Coral photophysiology in response to thermal stress, nutritional status and seawater electrolysis

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List of Papers

This thesis is based on the scientific publications listed below. The specific contributions of each of the authors in terms of idea and concept development, data acquisition and analysis, as well as manuscript writing for the respective publications are indicated.

Publication I
Title: Feeding sustains photosynthetic quantum yield of a scleractinian coral during thermal stress

Authors: Borell, EM, Bischof, K
Journal: Oecologia (in press, doi: 10.1007/s00442-008-1102-2)
The original idea and concept of this publication was developed by E. Borell, who also independently conducted all of the fieldwork and sample processing. Data analyses was carried out by E. Borell, with input by R. Coleman and W. Wosniok. The manuscript was written by E. Borell, with revisions and improvements by K. Bischof and C. Richter.

Publication II
Title: The effect of heterotrophy on photosynthesis and tissue composition of two scleractinian corals under elevated temperature

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Contents

Summary

Coral bleaching, the dissociation of corals and their zooxanthellae (endosymbiotic dinoflagellates of the genus *Symbiodinium*) and/or loss of chlorophyll in response to elevated seawater temperature and anthropogenic activities in coastal areas can lead to extensive coral mortality and loss in biodiversity. It is generally accepted that the foremost causes of bleaching involve photoinhibition of zooxanthellae and subsequent formation of damaging reactive oxygen species. Photoinhibition is coupled to levels of antioxidant activity and rates of protein repair, processes which incur high metabolic costs in terms of nitrogen demands and energy expenditure. Since heterotrophic abilities of corals can vary considerably between reefal habitats and species, understanding how food availability affects the bleaching susceptibility of corals could provide important information aiding the prediction of the effects of increasing temperature on coral community structure in relation to water quality.
Many destroyed coral reefs often do not recover naturally without active coral reef restoration efforts involving coral transplantation and the construction of artificial reefs. While reef degradation resulting from mass coral bleaching is often so widespread in extent, that restoration efforts are not feasible, considerable efforts are put into the restoration of coral reef environments subjected to anthropogenic degradation, which generally occur over much smaller scales. However there is still much uncertainty concerning the interactions between artificial substrates, the ecology of transplanted corals, and the environment into which they are placed, often resulting in high mortality and low growth rates of coral transplants. The mineral accretion technology as a method for coral reef restoration involves the accumulation of mineral ions in the vicinity of an underwater cathode and the precipitation of carbonate onto the cathode which serves as a substrate for coral transplants. The electrochemical processes around the cathode have been proposed to increase survival and growth of coral transplants through reinforced substrate stabilization, mineral accretion and enhanced metabolic efficiency. Despite the increasing popularity of 'electric' reefs, evidence supporting these claims however remains largely anecdotal. Using different methodological approaches, the objectives of this thesis were to1) test the prediction that exogenous food increases the thermal tolerance of zooxanthellae photosynthesis and to 2) evaluate the proposed mechanisms of enhanced survival and skeletal growth rates of coral transplants in the presence of mineral accretion.

To test the effect of food on the thermal tolerance of the symbiotic association, two feeding experiments were carried out at the Hasanuddin University Marine Field Station in southwest Sulawesi, Indonesia between June and October 2005. In both experiments corals were either starved, i.e. deprived of organic particles >0.5 μm or fed daily with freshly hatched *Artemia salina* nauplii. In Experiment 1 the effect of zooplankton feeding versus starvation on the bleaching susceptibility, photosynthetic activity of photosystem II (PSII) and non-photochemical quenching (NPQ) of zooxanthellae in the temperature sensitive species *Stylophora pistillata* under elevated temperature was assessed over a period of 10 days employing pulse-amplitude-modulated (PAM) chlorophyll fluorometry. Experiment 2 was run for 15 days in order to investigate the potential interactions between zooplankton feeding, basal metabolic functions (photosynthesis and respiration), energy status (lipid concentrations), total protein concentrations, and the bleaching susceptibility of *S. pistillata* and the more temperature tolerant species *Galaxea fascicularis*.
The effects of mineral accretion on the physiology and rates of mortality of coral transplants were investigated in North Sulawesi, Indonesia between April and July 2006, using the congeneric species *Acropora yongei* and *A. pulchra*. In order to discriminate between the effects of electrochemical processes in the vicinity of a cathode and those of an electric field on coral performance, coral transplants were grown under 3 treatment conditions: 1) on a cathode in the presence of mineral accretion, 2) on bamboo inside an electric field, or 3) on bamboo outside the experimental matrix (control), for 4 months. At the end of the treatment

phase coral survival, growth, zooxanthellae population characteristics and chlorophyll fluorescence responses of the two species were determined.

The overall results of the two feeding experiments show that the thermal stress tolerance in terms of photosynthetic activity and pigmentation of both species was coupled to food availability. Zooxanthellae in fed corals in experiment 1 maintained high photosynthetic activity while starved corals displayed strong signs of chronic photoinhibition which was reflected by a progressive decline in the daily maximum quantum yield (F_v/F_m) of PSII and nocturnal recovery rates of PSII, which was manifest by a significant decrease in F_v/F_m, effective quantum yield ($\Delta F/F_m'$) and relative electron transport rate (rETR). This was paralleled by the progressive inability to dissipate excess excitation energy via non-photochemical quenching (NPQ).In addition, both gross (Pg) and net (Pn) primary production of starved *S. pistillata* and *G. fascicularis* in experiment 2 decreased significantly over the course of 15 days. Fed corals by comparison maintained higher levels of NPQ, which coincided with significantly higher rates of nocturnal PSII recovery. Sustained photosynthesis of fed corals was accompanied by a reduced loss in pigmentation compared to starved corals. After 10 and 15 days of exposure to elevated temperature the tissue of starved corals appeared pale relative to fed corals. While the loss in pigmentation of starved *S. pistillata* was characterized primarily by a loss of zooxanthellae, starved *G. fascicularis* displayed a substantial reduction in both zooxanthellae densities and chl a + c_2 concentrations. Furthermore, the results of experiment 1, in which the mitotic indices of zooxanthellae in fed *S. pistillata* were significantly higher than the mitotic indices of starved corals, indicate that zooxanthellae of starved corals exhibited cellular degradation.

Respiration rates of *S. pistillata* over 15 days displayed a similar pattern to that of photosynthetic rates. Starvation resulted in a significant decrease in respiration of starved corals while there was no change in respiration for fed corals. By contrast there were no significant changes in rates of respiration between fed and starved *G. fascicularis.* Furthermore, the amount of lipid per unit surface area in starved *S. pistillata* was significantly lower than in fed *S. pistillata* while feeding treatment had no significant effect on lipid concentrations of *G. fascicularis*. In both species the amount of protein per unit surface area however was significantly lower in starved than in fed corals. The combined results of these experiments indicate that exogenous food can play an important role in reducing the photophysiological damage of zooxanthellae that typically leads to bleaching. The outcome of this study therefore presents an interesting consideration for coral communities in turbid, nearshore waters, which often comprise a potentially rich heterotrophic environment, that could benefit some coral species under bleaching conditions.

The three most relevant findings of the investigation evaluating the proposed mechanisms of enhanced survival and skeletal growth rates of coral transplants in the presence of mineral accretion show that 1) the electric field and not the cathode resulted in the highest growth rates, 2) corals grown under the presence of mineral accretion can exhibit low growth and reduced health , and 3) the suitability of the mineral accretion technology for coral transplantation can vary greatly between closely related species. Contrary to previous observations, the presence of mineral accretion had either no or even a negative effect on coral survival over a period of 4 months. Mortality rates of *A. yongei* after 4 months were significantly higher for corals grown on the cathode than for those inside the electric field and on the control. By contrast there were no significant differences in mortality between treatments for *A. pulchra*. Coincident with high rates of mortality, *A. yongei* exhibited the lowest growth rates on the cathode while growth rates of *A. pulchra* were lowest for corals in the control treatment. Moreover the results show that F_v/F_m and rETRs of zooxanthellae in *A. yongei* were significantly lower for corals grown on the cathode compared to corals inside the electric field and on the control while there were no significant differences between treatments for F_v/F_m and rETRs of zooxanthellae in *A. pulchra*. The results clearly

demonstrate that the cathodic environment had an overall negative effect on the health of *A. yongei*. This is corroborated by the differences in zooxanthellae densities between treatments. *A. yongei* on the cathode contained significantly lower zooxanthellae densities than corals inside the electric field and on the control treatment. *A. pulchra* displayed the opposite pattern, containing significantly higher zooxanthellae densities on the cathode than inside the electric field and on the control treatment. Thus the results do not support the proposed merits of the mineral accretion technology as an efficient method for reef restoration.

The overall outcome of this study elucidates the convoluted nature of the interaction between photosynthetic performance of in *hospite* zooxanthellae and associated fitness of corals and their immediate environment. The results illustrate that high interspecific physiological variability adds further complexity to such interactions. This underscores that both the prediction of ecological consequences of future bleaching events as well as the efforts to restore reef habitat require multivariate consideration and sound knowledge of the ecology of the coral species involved.

Zusammenfassung

Das Phänomen der Korallenbleiche, d. h. der Zusammenbruch der Symbiose zwischen Korallen und ihren Zooxanthellen (endosymbiotische Dinoflagellaten der Gattung *Symbiodinium*) und/oder eine Reduzierung der Chlorophyllgehalte infolge erhöhter Seewassertemperaturen und anthropogener Einflüsse in Küstengebieten, kann großflächige Korallenmortalität und Biodiversitätsverlust verursachen. Hauptursache der Korallenbleiche sind vornehmlich die Photoinhibition der Zooxanthellen und die dadurch erhöhte Bildung von reaktiven Sauerstoffspezien. Die Photoinhibition ist v. a. abhängig von dem Ausmaß der antioxidativen Aktiviäten und den Raten der Proteinregenerierung. Diese Prozesse sind jedoch energetisch sehr aufwendig und erfordern zudem eine hohe Stoffwechselaktivität insbesondere in Bezug auf Stickstoff. Da das Ausmaß der Korallen hinsichtlich ihrer heterotrophischen Aktivitäten nicht nur zwischen unterschiedlichen, sondern auch innerhalb einer Art und zwischen verschiedenen Riffhabitaten beachtlich variieren kann, ist das Verständnis über den Einfluss der Futterverfügbarkeit auf die Anfälligkeit zu bleichen von großer Bedeutung. Dies wiederum kann wertvolle Informationen bezüglich möglicher ökologischer Folgen von Temperaturerhöhungen auf die Strukturen von Korallengemeinschaften in Abhängigkeit der Wasserqualität liefern.

Zerstörte Korallenriffe können sich nicht immer auf natürliche Weise regenerieren, was zur Folge hat, dass rigorose Korallenriffrestaurationsmaßnahmen notwendig sind. Solche Maßnahmen beinhalten die Transplantation von Korallen und die Konstruktion von künstlichen Riffen. Eine Riffdegradierung, die als Konsequenz einer Massenkorallenbleiche auftritt, kann auf Grund ihrer Großflächigkeit durch Restaurationsmaßnahmen nicht aufgefangen werden. Der Fokus von Riffrestaurationsmaßnahmen liegt infolgedessen weitestgehend auf durch anthropogene Einflüsse degradierten Riffregionen, deren Ausmaß grundsätzlich geringer ist.

Die Interaktionen zwischen dem künstlichen Substrat, der Ökologie der transplantierten Korallen und ihrer Umgebung ist jedoch noch unzureichend erforscht. Oft sind die Transplantationen von Korallen durch hohe Mortalitäts- und niedrige Wachstumsraten gekennzeichnet. Die Technologie der Mineralienablagerung wird häufig in der Korallenriffrestauration eingesetzt und umfasst die Anreicherung von Ionen in der unmittelbaren Umgebung einer Unterwasserkathode. Dies führt zu einem Niederschlag von Karbonat auf der Kathode, welche den transplantierten Korallen als Substrat dient. Es wird vermutet, dass die elektrochemischen Prozesse an der Kathode durch verstärkte Substratstabilisierung infolge der Ablagerung von Mineralien und einer erhöhten metabolischen Effizienz zu erhöhten Überlebens- und Wachstumsraten führt. Trotz weltweit wachsender Popularität der elektrischen Riffe ist die aktuelle Beweislage für die mutmaßlichen positiven Auswirkungen dieser Technologie auf das Wachstum und die Mortalitaet unfundiert.

Die Ziele dieser Arbeit waren daher wie folgt: 1) Untersuchung der Hypothese, dass eine exogene Nahrungszufuhr die Temperaturtoleranz der Photosynthese der Zooxanthellen erhöht, 2) Evaluierung der Auswirkungen der Mineralienablagerungstechnologie, die erhöhte Wachstums- und Überlebensraten zur Folge haben soll. Um den Effekt der Heterotrophie auf die Temperaturtoleranz der symbiotischen Assoziation zu untersuchen, wurden zwei Fütterungsversuche an der Hasanuddin University Marine Field Station in Südwest-Sulawesi, Indonesien, zwischen Juni und Oktober 2005 durchgeführt. In beiden Experimenten wurden die Korallen entweder ausgehungert, d.h. Partikel >0,5 µm wurden ausgeschlossen, oder täglich mit frisch geschlüpften *Artemia salina*-Nauplien gefüttert. In Experiment 1 wurde der Effekt der exogenen Nahrungsergänzung (Zooplankton) auf die Bleichempfindlichkeit, die photosynthetische Aktivität des Photosystem II (PSII) und das Non-photochemical quenching (NPQ: die Ableitung überschüssiger Energie als Waerme) von Zooxanthellen in der temperatursensitiven Art *Stylophora pistillata* unter erhöhten Temperaturen über einen

Zeitraum von 10 Tagen mit Hilfe von Chlorophyll-Fluorometrie ermittelt. In Experiment 2 wurden die potentiellen Interaktionen zwischen der Aufnahme von Zooplankton, den grundlegenden metabolischen Funktionen (Photosynthese und Respiration), dem Energiezustand (Lipidgehalt), dem Gesamtproteingehalt und der Bleichempfindlichkeit von *S. pistillata* und der temperaturtoleranteren Art *Galaxea fascicularis* über einen Zeitraum von 15 Tagen analysiert.

Die Effekte der Mineralablagerung auf die Physiologie und Mortalitätsraten von Korallentransplantaten wurden von April bis Juli 2006 an den Arten *Acropora yongei* und *A. pulchra* in Nord-Sulawesi, Indonsien, untersucht. Um zwischen den Effekten unmittelbar an der Kathode und denen eines elektrischen Feldes auf das Wachstum und die Mortalität der Korallentransplantate zu unterscheiden, wurden die Transplantate über einen Zeitraum von vier Monaten den folgenden drei verschiedenen Behandlungen ausgesetzt: 1) Direkter Kontakt mit der Kathode, 2) Verpflanzung auf Bambus in einem elektrischen Feld, 3) Verpflanzung auf Bambus außerhalb der experimentellen Matrix (Kontrolle). Am Ende des Versuches wurden die Überlebensraten, das Wachstum, die Zooxanthellenpopulationparameter und die Chlorophyllfluoreszenz der beiden Arten bestimmt.

Die Ergebnisse zeigen, dass die Temperaturtoleranz in Bezug auf photosynthetische Aktivität und Pigmentierung beider Arten an die Verfügbarkeit von Zooplankton gekoppelt war. Die Zooxanthellen in gefütterten Korallen hielten ihre photosynthetische Aktivität aufrecht, wohingegen ausgehungerte Korallen deutliche Anzeichen chronischer Photoinhibition zeigten. Dies manifestierte sich in einem fortschreitenden Verlust der maximalen Quantenausbeute (F_v/F_m) von PSII sowie nächtlicher Regenerationsraten von PSII, die durch eine Reduzierung der Parameter F_v/F_m, effektiver Quantum yield ($\Delta F/F_m'$) sowie relative elektronische Transportrate (rETR) gekennzeichnet waren. Zudem verringerte sich die Bruttoprimärproduktion (Pg) während des Verlaufes von Experiment 2. Parallel dazu nahm die Fähigkeit zum NPQ ab. Im Vergleich dazu konnten gefütterte Korallen ein höheres Niveau an NPQ aufrechterhalten, was mit signifikant höheren nächtlichen Regenerationsraten von PSII korrellierte. Nach 10 und 15 Tagen war das Gewebe beider Arten, welche ausgehungert worden, bleich, was den höheren Verlust in Pigmentierung in ausgehungerten Korallen im Gegensatz zu gefütterten Korallen reflektiert. Während die Abnahme der Pigmentierung in ausgehungerten *S. pistillata* vorwiegend durch die Reduzierung von Zooxanthellen gekennzeichnet war, war dies in ausgehungerten *G. fascicularis* neben der Reduzierung von Zooxanthellendichten auch auf eine Abnahme des Chlorophyllgehaltes zurückzuführen. Des Weiteren waren die mitotischen Indexe der Zooxanthellen in gefütterten *S. pistillata* signifikant höher als in ausgehungerten Korallen, was auf eine Zersetzung der Zellstruktur der Symbionten in ausgehungerten *S. pistillata* schließen lässt.

Die Respirationsraten von *S. pistillata* über eine Periode von 15 Tagen zeigten einen ähnlichen Trend wie die photosynthetischen Aktivitäten. Während die Aushungerung eine signifikante Abnahme in Sauerstoffverbrauch zur Folge hatte, blieben die Respirationsraten in gefütterten Korallen unverändert. Im Gegensatz dazu waren keine signifikanten Veränderungen in Respirationsraten zwischen gefütterten und ausgehungerten *G. fascicularis* zu beobachten. Darüber hinaus war der Lipidgehalt in ausgehungerten *S. pistillata* signifikant geringer als in gefütterten Korallen, während die Zooplanktonaufnahme keine Auswirkungen auf den Lipidgehalt in *G. fascicularis* hatte. Der Proteingehalt in beiden Arten war signifikant geringer in gefütterten als in ausgehungerten Korallen. Zusammengefasst deuten die Ergebnisse dieser beiden Experiment darauf hin, dass die exogene Nahrungsergänzung den photophysiologischen Schaden in Zooxanthellaen, welche charakteristischerweise zur Korallenbleiche führt, reduzieren kann.

Dies kann positive Konsequenzen für Korallengemeinschaften in trüben, küstennahen Gewässern haben, da diese oft eine potentiell nährstoffreiche Umgebung darstellen.

Die drei wichtigsten Ergebnisse der Studie zur Untersuchung der potentiellen Mechanismen, die zu erhöhten Überlebensraten und erhöhtem Wachstum in einer mineralreichen Umgebung führen, zeigten dass, 1) die höchsten Wachstumsraten beider Arten in dem elektrischen Feld und nicht in der mineralstoffreichen Umgebung der Kathode stattfanden, 2) die Kathode einen negativen Effekt auf Wachstum und generelle Gesundheit von Korallen haben kann, und dass 3) die Eignung der Mineralablagerungstechnologie in Bezug auf Korallentransplantation stark auch zwischen verwandten Arten variieren kann. Im Gegensatz zu vorherigen Studien hatte die Mineralablagerung in dieser Studie entweder keinen oder sogar einen negativen Effekt auf die Überlebensraten über einen Zeitraum von 4 Monaten. Die Mortalitätsraten von *A. yongei* nach 4 Monaten waren signifikant höher für die Korallen, welche auf der Kathode wuchsen, verglichen mit den Korallen in dem elektrischen Feld und denen in der Kontrolle. Im Unterschied dazu, waren keine signifikanten Unterschiede in Mortalitätsraten zwischen den drei Behandlungen für *A. pulchra* zu beobachten. Hohe Mortalitätsraten von *A. yongei* auf der Kathode gingen einher mit den niedrigsten Wachstumsraten, die für *A. yongei* gemessen wurden, während die niedrigsten Wachstumsraten von *A. pulchra* in der Kontrollbehandlung gemessen wurden. Außerdem zeigten die Ergebnisse der Fluoreszenzmessungen, dass F_v/F_m und rETRs der Zooxanthellen in *A. yongei* auf der Kathode signifikant niedriger waren als in *A. yongei* in dem elektrischen Feld und der Kontrolle. Demgegenüber wurden keine signifikanten Unterschiede dieser Parameter wurden für Zooxanthellen in *A. pulchra* gemessen. Die Ergebnisse zeigen, dass sich das unmittelbare Umfeld der Kathode negativ auf den allgemeinen Gesundheitszustand von *A. yongei* auswirkte. Dies wird zusätzlich dadurch unterstrichen, dass die Zooxanthellendichten von *A. yongei* auf der Kathode signifikant niedriger waren als in Korallen in dem elektrischen Feld und in der Kontrolle. Im Gegensatz dazu wurden die höchsten Zooxanthellendichten in *A. pulchra* in den Korallen auf der Kathode gemessen. Die Ergebnisse widerlegen die mutmaßlichen Vorzüge der Mineralablagerungstechnologie als eine effiziente Methode der Riffrestauration.

Zusammengefasst zeigen die Ergebnisse dieser Arbeit die komplexe Natur der Interaktionen zwischen der photosynthetischen Aktitität von *in hospite* Zooxanthellen, der assoziierten Fitness von Korallen und den vorherrschenden Milieubedingungen. Weiterhin illustrieren die Ergebnisse, dass eine hohe physiologische Variabilität sowohl zwischen wie auch innerhalb der Arten bestehen kann, was die Komplexität dieser Interaktionen weiter erhöht. Dies unterstreicht, dass sowohl die Prognosen der ökologischen Konsequenzen von Korallenbleichen als auch die Maßnahmen zur Riffrestauration einen multivariaten Ansatz benötigen und folglich ein fundiertes Wissen der Ökologie der involvierten Korallenarten voraussetzen.

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*

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Overview

Coral reefs are diverse and productive biological communities which thrive in shallow tropical marine environments. Scleractinian corals form an obligate symbiosis with endocellular dinoflagellates, mostly of the genus *Symbiodinium* (Coffroth and Santos 2005), commonly referred to as zooxanthellae. This symbiotic association is an essential attribute of modern coral reefs (Glynn 1996), allowing corals to thrive in tropical waters (Muscatine and Porter 1977, Rahav et al. 1989) which are generally very low in dissolved inorganic nutrients (oligotrophic) with concentrations ranging between 0.1-0.5 µM nitrate, 0.2-0.5 µM ammonium and less than 0.3 µM phosphorous (Furnas 1991). The zooxanthellae are located in vacuoles within the endoderm cells of the host where they mediate the flux of carbon and nutrients between host and the environment (Trench 1993, Muscatine 1990). At a density of more than 10^6 cells cm^{-2} of coral skeletal surface, depending on species and geographical area, zooxanthellae are among the dominant primary producers in tropical reef communities (Muscatine 1990). Scleractinian corals are essentially mixotrophs. Although they receive the bulk of metabolic energy requirements, and other important organic compounds, from photosynthetically fixed carbon translocated by the zooxanthellae, they are also active suspension feeders and may obtain additional nutrients and energy from captured prey and suspended organic particles (e.g. Falkowski et al. 1984, Sebens et al. 1996, Anthony and Fabricius 2000). While scleractinian corals are not necessarily the most abundant or diverse faunal components of coral reefs, they produce the majority of the habitat structure for other reef organisms (Richmond 1993).

Traditionally, coral reefs were thought of as diverse oases in an oceanic desert, with the prevailing opinion that reefs were closed, fragile climax systems found in areas with only little environmental fluctuation (Hatcher 1997). This perception has altered over time and coral reefs are now considered as dynamic systems subject to natural disturbances (Connell 1997, Buddemeier and Smith 1999). However, over the past few decades the nature and temporal pattern of disturbances have changed severely coinciding with global climate change (Hoegh-Guldberg 1999) and increasing anthropogenic activities in coastal areas (Richmond et al. 1993). The extent of disturbances often exceed the regenerative capacity of coral reefs and have lead to vast reef degradation and a worldwide reduction of biodiversity (Sebens 1994, Bellwood et al. 2004).

Although the symbiotic association can be seen as a necessary adaptation to oligotrophic conditions, its functional persistence is highly labile. Various environmental stressors can lead to the break-down of the symbiosis (bleaching) including, but not limited

to, deceased salinity (Coles and Jokiel 1992), temperature anomalies (Gates et al. 1992, Hoegh-Guldberg 1999), sedimentation (Bak 1978), competition with benthic algae (Titlyanov et al. 2007), solar radiation (Brown et al. 1994) and cyanide (Jones et al. 1999). Since the photosynthetic potential of a coral invariably dictates its success, any reduction in the photosynthetic performance of the colony is an ecologically significant response as it reduces coral fitness, which often culminates in the death of the coral host (Glynn and D'Croz 1990). Reef corals have long been described as living at temperatures near their upper thermal tolerance limits (Mayer, 1914; Edmondson, 1928 *loc cit* Fitt et al. 2001), and maximum temperatures that have occurred in the tropics over the past two decades have coincided with episodes of coral bleaching that exceeded previous bleaching events in both frequency and magnitude (Coles and Brown 2003). The ecological consequences of coral bleaching in response to increasing seawater temperatures have thus become an accelerating environmental concern over the past decade (Hoegh-Guldberg 1999, Donner et al., 2005). An increase in the world's mean ocean temperature due to global warming (Pittock, 1999) has resulted in recurrent widespread mass bleaching and concomitant coral mortality, which has lead to a significant decline of coral reefs around the world (Fitt et al. 2001).

Growing concern about the structural integrity of coral reefs has also arisen from increased anthropogenic activities including coastal development, sewage, dredging, ship groundings, dynamite fishing and damage by tourists (Richmond 1993). Especially in countries in Southeast Asia, such as Indonesia, which has more coral reef than any other country, the pressure on coral reefs is increasing exponentially as a result of a rapid population increase and weak management capacity (Bryant and Burke 1998, Tomascik et al. 1997, Cesar et al. 1997). The fatal changes to Indonesian reefs over the last century is well captured by a description of the marine community around the resort island of Leiden (now Nyamuk besar) in Jakarta Bay. In 1928 Umbrove wrote (*loc cit* Tomascik et al. 1997): 'The unrivalled splendour and wealth of forms and the delicate tints of the coral structures, the brilliant colours of fishes, clams and sea anemones, worms, crabs, star fish and the whole rest of the reef animals are so attractive and interesting that it seems impossible to give an adequate description of such a profusion of serene and fascinating beauty.' By 1985, the coral cover around this island was down to less than 1% of the previous cover (Scoffin 1986, Tomascik et al. 1997).

