to exposure by other ROS. Both the chloroplast and mitochondria (Lesser 2006; Rhoads et al. 2006), employ AEF pathways that result in transient formation of superoxide (O_2^-) and/or hydrogen peroxide (H_2O_2) (see Fig. 30.1). Whilst the very nature of reduced quinone molecules within these organelles can act as antioxidants (Lutz et al. 2014), excessive ROS production will occur where the key quenching molecules such as superoxide dismutases and peroxidases cannot match O_2^- and H_2O_2 production rates (Das et al. 2015; Lesser 2006). At face value therefore, use of AEF pathways would seem a risky strategy to minimize photodamage since highly reactive O_2^- and/or H_2O_2 can attack numerous cellular components, including proteins, nucleic acids and lipid membranes (see Lesser 2006); for example the thylakoid membranes of *Symbiodinium* can become particularly destabilized during ROS-induced stress (Tchernov et al. 2004). However, these ROS are also increasingly recognized as important signaling molecules that not only play a role in maintaining optimum photosynthetic operation via redox homeostasis in other photosynthetic organisms (Das et al. 2015; Rhoads et al. 2006; Triantaphylidès and Havaux 2009), and thus are effectively part of the photoacclimation process, but also the wider cellular network for population control (autophagy) (Pérez-Pérez et al. 2012). Not only is this ROS component of photoacclimation and photoprotection still largely unexplored for most microalgae, but how it links to cellular processes via metabolites and signaling, i.e. the ROS production "network" that is well established in terrestrial plants, unknown. This latter point is particularly important given the increasingly recognized role of volatile biogenic gases, which are produced in abundance by many microalgae, but especially *Symbiodinium* (Exton et al. 2015), in the antioxidant network.

Given the dynamic and complex network of processes inherent to photoacclimation and adaptation, we still know surprisingly little as to how these processes operate and interact to determine the net measureable outcomes, e.g. photosynthesis- and quantum yield-light response curves. Instead most detailed studies to date have focused on specific pathways and processes that appear to govern photosynthetic optimisation and thus how they act as 'pinch points" under stressors and/or low resource availably. In our next section we consider these main areas of focus for *Symbiodinium* to date.

30.3 Specific Cases

30.3.1 The Role of the Light-Harvesting Complex in Photosynthetic Downregulation

As with other dinoflagellates, the light-harvesting complex (LHC) that is coupled to the photosynthetic reaction centres of *Symbiodinium* binds Chlorophyll*a* and Chlorophyll*c2*,

with peridinin as the primary "accessory" carotenoid (Venn et al. 2006; Boldt et al. 2012). Peridinin is associated with both water-soluble peridinin-Chl*a* protein complex (PCP) located on the lumen side of the thylakoid membranes as well as the integral Chl*a*-Chl*c2*-peridinin protein complex (acpCP) (Boldt et al. 2012; Kanazawa et al. 2014, and references therein). Together these harvest light across a relatively broad spectrum but with primary absorption across the blue green region of the visible spectrum (ca. 420–520 nm, Venn et al. 2006). An array of secondary carotenoids, e.g. dinoxanthin, diatoxanthin, diadinoxanthin, β-carotene and phaeophytin*a*, are also present to enhance absorption across the blue-green absorption band (see Venn et al. 2006). Importantly, these various pigments govern the transfer efficiency with which absorbed light is passed to photochemistry or re-emitted as heat; for example, the transfer efficiency for chlorophylls, peridinin and diadinoxanthin is higher than that for diatoxanthin and β-carotene, so these two pigment groups are often referred to as "photosynthetically active" and "photoprotective" respectively. However conformational changes in binding of the "photosynthetically active" pigments and protein complexes with one another can further influence their overall transfer efficiency (Kanazawa et al. 2014; Suggett et al. 2004).

The type of pigments present and how they are arranged within the thylakoids (packaged, binding state), together determine the effective antennae size of light harvesting for photochemistry (σ_{PSII}, Sect. 30.2). Growth under higher light intensities typically increases the ratio of photoprotective-to-photosynthetically active pigments (as well as reduced packaging) and thus reduced σ_{PSII} (Hennige et al. 2009; Suggett et al. 2004, 2007, 2009); however two key properties of *Symbiodinium* play fundamental roles in further modifying σ_{PSII} within the LHC:

Firstly, transient exposure to high light induces "xanthophyll cycling" (Brown et al. 1999; Ragni et al. 2010; Warner and Berry-Lowe 2006). This process up-regulates the proportion of absorbed light re-emitted as heat (non-photochemical quenching or NPQ) and is signaled by increases in transthylakoid pH (ΔpH). An enzymatic process removes epoxy groups from diadinoxanthin (DD) to produce diatoxanthin (DT) (Goss and Jakob 2010) and hence the extent of xanthophyll cycling is often referred to as the "de-epoxidation state" (DPS = DT/[DD+DT]); as such, the transfer efficiency of the LHC (and therefore σ_{PSII}) is lowered. Many studies have now established that xanthophyll cycling is an important means of photoprotection for *Symbiodinium*, whereby xanthophyll activity tracks natural light-dark cycles for cells both in culture (Sorek et al. 2013) and *in hospite* (Brown et al. 1999; Ferrier-Pagès et al. 2013; Ulstrup et al. 2008; Warner and Berry-Lowe 2006) and remains high where cells are exposed to continuous light (Sorek et al. 2013). The extent with which this activity operates in response to transient light availability is not the same for all *Symbiodinium* sp. *in hospite* (Warner and Berry-Lowe 2006; Ulstrup et al. 2008; Venn et al. 2008). Xanthophyll cycling capacity does appear to differ between *Symbiodinium* genotypes (Krämer et al. 2013), which may in part reflect differences in pigment pool sizes pre-transient exposure (e.g. Hennige et al. 2009; Ragni et al. 2010) and thus cell size (Suggett et al. 2009, 2015); however, the modification of the light field by host properties incident to *Symbiodinium* when *in hospite* clearly plays a major role as to why extent of xanthophyll activity cannot be easily reconciled with *Symbiodinium* genotype present (Brown et al. 1999; Dove et al. 2006; Venn et al. 2006; Warner et al. 2006; Ulstrup et al. 2008; Krämer et al. 2012) . Enhanced xanthophyll cycling is also observed when cells experience greater excitation pressure associated with transient heat stress (Dove et al. 2006; Venn et al. 2006; Roth et al. 2012; Krämer et al. 2013) as well as cold stress (Roth et al. 2012). Importantly, such enhancement is not observed where thermal stressors are applied relatively slowly/gradually (e.g. Brown et al. 2002; Middlebrook et al. 2010), presumably since cells invest in other downregulation pathways that operate across longer time scales.

