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Photosynthesis and photoinhibition at low temperatures: physiological responses of Antarctic rhodophytes

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Cuando llegaste, apenas me conociste.
Cuando te vayas, me llevaras contigo.

”Als Du ankamst, hast Du mich kaum gekannt.
Wenn Du gehst, wirst Du mich mit Dir nehmen.”

Über die Antarktis. Unbekannter Autor.

Summary

The environment of Antarctica represents one of the most challenging and harshest ecosystems, which is characterized by very low temperatures and a strong seasonality in light availability. Due to the particular abiotic conditions, inhabiting organisms are highly adapted to their habitat and possess the ability to cope flexibly with changing environmental factors. In the context of global climate change, the Antarctic and especially the Antarctic Peninsula undergo the most rapid and significantly changing regions worldwide. These changes become visible in retreating glaciers and extented amounts of icebergs as well as increased precipitation and higher wind speeds. The drastic increase in annual mean temperature is regarded as the most apparent change (3°C within the last 50 years). This results in sea-ice thinning and earlier break-up, thus, hight light intensities may penetrate earlier within the season deeply into the water column.

Macroalgal communities settle on hard bottom substrate and build up extense biomass. These communities have to cope with a high variability in abiotic and biotic factors, which can be due to seasonal changes as well as a result of the global climate change. So far, species have been studied with respect to temperature demands and growth pattern, UV-susceptibility and succession. This study focusses on the acclimation potential of two Antarctic rhodophytes under changing light and temperature levels and the possible interaction of these two factors. As during global climate warming melt water influx will increase, investigations on the salinity tolerance are included in this study.

The combination of low temperatures and high light intensities are challenging conditions for photosynthetic organisms, as low temperatures reduce for instance enzymatic processes and the turn-over of the D1 centre protein of photosystem II. This protein plays a crucial role in photosynthetic function and is highly sensitive towards any pertubation. Low water temperatures also decrease membrane fluidity, resulting in an impairment of transfer processes for instance through the thylakoid membrane of chloroplasts. In addition, photosynthetic activity is particularly sensitive to low temperatures, as enzymatic secondary reactions are strictly temperature-dependent, while primary reactions are not. Therefore, the possibility of the generation of reactive oxygen species is enhanced at elevated light intensities, which may lead to degraded D1-protein. Thus, the likelihood of chronic photoinhibition increases.

The present thesis investigated the physiological performance, the acclimation potential and tolerance limits of the endemic Antarctic rhodophyte *Palmaria decipiens*. The studies were carried out on King George Island, South Shetland Islands, Antarctica. In various experiments conducted under laboratory and field conditions, the alga was exposed to changing light and temperature levels. It was hypothesized that in particular the combination of photosynthetically active radiation (PAR, 400-700 nm) and low temperatures lead to stress responses in the organism. To estimate the responses of *P. decipiens*, the alga was exposed over different periods of time to natural and artificial radiation. The artificial irradiances ranged between dim light conditions of 53 μmol photons m^{-2} s^{-1} and high irradiances of 650 μmol photons m^{-2} s^{-1}.

The exposure of *P. decipiens* to the combination of two temperatures (0°C und 8°C)

and two PAR-intensities (200 und 400 μmol photons m^{-2} s^{-1}) resulted in an impairment of photosynthetic performance at low temperatures and high irradiances as well as decreasing pigment concentrations. Overall, this indicates that elevated temperatures may compensate for high light stress within the species-specific tolerance limit. In general, high intensities led to a decline in photosynthetic performance of *P. decipiens*, and especially under the exposure to 650 μmol photons m^{-2} s^{-1} revealed a pronounced reduction in phycobiliprotein concentration. These pigments are generally sensitive to pertubations and degrade rapidly.

P. decipiens specimens from two different shore levels, the intertidal and the subtidal, were exposed to the natural solar radiation and two temperature for three weeks. This resulted in decreasing photosynthetic efficiency and capacity as well as a reduction of chlorophyll *a* and the phycobiliproteins. In contrast to the intertidal specimens, a decline in D1-protein concentration was observed in subtidal specimens exposed to low temperatures. Regarding the fatty acid composition, it was observed that intertidal specimens acclimated to changing conditions by increasing the content of saturated fatty acids, probably to maintain membrane fluidity. In this context, the concentration of polyunsaturated seems to be important to prevent from photoinhibition and to maintain the integrity of the D1. As these changes only occurred at elevated temperatures, it is assumed that the adjustment of fatty acid composition is triggered by temperature solely.

Due to global warming, fresh water input and precipitation will increase and therefore, salinity tolerance of *P. decipiens* was investigated. The alga inhabits a broad vertical range within the phytal zone and especially in high abundances in close proximitry to the glacier and thus, within higher variances in the salinity. Therefore, a high salinity tolerance of the organisms was expected and confirmed by the experiments. Specimens from the subtidal and the intertidal were exposed to salinities between 5 to 28 PSU and photosynthetic performance was measured over five days. Pronounced effects on photosynthesis were observed under 5 and 10 PSU with the subtidal specimens being more affected and died after two days. Nevertheless, as a truly marine species *P. decipiens* posses a wide salinity tolerance and may be regarded as euryhaline.

A comparative study between the two rhodophytes *Iridaea cordata* and *Palmaria decipiens* at elevated temperatures and light intensities revealed a slightly higher tolerance towards these factors as *P. decipiens*, as photosynthetic values remained at a constant higher level. This pattern may be reflected within the geographic distribution of the two species. *I. cordata* inhabits more northern subantarctic waters, whereas *P. decipiens* can only be found on a few subantarctic island and may therefore be considered as endemic.

The macroalgae investigated in the present study revealed a relatively high acclimation potential within their species-specific tolerance ranges. Obviously, this tolerance is limited and particularly rapid and pronounced changes can impact seaweeds negatively. To obtain realistic predictions in a more ecological context, investigations should involve interactive effects of the abiotic and biotic conditions. The present study shows the importance of experiments with combined factors, as results of single factor experiments may lead to misinterpretations in one or the other way. Especially the combination between light and temperatures clearly shows how both factors interact and thus, multiply or compensate each other.

In summary, this study demonstrates that Antarctic rhodophytes are very well adapted to their environment and are, in spite of this, still able to acclimate and cope with certain changes in the abiotic conditions. The results obtained during the present study also

indicate that the combined effects of environmental conditions is an important issue that has to be considered when adressing ecological questions.

Zusammenfassung

Der Lebensraum Antarktis stellt eines der schwierigsten und rauhesten Ökosysteme dar, welches sich durch extrem niedrige Temperaturen und eine stark ausgeprägte Saisonalität im Lichtklima auszeichnet. Die vorherrschenden abiotischen Bedingungen erfordern ein hohes Maß an genetischen Adaptationen der Organismen sowie zugleich die Fähigkeit, sich flexibel an variierende Umweltfaktoren anzupassen. Im Zuge des globalen Klimawandels gehört die Antarktis und insbesondere die antarktische Halbinsel zu den sich am schnellsten und deutlichsten verändernden Regionen. Dies zeigt sich in zurückgehenden Gletschern, vermehrter Eisbergbildung, erhöhten Niederschlägen und zunehmenden Windgeschwindigkeiten. Die gravierendste Veränderung dürfte aber die rapide angestiegene Jahresmitteltemperatur (3°C in den letzten 50 Jahren) sein. Dies hat zur Folge, dass das Meereis dünner wird und schneller aufbricht, wodurch die hohe Sonnenstrahlung bereits eher im Jahr tief in die Wassersäule eindringen kann.

Makroalgengemeinschaften bilden sich auf den vorhandenen Hartsubstratböden der Küstenzonen heraus und können eine hohe Biomasse von bis zu 74.000 Tonnen pro Jahr erreichen. Diese Gemeinschaften unterliegen großer abiotischer und biotischer Variabilität. Diese können sowohl im jahreszeitlichen Verlauf als auch als Folge des voranschreitenden globalen Klimawandels auftreten. Bisher wurden die in der Antarktis vorkommenden Arten unter anderem bezüglich ihrer Temperatur- und Wachstumseigenschaften, ihrer Empfindlichkeit gegenüber UV-Strahlung sowie ihrer Sukzession untersucht. Im Rahmen dieser Studie wird die Anpassungsfähigkeit von zwei antarktischen Rotalgen hinsichtlich der beiden abiotischen Faktoren Temperatur und Licht und ihrer möglichen Interaktion erforscht. Außerdem wird im Zusammenhang mit der globalen Erwärmung der Schmelzwassereintrag zunehmen, so dass zusätzliche Untersuchungen zum Einfluß des Salzgehaltes auf die Photosynthese der Alge durchgeführt wurden.

Die Kombination von tiefen Temperaturen und hoher Sonnenlichtintensität stellt photosynthetische Organismen vor besondere Herausforderungen: unter niedrigen Temperaturen verlangsamen sich enzymatische Prozesse sowie Auf- und Abbau des D1-Proteins im Photosystem II. Dieses Protein ist für die Funktionalität der Photosynthese von zentraler Bedeutung und zugleich äußerst sensibel und angreifbar. Desweiteren setzen niedrige Temperaturen die Membranfluidität herab, wodurch Transferprozesse beispielsweise durch die Thylakoidmembran der Chloroplasten beeinträchtigt werden kann. Insbesondere die photosynthetische Aktivität reagiert temperaturempfindlich, weil die sekundären Enzymreaktionen temperaturabhängig sind, hingegen die primären Lichtreaktionen unabhängig von der Umgebungstemperature stattfinden. Insofern ist unter erhöhten Lichtintensitäten die Entstehung von reaktiven Sauerstoffspezies wahrscheinlicher, und somit auch das Risiko einer chronischen Photoinhibiton infolge der Beschädigung und Zerstörung des D1-Proteins steigt.

In dieser Arbeit wurde das physiologische Potential, sowie die Anpassungsfähigkeit und Toleranzgrenzen der endemisch-antarktischen Rotalge *P. decipiens* auf King George Island, Südshetlandinseln (Antarktis) untersucht. Hierzu wurde die Alge in verschiedenen Experimenten, die sowohl unter Labor- als auch unter Freilandbedingungen stattfanden,

veränderten Strahlungs- und Temperaturbedingungen ausgesetzt. Es wird davon ausgegangen, dass die sogenannte "photosynthetisch aktiven Strahlung" (PAR, 400-700 nm) in Kombination mit den niedrigen Temperaturen vermehrt zu Stressreaktionen der Alge führt. Um die Auswirkungen auf *P. decipiens* einschätzen zu können, wurde sowohl mit natürlichen als auch mit teilweise stark erhöhten Lichtintensitäten gearbeitet, denen die Alge über unterschiedliche Zeiträume ausgesetzt war. Die eingesetzten Lichtintensitäten lagen zwischen 53 μmol photons m^{-2} s^{-1} und 650 μmol photons m^{-2} s^{-1}.

In einem Experiment, bei dem *P. decipiens* zwei Temperaturen (0°C und 8°C) mit zwei verschiedenen Strahlungsintensitäten (200 und 400 μmol photons m^{-2} s^{-1}) kombiniert wurden, konnte gezeigt werden, dass unter niedrigen Temperaturen und gleichzeitig erhöhter Strahlungsintensität, die photosynthetische Leistung eingeschränkt ist. Außerdem konnte eine Abnahme im Pigmentgehalt beobachtet werden. Insgesamt deuten diese Ergebnisse daraufhin, dass höhere Temperaturen innerhalb der artspezifischen Toleranzgrenzen Strahlungsstress kompensieren können. Generell führten erhöhte Lichtintensitäten zu abnehmender Photosyntheseleistung bei *P. decipiens*. Insbesondere unter 650 μmol photons m^{-2} s^{-1} nahm die Phycobiliproteinkonzentration ebenfalls deutlich ab, diese akzessorischen Pigmente gelten allgemein als stressempfindlich und werden leicht abgebaut.

In einem Mesokosmosversuch wurde *P. decipiens* aus zwei unterschiedlichen Tiefenstufen, dem Sublittoral und dem Gezeitenbereich, unter natürlichen Strahlungsbedingen und zwei Temperaturen über den Zeitraum von drei Wochen exponiert. Dies resultierte in einer Einschränkung der Photosyntheseleistung mit gleichzeitiger Reduktion der Photosynthesepigmente Chlorophyll a und der Phycobiliproteine. Im Gegensatz zu den Algen aus dem Gezeitenbereich nahm jedoch die Konzentration des D1-Proteins der sublittoralen Algen bei kalten Temperaturen ab. Hinsichtlich der Fettsäurezusammensetzung ließ sich feststellen, dass die Algen aus dem Gezeitenbereich eine wesentlich höhere Anpassungsfähigkeit aufwiesen und den Anteil der gesättigten Fettsäuren am Gesamtlipidgehalt erhöhten, möglicherweise um die Membranfluidität beizubehalten. In diesem Zusammenhang scheint der Gehalt von mehrfach ungesättigten Fettsäuren eine bedeutende Rolle zu spielen, um Photoinhibition vorzubeugen und die Funktionsfähigkeit des D1-Proteins beizubehalten. Diese Veränderungen waren lediglich unter erhöhten Temperaturen zu beobachten. Deshalb kann davon ausgegangen werden, dass der Umbau der Fettsäuren temperaturabhängig ist und die Lichtintensität eine untergeordnete Rolle zu spielen scheint.

Da es im Zuge der globalen Klimaerwärmung vermehrt zu Süßwassereintrag durch erhöhten Schmelzwasserzufluss sowie einer Zunahme der Niederschläge kommen wird, ist *P. decipiens* zusätzlich auf ihre Salinitätstoleranz hin untersucht worden. Aufgrund der vertikalen Verbreitung der Alge im Phytal und insbesondere den hohen Abundanzen in Gletschernähe, die mit größeren Salinitätsschwankungen einhergehen, ist anzunehmen, dass *P. decipiens* eine hohe Toleranz aufweist. Dies konnte in den durchgeführten Experimenten bestätigt werden. Hierzu wurde die Alge aus dem Sublittoral und aus dem Gezeitenbereich Salinitäten im Bereich von 5 bis 28 PSU ausgesetzt und die Photosyntheseleistung über einen Zeitraum von fünf Tagen gemessen. Deutliche Beeinträchtigungen zeigten sich lediglich bei 5 und 10 PSU, wobei die Organismen aus dem Sublittoral sensitiver reagierten und bereits nach zwei Tagen abstarben. Dennoch verfügt *P. decipiens* als eine rein marine Art über eine hohe Salinitätstoleranz, so dass die Alge als eine euryhaline Art angesehen werden kann.

Ein Vergleich der beiden Rotalgenarten *Iridaea cordata* und *Palmaria decipiens* unter erhöhten Strahlungsintensitäten und erhöhten Temperaturen führte zu der Erkenntnis,

dass *I. cordata* einen leicht größeren Toleranzbereich aufweist als *P. decipiens*. Dieses Muster ist ebenfalls in der Verbreitung der beiden Arten zu erkennen, da *I. cordata* auch in nördlicheren subantarktischen Gewässern vorkommt, während *P. decipiens* nur auf einigen subantarktischen Inseln zu finden ist und daher als endemisch eingestuft werden kann.

Die in dieser Studie untersuchten Makroalgen zeigten innerhalb ihrer artspezifischen Toleranzgrenzen ein relativ hohes Anpassungsvermögen. Dabei ist zu beachten, dass diese Toleranzgrenzen limitiert sind und sich vor allem schnelle und starke Veränderungen nachteilig auf die Organismen auswirken können. Um zuverlässige und realistische Aussagen im ökologischen Kontext treffen zu können, sind Untersuchungen von interaktiven Effekten nicht nur zwischen der abiotischen, sondern auch der biotischen Umwelt notwendig. Diese Studie belegt die Wichtigkeit solcher Experimente, da Einzeleffekte unter Umständen zu einer fehlerhaften Einschätzung sowohl in positiver als auch in negativer Hinsicht führen. Gerade die Kombination zwischen Temperatur und Licht zeigt deutlich, wie sich beide Parameter gegenseitig beeinflussen und dementsprechend verstärken oder abschwächen können.

Schlussfolgernd zeigt diese Arbeit, dass antarktische Rotalgen auf der einen Seite extrem an ihren Lebensraum angepasst sind, aber dennoch eine gewisse Toleranz gegenüber veränderten Umweltbedingungen besitzen. Diese Studie zeigt außerdem, dass der Effekt von kombinierten Umweltfaktoren unbedingt zu berücksichtigen ist, da nur somit die Komplexität der wirkenden natürlichen Bedingungen im ausreichendem Maße erfasst werden kann.

Contents

1 Introduction

1.1 Antarctic coastal ecosystems and the role of macroalgae

Polar marine ecosystems belong to the most productive regions worldwide, containing highest densities of phytoplankton and huge stocks of macroalgae along rocky shore lines (Wängberg et al., 1996). The Arctic and Antarctic region are characterized by an extreme seasonal variation in light availability, reaching from six month of total darkness to polar day at high latitudes (Lüning, 1990, et al., 2009). In spite of this, other abiotic factors such as salinity or nutrient availability are very constant, and especially in Antarctica macroalgal growth is not nutrient-limited (Quartino et al., 2005, Nedzarek and Rakusa-Suscezewski 2004). Nevertheless, Polar Regions are very susceptible to environmental changes and thus, changes observed in Polar waters and coastal zones may be indicative for the onset of global climate change (IPCC 2007, Turner et al., 2009).

Antarctic coastal ecosystems do also represent one of the most stable environments in terms of water temperature, since the cold water history of the Southern Ocean began 30 million years ago. Since then, Antarctica has been isolated due to its oceanographic peculiarities within the Polar Front (Rintoul et al., 2001). The oceanography of the Antarctic continent is characterized by strong westerly winds that drive the Antarctic circumpolar current clockwise around the landmass and hence, isolates the inner part from the surrounding Pacific, Atlantic and Indian Ocean. Thus, a strict temperature regime developed and it is hypothesized that this particular temperature regime is the reason why invasive species are so far unable to migrate into this isolated region. As any pertubation connected to global climate change manifests itself rapidly in the Polar Regions, the Antarctic is regarded as a sensitive indicator for climatic alterations.

With respect to global climate change, studies have shown that a variety of abiotic factors (e.g. temperature increase, retreating glaciers with increased calving, changing radiation climate with increasing UV-intensities due to seasonal depletion of stratospheric ozone, CO_2 rise in the atmosphere and the seawater), will influence the coastal zones in the Southern Ocean. The Western Antarctic Peninsula (WAP) is affected the most and belongs to the regions worldwide with the most rapid and drastic changes (Turner et al., 2005, Johnston et al., 2007, Barnes and Peck, 2008). It has been shown that during the last 50 years air temperatures on the WAP increased by 0.56°C per decade and even by 1.09°C per decade during winter (Turner et al., 2005, Barnes et al., 2006). These immense changes will impact Antarctic coastal zones, in particular shallow benthic communities (as reviewed by Barnes and Conlan, 2007). In that context, the uniquely adapted flora of Antarctica is believed to be extremely vulnerable to climate shifts (e.g. IPCC, 2007, Turner et al., 2009). Thus, research activities have been undertaken and will continue intensively to shed light on Antarctic coastal ecosystem function, in which benthic primary producers play an essential role.

The benthic communities may be divided into two mayor groups with regard to the type of substrata found: the soft bottom, characterized by sand and fine sediments found in close proximity to glaciers or river mouths, and hard bottom substrate consisting of

pebbles, rocks or solid boulders (Tatián et al., 2008a). Generally, hard bottom substrate is rare in the Antarctic, as in most regions, glaciers enter the ocean directly, making intertidal platforms especially scarce. Soft bottoms are mostly inhabited by filter feeders like e.g. ascidians or mollusks (Tatián et al., 2008b, Mercuri et al., 2008) and other types of invertebrates (star fish, ophiurians, limpets; Sahade et al., 1998). Within these regions, macroalgae are rare and only benthic diatoms may be found. The rocky hard bottom areas of Antarctica are covered by huge stands of all kinds of macroalgae showing a typical depth zonation (Klöser et al., 1993, 1996, Quartino et al., 2001). Interestingly, filamentous macroalgae are missing in Antarctic subtidal zones (as reviewed by Amsler et al., 2009).

Seaweeds are important structural components in shallow benthic communities, providing habitat, food and shelter for associated animals such as mollusks, amphipods, copepods or fish (Iken et al., 1999, Huang et al., 2006, Amsler et al., 2005, 2009). Macroalgae provide structure within habitats by stabilizing sediments or by settling new ice free areas as pioneer species (Quartino et al., 2005). In Admiralty Bay on King George Island macroalgal biomasses can reach values up to 74.000 tons per year, which serves as a huge nutrient source when the algal material decomposes (Nedzarek and Rakusa-Suszczewski, 2004, Oliveira et al., 2009).

1.1.1 Coastal zones of King George Island

Coastal benthic communities are very well adapted to their particular environment, either the intertidal or the subtidal zone. The subtidal, which is always covered by the water column, is characterized by little variation within water temperatures and salinity, and is thus, regarded as a rather stable habitat. In contrast, Antarctic intertidal zones provide one of the most challenging habitats on Earth: during the course of the tides, inhabiting organisms are exposed to a wide temperature range reaching from far subzero temperatures up to +18°C (in tide pools in the Gerlach Strait, G. Ferreyra, pers. comm.). Tide pools on King George Island may reach temperatures up to +8°C (Abele et al., 1999). Additionally, they are exposed to drought and freezing, to drastic salinity changes ranging between full marine to 20 PSU and ice scouring. A major factor is the pronounced seasonality within the light regime (see section 1.2), which varies between five hours during the winter season to 20 hours in summer at the study site of Potter Cove (King George Island, South Shetlands; Wiencke, 1990, Zacher et al., 2009).

On King George Island, the upper parts of the sublittoral are commonly inhabited by foliose rhodophytes of the genus *Iridaea* or *Palmaria*, as well as phaeophytes such as *Desmarestia anceps*. Interestingly, the worldwide dominant order Laminariales is completely absent in the Southern Ocean and is replaced by species belonging to the order Desmarestiales, which can form dense stocks and may build up to 80% of the summer biomass (Wiencke and Clayton, 2002, Quartino and Boraso de Zaixso, 2008). These species often have an undergrowth of encrusting rhodophytes. In deeper depths, huge brown algae like *Himanthothallus grandifolius* or *Desmarestia menziesii* become dominant and can be found down to depths of 50 m (Klöser et al., 1996, Wiencke and Clayton, 2002, Wulff et al., 2009).

The intertidal zones are covered by extensive stands of *Ascoseira mirabilis*, a phaeophyte, as well as filamentous rhodophytes such as *Plocamium* species or species belonging to the Gigartinales. In general, the degree of endemism is extraordinarily high in Antarctic waters; in the marine algal flora of the West Antarctic 35% of the species are endemic and within

the rhodophytes there are 37 species exclusively inhabiting Antarctica (Wiencke and tom Dieck, 1989, Wiencke and Clayton, 2002, Hommersand et al., 2009). As endemic species are presumably high adapted to their specific environment, insights into their physiology and response towards changing abiotic factors provides valuable information about their particular adaptation and acclimation potential as well as the general ecosystem function.

Palmaria decipiens is a very common alga in Potter Cove on King George Island, the study site, where the alga may become the dominating species in terms of density (Klöser et al., 1996, Quartino et al., 2001). In Admiralty Bay that borders on Potter Cove, *P. decipiens* may also become dominant in terms of biomass (Oliveira et al., 2009 and references therein). It is regarded as a pioneer species that may rapidly colonize disturbed areas (M.L. Quartino, pers. comm.). Amphipods are often associated with *Palmaria decipiens* and feed directly on the alga, as well as high densities of the limpet *Nacella concinna*, which feeds on epiphytic microalgae of *P. decipiens*. Rhodophytes are rich in nitrogen and carbon and are of high nutritional value (Peters et al., 2005). Taken that into account in combination with the high biomass and its importance for ecosystem function, *P. decipiens* may be regarded as one of the key species in shallow Antarctic benthic communities.

Thus, the present study focuses on the physiological acclimation potential of *Palmaria decipiens*, a very abundant rhodophyte in Antarctica and sub-Antarctic islands, to combined abiotic (stress-) factors.

1.2 Irradiance and temperature characteristics

The most obvious abiotic factor characterizing Polar Regions is the strong seasonal variability of light. At latitudes higher than 80°, six months of total darkness during the polar night and six months of 24 h daylengths occur. Due to solar angle, light climate at King George Island (62°14‘S, 58°38‘W) still varies broadly, from only five hours of daylight in winter to a maximum of 21 hours in austral midsummer. During winter months, where additional sea-ice cover and snow layers may further reduce light availability and prolong periods of darkness in the subtidal zone, algae have developed survival mechanisms, for example by decreasing the concentration of photosynthetic pigments and live off storage compounds synthesized in summer, e.g. floridean starch in *P. decipiens* (Weykam et al., 1997, Lüder et al., 2001, 2002). After sea-ice break up, algae are suddenly exposed to high light intensities, as the water body is most transparent and light penetrates into great depths (Drew and Hastings 1992, McMinn et al. 2004).

Global climate warming may reduce sea-ice formation and snow cover resulting in a earlier break up of sea-ice. Thus, periods of darkness or dim light conditions are shortened and macroalgae may experience high irradiances earlier in the season. As *P. decipiens* is a season anticipator with its maximum growth in late winter (Wiencke, 1990, 1996), very young thalli might be exposed to high irradiances immediately after sea-ice break up. As glacier melting and river run-off may increase, additional sediments will be washed into the bay, and an additional reduction in light availability after onset of melting might occur. Depending on how flexible organisms can adjust their photosynthetic performance, this discrepancy between too little light and excessive light intensities might contribute to the formation of defined zonation patterns. A detailed overview on the radiation climate at King George Island is available by Richter et al. (2008) and for photosynthetically

active radiation (PAR) in publication II. Maximum PAR-intensities reach values up to 1500 μmol photons m^{-2} s^{-1} on a sunny day (see table 1 in publication II).