1.1. The symbiosis

Photosynthetically fixed carbon constitutes the principal source of energy covering up to 100% of the coral's daily energy requirements (e.g. Falkowski et al. 1984, Muscatine et al. 1984, Davies 1984, Muscatine 1990). Moreover, zooxanthellae play an essential role in the conservation of limited nutrients, especially nitrogen (Rahav et al. 1989), as well as synthesis of fatty acids, and supplying the host with essential amino acids and metabolites necessary for lipogenesis (Kellogg and Patton 1983, Harland et al. 1993, Markell and Trench 1993, Wang and Douglas 1999, Oku et al. 2003, Shick et al. 2005). Furthermore, zooxanthellae photosynthesis significantly enhances coral calcification (Gattuso et al. 1999); in fact, without the coral–algal symbiosis there would be no coral reefs as we know them today. Scleractinian corals comprise one of the major calcifying groups of animals (Chave et al. 1975), representing almost half of the world's $CaCO_3$ precipitation (Smith 1978). The exact mechanism underlying photosynthesis enhanced (light enhanced) calcification is still not fully understood, but recent evidence suggests that photosynthesis plays an important role in balancing the pH inside the coelenteron of the coral, and supplies precursors for the organic matrix which is associated with skeletal formation (Allemand et al. 1998, Moya et al. 2006).

A second potential energy source for the coral host is heterotrophic nutrition. Corals are passive suspension feeders with the potential for utilizing a wide range of food sources including suspended particulate matter (Anthony 1999, Anthony and Fabricius 2000), sediment (Stafford Smith and Ormond 1992, Rosenfeld et al. 1999), bacteria (Sorokin 1991, Bak et al. 1998) and zooplankton (Johannes et al. 1970, Lewis 1992, Sebens et al. 1996, Ferrier-Pages et al. 2003). The classic experiment by Yonge and Nicholls (1931) showed that when aposymbiotic corals (corals without zooxanthellae) were fed with zooplankton, they survived equally well relative to their symbiont containing conspecifics.

Although the amount of fixed carbon released by the zooxanthellae to the coral host can potentially satisfy the animal's daily respiratory carbon requirements, the relative contribution of zooxanthellae to the host's metabolic needs will ultimately depend on photosynthetic rates, which can vary with temperature (Al-Sofyani and Davies 1992, Castillo and Helmuth 2005), symbiont numbers (Houlbreque et al. 2004), chlorophyll $a+c_2$ (chl $a+c_2$) concentrations and light (McCloskey and Muscatine 1984, Porter et al. 1984, Anthony and Hoegh-Guldberg 2003), as well as food available to the coral host (Taylor 1978, Szmant-Froelich 1981, Cook et al. 1992, McAuley and Cook 1994, Houlbreque 2003, 2004, Davy et al. 2006). In clear, shallow waters, autotrophy alone may meet all the nutritional requirements (Falkowski et al. 1984) while corals living in deeper water or turbid environments are more

dependent on heterotrophy (Tomascik and Sander 1985, Anthony 2000, Fabricius and Domisse 2000). Furthermore, it has been shown that some species can exhibit great trophic plasticity and are capable of adjusting to changes in light conditions and water quality (Wellington 1982, Anthony and Fabricius 2000).

The relative importance of autotrophic versus heterotrophic nutrition has been a source of considerable controversy (Goreau et al. 1971, Porter 1976, Wellington 1982, Edmunds and Davies 1986, Sebens and Johnson 1991, Lesser et al. 2000), but it is generally accepted that particulate food, and zooplankton in particular, provides a major source of essential nutrients (Corner and Davies 1971, Kellogg and Patton 1983, Rahav et al. 1989, Sebens et al. 1996, Fitt and Cook 2001, Ferrier-Pages et al. 2003). Probably the most important of these essential nutrients is nitrogen, which is excreted as ammonium by the host (Rahav et al. 1989, Falkowski et al. 1993) and then assimilated via the glutamine synthetase-glutamate synthase pathway by the zooxanthellae (McAuley and Cook 1994, Yellowlees et al. 1994). Contrary to the traditional perception that nutrients derived from prey capture are available to the zooxanthellae only after host catabolism, prey nitrogen digested within the coelenteron has been shown to be able to be taken up directly by the zooxanthellae (Piniak et al. 2003). Zooxanthellae may either use this nitrogen for growth (Rahav et al. 1989, McAuley and Cook 1994, Fitt and Cook 2001) or release some of it back to the host in the form of amino acids (Sutton and Hoegh-Guldberg 1990, Wang and Douglas 1998).

Thus the mixotrophic feeding ecology of the intact holobiont (coral – zooxanthellae association) can be summarized as a semi-closed system where the nutrient (nitrogen) and energy (carbon) status of both zooxanthellae and host is the combined function of photosynthesis and the recycling of excretory nitrogen compounded by host feeding (Fig. 1).

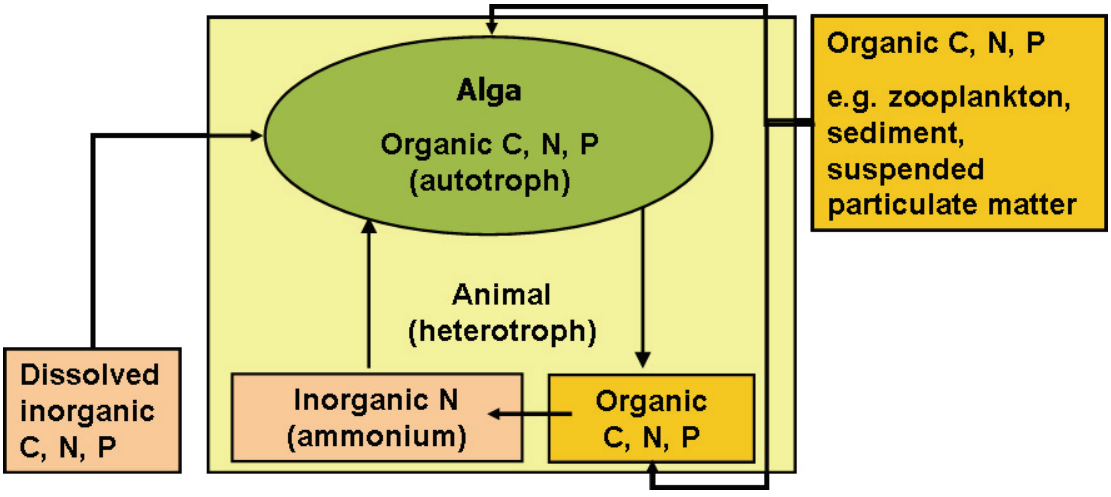

Fig. 1 Schematic illustration of the cycling of organic and inorganic nutrients (C = carbon, N = nitrogen, P = phosphate) within the holobiont.

1.2. Coral bleaching

The dissociation of the coral host and it's zooxanthellae, a classic stress response (Brown 1988), is referred to as coral bleaching and was first described in detail by Yonge and Nicholls (1931a) as a reduction in cellular densities of symbiotic zooxanthellae in corals that had been exposed to elevated temperature at Low Islands, Great Barrier Reef, Australia. Today, the term bleaching pertains to both the loss of zooxanthellae and/or loss of chlorophyll (chl) (Glynn 1993) and has been most frequently linked to increases in seawater temperatures. While bleached corals following warm-water bleaching events typically contain lower zooxanthellae densities than unbleached corals (e.g. Porter et al. 1989, Fitt et al. 1993, Fitt and Warner 1995, Jones 1997, Jones and Yellowlees 1997), analyses of zooxanthellae chl concentrations have produced equivocal results. Some studies (e.g. Kleppel et al. 1989, Porter et al 1989, Jones 1997) found that zooxanthellae of bleached corals contained lower chlorophyll a (chl a) concentrations while others (Hoegh-Guldberg and Smith 1989, Fitt et al. 1993, Edmunds et al. 2003) reported that bleached corals exhibited higher chl a concentrations compared to unbleached corals.

Global warming has resulted in an increase of the world's mean ocean temperature by approximately 0.5°C over the last century (Pittock, 1999), and various atmospheric models predict another l-3% rise worldwide by the mid-21st century (Boesch et al., 2000, Donner et al. 2005). The most widespread and severe bleaching occurred during 1997-1998 (El Nino) when reefs in over 42 countries were affected, with massive coral mortality being recorded in southern Japan (Fujiokka 1999), Sri Lanka, the Maldives, India, Kenya, Tanzania, the Seychelles, and various other sites in the IndoPacific (Wilkinson et al. 1999).

Since zooxanthellae provide photosynthetically fixed carbon (Muscatine 1990), mediate elemental nutrient flux (Rahav et al. 1989) and enhance calcification (Gattuso et al. 1999), bleaching significantly reduces coral fitness, often resulting in the death of the coral host (Glynn and D'Croz 1990). Typical consequences of prolonged bleaching include reduced coral growth (Goreau and McFarlane 1990), reduced tissue biomass and fecundity (Porter et al. 1989, Szmant and Gassman 1990, Fitt et al. 1993) and reduced lipid concentrations (Grottoli et al. 2004, Bachok et al. 2006).

1.3. Photoinhibition precedes the bleaching response

The molecular, cellular, and physiological mechanisms associated with coral bleaching are yet nut fully understood. Since the discovery that zooxanthellae photosynthesis is significantly reduced by elevated temperature, however, considerable attention in recent years has been directed towards research of the symbionts (Warner et al. 1996, Jones et al. 1998, Warner et al. 1999, Bhagooli and Hidaka 2003, Takahashi et al. 2004). The reduction in photosynthetic efficiency translates into a decline in population of functional reaction centres via a process known as photoinhibition (Long et al. 1994) and precedes the loss of algae from the coral tissue (Franklin et al. 2004). Elevated temperature can act at one or more levels of the photosynthetic machinery, alone or in synergy with light (Lesser and Farrell 2004), leading to suppressed Calvin cycle activity (Crafts-Brander and Salvucci 2000, Jones et al. 1998) and degradation of the photosystem II (PSII) D1protein (Warner et al. 1999, Jones and Hoegh-Guldberg 2001, Takahashi et al. 2004). This results in an over-reduction of the electron transport chain (Jones et al. 1998), and concomitant decrease in rates of photosynthesis, which in turn increases the extent to which absorbed light becomes excessive (Demmig-Adams and Adams 1996). This process leads to an increased production of reactive oxygen species (ROS) within the symbionts (Lesser 1997), which have been implicated as a principal cause of thermal bleaching (Brown 1997, Fitt et al. 2001, Brown et al. 2002a, Franklin et al. 2004, Lesser 2004, Tchernov et al. 2004).

Photoinhibition may be dynamic or chronic in nature (Long et al. 1994). Dynamic photoinhibition denotes *reversible* inhibition of photosynthesis and falls under the category of protective mechanisms. Just like higher plants, zooxanthellae are able to regulate excess excitation energy through photoprotective non-photochemical quenching (NPQ) processes. A major NPQ mechanism is associated with xanthophylls whereby cycle-dependent thermal energy dissipation of excess light occurs within the light-harvesting complex via the de-epoxidation of the xanthophyll cartenoids diadinoxanthin to diatoxantin (Ambarsari et al. 1997, Brown et al. 1999). This process alleviates PSII from excitation pressure and as such wards off oxidative damage to the photosynthetic apparatus under bleaching conditions (Long et al. 1994). However, the level of photoinhibition can increase by positive feedback, which may lead to chronic photoinhibition. Chronic photoinhibition refers to *non-reversible* photodamage, which occurs when the capacity of photoprotective mechanisms are exhausted ensuing in damage to PSII reaction centres. The level of chronic photoinhibition is determined by differential rates of photodamage versus protein repair and usually requires *de novo*

synthesis of the D1 reaction centre protein, in order to resume photochemistry (Kyle 1987, Warner et al. 1999, Takahashi et al. 2004).

1.4. Bleaching differences

The ecological consequences of coral bleaching are difficult to quantify and predict because thermal tolerances and resulting bleaching susceptibility of corals for any given bleaching event is not uniform, but can vary greatly, not only between but also within species (Edmunds 1994, Hoegh-Guldberg and Salvat 1995, Marshall and Baird 2000, Fitt et al. 2001, Loya et al. 2001, Stimson et al. 2002, Brown et al. 2002b, Douglas 2003). The functional mechanisms underlying these variations are the subject of considerable controversy. In the past, differences in bleaching susceptibility were largely addressed within the context of either (i) differential stress responses in the coral host (Gates et al. 1992) or (ii) the varying thermal tolerances of different genetic strains of zooxanthellae (Baker et al. 1997, Rowan et al. 1997). However, extensive research in this field over the past decade, bridging gaps between molecular biology and ecophysiology, reveal that thermal tolerance and associated bleaching is not a unilateral stress response but rather a function of the combined relative stress tolerances of the algal versus the animal components of the symbiosis.

Cellular oxidative stress, albeit broadly associated with the endosymbiont, has been shown to occur simultaneously in the host and zooxanthellae (Lesser and Shick 1989, Downs et al. 2002). Although ROS are predominantly produced in the chloroplasts, hydrogen peroxide can diffuse into the coral cytoplasm where it is either neutralized via antioxidant processes or further converted into potent hydroxyl radicals. Elaborate biochemical defences against oxidative stress and associated cellular damage exist in all cells of both corals and zooxanthellae, including antioxidant enzymes (Lesser and Shick 1989, Shick et al. 1995, Nii and Muscatine 1997, Brown et al. 2002a, Downs et al. 2002, Richier et al. 2005, Levy et al. 2006), heatshock proteins, also referred to as ‘stress proteins’ (Black et al. 1995, Gates and Edmunds 1999, Brown et al. 2002a, Downs et al. 2002), and certain mycosporine-like amino acids (Yakovleva et al. 2004). A strong positive correlation between reduced thermal stress and high rates of antioxidant enzyme activity has been demonstrated by several authors (Brown et al. 2002a, Down et al. 2002, Yakovleva et al. 2004) and there is evidence of a quantitative relationship between concentrations of heatshock proteins and thermal tolerance in the coral host (Landry et al. 1987). A study by Richier et al. (2005) indicated that antioxidant enzymes in the sea anemone *Aiptasia viridis* may in fact protect its symbionts from oxidative damage under thermal stress.

At the physiological and behavioural level, host thermal sensitivity may be explained in terms of morphology *sensu* metabolic rates and tissue thickness. Loya et al. (2001) documented a positive correlation between bleaching severity and colony morphology. A survey carried out one year after the mass bleaching event in 1998 at Sesoko Island, Japan revealed that branching, fast growing and thin-tissued corals such as *Acropora* and pocilloporid species were significantly more impacted than massive, slow growing or thick-tissued species. Such variation is believed to result from: 1) differences in metabolic rates, i.e. fast growing species, with lower metabolic rates will have lower rates of protein turnover than slow growing species, and therefore reduced capacity to acclimatize (Gates and Edmunds 1999), or 2) differences in tissue retraction, i.e. polyp contraction of thick-tissued species can provide rapid protection against irradiation by moving zooxanthellae deep into coral calices (Brown et al. 2002c). A protective function of the host in the stress susceptibility of zooxanthellae was also suggested by Bhagooli and Hidaka (2003), who recorded conspicuous differences in photosynthetic efficiency among *in hospite* and isolated zooxanthellae of five coral species during exposure to increased temperature. Other important factors, which could produce varying spatial effects include small scale hydrodynamics. Enhanced water flow is thought to increase mass transfer processes, which facilitates the removal of oxygen from coral tissue during periods of maximal photosynthesis (Nakamura and van Woesik 2001, Finelli et al. 2006). Furthermore, variation in coral tissue darkness can create a microenvironment in temperature differences between corals and may thus lead to differential bleaching of conspecifics in the same location (Fabricius 2006).

1.5. Coral reef restoration

In recognition that many destroyed coral reefs do often not recover naturally without manipulation, the scientific discipline of reef restoration has drawn much attention over the past decade (Rinkevich 2005). While reef degradation resulting from mass coral bleaching is often so widespread and catastrophic in extent that restoration efforts are not feasible, considerable efforts are put into the restoration of coral reef environments subjected to anthropogenic disturbances and degradation, which generally occur over much smaller scales (Spieler et al. 2001).

Various restoration methods aimed at improving live coral cover, biodiversity, and topographic complexity of denuded parts of reef, have been proposed to date including the construction of artificial reefs. These involve the transplantation of coral nubbins, fragments or whole coral colonies onto three- dimensional artificial substrates, often comprised of

concrete or natural rock (Clark 2002, Zimmer 2006). Coral transplantation as a means of accelerating reef recovery is, however, only effective if coral transplants feature high survival rates and grow fast.

Despite considerable research efforts in this field, there is still much uncertainty concerning the interactions between artificial substrates, the ecology of transplanted organisms, and the environment into which they are placed, often resulting in high mortality and low growth rates of the coral transplants (Clark 2002, Rinkevich 2005). Firm attachment of coral transplants, even in low energy environments, is a prerequisite for high survivorship as securely fixed corals are likely to recover faster from the stress of transplantation as they may allocate more energy to lesion repair and subsequent growth (Bowden-Kerby 1997, Lindahl 1998, Ammar et al. 2000). Furthermore, it has been shown that the survival rates of corals are greatly influenced by the initial size of the transplants. Small coral fragments usually exhibit higher mortality rates than larger transplants or even whole colonies (Rinkevich 2005). Removal of whole colonies, however, invariably entails a substantial loss of coral material from the donor reef area (Edwards and Clark 1998). In an attempt to minimize this negative impact, several workers have promoted the use of coral nurseries or coral farms, where sexually or asexually produced juveniles are reared to a certain size in a protected environment before being transplanted to the recipient site; an effort which is very labour intensive and time consuming (Rinkevich 1995, Bowden-Kerby 1997, Oren et al. 1997, Franklin et al. 1998, Heeger et al. 1999, Borneman & Lowrie 2001, Epstein et al. 2001, Omori & Fujiwara 2004).

The mineral accretion technology described by Hilbertz (1992) has been advocated as an effective method for coral reef rehabilitation, proposing to increase survival, growth, and coral fitness through reinforced substrate stabilization and enhanced metabolic efficiency of the coral transplants (Hilbertz and Goreau 1996, Goreau et al. 2004).

The principle of this technology follows that of a galvanic cell, which involves passing a low voltage of direct electrical current through a cathode and an anode to induce electrolysis of the seawater. Seawater is split into hydrogen gas and two molecules of hydroxide anions leading to a rise in pH around the cathode, onto which the corals are transplanted. Ca^{2+} and Mg^{+} combine with dissolved HCO_3^- and OH^- and precipitate as $CaCO_3$ (aragonite) or $Mg(OH)_2$ (brucite) on the cathode (Hilbertz 1992). Coral fragments on the cathode are thus quickly cemented to the structure by the accreted carbonate material (van Treeck and Schuhmacher 1997) which has previously been demonstrated to enhance survival of *Porites cylindrica* transplants (Sabater and Yap 2002, 2004).

Since zooxanthellae photosynthesis and coral calcification both require a continuous supply of inorganic carbon and share HCO_3^- and CO_2 as major substrates (de Beer et al. 2000, Furla et al. 2000a, Al-Horani et al. 2003), competition for inorganic carbon has been suggested to lower the rate of calcification (Marubini and Thake 1999). Furthermore at ambient seawater Ca^{2+} concentrations, calcification rates of some species are saturated (Chalker 1976, Tambutte et al. 1996) while Ca^{2+} appears to be limiting in others (Chalker 1976, Krishnaveni et al. 1989). Based on the assumption that the calcification process is greatly affected by the availability of mineral ions in the surrounding seawater, the electrolysis of seawater and resulting increase in concentrations of mineral ions in the external seawater medium are thought to increase calcification and thus accelerate skeletal growth (Hilbertz and Goreau, 1996). In an US patent, Hilbertz and Goreau (1996) specifically proposed the following three hypotheses of underlying mechanisms responsible for increased survival and growth of corals under a low current state: 1) the electric field enables carbonate accretion and causes the precipitated carbonates to attach directly to the coral skeleton of the transplants leading to increased survival rates of the transplants; 2) a low current induces an increase in the concentration of mineral ions in the immediate vicinity of the coral thereby enhancing natural calcification; and 3) excess production and release of electrons due to electrochemical processes might provide extra electrons for ATP production thereby enhancing metabolic efficiency of the organism leading to increased growth and fecundity.

2. Scope of this thesis

Within the above context, using different methodological approaches, this thesis investigates coral performance with particular relevance to the photophysiology of in *hospite* zooxanthellae in responses to challenging environmental conditions. **Paper I** and **II** deal with the issue of thermally induced bleaching and food availability as a potential factors contributing to thermal tolerance of corals. **Paper III** investigates coral performance in response to different reef restoration methods.

The effect of zooplankton feeding on the photophysiology and thermal tolerance of scleractinian corals

The expression and regulation of biochemical defences and resulting potential to adapt or acclimatize to stressful environmental conditions underlie the intrinsic protein metabolism of the holobiont (Gates and Edmunds 1999, Coles and Brown 2003, Koehn and Bayne 1989). High protein turnover, measured as the sum of protein degradation and synthesis required to maintain cellular homeostasis, however, incurs high metabolic costs in terms of nitrogen demands and energy expenditure (Koehn and Bayne 1989). Thus, under bleaching conditions where the photosynthetic performance of the coral colony is greatly reduced (Jones et al. 2002, Warner et al. 2002), nutritional interactions of the symbiotic association are likely to become corrupted, potentially rendering the coral host into a state of starvation, which can lead to changes in biochemical composition and reduced energetic status of the colony (Glynn and D'Croz 1990, Grottoli et al. 2004, Szmant and Gassman 1990, Fitt et al. 1993, Bachok et al. 2006). While it is well established that under non-bleaching conditions, food availability tends to increase protein levels of host tissue, zooxanthellae densities, chl a concentrations (Titlyanov et al. 2001, Ferrier-Pages et al. 2003, Houlbreque 2003, 2004) and rates of photosynthesis (Houlbreque et al. 2003, Davy et al. 2006), the interaction between food availability, coral tissue composition and physiological functioning of corals under thermal stress is unknown. Understanding how food availability influences bleaching susceptibility and severity could, however, provide important information aiding the prediction of the effects of increased temperature on coral community structure along environmental gradients. Coastal reefs, for example, often feature high loads of suspended particulate matter and resuspended sediment, which on one hand reduces the light for photosynthesis (Rogers 1990) but on the other offers a rich source of nutrients and additional energy (Anthony and Fabricius 2000, Anthony 2006). The rationale of **paper I** (experiment 1) and **paper II** (experiment 2)

was to test the hypothesis that exogenous food increases the thermal tolerance of zooxanthellae photosynthesis through the provision of additional resources required for metabolic processes involved in protein synthesis or repair and the photoprotective mechanisms of the holobiont (coral-symbiont association).

The aim of experiment 1 (**paper I**) was to investigate the effect of zooplankton feeding on bleaching susceptibility (loss of zooxanthellae and/or chl), photosynthetic activity of PSII and NPQ kinetics of zooxanthellae in the coral *Stylophora pistillata*, a thermally sensitive species (Yakovleva et al. 2004), under elevated temperature, employing pulse-amplitude-modulated (PAM) fluorometry. Specifically, the following hypotheses were tested: (1) PSII of zooxanthellae in fed corals will maintain higher photosynthetic efficiency during prolonged exposure to elevated temperatures than PSII of zooxanthellae in starved corals; (2) zooxanthellae in fed corals will retain higher levels of NPQ during prolonged exposure to elevated temperatures than zooxanthellae in starved corals; (3) fed corals will have higher zooxanthellae densities, higher mitotic indices, and higher chlorophyll a and c_2 concentrations per algal cell than zooxanthellae in starved corals following exposure to thermal stress.
The objective of experiment 2 (**paper II**) was to examine the functional interactions between food availability, basal metabolic functions (photosynthesis and respiration), energy status (lipid concentrations), protein concentrations and the bleaching susceptibility (loss of zooxanthellae and/or chl) of two scleractinian coral species, *S. pistillata* and the more temperature tolerant species *Galaxea fascicularis* (Stimson et al. 2002, Bhagooli and Hidaka 2003) from a turbid inshore reef.

The effect of seawater hydrolysis on the coral photosynthetic performance and skeletal growth

Despite increasing popularity of 'electric' reefs as a tool for reef restoration world wide (e.g. Schuhmacher et al. 2000, Goreau et al. 2004, various reports by Goreau, unpublished), quantitative evidence supporting the hypotheses of electrochemically enhanced survival, growth and increased coral fitness remain largely anecdotal. It is well established that coral calcification is closely coupled to photosynthetic processes of the zooxanthellae (Furla et al. 2000, Moya et al. 2006). In order to evaluate the potential underlying mechanisms of enhanced survival and skeletal growth rates of coral transplants pertaining to the proposed hypotheses by Hilbertz and Goreau (1996) **paper III** investigates the direct effect of a cathode versus that of an electric field on the potential functional interactions between coral

survival, skeletal growth, zooxanthellae densities, chl a concentrations and photosynthetic performance of two *Acropora* species.

3. Summary of materials and methods

3.1. Feeding experiments

Collection and maintenance The feeding experiments (**paper I, II**) were carried out outdoors, under natural sunlight at the Hasanuddin University Marine Field Station on Barang Lompo island (05^{o} 03' S, 119^{o} 19' E; Spermonde Archipelago, southwest Sulawesi) between June and October 2005. For logistical reasons each experiment was run in 3 consecutive, independent repeat trials.