Secondly, peridinin itself has been proposed as a "photoprotective" pigment under some circumstances. Not only can peridinin instantaneously quench Chl*a* triplet excitation, which otherwise forms $^1O_2$ (Niedzwiedzki et al. 2013), but also acts to dramatically lower the transfer efficiency during stress. Early evidence for the latter (McCabe Reynolds et al. 2008) resulted in a "detachment model" whereby the PCP complex was thought to dissociate or move between different membrane components. Such dissociation, however, is not analogous to the subsequent binding and redistribution of light toward the PSI reaction centre or state transition noted in terrestrial plants and until recently, chlorophytes (Ünlü et al. 2014; Lemeille and Rochaix 2010). This model was developed from observations of increased 77 K fluorescence emission attributable to PCP (672–675 nm) from both free living and *in hospite Symbiodinium* genotypes undergoing light (McCabe Reynolds et al. 2008) and heat (Hill et al. 2012) stress. However, the "detachment model" has recently been rejected in favour of a "quenching model" (Kanazawa et al. 2014), whereby the enhanced emission appears to in fact originate from strong non-photochemical quenching (NPQ) of longer wavelength band(s) (686 nm) by the membrane antenna complexes associated with acpPC and reaction centers. Just how acpCP operates in *Symbiodinium* as a strong quencher is still unknown but may relate to conformational changes associated with the inherent pigment protein complexes (e.g. Ünlü et al. 2014); indeed, acPCP sequences appear highly variable both within and between algal genotype (Boldt et al. 2012; Jiang et al. 2014). De novo synthesis of acpCP is impaired by heat stress in some *Symbiodinium*,

further highlighting the key role this complex plays in photoprotection (Takahashi et al. 2008).

30.3.2 The Role of Reaction Centre Stability and Turnover

The PSII reaction centre has been a primary focal point in previous and current investigations of environmental stress in reef corals and in the study of coral bleaching in particular. Bleaching, a common phenomena whereby corals lose a significant number of symbionts (and less frequently a loss in chlorophyll algal cell^{-1}) following periods of exposure to high water temperature, has long been linked to a loss in photosynthetic activity as measured by traditional oxygen exchange and PE curve analyses (Coles and Jokiel 1977) (described above). Subsequent work utilizing active chlorophyll *a* fluorescence noted a substantial loss in the maximal PSII fluorescence quantum yield (Φ_{PSII}^{max} or F_v/F_m) and established a link between the loss of symbionts and the decline in PSII photochemistry (Hill et al. 2004; Iglesias-Prieto et al. 1992; Jones et al. 2000; Warner et al. 1996). In many cases, PSII function declines prior to the significant loss of algal cells (Warner et al. 2002), however, such evidence is reliant on high temporal sampling, which is not always logistically possible. A common key feature of natural mass coral bleaching is that seawater heating is accompanied by extended periods of calm weather and hence greater light dose (Mumby et al. 2001). This factor leads to the notion that patterns of photodamage are similar or analogous to pathways of photoinhibition in terrestrial plants and other phytoplankton (Lesser and Farrell 2004). In particular, this process occurs when the rate of light absorption and photochemical reactions in the PSII reaction centre exceed the rate of energetic utilization for carbon fixation and/or rates of other sources of non-assimilatory electron flow; importantly, here the strict definition of photoinhibition is used to refer to reactions driven by high light alone and does not require damage to other components of photosynthesis (e.g. the Calvin Benson Cycle) or any manipulation of temperature per se (Adir et al. 2003). PSII damage and repair, whether related to high light alone or in combination with several stressors, involves a suite of possible triggers such as different reactive oxygen species as well as several modes of reaction centre degradation and assembly. For a more exhaustive review on the many facets of photoinhibition and the current debated topics therein, we direct the reader to other reviews (Roach and Krieger-Liszkay 2014; Nishiyama and Murata 2014). However, given the evidence for multiple stress pathways, and the dependence of heat and light in the context of coral bleaching, we use the broader term 'photoinactivation' to describe the general disruption to photosynthesis in *Symbiodinium*, while still acknowledging some clear analogs to the loss in PSII function by high light alone.

Earlier investigations with *Symbiodinium* noted a significant loss in a primary structural PSII reaction centre protein, known as D1 (also denoted as PsbA), during natural and experimental bleaching in thermally sensitive algae *in hospite* as well as in culture (Warner et al. 1999). By following the rate of photoinactivation at ambient and elevated temperature by chlorophyll *a* fluorescence and in the presence and absence of the chloroplast protein synthesis inhibitor lincomycin, Warner et al. (1999) noted a much higher rate of PSII turnover in a *Symbiodinium* isolate possessing a higher thermal tolerance, thus providing evidence that the reaction centre repair cycle was a key determinant for heat tolerance as well as a possible weak point for heat-sensitive algae. This hypothesis was further corroborated by studies with corals (Takahashi et al. 2004). For some *Symbiodinium*, there is an interesting link between their ability to withstand acute shifts to high light and their thermal tolerance. Comparing the rate of loss and recovery in PSII photochemistry following high light exposure in the absence and presence of lincomycin permits calculation of an index of net vs. gross photoinhibition (Ragni et al. 2010). Such comparisons have indeed shown how one cultured alga (*Symbiodinium microadriaticum*, ITS2-type A1) sustains a higher rate of PSII repair as compared to a closely related yet thermally sensitive alga *Symbiodinium necroappentens* (formerly ITS2-type A13; LaJeunesse et al. 2015; Ragni et al. 2010), and these differences match the previously determined respective thermal tolerances of these two isolates (Robison and Warner 2006). Subsequent studies using similar protocols with various corals also provide a close link between more rapid PSII repair rates and historical resistance to thermal bleaching (Hennige et al. 2011). In contrast to one study, where PSII repair declined in a cultured isolate held above 31 °C (Takahashi et al. 2009), other studies to date with *in hospite Symbiodinium* across a range of coral species have demonstrated substantial elevated PSII repair under elevated light and/or temperature (Hennige et al. 2011; Hill et al. 2011; Krämer et al. 2013). Thus, in several thermally sensitive *Symbiodinium* spp., rather than chloroplast protein repair pathways being directly damaged, the rate of PSII repair cannot keep pace with the simultaneous rate of photodamage during thermal stress. However, it should be stressed that it is not easy to compare results across many of the aforementioned studies, given the use of cultures vs. intact corals and different species of corals, as well as different heating and light treatments. Thus, there is a strong probability that some thermally sensitive algal species may suffer from direct damage to protein repair pathways, while other algal species are essentially losing a race to maintain adequate repair.