It is accepted that temperature is the crucial factor determining geographic distribution of macroalgal species (Lüning, 1990). Endemic species have a particularly narrow range of tolerances to abiotic (and biotic) changes. For *P. decipiens*, the northern distribution limit on the sub-Antarctic Islands Macquarie (54°S) and the Kerguelen (50°S) lies within subantarctic regions and coincides well with the upper temperature limit for growth of 10°C (Wiencke and tom Dieck, 1989, Wiencke and Clayton, 2002). A detailed summary of temperature adaptations of *P. decipiens* can be found in publication III.

1.3 Impacts of abiotic (stress-) factors on macroalgal communities

The variety of abiotic and biotic (stress-) factors on macroalgal communities range from osmotic stresses under changing salinities (especially in the intertidal zone), changing radiation climate with enhanced intensities within the UV-range as well as an increase in PAR-availability (Franklin and Forster, 1997, Bischof et al., 1999, 2002, Karsten, 2007). Additionally, seaweeds have to cope with mechanical stress through wave action and storm actitivities to intra- and interspecific competition and grazing pressure (Hurd, 2000, Zacher and Campana, 2008, Zacher et al., 2009, Amsler et al., 2009). Generally, the abiotic environment of specific habitats shapes depth zonation and community structure (e.g. Bischof et al., 2006).

The influence of single abiotic factors on macroalgae has been investigated intensively and was reviewed recently by Wiencke et al. (2009). Insights in acclimation potential and stress tolerance limits of a variety of macroalgal species (e.g. *Desmarestia anceps*, *Ulva sp.* by Rautenberger and Bischof, 2006) and community structure (Zacher et al., 2007, Zacher and Campana, 2008) have been obtained, however, investigations of the effect of combined (stress-) factors are still scarce. As ecological parameters are always interacting, the present study tries to evaluate the impact of a combination of abiotic factors (temperature and light intensity) on the physiological performance of an Antarctic rhodophyte.

1.4 Protective mechanisms

To adjust to changing environmental factors, macroalgae developed a variety of protective mechanisms. Protective mechanisms can be either genetically determined through long evolutionary processes (adaptation) or a certain flexibility to respond to a changing environment (acclimation). The ability to acclimate is of great importance as it enables optimization of photosynthetic performance and hence, growth and reproduction, for example in response to seasonal changes in water temperatures (Kuebler et al., 1991 and references therein). Protective mechanisms with respect to high irradiances, UV-radiation and low temperatures may include e.g. a rapid DNA repair (Karentz et al., 1991), adjustment of fatty acid composition (Gombos et al., 1994, Harwood and Guschina, 2009), high turnover rates of important proteins such as the D1 centre protein of photosystem II (PS II; Mattoo et al., 1999, Aro et al., 2005), variation of pigment content (Kuebler et al., 1991, Maxwell et al., 1994, Grabowski et al., 2001, Lüder et al., 2001, 2002) or the accumulation of certain metabolites such as mycosporine-like amino acids (MAAs) as UV-protective solutes (Hoyer et al., 2002, Shick and Dunlap, 2002, Oren and Gunde-Cimerman, 2007).

1.4.1 Adaptations to photosynthetically active radiation - PAR

Generally, photosynthetic organisms need to harvest sufficient light to drive photosynthesis and thus, maintain metabolic function. Depending on species-specific demands and geographic distribution, photosynthesis withstands only a certain increase of radiation and becomes saturated at relatively low irradiances. Light harvesting complexes and antennae pigments absorb light radiation, which is transferred to the chlorophyll *a* reaction centres of photosystem II. Absorbed excitation energy is partioned between photochemistry, heat dissipation (mostly in antennae) and chlorophyll fluorescence (Schreiber et al., 1994, Franklin et al., 2003). A surplus of excitation energy can inactivate PS II in two different ways as tested *in vitro* by Bouchard et al. (2006). First, damage on the acceptor-side of PS II may occur under light intensities above saturation, which, in the end, leads to the formation of triplet chlorophyll. This highly reactive chlorophyll triplet may react with molecular oxygen, resulting in the generation of reactive oxygen species (ROS). ROS and particularly singlet oxygen may damage the D1 centre protein of PS II. The second way occurs on the donor side of PS II. Here, PS II is unable to withdraw electrons from P_{680} and thus, highly oxidizing radicals can be generated. In that case, radicals may damage proteins and inactivate *de novo* synthesis of the D1-protein (Bouchard et al., 2006 and references therein). *In vivo*, these two ways are not easily distinguished, and a more complex pattern is found. *In vivo*, the repair of damaged D1 takes place simultaneously with the photoinactivation and the repair processes themselves are subject to metabolic and environmental influences. High intensities of PAR radiation do not only impair PS II: if the drain-off of the electrons is disturbed, PS I is also vulnerable due to increased concentrations of H_2O_2 (He et al. 2002). Hence, ROS are generated and several mechanisms to detoxify cells from ROS have developed, for instance through the water-water-cycle (including H_2O_2 via the enzyme superoxide dismutase, SOD), in which electrons participate in the Mehler-reaction or the cyclic electron transport (Asada, 1999, 2000).

The initiation of ROS generation through excessive light absorption may cause protein degradation, lipid peroxidation of thylakoid membranes and inactivation of enzymes involved in carbon metabolization (Asada, 2000, He et al., 2002). Especially at low temperatures, where enzyme activities are slowed down and thus, electron transport under excessive light may become unbalanced, higher amounts of electrons participate in the Mehler reaction and the likelihood for ROS generation is increased. The primary target of ROS is the D1-protein complex in the centre of PS II, which may degrade and lose its functionality (e.gFranklin et al., 2003, Aro et al., 2005, Bouchard et al., 2006). Generally, the PS II reaction centres consist of a heterodimer of D1- and D2-protein. D1 is characterized by a rapid turn-over rate, i.ethe exchange of damaged and re-synthesized protein (Aro et al., 1993, 2005). This turn-over is due to its location in one of the most energetically charged environments in living organisms ("the suicide protein", see Franklin et al., 2003). By increasing the repair rate of damaged D1, photosynthetic organisms such as macroalgae reduce the risk of chronic photoinhibition. Only if the repair rate is slower than the degree of degradation under high irradiances, algae become chronically photoinhibited. Hence, the organisms have to balance between absorbing enough light to maintain photosynthesis and to protect exactly that machinery from excessive irradiances to avoid photodamage.

By varying the amount of pigments such as chlorophyll *a* or the antennae pigments of the phycobilisomes, rhodophytes can adjust their photosynthetic apparatus to the respective light regime (Beach et al., 2000, Lüder et al., 2001, 2002). Light harvesting complexes

(LHC) and xanthophylls such as zeaxanthin in rhodophytes, enhance the ability to dissipate over-absorbed light energy as harmless heat (Hanelt et al., 1994, Grabowski et al., 2001). Thus, an increase in xanthophylls might be an advantage in habitats exposed to high PAR intensities, taken into account that carotenoids may also serve as antioxidants or passive UV-sunscreens (Franklin and Forster, 1997).

1.4.2 Adaptations to low temperature

Antarctic waters and the coastal zone provide constant, but very low water temperatures (Abele et al., 1999, Wiencke et al., 2007, and see Figure 5.1). In that context organisms have to adapt their biochemical processes to maintain function at a mean water temperature of ± 0°C. Overall, enzymatic pathways and reactions are slowed down in the cold, influencing secondary enzymatic reactions of photosynthesis. To compensate for slower enzymatic rates, protein concentrations of for instance the carbon-fixation protein RubisCO might be enhanced or the density of mRNA transcript might be increased. Thus, high photosynthetic rates can be maintained at low temperatures (Kuebler et al., 1993). With respect to freezing temperatures, the most dramatic consequence for organisms would be the formation of intracellular ice-crystals. Therefore, a lot of micro- and macroalgae may synthesize low molecular weight compounds as cryoprotectants to avoid intracellular freezing (e.g. mannitol in *Mastocarpus stellatus*, Lohrmann et al., 2004).

In macroalgae from low temperature environments, a higher degree of unsaturation of membrane lipids, especially in the thylakoid membrane lipid composition, seems important to maintain membrane fluidity and function (Graeve et al., 2002, Harwood and Guschina, 2009). Apparently, photoinhibition is less severe at low temperatures, depending on the degree of unsaturation of glycerolipids. It was shown in cyanobacteria, that a high unsaturation degree of fatty acids accelerates the recovery processes from photoinhibition at low temperatures (Gombos et al., 1994). In this context, a remarkable adaptation of Antarctic endemic macroalgae is evident, since their temperature demands for growth and photosynthesis are very low. Nevertheless, comparable metabolic and oxygen production rates are reached as in temperate species (Wiencke and tom Dieck, 1989, Wiencke, 1990, Eggert and Wiencke, 2000) and it is hypothesized that membrane lipid composition may play a key role in metabolic maintenance (Gombos et al., 1994, Murata and Los, 1997).

1.4.3 High light and low temperatures - what a mixture!

The main challenge of photosynthetic organisms inhabiting Polar coastal zones is the combination of very low water (and air) temperatures with high irradiances. In these regions, macroalgae adapted to their environment, which amongst others resulted in a higher concentration of unsaturated fatty acids in their thylakoid membranes (Murata and Los, 1997, Harwood and Guschina, 2009). Due to this, membranes of cold-adapted seaweeds may obtain similar membrane fluidity as temperate species. Therefore, the transfer of degraded D1-protein through the thylakoid membranes should be achieved at similar rates. Nevertheless, high PAR-irradiances can become harmful, and may induce ROS generation with a subsequent fragmentation of D1. Generally, under low temperatures enzyme activity is reduced. This may affect *de novo* synthesis of D1-protein and ROS scavenging by antioxidant enzymes as well as possibly influencing the carbon fixation enzyme RubisCO and consequently, CO_2 assimilation drops (Bischof et al., 2000). Simultaneously high irradi-

ances are still absorbed and regular transfer may be impaired, resulting in ROS generation as stated above. To investigate how the physiology of Antarctic macroalgae is adapted to that very particular environment is the major task of the present thesis.

1.5 Research questions

Hitherto, most information available on the physiology of *Palmaria decipiens* was obtained in unifactorial experiments and is summarized in publication III. Hence, the present study addresses the effects of combined abiotic stressors. Abiotic factors such as light and temperature may interact and interpretation of results of single-factor experiments can lead to over- or underestimation of the impact on physiological and metabolic parameters. Thus, to predict more realistic physiological responses, two factors are included and their eventual synergistic effect will be investigated and discussed.

The aim of the present study was to answer the following research questions:

(I) Is photosynthetic performance of *Palmaria decipiens* more impaired by high PAR radiation at low temperatures in comparison to moderate PAR intensities?

(II) May elevated temperatures enhance the ability of *Palmaria decipiens* to protect from photoinhibition?

(III) Since *Palmaria decipiens* inhabits a broad vertical range in the phytal zone, physiological responses of specimens of *P. decipiens* from two different shore levels were investigated under the combination of natural solar radiation and changing temperature levels. Do specimens from different shore levels react differently?

(IV) Rhodophytes are very abundant in Antarctic shallow benthic communities, therefore the species *Iridaea cordata* was included. It inhabits a similar vertical range and its responses to changing environmental factors in comparison with those from *P. decipiens* may provide more insights for community structure and ecosystem function. Do two rhodophyte species, *I. cordata* and *P. decipiens*, respond differently to elevated temperatures in combination with enhanced irradiances?

(V) Is either of the two species better adapted to the same environment or reveals a higher acclimation potential and may thus, outcompete the other?

(VI) As this study was performed within the International Polar Year (IPY) and the Climate Change on Western Antarctic Peninsula-Initiative (ClicOPEN), addressing particularly climate change topics on the western Antarctic Peninsula, photosynthetic responses of *Palmaria decipiens* towards salinity changes were investigated. To what extend may *P. decipiens* cope with freshwater input due to increased glacier melting and river run-off?

2 Publications

2.1 List of publications

The present thesis is based on the following publications, to which is referred to in the text by their Roman numbers:

I **Becker S**, Walter B, Bischof K. 2009. Freezing tolerance and photosynthetic performance of polar seaweeds. Botanica Marina 52:609-616.

II **Becker S**, Graeve M, Bischof K. 2010. Photosynthesis and lipid composition of the Antarctic endemic rhodophyte *Palmaria decipiens*: Effects of changing light and temperature levels. Polar Biology, DOI 10.1007/s00300-010-0772-5.

III **Becker S**, Quartino ML, Campana GL, Bucolo P, Wiencke C, Bischof K. (to be submitted). The Biology of an abundant Antarctic rhodophyte, *Palmaria decipiens*: An overview of recent advances. Antarctic Science.

2.2 Contribution to publications

Publications I, II and parts of publications III are based on my own laboratory and field experiments, which were planned, organized, conducted and analysed by myself in close cooperation with my supervisor K. Bischof. The first version of all manuscripts was written by myself and improved in close cooperation with the respective co-authors. Parts of publication I are based on data of the diploma thesis of B. Walter, who has contributed data analysis and parts of the manuscript as well. Technical assistents supported GC measurements of fatty acid analysis for publication II. The idea and cooperations for publication III were initiated by myself, improvement of the manuscript was achieved in close cooperation with co-authors, C. Wiencke and K. Bischof.

3 Material and Methods

3.1 The marine environment of King George Island

Collecting of sample material as well as field and laboratory experiments were performed on King George Island located on the Western Antarctic Peninsula (see Figure 3.1). The scientific station, Dallmann Laboratory is an annex to the Argentinean base Teniente Jubany and is facing Potter Cove, a small inlet of Maxwell Bay. On the northeastern part of the bay, the Fourcade glacier arises, a type of glacier that rarely calves huge icebergs, but smaller pieces of ice are often found in the bay. The beach part on the station side is especially influenced by its ice scouring (Klöser et al., 1993, 1996). Within the bay, a clockwise streaming occurs, therefore, ice pieces are transported towards the beach on the western shore and leave Potter Cove with the streaming at Punta Elefante and into the Bransfield Strait. Due to the streaming as well as river run-off and melting events from the glacier and higher located lagoons in summer, the western side becomes more turbid than the eastern shore. During these melting events, the underwater light climate and salinity may change drastically. Nevertheless, it has been shown by Klöser et al. (1996) that stratification is rare as water becomes quickly mixed by frequent winds and the turbid water leaves the bay relatively fast. In general, water temperature and salinity are regarded to be quite conservative in an oceanographic point of view, it is not surprising that these two factors are very stable and do not vary broadly within the season. Salinity usually ranges between 34 PSU and 32 PSU (Schloss et al., 2002, Klöser et al., 1993, 1996), depending on melt water input during summer. Water temperatures in the subtidal vary between -1.8°C in winter and +2°C in summer (Wiencke, 1990, Zacher et al., 2009), although it is noticeable that within tide pools temperatures may reach up to +8°C (Abele et al., 1999).

Benthic communities differ with the respective substrate characteristics: hard bottom occurs in the mouth of the bay, the intertidal zone outside the bay and directly underneath the glacier foot. The rest of the bay is characterized by soft bottoms such as mud and sand, each with its own typical communities, composed of e.g. ascidians, benthic diatoms and sea stars. Macroalgae may be found on hard bottom substrates in depths up to 30 m (Klöser et al., 1996, Gomez et al., 1997, Quartino and Boraso de Zaixso, 2008) and might reach high densities with a total of 38 species (Quartino and Boraso de Zaixso, 2008).

All experiments of the present thesis have been carried out at the Dallmann Laboratory during two summer campaigns. The first one took place from the beginning of January until end of March 2007, this being from midsummer to early fall in the southern hemisphere. The second expedition lasted from mid-October 2007 until beginning of March 2008, covering the seasons from late winter, spring and summer. Thus, the respective samples are related to a specific season which may have an influence on the discussed data. Experiments have been either carried out in the laboratory facilities of the station or directly on the shoreline of the bay.

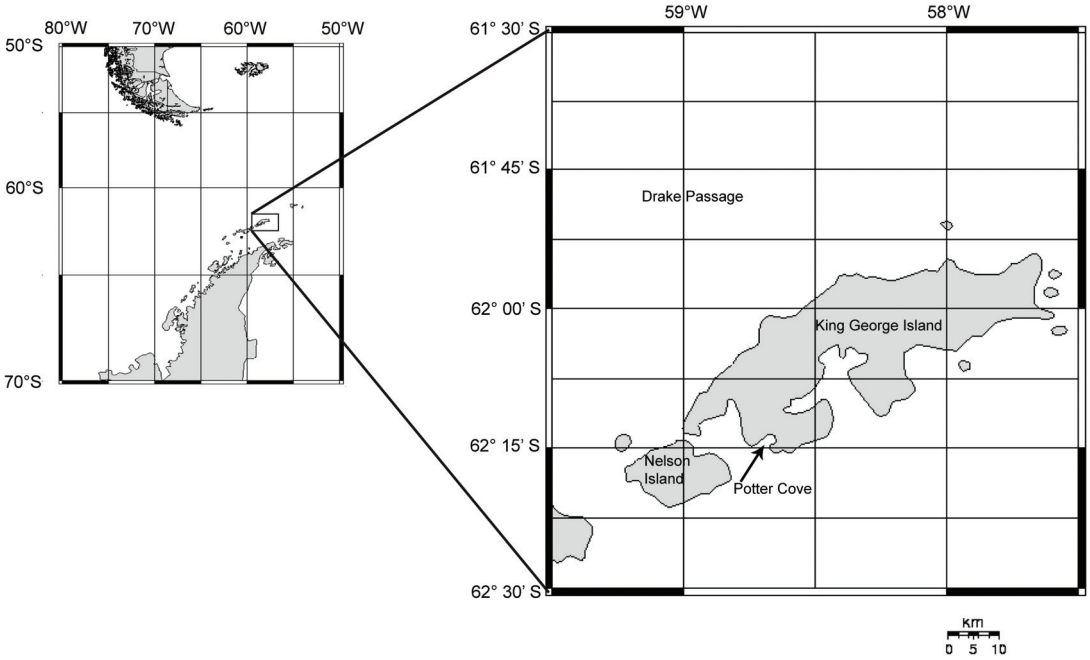

Figure 3.1: Geographic overview of the location Potter Cove, King George Island, South Shetland Islands, Antarctica (www.aquarius.geomar.de)

3.2 Algal material

The vast majority of the experiments in the present study were performed on *Palmaria decipiens*, a rhodophyte belonging to the order Palmariales. Its blades are lanceolate up to 70 cm long and a width of 15 cm, with a purplish red to pink color and a glossy surface (see Figure 3.2). It inhabits intertidal zones and rock pools, as well as subtidal zones down to 30 m (Wiencke and Clayton, 2002, for details). A detailed summary on the biology of the species can be found in publication III. Experiments were performed on intertidal and subtidal specimens of the alga.

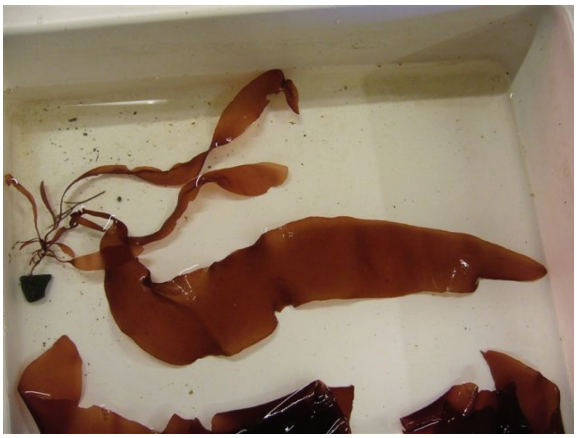

Figure 3.2: Thallus of *Palmaria decipiens*.

Additionally, some investigations were conducted on *Iridaea cordata*, and some exemplary results are included in this thesis. *Iridaea cordata* (Turner) Bory (1826) belongs to the *Gigartinaceae* within the order *Gigartinales*. Its fronds are dark purplish red with an ovate to lanceolat shape, up to 50 cm in diameter. Older plants often become perforated. Characteristically are the anticlinal chains of small, almost isodiametrical pigmented cells of the cortex (see Figure **??**, left). It inhabits intertidal zones together with *P. decipiens* and *Adenocystis utricularis*, but is more common in subtidal zones down to 30 m. *I. cordata* is, in contrast to *P. decipiens*, a season responder with highest growth rates during the austral summer. It is also strongly shade- and cold-adapted (Wiencke and Clayton, 2002). Experiments were performed on subtidal specimens of *Iridaea cordata* from depths of 8 to 10 m.

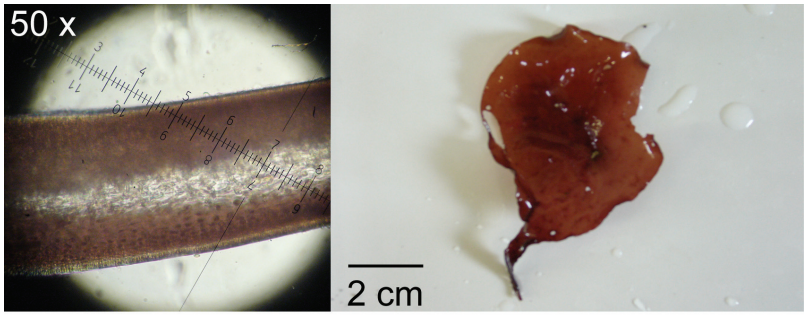

Figure 3.3: Young thallus of *Iridaea cordata* (right). Characteristic, anticlinal chains of pigmented cells within the cortex are presented in microscopic view with a magnification of 50 (left).

3.3 Experimental set-up

In all experiments performed in Antarctica, algae specimens from the subtidal were collected by scuba diving in front of the Fourcade glacier facing the station. For each experiment, 15 to 20 individuals of *Palmaria decipiens* or *Iridaea cordata* with a size of 30 to 50 cm were collected by scuba divers at 8 to 10 m water depth and brought to the laboratory in light-protected black boxes to avoid light stress during the transfer. For experiments conducted with specimens from the intertidal, algal material was collected during low tide conditions and brought back to the station in a bucket filled with seawater and covered with black gauze. If cultivation was necessary prior to the experiments, algae were kept under low light conditions (15 μmol photons m^{-2} s^{-1}) in a seawater tank with ambient water temperature (approx. +2°C). Water was aerated and replaced every second day. An overview of the performed experiments is given in Table 3.1.

Table 3.1: Overview of the applied conditions on the two alga species *Palmaria decipiens* and *Iridaea cordata*. Sampling depth is indicated by the letters subtidal (s) and intertidal (i). Light intensities are values of photosynthetically active radiation (PAR) in μmol photons m^{-2} s^{-1}, salinity is indicated by practical salinity unit (PSU). Duration of the treatment in days as well as potential recovery period in hours are supplied. Measured and analysed physiological factors are indicated by a cross.

	Species and depth	PAR	PSU	(°C)	Duration (d)	Recovery (h)	Fv/Fm	rETRmax	content of Pigment	D1	FA
laboratory experiments	*Palmaria decipiens*, i	53	5-28	2	3	48	x	x			
	P. decipiens, s	53	5-28	2	3	48	x	x			
	P. decipiens, s	200	34	0	4	24	x	x	x		
	P. decipiens, s	400	34	0	4	24	x	x	x		
	P. decipiens, s	200	34	8	4	24	x	x	x		
	P. decipiens, s	400	34	8	4	24	x	x	x		
	P. decipiens, s	650	34	2	45 hours	-	x	x	x		
	P. decipiens, s	600	34	0	3	48	x	x			
	P. decipiens, s	600	34	5	3	48	x	x			
	P. decipiens, s	600	34	8	3	48	x	x			
	P. decipiens, s	300	34	5	3	48	x	x			
	Iridea cordata, s	300	34	5	3	48	x	x			
field exp.	*P. decipiens*, s	ambient	34	2-5	23	-	x	x	x	x	x
	P. decipiens, s	ambient	34	5-10	23	-	x	x	x	x	x
	P. decipiens, i	ambient	34	2-5	23	-	x	x	x	x	x
	P. decipiens, i	ambient	34	5-10	23	-	x	x	x	x	x

3.3.1 Laboratory experiments

For all laboratory experiments conducted during the two campaigns, collected algal material was cleaned, cut into discs of approximately 50 mm in diameter taken from the middle part of the fronds and haphazardously distributed among the experimental units. The experimental units were either glass or plastic beakers of 750 mL to 1 L in volume. Three of these beakers were placed into temperature-controlled water tanks (39 L, Bürkle GmbH, Germany) providing the respective temperatures used for the experiments. Temperature was maintained via cryostats (model 1160S, VWR International GmbH, Germany) and controlled by temperature loggers (Testo 175-T1, Testo AG, Lenzkirch, Germany) as well as additionally checked with a handheld digital thermometer. Two of these tanks were established for each temperature treatment (see Figure 3.4).

Light treatments were applied by six halogen spot lights (EXN-P, 50W, SP581, Conrad, Germany) mounted on an aluminum frame (500 x 300 mm). Individual frames were arranged above each water tank. Light intensity was adjusted to different intensities of PAR by varying the distance to the beakers or by using black net gauze to provide shade or dark conditions. PAR ranged from dim light conditions of 53 μmol photons m^{-2} s^{-1} to a maximum of 650 μmol photons m^{-2} s^{-1}.

Different salinities were provided by diluting the ambient filtered bay water with freshwater until the respective salinity was reached. Salinities varied from 34 PSU (ambient salinity of Potter Cove, Schloss et al., 2002) to nearly freshwater with a salinity of 5 PSU. Salinities were adjusted and checked with a refractometer (SE-10, Atago, Tokyo, Japan).