For experiment 1 (**paper I**), coral samples of *S. pistillata* were collected on the fringing reef around Barang Lompo at a depth of 3 m. For each trial 3 terminal branches (surface area approximately 25-30 cm^2) were cut from each of two spatially distant (> 30 m) parent colonies, assumed to be genetically distinct. In order to minimize a bias effect of feeding treatment on the photophysiological responses due to genotype differences as the number of replicates in each trial was small (n = 2), for each treatment fragments from the same parent colony were used.

For experiment 2 (**paper II**), coral samples of *S. pistillata* and *G. fascicularis* were collected at ~2 m depth around the inshore reef of Kayangang island located in the vicinity of Makassar harbour. For each trial, twelve fragments of each species (surface area approximately 25-50 cm^2 for *S. pistillata* and 150-300 cm^2 for *G. fascicularis*) from widely separated (> 10 m) colonies were collected on 2 consecutive days. Because of the small size of coral colonies at Kayangang island, only 1 fragment was sampled from a colony yielding 12 independent coral samples of each species per trial. A portion of sampled fragments was frozen immediately at -20 ^{o}C and used as reference corals in later tissue analysis.

Experimental design Each fragment was supplied independently with oligotrophic (<0.3 µM total inorganic nitrogen), filtered (>0.5 µm) seawater (0.45 l min $^{-1}$), which was aerated continuously. Acrylic covers were used to prevent contamination with airborne particulates and to reduce ultraviolet (UV) radiation. Each tank was screened with black plastic netting (1 mm mesh) in order to attain an irradiance regime, similar to prevailing conditions *in situ* at 3 m (experiment 1, PAR max. 500-600 µmol m^{-2} s^{-1}) and 2 m (experiment 2, max. 600-700 µmol m^{-2} s^{-1}) depth under cloudless conditions. The daily water temperature in the experimental tanks increased from 28-29^{o}C (ambient temperature *in situ*) just after sun rise to 32.5 ± 0.05 ^{o}C (experiment 1, n = 120; mean ± SE) and 32.7 ± 0.02 ^{o}C (experiment 2, n = 720; mean ± SE) around noon following the solar zenith, and decreased slowly with

decreasing irradiance to pre-dawn levels at ~19:00 hrs (Fig. 2). Water temperature and light in each tank were recorded hourly between 05:00-21:00 hrs and 06:00-18:00 hrs respectively. Salinity was recorded daily and tanks were cleaned several times per week to prevent algal growth. Each trial was run for 10 (experiment 1) and 15 days (experiment 2).

Corals were acclimated to experimental conditions for 4 days in unfiltered seawater at subdued light (~400 µmol m^{-2} s^{-1}) with max. daily temperatures ≤31°C. After the acclimation period, one fragment of each colony was assigned to one of the following treatments: (1) Starvation: starved corals were deprived of organic particles >0.5 µm; (2) Feeding: fed corals were provided daily with freshly hatched *Artemia salina* nauplii.

Fluorescence measurements Photophysiological responses of zooxanthellae to either feeding or starvation under elevated temperature were assessed by chl fluorescence analyses using a pulse amplitude modulated fluorometer (Diving-PAM) inside the experimental tanks.

The effect of feeding treatment on the activity of PSII and the NPQ kinetics of *S. pistillata* was explored in two different ways. Firstly, the changes in maximum potential quantum yield (F_v/F_m), a proxy for the photosynthetic efficiency of PSII, over time were recorded in 2 day intervals between 10:00-11:00 hrs at an average temperature of 31.2 ± 0.05 °C (n = 120; mean ± SE) and average light intensity of 345 ± 0.05 µmol m^{-2} s^{-1} (n = 120; mean ± SE). Secondly, nocturnal recovery and of the photosynthetic apparatus from daily exposure to elevated temperature NPQ were determined before dawn (05:00–06:00 hrs) following a 10-h recovery period under dim light (~5 µmol m^{-2} s^{-1}) and *in situ* temperature (29°C). Recovery rates of the photosynthetic apparatus were derived from measurements of F_v/F_m, effective quantum yield ($\Delta F/F_m$) and relative electron transport rates (rETR).

Photosynthesis and respiration measurements Changes in rates of net primary production (Pn) and respiration in darkness *S. pistillata* and *G. fascicularis* were determined by Winkler titration measuring oxygen evolution and uptake between 10:00-11:00 hrs on day 1, 5, 8, 12 and 15 of each trial at an average temperature of 31.6 ± 0.03°C (n = 240; mean ± SE) and light regime of 484.83 ± 7.44 µmol m^{-2} s^{-1} (n = 240; mean ± SE). Coral fragments of each treatment were allocated to either one of four transparent or dark tinted, sealed plexiglas chambers (1.8 l), which were kept in running water inside a seawater table with a white reflective interior. The seawater in the chambers was circulated with a multi-channel peristaltic pump (521VK, Watson-Marlow, UK) through silicon hoses (Ø 8 mm) connected to an in-and outlet fitted to the chamber lids. Temperature and irradiance within the chambers

were measured at the beginning and end of each incubation run. Water samples were taken before and after each 1 hour run to obtain Pn and rates of respiration. Rates of gross primary production (Pg) were estimated by adding Pn to respiration. At the end of each trial corals were frozen at -20°C pending tissue analyses.

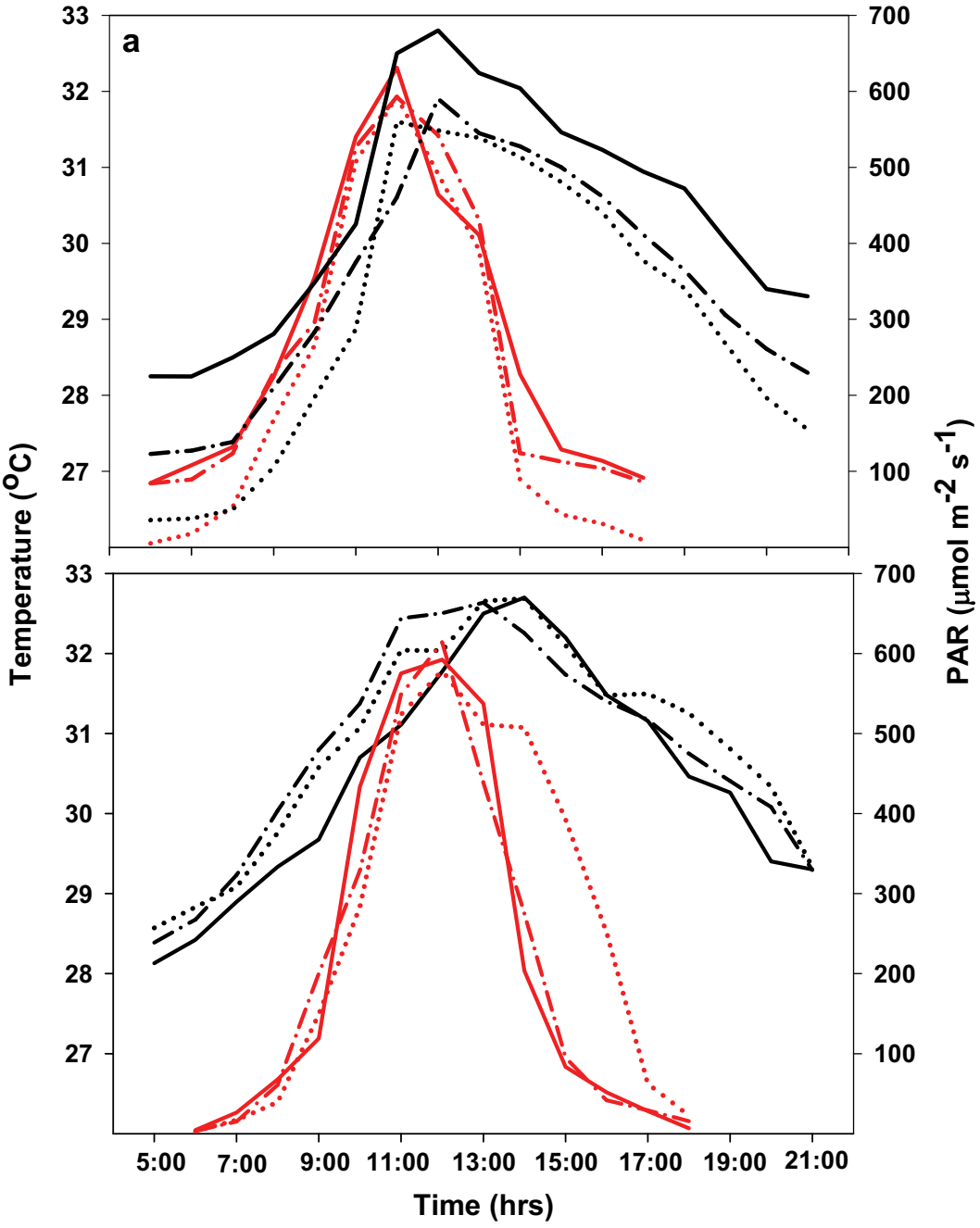

Fig. 2 Average daily temperature (°C) and light (PAR, µmol m^{-2} s^{-1}) of 3 repeat trials during a) experiment 1 and b) experiment 2. Black lines show temperature and red lines show PAR of trial 1(——), trial 2 (— · —), and trial 3 (·····).

Tissue analyses Coral tissue was stripped from the skeletons under dim light using an airbrush gun and a phosphate buffered saline solution (Sigma Aldrich). The slurry (~100 ml) was homogenized in a hand potter and subsamples of 20 ml frozen for chlorophyll a and c_2 analyses. A 10 ml aliquot of the homogenate was preserved in 4% formalin and used to determine zooxanthellae densities (8 replicate counts) using a Neubauer haemocytometer. The mitotic indices were determined from the number of cells appearing as doublets in two samples of 1000 cells (Jones and Yellowlees 1997). Chlorophyll a and c_2 were extracted as described by Gardella and Edmunds (1999) and concentrations calculated according to the equation of Jeffrey and Humphrey (1975). Total protein content was determined by Kjeldahl digestion as described in Chow et al. (1980). Lipids were extracted using the standard method of Folch et al. (1957) and the total lipid content of each sample determined gravimetrically. Oxygen flux, proteins, lipids and zooxanthellae densities were normalized to skeletal surface area and chl concentrations expressed as μg x 10^6 $cells^{-1}$. For experiment 1 coral surface area was determined photometrically using the dye-dipping method of Hoegh-Guldberg (1988). For experiment 2 the surface area was measured using the paraffin wax technique (Stimson and Kinzie 1991).

Data analysis All data were checked for homogeneity of variances using Cochran's C-test and if necessary ln(x)-transformed. The variances of maximum temperatures and light intensities among experimental tanks were analyzed by a repeated measurements two-way ANOVA (Sokal and Rohlf 1995) using WinGMAV (EICC, University of Sydney, Australia). The data variances of both temperature and light in experiment 1 (**paper I**) were heterogeneous and failed to be stabilized. However, since the data set was large (n = 120) and there were no significant differences in daily maximum temperature and light intensity between individual tanks, the probability of a Type I error was omitted and the analysis thus regarded reliable (Underwood 1997).

The variances of maximum temperatures and light intensities among experimental tanks and between species of experiment 2 (**paper II**) were analyzed by three-way ANOVA, using a repeated measurements without replication model (Sokal and Rohlf 1995). To verify that there were no significant differences in mean temperature between light and dark chambers and mean light intensity between light chambers, the temperature and light data recorded at the beginning and end of each incubation run were averaged and analyzed by three-way and two-way ANOVA respectively. A separate two-way ANOVA was performed

to confirm that there was no significant difference in temperature and light intensity during the chamber incubations between species (see appendix I for statistical tables).

The effect of feeding versus starvation on changes in the fluorescence responses of zooxanthellae in *S. pistillata* and biomass characteristics after 10 days were analyzed using a general linear model with a three-factorial nested design and *post–hoc* SNK testing using Statistica. The effect of treatment on the overall change of photosynthesis and respiration of *S. pistillata* and *G. fascicularis* after 15 days was analyzed by two-way ANOVA. Tissue variables in experiment 2 were analyzed for significant differences between fed and starved corals by three-way ANOVA. To test for significant differences in tissue variables between reference and fed corals, additional two-way ANOVA were performed. *Post–hoc* SNK tests were run for separation of significant factors. All ANOVA data were analyzed using WinGMAV (EICC, University of Sydney, Australia).

3.2. Electric reef experiment

The experiment was carried out in North Sulawesi, Indonesia (01° 45' N, 125° 3' E) between April and July 2006, using the congeneric, species *Acropora yongei* and *A. pulchra*, both featuring high growth rates (Harriott 1999) and occurring at high abundance in the Indo-Pacific (Veron 2000) making this genus particularly relevant for coral reef restoration projects.

Experimental design The experimental matrix (cathode) consisted of a 10 m^2 steel frame which was subdivided by steel bars into a grid of 10 squares (1m^2). Five squares were intersected by additional steel bars running parallel to each other alternating with 5 squares which contained bamboo boards (electric field). Titanium mesh anodes were placed in close proximity on each side of the experimental matrix (Fig. 1, **paper III**) and each electrode was connected to a separate cable in order to minimize the resistance and create an evenly distributed electrical field. An additional bamboo grid comprising 4 squares (1 m^2) was deployed outside the experimental matrix to serve as a control. The experiment was run for 4 months, during which the effect of mineral accretion has been shown to be most pronounced (Sabater and Yap 2004).

Collection and preparation of samples A total of 350 coral fragments of each species with a length of 6-8 cm (Soong and Chen 2003) were collected from parent colonies in close proximity to the experimental site and were transplanted onto the experimental matrix on a

shallow rubble field, protected from currents at 5 m depth. The coral fragments of each species were interspersed on each square (n = 25) to minimize the effect of location. Fragments on the cathode were placed into adjustable metal tubes, which were welded onto the cathode, while the rest of the fragments were glued into concrete cups using underwater epoxy and then tied to the bamboo boards with cable ties, either inside the electric field or outside the experimental matrix.

Survival and skeletal growth After 4 months, fragments were considered as dead when no live tissue was noticeable (90% tissue necrosis) or when fragments were lost due to wave action or removal by fish. To determine linear skeletal growth rates, the initial length of each fragment was measured from the top of the cup to the coral tip using a pair of callipers (accurate to 0.01 mm). After the experimental treatment phase, all fragments which had survived were measured again to determine the vertical skeletal extension rates as an estimate of coral growth over 4 months.

Fluorescence measurements The effect of treatment on the relative photosynthetic performance of zooxanthellae was determined by measuring F_v/F_m and rETR during 5 consecutive, cloudless days just before the end of the treatment phase (as described in 3.1.1)

Tissue analyses Zooxanthellae densities, mitotic indices, and chl a concentrations, were determined as described in 3.1.3. Coral surface area was measured using the paraffin wax technique (Stimson and Kinzie 1991).

Data analyses The data were checked for homogeneity of variances using Cochran's C-test (Underwood 1997). To test if treatments had an effect on the skeletal extension rates, 26 replicates were chosen randomly from each treatment group, adjusted to the group with the lowest survival rates, and analyzed by one-way ANOVA and *post–hoc* SNK testing. To test for significant effects of treatment on coral survival, we used a likelihood (G) test with William's correction (Sokal and Rohlf 1995). The effect of treatments on fluorescence parameters, growth, zooxanthellae density and chl a were analyzed separately for each species by one-way ANOVA and *post–hoc* SNK test separation of significant factors. All data were analyzed using WinGMAV (EICC, University of Sydney, Australia).

4. Results

Effects of food availability versus starvation on the symbiotic association For both experiments there were no significant differences in daily max. temperature and light intensity between treatments within each trial (see appendix 1 for ANOVA tables). Thus, the experimental conditions were well suited to test the effect of feeding versus starvation on the photophysiology and tissue characteristics of *S. pistillata* and *G. fascicularis* under elevated temperature.

Overall the results show that the stress tolerance in terms of photosynthetic activity and pigmentation of both species was coupled to food availability. Zooxanthellae in fed corals maintained high photosynthetic activity while starved corals displayed strong signs of chronic photoinhibition which was reflected by a progressive decline in F_v/F_m and Pg between 10:00-11:00 hrs over the course of experiment 1 (**paper I**) and 2 (**paper II**) respectively.

Chronic photoinhibition of starved *S. pistillata* in experiment 1 coincided with a decline in nocturnal recovery rates of PSII over the course of 10 days as was manifest in a significant decrease in F_v/F_m, $\Delta F/F_m$', rETR. This was paralleled by the progressive inability to dissipate excess excitation energy via non-photochemical quenching (NPQ). High rates of nocturnal PSII recovery of fed corals by comparison were accompanied by high levels of NPQ. Sustained photosynthesis of fed corals was accompanied by a reduced loss in pigmentation compared to starved corals (**paper I**). After 10 (**paper I**) and 15 (**paper II**) days of exposure to elevated temperature the tissue of starved corals appeared pale relative to fed corals. While the loss in pigmentation of starved *S. pistillata* was characterized primarily by a loss of zooxanthellae, starved *G. fascicularis* displayed a substantial reduction in both zooxanthellae densities and chl a + c_2 concentrations. Yet the results of experiment 1 clearly demonstrate that mitotic indices of zooxanthellae in fed *S. pistillata* were significantly higher than the mitotic indices of starved corals (**paper I**). Respiration rates of *S. pistillata* over 15 days displayed a similar pattern to that of photosynthetic rates. Starvation resulted in a significant decrease in respiration of starved corals while there was no change in respiration for fed corals. By contrast, there were no significant changes in rates of respiration between fed and starved *G. fascicularis*. Overall the oxygen consumption of *S. pistillata* for both treatments was about 39-67% higher than the consumption of *G. fascicularis* suggesting that *S. pistillata* had higher metabolic rates than *G. fascicularis* (**paper II**). The results further show that the amount of lipid per unit surface area in starved *S. pistillata* was significantly lower than in fed *S. pistillata*, while feeding treatment had no significant effect on lipid

concentrations of *G. fascicularis*. In both species, however, the amount of protein per unit surface area was significantly lower in starved than in fed corals.

Effects of mineral accretion versus electric field on coral performance Mortality rates of *A. yongei* after 4 months were significantly higher for corals grown on the cathode (32%) than for those on the control (1%) and inside the electric field (1%). In contrast, there were no significant differences in mortality between cathode (17%), electric field (9%) and control corals (13%) for *A. pulchra*. Treatment had a significant effect on the vertical skeletal extension rates. For both species, growth rates were significantly higher inside the electric field compared to cathode and control corals. Coincident with high rates of mortality, *A. yongei* exhibited the lowest growth rates on the cathode while growth rates of *A. pulchra* were lowest for corals in the control treatment. Moreover, the results show that F_v/F_m and rETRs of *A. yongei* were significantly lower for corals grown on the cathode compared to corals inside the electric field and on the control, while there were no significant differences between treatments for F_v/F_m and rETRs of zooxanthellae in *A. pulchra*. *A. yongei* on the cathode contained significantly lower zooxanthellae densities than corals inside the electric field and on the control treatment while *A. pulchra* displayed the opposite pattern, containing significantly higher zooxanthellae densities on the cathode than inside the electric field and on the control treatment. The effect of treatment on chl a was identical for both species. Zooxanthellae in corals inside the electric field contained significantly less chl a than cathode and control corals.

5. Discussion

Effects of food availability versus starvation on the symbiotic association Zooxanthellae of the genus *Symbiodinium* comprise different genotypes with different thermal sensitivities (Rowan 2004). *S. pistillata* and *G. fascicularis* from southern Taiwan and Okinawa, for example, have been both shown to contain clade C types (Chen et al. 2005, LaJeunesse et al. 2004) while *G. fascicularis* from the Great Barrier Reef has been associated with clade D, a more stress tolerant type than clade C (LaJeunesse et al. 2004). Unfortunately, there is no information regarding the genotype inhabiting the corals used in this study. However, the results show that the thermal stress tolerance in terms of photosynthetic activity and loss of pigmentation of both species was coupled to food availability.

As predicted, fed corals maintained high photosynthetic activity while starved corals displayed strong signs of chronic photoinhibition, which was reflected in the continuous decline in both F_v/F_m and Pg of over a period of 10 (**paper I**) and 15 (**paper II**) days, paralleled by a decrease in pre-dawn values for F_v/F_m, $\Delta F/F_m$', ETR and a decline in NPQ processes. This pattern is consistent with previous observations (Jones et al. 1998, Jones and Hoegh-Guldberg 2001, Franklin et al. 2004), that photoprotective downregulation of photosynthesis culminates in chronic photoinhibition if nocturnal recovery is incomplete.

The relative importance of heterotrophy as a means of energy and nitrogen acquisition for both partners of the cnidarian symbiotic association has been well documented (e.g. Cook et al. 1994, Fitt and Cook 2001, Piniak and Lipschultz 2004). Since the thermal tolerance of corals is directly related to levels of photosynthetic activity (Warner et al. 1996), levels of antioxidant activity and rates of protein turnover (Downs et al. 2002, Yakovleva et al. 2004) involving high demands of energy and nitrogen, food availability was hypothesized to lower the bleaching susceptibility of corals under elevated temperature conditions.

While the loss in pigmentation of starved *S. pistillata* was characterized primarily by a loss of zooxanthellae, starved *G. fascicularis* displayed a substantial reduction in both zooxanthellae densities and chl a + c_2 concentrations. It is therefore possible that zooxanthellae in *G. fascicularis* were more sensitive to solar radiation than zooxanthellae in *S. pistillata* (Brown et al. 1991, 1997). For *S. pistillata* the effect of treatment on zooxanthellae densities was significantly more pronounced in experiment 2 than experiment 1. It is possible that this difference was due to temperature and light differences between experiment 1 and 2 (see Fig. 2) but also suggests that an experimental period of 10 days was insufficient to determine clear treatment-dependent changes in zooxanthellae populations. Furthermore, the results of experiment 1, showing that the mitotic indices of zooxanthellae in

fed *S. pistillata* were significantly higher than the mitotic indices of starved corals, corroborate the general tenet that temperature stress and chronic photoinhibition result in cellular degradation and death of zooxanthellae due to oxidative stress (Franklin et al. 2004, Dunn et al. 2002).

Contrary to natural bleaching conditions, where increased temperatures prevail for extended periods of time (Gleeson and Strong 1995), corals in this study were allowed to recover during the night at ambient temperatures (29^{o}C). Nonetheless, the present results indicate that the thermal tolerance of the symbiotic association is to some extent a function of the coral's resource environment. Although the results of this study provide no indication of the underlying biochemical mechanisms as to how additional resources could have modulated the thermal resistance of the photosynthetic apparatus, it is conceivable that zooplankton provided a direct source of nitrogen to the zooxanthellae (Piniak et al. 2003) facilitating enhanced rates of protein repair and re-synthesis of the PSII D1 protein (e.g. Ohad et al. 1984, Warner et al. 1999, Takahashi et al. 2004, but see Smith et al. 2005 for review) or reduced photophysiological damage of the zooxanthellae indirectly by enhancing the capacity of either symbiotic partners to synthesizze antioxidant compounds or heat-shock proteins or both (e.g. Downs et al. 2002, Yakovleva et al. 2004, Shick et al. 2005).

Overall the oxygen consumption of *S. pistillata* for both treatments was about 39-67 % higher than the oxygen consumption of *G. fascicularis*. The lack of effect of treatment on the respiration of *G. fascicularis* could have therefore been the result of low metabolic rates relative to *S. pistillata*. This hypothesis is consolidated by the observed changes in lipid and protein concentrations. The results show that the amount of lipid per unit surface area in starved *S. pistillata* was significantly lower than in fed corals while feeding treatment had no significant effect on lipid concentrations in *G. fascicularis*. For *S. pistillata* total protein concentrations were about 50 % lower than for fed corals, whereas starved corals of *G. fascicularis* lost only about 10 % of tissue proteins compared to fed corals. The low metabolic rates of *G. fascicularis* thus may have allowed starved corals to conserve their energy reserves, and *G. fascicularis* may have preferentially used their protein stores to support their metabolic demands. This is corroborated by the results of Grottoli et al. (2004) who showed that there were no significant differences in total lipid concentrations between bleached and unbleached *Montipora verrucosa*, while bleached *Porites compressa* contained significantly lower lipid concentrations than unbleached colonies, which coincided with lower respiration rates of *M verruscosa* compared to *P. compressa.*

Zooxanthellae are believed to utilize and recycle ammonium produced by host catabolism, but it is clear that the internal recycling of ammonium can not proceed indefinitely without 'new' nitrogen input from the outside (see 1.1). Thus, the absence of external food may lead to a *ciculus vitiosus*, where the thermal tolerance of corals is lowered as additional stressors such as high temperature are likely to further constrain physiological processes due to increased metabolic costs.

Effects of mineral accretion versus electric field on coral performance The three most relevant findings of this study show that 1) the electric field and not mineral accretion resulted in the highest growth rates, 2) mineral accretion can reduce coral health and growth, and 3) the suitability of the mineral accretion technology for coral transplantation can vary greatly between closely related species. Contrary to previous observations by Sabater and Yap (2002, 2004), mineral accretion had either no or even a negative effect on coral survival over a period of 4 months.

The results demonstrate that the cathodic environment had an overall negative effect on the health of *A. yongei*. This is corroborated by the differences in zooxanthellae densities between treatments. *A. yongei* on the cathode contained significantly lower zooxanthellae densities than corals inside the electric field and on the control treatment. A reduction in zooxanthellae density and decrease in F_v/F_m are good indicators of physiological stress in corals and their symbionts in response to abnormal environmental conditions (Jones 1997, Jones et al. 1999, Warner et al. 1999). *A. pulchra* displayed the opposite pattern, containing significantly higher zooxanthellae densities on the cathode than inside the electric field and on the control treatment.

A loss of zooxanthellae, as well as reduced photosynthetic efficiency as was observed for *A. yongei* on the cathode, would have invariably resulted in a decrease in photosynthetic potential of the coral colony. This may have greatly impacted the colony's energy balance (Fitt et al. 2000), leading to a reduction or even cessation in growth (Porter et al. 1989, Goreau and MacFarlane 1990) and subsequent mortality (Anthony et al. 2007).

