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Symbiotic dinoflagellate functional diversity mediates corals survival under ecological crisis

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Symbiotic dinoflagellate functional diversity mediates corals survival under ecological crisis

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Abstract

Coral reefs have entered an era of "ecological crisis" as climate change drives catastrophic reef loss worldwide. Coral growth and stress susceptibility are regulated by their endosymbiotic dinoflagellates (genus *Symbiodinium*). Phylogenetic diversity of *Symbiodinium* frequently corresponds with patterns of coral health and survival, but knowledge of functional diversity is ultimately required to reconcile broader ecological success over space and time. Here we explore functional traits underpinning the complex biology of *Symbiodinium* that spans free-living alga to coral endosymbiont. In doing so, we propose a mechanistic framework integrating the primary traits of resource acquisition and utilisation as a means to explain *Symbiodinium* functional diversity, and resolve the role of *Symbiodinium* in driving the stability of coral reefs under an uncertain future.

Global deterioration of reefs through coral bleaching

Coral reefs have become a global ecological casualty of the Anthropocene Epoch. Ecosystem services provided by coral reefs sustain nearly 10% of all people on Earth and support billion dollar industries in tourism and fisheries [1]. However, coral reefs worldwide have moved into an era of "ecological crisis" from accelerating over-exploitation and persistent anthropogenic threats. Elevated seawater temperature from climate change poses the greatest threat, driving mass coral bleaching (see Glossary) and associated mortality across entire regions with increasing frequency and intensity [2].

The ecological foundation of coral reefs rests on the symbiosis between reef-building corals and dinoflagellate microalgae ("zooxanthellae") of the genus *Symbiodinium* (Dinophyceae, Suessiales). When surface seawater temperature (SST) exceeds the long-term maximum monthly mean for extended durations [3] this symbiosis disassociates leading to rapid loss of *Symbiodinium* cells and/or their pigmentation. Bleaching is defined by the conspicuous whitening of the coral tissue [4], and whilst there is a clear hierarchy of bleaching susceptibility among coral species [5] (e.g., Fig. 1), the broad scale impacts of elevated SST on individual coral colonies are modified by a variety of physical and biological processes, including inherent properties of the coral host and *Symbiodinium* [6, 7]. Together these processes result in complex bleaching mosaics across reefs with distinct inter- and intra-specific responses (Fig. 1) that modify bleaching thresholds and hence the impact of heating. Consequently, mass bleaching manifests when ecologically dominant coral-*Symbiodinium* associations are exposed to temperatures above their thermal thresholds [2, 5] (Fig. 1).

Heat stress induced coral bleaching characterises continual progression of the symbiosis disassociation that is governed by a variety of host and/or *Symbiodinium* responses (Fig. 1), but distilling this process into a generalised response for *Symbiodinium* is still particularly unresolved (Box 1). Unlike in higher plants where thermal stress primarily affects RuBisCO activase [8], the cellular target broadly underpinning *Symbiodinium* thermal stress susceptibility is still unknown. Various putative sites, including, light harvesting complexes, the reaction centre complex of photosystem II, thylakoid membranes, RuBisCO and carbon concentrating mechanisms (CCMs), have all been described as targets of heat stress [9-12], implying broad functional diversity with which cellular networks have evolved to govern stress susceptibility across the genus [13]. However, potentially unifying traits that govern stress tolerance have still not been identified (Box 1), thereby highlighting that second-order traits governing stress tolerance may simply be poor metrics to represent the complex physiologies ultimately regulating broad ecological success. Instead we propose functional diversity underpinning stress susceptibility should be based on the key first-order traits that govern *Symbiodinium* metabolic functioning, and thus the growth and performance of their coral hosts, under both optimum and sub-optimum environmental conditions.

Ecosystem stability from *Symbiodinium* diversity

Molecular level markers have established exceptional phylogenetic diversity inherent within the genus *Symbiodinium* (Box 2), which plays a major role in whether and how coral reef ecosystems respond to environmental perturbations [26]. Molecular ecological-based studies have now repeatedly demonstrated that viability of the entire coral symbiosis over space and time [14, 27-29] often corresponds with the species (or genetic variant) of *Symbiodinium* present. Similarly, corals populating relatively unfavourable environments, such as hot-acidic lagoons [36] or hot-saline catchments [31], typically associate with specific *Symbiodinium* taxa (Box 2). Continued improvements to molecular tools have therefore unquestionably established a central role for *Symbiodinium* diversity in shaping environmental thresholds for coral productivity and ultimately reef growth.

