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SUMMARY

Coral reefs are among the most biologically diverse ecosystems on Earth and have a great economic value for human populations inhabiting tropical coastal areas. However, the foundation for coral reef ecological success in the nutrient-poor tropical waters also constitutes its weaker link: a highly stress-sensitive mutualistic symbiosis between the reef-building animal host (scleractinian coral) and photosynthetic dinoflagellates of the genus *Symbiodinium*. Together, host and symbiont constitute the holobiont, whose ecological and evolutionary resilience depend on the integrated physiology of the symbiotic partners. Light constitutes the main energy resource in the ecosystem and its intensity is dramatically reduced with increasing depth over reef slopes.

The main objective of this PhD thesis is to understand how coral holobionts thrive across the large light gradients that exist over reef slopes. Because the endosymbiont plays a crucial nutritional role in host maintenance and skeletal calcification, it has been hypothesised that the most significant feature of holobiont response to light is the occurrence of taxonomically and functionally diverse algal symbionts establishing more or less specific associations with the numerous coral species that form a reef. This thesis focuses on the phylogenetic and functional diversity of coral-symbiont associations and their adaptive strategies to cope with large light gradients.

Throughout this study the widely depth distributed (2 to > 100 m) brooding coral genus *Madracis* Milne Edwards and Haime 1849 and its symbiont populations were used as biological models. All sample collection took place at the CARMABI Buoy One reef site in Curaçao, Netherlands Antilles.

What is the genetic variation among *Symbiodinium* in the coral genus *Madracis* over large depth ranges, and is there a relationship between the composition of *Symbiodinium* genotypes and the depth distribution of the host?

Given the scarce amount of data available on *Symbiodinium* variation below depths of 10 - 15 m, the functional diversity of coral-algal associations distributed over large vertical ranges constitutes an overlooked issue. In **Chapter 2** I address the genetic variation of *Symbiodinium* in the six currently recognised Caribbean morphospecies of the coral genus *Madracis*, over a large depth range from 5 to 60 m and two different colony surface positions. Coupling ITS2 rDNA polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) three *Symbiodinium* genotypes were consistently identified. ITS2 type B7 occurred in all six *Madracis* morphospecies and at all depths, thus considered the generalist symbiont in the genus. Type B13 was restricted to some colonies of the shallow water specialist *M. mirabilis*. Type B15 was typical of deep reef habitats and gradually replaced B7 in the depth generalist coral *M. pharensis*. Intracolony variation in symbiont composition was absent. Thus, *Madracis* symbiont composition is host-specific and relates to depth-zonation. Results are discussed in relation to measured physical parameters such as irradiance intensity, the underwater light spectrum, and temperature. Overall results strongly suggest ecological niche partitioning among the symbionts, consistent with other recent studies, and confirm the applicability of the rDNA ITS region for studies on coral symbiont ecology and evolution.

What is the physiological plasticity hold by the symbiotic associations in *Madracis* across the reef slope, and what are the respective roles of host and symbiont in the holobiont response?

In **Chapter 3** I study the *in situ* integrated photobiology of *Madracis* symbiotic associations over large depth ranges by applying an innovative holistic multivariate approach. Parameters such as photosynthetic activity (assessed through chlorophyll *a* fluorescence techniques), pigment composition (measured by high-performance liquid chromatography), symbiont densities (estimated using flowcytometry) and cell size (determined by microscopy) were measured for four *Madracis* morphospecies (*M. mirabilis*, *M. pharensis*, *M. senaria*, *M. formosa*) and their three hosted symbiont genotypes (B7, B13 and B15), across a large depth range (5 – 40 m). Results stress the important role of irradiance on coral physiology and reveal that photobiological mechanisms are either regulated by the environment or genetically constrained. Photochemical efficiencies and symbiont cell sizes constitute respective examples. Different holobiont strategies thought to relate to symbiont cell density in the host tissue vary in their optimization of light-harvesting (light collecting pigments) or photoprotective mechanisms (non-photochemical quenching) and are proposed to relate to host-species vertical distribution and dominance over the reef slope. Overall, symbiont functional diversity does not appear to explain host distribution patterns. Instead, this study highlights the role of host properties in the adjustment of the internal environment available for the endosymbionts and in control of the photobiology of the intact symbiosis.

What is the functional variation between symbiont genotypes in *Madracis pharensis*, and is there a role for colourful niches in regulating symbiont distribution?

In the context of host properties, and also because the previous study provided inconclusive evidence for functional differences between symbiont ITS2 types, in **Chapter 4** I re-address the distribution and the role of symbiont variation in holobiont photobiology for a single host species, *M. pharensis*. This species occurs in three main colour morphs: brown, green and purple. Host tissue colours are known to relate to the presence of fluorescent proteins, which are suggested to either shade or amplify the light reaching the endosymbionts. *Symbiodinium* ITS2 distribution in *M. pharensis* was studied for two depths (10 and 25 m), two different light habitats at each depth (exposed and shaded) and the three colour morphs. Symbiont distribution was depth and colour morph dependent. ITS2 type B15 was predominantly present on the deeper reef and in green and purple colonies. Type B7 was mostly present in shallow environments and brown colonies. Light microhabitats at fixed depth had no effect on symbiont occurrence. This ecological distribution indicates that symbiont presence may be at least partially regulated by spectral light niches. Most functional variation (several parameters measured only for 25 m) was based on two photobiological components that relate to either symbiont genotype or light microhabitat, reflecting adaptation and acclimatisation mechanisms, respectively. ITS2 type B15 occurred with significantly lower cell densities but larger cell sizes, higher cellular pigment concentrations, higher peridinin to chlorophyll *a* ratio and smaller size of the photoprotective xanthophyll-pool than type B7, respectively. Thus, type B15 is potentially better adapted to darker or deeper habitats than type B7, a suggestion consistent with symbiont distribution with depth. Symbiont cell size may play an important role in the adaptation of coral holobionts to the deeper reef. The unambiguous differences between several *in situ* symbiont functional parameters indicate distinct ecological lineages

based on light adaptation and confirm the utility of the ITS2 marker for *Symbiodinium* ecological studies.