Increased availability of mineral ions created by seawater hydrolysis and consequent increases of ions available for active uptake via the transcellular pathway of calcification has been claimed a major advantage for reef restoration (Hilbertz and Goreau, 1996). However, in branching corals, linear skeletal extension occurs through enhanced calcification in the apical region of the branch (Goreau and Goreau 1959, Oliver 1984). A small increase in mineral ions at the base of the coral seems therefore unlikely to enhance linear growth rates of corals.

This hypothesis is also in conflict with the pronounced divergent growth rates, which were observed for *A. yongei* and *A. pulchra* on the cathode while both species displayed highest growth rates inside the electric field. The results, therefore, strongly suggest that factors other than mineral ions were stimulating skeletal growth. Likewise, the hypothesis that provision of additional electrons could enhance the coral's bioenergetics, presumably facilitating both increased skeletal and tissue growth (Hilbertz and Goreau 1996, Goreau et al. 2004) contradicts the apparent negative effect of the cathode on the overall health of *A. yongei*. Even though calcification is primarily a physiochemical process, it is mediated by the synthesis of an organic matrix (Allemand et al. 1998). Results by Allemand et al. (1998) suggest that skeletal growth is limited by the biosynthesis of the organic matrix rather than the deposition of calcium. The relevance of photosynthesis for the skeletal organic matrix biosynthesis has long been recognized (Muscatine and Cernichiari 1969, Barnes and Crossland 1978), but it is now established that heterotrophic sources also play an important role in the acquisition of both precursors and energy necessary for the synthesis of the organic matrix (Allemand et al. 1998, Houlbreque et al. 2004). Although there is, at present, no direct evidence of electrosensory organs in zooplankton, due to a lack of studies it can not be ruled out that zooplankton are sensitive to electric or magnetic cues (Bullock 1996). Alternatively zooplankton migratory behaviour may have been altered indirectly in response to changes in the presence of planktivores (McKelvey and Forward 1995).

The overall outcome of this study elucidates the convoluted nature of the interaction between photosynthetic performance of in *hospite* zooxanthellae and associated fitness of corals and their immediate environment. The results illustrate that high interspecific physiological variability adds further complexity to such interactions. This underscores that both the prediction of ecological consequences of future bleaching events, as well as the efforts to restore reef habitat, require multivariate consideration and sound knowledge of the ecology of the coral species involved.

6. Further considerations and concluding remarks

The underlying mechanisms of the functional interactions between food availability and increased thermal tolerance of corals as inferred from the current data are only implicit. To verify the hypothesis that exogenous food facilitates an increased capacity for enzyme synthesis and protein repair certainly calls for further investigation, which explicitly address the potential biochemical and molecular processes pertaining to the increased thermal tolerance of the photosynthetic apparatus in relation to food availability.

In addition, there are two questions that have transpired from experiment 1 and experiment 2 which merit further attention. First, the results from experiment 1 show a strong correlation between the absences of external food, a decrease in the thermal tolerance of PSII functioning and NPQ processes, which points to a functional interaction between zooplankton feeding and the functional integrity of the thylakoid membranes of the zooxanthellae. The regulation of excess excitation energy via NPQ is associated with xanthophyll cycle-dependent thermal energy dissipation of excess light via the de-epoxidation of the xanthophyll cartenoids diadinoxanthin to diatoxantin (Ambarsari et al. 1997, Brown et al. 1999). However, the de-epoxidation of diadinoxanthin requires a high proton concentration within the thylakoid membrane (Gilmore and Yamamoto 1993). The significant decline in both rETR and NPQ of starved corals following nocturnal recovery therefore indicate damage to the thylakoid membranes. Thus, proton leakage may have prevented the generation of a transthylakoid pH gradient upon exposure to high light. This hypothesis would be in agreement with Tchernov et al. (2004) who showed a strong correlation between high bleaching susceptibility of *S. pistillata*, changes in zooxanthellae membrane integrity, loss of photosynthetic activity and increased production of ROS. High levels of NPQ of fed corals, by contrast, would therefore suggest that the thylakoid membranes of zooxanthellae remained functionally intact.

Analyses of zooxanthellae thylakoid lipids, from a range of coral species with differing thermal sensitivities to bleaching, revealed that thermally tolerant zooxanthellae feature a markedly lower content of the major polyunsaturated fatty acid Δ6,9,12,15-*cis*-octadecatraenoic acid relative to Δ9-*cis*-octadecatraenoic acid. The higher relative concentration of Δ9-*cis*-octadecatraenoic acid is thought to enhance the thermal stability in eukaryotic thylakoid membranes (Hazel 1995) and simultaneously reduce the susceptibility to lipid peroxidation (Gombos et al. 1994). If indeed, as has been suggested by Tchernov et al. (2004), the lipid composition of the thylakoid membranes of zooxanthellae determine the thermal sensitivity of a coral species, a testable hypothesis would be that enhanced food

availability decreases the bleaching susceptibility of temperature sensitive species by facilitating increased production of antioxidant enzymes thereby promoting the functional integrity of zooxanthellae thylakoid membranes.

Second, it would be pertinent to investigate the potential factors underlying the observed differences in the changes in pigmentation between starved *S. pistillata* and *G. fascicularis.* Since loss of chlorophyll may be attributed to both photodegradation and active oxygen radicals (Jen and Mackinney 1970, Lesser and Farrell 2004), the significant decrease in chlorophyll of starved relative to fed *G. fascicularis* could imply differences in the sensitivity to ultraviolet radiation (UVR) and PAR between the two species. Differential sensitivity of marine invertebrates to solar radiation can, to some extent, be explained in terms of depth related differences in the total content of mycosporine-like amino acids (MAAs), of which some in addition to their protective function against solar irradiance have antioxidant properties (Dunlap and Yamamoto 1995, Yakovleva et al. 2004). Indeed, it has been shown that shallow water corals contain higher concentrations of MAAs than sensitive conspecifics living at greater depth (Gleason 2001, Shick and Dunlap 2002). However, since both species were sampled at the same depth and therefore had a similar light history in the context of accumulation of MAA compounds, the variation in chlorophyll concentrations may be attributed to genetic differences in the symbiotic algae (Rowan et al. 1997, LaJeunesse 2001) or differences in composition of individual MAAs associated with the genetic adaptation of the species (Yakovleva and Hidaka 2004). Tissue retraction is thought to act as protective mechanisms against high irradiance (Brown 2002c; Stimson et al. 2002). Calices of *S. pistillata*, however, are much shallower than and the calices of *G. fascicularis*. Thus, it could be possible that *S. pistillata*, under bleaching conditions, where solar radiation invariably becomes excessive and the effects of UVR exacerbated, accumulates higher levels of certain MAAs than *G. fascicularis* as a compensation for the inability to 'withdraw' it's symbionts from damaging light conditions.

The ability of corals to utilize heterotrophic food sources depends largely upon successful capture of zooplankton and other food particles such as suspended and downward-fluxing particulate matter, which can differ considerably among coral species due to a suite of factors including light (Ferrier-Pages et al. 1998), water temperature (Palardy et al. 2005), water flow (Sebens and Johnson 1991, Sebens et al. 1998), zooplankton abundance and composition (Ferrier-Pages et al. 2003, Heidelberg et al. 2004, Palardy et al. 2006), and concentrations of suspended particulate matter (Anthony and Fabricius 2000). Whilst low to moderate water flow is likely to facilitate the capture of SPM (Sebens and Johnson 1991,

Helmuth and Sebens 1993), zooplankton capture rates decrease under low flow conditions (Johnson and Sebens 1993, Heidelberg et al. 1997, Sebens et al. 1998, Levy et al. 2001). A number of studies have demonstrated that heterotrophic abilities are positively correlated to zooplankton concentrations (Ferrier-Pages et al. 2003, Palardy et al. 2006) and levels of SPM (Anthony 2000). Zooplankton abundance on a reef, however, is often low (Johannes et al. 1970) and highly variable in space and time (Allredge and King 1980, Heidelberg et al. 2004, Palardy et al. 2005). Similarly SPM loads can fluctuate greatly between locations (Larcombe et al. 1995, 1999) and in nutritional quality (Wotton 1994). Within this context, the results of this thesis indicate that high fluctuations of available nitrogen and fixed carbon may, to some extent, explain the often observed variability in bleaching susceptibility and severity, not only between species, but also within conspecifics.

Furthermore, Anthony et al. (2007) showed that lipid concentrations make good predictors of coral survivorship under bleaching conditions. The results of this thesis expand on their findings, indicating that lipid concentrations, irrespective of resource environment and bleaching status, were linked to metabolic rates. Although survivorship was not determined in this study, the observed differences in tissue composition between *S. pistillata* and *G. fascicularis* suggest that under the current experimental conditions coral survivorship would be greater for *G. fascicularis* than for *S. pistillata*. Protein synthesis is a fundamental component of physiological acclimation to change in temperature (Hazel and Prosser 1974), *ergo* species with a high inherent rate of protein synthesis might have a fitness advantage under thermal stress. Koehn and Bayne (1989) proposed the stress susceptibility of an organism to vary with the relative efficiency with which metabolic requirements are met. Thus, under bleaching conditions, where the coral's metabolic demands are expected to be greatly increased, the stress tolerance of species with low metabolic efficiency will be compounded by the supply of energy and nutrients, while species with high metabolic efficiency, as might be the case for *G. fascicularis*, energy and nutrient availability may constitute a quantitatively minor factor affecting coral fitness. This presents an interesting consideration for the general stress (temperature, light, salinity, bacterial- and viral infections) susceptibility and associated mortality of corals along environmental gradients:

The general perception that clear oceanic waters provide optimal growth conditions for reef building corals is to some extent tied to the large body of pioneering work on the energetic relationships between hermatypic corals and their endosymbionts in the 90s (e.g. Muscatine et al. 1981, 1984, Falkowski et al. 1984, Edmunds and Spencer Davies 1986, 1989). Nearshore environments are often characterized by heavy loads of terrestrial

discharges of nutrients and sediment, which results in turbid, nutrient-rich waters (Mitchell and Furnas 1997, Larcombe et al. 1995,1999, Fabricius 2005) smothering coral tissues (Rogers 1990) and reducing light penetration, the key resource of scleractinian corals (Muscatine 1990). As such, turbid waters are commonly considered a stress factor for corals (Rogers 1990, Szmant 2002). Although coral populations can experience high levels of mortality in regions with high nutrient loads (e.g. Maragos et al. 1985), this is not necessarily always the case. Nearshore fringing reefs frequently feature corals which are adapted to thrive in this type of environment, yielding high coral cover, coral growth rates and coral diversity (e.g. Edinger 1998). Suspended sediment and inorganic nutrients can be rapidly converted into organic particulate material (Furnas 2005) and therefore provide a potentially rich heterotrophic environment (Anthony 2006). Edinger (1998) has referred to the balance between coral growth, reef growth and nutrient concentration as the 'Janus Effect', after the two-faced Roman guardian of entrances and exits. Against the background of rapid environmental change, turbid coastal environments may therefore buffer the stress susceptibility and enhance the fitness of some coral species. This would have important ecological implications for the persistence and dynamics of coral reef communities in the future.

These results, documented in **paper III,** illustrate that the proposed benefits of the mineral accretion technology to meet important objectives of reef rehabilitation should be reconsidered. Whether food availability was involved as a factor effecting coral growth on the experimental matrix as described in **paper III**, of course is ambiguous. In view of the uncertainties associated with the effect of electrochemical processes on coral fitness, it is evident that further studies are warranted in order to gain a better understanding of possible interactions between electrochemical processes and the environment and how they effect physiological processes of the coral colony. Although further investigations under controlled conditions would, no doubt, yield some very interesting information, its relevance is questionable. Firstly, there are methods which appear to be by far cheaper and less labour intensive in terms of construction and maintenance than the mineral accretion technology (Clark 2002, Zimmer 2006, Ferse 2008). Secondly, steel structures, cables and car batteries scattered over coastal areas beg the question as to whether this may not actually present a form of environmental pollution. The fact that there is hardly any 'hard' evidence of coral performance under the influence of mineral accretion, least of all from the inventors of this method, who are vigorously promoting the mineral accretion technology nonetheless, ultimately raises an air of suspicion. Further research in this area, at least within the context of

reef restoration, would therefore squander money that could be used for something more viable.

7. References

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Paper I

Feeding sustains photosynthetic quantum yield of a scleractinian coral during thermal stress

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Feeding sustains photosynthetic quantum yield of a scleractinian coral during thermal stress

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Abstract

Thermal resistance of the coral – zooxanthellae symbiosis has been associated with chronic photoinhibition, increased antioxidant activity and protein repair involving high demands of nitrogen and energy. While the relative importance of heterotrophy as a source of nutrients and energy for cnidarian hosts, and a means of nitrogen acquisition for their zooxanthellae is well documented, the effect of feeding on the thermal sensitivity of the symbiotic association has been so far overlooked. Here we examine the effect of zooplankton feeding versus starvation on the bleaching susceptibility and photosynthetic activity of photosystem II (PSII) of zooxanthellae in the scleractinian coral *Stylophora pistillata* in response to thermal stress (daily temperature rises of 2-3^{o}C) over 10 days, employing pulse-amplitude-modulated (PAM) chlorophyll fluorometry. Fed and starved corals displayed a decrease in daily maximum potential quantum yield (F_v/F_m) of photosystem II (PSII), effective quantum yield ($\Delta F/F_m$') and relative electron transport rates (rETR) over the course of 10 days. However after 10 days of exposure to elevated temperature, F_v/F_m of fed corals was still 50–70 % higher than F_v/F_m of starved corals. Starved corals showed strong signs of chronic photoinhibition, which was reflected in a significant decline in nocturnal recovery rates of PSII relative to fed corals. This was paralleled by the progressive inability to dissipate excess excitation energy via non-photochemical quenching (NPQ). After 10 days, NPQ of starved corals had decreased by about 80% relative to fed corals. Feeding treatment had no significant effect on chlorophyll a and c_2 concentrations and zooxanthellae densities, but the mitotic indices were significantly lower in starved than in fed corals. Collectively the results indicate that exogenous food may reduce the photophysiological damage of zooxanthellae that typically leads to bleaching and could therefore play an important role in mediating the thermal resistance of some corals.

Key Words: Bleaching, Heterotrophy, Photosystem II, *Stylophora pistillata*, Zooxanthellae

Introduction

Coral bleaching, the dissociation of corals and their zooxanthellae (endosymbiotic dinoflagellates of the genus *Symbiodinium*) in response to thermal stress is a global threat to coral reefs (Hoegh-Guldberg 1999). Coral responses to elevated temperatures are however not uniform but often display high variation in bleaching severity, differential mortality and subsequent recovery not only between different species but also among and within conspecific colonies in the same reefal habitat (e.g. Brown and Suharsono 1990; Marshall and Baird 2000). Recent advances in chlorophyll fluorescence and molecular techniques have led to several lines of evidence which implicate coral bleaching with chronic photoinhibition of photosynthesis and the subsequent increase in reactive oxygen species within both the zooxanthellae and coral host (for review, see Smith et al. 2005). Both symbiotic partners however feature elaborate protective mechanisms against oxidative cellular damage, including antioxidant enzymes, heat shock proteins (e.g. Downs et al. 2002; Richier et al. 2005) and certain mycosporine-like amino acids (Yakovleva et al. 2004). In addition, zooxanthellae are able to regulate excess excitation energy via photoprotective non-photochemical quenching (NPQ) processes, associated with xanthophyll cycle-dependent thermal energy dissipation of excess light via the de-epoxidation of the xanthophyll cartenoids diadinoxanthin to diatoxantin (Ambarsari et al. 1997; Brown et al. 1999). The evidence derived from studies by Warner et al. (1996, 1999) and Hill et al. (2005) strongly suggest that those species more capable engaging in NPQ processes are less susceptible to thermal bleaching. The work by Warner et al. (1999) and Takahashi et al (2004) indicate that bleaching susceptibility is determined by the rate of protein turnover of the photosystem II (PSII) D1 protein and efficiency of the photosynthesis repair machinery. Conversely thermally sensitive species have been shown to exhibit higher levels of antioxidant enzyme activities than thermally tolerant species (Yakovleva et al. 2004). Thus maintaining cellular homeostasis during thermal stress can incur high metabolic costs and divert resources away from growth and reproduction (Hawkins 1991). Recent findings by Grottoli et al. (2006) indicated that coral species, that were capable of increasing their heterotrophic carbon input recovered faster from bleaching than species, that remained largely dependent on photosynthate translocates. In healthy corals, photosynthetically fixed carbon is translocated from the zooxanthellae to the coral host covering up to 100% of the host's daily energy requirements (e.g. Falkowski et al. 1984) while substantial amounts of the host's nitrogen are derived from heterotrophic sources (Cook et al. 1994; Anthony and Fabricius 2000; Fitt and Cook 2001). It is well established that under steady-state conditions, food availability tends to increase protein levels of host

tissue, and chlorophyll concentrations of zooxanthellae (Titlyanov et al. 2001; Ferrier-Pages et al. 2003; Houlbreque 2003, 2004). Furthermore Piniak et al. (2003) have demonstrated that zooxanthellae can take up zooplankton nitrogen digested within the coelentron directly, which underscores the importance of a heterotrophic diet as a direct source of nitrogen to the endosymbiont. The heterotrophic abilities of corals can vary considerably between reefal habitats due to factors including light (Ferrier-Pages 1998; Titlyanov et al. 2000), water temperature (Palardy et al. 2005), ambient zooplankton abundance (Ferrier-Pages et al. 2003; Palardy et al. 2006) and concentrations of suspended particulate matter (Anthony and Fabricius 2000). Furthermore factors such as water flow (Sebens and Johnson 1991; Sebens et al. 1998, 2003) and escape behaviour of zooplankters (Sebens et al. 1996) may create high levels of small scale variability of available food among individual colonies, which may contribute to the often observed conspecific variation in bleaching susceptibility and severity. Feeding may thus present an important, yet so far overlooked, factor that contributes to differential bleaching susceptibilities not only between but also within species.

The rationale of this study was to test the prediction that exogenous food increases the thermotolerance of zooxanthellae photosynthesis through the provision of additional resources required for metabolic processes involved in protein synthesis or repair and the photoprotective mechanisms of the holobiont (coral-symbiont association). The effect of zooplankton feeding on the photosynthetic activity of PSII of zooxanthellae in the coral *Stylophora pistillata*, a thermally sensitive species (Yakovleva et al. 2004), in response to thermal stress (daily temperature rises of 2-3^{o}C) over a period of 10 days was investigated experimentally employing pulse-amplitude-modulated (PAM) fluorometry. Specifically the following hypotheses were tested: (1) PSII of zooxanthellae in fed corals will maintain higher photosynthetic efficiency during prolonged exposure to elevated temperatures than PSII of zooxanthellae in starved corals; (2) zooxanthellae in fed corals will retain higher levels of NPQ during prolonged exposure to elevated temperatures than zooxanthellae in starved corals; (3) fed corals will have higher zooxanthellae densities, higher mitotic indices and higher chlorophyll a and c_2 concentrations per algal cell than zooxanthellae in starved corals following exposure to thermal stress.

Materials and methods

This study was carried out at Hasanuddin University Marine Field Station on Barang Lompo island (05^{o} 03’ S, 119^{o} 19’ E; Spermonde Archipelago, southwest Sulawesi) during the dry season in July-August 2005. Feeding experiments using the scleractinian coral *S. pistillata*

were conducted outdoors under natural sun light. For logistical reasons experiments were run in 3 consecutive, independent repeat trials. Each trial was run for 10 days. Coral samples were collected on the fringing reef around Barang Lompo at a depth of 3 m. For each trial two spatially distant (> 30 m) parent colonies (brown colour morphs), assumed to be genetically distinct, were randomly selected. In order to minimize a bias effect of feeding treatment on the photophysiological responses due to genotype differences as the number of replicates in each trial was small (n = 2, one per colony), we used fragments from the same parent colony for each treatment. From each of the two colonies, three terminal branches (surface area approximately 25-30 cm^2) were cut, placed into dark containers and transported to the field station within 30 min of collection. One fragment of each colony was frozen immediately at -20 ^oC and used as reference coral in later tissue analysis.

Coral maintenance and experimental design

The four remaining fragments were glued to PVC nuts using non toxic, two component underwater epoxy, and mounted on PVC screws attached to perspex blocks. Each individual fragment was thus placed into one of four transparent 14-l plastic tanks. The tanks were supplied independently with oligotrophic (<0.3 µM total inorganic nitrogen), filtered (>0.5 µm) seawater (0.45 l min^{-1}), and aerated continuously. Acrylic covers reduced contamination with airborne particulates and ultraviolet (UV) radiation. Each tank was covered with black plastic netting (1 mm mesh) to attain an irradiance regime, similar to *in situ* conditions at 3m depth (max. 500-600 µmol m^{-2} s^{-1} under cloudless conditions). Seawater was pumped from a depth of 30 m into an intermediate reservoir, from where it was subsequently pumped into the experimental tanks. The water temperature in the reservoir and tanks increased from 28-29^oC (temperature *in situ*) just after sun rise to 32.5 ± 0.05 ^oC (n = 120; mean ± SE) at 12:00 hrs following the solar zenith (max. PAR 572 ± 5 µmol m^{-2} s^{-1}; n = 120; mean ± SE) and decreased slowly with decreasing irradiance back to *in situ* temperatures at ~19:00 hrs. Water temperature and light in each tank were recorded hourly between 05:00-21:00 hrs and 06:00-18:00 hrs respectively. Temperature was measured using digital thermometers (accuracy ± 0.1^oC) and light levels (photosynthetic active radiation, PAR) were measured with the fibre quantum sensor of the Diving-PAM fluorometer (Diving-PAM, Walz, Effeltrich, Germany; µmol quanta m^{-2} s^{-1}, calibrated against a LI-192SA quantum sensor). Salinity was recorded daily and tanks were cleaned several times per week to prevent algal growth.

Feeding experiments

Corals were acclimated to experimental conditions for 2 days in unfiltered seawater at subdued light (~400 μmol m^{-2} s^{-1}) with max. daily temperatures <30°C. No changes in mucus production and tissue coloration were noticed and all corals displayed full polyp extension at night. Water supply was switched to filtered seawater 12 h prior to the start of the experiment, allowing sufficient time for food digestion (Rossi et al. 2004). One fragment of each colony was assigned to one of the following treatments: (1) Starvation: corals were kept in filtered seawater, deprived of organic particles >0.5 μm; (2) Feeding: corals were provided daily with freshly hatched *Artemia salina* nauplii (1070±110 ind. L^{-1}), and were allowed to feed for 3 h. During each feeding bout the water flow was discontinued in all tanks, while aeration was maintained, to keep the water well oxygenated and zooplankton in circulation (Sebens and Johnson 1991). At the end of each feeding period, the tanks of both fed and starved corals were rinsed and the water flow continued. At the end of each trial corals were frozen at -20°C pending tissue analyses.

Fluorescence measurements

Photophysiological responses of zooxanthellae were assessed by chlorophyll fluorescence analyses using a pulse amplitude modulated fluorometer (Diving-PAM) inside the tanks. All corals were dark adapted prior to each measurement. Hoegh-Guldberg and Jones (1999) showed that most changes in dark-adapted quantum yield of *S. pistillata* occurred after 10 min. We tested the change in quantum yield following different periods of dark adaptation (10 – 20 min) prior to the start of the experiment and found that a 15 min period of dark-adaptation was suitable for subsequent measurements of dark-adapted quantum yield. To account for a decrease in chlorophyll fluorescence of some fragments due to the loss of pigmentation over the course of 10 days, all measurements were carried out using high instrument settings for both 'measuring light intensity' and 'electronic signal gain'. Due to localized differences in photoacclimation and small-scale variability of the zooxanthellae within the host tissue (Levy et al. 2004), changes in minimal (F_o) and maximal (F_m) fluorescence yields relevant to photoinhibitory processes can be only interpreted meaningfully if repeated measurements are carried out at exactly the same location. This however, can lead to localized bleaching (personal observation). To avoid excessive exposure to high light and associated development of photolesions, all measurements were conducted in 2 day intervals. The fiber-optic probe of the PAM was clipped to the upper region of the fragment (1 cm

below the tip of the branch) using a 'darkening adapter' and modified 'dark leaf clip' (Diving-PAM accessories) while the position of the clip on the coral was marked by three bearing points on the perspex blocks and side of the tanks.

The effect of feeding treatment on the activity of PSII and the NPQ kinetics of *S. pistillata* was explored in two different ways. Firstly, the changes in maximum potential quantum yield ($F_v/F_m = (F_m - F_o)/F_m$), a proxy for the photosynthetic efficiency of PSII, over time were recorded consistently between 10:00-11:00 hrs at an average temperature of 31.2 ± 0.05 ^oC (n = 120; mean ± SE) and average light intensity of 345 ± 0.05 μmol m^{-2} s^{-1} (n = 120; mean ± SE), just before the onset of photoinhibition (established in forgoing measurements). Each fragment was pulsed with a weak (<1 μmol m^{-2} s^{-1}) red light to obtain F_o, followed by a 1 s pulse of saturating actinic light (>5000 μmol m^{-2} s^{-1}) to determine F_m. F_v/F_m was calculated in the conventional manner as $F_m – F_o/F_m = F_v/F_m$ (Schreiber 2004).

Secondly, nocturnal recovery of the photosynthetic apparatus from daily exposure to elevated temperatures of 2-3^oC above temperatures *in situ* was determined before dawn (05:00–06:00 hrs) following a 10-h recovery period under dim light (~5 μmol m^{-2} s^{-1}) and *in situ* temperature (29^oC). F_o, F_m and F_v/F_m were assessed as above. Followed by a 40 s period of darkness, each fragment was exposed to saturating light for 4 min (1000 μmol m^{-2} s^{-1} PAR), after which the fluorescence had reached steady state. The minimum and maximum fluorescence (F' and F_m' of light adapted samples) were recorded and the effective quantum yield determined as the ratio $\Delta F/F_m$' where $\Delta F = F_m' - F'$. The relative electron transport rates (rETR) of PS II were then calculated as rETR = $\Delta F/F_m$' x PAR x 0.5 (Hoegh-Guldberg and Jones 1999) and NPQ as NPQ = F_m-F_m'/F_m' (Schreiber 2004).