Basic evolutionary theory requires that the maintenance of phylogenetic diversity must be driven by functional differences in *Symbiodinium*. However, we now lag far behind molecular ecology with any comprehensive understanding of *Symbiodinium* primary physiological attributes that determine coral ecological success given the alga's immense phylogenetic diversity. This is clearly problematic where a change (or maintenance) in function does not reflect a parallel change to phylogenetic diversity. Notably, convergent evolution across bioregions such as the Caribbean (dominated by clades A, B) versus Indo-Pacific (dominated by clades C, D), to common environmental histories selecting for the same functional responses, e.g. high versus low light "ecotypes" [37] that are genetically distinct. Conversely, divergent evolution due to local scale environmental differences select amongst closely related genotypes and/or populations. [33-34]. The net outcome is that clades, species and even genotypes differ in their functional responses to changes in key resources, such as light [37] and CO_2 [38], but also temperature stress [39]. Phylogeny thus cannot provide an exclusive currency with which to resolve *Symbiodinium* diversity with ecological function.

Disciplines ranging from oceanography [40-41] to plant ecology [42] have overcome such difficulties linking diversity to ecological functioning, by turning to the inherent traits ("emergent properties" of individual organisms) that ultimately govern the processes defining ecosystem health. Functional traits provide a standardised measure of the biogeochemical role organisms play, e.g. photosynthetic rate or nutrient turnover, but also capture fundamental trade-offs with fitness such as investing energy into cellular maintenance versus growth or nutritional mode [40-43]. Functional traits thus define the ecological success of species, and hence overcome the uncertainties associated with phylogenetic resolution and how it is applied to reconcile ecological success. The central concept of functional diversity is not new to coral ecology, and has in fact been recently considered a likely key operational unit driving ecological success of corals [44] and coral-*Symbiodinium* associations [45]. Coral reef management is increasingly turning to knowledge of key traits that regulate (or are indicative of) coral health for innovative management practices [46], whilst state-of-the-art ecological models that can evaluate winners and losers under complex environmental conditions rely on knowledge of quantifiable traits governing competitive ability [43, 47]. However, fundamentally, the 'choice' of trait(s) that best defines *Symbiodinium* functional diversity still remains largely unexplored.

***Symbiodinium* spp. fitness traits and trade-offs**

Understanding the functional roles that underpin the ecological success of *Symbiodinium* spp. within the holobiont landscape ("what makes a good endosymbiont"?) demands knowledge of resource acquisition and utilisation. Algal [41] and plant [42] trait-based models commonly rest on end-to-end tracking of resources that govern growth and cellular maintenance, and thus provide a logical conceptual framework. *Symbiodinium* spp. genetic variants have clearly adapted to thrive across a broad range of habitats and host associations, where resource availability will differ (Fig. 2). Such diversity of ecological niche exploitation and optimisation would suggest major selection pressure for trade-offs amongst key traits [43, 47]; for example, broad ecological success of phytoplankton can generally be explained via an evolved continuum of "r *vs* k" strategies [48, 49], whereby cell size operates as a "master trait" governing allometric scaling rules for light harvesting [47] and inorganic nutrient assimilation [43, 50]. *Symbiodinium* spp. genetic variants in fact exist across a cell size continuum, albeit in a relatively narrow range (ca. 7-14μm), that appears to explain variation in light harvesting but not utilisation capability [32, 37]; however, whether this central principle similarly applies to inorganic nutrient acquisition is as yet unexplored.

Dinoflagellates have particularly acquired a broad spectrum of physiological and life history traits that have enabled ecological diversification beyond boundaries set by allometric scaling rules (“dirty tricks”, *sensu* [51]). An array of strategies associated with light harvesting and photoprotection [52-53] have been relatively well described for *Symbiodinum* spp.. However, partitioning *Symbiodinium* spp. genetic variants according to differences in light harvesting and utilisation actually results in few functional groups [13, 37] suggesting trade-offs associated with nutrient acquisition and allocation strategies may in fact be pivotal in explaining their diverse niche exploitation [54] (Fig. 2). Such strategies in other microalgae include plasticity of: (i) the number of inorganic nutrient uptake (“porter”) sites [50, 55], which in the case of inorganic carbon is further complicated by the nature of CCMs and RuBisCO affinity (including *Symbiodinium* spp., [38]); (ii) minimum cellular requirements for different inorganic macro and micro nutrients [56], reflecting both pool size (active and stored) and turnover of key constituents that support cellular growth *vs* maintenance; and (iii) supplementing cellular energy (ATP) production through heterotrophy. *Symbiodinium* spp. are notably active mixotrophs that can supplement their phototrophic metabolism by feeding on bacteria [57] and simple sugars [58].