Further examination of the photochemical response by active chlorophyll *a* fluorometry and tracking core PSII reaction centre proteins has led to a suite of reactions across different corals and *Symbiodinium*, most likely reflecting the fact that a range of damage and repair processes are occurring and there is not a "one-size-fits-all" model of photodamage across this genus. For example, Jeans et al. (2014) noted a significant drop in PsbA and PsbD cell^{-1} (the D1 and D2 proteins of the PSII reaction centre respectively) in *Symbiodinium* remaining within a coral subjected to short-term heating, yet F_v/F_m remained unchanged. However, expelled algae over this time period displayed the opposite trend. By 14 h of heating, net F_v/F_m declined while PSII reaction centre protein content of the remaining *in hospite* symbionts increased, possibly representing a portion of inactive reaction centres. Some work has shown a close correlation between the rate of loss in photochemistry and D1 protein abundance in heated corals and algal cultures (Hill et al. 2011; Robison and Warner 2006). However, continued efforts to measure recovery in the presence/absence of PSII reaction centre repair following thermal and light stress have shown that well over half of the photochemical recovery was independent of de novo chloroplast protein repair in one temperature sensitive coral (*Pocillopora damicornis*), while protein repair was responsible for over 90% of PSII photochemical recovery in symbionts within another bleaching-sensitive species (*Acropora millepora*). Further, there was minimal increase in D1 protein content in either of these corals in the absence of protein synthesis inhibitors.

Interestingly, while the PSII core and its de novo assembly are incredibly conserved from cyanobacteria to terrestrial plants, there are marked differences in comparison to PSII repair pathways (Nickelsen and Rengstl 2013). For example, in plants several light harvesting and core PSII proteins, including D1 and D2, have a redox regulated phosphorylation cycle that is critical for proper migration of damaged PSII complexes from grana to stromal lamellae (Tikkanen et al. 2008), while such biochemical pathways and segregation of chloroplast thylakoids are not present in dinoflagellates. Further, in the chlorophyte, *Chlamydomonas reinhardtii*, repair-related synthesis and de novo assembly synthesis of D1 are two very different pathways that are spatially segregated, and de novo assembly is controlled at the level of translation initiation while synthesis for repair seems to be regulated at the peptide elongation step (Nickelsen and Rengstl 2013). Similarly, recent studies with cyanobacteria and plants have noted a myriad of specific proteases, including ATP-dependent FtsH metalloproteases and ATP-independent Deg endoproteases required for the proper degradation and clearance of D1 (Kato et al. 2012; Nixon et al. 2010). Recent efforts have noted an excellent correlation between the rate constant for removal of D1 and the content of an $FtsH_6$ protease in the diatom *Thalassiosira pseudonana* (Campbell et al. 2013). Furthermore, an offshore strain of *T. pseudonana*, which had evolved from a more stable light environment, displayed a lower FtsH content and slower rate constants for removal of D1 as compared to a coastal strain which had evolved in a much more variable light environment (Campbell et al. 2013). Given some similarity in thylakoid topology between diatoms and dinoflagellates, these results point to the possibility that perhaps similar protease systems could function in an analogous fashion in the PSII repair cycle in *Symbiodinium* spp.

Substantial gaps are still evident for our knowledge of how dinoflagellates maintain chloroplast protein assembly and repair and these are grounded in the current mystery surrounding the cellular and molecular level of their control. Like all peridinin-based dinoflagellates examined to date, *Symbiodinium* possess a chloroplast genome that is encoded on several small minicircles approximately 2–3 kbp in size, with typically one gene per minicircle (Mungpakdee et al. 2014; Howe et al. 2008). While the majority of the original chloroplast encoded genes have been transferred to the nucleus, a set of 14 genes remain in these minicircles within the *Symbiodinium minutum* chloroplast that encode core functional proteins involved in the photosynthetic electron transport (PET) chain (Mungpakdee et al. 2014). An enticing hypothesis for why these particular genes have been retained in the chloroplast while so many more reside in the nucleus is that they are needed for rapid regulation in response to environmental change that also affects the redox state of the PET chain (Allen 1995). This Co-location for Redox Regulation (CoRR) hypothesis would seem germane, especially in light of the rapid loss in electron transport and plastoquinone reduction noted in *Symbiodinium* undergoing thermal and light stress. However, attempts to test co-regulation of specific PET components by manipulating the redox poise of the plastoquinone pool in cultured *Symbiodinium*, either by light or with specific chemicals (sensu Pfannschmidt et al. 2009), has provided mixed results at best (McGinley et al. 2013; McGinley 2012). Despite evidence for thermal loss in *Symbiodinium* chloroplast encoded genes (e.g. *psbA* and *psaA*) (McGinley et al. 2012), and while posttranscriptional control of such genes likely plays a larger role, substantial differences in gene expression were recently noted across two different types of *Symbiodinium* (Barshis et al. 2014).