Biomass analysis

Coral tissue was stripped from the skeletons under dim light using an airbrush gun and a phosphate buffered saline solution (Sigma Aldrich). The slurry (~100 ml) was homogenized in a hand potter and subsamples of 20 ml frozen for chlorophyll a and c_2 analyses. A 10 ml aliquot of the homogenate was preserved in 4% formalin and used to determine zooxanthellae densities (8 replicate counts) using a Neubauer haemocytometer. The mitotic indices were determined from the number of cells appearing as doublets in two samples of 1000 cells (Jones and Yellowlees 1997). Chlorophyll a and c_2 were extracted as described by Gardella and Edmunds (1999) and concentrations calculated according to the equation of Jeffrey and Humphrey (1975). Zooxanthellae densities were normalized to surface area (cells cm^{-2}) and chlorophyll concentrations expressed as μg (10^6 $cells^{-1}$). The surface area of the coral

skeletons were determined photometrically using the dye-dipping method of Hoegh-Guldberg (1988).

Data analyses

All data were checked for homogeneity of variances using Cochran's C-test and if necessary ln(x)-transformed. The variances of maximum temperatures and light intensities among tanks were analyzed by a repeated measurements two-way ANOVA (Sokal and Rohlf 1995) using WinGMAV (EICC, University of Sydney, Australia). The data variances of both temperature and light were heterogeneous and failed to be stabilized. However since the data set was large (n = 120) and the analysis revealed no significant differences in daily maximum temperature and light intensity between individual tanks, the risk of committing a Type I error was eliminated (Underwood 1997), and the analysis thus regarded reliable. The effect of feeding versus starvation on changes in the fluorescence responses of zooxanthellae in *S. pistillata* and biomass characteristics after 10 days were analyzed using a general linear model with a three-factorial nested design and post–hoc SNK testing using Statistica.

Results

As would be expected, daily max. temperatures ($F_{2,108}$ = 26.28, P = 0.001) and light intensities ($F_{2,108}$ = 39.44, P = 0.001) of experimental tanks varied significantly among trials. However there was no significant variation in temperature ($F_{3,108}$ = 0.39, P = 0.75) or light ($F_{3,108}$ = 2.01, P = 0.11) regime among tanks within each trial.

Effect of feeding on zooxanthellae chlorophyll fluorescence

There were no significant differences in the effect of feeding treatment between trials and colonies and no significant interaction was detected between trial and treatment. (see Fig. 1 and 2). After 10 days, F_v/F_m values measured between 10:00-11:00 hrs were significantly lower for starved than for fed corals (Fig. 1a).

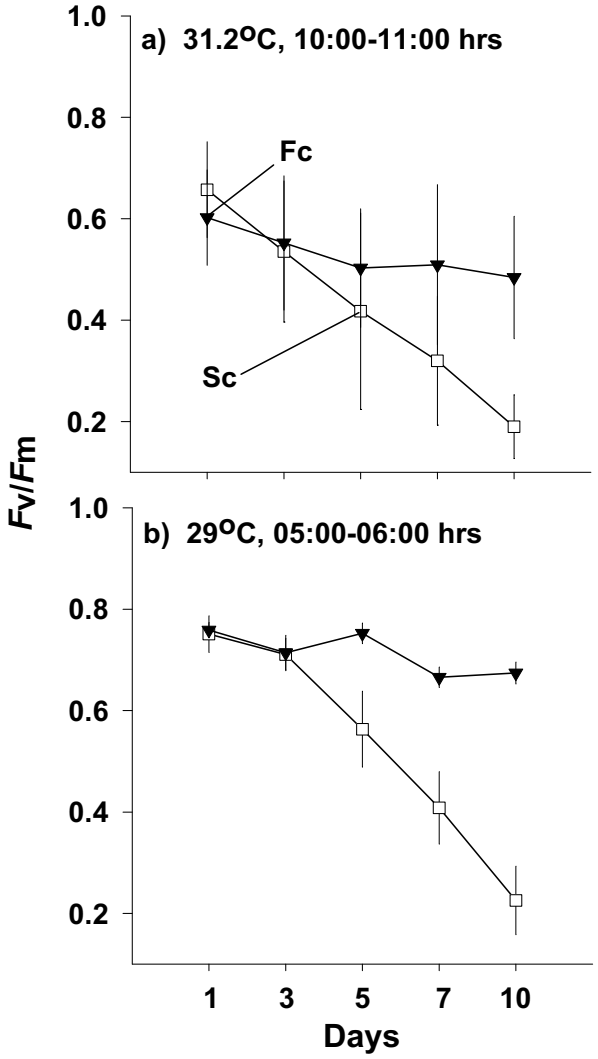

Fig. 1 Maximum potential quantum yield (F_v/F_m) of zooxanthellae in dark-adapted *Stylophora pistillata* at a) 31.2 ± 0.05 °C between 10:00 – 11:00 hrs and b) before dawn (05:00 – 06:00 hrs) at ~29°C after daily exposure to elevated temperature for 10 days in three repeat trials. Corals were exposed to either feeding (Fc) or starvation (Sc). Shown are means (n = 6) and 95% confidence limits. Effects of feeding treatment on F_v/F_m after 10 days were significant for a) 10:00 – 11:00 hrs ($F_{1,3}$ = 461.08, P = 0.002) and b) 05:00 – 06:00 hrs ($F_{1,3}$ = 117.26, P = 0.008). There was no effect of colony and trial (10:00 – 11:00 hrs: $F_{3,3}$ = 2.84, P= 0.20; 05:00 – 06:00 hrs: $F_{3,3}$ = 0.77, P = 0.58) and no significant trial by treatment interactions (10:00 – 11:00 hrs: $F_{2,3}$ = 0.38, P = 0.70; 05:00 – 06:00hrs: $F_{2,3}$ = 1.12, P = 0.43)

Starvation resulted in a marked decrease in F_v/F_m with values dropping from ~0.6 at day 1 to ~0.2 at day 10 while F_v/F_m for fed corals did not decrease below ~0.5. Predawn values of F_v/F_m were similar for starved and fed corals during the first 3 days of the experiment indicating high rates of nocturnal recovery of the photosynthetic apparatus in all corals. Thereafter F_v/F_m values of starved corals declined monotonically resulting in a ~70% decrease relative to F_v/F_m of fed corals, which remained relatively constant for 10 days Fig. (1b). At day 10 F_v/F_m was significantly lower for starved than for fed corals (Fig. 1b).

Values measured for $\Delta F/F_m$' before dawn decreased markedly over time regardless of treatment (Fig. 2a). However while $\Delta F/F_m$' of starved corals declined from ~0.35 to almost 0 over the course of 10 days, $\Delta F/F_m$' for fed corals declined by only 50% resulting in a significant difference between fed and starved corals at day 10 (Fig. 2a). Similarly, rETRs of both fed and starved corals declined an order of magnitude over time (Fig. 2b). At the end of each trial rETRs of starved corals however were significantly lower than rETRs of fed corals (Fig. 2b). Feeding significantly increased the coral's ability to engage in NPQ (Fig. 2c). Fed

corals sustained high NPQ whereas starved corals displayed a progressive inability to engage in NPQ, culminating in a ~80% decline in NPQ by day 10 relative to fed corals (Fig. 2c).

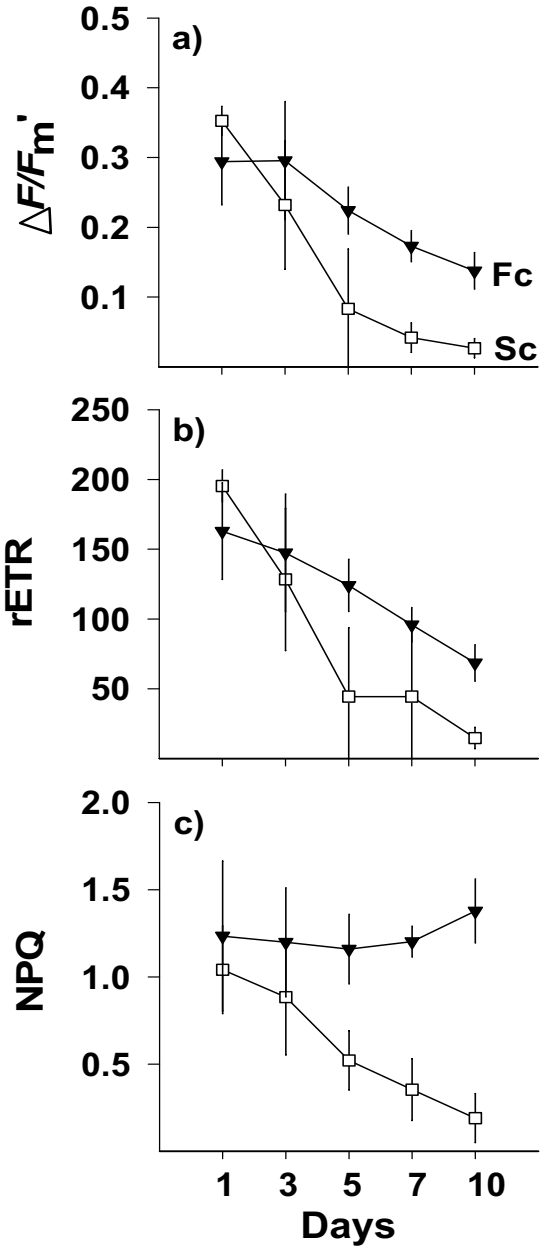

Fig. 2 *Stylophora pistillata*. Changes in a) effective quantum yield ($\Delta F/F_m$'), b) relative electron transport rate (rETR), and c) non-photochemical quenching (NPQ) of corals before dawn (05:00 – 06:00 hrs) following 4 min exposure to >1000 µmol m^{-2} s^{-1} over 10 days in three repeat trials. Corals were subjected to either feeding (Fc) or starvation (Sc). Shown are means (n = 6) and 95% confidence limits. Effects of feeding treatment after 10 days were significant for a) $\Delta F/F_m$' ($F_{1,3}$ = 1588.77, P = 0.000), b) rETR ($F_{1,3}$ = 1274.60, P = 0.000), and c) NPQ ($F_{1,3}$ = 20.68, P = 0.04). There was no effect of colony and trial ($\Delta F/F_m$': $F_{3,3}$ = 0.374, P = 0.77; rETR: $F_{3,3}$ = 0.377, P = 0.77; NPQ: $F_{3,3}$ = 0.376, P = 0.77) and no significant trial by treatment interactions ($\Delta F/F_m$': $F_{2,3}$ = 0.014, P = 0.98; rETR: $F_{2,3}$ = 0.015, P = 0.98; NPQ: $F_{2,3}$ = 2.85, P = 0.20)

Effect of feeding on zooxanthellae densities, mitotic indices and chlorophyll concentrations

For zooxanthellae densities and mitotic indices there were no significant effects of feeding treatment between trials or colony, nor was there an interaction between trial and treatment. (Fig. 3). Zooxanthellae densities were significantly lower in both fed and starved corals than in reference corals. Although the zooxanthellae densities in fed corals were about 40% higher than zooxanthellae densities in starved corals, this difference was not statistically significant (Fig. 3). By contrast, the mitotic indices of zooxanthellae in fed corals were significantly higher than in both starved and reference corals but there were no significant differences between starved and reference corals (Fig. 4). Significant trial by treatment interactions were detected for both chlorophyll a and c_2 , which makes it difficult to evaluate an effect of feeding treatment. Post-hoc analyses still distinguished the following differences for chlorophyll a: trial 1 and 2, Reference = Feeding = Starvation; trial 3, Reference = Feeding < Starvation and for chlorophyll c_2: trial 1, Reference > Feeding = Starvation; trial 2, Starvation > Reference = Feeding; trial 3, Reference = Feeding = Starvation.

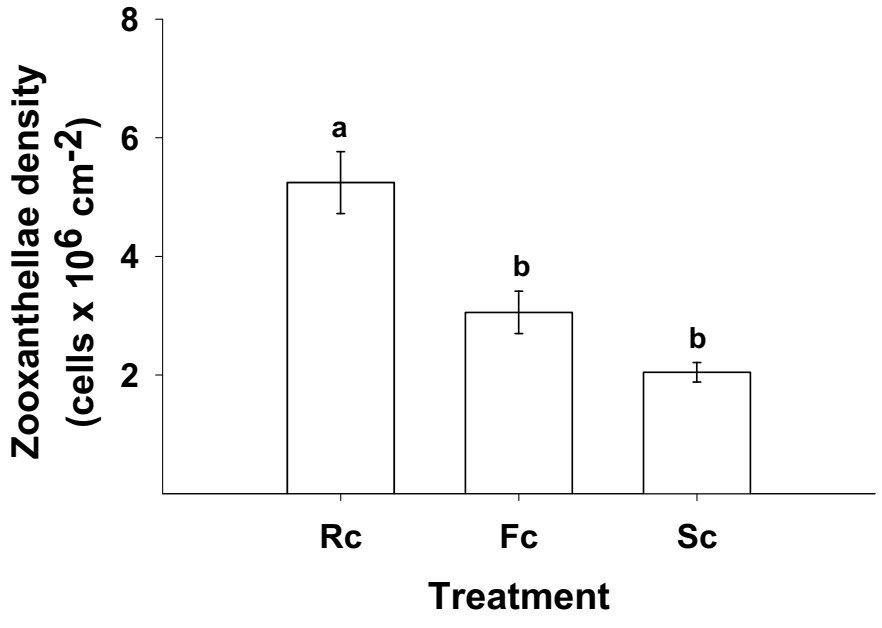

Fig. 3 Zooxanthellae densities (cells x 10^6 cm^{-2}) of *Stylophora pistillata* in reference corals (Rc) and corals after 10 days of exposure to elevated temperature and subjected to either feeding (Fc) or starvation (Sc) in three repeat trials (mean ± SE, n = 6). Effects of feeding treatment were significant ($F_{2,6}$ = 78.31, P = 0.000). There was no effect of colony and trial ($F_{3,6}$ = 2.24, P = 0.18) and no significant trial by treatment interactions ($F_{4,6}$ = 0.24, P = 0.90). Bars that share the same letters are not significantly different (SNK, P < 0.05)

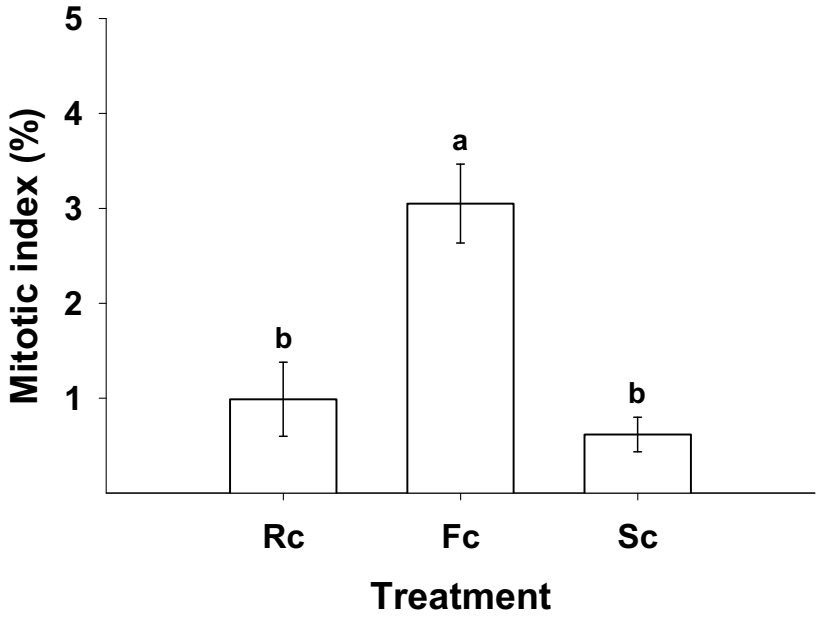

Fig. 4 Mitotic indices (%) of zooxanthellae of *Stylophora pistillata* in reference corals (RC) and corals after 10 days of exposure to elevated temperature and subjected to either feeding (FC) or starvation (SC) in three repeat trials (mean ± SE, n = 6). Effects of feeding treatment were significant ($F_{2,6}$ = 13.90, P = 0.01). There was no effect of colony and trial ($F_{3,6}$ = 0.74, P = 0.56) and no significant trial by treatment interactions ($F_{4,6}$ = 1.25, P = 0.38). Bars that share the same letters are not significantly different (SNK, $P < 0.05$)

Discussion

This is the first experimental study to examine the thermal tolerance of corals in response to exogenous food using chlorophyll fluorescence as a proxy for the photosynthetic activity of PSII of zooxanthallae in *S. pistillata*. Overall our results show a strong correlation between the absences of external food, a decrease in the thermal tolerance of PSII functioning and NPQ processes. As predicted, zooxanthellae in fed corals maintained high photosynthetic efficiency of PSII while starved corals displayed strong signs of chronic photoinhibition. This was reflected in the significant decline in F_v/F_m values between 10:00-11:00 hrs and the concomitant decrease in pre-dawn values for F_v/F_m, $\Delta F/F_m$', rETR. This pattern is consistent with previous conclusions (Jones et al. 1998; Jones and Hoegh-Guldberg 2001; Franklin et al. 2004), that photoprotective downregulation of photosynthesis culminates in chronic photoinhibition if nocturnal recovery is incomplete. Although zooxanthellae in fed corals also exhibited signs of loss in overall photosynthetic activity, the high levels of NPQ over time indicate that fed corals sustained the ability to dissipate excess excitation energy as heat, which may have prevented the photoinactivation and photodamage to PSII (Warner 1996,

1999) by reducing the generation of reactive oxygen species (Demmig-Adams and Adams 2002).

Zooxanthellae are believed to utilize and recycle ammonium produced by host catabolism as a nitrogen source (e.g. Falkowski et al. 1993). However this internal recycling of ammonium can not proceed indefinitely without 'new' input from the outside and the relative importance of heterotrophy as a means of nitrogen acquisition for cnidarian zooxanthellae has been well documented (McAuley and Cook 1994; Fitt and Cook 2001; Piniak et al. 2003). Host starvation is thought to reduce ammonium assimilation in the zooxanthellae as well as photosynthetic rates and has been shown to increase cell degradation and reduce cell division and numbers (Fitt and Cook 2001; Titlyanov et al. 2000, 2001). Corals typically maintain high levels of lipids derived from excess photosynthetically fixed carbon and receive substantial amounts of essential fatty acids (Harland et al. 1993), amino acids (Wang and Douglas 1999) and mycosporine-like amino acids (Shick et al. 2005) from their zooxanthellae. In addition the coral host is believed to obtain essential nutrients for tissue synthesis from heterotrophic sources (Dubinsky and Jokiel 1994; Mueller-Parker et al. 1994; Anthony et al. 2002). Under bleaching conditions where the photosynthetic efficiency of zooxanthellae is greatly reduced (Jones et al. 2000; Warner et al. 2002), trophic interactions of the symbiotic association may be therefore uncoupled resulting in changes in the biochemical composition (Szmant-Froelich 1981; McAuley and Cook 1994) and energy balance of the coral colony (Clayton and Lasker 1984). Thus in the absence of external food, the temperature tolerance of corals may be lowered as additional stressors such as high temperature are likely to further constrain physiological processes due to increased metabolic costs (Calow 1989, Koehn and Bayne 1989). Unfortunately our results provide no indication of the underlying biochemical mechanisms of how additional resources could have modulated the thermal resistance of the photosynthetic apparatus. We can only speculate that zooplankton may provide a direct source of nitrogen to the zooxanthellae facilitating enhanced rates of protein repair and re-synthesis of the PSII D1 protein (e.g. Ohad et al. 1984; Warner et al. 1999, Takahashi et al. 2004, but see Smith et al. 2005 for review) or reduce photophysiological damage of the zooxanthellae indirectly by enhancing the capacity of either symbiotic partners to synthesize antioxidant compounds or heat-shock proteins or both (e.g. Downs et al. 2002, Yakovleva et al. 2004).

Contrary to our prediction that fed corals would be less susceptible to bleaching than starved corals, there was no clear correlation between feeding regime, zooxanthellae densities and chlorophyll concentrations. High rates of cell division as were observed for fed corals can be

characteristic for the recovery of *in hospite* zooxanthellae populations due to increased space and nutrient availability (Suharsono and Brown 1992, Jones and Yellowlees 1997). Considering that zooxanthellae numbers in fed corals had decreased by 50% compared to reference corals but displayed rates of cell division that were three times as high as in reference corals, the high mitotic indices in fed corals appear odd. The most parsimonious explanation for this observation would be that zooxanthellae of fed corals were dividing at a faster rate after they had been released into the coelenteron compared to those remaining in the tissue (Suharsono and Brown 1992). This suggests that zooxanthellae were released from their hosts at similar rates regardless of feeding treatment but that zooxanthellae in fed corals were healthier than those expelled from starved corals. Thus zooxanthellae in starved corals may have suffered cellular degradation as has been reported for zooxanthellae in corals during thermal stress in previous studies by Dunn et al. (2002) and Franklin et al. (2004). The low mitotic indices in reference corals by contrast are consistent with values reported for healthy *S. pistillata* (Muscatine et al. 1984).

An experimental period of 10 days may have been insufficient to determine clear treatment-dependent changes in zooxanthellae densities and chlorophyll content. Also the water temperatures of the experimental protocol in this study did not entirely match increases of seawater temperatures during natural bleaching events, which commonly feature less diel fluctuations exceeding periods of 10 days (e.g.Gleeson and Strong 1995). The combined results of this study however provide indication that exogenous food can play an important role in reducing the photophysiological damage of zooxanthellae that typically leads to bleaching. This could present an interesting consideration for coral communities in turbid, near shore waters which often provide a potentially rich heterotrophic environment and may therefore benefit some coral species under bleaching conditions (Anthony 2006; Anthony et al.2007).

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Paper II

The effect of heterotrophy on photosynthesis and tissue composition of two scleractinian corals under elevated temperature

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The effect of heterotrophy on photosynthesis and tissue composition of two scleractinian corals under elevated temperature

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Abstract

Exogenous food can increase protein levels of coral host tissue, zooxanthellae densities, chlorophyll (chl) concentrations and rates of photosynthesis and is thought to play an important role in the resilience of bleached corals. There is however no information about the effect of heterotrophy on the bleaching susceptibility of corals under elevated temperature conditions. This study investigates potential interactions between food availability, basal metabolic functions (photosynthesis and respiration), energy status (lipid concentrations), total protein concentrations and the bleaching susceptibility (loss of chl and/or zooxanthellae) of the scleractinian corals *Stylophora pistillata* (Esper) and *Galaxea fascicularis* (Linnaeus) in response to elevated temperature (daily temperature rises of 3-4^{o}C) over 15 days. Feeding experiments were carried out in which the corals were either fed daily with zooplankton or starved. Compared to fed corals, starvation of both species resulted in a significant decrease in daily photosynthetic oxygen evolution over time. Gross (Pg) and net (Pn) photosynthetic production of starved corals of both species between 10:00-11:00 hrs had declined by ~50 % at day 15 while there were no marked change in Pg and Pn of fed corals. After 15 days, starved *S. pistillata* contained significantly lower zooxanthellae densities, lipid and protein concentrations than fed corals. Starved *G. fascicularis* also displayed a decrease in zooxanthellae densities which was accompanied by a significant decline in algal chl concentrations. Contrary to *S. pistillata* feeding treatment had no effect on the lipid concentrations of *G. fascicularis*. Total protein concentrations however were significantly lower in straved than in fed *G. fascicularis*. Furthermore starvation resulted in a significant decrease in respiration of *S. pistillata* during the last four days of the experiment while treatment had no effect on the respiration rates of *G. fascicularis*. Overall the oxygen consumption of *S. pistillata* of both treatments was about 39-67 % higher than the respiration of *G. fascicularis* indicating that low metabolic rates may have allowed starved *G. fascicularis* to conserve energy reserves over the course of the experiment. The combined results reveal a strong positive relationship between food availability, sustained photosynthetic activity and reduced loss in pigmentation of both species under elevated temperature conditions.

Key words: Bleaching, Lipid, Protein, Respiration, Zooplankton, Zooxanthellae

Introduction

The ecological consequences of coral bleaching (loss of symbiotic zooxanthellae and/or chlorophyll (chl)) in response to seawater temperature anomalies continue to be a major environmental concern (Donner et al., 2005). The thermal tolerance and resulting bleaching susceptibility of corals for any given bleaching event is not uniform but can vary greatly not only between but also within species (Edmunds, 1994; Fitt et al., 2001; Stimson et al., 2002), which makes it difficult to predict bleaching related shifts in coral population and community structure. It is generally accepted that the foremost causes of bleaching involve photoinhibition of zooxanthellae photosynthesis and subsequent formation of damaging reactive oxygen species (Franklin et al., 2004; Smith et al., 2005). Increasing evidence suggests that the extent of photosynthetic impairment is coupled to levels of antioxidant activity and rates of protein repair (Downs et al., 2002; Takahashi et al., 2004; Yakovleva et al., 2004), processes which incur high metabolic costs in terms of nitrogen demands and energy expenditure (Koehn and Bayne, 1989).