Accounting for these various factors associated with resource acquisition and utilisation introduces immense functional complexity, but it is possible to initially distil this complexity to several first-order measurable traits, as commonly employed for Dynamic Energy Budget (DEB) modelling [59]; specifically, nutrient uptake kinetics, and cellular nutrient content relative to growth rate and cell size (see Fig. 2), which together describe nutrient competitive ability [43]. Algae can preferentially trade-off these resources into opportunistic growth versus persistent maintenance (a classical view of r *vs* k selection; e.g. [42]); however, *Symbiodinium*, as with many other dinoflagellates [20], can potentially short-circuit this trade-off through additional secondary traits that likely disproportionately alter their competitive fitness. Examples of such secondary traits include the extent to which fixed inorganic nutrients are either excreted as dissolved organics [60], including by pathways such as photorespiration that effectively aid photoprotection, or stored as particulate organics for mobilisation during transient resource limitation [55-56]. Whilst differences in the biochemical foundation for cellular fitness can be established from knowledge of cellular nutrient (elemental) stoichiometry [56] and hence first-order traits, allocation to specific constituents that enhance fitness are arguably secondary traits of interest. For example, *Symbiodinium* spp. tolerance to stressors that promote bleaching is enhanced by increasing protein pools that dissipate reactive oxygen species, ROS (Box 1), but also through production of biogenic volatile signalling molecules [20].

Metabolic coupling of coral-*Symbiodinium* associations

Functional traits of interest need to span the complex life history dynamics of *Symbiodiunium*, where environmental constraints on fitness posed by life in symbiosis are very different from those for free-living algal cells. A defining characteristic to consider initially is the degree of specificity between certain *Symbiodinium* species and their coral hosts, as well as mode of symbiont acquisition (vertical *vs* horizontal transmission). Most coral species as adults associate with a single *Symbiodinium* type (or share a few closely related types) [26], although some exceptional coral species may host as many as 5–7 distinct types, as co-dominant [61] or rare [62] populations. Consequently, genetically unique *Symbiodinium* populations may fluctuate in certain coral species or persist across others [26]. Even so, types that contribute minimally to the total population pool may ultimately yield a low net metabolic contribution to their host [63]. Coral species that do harbour multiple *Symbiodinium* types in abundance within a single colony appear to reflect complex algal-derived niche partitioning (e.g., photoacclimation to different light levels) [62]. Similarly, for coral species with shifts in dominant *Symbiodinium* type, ‘shuffling’ is best described in the context of environmental history, e.g. the thermal trends driving bleaching and subsequent recovery [54, 64-65], or complex multivariate interactions of several physical-chemical (temperature, light, nutrient availability) and biological factors acting in tandem [66] that are rarely fully characterised. Unique *Symbiodinium* populations, especially within horizontally transmitted systems, may further represent true localized adaptive radiations to specific *in hospite* environmental conditions [26, 31, 34]. Thus trait-based characterisation of *Symbiodinium* functional performance is equally appropriate to best describe their realised niche space when *in hospite* as for cells that are free-living.

Metabolic coupling within the coral-*Symbiodinium* relationship is exceptionally complex and likely extends further to the milieu of constituents representing the true holobiont (i.e., bacteria, archaea, fungi and viruses) [67]. Historically, efforts to understand this coupling have focussed on photosynthetically derived carbon translocation from *Symbiodinium* to coral, in the context of host 'control' over algal populations via nitrogen and metabolite (“host release factor”) exchange (see [68] for an extensive review; [69]) (Fig. 2). Much of this work has originated from other symbioses (e.g., *Hydra* and the green alga *Chlorella* sp. or in anemone-*Symbiodinium* systems) but provides important evidence for host-controlled nitrogen limitation as a source of slower algal mitotic division [68]. First principles would suggest N-limitation to cause an imbalance in the carbon-nitrogen ratio (and hence C:N:P [56]) and in turn reduced symbiont growth yet continued translocation of photosynthetically fixed carbon [69]. However, exposure to inorganic nutrient supplements intriguingly leads to a rebalance in symbiont C:N ratios toward nutrient sufficiency but sustained algal growth arrest [70-71]. External eutrophication events can drive elevated *Symbiodinium* N:P ratios as a result of direct inorganic N stimulation of the alga [71] or fuelling the corals' nitrogen fixing bacterial community via indirect DOC enrichment [72]. Such N enrichment drives P-starvation (higher N:P) to result in significant *Symbiodinium* photoinhibition that exacerbates thermally-induced coral bleaching [71]. Conversely, host feeding post "starvation" re-establishes *Symbiodinium* nutrient quotas and algal growth [70] and substantially ameliorates photoinhibition and coral bleaching during thermal stress [73].

Existing evidence of changes to *Symbiodinium* physiological performance from altered nutrient availability would suggest that cellular nutrient content relative to cell size could provide a first-order measurable trait to consider *Symbiodinium* functional diversity and competitive ability *in hospite*, and hence importantly a direct comparison currency with cells that are free-living (Fig. 2). Bulk elemental stoichiometries could not only encapsulate how growth environment regulates resource availability relative to inherent requirements across different *Symbiodinium*, but also overcome the challenge in balancing symbiont type versus population size (cell size and number of a single genetic variant) to fulfil overall translocation demands [74-75]. Corals that maintain flexibility in association with more than one *Symbiodinium* type [Kemp], would require different population sizes to offset any differences in nutrient uptake and/or release across types to ensure translocation output is sustained.