Single turnover chlorophyll *a* fluorescence techniques such as Fluorescence-Induction and Relaxation (FIRe) and Fast Repetition Rate fluorometry (FRRf) have provided yet more detail regarding the possible change in energy balance between light harvesting and photochemistry during thermal stress. In some cases the functional absorption cross section of PSII may not change while F_v/F_m is stable or declines under thermal treatment (Jeans et al. 2014; Lesser and Farrell 2004), thus light harvesting may remain balanced with PSII

photochemistry or a substantial rise in nonphotochemical quenching (NPQ) of chlorophyll *a* fluorescence may be the result of damaged or down-regulated PSII reaction centers acting as quenchers (Matsubara and Chow 2004). Likewise, there is evidence for substantial xanthophyll de-epoxidation even after prolonged dark acclimation and hence this may also contribute to long-lived nonphotochemical quenching and confound the interpretation of a decline in PSII photochemistry (Middlebrook et al. 2010). While no significant change in σ_{PSII} suggests minimal contribution from antennae-based down regulation (e.g. xanthophyll cycling; see Sect. 30.3.1) other studies have noted a significant rise in σ_{PSII} and a decline in photochemistry (F_v/F_m) within cultured and *in hospite Symbiodinium* spp. (Ragni et al. 2010; McGinley et al. 2012). This particular pattern of damage likely reflects a greater loss of core PSII reaction centre proteins while the antennae bed remains more intact, thereby enhancing the interactive effects of combined thermal and light stress, as light harvesting complexes continue to move excitation energy toward damaged reaction centers. Importantly, such differences in photochemical metrics may also be driven by the constraints inherent in a particular experimental design, such as the range and rate of heating, as well as the different response. How such trade-offs between LHC and reaction centre maintenance during thermal stress relate to the fundamental constraints of "σ-type" versus "n-type" strategies for photoacclimation (see Sect. 30.3.1) remain an open area ripe for new discoveries that could provide more detail in forecasting the resilience of a particular *Symbiodinium*/host combination.

30.3.3 Interactions with Nutrient Limitation

An emerging theme amongst studies examining *Symbiodinium* photochemistry in the context of a changing environment is possible interacting roles from different sources of limiting compounds, including inorganic carbon as well as organic and/or inorganic nitrogen and phosphorous. Despite earlier assertions (Burris et al. 1983), there is now considerable evidence for some form of carbon limitation in many organisms harboring *Symbiodinium*. For example, Goiran et al. (1996) noted that the half saturation constant (K_m) for HCO_3^- was considerably lower in *Symbiodinium* freshly isolated from a coral as compared to the alga following isolation and culture (71 vs 178 μM, respectively); however, it bears noting that it was not confirmed that the alga in culture was indeed representative of the dominant alga *in hospite*. Other work has identified a significant increase in gross photosynthesis (normalized to chlorophyll or algal cell number) when comparing *in hospite* vs. freshly isolated algae from a high light acclimated hydroid (Fitt and Cook 2001), and elevated photosynthesis in several coral species when HCO_3^- is supplied above typical levels of ambient seawater DIC (~2 mM) (Herfort et al. 2008). Whilst photosynthesis may be saturated above ambient DIC, greater sensitivity of *in hospite Symbiodinium* as compared to cultured algae is evident when DIC is experimentally limited, leading to a reduction in PSII electron flow and net oxygen production (Buxton et al. 2009).

Further evidence for the high carbon demands of these symbioses stems from the fact that the dissolved CO_2 necessary for carbon fixation in the Calvin-Benson cycle (CBC) is a small constituent in seawater and cannot meet the demands of photosynthesis by passive diffusion alone. This problem is solved, in part, from the enhanced transcriptional up-regulation, elevated activity and cellular localization of the host-derived carbon concentrating enzyme carbonic anhydrase (CA), an enzyme that catalyses the interconversion of HCO_3^- and CO_2 (Bertucci et al. 2013, 2011; Weis and Reynolds 1999; Weis 1993). Like many algae, *Symbiodinium* also minimises CO_2 limitation by investment in several possible carbon concentrating mechanisms (CCMs), including internal and external CAs, an H^+-ATPase pump, and Na^+/HCO_3^- transporters (Al-Moghrabi et al. 1996; Leggat et al. 1999; Yellowlees et al. 1993; Bertucci et al. 2010). Attempting to understand the possible role(s) of C-limitation is further confounded by variability or alternative preference in DIC utilised within the intact symbiosis as well as for algae in vitro and how such preferences affect particular CCMs. Strong preference for HCO_3^- as well as CO_2 has been exemplified in some *Symbiodinium* in corals (Goiran et al. 1996), while algae within the giant clam, *Tridacna gigas*, utilise CO_2, but then rapidly covert to a greater dependence for HCO_3^- shortly after going into culture (Leggat et al. 1999). Brading et al. (2013) further recently documented different preferences of carbon type amongst two *Symbiodinium* genotypes in culture.

The importance of CO_2 limitation as well as DIC utilization in *Symbiodinium* symbioses has been highlighted in two areas related to global climate change: thermal anomalies that result in coral bleaching and increased human production and dissolution of CO_2 resulting in ocean acidification. For coral bleaching, Jones and colleagues (1998) proposed that some unknown aspect of the CBC, possibly at the point of carboxylation, was the initial site of thermal perturbation in the algal chloroplast, which then leads to over-reduction of the PET chain and eventual damage to the PSII reaction centre as detailed above. Subsequent investigations have attempted to further validate this hypothesis by other measures such as by tracking Rubisco content and the photochemical response following chemical inhibition of the CBC as well as electron transport inhibitors. No studies to date have documented a significant loss in total RbcL content or Rubisco enzyme activity in *Symbiodinium* that corresponds to ecologically relevant temperatures for many reef locations

(inhibition of activity is typically >34 °C) (Hill et al. 2011; Leggat et al. 2004; Lilley et al. 2010). However, studies inhibiting carbon fixation by low levels of inhibitors (e.g. glycolaldehyde or potassium cyanide) or exogenous competitive electron acceptors (e.g. methyl viologen) have corroborated the original hypothesis of some limitation to the dark reactions leading to a loss of photochemistry and a bleaching response for the same coral species (*Stylophora pistillata*) and collection location investigated by Jones et al. (1998) (Bhagooli 2013); however, such a response has not been replicated for other coral-symbiont associations (Hill et al. 2014). As such, the role of the CBC in regulating photobiological stressors during heat stress is still largely unresolved, but may be important for a small number of host-*Symbiodinium* combinations. Additional hypotheses concerning C-limitation and bleaching are that either the symbiont and/or host CCM pathways are damaged by excess temperature or the loss of photosynthate itself would limit the host energetic demands to maintain high CCM activity (Wooldridge 2009). All of these scenarios could lead to a similar sink limitation resulting in eventual PSII photoinactivation as previously outlined in Jones et al. (1998), nevertheless, direct evidence for thermal inhibition of host CCM mechanisms is currently lacking, and Oakley et al. (2014) recently demonstrated a decline in the half-saturation constant of photosynthesis vs. DIC concentration (a good predictor of CCM function) with elevated temperature among several cultured *Symbiodinium*; thus algal CCM components do not appear to be likely candidates for the point of initial thermal liability. While determining the ultimate vs. proximal cues of photostress in the context of bleaching remains a formidable challenge, the exacerbation of temperature with high light and common links to ROS formation (see Sect. 30.3.4 below) suggests several shared physical and chemical pathways across many different symbioses.