In healthy corals, photosynthetically fixed carbon constitutes the principal source of energy covering up to 100% of the coral's daily energy requirements (Falkowski et al., 1984; Davies, 1984). In addition, corals receive substantial amounts of important amino and fatty acids (Patton and Burris, 1983; Harland et al., 1993; Wang and Douglas, 1999; Shick et al. 2005) from their zooxanthellae. Thus under bleaching conditions where the photosynthetic activity of zooxanthellae is greatly reduced (Warner et al., 2002), the energetic and nutritional balance of the symbiotic association becomes corrupted, potentially rendering the coral host into a state of starvation, which can lead to changes in biochemical composition and reduced energetic status of the colony (Fitt et al., 1993; Grottoli et al., 2004; Szmant and Gassman, 1990; Bachok et al., 2006). However, corals can also use energy from heterotrophic sources to balance their metabolic needs (Fitt and Pardy, 1981; Wellington, 1982; Anthony and Fabricius, 2000) and for both, the coral host and zooxanthellae, heterotrophy constitutes an important means of nitrogen acquisition (Steen, 1987; McAuley and Cook, 1994; Fitt and Cook, 2001; Piniak et al., 2003). While it is well established that under non-bleaching conditions, food availability tends to increase protein levels of host tissue, zooxanthellae densities, chl a concentrations (Titlyanov et al., 2001; Ferrier-Pages et al., 2003; Houlbreque et al., 2003, 2004) and rates of photosynthesis (Houlbreque et al., 2003; Davy et al., 2006), less is known about the interactions between heterotrophy, coral tissue composition and physiological functioning of corals under thermal stress. There is evidence showing that feeding can sustain the photosynthetic quantum yield of *Stylophora pistillata* under thermal

stress (Borell and Bischof, 2008). Grottoli et al. (2006) demonstrated that bleached species which are capable of satisfying their energy demands through feeding feature higher rates of recovery than those which remain primarily autotrophic. Thus far however there have been no physiological studies, which explicitly investigate the effect of heterotrophy on the bleaching susceptibility of corals. Nearshore reefs often feature high loads of suspended particulate matter and resuspended sediment, which on one hand reduces the light for photosynthesis (Rogers, 1990) but on the other may offer a rich source of nutrients and additional energy (Anthony and Fabricius, 2000; Anthony, 2006). Since many corals are able to exploit a wide range of food sources including suspended particulate matter (Anthony and Fabricius, 2000), sediment (Rosenfeld et al., 1999), bacteria (Sorokin, 1973) and zooplankton (Sebens et al., 1996; Ferrier-Pages et al., 2003), an understanding of how food availability influences the thermal stress susceptibility could therefore provide important information aiding the prediction of the effects of increasing temperature on coral community structure along environmental gradients.

The objective of this work was to test the effect of food availability on basal metabolic functions (photosynthesis and respiration), energy status (lipid concentrations) and protein concentrations and the bleaching susceptibility (loss of zooxanthellae and/or chl) of two coral species from a turbid nearshore reef under elevated temperature conditions (daily temperature rises of 3-4^{o}C).

Materials and methods

This study was carried out at Hasanuddin University Marine Field Station on Barang Lompo island (05^{o} 03' S, 119^{o} 19' E; Spermonde Archipelago, southwest Sulawesi, Indonesia) between September and October 2005. Feeding experiments at elevated seawater temperature were conducted outdoors under natural sun light using samples of *Galaxea fascicularis* (Linnaeus) and *S. pistillata* (Esper). *G. fascicularis* is a massive-submassive species, that has been previously shown to be more temperature tolerant than the branching species *S. pistillata,* (Stimson et al., 2002; Bhagooli and Hidaka, 2003). For logistical reasons experiments were run in 3 consecutive, independent repeat trials. Each trial was run for 15 days. All coral samples were collected at ~2 m depth around the inshore reef of Kayangang island, located in the vicinity of Makassar harbor. For each trial, twelve independent fragments were sampled; one fragment from each of twelve spatially distant (> 10 m) and randomly selected parent colonies of each species (surface area approximately 25-50 cm^2 for *S. pistillata* and 150-300 cm^2 for *G. fascicularis*), assumed to be genetically distinct, were

collected, placed into dark containers and transported to the field station within 30 min of collection. Four fragment of each species were frozen immediately at -20 ^{o}C and used as reference corals in later tissue analysis.

Coral maintenance and experimental design

The eight remaining fragments were glued to PVC nuts using non toxic, two component underwater epoxy, and mounted on PVC screws attached to perspex blocks. Each fragment was thus placed into one of eight transparent 14-l plastic tanks. All tanks were supplied independently with oligotrophic (<0.3 µM total inorganic nitrogen), filtered (>0.5 µm) seawater (0.45 l min $^{-1}$) and were aerated continuously. Acrylic covers were used to prevent contamination with airborne particulates and to reduce ultraviolet (UV) radiation. Simulated *in situ* irradiance at 2 m depth (max. 600-700 µmol m^{-2} s^{-1} under cloudless conditions) and elevated temperatures (max. 3-4°C above *in situ* temperature) were attained by screening with black plastic netting (1 mm mesh). Water temperature and light in each tank were recorded hourly between 05:00-21:00 hrs and 06:00-18:00 hrs respectively. Temperature was measured using digital thermometers (accuracy ± 0.1^{o}C) and light levels (photosynthetic active radiation, PAR) were measured with a LI-192SA quantum sensor (µmol quanta m^{-2} s^{-1}). The daily water temperature rose rapidly from 28-29^{o}C (temperature in situ) just after sun rise to 32.7 ± 0.02 ^{o}C (n = 720; mean ± SE) around noon (max. PAR 633 ± 2.31 µmol m^{-2} s^{-1}; n = 720; mean ± SE) and decreased slowly with decreasing irradiance to pre-dawn levels at ~19:00 hrs. Salinity was recorded daily and tanks were cleaned several times per week to prevent algal growth.

Corals were acclimated to experimental conditions for 4 days in unfiltered seawater at subdued light (~400 µmol m^{-2} s^{-1}) with max. daily temperatures ≤31^{o}C. No changes in mucus production and tissue coloration were noticed and all corals displayed full polyp extension at night. Water supply was switched to filtered seawater 12 h prior to the start of the experiment. Coral fragments were haphazardly assigned to one of the following treatments: (1) Starvation: starved corals were deprived of organic particles >0.5 µm; (2) Feeding: fed corals were provided daily with freshly hatched *Artemia salina* nauplii (1203±89 ind. l^{-1}) and were allowed to feed for 3 h during which the water flow was discontinued in all tanks. Aeration was maintained, to keep the water well oxygenated and zooplankton in circulation (Sebens et al., 1998). At the end of each feeding period, the tanks of both fed and starved corals were rinsed and the water flow continued.

Photosynthesis and respiration measurements

Changes in rates of net primary production (Pn) and respiration in darkness were determined by measuring oxygen evolution and oxygen consumption between 10:00-11:00 hrs on day 1, 5, 8, 12 and 15 of each trial at an average temperature of 31.6 ± 0.03°C (n = 240; mean ± SE) and light regime of 484.83 ± 7.44 μmol m^{-2} s^{-1} (n = 240; mean ± SE). Coral fragments of each species and each treatment (n = 2 per trial) were allocated to either one of four transparent or dark tinted, sealed plexiglas chambers (1.8 l) in order to measure Pn and respiration respectively. The seawater in the chambers was circulated with a multi-channel peristaltic pump (521VK, Watson-Marlow, UK) through silicon hoses (Ø 8 mm), which were connected to an in-and outlet fitted to the chamber lids. Temperature and irradiance within the chambers were measured at the beginning and end of each incubation run as described above. The oxygen flux was determined in triplicates by Winkler titration using a modified method after Grasshoff (1983). Rates of gross primary production (Pg) were estimated by adding Pn to respiration. To control for photosynthesis of micro-organisms, two chambers in each run were left empty. All data were standardized to surface area. At the end of each trial corals were frozen at -20°C pending tissue analyses.

Tissue analyses

Coral tissues were stripped from the skeletons under dim light using an airbrush gun and phosphate buffered saline solution (Sigma Aldrich). The slurry (~100 ml) was homogenized in a hand potter and subsamples were frozen for later chl a and c_2 (20 ml), protein and lipid (55 ml) analyses. In addition, a 10 ml aliquot of the homogenate was preserved in 4% formalin and used to determine zooxanthellae density (8 replicate counts using a Neubauer haemocytometer). Total chl was extracted as described by Gardella and Edmunds (1999) and concentrations calculated according to the equation of Jeffrey and Humphrey (1975). Total protein content was determined by Kjeldahl digestion as described in Chow et al. (1980). Lipids were extracted using the standard method of Folch et al. (1957) and the total lipid content of each sample determined gravimetrically. In order to compare physiological parameters between coral species, data should ideally be normalized to surface area as well as biomass (Edmunds and Gates, 2002). Since the main focus of this study was to investigate the relative change in tissue composition in response to the feeding treatments of the two species,

proteins, lipids and zooxanthellae densities were normalized to skeletal surface area. Chl concentrations were expressed as μg x 10^6 $cells^{-1}$. Coral surface area was measured using the paraffin wax technique (Stimson and Kinzie, 1991).

Data analyses

All data were checked for homogeneity of variances using Cochran's C-test and if necessary ln(x)-transformed. The effect of feeding versus starvation on changes in the photosynthetic production and respiration of *S. pistillata* and *G. fascicularis* after 15 days were analyzed by two-way ANOVA with treatment and trial as fixed factors. To ensure that there was no bias effect of chamber incubations, the effect of feeding treatment on the tissue composition of coral samples after 15 days was analyzed by three-way ANOVA with treatment, trial and chambers (dark/light) as fixed factors. As there was no significant effect of chamber incubations, the data for fed corals (Table 2) were pooled (n = 12) and additional two-way ANOVAs with treatment and trial as fixed factors were performed to test for significant differences in tissue composition between reference and fed corals. Post–hoc SNK tests were run for separation of significant factors. All data were analyzed using WinGMAV (EICC, University of Sydney, Australia).

Results

Effect of feeding regime on photosynthesis and respiration

Zooplankton feeding versus starvation had a significant effect on both oxygen evolution and consumption of *S. pistillata* over time (Fig. 1a, b). Pg and Pn of starved compared to fed corals declined significantly after day 12 and had decreased to about 50% of initial values by the end of the experiment (Fig. 1a, b). Fed *S. pistillata* by contrast maintained high levels of Pg and Pn over the course of the experiment (Fig. 1a, b). ANOVA showed a significant trial by treatment interaction for the overall change in both Pg and Pn over time, i.e. the effect of treatment varied with trial (Table 1a, b). Post-hoc analyses however revealed that this interaction was due to higher oxygen concentrations of fed corals in trial 3 compared to trial 1 and 2 and that Pg and Pn of fed corals in all trials was significantly higher than for starved corals. Respiration rates of *S. pistillata* over 15 days displayed a similar pattern to photosynthetic rates. Starvation resulted in a significant decrease in respiration (Fig. 1c, Table 1c) while there was no change in respiration of fed corals (Fig. 1c).

Feeding treatment also had a significant effect on the photosynthetic production of *G. fascicularis* over time (Fig. 1d, e). As for *S. pistillata*, starvation of *G. fascicularis* had resulted in a ~50% decrease in Pg and Pn after 15 days while there was no marked change in the oxygen evolution of fed corals (Fig. 1d, e). Although confidence intervals indicate that Pg at the end of the experiment was significantly lower for starved than for fed corals, the treatment effect was not strong enough to be detected by ANOVA (Table 1a). However Pn of starved *G. fascicularis* was significantly lower than Pn of fed *G. fascicularis* (Table 1b). There were no significant changes in rates of respiration between fed and starved *G. fascicularis* (Fig. 1f, Table 1c). Overall the oxygen consumption of *S. pistillata* for both treatments was about 39-67 % higher than the oxygen consumption of *G. fascicularis*.

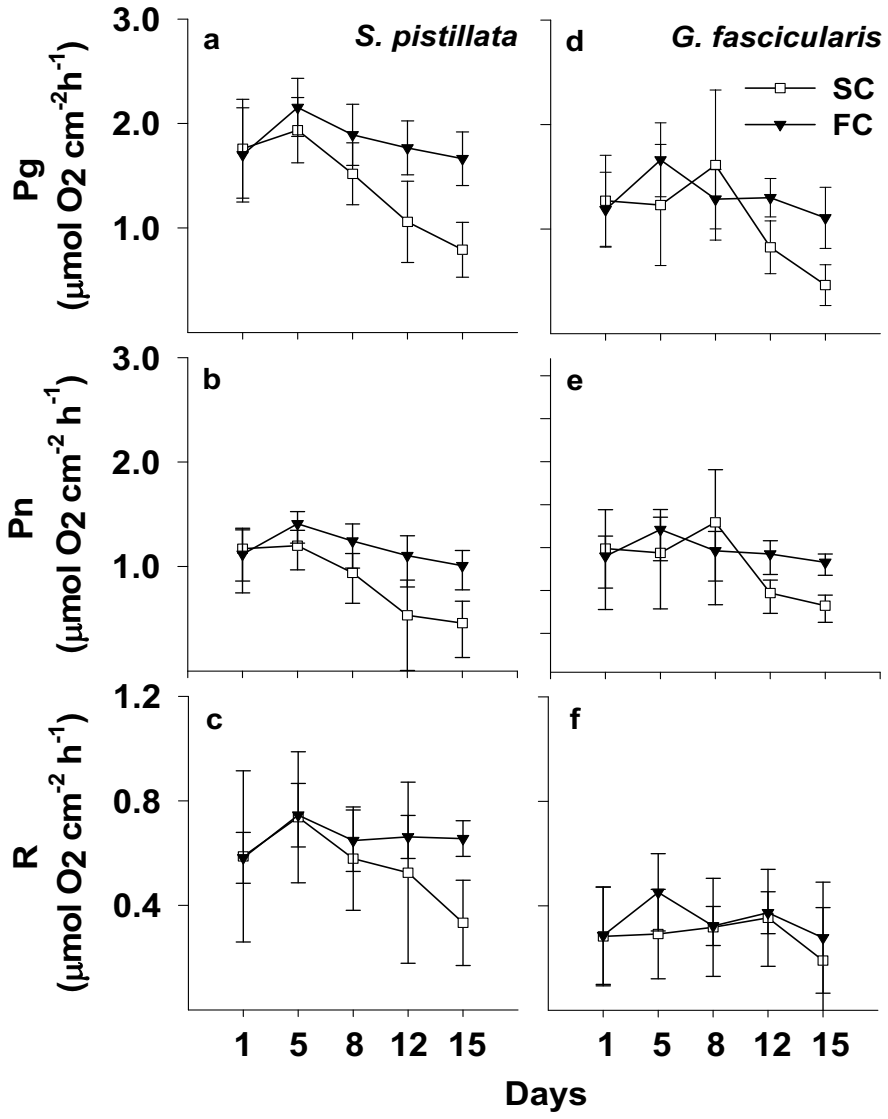

Fig. 1 Change in gross photosynthetic production (Pg), net photosynthetic production (Pn) and respiration (R) of Stylophora pistillata (a,b,c) and Galaxea fascicularis (d,e,f) at ~31.5oC between 10:00-11:00 hrs over 15 days of exposure to either feeding (FC) or starvation (SC) in three repeat trials. Shown are means (n = 6) and 95% confidence limits.

Table 1 ANOVA of the change in a) gross photosynthetic production (Pg), b) net photosynthetic production (Pn) and c) respiration of *Stylophora pistillata* and *Galaxea fascicularis* at ~31.5°C between 10:00-11:00 hrs over 15 days of exposure to either feeding or starvation in three repeat trials. ns = p value >0.05, * = p<0.05, ** = p<0.01, *** = p < 0.001.

		S. pistillata			*G. fascicularis*		
Source of variation	**df**	**MS**	***F***	***P***	**MS**	***F***	***P***
a) Pg (µmol O_2 cm^{-2} h^{-1})							
Treatment (T)	1	1.087	160.24	***	1.593	5.37	ns
Trial (Tr)	2	0.388	57.29	***	0.159	0.54	ns
T x Tr	2	0.135	19.95	**	0.334	1.12	ns
Residual	6	0.006			0.269		
b) Pn (µmol O_2 cm^{-2} h^{-1})							
Treatment(T)	1	1.087	160.24	***	0.933	6.48	*
Trial (Tr)	2	0.388	57.29	***	0.004	0.03	ns
T x Tr	2	0.135	19.95	**	0.182	1.26	ns
Residual	6	0.006			0.144		
c) Respiration (µmol O_2 cm^{-2} h^{-1})							
Treatment (T)	1	0.322	28.16	**	0.021	0.45	ns
Trial (Tr)	2	0.237	20.72	ns	0.363	7.58	*
T x Tr	2	0.033	2.89	ns	0.058	1.21	ns
Residual	6	0.011			0.047		

Effect of feeding on coral tissue composition

Zooxanthellae densities after 15 days of exposure to elevated temperature were significantly lower in starved than in fed *S. pistillata* (Table 2a, Fig. 2a). Although a decrease in zooxanthellae densities was evident for starved *G. fascicularis*, ANOVA revealed no significant differences between treatments (Table 2a, Fig. 2a). Zooxanthellae densities in fed corals of both species were not significantly different from reference corals (Table 3a, Fig. 2a). Chl a + c_2 concentrations of *G. fascicularis* were significantly lower in zooxanthellae of starved relative to fed corals whereas no significant differences were detected between fed and starved *S. pistillata* (Table 2b; Fig. 2b). ANOVA revealed no significant differences in chl concentrations between fed and reference corals for *G. fascicularis* (Table 3b, Fig. 2b) For *S. pistillata* significant trial by treatment interactions were detected for chl concentrations of fed and reference corals (Table 3b). Post-hoc analysis showed that chl concentrations in trial 1 were significantly lower in reference corals than in fed corals while there were no significant differences between reference and fed corals in trial 2 and trial 3.

The amount of lipid per unit surface area in starved *S. pistillata* was significantly lower than in fed corals (Table 2c; Fig. 3). By contrast, treatment had no significant effect on the lipid concentrations of *G. fascicularis* (Table 2c, Fig. 3). For both species the amount of

protein per unit surface area was significantly lower in starved than in fed corals (Table 2d; Fig. 4). Lipid and protein concentrations in fed *S. pistillata* after 15 days were significantly lower compared to concentrations in reference corals (Table 3c,d; Fig. 3,4) but no significant differences were detected between fed and reference *G. fascicularis* (Table 3c,d; Fig. 3, 4).

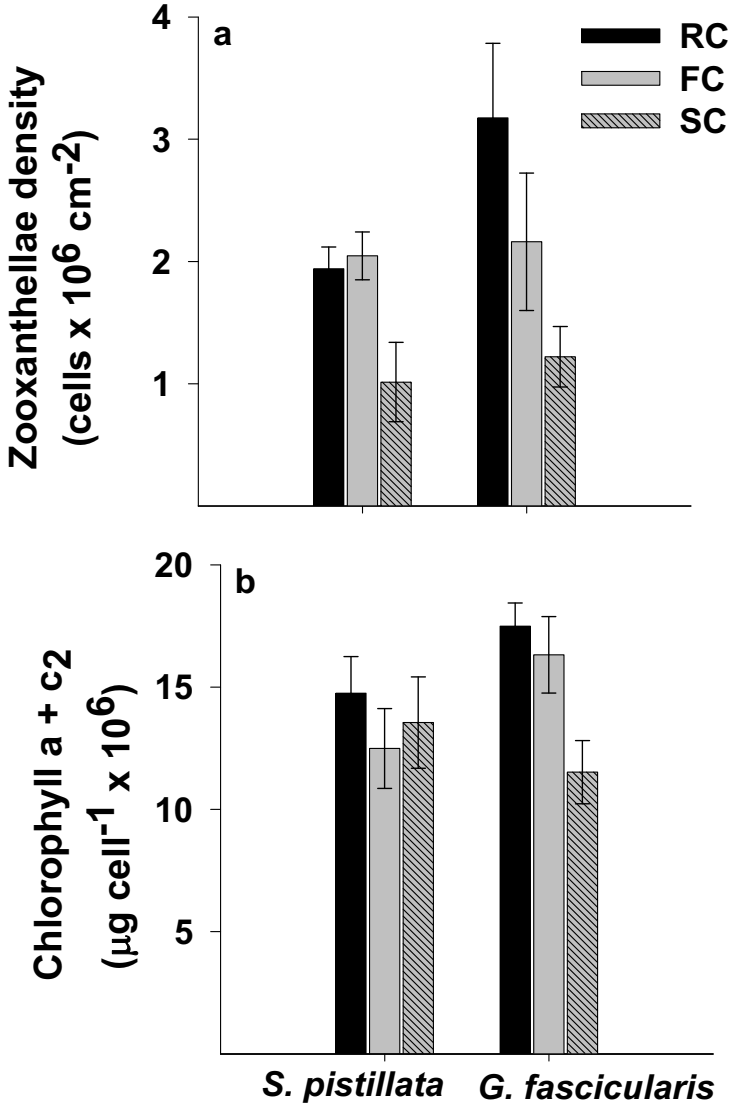

Fig. 2 Mean zooxanthellae densities and chlorophyll $a+c_2$ concentrations (n = 12, ± SE) of *Stylophora pistillata* and *Galaxea fascicularis*. Shown are data of reference corals (RC) and corals after 15 days of exposure to elevated temperature and subjected to either feeding (FC) or starvation (SC) in three repeat trials.

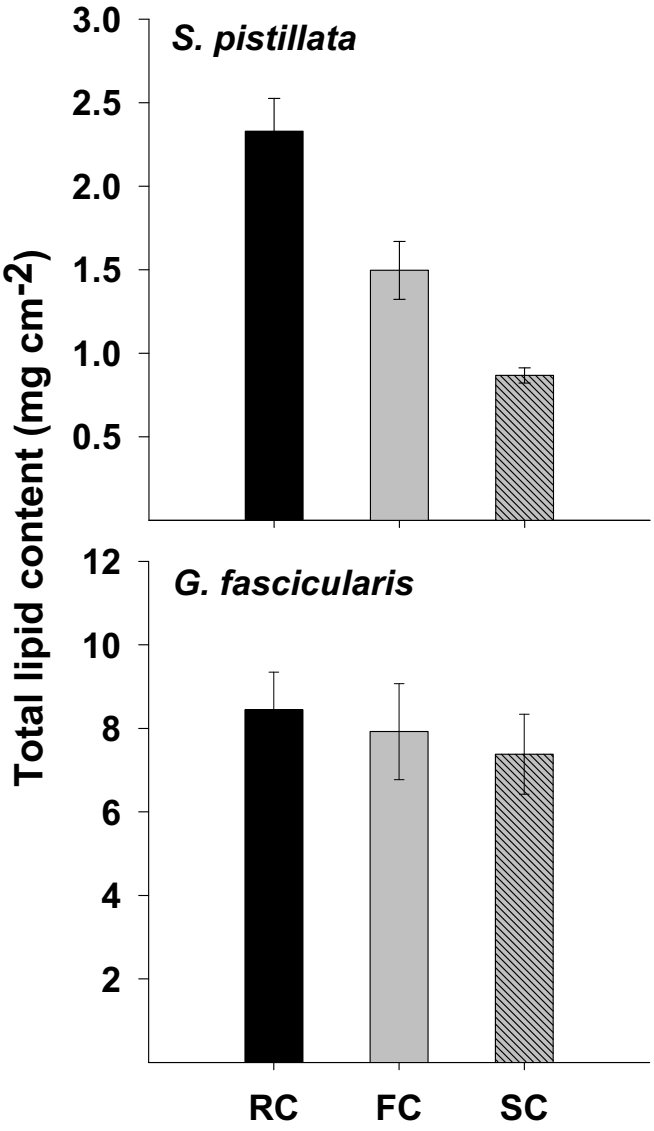

Fig. 3 Mean lipid concentrations (n = 12, ± SE) of *Stylophora pistillata* and *Galaxea fascicularis*. Shown are reference corals (RC) and corals after 15 days of exposure to elevated temperature and subjected to either feeding (FC) or starvation (SC) in three repeat trials.

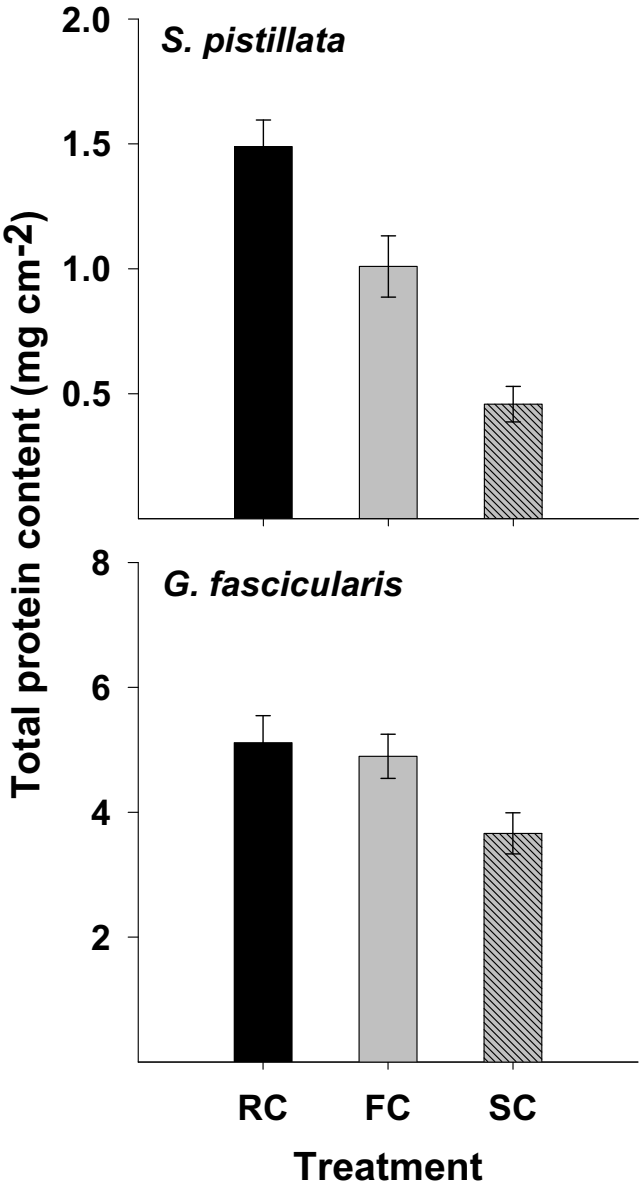

Fig. 4 Mean protein concentrations (n = 12, ± SE) of *Stylophora pistillata* and *Galaxea fascicularis*. Shown are data of reference corals (RC) and corals after 15 days of exposure to elevated temperature and subjected to either feeding (FC) or starvation (SC) in three repeat trials.