Host corals regulate both light [4, 76] and CO_2 (dissolved inorganic carbon, DIC) [77] availability for *Symbiodinium* photosynthesis. In fact, the host may regulate DIC delivery more heavily than N or P. When *Symbiodinium* are present, numerous symbiotic anthozoans show substantial transcriptional upregulation for carbonic anhydrase (CA), the enzyme responsible for interconverting CO_2 and HCO_3^- and providing DIC for photosynthesis as well as calcification. Recent work has confirmed both external as well as internal CA activity in several corals [78-79], and corals harness a sharp proton gradient to significantly lower the pH (down to ~ 4.0) surrounding the symbiont sitting within the host-derived membrane, or "symbiosome", via a vacuolar H^+-ATPase [77]. Hence substantial energetic investment by the host supports the DIC demands of photosynthesis [79]. Whilst the dynamics describing the light dependency of *Symbiodinium* photosynthesis *in hospite* are generally well described [76], those describing DIC (indeed other dissolved inorganic nutrients, e.g. N, P) dependency are not; clearly these therefore also represent promising first order traits with which to define *Symbiodinium* functional performance and how it alters over space and time (Fig. 2).

Fundamentally, we have sparse knowledge regarding cellular nutrient quotas and uptake kinetics for *Symbiodinuium*; however, as has been repeatedly demonstrated across other microalgae [40, 43], these traits inherently modulate the physiological and competitive response of cells. Clearly this represents an area ripe to explore the ever-widening gap in knowledge between diversity and ecological success for *Symbiodinium*, which *in hospite* likely drive metabolic trade-offs for the host coral. Inherent nutrient supply, along with *in hospite* light and thermal conditions, may be key attributes in determining the interspecific competitive outcome among different yet compatible symbionts. Thus, “shuffling” of compatible symbionts may have less to do with specific host ‘control’ but rather reflect an outcome of shifting host metabolic processes [67], and hence a function of trade-offs amongst first-order algal traits (e.g., cell size, macro and micro nutrient/elemental quotas, and strategies for light-temperature acclimation) [54]. Variation in the first-order traits that determine the cellular energy budget drives broad niche exploitation [59] and hence the scope for functional diversity across *Symbiodinium* genetic variants. However, the exact first-order trait profile will determine the ecological success of any given symbiont; specifically: how, when, and to what extent *Symbiodinium*-coral associations are sustained and the capacity for *Symbiodinium* to thrive ex-hospite. In focussing on second-order traits of interest e.g. ROS production (Box 1), for some time as key factors influencing *Symbiodinium*-coral fitness, we have in fact overlooked the first-order traits that functionally connect *Symbiodinium* to their surrounding environments (Fig. 2).

Concluding Remarks

Understanding how *Symbiodinium* spp. are optimised to function across different host corals and reef environments is more critical than ever as reefs face global “ecological crisis”. Functional diversity theoretically mediates the response of *Symbiodinium* to changing environmental conditions, and provides a means to reconcile (and complement) the growing wealth of knowledge aligning *Symbiodinium* phylogenetic diversity with coral ecological success. In evaluating key traits that govern cellular growth and physiology, we have proposed a mechanistic physiological framework (Fig. 2) that directly complements the rapid uptake of molecular-based descriptors of both *Symbiodinium* phylogeny and function. Trait-based models provide a means to evaluate this physiological framework against ecological success but only through measuring the key first-order traits. We therefore call for renewed focus into resource acquisition and utilisation as a fundamental regulator of competitive ability (see Outstanding Questions), as a first step to resolve *Symbiodinium* spp. niche boundaries across habitats and specific host-symbiont associations, and hence the role of *Symbiodinium* in driving productive and diverse coral reefs as they enter an uncertain future.

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Text Box 1: *Symbiodinium* as cellular sources of coral stress

Coral host-specific responses that operate to effectively increase thermal tolerance [7] have become increasingly well described; notably reduced oxidative stress via mitochondrial excitation pressure that produces reactive oxygen species (ROS) (e.g. fluorescent proteins, tentacular retraction [15]), increased production of ROS detoxifying proteins and organelle stability (e.g. mitochondria [16]) or silencing of ROS-triggered caspases that in turn induce apoptosis [17]. ROS is produced by the coral's microbial community, in particular by *Symbiodinium* photosynthetic dysfunction and associated bacterial metabolism, as well as host mitochondria [18]. However, how physiological dysfunction initiates and progresses to drive ROS emissions remains unresolved (but see [19]); in the case of *Symbiodinium* this has reflected challenges in utilising genomics to unlock the inherent cellular networks and how they are regulated.