Nearly each experiment was carried out with specimens from two different shore levels, namely the intertidal region of Peñon Uno and depths of 8 to 12 m in front of the Fourcade glacier facing the station (see Figure 3.1). Exposure and recovery time differed

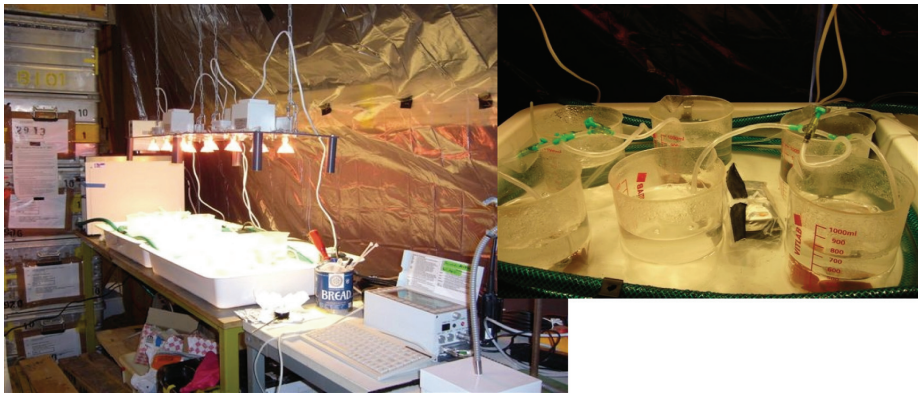

Figure 3.4: Set-up of the laboratory experiments.

within experiments with respect to the specific conditions applied as well as alga sensitivity regarding different habitats (see Table 3.1).

3.3.2 Field experiments

Field experiments were carried out in two mesocosms (100 x 200 x 35 cm plastic tanks) placed on the shore line of Potter Cove within the area of the station (see Figure 3.5). One of these tanks was temperature controlled by a cryostat to provide temperatures between 5 to 10°C whereas the second one maintained ambient water temperatures between 2 to 5°C. Temperature was controlled by underwater temperature loggers (Testo 175-T1, Testo AG, Lenzkirch, Germany) and salinity was checked with a refractometer (SE-10, Atago, Tokyo, Japan). Within these tanks three water-permeable plastic cages were installed for specimens from each shore level serving as the experimental unit. A submersed seawater pump provided a constant flux of bay water inside the tanks. Occasionally, the pump had to be taken out in cases of extreme low tide or ice scouring. Algal material was exposed in triplicates to ambient radiation conditions during the exposure. Experiments were carried out as mentioned above. One may note that in long-term experiments, subtidal specimens were covered with black net gauze to ensure survival.

Radiation measurements were conducted in air with a LiCor 1400 Data Logger equipped with a flat-head cosine corrected PAR quantum sensor (LICOR 190 SA, Li-Cor, Lincoln, NE, USA) at least four times daily and always at noon (see Table 1 in publication II). Additionally, underwater radiation spectra were recorded at the sampling site within Potter Cove using a broadband spectroradiometer (RAMSES, UV-VIS, TriOS, Oldenburg, Germany). In depths between 0 and 12 m, measurements of PAR, ultraviolet radiation-A (UVA, 320-400 nm) and ultraviolet radiation-B (UVB, 280-320 nm; Zacher et al., 2009) radiation were performed as well as spectra in each respective depth. This underwater light data was used to calculate where artificial intensities used in the experiments would occur in nature and also to determine the 1% depth using the formula of Kirk (1994). Salinity was checked daily with a handheld refractometer, although a little variation had to be accepted, as freshwater input could not be avoided.

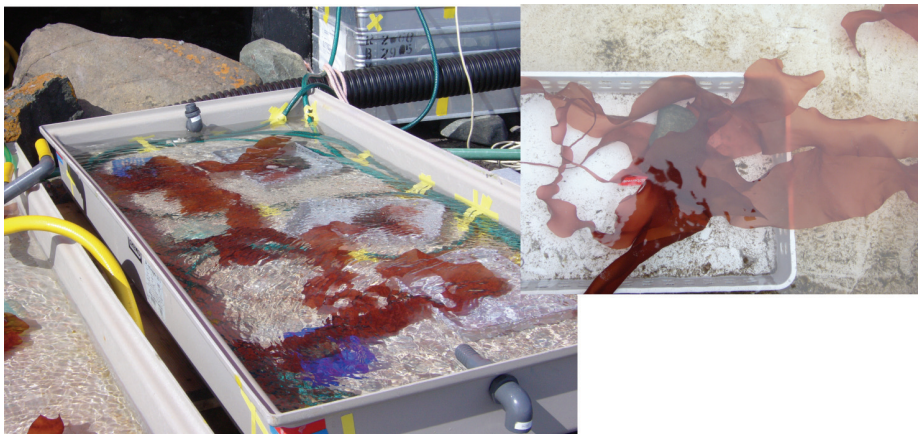

Figure 3.5: Set-up of the mesocosms for the field exposure of *Palmaria decipiens* on the shore line of Potter Cove.

For sampling, algal pieces of approximately 50 mm in diameter were haphazardously cut out of the exposed squares and transferred in darkness to the laboratory. After PAM-measurements, sample material was immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

3.4 Investigated Parameters

The variety of investigated parameters within this study will be described briefly in the following paragraphs. A detailed description may be found in the respective publications and cited references (see attached publications).

3.4.1 Pulse amplitude modulated Chlorophyll Fluorometry - PAM

Determination of photosynthetic performance, measured as fluorescence of photosystem II (PS II) by using a PAM 2100 chlorophyll fluorometer (Walz, Effeltrich, Germany) was performed *in vivo* in exposed algal samples. During the experiments, photosynthetic parameters such as maximum quantum yield (Fv/Fm) and photosynthesis versus irradiance curves (PI-curves) were recorded in order to estimate the physiological state of the alga. The internal LED was used as light source, emitting intensities ranging from 21.3 to 630 μmol photons m^{-2} s^{-1}. As previously described by Schreiber et al. (1994) and Hanelt et al. (1997), samples were dark-adapted for five minutes prior to the measurement. By measuring the mentioned parameters, information regarding photosynthetic efficiency (Fv/Fm), photosynthetic capacity and light saturation points are obtained. While maximum quantum yield is immediately provided by the PAM device, the latter factors have to be calculated after recording PI-curves by multiplying yield with PAR-intensity (Büchel and Wilhelm, 1993, Schreiber et al., 1994). Photochemical capacity, given as the maximal relative electron transport rate (rETRmax), is the result after curve fitting by either Eilers and Peeters (1985) or Jassby and Platt (1976), depending whether light saturation was reached.

PAM-fluorometry is a widespread method in botany and phycology (as reviewed by Baker, 2008), through which one may obtain fast indications of the photosynthetic per-

formance of the studied organism. This is especially neat and useful in field experiments. Nevertheless, it should be taken into account that this method only provides relative information about the complex physiology of the underlying photochemistry.

3.4.2 Photosynthetic pigments

Extraction of photosynthetic pigments was conducted using the following protocols: for determination of chlorophyll *a* content frozen samples were transferred to 5 mL of N,N-dimethylformamide and kept in darkness at 4°C for four days. The supernatant was measured spectrophotometrically (UV-2401 PC, Shimadzu, Tokyo, Japan) at wavelengths of 664 nm (Inskeep and Bloom, 1985, modified by Lüder et al., 2001). Determination of the phycobiliproteins phycoerythrin and phycocyanin was achieved after grinding frozen sample material for two minutes at 1500 rpm with a dismembrator (Mikrodismembrator U, B. Braun, Biotech International, Germany). Powdered samples were transferred into 5 mL of 100 mM phosphate buffer and centrifuged for 20 min at 5000x*g*. The supernatant was measured spectrophotometrically (455 nm, 564 nm, 592 nm, 618 nm and 645 nm) as described by Beer and Eshel (1985).

3.4.3 Protein analyses

Changes in the concentration of D1-protein were determined by SDS-PAGE after Laemmli (1970) using ready gels with 8 -16% HCl (50 μL, BioRad Laboratories, Canada). Subsequent Western Blotting was performed according to Bischof et al. (2000), using a D1 specific primary antibody (AS 01016 Chicken Anti PsbA, Agrisera, Vännäs, Sweden) and ab6754, Abcam, for secondary immunodecoration (Cambridge, United Kingdom).

3.4.4 Lipid content and fatty acid composition

Lyophilized sample material was homogenized and according to the protocol of Folch et al. (1957), dichloromethane:methanol (2:1, v/v) was used for extraction. Prior to the extraction, an internal standard was added (23:0 fatty acid methylesters, FAMEs) and samples were crushed by ultrasonification for ten minutes. To allow gas liquid chromatographic determination of the fatty acids, methyl esters were prepared by transesterification with 3% concentrated sulphuric acid in methanol for 4 h at 80°C. After extraction with hexane, FAMEs were analyzed with a gas-liquid chromatograph (HP 6890, Hewlett-Packard GmbH, Waldbronn, Germany) on a capillary column (30 m x 0.25 mm I.D.; film thickness: 0.25 μm; liquid phase: DB-FFAP, J&W, Cologne, Germany) using temperature programming after Kattner and Fricke (1986). Known standard mixtures were used to identify FAMEs, which was occasionally confirmed additionally by GC-MS measurements. The total lipid content refers to the sum of total FAMEs.

3.5 Statistical data treatment

Laboratory as well as mesocosms experiments in the field were set up as split-plot designs. This special type of design is used in experiments testing two or more variables in order to reduce replicates and work amount to an appropriate level (Sokal and Rohlf, 1995). Therefore, replicates are placed into the respective treatment in a specific pattern and thus, may be treated as experimental units.

All experiments were conducted in triplicates, from which mean values and standard deviation were calculated. In cases of percentages and ratios, standard error was used. For statistical analyses, percentages and ratios were arcsin transformed to achieve normality and homogeneity of variances. Normality was tested using Shapiro Wilk's test, homogeneity of variances was tested by Bartlett's test. After transformation, data were subjected to a full factorial two-way ANOVA (mean vs. light and temperature and light x temperature or depth and temperature and depth x temperature, respectively) and a subsequent posthoc test on differences between means was conducted (Tukey-Kramer HSD, $p \leq 0.1$ or $p \leq 0.05$, Sokal and Rohlf, 1995). Data analysis was performed using the JMP 5.1 software (JMP Inc. USA).

4 Summary of results

The following paragraphs provide a summary of the results obtained during this study with some exemplary figures. In cases of already published data, results will be briefly described and further information can be found in the attached publications (I, II, III).

4.1 Photosynthetic performance under changing light and temperature levels

Various experiments regarding the physiological performance of *Palmaria decipiens* under changing radiation and temperature conditions have been performed in laboratory and mesocosm experiments in the field (see Table 3.1). The photosynthetic parameters of specimens exposed to 0 or 8°C, respectively, are shown in Figure 1 in publication I as an example of an experiment performed under controlled laboratory conditions. It was shown that photosynthetic efficiency (Fv/Fm) differed significantly on day 3 of exposure at 0°C between the two light treatments consisting of 200 μmol photons m^{-2} s^{-1} and 400 μmol photons m^{-2} s^{-1}, indicating that in particular, the combination of high light intensities and low temperature affects the photosynthetic performance of *P. decipiens*. A similar decrease under high light conditions and 8°C was observed in rETRmax values (see Figure 2 in publication I), where values decreased to 92% of the initial value and a significant time x temperature interaction was observed.

A short-term experiment for the duration of 45 h under high light conditions (650 μmol photons m^{-2} s^{-1}) and 2°C led to decreasing photosynthetic parameters (see Figure 4.1). Initial Fv/Fm values decreased from 0.52 relative units (r.u.) to significantly lower 0.32 r.u. (Tukey-Kramer posthoc test with $p \leq 0.05$) after 45 h. The same pattern was found for relative maximum electron transport rate (rETRmax), where values decreased to 53% of the initial value by the end of the exposure. The strongest decline of rETRmax values was observed after 17 h.

Exposure to high radiation intensities of 600 μmol photons m^{-2} s^{-1} for three days at three different temperatures (0°C, 5°C and 8°C) with a 40 h recovery period in dim light conditions, revealed decreasing photosynthetic efficiency (Fv/Fm). Specimens exposed to the highest temperature were the most affected after the recovery (see figure 4.2). Here, values decreased to about 40% of the initial value, whereas specimens exposed to 5°C decreased to 55%, and those at 1°C decreased to just 60%. During the exposure, few significant differences were found within each temperature treatment: by comparison significant differences to the initial percentage were found at all three temperatures on day 3 of exposure as well as after the recovery period. At 5°C, a significant difference was already detected on day 2 of exposure (see Figure 4.2).

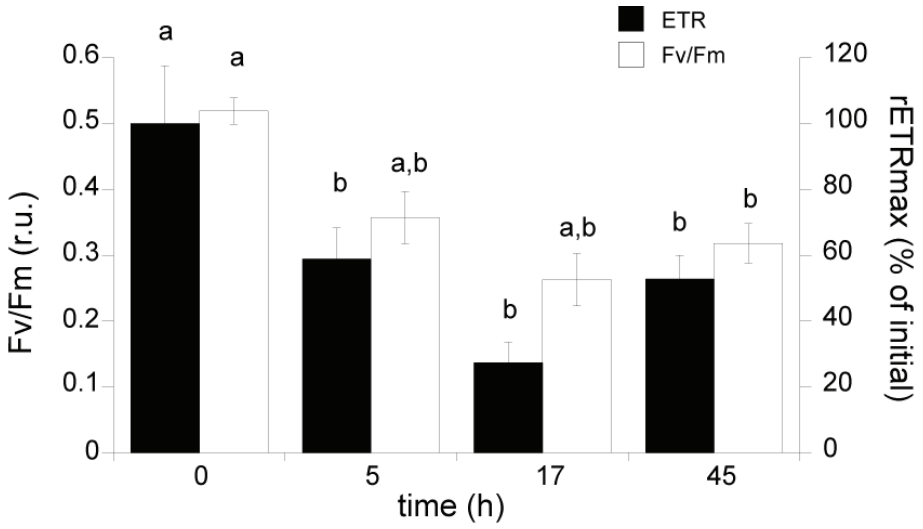

Figure 4.1: Photosynthetic parameters of *Palmaria decipiens* exposed to high radiation conditions of 650 μmol photons m^{-2} s^{-1} and 2°C for 45 h. White bars represent Fv/Fm values (left axis) as relative units (r.u.), black bars represent relative ETRmax values (right axis) as percent of the initial value. Bars represent means of triplicates with standard error bars. Different letters mark significant differences over time (Tukey-Kramer posthoc test with $p \leq 0.05$.)

Differences between temperature treatments occurred on day 2 of exposure, and the most apparent impact on Fv/Fm values occurred in specimens exposed to the intermediate 5°C treatment. In that case, Fv/Fm values differed significantly from those exposed to 0°C. After the 40 h recovery period in dim light conditions, the photosynthetic efficiency of specimens exposed at 8°C was significantly lower in comparison with values measured in specimens exposed at 0°C (see asterisks in Figure 4.2).

Regarding the relative maximum electron transport rate (rETRmax), differences in rETRmax values during the exposure were only observed at 8°C, where values differed significantly after the recovery period (see Figure 4.3). Overall, values decreased during the exposure until day 3 to about 73% at 0°C and 63% at 8°C, respectively. Algal specimens exposed to 5°C were an exception, as rETRmax values increased to about 140%.

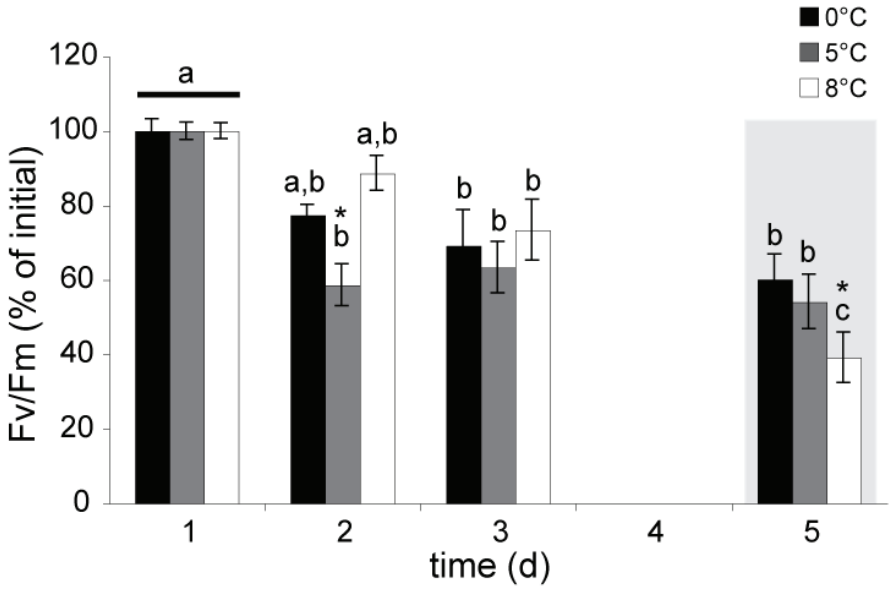

Figure 4.2: Photosynthetic efficiency (Fv/Fm) of *Palmaria decipiens* specimens exposed to 600 μmol photons m^{-2} s^{-1} PAR at three different temperatures (0°C, black bars, 5°C, grey bars, 8°C, white bars). Bars represent means of triplicates and are expressed as the percentage of the initial Fv/Fm with standard error bars. Grey shading marks values measured after 40 h of recovery in dim light conditions. Asterisks mark significant differences on the respective day in comparison to the 0°C-treatment, different letters mark significant differences within a temperature treatment over time (Tukey-Kramer posthoc test with $p \leq 0.05$).

In the mesocosm field experiments, photosynthetic performance was recorded for specimens from two shore levels (intertidal and subtidal) exposed for 23 days to two different temperatures (2-5°C and 5-10°C) and elevated light levels compared to their original habitat was rather variable. During the first half of the experiments, Fv/Fm values of the higher temperature treatment were slightly higher, while this pattern became unclear with ongoing exposure. On average, photosynthetic efficiency was slightly higher in subtidal specimens (see publication II).

4.2 Effects of abiotic factors on the physiology of Palmaria decipiens

As photosynthetic performance under varying abiotic scenarios may provide an indication of the physiological condition of the alga, additional changes in photosynthetic pigments and in D1-protein concentration were investigated (publication I and II). New insights into the importance of the ability of the alga to adjust fatty acid composition were obtained by analysing changes in total lipid content and fatty acid composition (publication II).

4.2.1 Photosynthetic pigments

Short-term exposure (45 h) to high irradiances (650 μmol photons m^{-2} s^{-1} led to decreasing concentrations of phycobiliproteins in *Palmaria decipiens*(see figure 4.4). Initial

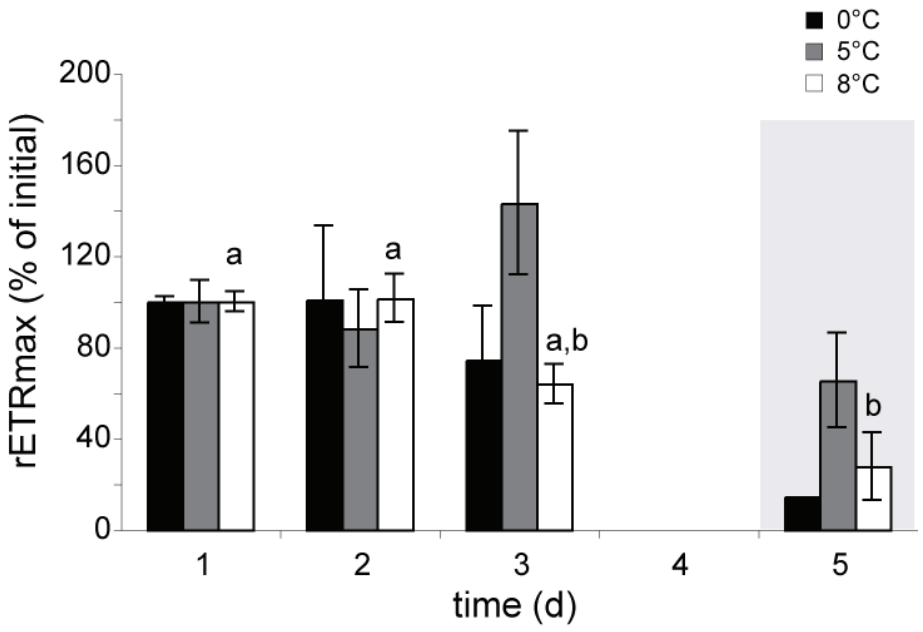

Figure 4.3: Relative maximum electron transport rate (rETRmax) of *Palmaria decipiens* specimens exposed to 600 μmol photons m^{-2} s^{-1} PAR at three different temperatures (0°C, black bars, 5°C, grey bars, 8°C, white bars). Bars represent means of triplicates and presented as the percentage of the initial rETRmax with standard error bars. Grey shading marks values measured after 40 h of recovery in dim light conditions. Different letters mark significant differences within a temperature treatment over time (Tukey-Kramer posthoc test with $p \leq 0.05$).

concentrations of phycoerythrin decreased from 0.6 mg mL^{-1} g FW^{-1} to significantly lower value of 0.33 mg mL^{-1} g FW^{-1}($p \leq 0.05$). On a smaller scale, the same decrease was observed for phycocyanin, where initial concentrations of 0.033 mg mL^{-1} g FW^{-1} dropped significantly to 0.023 mg mL^{-1} g FW^{-1}.

Exposure of *P. decipiens* specimens to controlled, artificial conditions (0°C and 8°C in combination with either 200 μmol photons m^{-2} s^{-1} and 400 μmol photons m^{-2} s^{-1}) resulted in only slight differences in pigment content. Total phycobiliprotein content decreased in all experimental treatments, however, the decline was greater at 0°C (see publication I, table 1). Within total chlorophyll *a* concentration, a mixed pattern occurred: at 8°C, concentrations declined to 68% of the initial concentration during the exposure, whereas an increase to 128% was observed under high radiation conditions. The opposite pattern was found at the more ambient 0°C treatment, where the decline to 88% occurred at 400 μmol photons m^{-2} s^{-1} and an increase to 122% was detected under the lower light intensity. Overall, the ratio of phycobiliproteins to chlorophyll *a* decreased during the exposure, and was slighty more pronounced in specimens exposed to 200 μmol photons m^{-2} s^{-1} at both temperatures.

In field experiments, the concentration of chlorophyll *a* and phycobiliproteins was investigated in specimens exposed to the combined factors of light and temperature. Generally, it was found that under increased light intensities and temperatures, the total content of chlorophyll *a* and phycobiliproteins decreased. Differences were also observed in initial

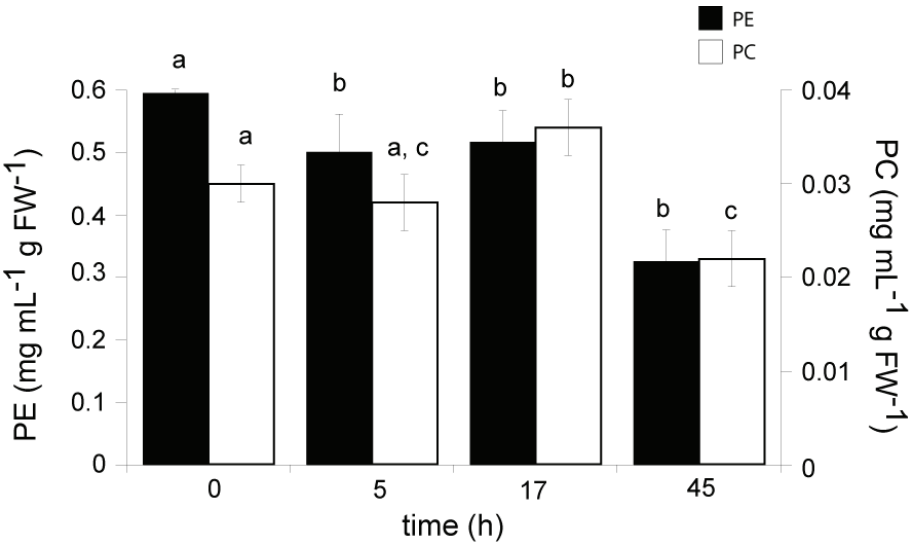

Figure 4.4: Concentration of phycobiliproteins (phycoerythrin, PE and phycocyanin, PC) of *Palmaria decipiens* exposed to 650 μmol photons m^{-2} s^{-1} for 45 h given as mg mL^{-1} g FW^{-1}. Black bars and left axis represent concentrations of PE, white bars and right axis represent concentrations of PC. Bars represent means of three replicates with standard deviation bars. Different letters mark significant differences between sampling time for each pigment in comparison to the inital concentration (Tukey-Kramer posthoc test, $p \leq 0.05$).

values of specimens from different shore levels, where subtidal specimens obtained a higher concentration of antennae pigments like phycobiliproteins compared to specimens from the intertidal. The decline of the phycobiliprotein to chlorophyll *a* ratio was more pronounced in subtidal specimens, where values decreased by 85% in the colder treatment and even still by 79% in the warmer treatment. For intertidal specimens, a decrease by 30% was observed at 2-5°C and by 75% at 5-10°C. However, these differences were not significant ($p \geq 0.1$). For details, see text and Figure 3 in publication II.

4.2.2 D1-protein

The concentration of the D1-protein was measured in algal material exposed in the mesocosm experiments for the duration of 23 days (publication II). *P. decipiens* specimens were exposed to the natural solar radiation at two different temperature treatments. No significant differences were found in specimens sampled in the intertidal in both temperature treatments, as well as in specimens collected in 8 to 10 m depth exposed at 5-10°C (see figure 4.5). A significant decline was detected in specimens originating from the subtidal exposed to 2-5°C, where values decreased to 68% of the initial concentration (see also publication II for details).