Ocean acidification has driven renewed recent interest in carbon assimilation processes in *Symbiodinium* and how they are potentially connected to photochemical performance. Laboratory culture studies have demonstrated that genotypes within clade A exhibit improved photosynthesis or growth rates when grown under "future" CO_2 scenarios (Brading et al. 2011), which appears to reflect differences in inorganic carbon acquisition and assimilation pathways (Brading et al. 2013, above); specifically under elevated pCO_2, some isolates within clade A undergo enhanced growth, interpreted as a relief of the energetic costs imposed through use of a CCM, whilst others undergo enhanced rates of productivity, which is interpreted as relief of carbon limitation within the CBC (see Brading et al. 2011). Similarly, ITS2-type A19 shows enhanced electron transfer and CO_2-uptake rates when *in hospite* in the anemone *Anemonia viridis* under naturally elevated CO_2 conditions, leading to enhanced ecological success of the host anemone (Suggett et al. 2012a). Not surprisingly, such a response is not solely restricted to algae within a particular clade, with enhanced symbiont productivity and host growth recently observed for an ITS2-type B1 (most likely *Symbiodinium minutum*) within the sea anemone *Aiptasia* sp. (Gibbin and Davy 2014).

An important element of these OA-induced responses appears to be the interactive role of light availability (Suggett et al. 2013). Whilst several recent studies from free-living phytoplankton indicate an inhibitory role of OA on photosynthesis (Gao et al. 2012) or, in some cases, enhanced photosynthesis (Trimborn et al. 2014; Hutchins et al. 2007; Fu et al. 2007), Suggett et al. (2013) demonstrated that higher light availability increases the symbionts' demand for CO_2 when *in hospite* in common Indo-Pacific coral species, which is met through the supply of elevated CO_2. In turn, higher light promotes host calcification and dampens the effect of OA on coral growth. O_2 competes with CO_2 at the active site of RUBISCO such that cells undergo "photorespiration" where O_2 availability is high (CO_2 low). Not only does this process lower the quantum yield of carbon fixation but also is energetically expensive (five ATP and three NADPH per oxygenation event) (Badger et al. 2000), exceeding carboxylation events by two ATP and one NADPH. Thus, photorespiration also provides a means to consume excessive energy produced under high light (Silva et al. 2015) and hence can act as another source of alternative electron flow (detailed further in the next section below). Although *Symbiodinium* minimize CO_2 limitation at the site of RUBISCO through the use of CCM's as discussed above, several studies have documented biochemical and molecular evidence for a photorespiratory pathway (e.g. demonstration of phosphoglycolate phosphatase, as well as glycolate production and release) (Crawley et al. 2010; Trench 1993). In the context of OA, Anthony et al. (2008) and Crawley et al. (2010) have hypothesized declined photorespiration whilst under high light and excess CO_2 leads to a loss in the pathway's AEF-associated photoprotective role. Together these various lines of evidence to date would suggest that OA potentially relieves carbon limitation of steady state growth but at a cost how well *Symbiodinium* can respond to transient light stress; however, considerably more evidence is needed in order to confirm the general importance of photorespiration in *Symbiodinium*.

Overall, there appears to be a range in the photosynthetic response of *Symbiodinium* to OA, but in several cases, there is minimal change to PSII photochemistry (Wall et al. 2014), while elevated photosynthesis leads to enhanced carbon translocation to the host (Tremblay et al. 2013). However, in the few studies that have tested the combined affects of elevated temperature and OA with regard to photochemical response, excess CO_2 rather than alleviating or enhancing the negative affect of high temperature to *Symbiodinium* photosynthesis, largely has a minimal affect (Wall et al. 2014;

Sinutok et al. 2014; Hoadley et al. submitted, but see also Anthony et al. 2008).

Nutrient limitation, often in the context of nitrogen, has been inferred in many studies with animals hosting *Symbiodinium* (Dubinsky and Jokiel 1994; Muller Parker and D'Elia 1997). Historically, considerable focus has revolved around the physiological response of reef corals to large changes in inorganic nitrogen and phosphorus availability, and questions of nutrient demand between the host and the alga in the context of algal population control. In many cases, when a coral is exposed to excess nitrogen, chlorophyll *a* cell^{-1} as well as algal cell number increase significantly, providing clear evidence for nutrient limitation. For a typical healthy coral, the high density of *Symbiodinium*, low specific algal growth rate (doubling times ~60–100 days), yet sustained productivity means that algal cell growth is uncoupled from photosynthesis and *Symbiodinium* are essentially living in a state of extreme unbalanced growth (Dubinsky and Berman-Frank 2001). However, it is important to recognize two important points when considering nutrients such as nitrogen or phosphorus in the context of these symbioses: (i) nutrient limitation is not equivalent to nutrient starvation (see MacIntyre and Cullen (2005) and Moore et al. (2013) and references therein for a more thorough summary on this point) and (ii) *Symbiodinium* appear to be fully acclimatised to living in a unique state of unbalanced growth. While *Symbiodinium* release a significant portion of their photosynthetically fixed carbon to the host, they are still able to utilise the organic nutrients released by the host as well as some photosynthetically fixed carbon in order to carry out energetically expensive pathways of photoacclimation, protein repair and typical daily homeostatic functions. Photochemistry often declines in phytoplankton maintained in batch growth, as cells approach stationary phase and transition from N-limited to an N-starved state (Parkhill et al. 2001; Suggett et al. 2009). In contrast to previous assertions that N-limitation may decrease F_v/F_m in *Symbiodinium* (Falkowski et al. 1993), other work has shown that F_v/F_m is independent of external nitrogen concentration and influenced to a larger extent by light acclimation (Rodriguez-Roman and Iglesias-Prieto 2005). Thus, from a photobiological perspective, *Symbiodinium in hospite* appear more like a nutrient-limited alga in steady-state growth (Parkhill et al. 2001) despite the unbalanced growth, which they are clearly under while within the host. However, surprisingly few studies have yet examined *Symbiodinium* physiological adjustments and how they potentially feedback to photochemical operation, when under nutrient limitation in *ex-hospite* culture experiments.