Dinoflagellates as a group have a variety of unique characteristics [20], including permanently condensed chromosomes, extremely large genomes and a significantly higher reliance on post-translational regulation, in contrast to transcriptional regulation, compared to other organisms. Therefore, while the magnitude of gene expression changes is generally less than 2-fold, up to 30% of the transcriptome can alter [21, 22], making it difficult to identify specific responses at this scale. Even so, such tools have begun to highlight parallels with how the host responds to heat stress; notably, a major re-organisation of the ROS antioxidant network in heat tolerant *Symbiodinium* [21-23], which clearly reflects simultaneous physiological observations of reduced ROS emissions for more heat tolerant *Symbiodinium* [12, 13, 21]. Enhancing ROS detoxification capability in fact appears a key mechanism with which heat tolerance can be acquired trans-generationally in *Symbiodinium* populations [24]. Such responses may thus be core and hence impose cellular trade-offs to processes that are secondary in affording thermal tolerance; for example, under stress clear upregulation in heat shock proteins (HSPs) is noted for the coral host (>32 fold) whereas *Symbiodinium* may [25] or may not [23] downregulate HSPs. Therefore, whilst the “source” of stress is becoming well documented, understanding how this is driven by (or feeds back to) a unifying target regulating *Symbiodinium* cellular dysfunction remains unknown [9-13].

Text Box 2: Species diversity amongst the genus *Symbiodinium*

Molecular tools continue to unlock the immense phylogenetic diversity inherent to the genus *Symbiodinium* [26-29]. At the broadest scale *Symbiodinium* spp. is divided into 9 distinct evolutionary lineages (i.e. clades, A-I) via divergence of the small ribosomal subunit RNA (SSU). More variable DNA regions, including the internal transcribed spacer regions (ITS), chloroplast large subunit (cp23S) and cytochrome oxidase b (cob) have subsequently resolved immense subcladal diversity [27] typically classified alpha-numerically (e.g. C1, C3z). Of these, ITS2 has been most widely adopted, but requires consideration alongside additional rapidly evolving regions (e.g. psbA^{ncr}) to resolve evolutionarily distinct species (multilocus barcoding, [30-31]). Integration of barcoding-based phylogeny with fundamental biological (e.g. morphology, physiology) and ecological (e.g. host specificity) patterns has provided the core framework for novel *Symbiodinium* species descriptions [31-33]. Molecular platforms have recently transitioned to high throughput pyrosequencing for barcode retrieval, and added further depth to phylogenetic differentiation through more accurate detection of low-abundance background *Symbiodinium* [28-29, 34]. Here, phylogeny is considered within an operational taxonomic unit (OTU) framework to identify ecologically discrete entities [29, 34], including the role of intra-genomic variability, to resolve taxonomic sub-groups [35].

Analysis of genetic recombination has become an important complimentary tool to examine *Symbiodinium* species level diversity [36]. Using the biological species concept, populations that exchange alleles through sexual recombination are the same species, and hence population genetics based on allele frequency similarity across multiple loci delimit species over space and time. Such delimitation of species based on this ‘incompatible breeding’ may be complicated where populations frequently reproduce asexually but only very rarely sexually and/or conspecific populations that previously diverged in isolation of one another become mixed [34]. Even so, the approach has proved powerful for establishing novel *Symbiodinium* species boundaries that persist over broad geographic regions [26].

Molecular based technical advances thus continue to highlight immense phylogenetic variation and speciation. ITS2 variation alone suggests existence of 10s-100s of *Symbiodinium* species, but this is likely an underestimate. Multi-locus and high throughput techniques would suggest 100s-1000s of putative species, which are particularly changing our ecological view of *Symbiodinium* ITS2 types previously considered to be widespread generalist species, e.g. ITS2 type C3 harboured by many highly stress sensitive coral species of Acropora [31, 35].

Glossary Box

Coral bleaching: Process with which corals pale (whiten) from loss of *Symbiodinium* cells and/or pigmentation from host coral tissues.

Fitness: Capacity of an organism to pass its genes to successive generations, as determined by the ability to survive and reproduce by inherent competitive traits.

Photosystem II (PSII): Protein complex that generates electrons for photosynthesis by oxidizing water in algae. Dysfunction of PSII activity is a common assay of heat stress sensitivity in *Symbiodinium*.

Reactive Oxygen Species (ROS): Chemically active molecules containing oxygen (“free radicals”) produced via mitochondria and/or chloroplast metabolic pathways; notably, singlet oxygen, superoxide, and hydrogen peroxide.

RuBisCo: Ribulose-1,5-bisphosphate carboxylase/oxygenase is the enzyme involved in the first step of CO_2 fixation, and considered the most abundant enzyme on Earth. It is modulated by the catalytic chaperone RuBisCo activase.

Secondary metabolites: Organic compounds that are not directly required for growth and reproduction; for example, toxins and volatiles that negatively affect fitness of competitors.

Traits: Measureable characteristics of an organism that are inherited or environmentally controlled. Can encompass cellular, physiological, morphological and life history characteristics.