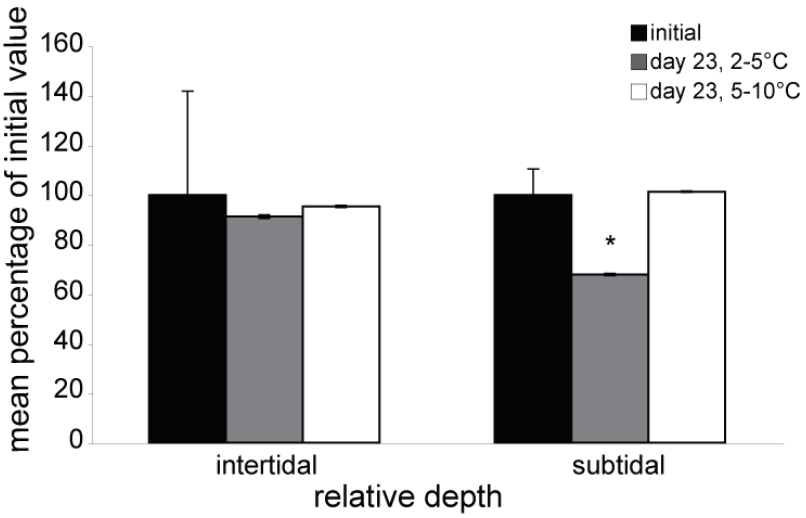

Figure 4.5: Changes in D1-protein concentration of *Palmaria decipiens* specimens from two different shore levels (intertidal and subtidal) exposed to two temperatures (2-5°C and 5-10°C) in mesocosm experiments over 23 days. Concentration values are given as a percentage of the initial concentration. Black bars represent the initial concentration, grey bars represent the concentration after 23 days at 2-5°C and white bars represent concentrations after 23 days at 5-10°C. Bars represent means of three replicates with standard error bars. Asterisks mark significant differences within a depth in comparison to initial values (Tukey-Kramer posthoc test, $p \leq 0.05$).

4.2.3 Lipid content and fatty acid composition

Analysis of lipid content and fatty acid (FA) composition was performed on algal material exposed to natural solar radiation and two temperature treatments, 2-5°C and 5-10°C on *Palmaria decipiens* specimens from two different shore levels (intertidal and subtidal, i.e. 8 to 10 m depths). Initial ratios of saturated to unsaturated FA differed significantly between specimens from the two shore levels: subtidal specimens had a higher concentration of saturated FA in comparison to specimens from the intertidal with a higher content of unsaturated FA (see Figure 4.6). Exposure to the mentioned conditions for 23 days led to an increase in saturated FA in *Palmaria* specimens from the intertidal, whereas the ratio remained the same in specimens from the subtidal. Due to sample limitation in the latter case, no data is available for the warmer treatment and for specimens from the subtidal. Information about the velocity of FA adjustment to changing environmental factors is given in Figure 4.6b. Specimens from the intertidal adjusted their FA content with increasing saturation degree after exposure for 11 days. Significant differences are indicated by different letters, for details see publication II.

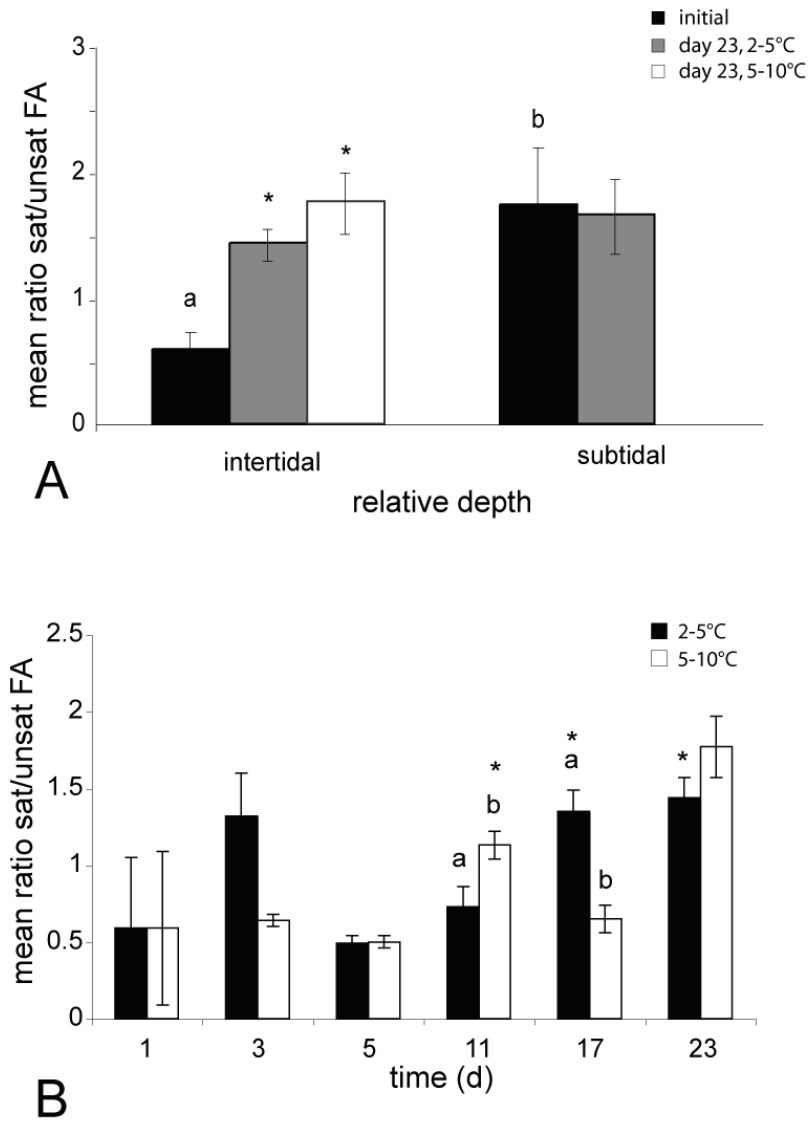

Figure 4.6: Fatty acid (FA) composition of *Palmaria decipiens* from two shore levels, exposed to natural solar radiation and two different temperatures (2-5°C and 5-10°C) for 23 days. **A:** Colour code for bars as mentioned in 4.5. Due to sample limitation, no data is available for specimens from the subtidal exposed at 5-10°C. Bars represent means of triplicates with standard error bars. Different letters mark significant differences between depths, asterisks mark significant differences between temperature treatments within a depth. **B:** FA content of *P. decipiens* from the intertidal. Black bars indicate ratios of specimens exposed at 2-5°C, white bars indicate ratios of specimens exposed at 5-10°C. Bars represent means of triplicates with standard error bars. Different letters mark significant differences between temperature treatments, asterisks mark significant differences within a temperature treatment in comparison to the initial ratio. Statistical difference after Tukey-Kramer posthoc test, $p \leq 0.05$.

4.3 Comparison of photosynthetic responses between two species

Exposure of specimens of the two species *Iridaea cordata* and *Palmaria decipiens* at 5°C and a radiation of 300 μmol photons m^{-2} s^{-1} for three days and a recovery period of 48 h led to decreasing Fv/Fm values in both species during the exposure and a slight increase after the recovery period (see figure 4.7). Initial Fv/Fm values of *P. decipiens* of 0.55 relative units (r.u.) decreased to 0.21 on day 3 and recovered only slightly. *I. cordata* specimens showed higher initial values (0.68 r.u.), which declined to 0.41 r.u. on day 3 and a Fv/Fm value of 0.48 r.u. was measured after the recovery period. Statistically significant differences between the two species were found on all days of the experiment (initial: p=0.0007, day 2: p=0.0089, day 3: p=0.0041 and after the recovery: p=0.0128, Tukey-Kramer posthoc test, asterisks in Figure 4.7). Within the decreasing Fv/Fm values of *P. decipiens*, a significant difference over time was found with p≤ 0.0001 (Figure 4.7, small letters), for *I. cordata* significant differences were found with p=0.0008 (Tukey-Kramer posthoc test p≤ 0.05).

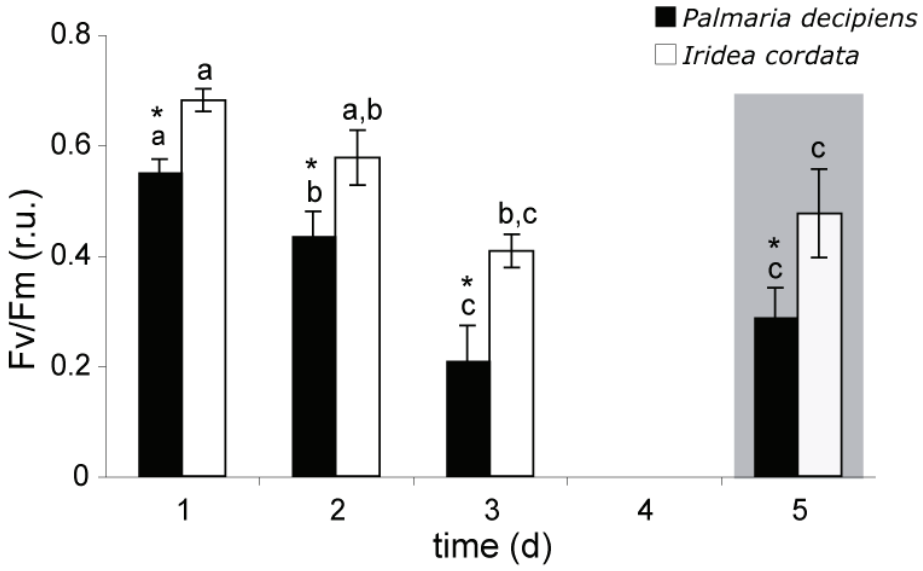

Figure 4.7: Fv/Fm values of *Palmaria decipiens* (black bars) and *Iridaea cordata* (white bars) exposed to 300 μmol photons m^{-2} s^{-1} at 5°C. Grey shading represents values measured after 48 h of recovery in dim light conditions. Bars represent means of triplicates with standard error bars. Different letters mark significant differences within a species, asterisks mark significant differences between species on the respective day (Tukey-Kramer posthoc test, p≤ 0.05).

Regarding the relative maximum electron transport rate (rETRmax), the two species showed a different pattern (see Figure 4.8): initial values of *P. decipiens* decreased from 32.6 r.u. to a minimum of 11.3 r.u. on day 2 and increased slightly afterwards and reached almost the initial level with a rETRmax of 27.3 r.u. after the recovery period. However, no significant differences over time were detected (p=0.3278). *I. cordata* had initial rETRmax values of 56.4 r.u., which further increased to a maximum of 68.2 r.u. on day 2. After decreasing to 42.2 r.u. by the end of the exposure, no recovery was detected on day 5 (11.9 r.u.), which is in contrast to the results of *P.decipiens*. Statistically sig-

nificant differences for rETRmax values of *I. cordata* were found with p=0.0101 between initial values, values measured on day 2 and after the recovery period (Tukey-Kramer posthoc test, p≤ 0.05). Comparing the two species, initial values (p=0.0447), values on day 2 (p=0.0475) and values at the end of the recovery period (p=0.0468) differed significantly, whereas no significant differences were found on day 3 of exposure (p=0.0765).

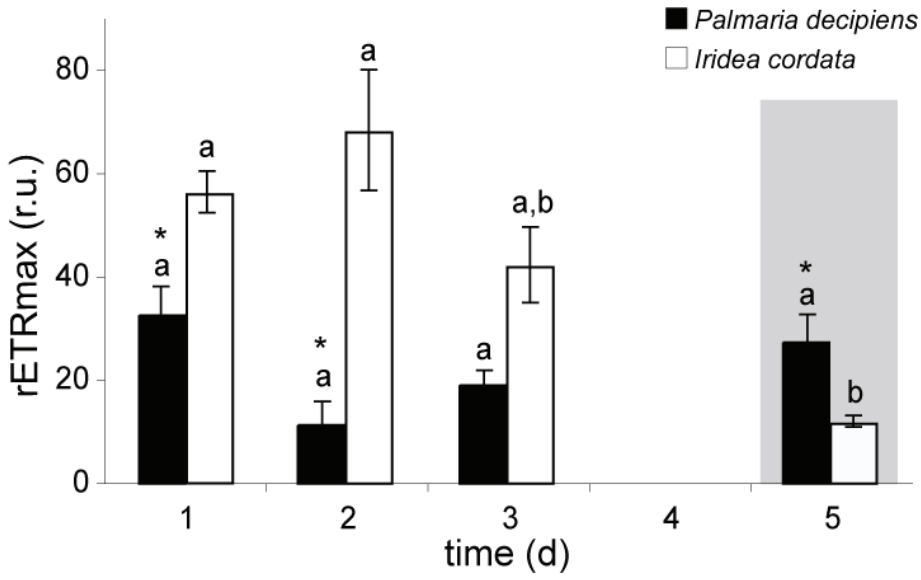

Figure 4.8: Relative maximum electron transport rate (rETRmax) of *Palmaria decipiens* (black bars) and *Iridaea cordata* (white bars) exposed to 300 μmol photons m^{-2} s^{-1} at 5°C. Bars represent means of triplicates with standard error bars. Grey shading represents values measured after 48 h of recovery in dim light conditions. Different letters mark significant differences within a species, asterisks mark significant differences between species on the respective day (Tukey-Kramer posthoc test, p≤ 0.05).

4.4 Photosynthetic responses to salinity changes

The photosynthetic performance of *Palmaria decipiens* from two different shore levels, the intertidal and subtidal (depths of 8 to 10 m), was measured at six artificially obtained salinities (5, 10, 15, 20, 25, 28 PSU). Specimens from both shore levels were highly tolerant towards freshwater input, as values of the maximum quantum yield (Fv/Fm) remained at the same level at each salinity except 5 PSU (see Figure 4.9, 4.10 and publication III). In the relative maximum electron transport rate (rETRmax), the same trend was observed. Algae exposed to 5 PSU from both shore levels were strongly bleached and had a softened structure, hardly any measurements were possible. Thus, no data could be obtained for subtidal specimens exposed at 5 PSU after day 2. Significant differences in Fv/Fm values of intertidal specimens were found between salinities of 5 PSU and 28 PSU on day 2 (p=0.0075), between 5, 15, 20 and 25 PSU on day 4 (p≤ 0.0001) as well as on day 5 (p=0.0039). Time effects were found for 5 and 10 PSU (see stars in Figure 4.9 with p=0.0058 and p=0.0599, respectively), in rETRmax values no significant differences were observed (p≥ 0.05). The influence of freshwater input on rETRmax values was significant

on day 2 between 15 and 25 PSU (p=0.0017) and on day 5 of exposure between 5 and 25 PSU (p=0.0299).

In subtidal specimens, Fv/Fm values differed less in different salinity treatments, except for a salinity of 5 PSU (see Figure 4.10A). In the latter case, values decreased to a value of 0.02 r.u. on day 4 of exposure and slightly increased on day 5 to a value of 0.15 r.u. These low values differed significantly to all other values measured in different salinity treatments with $p \leq 0.0001$ on day 4 and p=0.0140 on day 5, respectively. A significant effect of time was found in specimens exposed to 5 PSU on day 5 (p=0.0151), which were significantly lower than the initial value. No differences in rETRmax values were found between salinity treatments for subtidal specimens ($p \geq 0.05$), except for the 5 PSU treatment, where values were significantly lower on day 2 of exposure (p=0.0257) and not measurable afterwards (see Figure 4.10B).

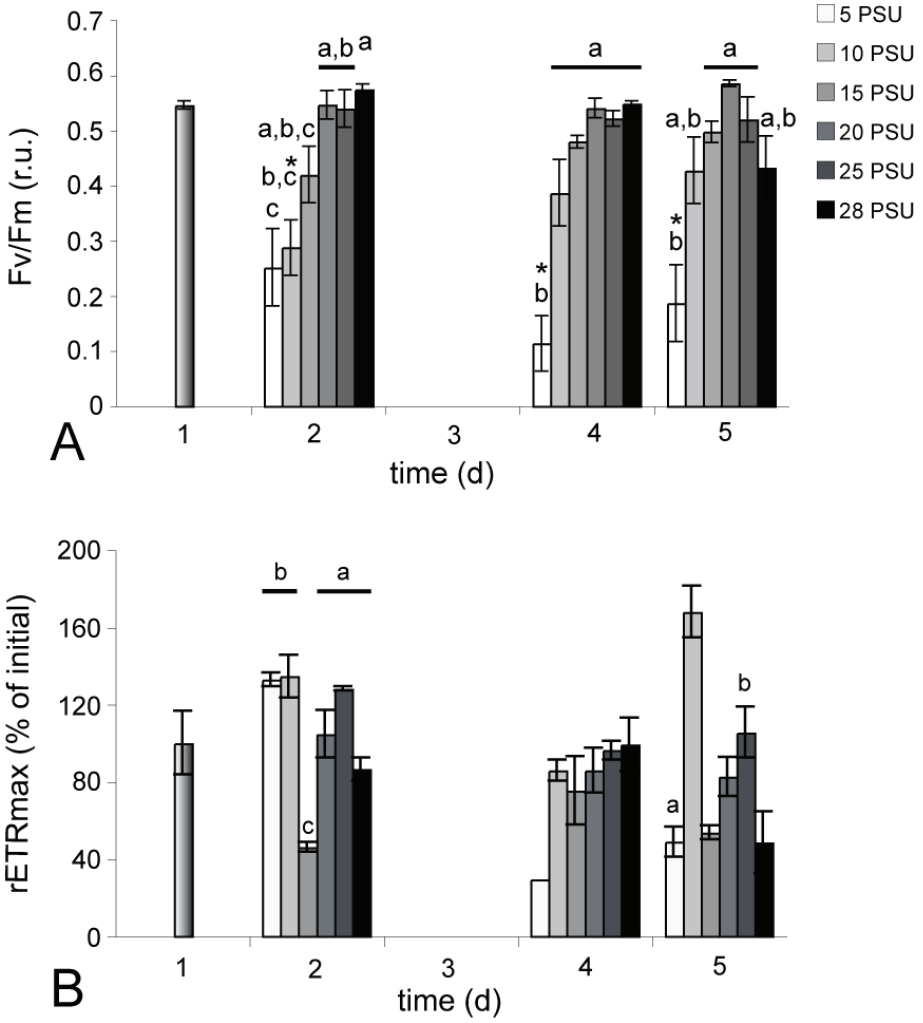

Figure 4.9: A: Photosynthetic efficiency (Fv/Fm) of intertidal specimens of *Palmaria decipiens* exposed to six different salinity treatments (5, 10, 15, 20, 25 and 28 PSU) for five days at 53 μmol photons m^{-2} s^{-1}. Shaded bar on day 1 represents initial values for all salinity treatments. Bars represent means of triplicates with standard error bars. Asterisks mark significant differences within a salinity treatment over exposure time, different letters mark significant differences between salinity treatments on the respective day (Tukey-Kramer HSD posthoc test with $p \leq 0.05$). B: Relative maximum electron transport rate (rETRmax) as percent of initial values. Details as described in A.

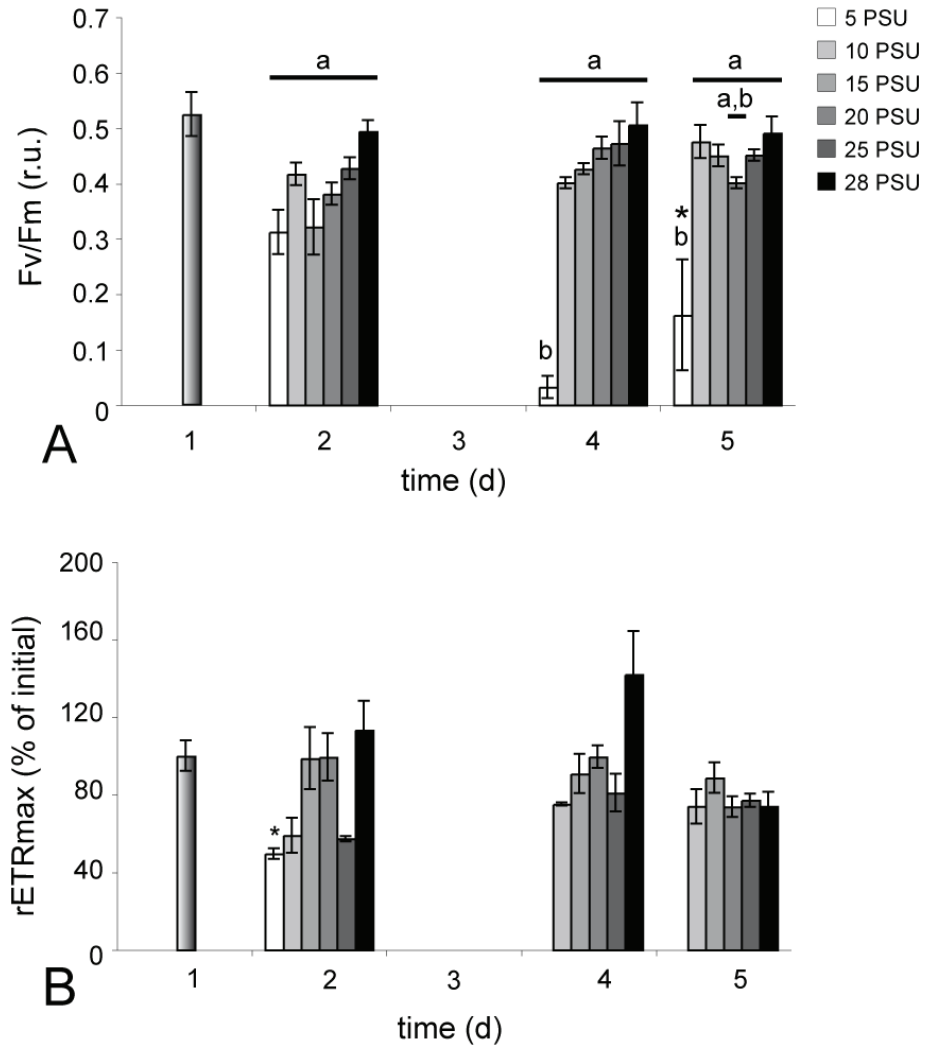

Figure 4.10: A: Photosynthetic efficiency (Fv/Fm) of subtidal specimens of *Palmaria decipiens* B: Relative maximum electron transport rate (rETRmax) as percent of initial values. Details as described in 4.9.

5 Synoptic discussion

5.1 The Antarctic environment - characteristics and adaptations

Both Polar Regions represent extreme abiotic environments, characterized mainly by low temperatures and a strong seasonality in the light regime. The most apparent difference between the Arctic and the Antarctic is, that Antarctica actually represents a ice-covered continent surrounded by the Southern Ocean, whereas the Arctic is a widely pack-ice covered sea surrounded by the northern regions of North America and Eurasia (as reviewed by Zacher et al., 2009). Consequently, different abiotic characteristics have developed, which have also generated distinct adaptational features in their respective fauna and flora. This thesis emphasizes on physiological adaptations of marine macroalgae to the Antarctic environment.

The special oceanography and a long history of cold-water masses led to the evolution of a high degree of endemism in Antarctica. Although the abiotic conditions are rather harsh in Antarctica, macroalgae may reach high densities (several thousand tons per year, as pointed out previously by Nezarek and Rakusa-Suszczewski, 2004) and especially phaeophytes form intense stocks (e.g. *Himanthothallus grandifolius*, *Desmarestia menziesii*, Wiencke and Clayton, 2002). Usually, these stocks develop in depths below the influence of ice-scouring (Wiencke and Clayton 2002, Zacher et al., 2009). Generally, Antarctic waters are not nutrient-limited in terms of macroalgal growth, and concentrations of nitrogen are consistently high throughout the year (Nedzarek and Rakusa-Suszczewski, 2004, Zacher et al., 2009). This is reflected in high tissue N contents and low C:N ratios in macroalgae (Weykam et al., 1996, Peters et al., 2005).

As macroalgae are photosynthetic organisms, they obviously depend on light availability to drive photosynthesis and thus, are able to grow and fulfill their respective life cycle. Light availability varies extremely towards the poles, which leads to six months of total darkness in latitudes higher than 80°. As a matter of fact, the southernmost distribution of macroalgae is reported for Cape Evans, Ross Sea, in the East Antarctic at 77°S, coinciding with the southernmost shoreline of the Antarctic continent (Schwarz et al., 2003). However, the northernmost tip of the Western Antarctic Peninsula is located at 62°S and therefore light is available around the year. Perennial and pseudo-perennial species may endure and survive prolonged periods of darkness by using up storage compounds such as floridean starch and by reducing their pigment content, such as phycobilisomes, which may be rich in nitrogen (Weykam et al., 1996, Lüder et al., 2001, 2002). Sea-ice cover with an additional snow layer further reduces light intensity that may reach the bottom. The present study has been performed on King George Island (South Shetland Islands) on the western Antarctic Peninsula. Due to its geographic position at 62°S, a minimum day time of 5 h occurs during winter and reaches a maximum of 20 h during the austral summer (Wiencke, 1990, Richter et al., 2008). Immediately after sea-ice break up, the water column is very clear and highly transparent. During this relatively short period of two months, the 1% depth for PAR radiation can be found down to 29 m, as measured on Signy Island (South Orkney Islands, Antarctica, Brouwer, 1996). After onset of glacier melting, the water becomes

more turbid, leading to a decreasing 1% depth, so that even PAR intensities (in air) of 1592 μmol photons m^{-2} s^{-1} are quickly attenuated and the 1% depth is reached at about 14 m (calculated after Kirk, 1994). An overview of minimum and maximum PAR intensities measured during the campaign 2007/08 can be found in publication II, table 1.