Table 2 ANOVA of a) zooxanthellae densities, b) chlorophyll concentrations, c) lipid and d) protein concentrations of *Stylophora pistillata* and *Galaxea fascicularis* after 15 days of exposure to elevated temperature and subjected to either feeding or starvation in three repeat trials. Data of zooxanthellae densities were homogenous after log (ln) transformation. Cochran's C = 0.5078 (*S. pistillata*), C = 0.2975 (*G. fascicularis*). ns = p value >0.05, * = p<0.05, ** = p<0.01, *** = p<0.001).

		S. pistillata			*G. fascicularis*		
Source of variation	**df**	**MS**	***F***	***P***	**MS**	***F***	***P***
a) zooxanthellae density (cell cm^{-2})							
Treatment (T)	1	5.343	7.53	*	1.826	3.59	ns
Light/Dark (L/D)	1	0.581	0.82	ns	0.111	0.22	ns
Trial (Tr)	2	0.053	0.07	ns	0.924	1.82	ns
T x L/D	1	0.020	0.03	ns	0.825	1.62	ns
T x Tr	2	0.010	0.01	ns	0.113	0.22	ns
Tr x L/D	2	0.263	0.37	ns	0.006	0.01	ns
T x Tr x L/D	2	0.335	0.47	ns	0.076	0.15	ns
Residual	12	0.709			0.508		
b) chlorophyll a + c$_2$ (µg cell^{-1} x 10^6)							
Treatment (T)	1	9.570	0.22	ns	115.144	5.74	*
Light/Dark (L/D)	1	25.622	0.58	ns	1.539	0.08	ns
Trial (Tr)	2	55.734	1.27	ns	57.465	2.86	ns
T x L/D	1	36.516	0.83	ns	13.938	0.69	ns
T x Tr	2	1.448	0.03	ns	27.539	1.37	ns
Tr x L/D	2	6.456	0.15	ns	0.526	0.03	ns
T x Tr x L/D	2	20.933	0.48	ns	42.568	2.12	ns
Residual	12	44.055			20.057		
c) Lipid (mg cm^{-2})							
Treatment (T)	1	1.932	38.09	***	1.765	0.22	ns
Light/Dark (L/D)	1	0.015	0.30	ns	47.575	6.01	ns
Trial (Tr)	2	0.235	4.65	ns	3.902	0.49	ns
T x L/D	1	0.083	1.64	ns	15.999	2.02	ns
T x Tr	2	0.098	1.94	ns	13.641	1.72	ns
Tr x L/D	2	0.069	1.91	ns	30.038	3.79	ns
T x Tr x L/D	2	0.009	0.20	ns	21.056	2.66	ns
Residual	12	0.050			7.918		
d) Protein (mg cm^{-2})							
Treatment (T)	1	20.357	61.22	***	34.271	21.23	***
Trial (Tr)	1	1.172	3.53	ns	7.026	4.35	ns
Light/Dark (L/D)	2	0.909	2.74	ns	1.927	1.19	ns
T x L/D	1	1.255	3.78	ns	0.277	0.17	ns
T x Tr	2	1.255	3.77	ns	2.284	1.42	ns
Tr x L/D	2	0.470	1.42	ns	2.921	1.81	ns
T x Tr x L/D	2	0.273	0.82	ns	2.702	1.67	ns
Residual	12	0.332			1.614		

Discussion

This is the first study which simultaneously investigates the effect of food availability on the tissue composition, photosynthetic activity and the bleaching susceptibility of *S. pistillata* and *G. fascicularis* under elevated temperature conditions. Zooxanthellae of the genus

Symbiodinium comprise different genotypes (Coffroth and Santos, 2005). *S. pistillata* and *G. fascicularis* from southern Taiwan and Okinawa for example have been both shown to contain clade C types while *G. fascicularis* from the Great Barrier Reef has been associated with clade D (Lajeunesse et al., 2004), which is believed to be a more stress tolerant type than clade C (e.g. Rowan et al., 2004). Unfortunately we have no information about the genotype inhabiting the corals used in this study. The results of this study however indicate that the thermal stress tolerance in terms of photosynthetic activity and loss of pigmentation of both species was coupled to food availability.

Heat-dependent photoinhibition is thought to result from damage to photosystem II and in particular the D1 reaction center protein (Warner, 1999). If protein damage exceeds the capacity of repair and recovery of PSII (Takahashi et al., 2004), zooxanthellae are likely to enter a state of chronic photoinhibition, which is characterized by a progressive decline in photosynthetic efficiency (Jones et al., 1998; Franklin et al., 2004). The pronounced decrease in Pg and Pn of starved corals of both species towards the end of the experiment may reflect such a scenario and is consistent with previous findings by Borell and Bischof (2008) indicating that starvation can result in a significant reduction in nocturnal recovery rates of the photosynthetic apparatus of zooxanthellae in *S. pistillata* under elevated temperature conditions. Host starvation can lead to a decrease in nitrogen supply to the zooxanthellae resulting in nitrogen stress of the symbionts as well as a reduction in metabolic processes associated with nitrogen assimilation and photosynthesis (Szmant-Froelich and Pilson, 1984; McAuley and Cook, 1994; Fitt and Cook, 2001; Davy et al., 2006). Thus a diminishing capacity for PS II recovery of zooxanthellae in starved corals, possibly as a result of nutrient insufficiency could have lead to chronic photoinhibition and subsequent decline in pigmentation.

While the loss in pigmentation of starved *S. pistillata* was characterized by a decrease in zooxanthellae, starved *G. fascicularis* displayed a substantial reduction in both zooxanthellae densities and chl a + c_2 concentrations. A stress related decline in zooxanthellae numbers decreases the competition for nutrients and thus facilitates an increase in chl concentrations per algal cell (Hoegh-Guldberg and Smith, 1989; Fitt et al., 1993; Jones and Yellowlees, 1997). This could explain the inverse relationship between zooxanthellae densities and chl concentrations of starved *S. pistillata*. It is possible that zooxanthellae in *G. fascicularis* were more sensitive to solar radiation than zooxanthellae in S. pistillata; perhaps due to genetic differences of the symbiotic algae or differences in composition of mycosporine-like amino acids (Yakovleva and Hidaka, 2004), of which some in addition to their protective function

against solar irradiance have antioxidant properties (Dunlap and Yamamoto, 1995). Alternatively it might be possible that *S. pistillata* and *G. fascicularis* have a different nitrogen metabolism and associated ammonium excretion rates (Szmant et al., 1990). Since ammonium excretion is a by-product of amino acid catabolism, lower rates of amino acid catabolism of *G. fascicularis* relative to *S. pistillata* could imply that less nitrogen was available to the zooxanthellae in *G. fascicularis* compared to zooxanthellae in *S. pistillata.* Although surface area can serve as a proxy for biomass (Edmunds and Gates, 2002), changes in tissue biomass over the course of the experiment make a comparison of metabolic rates between the two species difficult. However since the overall respiaration rates of *S. pistillata* were much greater than the respiration rates of *G. fascicularis* throughout the experiment regardless of treatment, it is conceivable that *S. pistillata* had higher metabolic rates than *G. fascicularis*. Differences in metabolic rates could also explain why lipids and proteins of fed *S. pistillata* had declined by ~30 % while there were no changes in protein and lipid concentrations in fed *G. fascicularis* relative to reference corals. The significant decline in lipid and protein concentrations of starved *S. pistillata*, is consistent with findings for bleached Carribean *Montastrea annularis* and *Agaricia lamarcki* showing a decrease in tissue biomass, total carbon, total lipid, carbohydrates, and total nitrogen (Porter et al., 1989; Szmant and Gassman, 1990; Fitt et al., 1993). It should be noted however that the overall change in tissue composition of corals in this study was probably exacerbated due to increased energetic expenses associated with tissue repair.

The increase in respiration rates of both starved and fed *S. pistillata* during the first three days of the experiment is in agreement with the results of Castillo and Helmuth (2005) showing that short-term exposure (3 h) of *M. annularis* to increased temperature was accompanied by an increase in oxygen consumption. Such an increase could imply increased energetic costs associated with damage repair, or increased rates of antioxidant activity. Short term exposure to thermal stress of *S. pistillata* has been shown to increase the rates of antioxidant enzyme activity and levels of mycosporine-like amino acids (Yakovleva et al., 2004). Prolonged exposure to elevated temperature by contrast can lead to a decrease in respiration, often concomitant with a decrease in photosynthetic efficiency (Porter et al., 1989; Fitt et al., 2001; Nystroem et al., 2001). Since the respiration of zooxanthellae contributes only 10-29 % to colony respiration (Davies, 1984; Edmunds and Davies, 1986; Davies, 1991), the decrease in respiration observed for starved *S. pistillata* may be largely attributed to the coral host and corroborates the significant decline in lipid and protein concentrations but may also represent an acclimation response that entails decreased

metabolic costs for maintenance to compensate for reduced primary production (Koehn and Bayne, 1989). Thus sustained photosynthesis and reduced bleaching of fed *S. pistillata* may be related to the provision of nitrogen and/or energy through zooplankton, which could have increased the symbiont's metabolic capacity for protein synthesis and damage repair thereby enabling photosynthesis to proceed. This in turn would have prevented rendering the coral into a state of starvation where the host ultimately has to rely upon protein and lipid reserves as an alternative energy source (Porter et al., 1989; Fitt et al., 1993; Grottoli et al., 2004).

The lack of effect of treatment on respiration rates and lipid concentrations of *G. fascicularis* by contrast suggests that starved *G. fascicularis* were able to conserve their energy reserves or preferentially used their protein stores to support their metabolic demands. This is supported by the lower protein concentrations in starved relative to fed corals. Similar observations were made by Grottoli et al. (2004) who found no significant differences in total lipid concentrations between bleached and unbleached *Montipora verrucosa* while bleached *Porites compressa* contained significantly lower lipid concentrations than unbleached colonies coincident with lower respiration rates of *M verruscosa* compared to *P. compressa.*

Prolonged starvation (20 – 40 days) of cnidarian hosts under non-bleaching conditions has been shown to result in reduced zooxanthellae densities, decreased chl a concentrations and decreased photosynthetic rates (Szmant-Froelich and Pilson, 1984; Cook et al., 1988; Rees, 1991; Titlyanov et al., 2000; Davy et al., 2006) while under short-term starvation, hosts are thought to support zooxanthellae cell division and growth through the supply of nutrients from their own reserves (Titlyanov et al., 2000). Furthermore several studies (Clayton and Lasker, 1984; Davy and Cook, 2001; Titlyanov et al., 2001; Leletkin 2005) have demonstrated that photosynthetic rates of zooxanthellae within cnidarian hosts exposed to starvation can remain constant for several weeks. The present results therefore suggest that coral bleaching can be exacerbated in the absence of external food and underscore the potential importance of external resources in mediating the stress susceptibility of the symbiotic association. Similar observations were made by Titlyanov et al. (2000) who showed that bleaching of starved corals was intensified in the presence of osmotic stress. A recent study by Anthony et al. (2007) showed that lipid concentrations make good predictors of coral survivorship under bleaching conditions. Although survivorship was not determined in this study, the observed differences in tissue composition between *S. pistillata* and *G. fascicularis* suggest that under the current experimental conditions coral survivorship would be greater for *G. fascicularis* than for *S. pistillata*.

Since heterotrophic abilities of corals may vary considerably due to a suite of factors including light (Ferrier-Pages et al., 1998), water temperature (Palardy et al., 2005), water flow (Sebens et al., 1998, 2003), zooplankton abundance and composition (Ferrier-Pages et al., 2003; Heidelberg et al., 2004; Palardy et al., 2006), concentrations of suspended particulate matter (Anthony and Fabricius, 2000), high fluctuations of available nitrogen and fixed carbon could be an important factor affecting the bleaching susceptibility of corals between and within reefal habitats.

Conclusion

The most important finding of this study shows that there was a strong positive correlation between zooplankton feeding, sustained photosynthetic activity and reduced loss in pigmentation of both species under elevated temperature conditions. Contrary to natural bleaching conditions, where increased temperatures prevail for extended periods of time (Gleeson and Strong, 1995), corals in this experiment were allowed to recover during the night at *in situ* temperatures (29^{o}C). Nonetheless the present results indicate that the thermal tolerance of the symbiotic association is to some extent a function of the coral's resource environment.

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Paper III

Differential physiological responses of two congeneric scleractinian corals to mineral accretion and an electric field

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Differential physiological responses of two congeneric scleractinian corals to mineral accretion and an electric field

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Abstract

The mineral accretion technology as a method for coral reef restoration involves the accumulation of mineral ions in the vicinity of an underwater cathode and the precipitation of carbonates and $Mg(OH)_2$ onto the cathode which serves as a substrate for coral transplants. The electrochemical processes around the cathode have been proposed to increase survival and growth of coral transplants through reinforced substrate stabilization, supersaturation of $CaCo_3$ and enhanced metabolic efficiency. Despite the increasing popularity of 'electric' reefs, evidence supporting these claims however remains largely anecdotal. This study simultaneously investigates the effects of electrochemical processes and those of an electric field on the potential functional interactions between coral survival, linear skeletal growth, zooxanthellae densities, chlorophyll a (chl a) and chlorophyll fluorescence of *Acropora pulchra* and *A. yongei*. Coral transplants were grown under 3 treatment conditions: 1) on a cathode in the presence of mineral accretion, 2) on bamboo inside an electric field, or 3) on bamboo outside the experimental matrix (control), for 4 months. Treatment had no effect on the survival of *A. pulchra* while mortality rates of *A. yongei* were significantly higher in the presence of mineral accretion compared to the electric field and control. High mortality rates of *A. yongei* coincided with low zooxanthellae densities, depressed photosynthetic efficiency (F_v/F_m), relative electron transport rates (rETR) and reduced growth rates. There were no differences in zooxanthellae densities, F_v/F_m and rETR between the electric field and the control. Contrary to *A. yongei*, zooxanthellae densities of *A. pulchra* were highest for corals on the cathode, which was however unrelated to growth. Highest growth rates of both species were observed inside the electric field and chl a concentrations of both species were inversely related to growth. All transplants inside the electric field had significantly lower chl a concentrations compared to the cathode and the control. Treatment had no effect on F_v/F_m and rETR of *A. pulchra*. The combined results demonstrate that 1) the electric field and not the cathode resulted in the highest growth rates, 2) corals grown under the presence of mineral accretion can exhibit low growth and reduced health, and 3) the suitability of the mineral accretion technology for coral transplantation can vary greatly between closely related species.

Key words: *A. pulchra*, *A. yongei*, cathode, chlorophyll fluorescence, growth, mortality, zooxanthellae

Introduction

Burgeoning anthropogenic and natural disturbances, separately or in synergy are causing coral reef degradation world wide (McClanahan et al., 2002; Bellwood et al., 2004). In recognition of the fact that many destroyed coral reefs do often not recover naturally without manipulation, the scientific discipline of reef restoration has drawn much attention over the past decade (Rinkevich, 2005). Various restoration methods aimed at improving live coral cover, biodiversity and topographic complexity have been proposed to date including the construction of artificial reefs. These involve the transplantation of coral nubbins, fragments or whole coral colonies onto three-dimensional artificial substrates, often comprised of concrete or natural rock (Clark, 2002; Zimmer, 2006).

There is still much uncertainty concerning the interactions between artificial substrates, the ecology of transplanted organisms and the environment into which they are placed, often resulting in high mortality and low growth rates of the coral transplants (Clark, 2002; Rinkevich, 2005). Firm attachment of coral transplants, even in low energy environments, is a prerequisite for high survival rates as securely fixed corals are likely to recover faster from the stress of transplantation and may allocate more energy to lesion repair and subsequent growth (Bowden-Kerby, 1997; Lindahl, 1998; Ammar et al., 2000). The mineral accretion technology described by Hilbertz (1992) has been advocated as an effective method for coral reef rehabilitation proposing to increase survival and growth through reinforced substrate stabilization, supersaturation of $CaCo_3$ and enhanced metabolic efficiency of the coral transplants (Hilbertz and Goreau, 1996; Goreau et al., 2004).

The principle of this technology follows that of a galvanic cell, which involves passing a low voltage and direct electrical current through a cathode and an anode to induce electrolysis of the seawater. Seawater is split into hydrogen gas and two molecules of hydroxide anions leading to a rise in pH around the cathode. Ca^{2+} and Mg^{+} combine with dissolved HCO_3^- and OH^- and precipitate as $CaCO_3$ (aragonite) or $Mg(OH)_2$ (brucite) on the cathode (Hilbertz, 1992). Coral fragments are transplanted onto the cathode and are quickly cemented to the structure by the accreted carbonate material (van Treeck and Schuhmacher, 1997). Since zooxanthellae photosynthesis and coral calcification both require a continuous supply of inorganic carbon and share HCO_3^- and CO_2 as major substrates (de Beer et al., 2000; Furla et al., 2000a; Al-Horani et al., 2003), competition for inorganic carbon has been suggested to lower the rate of calcification (Marubini and Thake, 1999). Although a study by Furla et al. (2000a) indicates that calcification is neither limited by external nor by coelentric concentrations of dissolved inorganic carbon implying that there is no competition for

substrate between these processes. Furthermore calcification rates may vary with the availability of mineral ions in the surrounding seawater (Gattuso et al., 1999). At ambient seawater Ca^{2+} concentrations, calcification rates of some species are saturated (Chalker, 1976; Tambutte, et al. 1996), while Ca^{2+} appears to be limiting in others (Chalker, 1976; Krishnaveni et al., 1989). An increase in the concentration of mineral ions could presumably induce a diffusional influx of Ca^{2+} into the coelenteron via the paracellular pathway, thereby increasing the availability of Ca^{2+} for active uptake via the transcellular route of calcification (Tambutte et al., 1996). Given that the calcification process is greatly affected by the availability of mineral ions and pH in the surrounding seawater (Tambutte et al., 1996; Al-Horani et al., 2003), an increase in concentrations of mineral ions resulting from the electrolysis of seawater around the cathode were suggested to increase calcification and thus accelerate skeletal growth (Sabater and Yap 2004).

Specifically, Hilbertz and Goreau (1996) proposed the following four mechanisms causing increased survival and growth of corals under a low current state: 1) carbonate accretion facilitates firm attachment of coral transplants to the substrate; 2) an increase in pH in the immediate vicinity of the cathode results in the supersaturation of $CaCO_3$, thereby enhancing natural calcification, 3) increased availability of electrons in the vicinity of the cathode will facilitate greater efficiency of uptake and internal transport of cations by the organism, and 4) excess production and release of electrons due to electrochemical processes will provide extra electrons for ATP production, thereby enhancing metabolic efficiency leading to increased growth of the coral.

However despite the increasing popularity of ‘electric’ reefs as a tool for reef restoration world wide, evidence supporting these hypotheses remains largely anecdotal. Although there are several accounts of increased survival rates of coral transplants grown in the presence of mineral accretion (Goreau et al., 2000; Schuhmacher et al., 2000; Sabater and Yap, 2002, 2004; various reports by Goreau, unpublished), there is only one published experimental study (Sabater and Yap 2004) confirming that growth rates of corals grown in the presence of mineral accretion were significantly higher than for corals grown in the absence of mineral accretion. The study though also showed that the effect was only significant during the first four months before the cathode became saturated by the accreted minerals, henceforth impeding the effect of the electrical current. Goreau et al. (2004), looking at zooxanthellae population characteristics in six species grown in the presence of mineral accretion for a period of twelve months, observed that on average, zooxanthellae densities were higher and chlorophyll concentrations lower in five species compared to

natural colonies; these differences, however, were statistically significant for only two species. While the authors interpreted their observation as evidence for electrically stimulated tissue growth and better health conditions of coral transplants, the lack of simultaneous measurements of skeletal growth leaves such a conclusion ambiguous.

It is well established that coral calcification is closely coupled to photosynthetic processes of the zooxanthellae (e.g., Furla et al., 2000a; Moya et al., 2006). In order to evaluate the potential underlying mechanisms of enhanced survival and skeletal growth rates of coral transplants pertaining to the hypotheses proposed by Hilbertz and Goreau (1996), the present study simultaneously investigates the effect of electrochemical processes and that of an electric field on the potential functional interactions between coral survivorship, skeletal growth rates, zooxanthellae densities, chlorophyll a (chl a) concentrations and chlorophyll fluorescence as a proxy for photosynthetic performance of two *Acropora* species.

Materials and methods

The experiment was carried out in North Sulawesi, Indonesia (01° 45' N, 125° 3' E) between March and July 2006, using the congeneric species *Acropora yongei* and *A. pulchra.* High growth rates, characteristic for the genus *Acropora* (Harriott, 1999), together with its high abundance in the Indo-Pacific (Veron, 2000) make this genus particularly relevant for coral reef restoration projects.

Experimental design

The experimental matrix (cathode) consisted of a 10 m^2 steel frame which was subdivided by steel bars into a grid of 10 squares ($1m^2$). Five squares were intersected by additional steel bars running parallel to each other alternating with 5 squares which contained bamboo boards (electric field). Titanium mesh anodes were placed in close proximity on each side of the experimental matrix in order to create an evenly distributed electrical field (Fig. 1). Three-conductor copper cables were used to connect the electrodes to a 12 V car battery on land over a distance of 120 m. Each electrode was connected to a separate cable. Electricity was generated 24 hrs, supplying the experimental matrix with a constant electrical current ~1 A m^{-2}. An additional bamboo grid comprising 4 squares ($1m^2$) that was deployed outside the experimental matrix served as a control. The experiment was run for 4 months, the time during which the effect of mineral accretion has been shown to be most pronounced (Sabater and Yap, 2004).

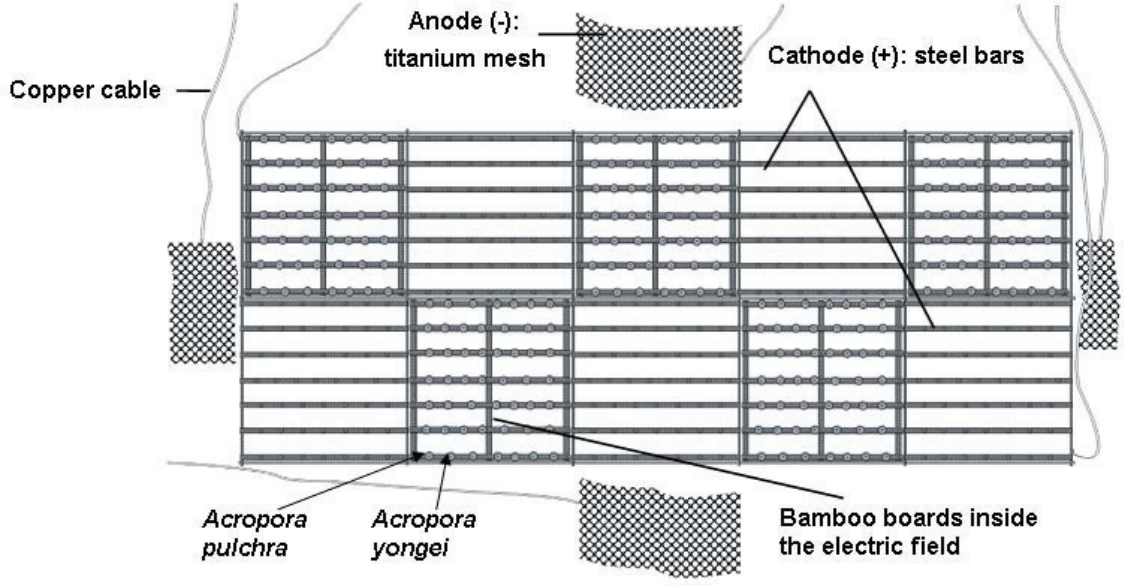

Fig. 1 Schematic diagram of the experimental matrix showing the major components: 1) cathode made up of steel bars, 2) anodes made up of titanium mesh, 3) bamboo boards inside the electric field, 4) copper cables, and 5) *Acropora pulchra* and *A. yongei* transplants.

Collection and preparation of samples

A total of 350 coral fragments of each species with a length of 6-8 cm (Soong and Chen, 2003) were collected from parent colonies in close proximity to the experimental site and were transplanted onto the experimental matrix on a shallow rubble field, protected from currents in 5 m depth. The coral fragments of each species were interspersed on each square (n = 25) to minimize the effect of location. Fragments on the cathode were placed into adjustable metal tubes which were welded onto the cathode, while the rest of the fragments were glued into concrete cups using underwater epoxy and then tied to the bamboo boards with cable ties either inside the electric field or outside the experimental matrix.

Measurement of survival and skeletal growth

After 4 months fragments were considered as dead when no live tissue was noticeable (90% tissue necrosis) or when fragments were lost due to wave action or removal by fish. To determine linear skeletal growth rates, the initial length of each fragment was measured from the top of the cup to the coral tip using a pair of calipers (accurate to 0.01 mm). After the experimental treatment phase, all fragments which had survived and remained undamaged

were measured again to determine the vertical skeletal extension rates as an estimate of coral growth over 4 months.

Fluorescence measurements

The effect of treatment on the relative photosynthetic performance of zooxanthellae was determined using the potential maximum quantum yield (F_v/F_m) as an estimate of the photosynthetic efficiency of photosystem II (PSII) and relative electron transport rates (rETR) as a measure of the photosynthetic capacity of PSII. Measurements were conducted during 5 consecutive, cloudless days just before the end of the treatment phase using a pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Walz, Effeltrich, Germany).