First- *vs* second-order traits: Traits that form the foundations for functioning are considered “central” (first-order) whereas other traits that arise from operation of first-order traits (or only under certain environmental conditions) are considered second-order.

Vertical *vs* horizontal transmission: In corals the process where larvae retain *Symbiodinium* cells from parent colonies (vertical transmission or “closed symbiosis”) versus uptake from the surrounding environment (horizontal transmission or “open symbiosis”).

Figure Legends

Figure 1. Generalised scheme of coral-*Symbiodinium* functional trait responses to thermal stress-induced bleaching. (A) Increased temperatures over-time induce a cascade of responses (Box 1) as the coral-*Symbiodinium* association is pushed towards the upper thermal thresholds for symbiosis viability ("point of no return"). These responses are either common to both coral and symbiont (upregulation of ROS detoxification networks, reduction to light harvesting through increased host fluorescence protein expression or decreased symbiont light harvesting complex (LHC) pigments, and induction of apoptosis), or specific to coral or symbiont (preferential alteration of heat shock proteins, HSPs; mitochondrial versus thylakoid stabilisation for host and symbiont, respectively). Sustained heating at or beyond the threshold causes mortality or requires acclimation or adaptation by selecting for upregulation of the various traits driving heat stress tolerance (in which case the upper threshold temperature alters by changing how traits providing thermal tolerance are expressed). Cooling can initiate recovery. How these processes operate across host-*Symbiodinium* associations in reef systems is highly dynamic and can manifest as different severities of bleaching at intra-species (e.g. (B) *Porites lutea* colonies) [34] and inter-species (e.g. (C) Alternate species within the same genus of *Acropora*; (D) Alternate genera, bleaching sensitive plating *Montipora* sp. *vs* species of *Acropora*) [2, 5] levels within any given reef area. Mono-specific host-*Symbiodinium* sp. associations manifest as mass bleaching (e.g. (E) *Acropora muricata* beds hosting *Symbiodinium* ITS2 type C3 in the Seychelles). Photographs (B-E) are courtesy of Emma Camp, University of Technology Sydney.

Figure 2. Generalised scheme of resource acquisition and utilisation underpinning *Symbiodinium* competitive fitness. External light and dissolved inorganic (DIC, DIN, DIP, S, Fe, etc.) and/or organic (POC, DOC) nutrient uptake drive cellular functioning, as well as determine the competitive outcome of phytoplankton in general [48]. Generation of energy (ATP) and reductant (NADP(H)) in *Symbiodinium* cell chloroplasts and mitochondria sustains active uptake and assimilation of nutrients into organic compounds. In turn these compounds are stored, drive formation of primary metabolites (e.g. formation of key carbon "skeletons"; lipids, carbohydrates and proteins) and secondary metabolites that may also act in signalling. According to theory developed for phytoplankton [40, 43] trade-offs in how these resources are acquired and utilised can explain ecological competitiveness, and generally accounted for using several key terms, highlighted in blue: Extent of light absorption (α), maximum photosynthesis rate (P^{max}); the maximum uptake rate (V_{max}), half saturation constant (K_N), and minimum quota (Q_{min}) for any one nutrient; quantity and hence stoichiometry of cellular particulate (POC:PON:POP:etc…), and excreted dissolved (DOC:DON:DOP:etc…) nutrients; as well as cell volume (V). Together these terms govern maintenance versus division, and hence the net achievable growth rate (μ), to reflect first order traits of competitive fitness. Such terms are governed by the growth environment and thus will be regulated when cells are *in hospite* (indicated by red lines and arrows) as well as free-living: Host corals also acquire external nutrients from both feeding and their broader microbial associations. Hosts modify the inherent light field [15] for *Symbiodinium* and have been suggested to excrete specific dissolved compounds [60] (signalling markers) that control delivery of inorganic nutrients back to the symbiont, i.e. distinct metabolites ("host release factors", [68]). Again, the net outcome is regulation of the net

achievable growth (μ), but *in hospite* will be relative to the overall population size required to meet host metabolic demands (*Symbiodinium* cell number, n). All aforementioned processes play a central role in the trade-off between cellular maintenance in the alga vs. the direct release of translocated material to the host coral.