Although salinity is regarded to be a rather conservative abiotic factor, onset of glacier melting may influence surface salinity. In this context, the onset of glacier melting refers to the seasonal phenomenon occurring each spring, whereas global warming aspects are discussed in section 5.4. To investigate water temperatures and salinity throughout the study, CTD-measurements were conducted (conductivity-temperature-depth, data kindly provided by O. Gonzalez, IAA/DNA, Argentina). Before the onset of glacier melting (begin of January 2008), water temperatures and salinity are very constant throughout the water column down to 20 m (see figure 5.1, left), with temperatures between +1.1 and +0.5°C with almost no variation from 5 m downwards. The salinity is even more stable at around 34 PSU, in accordance with the findings by Schloss et al. (2002). By mid-February, temperatures have risen and revealed a cooler surface layer, followed by some meters of warmer water of approximately +1.9°C (see figure 5.1, right). From 10 m and below, temperature stabilizes at +1.4°C. The influence of freshwater input due to increased glacier melting and river run-off is reflected by the salinity, which is around 32 PSU within the first three meters. Tide pools may be freshened to salinities down to 27 PSU in Potter Cove (Klöser and Arntz, 1994).

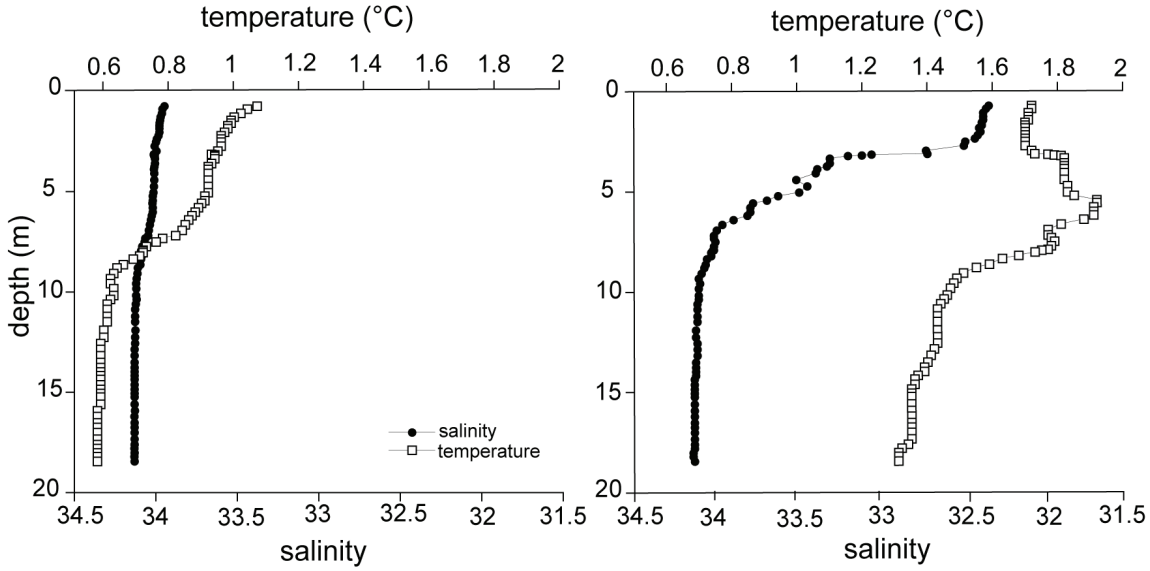

Figure 5.1: Salinity and water temperature measured via conductivity-temperature-depth (CTD) on January 5^{th}, 2008 (left) before onset of glacier melting, and on February, 14^{th}, 2008, after onset of glacier melting (right). Upper x-axis represents water temperature in °C (plain squares), lower x-axis represents salinity in PSU (filled black dots). Data kindly provided by O. Gonzalez, IAA/DNA, Argentina.

An important structuring factor in Antarctic coastal zones is the impact of sea ice and ice scouring on benthic communities. Ice is regarded as a key factor structuring algal depth zonation in Antarctica as a consequence of the mechanical damage caused by scrubbing and scouring (as reviewed by Gutt 2001, Gutt and Starmans, 2001, Zacher et al., 2009).

In winter, frozen sea ice (fast ice) may reach the bottom and thus, forming an ice foot that encloses biota (Barnes, 1999). However, some species, for example, *Iridaea cordata* are able to survive this enclosure, which was shown in McMurdo Sound, South Antarctica (Miller and Pearse, 1991).

In this context it becomes clear that species inhabiting this extreme environment of the Southern Ocean and its coastal zones require certain adaptations to cope with this particular habitat. This type of evolutionary adaptation occurred over generations by genetic changes and adjustments by the process of natural selection. Adaptation provides the physiological base and frame in which acclimation processes may take place. Acclimation involves more or less flexible, species-specific responses to changing environmental conditions, such as temperature or salinity changes or increased light intensities. This is also referred to as phenotypic plasticity. Usually, acclimation does not involve genetic changes and is thus, not heritable (Frankham and Kingsolver, 2004). Clearly, endemic species are strongly adapted to their particular environment and may therefore reveal a smaller acclimation potential than ubiquitous species.

In particular, Antarctic macroalgae have adapted to the special light regime at these high latitudes, resulting in very low light requirements for growth and to complete their life cycle (Wiencke, 1990, Weykam and Wiencke, 1996). Two general strategies are realized in Antarctic macroalgae to cope with the respective conditions of the light climate. One strategy is called a *season responder*, organisms that grow and reproduce during favourable conditions. In that sense, the alga responds for example to light availability. The second strategy is realized in the so-called *season anticipator*. Macroalgae following this strategy underlie a species-specific annual rhythm determining physiological processes. Growth, for example is not a response to environmental conditions, but is the response to a certain trigger, for instance day length (Kain, 1989).

In Antarctica, these concepts are realized in both species studied in this thesis. *Iridaea cordata* is regarded as a typical season responder, i.e. highest growth rates can be observed during the summer in favourable light conditions (Wiencke and Clayton, 2002). However, *Palmaria decipiens* requires just 47.1 mol photons m^{-2} y^{-1} to complete the life cycle and is regarded as very shade-adapted (Wiencke, 1990). Its strategy to start growth even in darkness and revealing highest growths rates in October is regarded as an adaptation to the light regime with its long winter period and very little light. Therefore, this species is considered as a season anticipator (*sensu* Kain, 1989, Wiencke, 1990, Wiencke and Clayton, 2002). As an additional feature in light adaptation, the content of antennae pigments is relatively high in Antarctic species compared to temperate macroalgae, hence, an optimal light harvesting is guaranteed. Interestingly, Lüder et al. (2001, 2002) have shown that *P. decipiens* drastically reduces the amount of phycobiliproteins and even loses its ability to perform photosynthesis during darkness. However, within 24 h of re-illumination, pigments are newly synthesized and photosynthetic performance recovers entirely. The authors suggested that by modulating the pigment content flexibly, the alga is able to respond quickly to the given irradiances in Antarctica. By reaching maximal pigment concentrations during spring, an optimal light utilization of favourable conditions is ensured and by decreasing the concentration during summer, photodamage by excessive light intensities is avoided (Lüder et al., 2001, 2002).

Another adaptation to the low temperature is displayed by the Antarctic macroalgae regarding optimum growth temperatures, which is due to their evolution in the long cold water history of the Southern Ocean. Upper temperature survival limits are usually rela-

tively low, although a limit of 13-17°C for *P. decipiens* is clearly above water temperatures encountered in Antarctica (Wiencke and tom Dieck, 1989, Wiencke et al., 1993, Eggert and Wiencke, 2000). Although the optimum temperature to drive photosynthesis is 15°C in *P. decipiens*, the carbon fixation rates at lower temperatures are comparable with temperate and/or tropical species at higher temperatures (Drew, 1977, Thomas and Wiencke, 1991). In general, the low temperature influences a variety of biochemical and metabolic components, such as reduced enzyme activity and synthetic pathways and decreased membrane fluidity, which is counterbalanced by enhancing the amount of unsaturated fatty acids (Graeve et al., 2002, Harwood and Guschina, 2009). The underlying mechanisms will be discussed in detail in section 5.3.

5.2 Photosynthesis in Antarctic rhodophytes and their acclimation potential to changing light and temperature levels

The particular environmental conditions in Antarctica with its low water temperatures and a strong light seasonality represent challenging conditions for photosynthesizing organisms. In the following paragraphs, first, the influence of the two factors light and temperature on photosynthetical performance of *Palmaria decipiens* will be outlined, and subsequently, the effects on physiological parameters are discussed.

5.2.1 Low temperatures and high light intensities

Low water temperatures in combination with high PAR intensities may severely stress macroalgae by impairing photosynthetical function, which may also affect pigment content, protein concentration or changes in lipid content and fatty acid composition. In this context, two different mechanisms may act synergistically: on the one hand, low water temperatures generally slow down enzymatic reactions and synthetic pathways as well as decreasing membrane fluidity (Gurr et al., 2002). Photosynthetic activity is especially sensitive to low temperatures due to the fact that enzymatic secondary reactions are strictly temperature-dependent, while primary reactions are not. Moreover, low temperature affects membrane fluidity and protein function. One of the most important components for photosynthetic function is the D1-protein, localized in the centre of the reaction centres of PS II (Gombos et al., 1994, Aro et al., 2005). This protein undergoes a continuous turnover, i.e. a constant exchange of degraded protein through *de novo* synthesized protein. D1 is characterized by a rapid turn-over rate of 30 ms. This turn-over includes fragmentation of degraded proteins, transfer through the thylakoid membrane, *de novo* synthesis of D1-protein, re-transfer through thylakoid membrane and finally, re-integration into the reaction centre (Aro et al., 1993, 2005). In case of exposure to low temperature or high irradiances as well as various other abiotic stress factors, this turn-over may be considerably reduced to 3 s (Aro et al., 1993). All processes involved in this turn-over are temperature sensitive.

Exposure to high PAR irradiances may result in photoinhibition, an either reversible dynamic response including down-regulation of photosynthesis, or a chronic response, which exhibits irreversible damage of the reaction centres of PS II (Demmig-Adams and Adams III, 1992, Hanelt et al., 1994). Most Antarctic macroalgae have been shown to be able to undergo dynamic photoinhibition in order to avoid photodamage by high light stress. In particular, organisms occurring in the intertidal such as *P. decipiens* or *Adenocystis*

utricularis, a phaeophyte, reveal a diurnal pattern, with down-regulated photosynthetic activities at noon (Hanelt et al., 1994). As soon as light irradiances reach non-harmful intensities, photosynthesis is up-regulated again.

Chronic photoinhibition, which involves severe damage of the photosystems themselves, is most likely due to an impaired D1 centre protein of PS II (Aro et al., 1993, 2005, Franklin et al., 2003). In that case, the damage rate of D1 is higher than its repair rate, and these underlying repair mechanisms may be impacted themselves (Franklin et al., 2003). Thus, photosynthetic organisms have to cope with balancing their energy supply through maintaining electron transport and compensate for respiratory losses in carbon fixation. That balance requires protection from detrimental effects of excessive irradiances while maintaining simultaneously certain amounts of ATP and NADPH for cellular metabolism function (Maxwell et al., 1994). Particularly under high irradiances, the primary target is the D1-protein within PS II (Aro et al., 1993, 2005, Mattoo et al., 1999, Bouchard et al., 2006). Here, excessive PAR intensities convert D1-proteins into an inactive form resulting in an inhibition of photochemical reactions of the PS II protein complex. As a consequence, this surplus of excitation energy is not regularly participating in the electron transport chain and electrons are transferred to oxygen instead of ferredoxine (Mehler reaction). Consequently, an increased production of reactive oxygen species (ROS) occurs. These ROS may cause damage to DNA, proteins and lipids, as well as to the D1-protein which may thus result in chronic photoinhibition (Asada and Takahashi, 1987, Asada, 1999, Andersson et al., 1992, Collén and Davison, 1999).

It has been shown by Maxwell et al. (1994) on *Chlorella sp.* that different mechanisms exist to maintain optimal photosynthesis at low temperatures. First, an reduction in light harvesting pigments avoids over-excitation. Secondly, increased rates of cyclic electron transport reduce the risk of reactive oxygen generation and increase photorespiration in the chloroplasts. Finally, organisms exposed to low temperatures may enhance their enzymatic activity within the Calvin cycle, which leads to higher rates of carbon fixation. Nevertheless, that study precluded genetic adaptation of stenotherm Antarctic organisms, in which an enhancement of enzymatic activity might be achieved via an increased quantity of enzymes present within the cell rather than via their activity.

Thus, exposure to high irradiances in combination with low temperatures may enhance chronic photoinhibition, as the sensitive balance between functioning reaction centres with impaired D1 centre protein and the re-integration of *de novo* synthesized D1 protein due to decreased membrane fluidity may become disturbed.

5.2.2 The challenge: photosynthesis under extreme conditions

The present study revealed that photosynthesis of subtidal specimens of *Palmaria decipiens* was significantly impaired by the combination of high PAR radiation and low water temperatures (publication I, Figures 1 and 2). It was shown that the slight increase to 400 μmol photons m^{-2} s^{-1}, which algae may experience in their natural habitat at 4 m depths, led to a dysfunctional photosynthetic performance. Nevertheless, this impairment appeared to be reversible, as for instance photosynthetic efficiency data display a certain recovery (see Figure 1a and b, publication I). It was investigated by Hanelt et al. (1994), *P. decipiens* possesses the ability to undergo dynamic photoinhibition and thus, avoids over-excitation.

Nonetheless, exposure to high PAR intensities of 600 μmol photons m^{-2} s^{-1} and three

different temperature treatments over three days resulted in a strong decline of the photosynthetic parameters Fv/Fm and rETRmax without any detectable recovery (Figure 4.2 and Figure 4.3). Interestingly, the impairment of photosynthetic efficiency was least at 0°C and strongest at 8°C. Studies by Kuebler et al. (1991) have shown that genetic adaptation plays an essential role in temperature tolerance and susceptibility to damage of the photosynthetic apparatus. In their study, two species of the rhodophyte genus *Lomentaria sp.* from different latitudes (boreal-temperate and temperate-subtropic) were compared in order to investigate if the acclimation and/or adaptation potential differs among close relatives of this genus. The authors found that light saturated photosynthetic rates were higher at low temperatures in the boreal-temperate species and photosynthesis was inhibited at temperatures adequate for the temperate-subtropical species. As both species have been cultured in equal conditions prior to the experiment for several months, these results point to a genetic adaptation (Kuebler et al., 1991). Several studies on the temperature tolerance limit of *P. decipiens* proved the alga to be a stenotherm with an optimum for growth at 5°C (Wiencke and tom Dieck, 1989, 1990, Bischoff-Bässmann and Wiencke, 1996, Eggert and Wiencke, 2000). Hence, *P. decipiens* is adapted to the Antarctic environment with its low water temperatures and may thus, be less impaired by high irradiances at low temperatures than at 8°C. Considering contrasting data of rETRmax in that particular experiment, in which least impact after the recovery occurred at moderate 5°C and strongest effects were observed at 0°C, it seems that apparently adaptation to low temperatures alone does not prevent from photoinhibition.

In fact, a concept has been proposed that higher temperatures may compensate for photoinhibition (Rautenberger and Bischof 2006). The authors could show by using Antarctic and cold-temperate green algae that the impact of harmful UV-and PAR radiation was mitigated at elevated temperatures and concluded subsequently, that higher temperatures might compensate for photoinhibition. This is because photosynthetic secondary reactions are temperature dependent and so, elevated temperatures might accelerate electron drain-off more efficiently. The likelihood of the generation of ROS within PS I is reduced, and consequently the susceptibility for photoinhibition (Rautenberger and Bischof, 2006, publication II). Of course it has to be taken into account that an acclimation potential has species-specific limitations within the respective adaptation state. The species-specific adaptation determines for instance the temperature tolerance limits, in which an organism might acclimate to changing temperatures. Declines within electron transport rates usually point to irreversible damages of the photosynthetic machinery (Franklin et al., 2003). The results of the present thesis might lead to this assumption, although data on D1-protein concentration is not available. In line with these results are data obtained during short-term exposure to 650 μmol photons m^{-2} s^{-1} and ambient temperatures, where the same pattern was observed (Figure 4.1).

Photosynthetic data gained during mesocosm experiments are rather variable. Here, specimens from the intertidal as well as from the subtidal were exposed to the natural solar radiation in combination with either ambient seawater temperatures or slightly increased water temperatures of 5-10°C. Overall, decreasing maximum quantum yield values were observed, with intertidal specimens exposed to the higher temperature treatment being the most affected (see publication II). At the end of the exposure, however, both photosynthetic parameters (Fv/Fm and rETRmax) were slightly higher in specimens exposed to elevated temperatures. As discussed above, the responses to higher temperatures might indicate a certain compensation of photoinhibition under high PAR intensities.

Intriguingly, specimens from different shore levels revealed significant differences already within their initial rETRmax, where intertidal specimens exhibited electron transport rates of about 40 r.u. higher than subtidal specimens (see Figure 2a and b in publication II). This might be indicative for different degrees of acclimation to the respective habitat along the depth gradient occurring in Potter Cove. In general, macroalgae possess the ability to acclimate to a given radiation regime along shore lines (Hanelt et al., 1997). Intertidal specimens are regularly exposed to high light intensities during low tide conditions, therefore, a higher tolerance towards high irradiances and temperature changes may be expected. Usually, intertidal specimens slightly differ morphologically from subtidal specimens, for instance in pigmentation and poses a thicker thallus (personal observation), which also may enhance their ability to cope with mechanical influences in the intertidal, e.g. wave action, tidal currents or ice scouring (Hurd, 2000, Zacher et al., 2009). Wiencke (1993) stated that low values for initial saturation (Ik) and a high initial slope (alpha) of PI-curves are typical for shade-adapted algae, nevertheless specimens from different depths may exhibit different states of acclimation within the same adaptation limits. This could be reflected in the data presented in this thesis.

Comparative studies on the two species *Iridaea cordata* and *P. decipiens*, both sampled in the intertidal, exposed to 5°C and 300 μmol photons m^{-2} s^{-1} resulted in decreasing photosynthetic performance, although *P. decipiens* seemed to be slightly more affected. Even after recovery conditions in dim light, no significant differences in rETRmax within each species were observed. Photosynthetic efficiency was different between species throughout the duration of the experiment. This may be due to the different thallus morphology of both species: *I. cordata* has a thicker thallus compared to the very delicate fronds of *P. decipiens* (Wiencke and Clayton, 2002). It has been argued whether thallus morphology does matter in terms of susceptibility to photoinhibition in the two intertidal red algae *Chondrus crispus* and *Mastocarpus stellatus*, but no clear pattern could be shown (Dudgeon et al., 1995). The authors suggested that life history stages might be more important rather than thallus morphology. However, the thallus of *C. crispus* is larger but more fragile than the one of *M. stellatus*, this leads to a higher biomass loss during harsh winter conditions (Dudgeon and Johnson, 1992, Lohrmann et al., 2004). In fact, after the sea ice melting in Antarctica, the intertidal platform at Peñon Uno was inhabited by thicker and more robust macroalgae such as *Adenocystis utricularis*, where as delicate thalli like the one of *P. decipiens* were absent (personal observation), which may be indicative for a similar phenomenon as in temperate regions. However, regarding the rETRmax values of both species after the recovery treatment, *P. decipiens* had significantly higher values than *I. cordata*, which might indicate a higher potential for withstanding unfavourable conditions with a faster recovery process. It has been supposed that a cold-induced increase in photosynthetic capacity could be interpreted as a compensatory effect of low temperatures on enzyme activity in the two species *M. stellatus* and *C. crispus* (Lohrmann et al., 2004). Thus, these higher enzyme concentrations could consequently compensate their reduced catalytic activity due to the low temperatures (Lohrmann et al., 2004).

Interestingly, *P. decipiens* and *I. cordata* compete within the same vertical range of the phytal zone (Quartino et al., 2005, Oliveira et al., 2009). As indicated by the present data, the photosynthetic machinery of *I. cordata* seems to be slightly less susceptible to high irradiances, which might be advantageous in terms of interspecific competition. In a comparative study performed by Collén and Davison (1999), the two intertidal rhodophytes *M. stellatus* and *C. crispus* were investigated in order to test their susceptibility towards

oxidative stress by exposing algal material directly to H_2O_2. The authors found that *M. stellatus* is less sensitive and consequently may settle in higher intertidal regions than *C. crispus*. In another year round study on these two species by Lohrmann et al. (2004), the findings of Collén and Davison were confirmed. *M. stellatus* is considered to be more tolerant to environmental stress, particularly during winter and thus, low temperatures. This is also reflected in the higher contents of ROS scavenging enzymes and the concentration of ascorbate (Lohrmann et al., 2004). Their data indicated that ROS scavenging is part of the winter acclimation, where low temperatures may enhance the generation of ROS. This is due to the fact that low temperatures slow down enzyme-catalyzed reactions of the Calvin cycle but temperature is not affecting light absorption itself. Hence, particularly at low temperatures light absorption might exceed photosynthetical utilization and thus, ROS may be generated (Asada, 2000, Lohrmann et al., 2004 and references therein). As Collén and Davison (1999) also included photosynthetic measurements, and obtained similar results as those from the two Antarctic species, it might be speculated that eventually the oxidative stress tolerance might also be higher in *I. cordata* than in *P. decipiens*.

5.3 Physiological responses to combined abiotic stress factors

The present thesis focused on physiological performances and the acclimation potential of *Palmaria decipiens* exposed to changing light and temperature levels. In addition to determining the photosynthetic performance, further analyses of pigment content, D1-protein content as well as lipid content and fatty acid composition were conducted. Therefore it is possible to make an estimation of the physiological state of seaweeds under current climatic conditions as well as eventual consequences under climate change conditions (while exercising caution when drawing precise ecologically relevant conclusions).

It is generally accepted, that photosynthetic pigments are reduced under high light intensities (as reviewed by Franklin et al. 2003), or that at low temperatures the content of unsaturated fatty acids is increased to maintain membrane functionability (Murata and Los, 1997, Harwood and Guschina, 2009). Charcteristical pigments of cyanobacteria and rhodophytes are phycobiliproteins. In particular, by using phycobiliproteins the range of the spectra inaccessible for chlorophyll *a* is absorbed and thus, light utilization becomes more efficient. However, these pigments are especially vulnerable to high light and UV-radiation and may degrade rapidly (Gómez et al., 2004). During the exposure of *P. decipiens* to high PAR intensities of 650 μmol photons m^{-2} s^{-1}, decreasing phycobiliprotein concentrations were observed (see publication I and II). Usually, these pigments increase the spectra of light available for photosynthetic performance, which is characteristical for deep growing species or those inhabiting light limited regions such as Antarctica. An increase in pigment content might indicate an increase in absorption cross-sectional areas of the photosynthetic centres (Weykam and Wiencke, 1996 and references therein). By reducing the content of antennae pigments, less light is absorbed and thus, the risk of photodamage is lowered.

This degradation of pigments as a response to changes in light availability, either excessive light intensities or very low intensities, has been observed on short time scales as well as long-term responses to changing seasons (Kuebler et al., 1991, Beach et al., 2000, Galland-Irmouli et al., 2000, Lüder et al., 2001, 2002, Aguilera et al., 2008, Martinez and Rico, 2008). Short-term acclimation includes daily cycles of pigment degradation and re-synthesis, which has been observed in the temperate rhodophyte *Porphyra umbilicalis*

(Aguilera et al., 2008). The authors exposed *P. umbilicalis* to fluctuating light/dark cycles while measuring photosynthetic performance as well as pigment content. As a result, it was observed that under excessive light intensities, the phycobiliprotein content decreased and recovered over night (Aguilera et al., 2008). These degradation processes are relatively fast, and especially under high light conditions phycoerythrin degrades faster than it is re-synthesized (Beach et al., 2000). Beach et al. (2000) investigated tropical red algal mats on Hawaii, by transplanting understorey specimens to the surface and *vice versa*. It was found that understorey specimens contained higher concentrations in phycobiliproteins as an acclimation to the specific light regime within the mat, and that these specimens acclimated faster by degrading their accessory pigments than those specimens from the surface transferred into greater depths (Beach et al., 2000). Furthermore, phycobiliproteins are known to degrade rapidly under exposure to high light and UV-radiation, while other pigment types are less susceptible (Tevini, 1994, Gómez et al., 2004). These relative quick responses indicate a certain acclimation potential to a changing light regime, in line with the data of mesocosm experiments with *P. decipiens* specimens from different shore levels of the present thesis. The results of *P. decipiens* exposed to high PAR intensities should rather be considered as an acclimation to the changing light regime than a result of photodamage as overall photosynthetic performance was hardly affected (see publication II, Figures 1, 2 and 3).

Long-term responses to seasonal changes in light availability have been studied in detail by Lüder et al. (2001, 2002). The underlying adaptation to long periods of extreme low light conditions includes a stepwise, complete degradation of the phycobilisomes. By the end of the winter, *P. decipiens* is unable to photosynthesize. Within 24 h of re-illumination, pigments are newly synthesized and within a couple of days growth is induced (Lüder et al. 2002). In *Palmaria palmata*, the northern congener species of *P. decipiens*, it has also been shown that pigment content varies seasonally (Galland-Irmouli et al. 2000). In this study, the authors focussed on isolated phycoerythrin and monitored the pigment content of *P. palmata* from Spain throughout a year. It was found that phycobiliprotein concentrations were lowest during the summer coinciding with highest irradiances. With decreasing light intensities through autumn, pigment concentration reached maximal values. Hence, it was concluded that light intensity and pigmentation are inversely correlated (Galland-Irmouli et al., 2000 and references therein). An important modulating factor in phycobiliprotein synthesis is the availability of nitrogen. Since during the Spanish summer nitrogen depleted conditions may occur, this deficiency leads to an inhibition in phycobiliprotein synthesis (Galland-Irmouli et al., 2000). Antarctic waters are rich in nitrogen and phosphate throughout the year, hence, it is rather unlikely that these nutrients become limiting for macroalgal growth and pigment synthesis. Nevertheless, phycobiliproteins are rich in nitrogen, and their degradation may fulfill N-demands under nitrogen-limited conditions as shown in sun-adapted *P. palmata* in nutrient-limited summer waters of Spain (Martinez and Rico, 2008). Similarly to *P. palmata*, *P. decipiens* endures and survives winter period of darkness mainly by using up floridean starch, a carbon reservoir, but possibly available nitrogen through pigment degradation will be used as well (Weykam and Wiencke, 1996, Hagen-Rodde et al., 2004).