The coral fragments were dark-adapted for 15 min prior to each measurement. Fragments (n = 6) of each species and treatment were chosen haphazardly and measured at random in order to minimize an effect of time. Seven fragments each were measured on the first 4 days and 6 fragments on day 5. Each fragment was pulsed with a weak (<1 µmol m^{-2} s^{-1}) red light to obtain the minimal fluorescence (F_o), followed by a 1 s pulse of saturating actinic light (>5000 µmol m^{-2} s^{-1}) to determine the maximal fluorescence (F_m). F_v/F_m was calculated in the conventional manner as $F_m - F_o/F_m = F_v/F_m$ (Schreiber, 2004). Followed by a 40 s period of darkness, each fragment was exposed to saturating light for 4 min (1000 µmol m^{-2} s^{-1} PAR), after which the fluorescence had reached steady state. The minimum and maximum fluorescence (F' and F_m' of light adapted samples) were recorded and the effective quantum yield determined as the ratio $\Delta F/F_m$', where $\Delta F = F_m' - F'$ (Schreiber, 2004), in order to calculate relative electron transport rates (rETR) of PS II as rETR = $\Delta F/F_m$' x PAR x 0.5 (Hoegh-Guldberg and Jones, 1999). All measured fragments were frozen for later tissue analyses.

Zooxanthellae density and chl a concentration

Coral tissues were stripped from the skeletons under dim light using an airbrush gun and phosphate buffered saline solution (Sigma Aldrich). The slurry (~100 ml) was homogenized in a hand potter and subsamples of 20 ml frozen for chl a analyses. A 10 ml aliquot of the homogenate was preserved in 4% formalin and used to determine zooxanthellae density from 8 replicate counts using a Neubauer haemocytometer. Chl a was extracted as described by Gardella and Edmunds (1999) and concentrations calculated according to the equation of Jeffrey and Humphrey (1975). Zooxanthellae densities were normalized to surface area (cells

cm^{-2}) and chl a concentrations expressed as µg (10^6 $cells^{-1}$). Coral surface area was measured using the paraffin wax technique (Stimson and Kinzie, 1991).

Data analyses

The data were checked for homogeneity of variances using Cochran's C-test (Underwood, 1997). To test if treatment had an effect on the skeletal extension rates, the number of replicates was adjusted to the group which had the lowest survival rate (n = 26). Fragments were selected at random and analyzed by one-way ANOVA and post–hoc SNK testing. To test for significant effects of treatment on coral survival, we used a likelihood (G) test with William's correction (Sokal and Rohlf, 1995). The effect of treatments on fluorescence parameters, growth, zooxanthellae density and chl a was analyzed separately for each species by one-way ANOVA and post–hoc SNK test separation of significant factors. All data were analyzed using WinGMAV (EICC, University of Sydney, Australia).

Results

Survival and growth

Mortality of *A. yongei* after 4 months was significantly higher for corals grown on the cathode (32%) than for those on the control (1%), and fragments grown inside the electric field (1%) displayed the lowest mortality (Table 1, Fig. 2a). By contrast there were no significant differences in mortality between cathode (17%), electric field (9%) and control corals (13%) for *A. pulchra* (Table 1, Fig. 2a). Treatment had a significant effect on the vertical skeletal extension rates of both species (Table 2, Fig. 2b). For both species, growth rates were significantly higher inside the electric field compared to cathode and control corals (SNK: *A. yongei*, $p < 0.01$; *A. pulchra*, $p < 0.05$) (Fig. 2b). Coincident with high rates of mortality, *A. yongei* exhibited the lowest growth rates on the cathode (SNK, $p < 0.01$), while growth rates of *A. pulchra* were lowest for corals in the control treatment (SNK, $p < 0.05$).

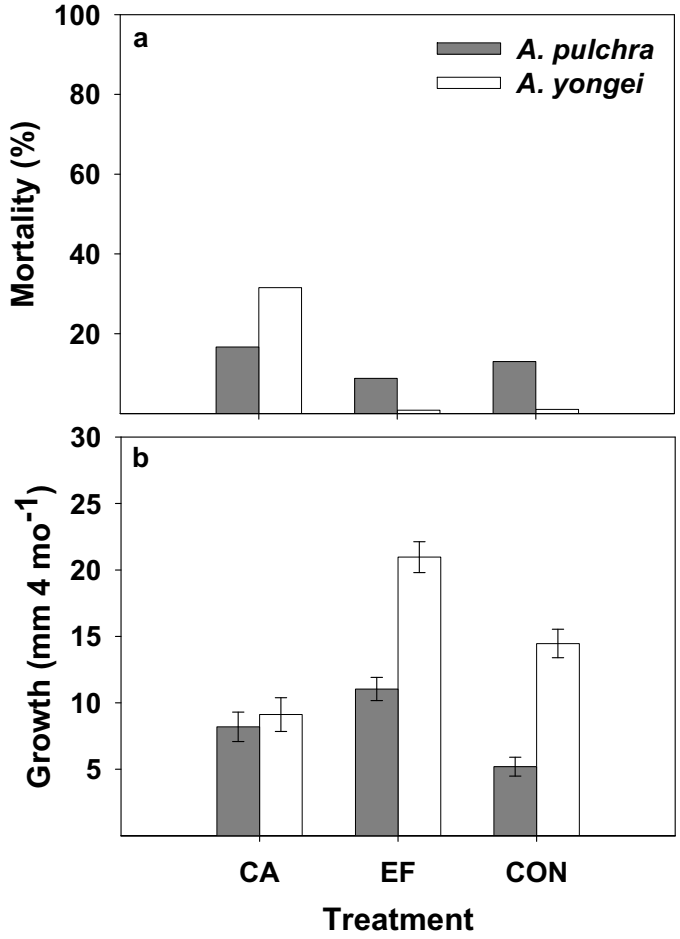

Fig. 2 (a) Mortality (%) and (b) mean (± SE, n = 26) linear growth rates (mm) of *Acropora pulchra* and *A. yongei* transplants after 4 months grown under 3 of the following treatments: 1) direct contact with the cathode (CA), 2) inside an electric field (EF), and 3) control, outside the experimental matrix (CON).

Table 1 Likelihood (G) tests with William's correction comparing mortality rates of *Acropora yongei* and *A. pulchra* after 4 months grown on a cathode, inside an electric field and outside the experimental matrix (control).

	A. yongei			*A. pulchra*		
Comparison	**G_{adj}**	**df**	***P***	**G_{adj}**	**df**	***P***
Cathode x Electric field	51.95	1	<0.001	2.80	1	>0.09
Cathode x Control	43.18	1	<0.001	0.36	1	>0.54
Electric Field x Control	0.02	1	>0.05	0.17	1	>0.91

Table 2 ANOVA of growth (skeletal extension) rates of *Acropora yongei* and *A. pulchra* after 4 months of exposure to 3 treatments: 1) direct contact with the cathode, 2) inside the electric field, 3) outside the experimental matrix (control).

		A. yongei			*A. pulchra*		
Source of variation	**df**	**MS**	***F***	***P***	**MS**	***F***	***P***
Treatment	2	915.038	25.68	<0.001	222.205	10.31	<0.001
Residual	75	35.627			21.560		

Chlorophyll fluorescence

The fluorescence response of zooxanthellae in *A. yongei* varied significantly with treatment (Fig. 3, Table 3). F_v/F_m was significantly lower for corals grown on the cathode compared to corals inside the electric field and on the control (SNK, $p < 0.01$: electric field > cathode = control) (Fig. 3a). Similarly rETRs were significantly lower for corals on the cathode than for corals in the control treatment and corals inside the electric field (SNK, $p < 0.001$: electric field < cathode = control) (Fig. 3b). There was no significant effect of treatment on both F_v/F_m and rETRs of zooxanthellae in A. pulchra (Table 3, Fig. 3a,b).

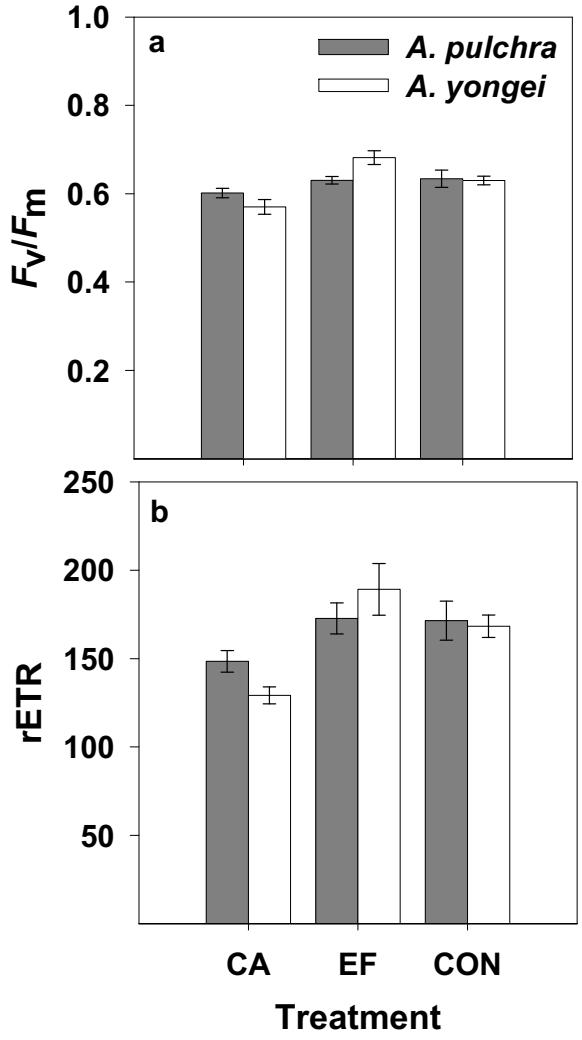

Fig. 3 Mean (± SE, n = 6) (a) maximum quantum yield (F_v/F_m) and (b) relative electron transport rates (rETR) of *Acropora pulchra* and *A. yongei* between 09:30 -11:00 hrs after 4 months grown under 3 of the following treatments: 1) direct contact with the cathode (CA), 2) inside an electric field (EF), and 3) control, outside the experimental matrix (CON).

Table 3 ANOVA of a) the maximum quantum yieled (F_v/F_m) and b) relative electron transport rates (rETR) of zooxanthellae in *Acropora yongei* and *A. pulchra* between 09:30 -11:00 hrs, after 4 months of exposure to 3 treatments: 1) direct contact with the cathode, 2) inside the electric field, 3) outside the experimental matrix (control). Data of rETR for *A. yongei* were homogenous after log (ln) transformation. Cochran's C = 0.6712, P > 0.05.

		A. yongei			*A. pulchra*		
Source of variation	**df**	**MS**	***F***	***P***	**MS**	***F***	***P***
a) F_v/F_m							
Treatment	2	0.010	15.33	<0.001	0.001	1.66	>0.22
Residual	15	0.000			0.001		
b) rETR							
Treatment	2	0.217	12.09	<0.001	1125.786	3.38	>0.12
Residual	15	0.018			427.165		

Zooxanthellae density and chl a concentration

Treatment had a significant effect on zooxanthellae densities in both species (Table 4, Fig. 4a). For *A. yongei*, densities were significantly lower in corals on the cathode compared to corals inside the electric field and in the control treatment (SNK, $p < 0.05$: cathode < electric field = control). Zooxanthellae densities in *A. pulchra* displayed the opposite pattern with significantly higher densities in corals on the cathode than in corals inside the electric field and in the control treatment (SNK, $p < 0.01$: cathode > electric field = control) (Fig. 4a). The effect of treatment on chl a was identical for both species (Table 4, Fig. 4b). Zooxanthellae in corals inside the electric field contained significantly less chl a than cathode and control corals (SNK, $p < 0.05$: electric field < cathode = control).

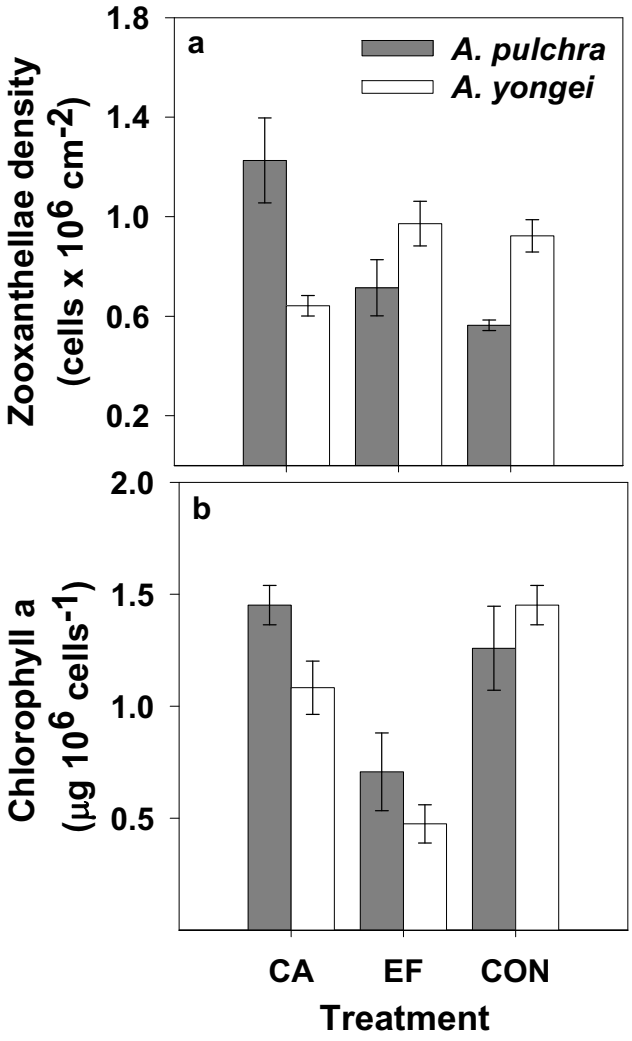

Fig. 4 Mean (± SE, n = 6) (a) zooxanthellae densities (cells cm^{-1}) and (b) chlorophyll a concentrations (µg 10^6 $cells^{-1}$) in *Acropora pulchra* and *A. yongei* after 4 months grown under 3 of the following treatments: 1) direct contact with the cathode (CA), 2) inside an electric field (EF), and 3) control, outside the experimental matrix (CON).

Table 4 ANOVA of a) zooxanthellae densities and b) chlorophyll a concentrations of zooxanthellae in *Acropora yongei* and *A. pulchra* after 4 months of exposure to 3 treatments: 1) direct contact with the cathode, 2) inside the electric field, 3) outside the electric field (control).

		A. yongei			*A. pulchra*		
Source of variation	**df**	**MS**	***F***	***P***	**MS**	***F***	***P***
a) Zooxanthellae density (cells cm^{-2})							
Treatment	2	4110471.648	6.82	<0.001	9873094.683	8.54	<0.01
Residual	15	3718100.964			4469368.765		
b) Chlorophyll a (µg 10^6 $cells^{-1}$)							
Treatment	2	0.831	8.00	<0.01	0.896	6.14	<0.05
Residual	15	0.104			0.146		

Discussion

The electrochemical deposition of $CaCO_3$ or $Mg(OH)_2$ onto a cathode reinforces coral attachment, which has previously been demonstrated to enhance survival of coral transplants (Sabater and Yap, 2002, 2004). This is in contrast to the current results showing that the presence of mineral accretion had either no or even a negative effect on coral survival over a period of 4 months. The electrical current density and voltage used in this study was lower than that used by Sabater and Yap (2002, 2004), which may have resulted in slower precipitation but does favor the deposition of $CaCO_3$, which forms a more solid crust than $Mg(OH)_2$ (Hilbertz, 1992; van Treek and Schuhmacher, 1997).

Seawater hydrolysis presumably increases available electrons and/or the pH in a thin boundary layer around the cathode which has been suggested to enhance uptake and internal transport of cations by the coral and to cause supersaturation of $CaCo_3$, thereby enhancing natural calcification, respectively (Hilbertz and Goreau, 1996). In addition it was suggested that an increase in ion availability may enhance active uptake of ions via the transcellular pathway of calcification (Sabater and Yap, 2004) However, in branching corals, linear skeletal extension occurs through enhanced calcification in the apical region of the branch (Goreau and Goreau, 1959; Oliver, 1984). A small increase in available electrons, pH or mineral ions at the base of the coral therefore seems unlikely to have resulted in increased linear growth rates of *A. pulchra*. The hypothesis is also in conflict with the pronounced divergent growth rates of *A. yongei* and *A. pulchra* on the cathode while at the same time, both species displayed highest growth rates inside the electric field. The results therefore indicate that factors other than electrochemical processes in the immediate vicinity of the cathode were stimulating growth. Likewise the hypothesis that provision of additional electrons could enhance the coral's bioenergetics, presumably facilitating both increased skeletal and tissue growth (Hilbertz and Goreau, 1996; Goreau et al., 2004), contradicts the observed poor performance of *A. yongei* on the cathode.

In a previous study of zooxanthellae responses under the influence of mineral accretion, Goreau et al. (2004) found no significant difference between zooxanthellae density in the tissue of *A. nasuta* grown on an electrical substrate and natural colonies, while the concentration of chl a per zooxanthellae was slightly reduced on the electrical substrate. By contrast, there was a significant effect on zooxanthellae density in the present study, and the response differed between species. A reduction in zooxanthellae density and decrease in F_v/F_m are good indicators of physiological stress in corals and their symbionts in response to abnormal environmental conditions (Jones, 1997; Jones et al., 1999; Warner et al., 1999). The

inverse relationship between zooxanthellae densities and chl a concentrations observed for *A. yongei* is consistent with previous observations that stress-related loss of zooxanthellae is accompanied by an increase in chl a concentration, likely as a result of decreased competition for nutrients (Hoegh-Guldberg and Smith, 1989; Jones and Yellowlees, 1997). A loss of zooxanthellae populations as well as reduced photosynthetic efficiency as was observed for *A. yongei* on the cathode invariably result in a loss of photosynthetic potential of the coral colony. This may greatly impact the colony's energy balance (Fitt et al., 2000), leading to a reduction or even cessation in growth (Porter et al., 1989; Goreau and MacFarlane, 1990) and subsequent mortality (Anthony et al., 2007), which corresponds to the high mortality and low growth rates of *A. yongei* on the cathode.

Even though calcification is primarily a physiochemical process, it is mediated by the synthesis of an organic matrix (Allemand et al., 1998). Results by Allemand et al. (1998) suggest that skeletal growth is limited by the biosynthesis of the organic matrix rather than the deposition of calcium. The relevance of photosynthesis for the skeletal organic matrix biosynthesis has long been recognized (Muscatine and Cernichiari, 1969; Barnes and Crossland, 1978) but it is now established that heterotrophic sources also play an important role in the acquisition of both precursors and energy necessary for the synthesis of the organic matrix (Allemand et al., 1998; Houlbreque et al., 2004). Indeed there are numerous examples in the literature documenting enhanced skeletal growth in response to zooplankton feeding (Jaques and Pilson, 1980; Wohlenberg Miller, 1995; Ferrier-Pages et al., 2003; Houlbreque et al., 2003, 2004). Food availability also tends to increase protein levels of host tissue, zooxanthellae densities, chl a concentrations (Titlyanov et al., 2001; Ferrier-Pages et al., 2003; Houlbreque et al., 2003, 2004) and rates of photosynthesis (Davy et al., 2006; Houlbreque et al., 2003) whereas starvation leads to a reduction in zooxanthellae densities, decrease in chl a concentrations and photosynthetic rate (Cook et al., 1988; Rees, 1991; Davy et al., 2006). Thus it may be possible that food availability was somehow modulated by the experimental matrix. Since heterotrophic abilities of corals can vary considerably with ambient zooplankton abundance and composition (Heidelberg et al., 1997; Heidelberg et al., 2004; Palardy et al., 2006), a change in zooplankton composition and/or abundance may have increased the food availability of *A. pulchra* and decreased it for *A. yongei* which could to some extent explain the differences in zooxanthellae densities and growth rates. Although there is at present no direct evidence of electrosensory organs in zooplankton, it can not be ruled out that zooplankters are sensitive to electric or magnetic cues as studies on this subject are still lacking (Bullock, 1996). Alternatively zooplankton migratory behaviour may have

been altered indirectly in response to changes in the presence of planktivores (McKelvey and Forward, 1995).

Although the exact cause for the observed differences in zooxanthellae numbers of corals on the cathode remains a matter of speculation, a functional relationship between growth and zooxanthellae densities is evident. Estes et al. (2003) found that carbonic anhydrase activity decreased with decreasing zooxanthellae densities. Carbonic anhydrase catalyzes the reversible dehydration of HCO_3^- to CO_2 and therefore plays a crucial role in the supply of inorganic carbon to both the site of photosynthesis and calcification (Al-Horani et al., 2003; Furla et al., 2000b). Moreover de Beer et al. (2000) showed that the inhibition of carbonic anhydrase results in a decrease of photosynthetic activity due to substrate limitation. If electron flow to the Calvin cycle is limited by CO_2 delivery, the electron transport chain can become over-reduced (Dietz et al., 1985). Thus the depressed rETR and low zooxanthellae densities of *A. yongei* may reflect such a development which could have resulted in an imbalance of interrelated physiochemical processes of photosynthesis and calcification.

Sebens et al. (1996) showed that more efficient predators feature higher cell specific algal densities than less efficient predators. Assuming that the zooxanthellae densities of both species inside the electric field and on the control treatment reflect the range of cell specific densities of natural colonies, it may well be possible that under natural conditions *A. yongei* has greater heterotrophic abilities than *A. pulchra*. Since the process of regeneration and lesion repair requires both energy and tissue reserves (Chadwick and Loya, 1990; Oren et al., 1997) differences in energy (photosynthesis) or nutrient (feeding) availability may in part account for the low mortality rates of *A. yongei* on the control treatment and inside the electric field as well as higher growth rates on the control treatment compared to *A. pulchra*.

An intriguing aspect about the current results however is the lack of relationship between growth rates and zooxanthellae densities of both species inside the electric field. The low chl a concentrations of both species inside the electric field indicate that zooxanthellae were nutrient limited (Rees, 1991). Anthony et al. (2002) showed that skeletal growth proceeds even when tissue growth is negative. This suggests that processes inside the electric field either directly or indirectly stimulated skeletal extension at rates exceeding the energetic or nutritional capacity for tissue growth. Coral growth rates are not necessarily proportional to the rate of calcification. Corals growing under potentially unfavorable conditions such as high sedimentation or nutrient loads have been shown to exhibit increased skeletal extension rates but decreased skeletal densities (Edinger et al., 2000; Cook et al., 2002) due to the so called

stretching modulation of skeletal growth (Carricart-Ganivet and Merino, 2001). Although these parameters were unlikely to vary between treatments, other factors may have evoked such a response.

Conclusion

The three most relevant findings of this study show that 1) the electric field and not the cathode resulted in the highest growth rates, 2) corals grown under the presence of mineral accretion can exhibit low growth and reduced health, and 3) the suitability of the mineral accretion technology for coral transplantation can vary greatly between closely related species. These results suggest that the proposed benefits of the mineral accretion technology to meet important objectives of reef rehabilitation should be reconsidered. Clearly further studies are warranted in order to gain a better understanding of possible interactions between electrochemical processes and the environment and how they affect physiological processes of the coral colony.

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Appendix

Statistical tables (paper II)

Table 1 ANOVA of a) daily mean maximum temperature and b) light intensity (PAR) between experimental tanks of *Stylophora pistillata* and *Galaxea fascicularis* over 15 days in three repeat trials. ns = p value >0.05, * = p<0.05, ** = p<0.01, *** = p 0.001.

		Temperature (°C)			PAR (µmol m^{-1} s^{1})		
Source of variation	**df**	**MS**	***F***	***P***	**MS**	***F***	***P***
Species (Sp)	1	0.012	0.04	ns	785.408	0.40	ns
Trial (Tr)	2	14.275	50.71	***	92075.939	47.31	***
Tank (Ta)	7	0.222	0.79	ns	644.112	0.33	ns
Sp x Tr	2	0.165	0.59	ns	1538.414	0.79	ns
Sp x Ta	7	0.437	1.55	ns	1511.259	0.78	ns
Tr x Ta	14	0.404	1.43	ns	2961.958	1.52	ns
Sp x Tr x Ta	14	0.423	1.50	ns	1190.922	0.61	ns
Residual	432	0.281			1946.028		

Table 2 ANOVA of a) mean light intensity (PAR) and b) temperature of incubation chambers of fed and starved *Stylophora pistillata* and *Galaxea fascicularis* during oxygen flux measurements in tree repeat trials. ns = p value >0.05, * = p<0.05, ** = p<0.01, *** = p 0.001.

		S. pistillata			*G. fascicularis*		
Source of variation	**df**	**MS**	***F***	***P***	**MS**	***F***	***P***
a) PAR (µmol m^{-1} s^{1})							
Treatment (T)	1	8230.959	1.30	ns	7442.634	1.09	ns
Trial (Tr)	2	2592.921	0.41	ns	46660.709	6.82	**
T x Tr	2	1947.691	0.31	ns	2721.134	0.40	ns
Residual	54	6347.321			6838.856		
b) Temperature (°C)							
Treatment (T)	1	0.065	0.68	ns	0.000	0.00	ns
Trial (Tr)	2	3.061	31.86	***	7.561	49.78	***
Light/Drak (L/D)	1	0.014	0.15	ns	0.172	1.14	ns
T x Tr	2	0.004	0.04	ns	0.126	0.83	ns
T x L/D	2	0.000	0.00	ns	0.371	2.45	ns
Tr x L/D	1	0.006	0.07	ns	0.042	0.28	ns
T x Tr x L/D	2	0.006	0.07	ns	0.066	0.43	ns
Residual	108	0.096			0.151		

Disclaimer

Gemäß §6 der Promotionsordnung der Universität Bremen für die mathematischen, nature- und ingenieurwissenschaftlichen Fachbereiche vom 14. März 2007 versichere ich, dass:

1. die Arbeit ohne unerlaubte fremde Hilfe angefertigt wurde

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Bremen, 24. Juli 2008

Esther Borell