While *Symbiodinium* appear to be well-acclimatised to living under nutrient limitation, recent evidence has shown that elevated inorganic nitrogen but low phosphate, i.e. imbalances in the N:P (see Moore et al. 2009) increase the susceptibility to light and thermal stress, and in turn coral bleaching (Wiedenmann et al. 2012). Specifically, as the N:P ratio falls out of balance under excess N supply, *Symbiodinium* numbers increase but the algae transition to a P-starved state as evidenced by elevated levels of alkaline phosphatase as well as the lipid sulphoquinovosyldiacylglycerol (SQDG) (Wiedenmann et al. 2012). Wiedenmann et al. (2012) hypothesised that this shift in structural lipids may create an ion imbalance in the thylakoid membrane thereby leading to a loss in PSII activity under high light or temperature. Intriguingly, trace metal limitation, such as by iron, also appears to increase photoinactivation in *Symbiodinium* in heated corals, possibly by destabilising pathways of ROS detoxification (Shick et al. 2011). Conversely, there is also an important link between nutrient availability and photoinactivation and coral bleaching, as many studies have documented higher photoprotection in fed vs. starved corals (Borell and Bischof 2008; Borell et al. 2008; Ferrier-Pagès et al. 2010; Tolosa et al. 2011). However, as Fabricius et al. (2013) note, the alleviation of thermal stress by nutrients is determined in part by the quality of the nutrient source, whereby high-quality food may provide an excellent source, while organically enriched water may also include a number of possible confounding components (e.g. sediments, detritus, microbial flocs or excess DOC) that are detrimental to the host animal. Clearly, the interplay of C, N, and P quotas and how they affect the photobiology of different *Symbiodinium* is yet another important avenue requiring more detailed research.

30.3.4 The Growing Importance of Alternative Electron Flow

The capacity to process absorbed excitation energy through the photosynthetic electron transport chain is a major determinant of how well microalgae can tolerate rapid shifts in light availability. In the marine environment, this paradigm appears to be particularly the case where nutrient limitation places high excitation pressure in PSII and thus large selective pressure for alternative electron flow (AEF) pathways in microalgae (Kana 1993; Behrenfeld et al. 2008; Mackey et al. 2008; Grossman et al. 2010). AEF decouples light harvesting from carbon fixation and so comes with a cost to the growth efficiency of cells (Wagner et al. 2006; Suggett et al. 2009; Brading et al. 2013) but provides the benefit of enhanced buffering against "light stress". AEF can be defined according to a number of central processes (Cardol et al. 2011; Fig. 30.1): (i) Regulation of electron flow at the PSI acceptor side by Mehler Ascorbate Peroxdase (MAP) activity, photorespiration (oxygenase activity of RUBISCO) and cyclic electron transport from PSI back to PSI donor side molecules (CET-PSI); (ii) Alternative electron flow processes around the PSII donor side by plastoquinone terminal

oxidase and cyclic flow amongst the PSII electron carriers (CET-PSII); and (iii) metabolic interactions between organelles, notably the chloroplast and mitochondria whereby photosynthetically generated reducing equivalents are consumed by the mitochondria (mediated by "shuttles") and can induce the operation of mitochondrial alternative oxidase (AOX). Whilst these various processes act to balance the availability of energy and reductant available for nutrient assimilation and reduction they can act as a direct sink for electrons. For MAP, PTOX and AOX the electrons convert photosynthetically generated O_2 back to water (and for photorespiration the O_2 is cycled back to CO_2 and NH_3). In contrast, CET-PSII reduces PSII charge capacity without loss of O_2 evolution capacity whereas CET-PSI maintains both O_2 evolution and PSII charge separation capacity.

Symbiodinium typically thrives in relatively nutrient limited environments and so it is perhaps unsurprising that considerable AEF is often observed for *Symbiodinium*; up to 50 % of gross O_2 produced by PSII is consumed (respired) in the light by *Symbiodinium* (Leggat et al. 1999; Badger et al. 2000), although this rate can be highly variable between types (Suggett et al. 2008; Brading et al. 2011; Roberty et al. 2014). However, perhaps most importantly, light-dependent O_2 consumption via AEF activity appears further up-regulated under high light conditions (Brading et al. 2013; Roberty et al. 2014) and under heat stress (Suggett et al. 2008; Oakley et al. 2014), strongly indicating an important role for AEF in regulating how *Symbiodinium* responds to stressors that are known to induce oxidative cascades and autophagy (Lesser and Farrell 2004, Sect. 30.3.2).

Recent studies have attempted to dissect the specific nature of the substantial light-dependent O_2 consumption inherent to *Symbiodinium* via pathway-specific inhibitors. Roberty et al. (2014) provided the first compelling evidence from several cultured *Symbiodinium* genotypes that maintenance of high PSII flow under high light conditions was sustained by high PSI activity and hence that the light-dependent O_2 consumption was largely from Mehler Ascorbate Peroxidase (MAP) activity. Such an outcome seems entirely logical since *Symbiodinium* is a high emitter of H_2O_2 in the light, which would be consistent with operation of MAP (e.g. Tchernov et al. 2004; Suggett et al. 2008) and how it interacts with the peroxidase and glutathione system activity via the Foyer-Halliwell-Asada cycle (see Krueger et al. 2014). However, it is somewhat contrary to additional evidence for significant operation of mitochondrial alternative oxidase (AOX) activity (Oakley et al. 2014) and chlororespiration in *Symbiodinium* (Badger et al. 2000; Hill and Ralph 2008; McCabe-Reynolds et al. 2008). In the latter case, chlororespiration acts to oxidise NADPH and catalyse the reduction of O_2 to water via reduction of the PQ pool and subsequent use of a terminal oxidase, and thus is analogous to plastoquinone terminal oxidase that operates in the light for some microalgae when PSII becomes over-reduced (PTOX, see Mackey et al. 2008; Cardol et al. 2011). Activity of AOX and PTOX would also be consistent with identification of domains for both pathways in ESTs of *Symbiodinium* (Oakley et al. 2014; Roberty et al. 2014). Similarly, PSI-CET has been observed for *Symbiodinium* via post-illumination PQ pool reduction (McCabe Reynolds et al. 2008) thereby suggesting not all PSI non-linear flow is directed to MAP.