Phycobiliprotein to chlorophyll *a* ratios were determined for specimens of *P. decipiens* after exposure to two different temperatures and two different light treatments (see publication I). In that case, higher irradiances and elevated temperature resulted in a slightly more pronounced decrease in pigment content, however, no significant differences were observed.

The same pattern was found in specimens from two shore levels exposed to natural solar radiation and elevated temperatures (see publication II). All treatments led to decreasing pigment ratios, although no significant temperature effect was detected. The reduction of pigment content is considered a response to the altered radiation conditions applied. As stated earlier, *P. decipiens* is known to adjust its pigment content flexible with respect to the prevailing light regime. Interestingly, initial ratios differed largely between specimens from the intertidal and from the subtidal (Figure 3, publication II). In specimens originating from the subtidal, the amount of phycobiliproteins was higher in comparison to those from the intertidal, indicating an acclimation to the respective habitat. That particular increase in antennae pigments maximizes effective light absorption in greater depths. A similar phenomenon has been observed in shade- and sun-adapted *P. palmata* in Spain (Martinez and Rico, 2008). The authors found higher concentrations of phycobiliproteins in understorey and shade-adapted specimens in comparison to the sun-adapted ones.

For instance, at low temperatures the freshwater green microalga *Chlorella sp.* is able to adjust the photosynthetic machinery, a reduction of chlorophyll *a* and LHC pigments reduce the probability of excessive light absorption and therefore the risk of photoinhibition (Maxwell et al., 2004). Taken together, *P. decipiens* may respond flexibly to changing light levels by modulating the pigment content and thus, ensures optimal light harvesting, while temperature had no apparent influence.

One of the most important factors for photosynthetic function is the integrity of the D1 centre protein of PS II (Aro et al., 1993, 2005, Osmond, 1994, Franklin et al., 2003). Especially increasing light intensities may severely damage the photosynthetic machinery and its repair rate is highly temperature dependent. Thus, the combination of high irradiances with low temperature might react synergistically and result in pronounced photoinhibition. In the present study, data obtained during mesocosm field experiments indicated that the photosynthetic integrity of *P. decipiens* was hardly impaired under elevated irradiances and different temperatures, as mirrored in only slight changes in D1-protein concentration (see Figure 4, publication II). Nevertheless, low temperatures and high irradiances led to a significant decrease in D1-protein concentration in subtidal specimens exposed to low temperatures. It seems that subtidal specimens cannot response as quickly as intertidal specimens to changing abiotic conditions, which can be connected to the very stable temperature and radiation conditions in 10 m depth. Apparently, intertidal specimens are more flexible in adjusting their physiological performance including D1-integrity as the intertidal is a highly dynamic habitat. Still, as these temperatures applied during the exposure are similar to *in situ* conditions for subtidal *P. decipiens* specimens, an interactive temperature effect is rather doubtful. It seems more likely that in the higher temperature treatments D1-fragmentation was counterbalanced due to higher repair rates at elevated temperatures.

In that context, membrane fluidity seems to play a crucial role as the fluidity is highly temperature dependent. A variety of alterations in cellular components including the extent of fatty acid (FA) unsaturation, the composition of glycerolipids, the positional redistribution of saturated and unsaturated fatty acids within lipid molecules, changes in the lipid/protein ratio, and activation of ion channels is induced by low temperatures (Guschina and Harwood 2006, and reference therein). Consequently, at a given temperature, membrane fluidity is determined by the respective degree of unsaturation of fatty acids. Generally, cold-adapted organisms contain higher amounts of unsaturated fatty acids to maintain membrane fluidity (Harwood, 1994). Apparently, a high amount of un-

saturated fatty acids may also result in a shift of tolerance limits towards abiotic stress factors. In cyanobacteria it was shown that not only photoinhibition is reduced at low temperatures, but also recovery processes are accelerated depending on the degree of unsaturation of glycerolipids in thylakoid membranes (Wada et a., 1994, 1990, Gombos et al., 1992, 1994, Murata and Los, 1997). By applying chilling temperatures on cyanobacteria mutants equipped with different unsaturation degrees in their membranes, Gombos et al. (1992, 1994) could show that the unsaturation degree plays a determining role in the protection from and tolerance of low temperature-induced photoinhibition. A similar result was obtained by Allakhverdiev et al. (2001) for the cyanobacterium *Synechococcus sp.*. The organisms revealed a higher tolerance towards salt stress with increasing amounts of unsaturated fatty acids. Thus, by adjusting fatty acid composition, particularly those of thylakoid membranes, photosynthetic organisms may respond and acclimate to changing environmental conditions.

A study by Graeve et al. (2002) investigated the lipid content and fatty acid composition in Polar macroalgae. The authors surveyed for the first time specific fatty acid characteristics of Antarctic macroalgae and found extremely high amounts of polyunsaturated fatty acids (PUFAs). It was shown that Chlorophytes contained mainly C_{18} unsaturated FA, which are typical for vegetative tissues or higher plants (Graeve et al., 2002). Phaeophytes had 18:4(n-3) and 20:5(n-3) as the most abundant FA and were thus, placed in an intermediate position between Chlorophytes and Rhodophytes. In Rhodophytes such as *P. decipiens*, the most abundant FA was 20:5(n-3). The authors concluded that the presence of 20:5(n-3) reflects are more "marine-like" and therefore more ancestral character (Graeve et al., 2002). The data gained during the mesocosm experiments of this thesis confirmed high contents of PUFAs and especially high concentrations of the FA 20:5(n-3)(see table 3, publication II).

An additional factor influencing fatty acid composition is light, which has been studied by Leu et al. (2006) on Arctic phytoplankton blooms. It was demonstrated how fatty acid composition shifted during the aging of the bloom. The early breakdown stage is characterized by a high light induced increase in the FA 18:0 and a subsequent decrease of PUFAs. FA composition of *P. decipiens* revealed increasing concentrations of 18:1(n-9) during the exposure to high light, which indicates a certain productivity, as these FA are induced under stressful conditions (Guschina and Harwood 2006, M. Graeve pers. comm.).

This increase in mono- and bisaturated FA of 18:X was stronger in specimens of *P. decipiens* exposed to low temperatures and elevated radiation in comparison to those exposed at 5-10°C. Such an increase of 18:0 FA had been reported previously for Antarctic waters by Fahl and Kattner (1993). The authors related this finding to the poor quality of organic matter in the Southern Ocean. In combination with the findings of Leu et al. (2006), an increase of 18:0 FA may point to phytoplankton in a poor physiological condition at the end of a bloom. Taken these previous finding together with the data of the present thesis, the changes in FA composition and the particular increase of 18:X FA indicates a declining physiological state of *P. decipiens*.

As *de novo* synthesis of fatty acids always starts with acetyl-CoA, which is elongated to 14:0 and 16:0 and then desaturated and further elongated by various elongases and desaturases in different steps, adjustments of FA are a rather slow response to changing environmental conditions (Graeve et al., 2002, Harwood and Guschina, 2009, and references therein). The present thesis could show that the adjustment of FA composition needs at

least 11 days considering the particular conditions applied in the mesocosm experiment (see Figure 4.6b).

Overall, the role of FA composition and membrane fluidity seems to be important for the re-integration of D1-protein and thus, for photosynthetic performance. PUFAs and especially phospholipids are considerably important for the functionality of photosynthetic membranes (Sanina et al., 2004, Aro et al., 2005). Presented data of the performed experiment imply that if FA composition is not adjusted under high light conditions and low temperatures, the D1 turn-over might be impaired and the risk for chronic photoinhibition is enhanced. In this case it remains unanswered, if the impairment of the photosynthetic performance was due to slowed down *de novo* synthesis of D1 or if *de novo* synthesized protein could not be integrated into the thylakoid membrane (see publication II).

5.4 Ecological conclusions and outlook on future research

In summary, results of the present thesis on the physiological performance of two Antarctic rhodophytes, *Palmaria decipiens* and *Iridaea cordata*, have shown a high adaptation to the particular environment and the ability to respond flexibly to changing abiotic factors. During the exposure to changing light and temperature levels it was observed that *P. decipiens* is able to cope with a certain increase in PAR intensity, but that particularly the combination of low water temperatures and high irradiances impacted photosynthetic performance as well as pigmentation.

These changes further influenced underlying biochemical processes such as D1 turn-over and membrane fluidity. Especially those two factors seem to depend on each other, as more rigid membranes at low temperatures may result in reduced turn-over rates, as re-integration of *de novo* synthesized D1-protein is affected. As global climate warming leads to high PAR-irradiances early within the season due to declining sea-ice formation and earlier sea-ice break up, algae might experience high PAR intensities over a longer period of time. As has been shown by the data of the present thesis, the combination of high PAR-irradiances with low water temperatures does affect photosynthesis and physiological parameters negatively. How these changes might influence for instance depth zonation or recruitment success of *P. decipiens* still has to be elucidated.

Nevertheless, it has been shown that *P. decipiens* is able to cope with changing abiotic conditions, if these changes do not exceed its particular tolerance limits. As stated by Wiencke and Clayton (2002), the alga is able to cope with high irradiances of up to 1500 μmol photonen m^{-2} s^{-1} occurring during low tide, which naturally is followed by a recovery period during high tide. Therefore, the conditions applied during the experiments were close to the natural range and results may reflect actual acclimation processes within *P. decipiens*.

For the first time it was shown that the role of fatty acid adjustment may be an important factor in the acclimation process of *P. decipiens* to changing abiotic conditions. Apparently this process is triggered by temperature solely, as high light intensities at nearly ambient temperatures did not result in FA adjustment. Interestingly, specimens from different shore levels revealed different content of FA and therefore an acclimation to the respective habitat. Overall, it has to be taken into account, that total lipid content and total fatty acid composition of the alga were investigated. If changes within the thylakoid membrane occurred during the treatment remained undetected in the present thesis. This would give valuable information regarding the D1 re-integration process and would allow more

precise conclusions about the interaction of membrane fluidity and D1 turn-over in cold- and shade-adapted algae.

During the last 50 years, a temperature increase of about 3°C was observed. This warming leads to glacier retreat, thinning of sea-ice and increased river and melt water run-off with concomitantly higher sediment loads (Turner et al., 2005). Data of the present thesis regarding the influence of freshwater input on *P. decipiens* have shown that the alga appears to be rather euryhaline, as photosynthesis was hardly impaired by decreasing salinities down to 5 PSU. The applied salinities (28-5 PSU) exceeded the natural range the algae might experience in their natural habitat (34-31 PSU according to Schloss et al., 2002). Still, particularly intertidal specimens might be exposed to rainfall during low tide and thus a high tolerance might benefit recruitment success in this harsh environment. On the other hand, glacier retreat also leads to new ice-free areas, mainly consisting of hard bottom substrate, which may serve as new areas for macroalgal settlement. It has been observed, that in this particular disturbed habitat with high water turbidity *P. decipiens* is the only alga that settles in high densities (M.L. Quartino, pers. comm.). Of course light availability is a crucial factor for photosynthetic organisms, but since *P. decipiens* is highly adapted to very low light intensities, it seems that at least in this case *P. decipiens* might take advantage of the new available habitat.

The comparison of the two rhodophyte species *I. cordata* and *P. decipiens* has shown, that the acclimation potential of the Antarctic-endemic species *P. decipiens* is slightly more limited than the one of *I. cordata*. This result is according to the respective geographic distribution of the two species. *I. cordata* is distributed up to the sub-Antarctic regions and reveals consequently a slightly broader tolerance limit than the well adapted endemic species. In that case it can be speculated whether thallus morphology matters and even may influence the ability to cope with oxidative stress (as in the two temperate species *Chondrus crispus* and *Mastocarpus stellatus*). It seems that in both Antarctic species the pre-dominant factor for physiological performance is temperature, when elevated temperatures were applied in combination with higher irradiances. As both species are regarded as strongly cold-adapted, even moderate increases in water temperature might reach the respective tolerance limit (Wiencke and Clayton, 2002).

Future studies should attempt to include not only adult thalli, but also study germlings and spores, which have been shown to be more susceptible and the most fragile link within the life cycle (e.g. Fredersdorf et al., 2009). For instance, germination as well as algal recruitment and settlement is reduced by increasing UV-intensities, and community shifts might occur under a changing radiation regime (Campana et al., 2008, Zacher and Campana, 2008, Zacher et al., 2008, 2009). If increases in PAR intensities impact these early life history stages to a greater extent than adult thalli, surely different conclusions of future ecological consequences will be drawn. In addition to physiological monitoring, future investigations should also consider molecular and gene expression patterns for different life history stages of macroalgae.

In terms of ecosystem function the dynamics of the biota in a changing environment play an important role, since patterns and frequency of grazing may vary under changing light regimes, as shown for e.g. amphipod grazing on *P. decipiens* (GL. Campana, pers. comm.). In a broader sense, biotic factors such as grazing or inter-/intraspecific competition may influence population structure and individual fitness as well.

Hence, the combined information of molecular techniques, ecophysiology and population ecology will contribute to understand the response of the highly dynamic shallow benthic

communities of Antarctica. This study provides a deeper insight on adaptation and acclimation potential of an abundant Antarctic rhodophyte and represents a first step towards a comprehensive understanding of the adaptive capabilities of *P. decipiens* in the changing Antarctic environment.

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8 Publications

Freezing tolerance and photosynthetic performance of Polar macroalgae

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Freezing tolerance and photosynthetic performance of polar seaweeds at low temperatures

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Running title: Photosynthesis at low temperatures

Abstract

Organisms populating benthic shallow water systems of both polar regions are adapted to a particularly harsh environment. We studied effects of freezing and the combination of high light intensities and low water temperatures on photosynthesis of key macroalgal species from the Arctic intertidal (*Fucus distichus*) and Antarctic subtidal (*Palmaria decipiens*). Photosynthetic activity of *F. distichus* specimens was monitored during the freezing process; there was a marked decrease in quantum yield with decreasing temperatures, and a rapid recovery as soon as temperatures increased again. Thus, under the experimental conditions tested, no indication of photodamage was found. Specimens of *Palmaria* were exposed to a combination of high light intensities and low water temperatures. A persistent impairment of photosynthetic activity occurred at temperatures 0 °C at light intensities of 400 µmol photons m^{-2} s^{-1}. In all treatments, there was a decreasing ratio of phycobiliproteins to chlorophyll *a*. Overall, the two studies provide baseline data for interpreting physiological responses of two important macroalgal species in an extreme environment, the polar coastal ecosystem.

Key Words

Palmaria decipiens, *Fucus distichus*, photosynthesis, high light, freezing

Abbreviations

ETR, electron transport rate; Fv/Fm, optimum quantum yield; FW, fresh weight; MDA, malondialdehyd; NPQ, non-photochemical quenching; PAR, photosynthetically active radiation; PB, phycobiliprotein; ROS, reactive oxygen species

Introduction

Species inhabiting the intertidal zone cope with drastic changes in the physical environment during the course of the tides. Moreover, in polar regions, intertidal organisms are frequently exposed to atmospheric temperatures substantially below 0°C (see Zacher, K., Rautenberger, R., Hanelt, D., Wulff, A. and Wiencke, C., unpublished data, 2009). However, as specimens are exposed to a variety of simultaneously changing abiotic factors, it is essential to understand how the combination of (stress-) factors influences organismic performance (see Gómez et al., 2009). For instance, Antarctic macroalgae are generally considered as being strongly shade-adapted (Wiencke, 1990, Lüder et al., 2001) and hence more susceptible to increasing light intensities. However, immediately after sea-ice break up, radiation may penetrate deeply into transparent waters (Drew and Hastings, 1992, McMinn et al., 2004), and even subtidal specimens will be exposed to elevated light intensities. In addition, photosynthetic activity is particularly sensitive to low temperatures, as enzymatic secondary reactions are strictly temperature-dependent, while primary reactions are not. Thus, exposure to high irradiances in combination with low temperatures may result in a surplus of electrons participating in the Mehler reaction and, consequently, in an increased production of reactive oxygen species (ROS), resulting in photo-oxidation (Asada and Takahashi, 1987, Andersson et al., 1992). Furthermore, low

temperatures strongly affect membrane fluidity and protein function, for instance in the turnover of the D1-protein, which is the central protein within photosystem II (Aro et al., 1993). Freezing has physiological effects similar to those of dehydration (Pearson and Davison, 1994), as the water potential within cells may be considerably reduced.

The Antarctic endemic rhodophyte *Palmaria decipiens* (Reinsch) Ricker is an excellent organism for studying the interactive effects of low temperatures and high light intensities; it has been studied previously for its temperature, as well as growth and light requirements (Wiencke and tom Dieck, 1989, Wiencke, 1990, Bischoff-Bäsmann and Wiencke, 1996, Wiencke et al., 1993, Lüder et al., 2001).

Along the coasts of the North Atlantic, the brown algal genus *Fucus* forms dense algal fringes within the intertidal zone. *F. distichus* Linnaeus is the species with the northernmost distribution and is, thus, the only member of the genus inhabiting the coasts of the Svalbard archipelago (Lüning, 1990). As a general pattern in polar regions, only very few algal species populate the intertidal zone, which is mostly attributed to the influence of ice scouring. *F. distichus* is almost the only intertidal macroalgal species along the Svalbard coastline (Kirst and Wiencke, 1995, Wiencke et al., 2004). In winter, it is exposed to subzero temperatures and, in extreme cases, survives being frozen in ice for several months (Kanwisher, 1957). However, earlier studies on *F. distichus* from more southerly locations reported that this species is less tolerant to freezing, desiccation or high light than other species of *Fucus* (Denton and Chapman, 1991, Collén and Davison, 1999). Thus, the ecological success of *F. distichus* and the absence of other species of *Fucus* in the European high Arctic remains an open field of enquiry.

We present here two separate studies on the effects of low temperature, light intensity and freezing on photosynthetic performance. The first was conducted in Antarctica, and focused on the combined effects of temperature and light on photosynthesis of the Antarctic endemic *Palmaria decipiens*. The second was conducted on the Arctic

Kongsfjord, Spitsbergen, where the effects of freezing on photosynthesis of *F. distichus* were studied in a set of repeated freezing and thawing experiments. These two individual studies focus on the adaptability of the photosynthetic apparatus to cold environments in a more eurythermal (*F. distichus*) and a strictly stenothermal species (*P. decipiens*).

Material and Methods

Study site, algal material and cultivation

Palmaria decipiens

Experiments were conducted at the German-Argentinean Dallmann Laboratory, Jubany Base, King George Island, South Shetland Islands, Antarctica (62°14'S, 58°40'W), during the Antarctic summer. A detailed overview on the physical and biological environment of the Potter Cove ecosystem is given by Wiencke et al. (2008). Specimens of *Palmaria decipiens* were collected by SCUBA diving between 20 January and 20 February 2007 offshore from the Fourcade glacier. Fifteen to 20 individuals 30 to 50 cm long were collected at 8 to 10 m water depth and brought to the laboratory in black boxes to avoid light stress during the transfer. Prior to the experiment, algae were kept under low light conditions (15 µmol photons $m^{-2}s^{-1}$) in a seawater tank at ambient water temperature (approximately 2°C). Water was aerated and replaced every second day.

Algal material was cleaned, cut into discs approximately 50 mm in diameter taken from the middle part of the thalli and randomly distributed among three 1l plastic beakers (Vitalab GmbH, Darmstadt, Germany) filled with ambient seawater. The beakers serving as three different experimental units were placed in temperature controlled water tanks providing temperatures of 0°C and 8°C. Temperature was maintained via cryostats (1160S, VWR International GmbH, Darmstadt, Germany) and controlled by temperature

loggers (Testo 175-T1, Testo AG, Lenzkirch, Germany). Two of these tanks were established for each temperature treatment.

Light treatments were applied by six halogen spot lights (EXN-P, 50W, SP581, Conrad, Hirschau, Germany) mounted on an aluminium frame (500x300 mm). Light intensity was adjusted by varying the distance to the beakers or by using black net gauze. Light intensity was set to 200 µmol photons $m^{-2}s^{-1}$ and 400 µmol photons $m^{-2}s^{-1}$ PAR. Light measurements were conducted with a LiCor 1400 Data Logger equipped with a flat-head cosine corrected PAR quantum sensor (LICOR 190 SA, Li-Cor, Lincoln, NE, USA). Samples were exposed to the continuous light treatment for three days, after which, algae were kept in dim light (15 µmol photons $m^{-2}s^{-1}$) for 48 h for recovery measurements.

Fucus distichus

The study was conducted in July 2004 in Ny Ålesund (78°55.5´N; 11°56.0´E) on the Arctic Kongsfjord (Spitsbergen, Norway). A detailed overview on the physical and biological environment of Kongsfjorden is provided by Svendsen et al. (2002) and Hop et al. (2002). During low tide conditions, young thalli from the uppermost part of the *Fucus* fringe were collected in Nansen-Bay and transferred to the laboratory. There, algal material was kept in running seawater at fjord temperature (approximately 4°C) and under permanent low light conditions (20 µmol photons m^{-2} s^{-1}) for about two days prior to the experiment. The base of the experimental set-up was a custom made “freezing table” consisting of a Pelletier element controlling the temperature of a steel surface of approximately 45 x 30 cm. The plate of the table may be cooled down to -20°C. Algal material was placed in Petri dishes (9 cm diameter, in the following referred to as “dish”) on the surface of the table. For measurements of photosynthetic activity, the fiber optic of a chlorophyll fluorometer (as described below) was fixed above randomly chosen thalli. In

this way, the effective quantum yield of photosynthesis (yield, ΔF/Fm´) was determined during the course of each experiment, and, in addition, maximum quantum yield (Fv/Fm) and photosynthesis vs. irradiance curves (PI-curves) were measured at given times.

Two light fields with identical irradiances were arranged (OSRAM HLX 15V, 150W, Munich, Germany) at the surface of the freezing table. We placed the dish for measuring photosynthetic performance in one of the fields; in the second field, we placed a dish containing algal material exposed for later biochemical analysis. The irradiance in these light fields was set to match the I_k-value of experimental specimens, i.e., 100 µmol photons m^{-2} s^{-1}, as determined by previously recorded PI-curves. Inside the dish, an external temperature sensor connected to the fluorometer provided continuous temperature measurements under water, or between immersed thalli in freezing experiments on non-submerged specimens.

Three experiments with different freezing and thawing conditions were conducted:

1. (Rapid freezing): Algal material was placed in a dish, submerged in seawater and arranged in the light fields on the surface of the table. After 30 min acclimation at ambient temperatures (approximately 8°C), the table was cooled down to the minimum temperature (-20°C). As soon as the temperature stopped falling, the table was held at this lowest temperature for about 10 min, and then reset to initial ambient temperatures. After ambient temperature was reached, the experiment was further continued for another 30 minutes. During the process of freezing and thawing, yield values were recorded every two minutes, Fv/Fm and PI-curves were measured after 30 minutes of pre-acclimation at the lowest temperature, at the end of the experiment, and after 36 h of recovery. As in the following experiments, Fv/Fm determinations were performed on three different thalli, whereas, due to limitations in time, PI-curves were recorded just once at each sampling time.

2. (Dry freezing): The setup was the same as in experiment 1, but samples were frozen in a dish without seawater. Fv/Fm and PI-curves were measured at the same time intervals as in the first experiment.

3. (Long term freezing): The algae were placed in a dish filled with seawater at the surface of the table. After a short period of acclimation to ambient conditions, the temperature was set to -1.5 °C, which was maintained for about 7 h. After that, the initial temperature was applied again for another 8 h.

In each experiment, reference samples were arranged in dishes as control treatments, which were maintained at ambient temperature and the same irradiance. At each measuring time, algal fragments from the second dish were collected for later biochemical analysis. For each experiment, there were three replicates.

Photosynthetic measurements

The photosynthetic parameters Fv/Fm, effective quantum yield and relative ETRmax were determined by measuring the variable chlorophyll fluorescence of PS II with a pulse-amplitude modulated fluorometer (PAM 2100, Walz, Effeltrich, Germany) as described in detail by Hanelt et al. (1997) and Bischof et al. (1998). PI-curves were conducted using an internal LED as light source at irradiances of 24 to 809 µmol photons m^{-2} s^{-1} PAR (*Fucus distichus*) and 30 to 713 µmol photons m^{-2} s^{-1} PAR (*Palmaria decipiens*), respectively. The product of quantum yield and photon fluence rate (PFR) allows calculation of relative electron transport rates (rel.ETR), as described by Schreiber et al. 1994 (rel. ETR = $\Delta F/F'm \times PFR$); maximum relative electron transport rate (ETRmax) was determined by curve fitting. Non-photochemical quenching was calculated according to the formula of Bilger et al. (1995):

$$NPQ = Fm - Fm'/ Fm'.$$

Biochemical analysis

As a marker of oxidative stress, the degree of lipid peroxidation was measured in *Fucus* samples by determining the concentration of malondialdehyde (MDA), according to Heath and Packer (1968) and Bischof et al. (2003).

Changes in the concentration of D1-protein of photosystem II were determined by SDS-PAGE and subsequent Western Blotting, according to Bischof et al. 2000.

The phycobiliprotein contents of *Palmaria* samples were determined after Beer and Eshel (1985). Chlorophyll *a* content was determined after Lüder et al. (2002) using the modified protocol of Inskeep and Bloom (1985).