Figure 1

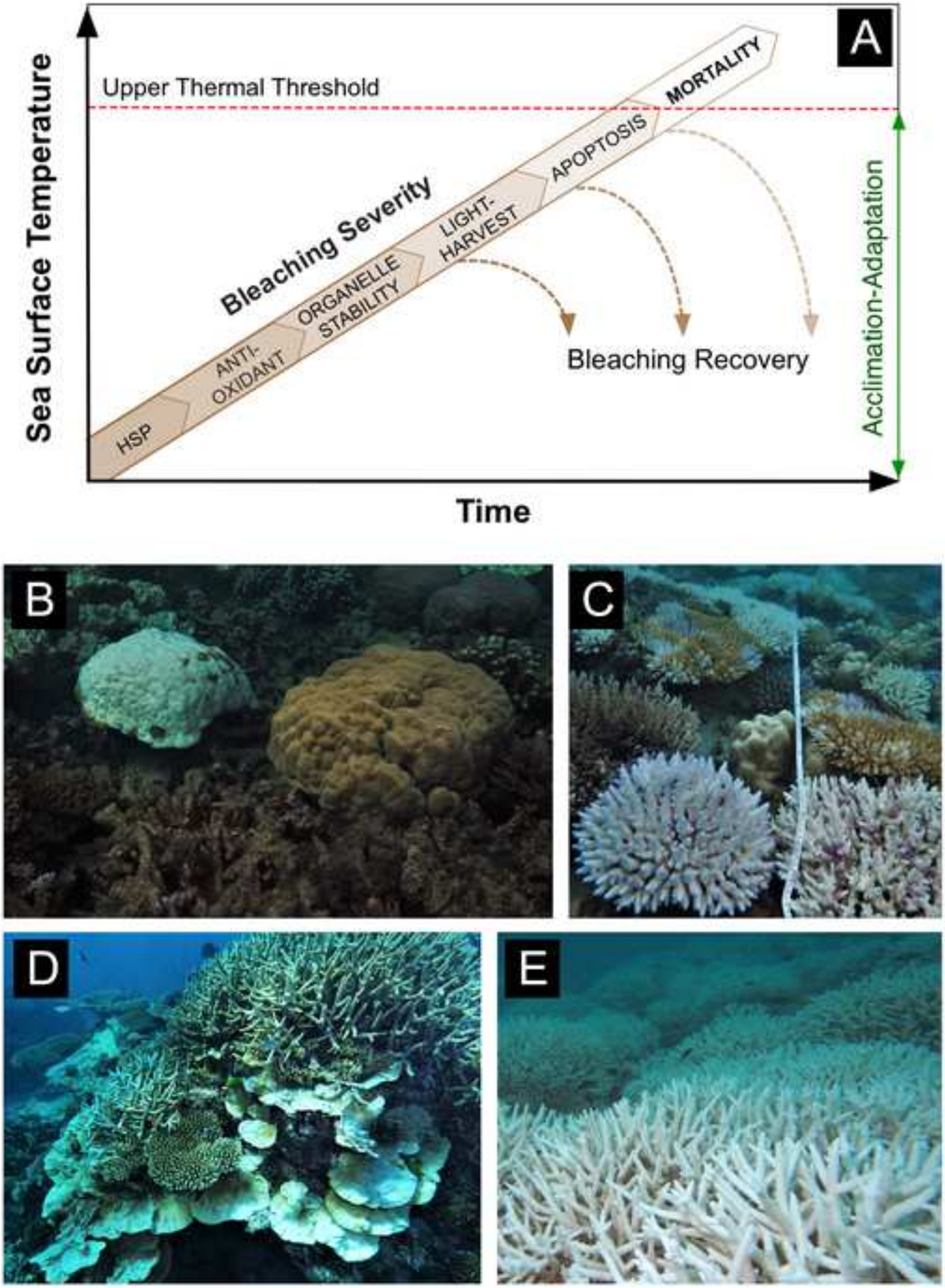
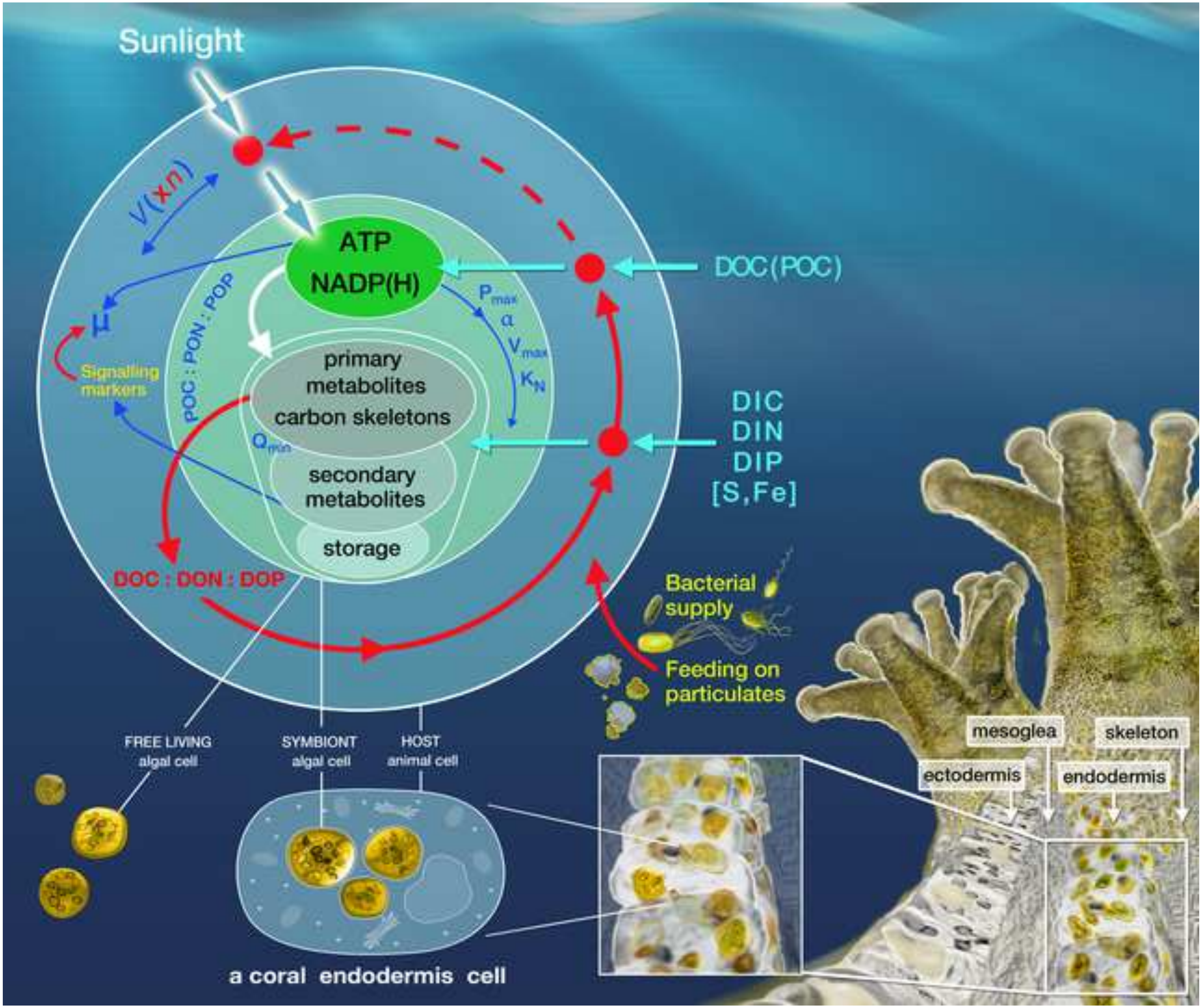