Activity of AOX, CET and chlororespiration (PTOX) have in fact only been evidenced to date in the dark for *Symbiodinium* (Hill and Ralph 2008; McCabe-Reynolds et al. 2008; Oakley et al. 2014) and thus raises the question of why these pathways do not appear to significantly occur in the light. The answer may simply reflect AEF to MAP far outweighs that to other electron and/or O_2 consuming pathways (Roberty et al. 2014). That said, the few studies to date that have examined specific AEF pathways have largely utilized very different symbiont genotypes and growth conditions (including media of different nutritional status) making reconciliation virtually impossible. Potential differences in the nutrient status of cells across studies as a source of variability in AEF observed is particularly intriguing: Lewitus and Kana (1995) demonstrated using mixotrophic microalgae that the extent of reliance on chloroplast versus mitochondrial activity to meet cellular energy demands governed the light-dependent O_2 consuming pathway at play. Recent evidence suggests that some *Symbiodinium* can feed heterotrophically on bacteria (Jeong et al. 2012), which in turn would influence the metabolic balance of the cells. Similarly, nitrogen and sulphur assimilation pathways clearly represent important energy/reductant sinks that can require up regulation of light dependent O_2 consumption (e.g. Weger and Turpin 1989) and in turn potentially influence production of additional ROS such as nitric oxide (Bouchard and Yamasaki 2008); therefore, the nutrient status ("quality") of the culture media used could further influence the nature and extent of AEF pathway expression. Such points certainly resonate when considering an additional role of photorespiration in AEF and light-dependent O_2 consumption. In culture, *Symbiodinium* does not appear to show any evidence of reduced light-dependent O_2 consumption in response to elevated CO_2 suggesting photorespiration is not significant (Leggat et al. 1999; Brading et al. 2011), an outcome that is perhaps not surprising given that *Symbiodinium* employs carbon concentrating mechanisms (CCMs) (Leggat et al. 1999; Brading et al. 2013). However, regulation of photorespiration is considered important in the *Symbiodinium*-cnidarian symbiosis (Yellowlees et al. 2008; see also Crawley et al. 2010; Mayfield et al. 2012), and appears to account for substantial light-dependent O_2 consumption when measured on intact corals (Schrameyer et al. 2014). As such, the predominant role of MAP observed from cultures may not accurately reflect how AEF ultimately

operates *in hospite*, i.e. where light and nutrient availability is very different compared to free living cultures. Both photorespiration (Fahnenstich et al. 2008) and AOX (Rhoads et al. 2006) can be significant producers of ROS, such as H_2O_2, suggesting that AEFs other than MAP could still potentially contribute to the central role of ROS production in governing the stress susceptibility of *Symbiodinium*.

30.4 Conclusions

Technical and conceptual advances that have predominantly stemmed from microalgal and terrestrial plant research fields have undoubtedly advanced our understanding of photobiological diversity and how this confers niche specialization in *Symbiodinium*. Even so, major uncertainty remains as to how adaptive variability in many photobiological traits as well as phenotypic plasticity can be reconciled (and how this variance potentially confer resilience to environment stressors), which in part is likely a consequence of the predominantly reductionist nature of past studies. Whilst experiments focusing on specific cellular components or pathways have been, and continue to be, at the forefront of advancing our state of knowledge, they inevitably can only capture the net outcome of the wider "operational network" at play (Fig. 30.2). Importantly, an array of signals, interactions and feedbacks govern the measurable outcome of any one component. At the cellular scale this operational network represents a con-