Are betaines abundant in reef-building corals, and if so, do they correlate to light gradients over the reef slope suggesting a role in oxidative stress defence?

Rapid climate change severely threatens coral reefs, particularly due to elevated seawater temperature, which in synergy with high light intensity often results in physiological stresses that culminate in coral bleaching and mortality. Coral bleaching, a loss or reduction of the symbiont populations or their photosynthetic pigments, is often related to the production of reactive oxygen species which damage protein and membrane function of both the photosymbiont and the host. Resistance of algal symbionts to environmental stress has often been related to several photoprotective mechanisms such as pigments involved in dissipating excessive excitation energy at the level of PSII. In vascular plants and free-living algae, however, betaines are well known metabolites that act as protective agents of PSII. These metabolites have been involved in successful genetic engineering attempts to ameliorate temperature and irradiance-related cellular stresses. In **Chapter 5**, presence and abundance of diverse betaines was screened by liquid chromatography – mass spectrometry (LC-MS) for ten reef-building coral species, including *Madracis*, over a depth range of 5 – 20 m. Glycine betaine, proline betaine and at least five other betaines occurred in concentrations of physiological significance in all coral species. Besides, betaine concentrations varied in depth-related patterns that confirm predictions of their role as molecules that defend photosynthesis against negative effects of high irradiance. Although these patterns did not relate to symbiont identity, they demonstrate that betaines in coral holobionts are phenotypically plastic and take part in acclimatisation. The study suggests that betaines may have a bleaching-protective role in reef-building corals and opens a new research line with potential future applicability.

Are morphology and molecular phylogenetics congruent in the coral genus *Madracis*, and is there evidence for a role of hybridization in the evolution of brooding corals?

A key issue to fully understand the ecology of coral-symbiont associations is to unravel the nature of the evolutionary units involved. For the animal host, many studies have shown incongruence between the morphologically described coral species and molecular phylogenetics. Scleractinians have been associated with low reproductive barriers and with interspecific hybridization events, which decouple morphology and DNA phylogenetic topologies. Although mass spawning events are known to maximize hybridization opportunities, introgressive hybridization has also been provisionally suggested for brooding corals. In **Chapter 6** I deal with *Madracis* species boundaries and the role of hybridization in brooding corals. Phylogenetic inference and population structure analyses were applied to sequence data obtained for three DNA intron markers for the six putative *Madracis* species. Recurrent nuclear DNA polyphyly among taxa were only challenged by *M. senaria*, the only monophyletic species within the genus. In fact, *M. senaria* is the only *Madracis* species with a lunar pattern of planulae release superimposed on the generic seasonal cycle. *Madracis pharensis* showed depth divergence suggesting strong population structure based on depth-related environmental factors. Moreover, this separation matched the vertical distribution of hosted symbionts (ITS2 type B7 in shallow colonies and type B15 in the deep colonies).

Overall results indicating non-monophyly of most *Madracis* taxa and high levels of shared polymorphism, combined with evidence for relatively old fossil record, suggest that introgressive hybridization is likely to have played a role in the evolution of *Madracis*. The study suggests that *Madracis* morphospecies remain recognizable either because hybridization is non-pervasive or because disruptive selection prevails over the homogenizing effect of gene flow. Depth divergence within *M. pharensis* suggests that symbiont functional differences may play a role in depth-based disruptive selection of the coral host and can partially explain patterns of holobiont niche diversification over reef slopes.

The main conclusions of this thesis are:

1. The genus *Madracis* provides an excellent model to study the functional diversity and photoecology of coral-symbiont associations and their adaptation to large depth ranges.
2. *Symbiodinium* ITS2 types are ecological and evolutionary lineages with distinct depth-based and host-specific distributions.
3. Constancy in symbiont populations along light gradients within single colonies suggests that in addition to light there are other factors controlling symbiont variation.
4. Holobiont light adaptive strategies are not necessarily explained by symbiont diversity but rather by a combination of host and symbiont physiological properties that, together, favour the enhancement of light harvesting in shaded habitats or provide photoprotection in light-exposed habitats.
5. Host species-specific morphological and physiological properties may play an important role in controlling holobiont vertical distribution patterns.
6. *Symbiodinium* ITS2 types differ in their *in situ* physiological activity and adaptation to particular light regimes, highlighting symbiont niche diversification and the role of symbiont diversity in the adaptation of reef corals to their environment.
7. Coral hosts associate with multiple symbiont assemblages allowing the occupation of distinct ecological niches.
8. Photosynthetic acclimatisation potential is large for single symbiont types but other parameters, such as cell size, are highly genetically constrained and likely the result of adaptation.
9. The ecological distribution of symbiont types over depth and host colour morphs suggests that spectral light niches may have a role in symbiont photobiology and adaptation.
10. Reef-building corals contain multiple abundant betaines that are phenotypically plastic and may protect against bleaching-related environmental stress.
11. The evolution of brooding corals, such as broadcast spawners, is likely to have been strongly affected by introgressive hybridization events.
12. Symbiont functional differences may play a role in depth-based disruptive selection of their coral hosts, therefore contributing to genetic divergence and niche diversification of reef-building corals.
13. Deep reefs hold crucial information on the diversity and functioning of coral-symbiont associations, and may be decisive to understand coral reef response to climate change.