Statistics

Experiments conducted on *Palmaria* were set-up as split-plot design with repeated measures and three replications. Mean values and standard deviations were calculated from the replicates and for each treatment. Photosynthetic data (Fv/Fm) were arcsine-transformed and a MANOVA was performed on the factors light and temperature and their interactions. Statistically significant differences were compared with Tukey's HSD (honestly significantly different) post-hoc test with $\alpha < 0.1$ (Sokal and Rohlf, 1995). Statistical analysis was performed with JMP software (JMP version 6.1., SAS Inc., USA).

Each experiment carried out on *Fucus* was performed three times. For each replication, one PI-curve and three Fv/Fm values were recorded and mean values as well as standard deviations were calculated. For experiment 2 statistical analysis on ETRmax was precluded due to limitation in sample number. An independent Samöels t-test was performed to test treatment differences. The values were accepted as significantly

different at $p < 0.05$. Statistic analysis was performed with SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Interactive effects of irradiance and temperature on photosynthesis of *Palmaria decipiens*

As an indicator of the physiological state of *Palmaria decipiens*, the optimum quantum yield (Fv/Fm) and PI-curves were recorded, indicating that particularly the combination of low water temperatures and high PAR irradiances affects photosynthesis of the alga. In the 400 µmol photons m^{-2} s^{-1} PAR treatment, Fv/Fm values decreased significantly at both temperatures ($p=0.0064$, see fig. 1a, b).

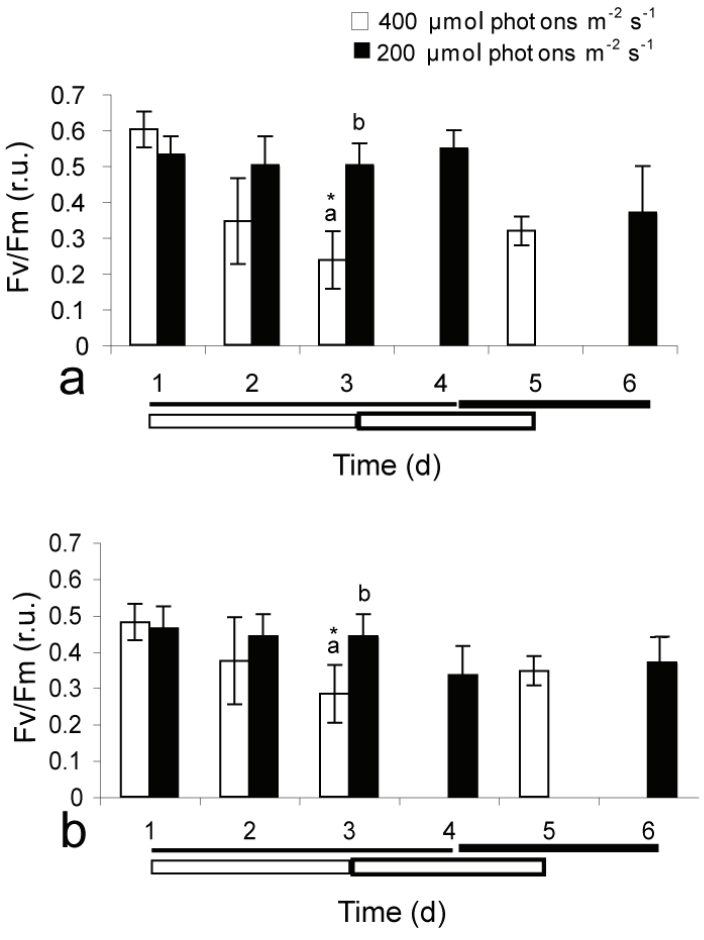

Fig. 1: *Palmaria decipiens*: **a:** optimum quantum yield (Fv/Fm) against exposure time (in days) at 0°C and two light treatments (200 and 400 µmol m^{-2} s^{-1}) and **b:** at 8°C and two light treatments as above. Bars beneath the x-axis represent exposure (thin line or thin framed box) or recovery conditions (bold line or bold framed box) at 400 µmol photons m^{-2} s^{-1} (boxes) or 200 µmol photons m^{-2} s^{-1} (lines) PAR treatments. In both cases, values are means of triplicate measurements, bars show standard deviations. *: significant differences ($p< 0.05$) within a light treatment on day three of exposure, different letters indicate statistically significant differences between treatments (Tukey's HSD, $p< 0.05$). r.u.: relative units

At 0°C and 400 µmol photons m^{-2} s^{-1} PAR, Fv/Fm values decreased from an initial value of 0.601 to 0.237 after three days exposure. Values for specimens exposed to the same temperature, but at 200 µmol m^{-2} s^{-1} remained at an almost constant level of 0.5 (see figure 1a). ANOVA showed a highly significant difference between light treatments on day three ($p=0.0012$). The combination of temperature, time and radiation level had a negative influence on Fv/Fm values ($p=0.0945$). For maximum electron transport rate (ETRmax), there was a significant time×temperature interaction between treatments ($p=0.0605$), but no differences in the combination of time×light ($p=0.8222$) or time×temperature×light were found ($p= 0.6574$, see also figure 2). In the 0°C/ high PAR-treatment, ETRmax decreased within three days to 69% of the initial value, whereas values at 0°C but with lower PAR decreased only slightly to 92% of the initial value.

Comparing the different temperature treatments at PAR intensities of 200 µmol photons m^{-2} s^{-1}, there was only a slight reduction in Fv/Fm values (initial value at 0°C and 8°C of 0.531 and 0.463, respectively, fig. 1a, b) to values of 0.502 and 0.442 after three days of exposure without any recovery after 36 h in dim light.

The same trend was observed in ETRmax values (values decreasing to 92% of initial values, see figure 2) and no recovery was detected. Comparing different light treatments at 0°C, there was a significant difference in ETRmax values on day three ($p= 0.0814$). ETRmax was negatively influenced by the 400 µmol photons m^{-2} s^{-1} PAR treatment compared to 200 µmol photons m^{-2} s^{-1} PAR, such that under these conditions both photosynthetic parameters decreased (see fig. 2).

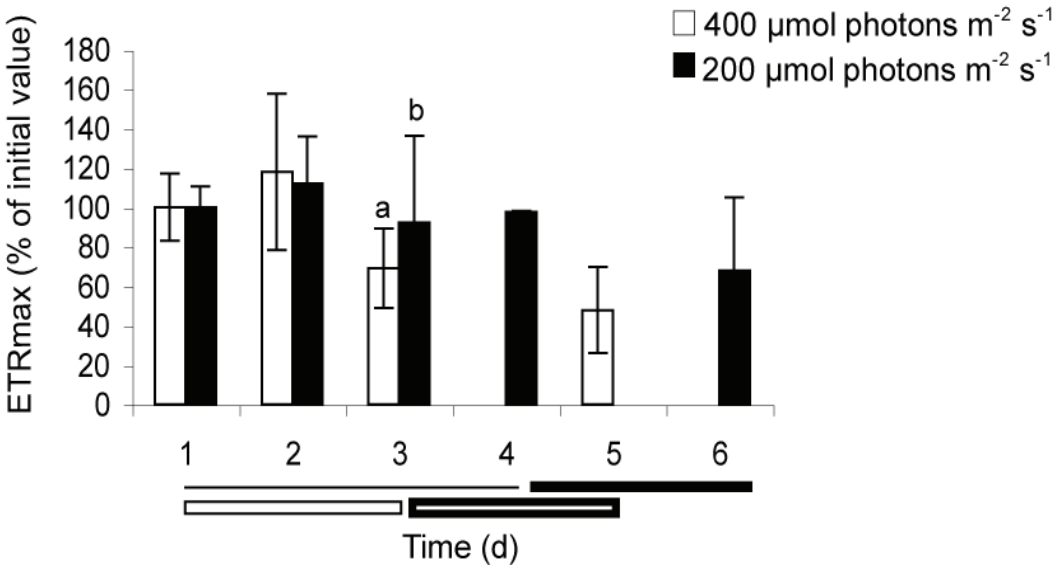

Fig. 2: *Palmaria decipiens*: Maximum electron transport rate (ETR_{max}) as percentage of the initial value against exposure time (in days) at 0°C. Bars beneath the x-axis represent exposure (thin line or thin framed box) or recovery conditions (bold line or bold framed box) at 400 µmol photons m^{-2} s^{-1} (boxes) or 200 µmol photons m^{-2} s^{-1} (lines) PAR treatments. Values are means of triplicate measurements, bars show standard deviation. Different letters indicate significant differences (Tukey's HSD) between treatments with p= 0.0814.

ETRmax values for specimens in the 8°C treatment decreased even during the recovery period (see above) to 13 % of the initial value, but no significant differences occurred (p=0.6877, data not shown). This was also the case for the 8°C treatment with varying light intensities, viz., no differences in the maximum quantum yield between treatments (data not shown).

Pigment analysis was performed for content of chlorophyll *a* and phycobiliproteins. In light and temperature treatments, the ratio of phycobiliproteins to chlorophyll *a* decreased substantially, more so under the lower PAR intensity at both temperatures (see table 1).

Table 1: *Palmaria decipiens:* Phycobiliprotein/ chlorophyll *a* ratio calculated from pigment content measured in samples at 8°C and 0°C and two different irradiances (200 and 400 µmol photons m^{-2} s^{-1}). Values are means of triplicate measurements ± SD; no statistically significant differences were found (ANOVA p> 0.1).

	200 µmol photons m^{-2} s^{-1}		400 µmol photons m^{-2} s^{-1}	
	initial	end	initial	end
0°C	4.05 ± 1.88	3.07 ± 1.04	3.38 ± 2	4.83 ± 1.74
8°C	5.35 ± 2.34	4.05 ± 1.36	4.68 ± 1.76	3.13 ± 1.93

Freezing effects on *Fucus distichus*

In each experiment, there was a pronounced reduction in effective quantum yield with decreasing temperature. A rapid decrease in temperature (experiment 1) from ambient to 0°C resulted in a marked reduction in yield values (Fig. 3a). After the water was completely frozen, a further reduction in temperature resulted in continuously decreasing yield values. At –12° C, quantum yield was reduced to 0.05 units. Subsequently, as soon as temperatures increased again, yield values also increased. The course of non-photochemical quenching (NPQ) followed a roughly reverse trend, with increasing NPQ at decreasing yield values. Temperature-dependent inhibition of photosynthesis and subsequent recovery was also observed when measuring Fv/Fm and ETRmax (Fig. 3b). Initial Fv/Fm values were 0.68, and at the lowest temperature, Fv/Fm was below 0.2. The recovery of Fv/Fm was not as rapid as the recovery of yield values, and a value of only 0.43 was reached. After a recovery of 36 h in low light and ambient temperature, initial values were reached again. Values for ETRmax showed followed an almost identical trend. However, the terminal value (43 relative units) was lower than the initial value (76 relative units), and had not recovered completely after 36 h, when 52 relative units were measured.

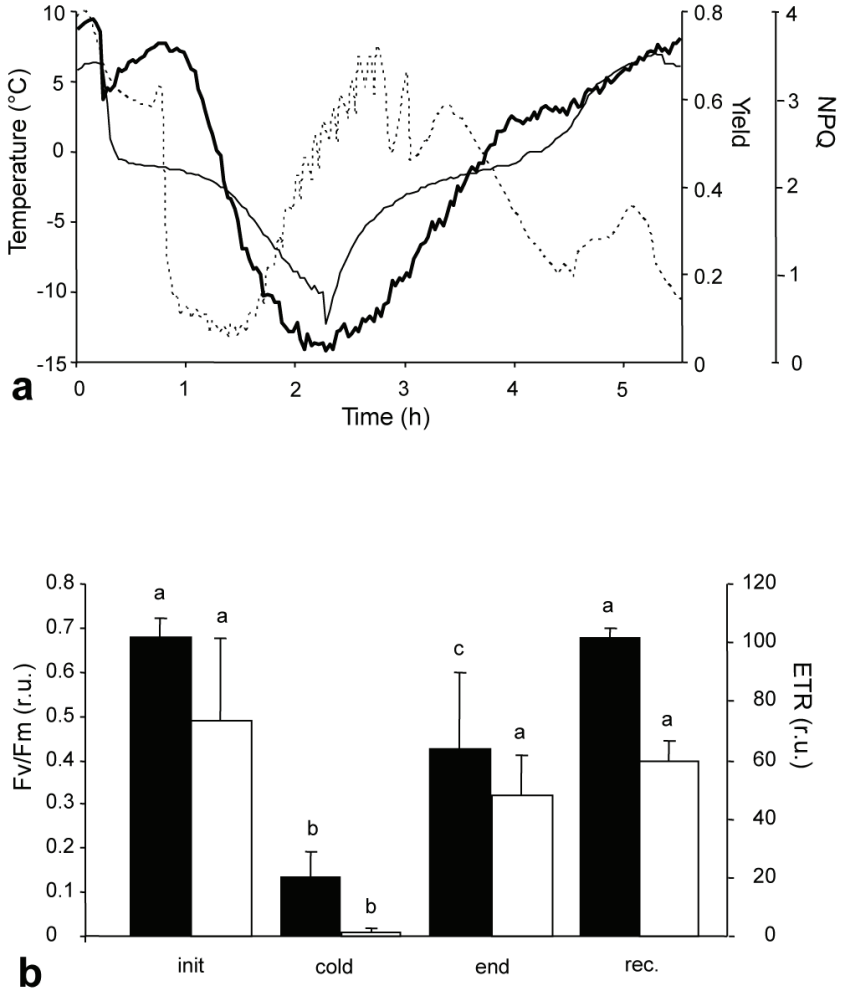

Fig. 3: *Fucus distichus:* Experiment 1 (fast and short freezing) with **a:** changes in temperature in °C (thin line), effective quantum yield (yield; bold line) and non-photochemical quenching (NPQ; dashed line) in relative units (r.u.) over time**; b:** optimum quantum yield (Fv/Fm; black bars) and maximum electron transport rate (ETRmax; white bars) measured after a short acclimation (init), at the lowest temperature (cold), at the end of the experiment (end), after a recovery of 36 h in dim white light and at fjord temperature (rec.). Different letters show statistically significant differences (Tukey's HSD $p < 0.05$). Values are means + SD (n=3)

In the second experiment, samples were subjected to freezing without immersion. A rapid and substantial decrease in yield values (Fig. 4a) was observed as soon as the temperature decreased below 0°C. When temperature further decreased, the yield

dropped to zero. However, yield increased again as soon as the temperature increased above –3°C, and recovered completely to initial values. Changes in NPQ in the course of the experiment were less pronounced than in the previous experiment, however, the general trend of increasing NPQ with decreasing yield was also observable.

Fv/Fm (Fig. 4b) decreased almost as strongly at the lowest temperature as in the previous experiment, but recovery proceeded faster than in the submerged specimens. Significant differences were found between the initial, cold and end values. The same trend was observed for ETRmax (Fig. 4b), which decreased down to a mean value of less than 10, and recovery of this parameter was rapid, although the initial value of ETRmax was not reached at the end of the experiment.

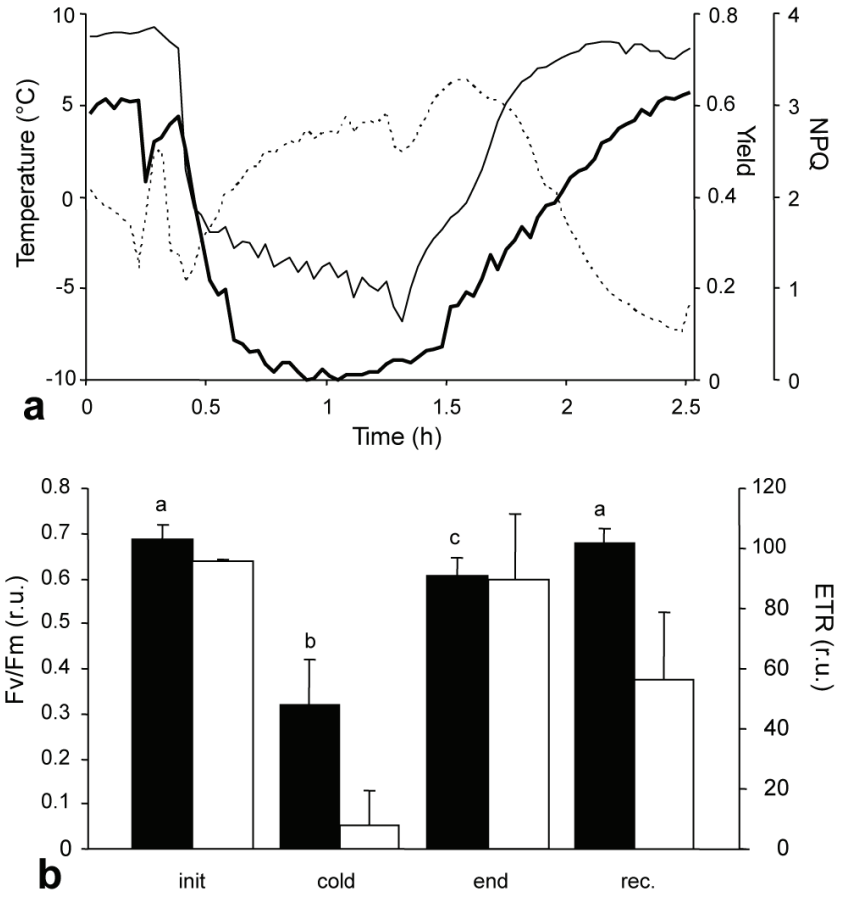

Fig. 4: *Fucus distichus:* Experiment 2 (dry freezing) with **a:** changes in temperature in °C (thin line), effective quantum yield (yield; bold line) and non-photochemical quenching in relative units (NPQ; dashed line) over time**;**

b: optimum quantum yield (Fv/Fm; black bars) and maximal electron transport rate (ETRmax; white bars) measured after a short acclimation (init), at the lowest temperature (cold), at the end of the experiment (end), after a recovery of 36 h in dim white light and at fjord temperature (rec.). Different letters show statistically significant differences for Fv/Fm values (Tukey's HSD $p < 0.05$). Values are means + SD (n=3). Due to limited sample size, no statistical analysis is available for ETRmax. r.u.: relative units

The results of the third experiment (long term freezing) are shown in Fig. 5. In this experiment, the temperature was maintained at –1.5 °C for almost 7 h. Yield values remained nearly constant at 0.6 (Fig. 5a). After almost 7 h of freezing, the yield decreased, probably due to an artefact while recording Fv/Fm and a PI-curve before moving samples into darkness. Subsequently, with an increase in temperature, the yield also increased. During subsequent exposure for 8 h at ambient temperature (approximately 4°C), yield values held at around 0.6 over the entire time period. The initial value of Fv/Fm (Fig. 5b) was 0.7 units and, by the end of the period of freezing, it decreased to 0.25. At the end of the experiment, the value was almost as high as the initial value. After a recovery time of 36 h, the initial value was reached again. ETRmax values decreased upon freezing and recovery proceeded more slowly than in experiments 1 and 2 (see fig. 5b). However, after a recovery period of 36 h, the values reached the the initial level.

Significant increases in low temperature-induced oxidative stress as determined by the MDA-assay were absent in all experiments (data not shown). Also, temperature related differences in D1-content were absent in all experiments (data not shown).

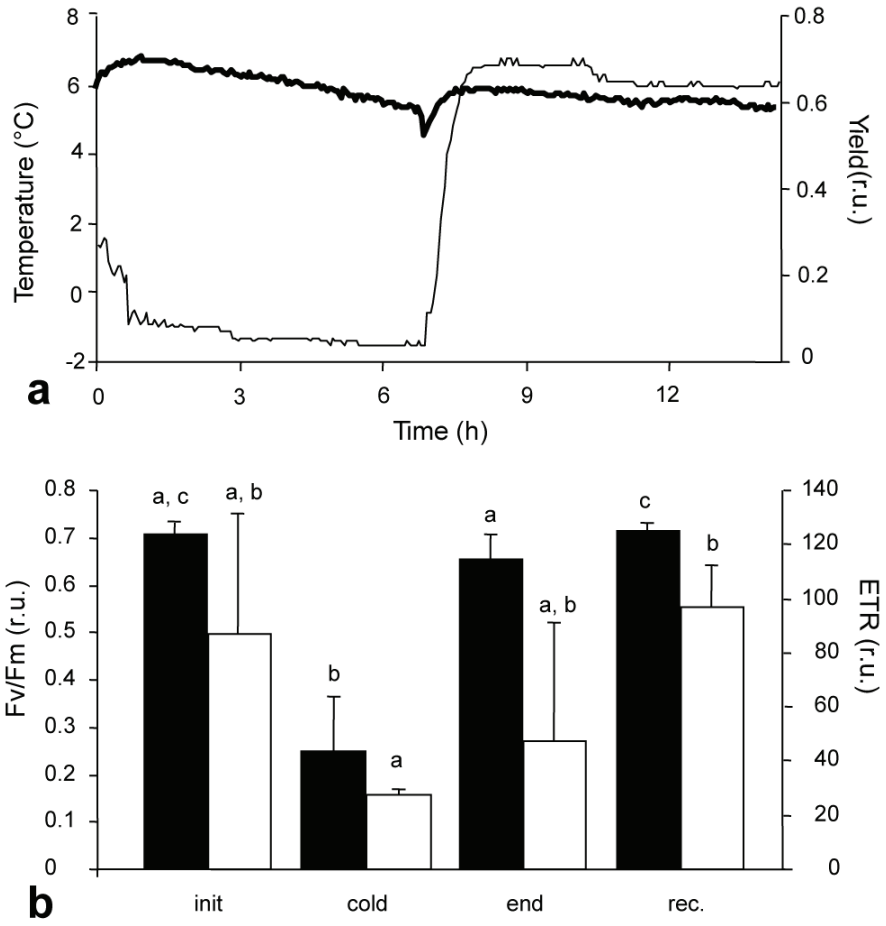

Fig. 5: *Fucus distichus:* Experiment 3 (long term freezing) **a:** changes in temperature (thin line) and effective quantum yield (yield; bold line) over time**; b:** optimum quantum yield (Fv/Fm) (white bars) and maximal electron transport rate (ETRmax) (white bars) measured after a short period of acclimation (init), at the lowest temperature (cold), at the end of the experiment (end), after a recovery of 36 h in dim white light and at fjord temperature (rec.). Different letters show statistically significant differences (Tukey's HSD $p < 0.05$). Values are means + SD (n=3). r.u.: relative units

Discussion

Low water temperatures in combination with high PAR intensities may severely stress algae by inhibiting photosynthetic performance and affect pigment content. This is thought to be due to the fact that high light at low temperature conditions slows enzymatic reactions with a consequent increased generation of reactive oxygen species. Furthermore *de novo* synthesis of previously degraded D1 protein and its reintegration into

the thylakoid membrane may be impaired by low temperatures due to reduced velocity of biosynthetic pathways and membrane fluidity (Aro et al., 1993).

Palmaria decipiens' photosynthesis was significantly impaired by high PAR irradiances in combination with low temperatures (see figures 1 and 2).

Although *P. decipiens* is thought to cope with high light intensities, since it may occur in Antarctic intertidal zones (Wiencke and Clayton, 2002), this combination of the specific experimental conditions was harmful. However, it is important to note that specimens used for the present experiments were collected in the subtidal and therefore may have been more sensitive than those inhabiting the intertidal. Specimens from the intertidal are thought to cope with high light conditions by down-regulating photosynthesis via dynamic photoinhibition (Demmig-Adams and Adams III, 1992). Hanelt et al. (1994) observed highest degrees of photoinhibition in Antarctic macroalgae floating near the water surface.

In our experiments, treatment of algal material under lower PAR intensities at 0°C had no negative influence on photosynthesis. These latter conditions are considered similar to those algae experience during Antarctic spring (Wiencke and tom Dieck, 1989, Klöser et al., 1993, Weykam and Wiencke, 1996). For instance, water temperatures rarely exceed +2°C in summer (Klöser et al., 1993, Wiencke, 1996) and favorable light conditions are available only immediately after sea-ice break up (Wiencke, 1996, Lüder et al., 2001). Based on our own light measurements conducted in Potter Cove, irradiances of about 200 and 400 µmol photons m^{-2} s^{-1} may be recorded down to water depths of 6 m and 4 m, respectively (calculated after Kirk, 1994) during summer conditions in February.

The lower distributional limit of *Palmaria decipiens* is set by a metabolic carbon fixation rate between 0.6 and 0.8 mg C g^{-1} FW d^{-1}, which allows growth to a maximum depth of 30 m (Wiencke et al., 2007 and references therein). Algae benefit from most favorable light conditions immediately after sea ice break up, when light intensities of up to 70 µmol m^{-2} s^{-1} are measured at 30m depth (Gómez et al., 1997). Like most of the Antarctic endemics,

P. decipiens is considered to be strongly shade adapted (Wiencke et al., 1993, Weykam et al., 1996, Weykam and Wiencke, 1996, Eggert and Wiencke, 2000) and has highest photosynthetic activity during spring (Weykam and Wiencke, 1996). However, temperature is thought to be the most important factor controlling the distribution of *P. decipiens* (Wiencke et al., 2007), as growth is suppressed at temperatures >10°C (Wiencke and tom Dieck, 1989), and optimum growth temperatures are between 0°C and 5°C (Bischoff-Bäsmann and Wiencke, 1996).

This illustrates the importance of our study on the interaction effects (compared to the previous single factor studies); even under favorable temperature conditions, light may easily become a crucial factor. As hypothesized, higher PAR levels generated photoinhibitory conditions at lower temperatures. However, in shallow waters and even in the intertidal, specimens are exposed to higher irradiances. Therefore, there is still a strong need to explore the underlying acclimation mechanisms under high light and low temperature stress in more detail. Our study also supports the concept that higher temperatures, even those close to the tolerance limit, may compensate for photoinhibition. Similar results have been obtained in Antarctic green macroalgae under PAR and UV radiation stress (Rautenberger and Bischof, 2006).