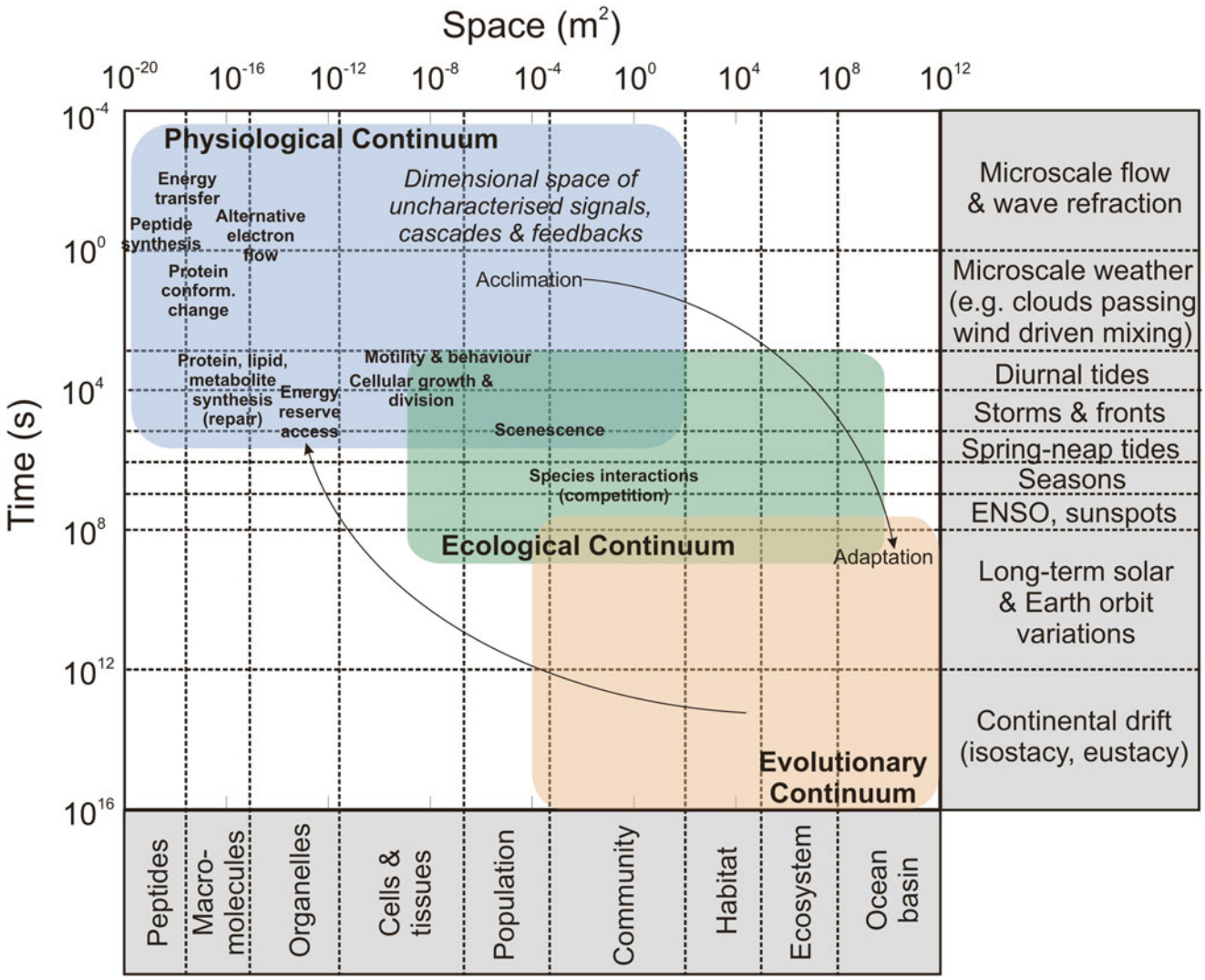

Fig. 30.2 Representation of the time- and space-scales that govern processes driving *Symbiodinium* physiological acclimation and genetic adaptation: Acclimation and adaptation processes occur across physiological, ecological and evolutionary continua within the space-time domain. Whilst scales of operation are shown for the predominant physiological and ecological components, each continuum box portrays the inherent network of (as yet largely uncharacterized signals, cascades and feedback) processes within and between each component. The time-space scale by which these various components are examined will therefore inevitably determine the net response via the extent to which other positive (synergy, cumulative) and/or negative (antagonist) interactions operate

tinuum of physiological processes (the "physiological continuum"), sensu Yellowlees and Warner (2003) that operate across various time-space scales, but ultimately couple across broader scales by ecological and evolutionary processes (Fig. 30.2); mutation rates serve as the feedback loop from the evolutionary continuum to the physiological continuum to alter resilience, and inherently reflects changes to the "topology" of the operational network itself (Chae et al. 2012; Fig. 30.2). Consequently, many fields have turned to 'systems' based approaches to overcome such fundamental limitations. Systems biology provides the computational means to model complex operational networks within a biological system, reducing the dimensionality of the complex layers of physiological and molecular information (Weston et al. 2012b). Central to this approach are network (re)construction tools, which have already transformed understanding of how metabolic pathways respond to environmental change in other microalgae (e.g. Chang et al. (2011) for *Chlamydomonas reinhardtii*; and Ashworth et al. (2013) and Park et al. (2014) for *Thalassiosira pseudonana*) but requires the means to annotate the complex "omics" data required. Transcriptomics (Baumgarten et al. 2013; Barshis et al. 2014; Palumbi et al. 2014), proteomics (Weston et al. 2015, 2012a) and metabolomics (Gordon et al. 2013) are all gathering pace, and it is becoming increasingly clear that these tools are needed in tandem, both with one another but also alongside key physiological "response metrics", to more fully resolve acclimation and acclimatisation processes (and their adaptive variability) within *Symbiodinium* and their hosts. Taking a systems approach for *Symbiodinium* in the near future will not be trivial, and certainly not possible to capture the vast phylogenetic diversity that exists; consequently, distilling *Symbiodinium*'s ever growing complex phylogenetic diversity into more meaningful functional groups may be a critical, if not an essential first step.

In parallel with systems biology, phenomics has emerged as a means to effectively capture the complex systems network at play through the net expression of traits that result from genome-environment interactions (e.g. Furbank 2009; Houle et al. 2010). In principal, phenomics operates as a platform to identify variability of both intrinsic (e.g. mechanistic properties) and/or extrinsic (emergent properties) expression of traits (Houle et al. 2010), and has become a key in industrial applications screening genotypes amongst a species to identify properties of commercial and/or societal interest (Furbank and Tester 2011). To date, this is perhaps best exemplified for algae from the wealth of studies now searching for high lipid yield mutants for biofuel applications. Importantly phenomics represents the practical step to screen for properties that define not only commercial value, but also ecological success where traits inherently impact or reflect ecosystem service provision (Matsubara et al. 2012), including key functional traits. On that basis and in the context of this chapter, *Symbiodinium* phenomics has in essence been growing in practice for decades, notably through screening genotypes for adaptive differences in photobiological traits (Hennige et al. 2009; Iglesias-Prieto and Trench 1994; Robison and Warner 2006; Suggett et al. 2015). However to effectively facilitate the use of phenomics as a platform in *Symbiodinium* research will first require that physiological-based studies begin to (i) inherently screen a much greater representation of the pool of possible *Symbiodinium* genetic diversity and (ii) identify properties that can not only be easily screened for (high throughput) but can accurately define ecological success. Such data will ultimately be pivotal for much-needed model development and parameterization into the future. For example, recent work by van Woesik et al. (2010) provides an exciting example where symbiont population fluctuations were predicted by a discrete time optimal-resource model that generated data similar to empirical results for some Caribbean coral species capable of hosting multiple types of *Symbiodinium*. Similarly, utilizing data for phytoplankton temperature optima for growth in eco-evolutionary as well as mechanistic species distribution models (i.e. models that use specific physiological traits to predict species abundance across environmental gradients) allowed Thomas et al. (2012) to show how algal distributions are driven by adaptive evolution to global temperature as well as the possibility for substantial range size reduction in tropical phytoplankton.

Appreciably, these challenges we have briefly summarized are not trivial to overcome and will only be solved through further technical innovation; for example, traits that define ecological success under steady state conditions may not be the same as those needed to define success under specific stressors. However, relatively easily measured photobiological traits have certainly proved central in explaining how *Symbiodinium* cell lines persist across ambient and stressed environments and thus seem a good candidate to provide a platform in some form for *Symbiodinium* phenomics and establish a basis for functional diversity. Integrating these layers of information into the development of better predictors of the success of particular *Symbiodinium* symbioses will be one of the grand challenges in coming years.

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