A
Sea Surface Temperature
Upper Thermal Threshold
Bleaching Severity
HSP
ANTI-OXIDANT
ORGANELLE STABILITY
LIGHT-HARVEST
APOPTOSIS
MORTALITY
Bleaching Recovery
Acclimation-Adaptation
Time
B
C
D
E

Figure 2


Sunlight
V(xn)
μ
Signalling markers
POC : PON : POP
ATP
NADP(H)
P_{max}
α
V_{max}
K_N
primary metabolites
carbon skeletons
Q_{min}
secondary metabolites
storage
DOC(POC)
DIC
DIN
DIP
[S,Fe]
DOC : DON : DOP
Bacterial supply
Feeding on particulates
FREE LIVING algal cell
SYMBIONT algal cell
HOST animal cell
a coral endodermis cell
mesoglea
skeleton
ectodermis
endodermis

Coral reef survival to ecological crisis through dinoflagellate functional diversity–

Suggett *et al.*

Highlights

Coral ecosystem health is strongly influenced by *Symbiodinium* diversity.

Ecological success of *Symbiodinium* cannot be resolved from phylogenetic diversity alone.

Traits describing resource acquisition and incorporation capture *Symbiodinium* functional diversity.

Symbiodinium species shifts reflect changing metabolic requirements of the host.

Functional diversity will determine the resilience of coral reefs to environmental change.

Coral reef survival to ecological crisis through dinoflagellate functional diversity–

Suggett *et al.*

Outstanding Questions

Can first-order traits (e.g. cellular uptake and allocation) alone explain niche breadth, *including* anomalous stress tolerance, or must they be considered alongside second order-traits (e.g. capacity to upregulate ROS detoxification pathways)?

How diverse are micro- (trace) relative to macro- (C, N, P, S) nutrient uptake and utilization properties in describing *Symbiodinium* ‘functional types’?

Does ecological resilience through “symbiont shuffling” reflect match/mis-match between changing host metabolic requirements and *Symbiodinium* consortia with alternate metabolic (nutrient uptake and allocation) profiles?

What is the extent of metabolic and resource trade-offs required to persist across alternate life history stages? Does *Symbiodinium* require “host resource surrogates” as a free-living alga (e.g. obligate associations with other microbes)?

How important are heterotrophic strategies for supporting *Symbiodinium* nutritional and/or metabolic requirements? Can *Symbiodinium* feed on host as well as microbial metabolites?

To what extent does functional diversity of nutrient strategy reflect evolutionary radiation (and/or potentially support phylogenetic re-constructions) of the genus *Symbiodinium*?

Proposed Cover Image