Palmaria decipiens is considered to be a season anticipator (Wiencke, 1990, Weykam and Wiencke, 1996), and thus has a life cycle perfectly adapted to Antarctic conditions. It starts growing under very low light conditions and daylength seems to be the trigger (Wiencke, 1990, Wiencke, 1996, Lüder et al., 2001, Wiencke et al., 2007). Its photosynthetic rhythm coincides with the production of pigments, which increase during spring (Lüder et al., 2001, 2002). Future studies should thus also focus on seasonal variation in species light requirements and temperature tolerance.

In our study on *Fucus distichus*, there were significant reductions in both optimum and effective quantum yield in response to decreasing temperatures. At the lowest temperatures applied, photosynthetic activity was completely halted (Fig. 3a, 4a). However, after release from freezing, quantum yield recovered almost completely, indicating that no persistent damage to the photosynthetic apparatus was induced. Rather, there may have been a down regulation of quantum yield in order to protect photosynthesis from increased production of reactive oxygen species (ROS). The ability to recover quickly from photoinhibition is referred to as “dynamic photoinhibition”, *sensu* Osmond (1994), or more recently as “photoprotection” (Franklin et al., 2003). This fast response might be part of a physiological protection strategy in a highly variable environment, such as the intertidal zone.

In *Fucus serratus* Linnaeus Huppertz et al. (1990) demonstrated a decline in Fv/Fm values as well as in oxygen production during low tide accompanied by high light intensities and a fast recovery as soon as thalli were submerged again. Dynamic photoinhibition is usually associated with an increase in non-photochemical quenching mediated by an increased activity of the xanthophyll-cycle (Demmig-Adams and Adams III, 1992), also operative in brown algae (Hanelt et al., 2003). Our results with *F. distichus* also showed an increase in NPQ when low temperatures induced a reduction in quantum yield (Fig. 3a and 4a), which might at least indicate that the above mentioned mechanisms of dynamic photoinhibition may also be deployed upon freezing as well. In accordance with the strict temperature dependence of light saturated photosynthesis, changes in ETRmax are more strongly pronounced than of Fv//Fm, as recovery from cold stress usually took longer.

Apart from the reduced activity of dark reactions, the respective concentration of D1-protein may have affected photosynthetic capacity upon freezing. However, no differences in D1-content were found in our studies. Similarly, only minor changes in ROS-mediated MDA formation were induced by our experimental treatments, which might either indicate

the high capacity of *Fucus* to protect itself from radical formation or that impinging radiation conditions were too low to induce oxidative stress.

In general, frost and drought have similar physiological effects (Pearson and Davison, 1994). In our experiments, the combination of drought and frost had no stronger effects on algal physiology than either factor alone. However, the effect of drought was less important at low temperatures because frozen water is not able to evaporate. Overall, the study showed that *Fucus distichus* is able to withstand temperatures down to –12° C and also the combination of drought and freezing for about 3 h without long-term damage to photosynthesis. Under field conditions in the intertidal zone of Svalbard, algae are frequently exposed to freezing temperatures during emergence at low tide (Hop et al., 2002, Svendsen et al., 2002).

The ability of *Fucus distichus* to rapidly down regulate photosynthesis under freezing and desiccating conditions and subsequently achieve full photosynthetic performance under submerged conditions is a major component of physiological adaptation to Arctic intertidal conditions. Curiously, in a related study on the effects of freezing, desiccation and high light stress on three different species of *Fucus* (*F. spiralis*, *F.evanescens*, *F. distichus*), *F. distichus* was shown to be the most sensitive species under investigation, responding to the experimental treatments with elevated production of ROS (Collén and Davison, 1999). However, in the latter study, an experimental irradiance of about 1600 µmol photons m^{-2} s^{-1} was applied, which is above the maximum irradiance encountered at Svalbard during summer (Svensen et al., 2002). Therefore, it might still be possible that in combination with very high PAR, *F. distichus*, as a species from high northern latitudes, becomes sensitive to drought and freezing.

Summing up, our study indicates that stenothermal *Palmaria decipiens* is well adapted to the current abiotic conditions encountered in Antarctica. Due to the long and stable cold-water history of Antarctica, its endemic species have developed suitable long-term

adaptations. However, in combination with the previous findings on the alga´s temperature tolerance, we propose that there is a very narrow range for its optimal physiological functioning; this may mean that this species will be sensitive to future environmental change. In contrast, as also indicated by its wide distributional range *Fucus distichus* is not likely to be impaired by increasing temperatures.

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Photosynthesis and lipid composition in the Antarctic endemic rhodophyte *Palmaria decipiens*: Effects of changing light and temperature levels

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Photosynthesis and lipid composition of the Antarctic endemic rhodophyte *Palmaria decipiens:* Effects of changing light and temperature levels

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Abstract

In coastal waters, Antarctic rhodophytes are exposed to harsh environmental conditions throughout the year, like low water temperatures ranging from -1.8°C to 2°C and high light during the summer season. Photosynthetic performance under these conditions may be affected by slowed down enzymatic reactions and the increased generation of reactive oxygen species. The consequence might be a chronic photoinhibition of photosynthetic primary reactions related to increased fragmentation of the D1 reaction centre protein in photosystem II. It is hypothesized that changes in lipid composition of biomembranes may represent an adaptive trait to maintain D1 turnover in response to temperature variation. The interactive effects of high light and low temperature were studied on an endemic Antarctic red alga, *Palmaria decipiens*, sampled from two shore levels, intertidal and subtidal, and exposed to mesocosm experiments using two levels of natural solar radiation and two different temperature regimes (2-5°C and 5-10°C). During the experimental period of 23 days, maximum quantum yield of photosynthesis decreased in all treatments, with the intertidal specimens exposed at 5-10°C being most affected. On the pigment level a decreasing ratio of phycobiliproteins to chlorophyll a was found in all treatments. A pronounced decrease in D1 protein concentration occurred in subtidal specimens exposed at 2-5°C. Marked changes in lipid composition, i.e. the ratio of saturated to unsaturated fatty acids, indicated an effective response of specimens to temperature change. Results provide new insights into mechanisms of stress adaptation in this key species of shallow Antarctic benthic communities.

Abbreviations

analysis of variance, ANOVA; fatty acid, FA; maximal electron transport rate, ETRmax; maximum quantum yield, Fv/Fm; photosynthetically active radiation, PAR; photosystem II, PS II; photosynthesis-irradiance curve, PI-curve; poly-unsaturated fatty acid, PUFA; ultraviolet radiation, UV radiation;

Introduction

Antarctic coastal ecosystems are characterized by low temperatures throughout the year (Wiencke and tom Dieck 1989), and intertidal organisms may be exposed to extreme subzero temperatures during low tides. This temperature regime has evolved by large-scale atmospheric and hydrographic conditions within the Antarctic convergence (Wiencke and tom Dieck 1989 and references therein). Organisms inhabiting the intertidal and shallow subtidal zones of Antarctic shores have to be strongly adapted to this regime, which is additionally characterized by a high seasonality of light availability. In particular sessile organisms may strongly rely on effective physiological adaptation mechanisms related to this specific abiotic environment.

In general, low temperatures slow down enzymatic reactions and synthetic pathways, and result in a decrease in membrane fluidity (Gurr et al. 2002; Peterson et al. 2007). To photosynthesizing organisms, as e.g. benthic macroalgae in Polar regions, the combination of low temperature and high radiation conditions is particularly challenging: because of reduced electron drainage as a result of the slowed down activity of Calvin cycle enzymes, the generation of reactive oxygen species may increase. Under these circumstances, the degradation of the D1 reaction centre protein of photosystem II is promoted (Aro et al. 1993; Bischof et al. 1999; Aro et al. 2005). D1 protein is characterized by a rapid and permanent turnover, which might become unbalanced under high radiation stress. A limiting factor in the reintegration of de-novo synthesized protein into the PS II reaction centres is the velocity of lateral diffusion through the thylakoid membrane (Aro et al. 2005). In this process, membrane fluidity represents the determinant factor, which is highly controlled by temperature. In particular low temperatures induce a number of alterations in cellular components, including the extent of fatty acid unsaturation the composition of glycerolipids, the positional redistribution of saturated and unsaturated fatty acids within lipid molecules, changes in the lipid/protein ratio, and activation of ion channels (Guschina and Harwood 2006, and reference therein). At a given temperature, membrane fluidity is determined by the respective degree of unsaturation of fatty acids.

It is generally accepted that in organisms from cold environments, membranes exhibit a higher amount of unsaturated fatty acids to maintain membrane fluidity

(Harwood 1994; shown for cyanobacteria and higher plants by Murata and Los 2007). However, studies on fatty acid composition in Polar macroalgae (e.g. Graeve et al. 2000) and especially studies addressing how the respective composition of fatty acids may be changed under varying conditions are scarce.

Especially species endemic to Antarctica as the red macrophyte *Palmaria decipiens*, should exhibit effective modes of adaptation to their environment, resulting in very low upper survival temperatures and a strong degree of shade adaptation (Wiencke et al. 1994; Bischoff-Bäsmann et al. 1996). Since physiological characteristics of *P. decipiens* have been gathered in the past, for instance regarding temperature and growth patterns (Wiencke and tom Dieck 1989; Wiencke 1990), pigment content (Lüder et al. 2001; 2002) or fatty acid composition (Graeve et al. 2002) and the fact that *P. decipiens* represents a dominant macroalgal species densely populating the intertidal and shallow subtidal along the Antarctic Peninsula (e.g. Quartino et al. 2005), the alga is thus considered an ideal candidate endemic to Antarctica to study mechanisms of adaptation.

Moreover, studies on radiation and temperature interactions, i.e. combined factors in general, in field experiments on Antarctic macroalgae are important to predict more precisely the effects of environmental changes on this very susceptible ecosystem. We conducted mesocosm experiments on King George Island (South Shetland Islands, Antarctica) exposing adult thalli of *Palmaria decipiens* from different shore levels to different temperatures in combination with the natural solar radiation. By measuring photosynthetic parameters such as maximum quantum yield (Fv/Fm) and recording photosynthesis versus irradiance-curves, as well as analysing changes in pigment composition, D1 protein content and fatty acid composition we investigated physiological responses to the combination of two (stress-) factors to test the following hypothesis: (I) subtidal specimens will exhibit higher susceptibility towards higher irradiances and elevated temperatures, leading to a decrease in D1 protein content and (II) intertidal specimens will maintain photosynthetic integrety and/or adjustment of fatty acids will occur. This will shed light to general mechanisms of adaptive traits of a dominant Antarctic endemic macroalgae.

Material and Methods

Study site and experimental set-up

The experiment was conducted during February and March 2008 at the Dallmann Laboratory/Jubany Base, King George Island, Antarctica (62°14'S, 58°40'W). A detailed description of the physical and biological environment of the study site located at Potter Cove is provided by Wiencke et al. (2008). Specimens of the endemic pseudoperennial rhodophyte (as decribed by Wiencke and Clayton 2002) *Palmaria decipiens* (Reinsch) RW Ricker (1987) were collected in the intertidal area of Peñon Uno during low tide conditions in about 80 cm depths. Twenty specimens, approximately 40 to 50 cm in size, were brought back to the station covered by water and black foil to avoid drought and light stress during transportation. Twenty subtidal specimens equal in size as intertidal specimens were collected by scuba diving in front of the Fourcade glacier in 8 to 10 m water depths and transferred to the station. Algal material was cleaned and cut into square-shaped fragments of about 15x25 cm, taken from the mid-thallus part. These fragments were exposed to the following conditions: two mesocosms (100x200x35 cm plastic tanks) were installed at the coastline in front of the station. A submersed seawater pump provided a constant flow of natural seawater to the tanks. In cases of extreme low tide or ice scoring the pump was removed occasionally. One of these tanks was temperature controlled by a cryostat to provide temperatures between 5 to 10°C whereas the second one maintained ambient water temperatures between 2 to 5°C. Temperature was monitored by underwater temperature loggers (Testo 177-T2, Lenzkirch, Germany) and salinity was checked with a handheld refractometer (Atago S-10E, Tokyo, Japan). Within these tanks three water-permeable plastic cages were installed for each set of specimens from different shore levels serving as the experimental unit. Algal material was exposed to the ambient light conditions during the exposure of 23 days with the subtidal specimens being covered with black net gauze, to prevent excessive high light stress and thus, to ensure survival. Light measurements were conducted in air with a LiCor 1400 Data Logger equipped with a flat-head cosine corrected PAR quantum sensor (LICOR 190 SA, Li-Cor, Lincoln, NE, USA) at least four times each day and always at noon. In the course of the experimental exposure, mean maximum irradiance of PAR reached 1516 µmol photons m^{-2} s^{-1} in air and a

mean minimum of 315 µmol photons m^{-2} s^{-1} was recorded. Total maximum PAR reached a value of 2068.3 µmol photons m^{-2} s^{-1} in February 2008. Detailed radiation conditions are given in table 1. Comparing the radiation condition in the tanks to the respective *in situ* conditions, minimum PAR was consistent with the irradiance intertidal specimens experience during low tide, and maximum PAR represented a four-fold increase compared to irradiance at 2 m water depths during high tide. For subtidal specimens, minimum PAR was nine times higher than at 8 m depths. Transferring especially subtidal specimens to shallow water depths leads to an additional shift not only within the PAR intensity but also changes the spectral radiation conditions in general, namely increasing UV-radiation intensities. We are aware of these changes, but our study focusses on possible different reactions of specimens of two shore levels to changing temperature and PAR levels.

Table 1: PAR (photosynthetically active radiation) radiation measurements in µmol photons m^{-2} s^{-1} taken in air throughout the duration of mesocosm experiment with a LiCor 1400 data logger equipped with a flat-head cosine corrected sensor (Li-190SA, LiCor). Values presented are measurements taken at 12:00 local time King George Island, South Shetland Islands, as well as mean daily radiation (n=10 per measurement, min. of n=4 per day).

	µmol photons m^{-2} s^{-1}	
Date	PAR at noon	Mean daily PAR
26.01.2008	1333.9	958.5
28.01.2008	270.7	315.6
30.01.2008	143.3	508.4
01.02.2008	706.8	798.3
03.02.2008	637.5	356.8
05.02.2008	266.1	244.3
07.02.2008	988.7	1029.4
09.02.2008	1492.4	1110.6
11.02.2008	1456.5	887.9
13.02.2008	437.3	520.2
15.02.2008	461.8	404.2
17.02.2008	1059.0	1186.2

Samples for photosynthetic measurements were taken every second day before noon (10 am to 11:30 am local time), and photosynthetic measurements were conducted immediately. For sampling, pieces of approximately 50 mm in diameter were randomly cut out of the exposed fragments and transferred in darkness to the laboratory. After measurements, samples were dried carefully, frozen in liquid nitrogen and stored at -80°C for later biochemical analysis. Samples for fatty acid analysis were lyophilised (Lyovac GT2 using a AMSO/FINN-AQUA pump) for 48 h and then kept dry at room temperature.

Photosynthetic measurements

Photosynthetic measurements such as the determination of maximum quantum yield of photosystem II (Fv /Fm) and photosynthesis vs. irradiance-curves (PI-curves) were conducted using a pulse amplitude modulated chlorophyll fluorometer (PAM 2100, Walz, Effeltrich, Germany) following the protocol by Hanelt et al. (1997) and Bischof et al. (1998) with pre-darkened samples. PI-curves were conducted by using the internal LED as light source emitting irradiances of 21.6 to 630.3 µmol photons m^{-2} s^{-1} PAR. According to Schreiber et al. (1994), relative electron transport rate (ETR) was calculated as the product of the respective effective quantum yield and photon fluence rate. Subsequently, ETRmax was determined by curve fitting after Jassby and Platt (1976). The maximum quantum yield (Fv/Fm) indicates the photosynthetic efficiency and thus, physiological performance of the alga, which might be effected by high light intensities or stress factors in general. Relative ETRmax is indicative for photosynthetic capacity and give hints in cases of chronic photoinbition and thus, effects on D1 protein (see Schreiber et al. 1994 for details; Bischof et al. 1998).

Biochemical analysis

The phycobiliprotein content was determined after Beer and Eshel (1985). Frozen sample material was ground in liquid nitrogen and transferred into 100 mM phosphate buffer and extraction of phycobiliprotein content was accomplished after 20 min. of centrifugation. The supernatant was measured spectrophotometrically and equations of Beer and Eshel (1985) were used. Chlorophyll a content was determined after Lüder et al. (2002) using the modified protocol of Inskeep and

Bloom (1985). Frozen samples were transferred into five ml of N,N-dimethylformamid and kept at 4°C for four days in the dark. Chlorophyll a concentration was calculated by measuring the supernatant spectrophotometrically (UV-2401 PC, Shimadzu) and by using the equation of Lüder et al. (2002).

Changes in the concentration of D1-protein of photosystem II were determined by SDS-PAGE and subsequent Western Blotting, according to Bischof et al. (2000), using a D1 specific primary antibody (AS 01016 Chicken Anti PsbA, Agrisera, Vännäs, Sweden) and ab6754, Abcam, for secondary immunodecoration (Cambridge, United Kingdom).

For fatty acid analyses a defined amount of lyophilized algal biomass was homogenized and extracted in dichloromethane: methanol (2:1, v/v) following the method described by Folch et al. (1957). Prior to extraction, an internal standard was added (23:0 FAME) and the samples were crushed by ultrasonification. For gas liquid chromatographic analysis of the fatty acids, methyl esters were prepared from aliquots of the extracted algae by transesterification with 3% concentrated sulfuric acid in methanol for 4 h at 80°C. After extraction with hexane, fatty acid methylesters (FAME) were analyzed with a gas–liquid chromatograph (HP 6890, Hewlett-Packard GmbH, Waldbronn, Germany) on a capillary column (30 m x 0.25 mm I.D.; film thickness: 0.25 µm; liquid phase: DB-FFAP, J&W, Cologne, Germany) using temperature programming (Kattner and Fricke 1986). FAMEs were identified by comparison with known standard mixtures. If necessary, identification of FAMEs was confirmed by GC-MS measurements. The total lipid concentration refers to the sum of total fatty acids methyl esters (TL).

Data treatment

Experiments were set-up as a split-plot design with three replicates for each treatment. Mean values and standard deviations and for values given as ratios and percentages standard errors were calculated per treatment. Photosynthetic data (Fv/Fm) and percentages were arcsine transformed and a two-way ANOVA was performed for the two factors depth and temperature and their interactions. Statistically significant differences were tested separately with Tukey-Kramer HSD

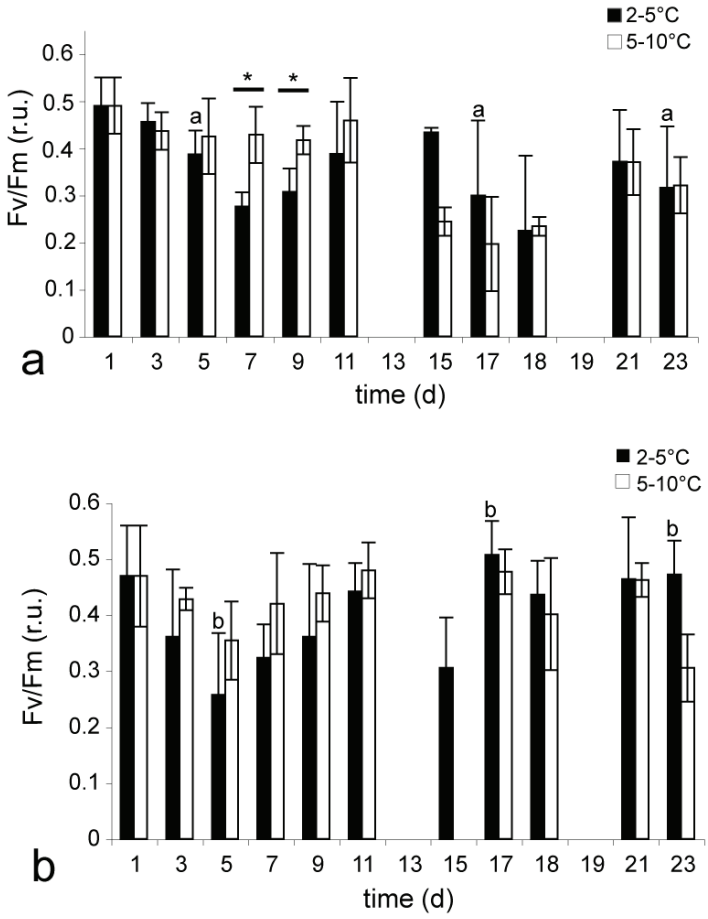
(honestly significant different) posthoc test with $p<0.1$ according to Sokal and Rohlf (1995). For more details, p-values are indicated where applicable. Statistical analyses were performed using the JMP 6.0 software (SAS, Cary, NC, USA).

294 Results

295

296 In the course of the experiment, maximum quantum yield of photosystem II (Fv/Fm)
297 exhibited little variation over time, however, without a consistent pattern (Fig. 1). In
298 the intertidal as well as in the subtidal specimens, initial Fv/Fm values of 0.47 relative
299 units were recorded. Slightly higher values were observed in the 5-10°C treatment
300 during the first 13 days of exposure. During the ongoing exposure, only a mixed
301 pattern was found, however, with on average higher values for subtidal specimens
302 (approx. 0.4 relative units) than for intertidal specimens (0.3 relative units). This
303 pattern was found for both temperature treatments (see fig. 1a and b). Post-hoc
304 analysis showed significant temperature effects only on day seven and nine for
305 intertidal specimens. Significant differences between the two depths were recorded
306 on days 5 (p=0.0878), 17 (p=0.0136), 18 (p=0.0417) and 23 (p=0.0334).

307



308

309 **Fig. 1 a:** Maximum quantum yield (Fv/Fm) in the course of exposure time (in days) at two
310 temperatures (2-5°C, black dots and 5-10°C, white dots) in specimens of intertidal *Palmaria decipiens*
311 **b:** subtidal specimens at the same temperature treatments as above. Values are means of triplicate
312 measurements, bars show standard deviations. Asterisks represent significant differences between

temperature treatments in specimens from the same shore level, different letters show significant differences in specimens from different shore levels within the same temperature treatment. r.u.: relative units

A similar pattern was observed for ETRmax values (see figure 2a and b). Values for intertidal *P. decipiens* specimens decreased from an initial of 110 relative units to a final value of only 35 relative units without any significant difference between temperature treatments. ETRmax of subtidal specimens showed less variation during the exposure and decreased only slightly from an initial of 70 relative units to about 40 relative units (see fig. 2b), exhibiting a significant temperature effect on day 21 (p=0.0302).

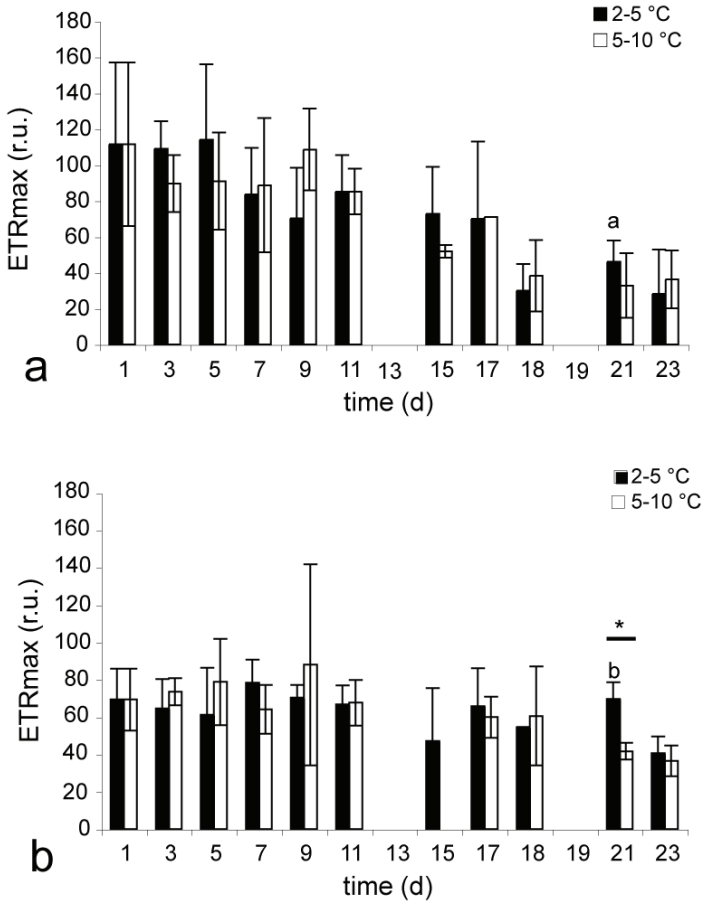


Fig. 2 a: Relative maximum electron transport rate (ETRmax) in the course of exposure time (in days) at two temperatures (2-5°C, black dots and 5-10°C, white dots) in specimens of intertidal *Palmaria decipiens* **b:** subtidal specimens at the same temperature treatments as above. Values are means of triplicate measurements, bars show standard deviations. Asterisks represent significant differences between temperature treatments in specimens from the same shore level, distinct letters represent significant differences in specimens from different shore levels in the 2-5°C-treatment. r.u.: relative units

The results of pigment analyses are presented as the ratio of phycobiliproteins to chlorophyll a and revealed a decrease in pigment concentration for specimens of both depths (see figure 3). In intertidal specimens the initial ratio decreased by 30% within the 2-5°C and by 75% at 5-10°C, respectively. For subtidal specimens an even more pronounced decline was observed: initial values decreased by 85% in the colder and by 79% in the warmer treatment. However, no significant differences were observed (p=0.1986 and p=0.6471, respectively). Total concentrations of chlorophyll a and phycobiliproteins decreased also, with a slightly more pronounced decline in subtidal specimens at 5-10°C (data not shown).

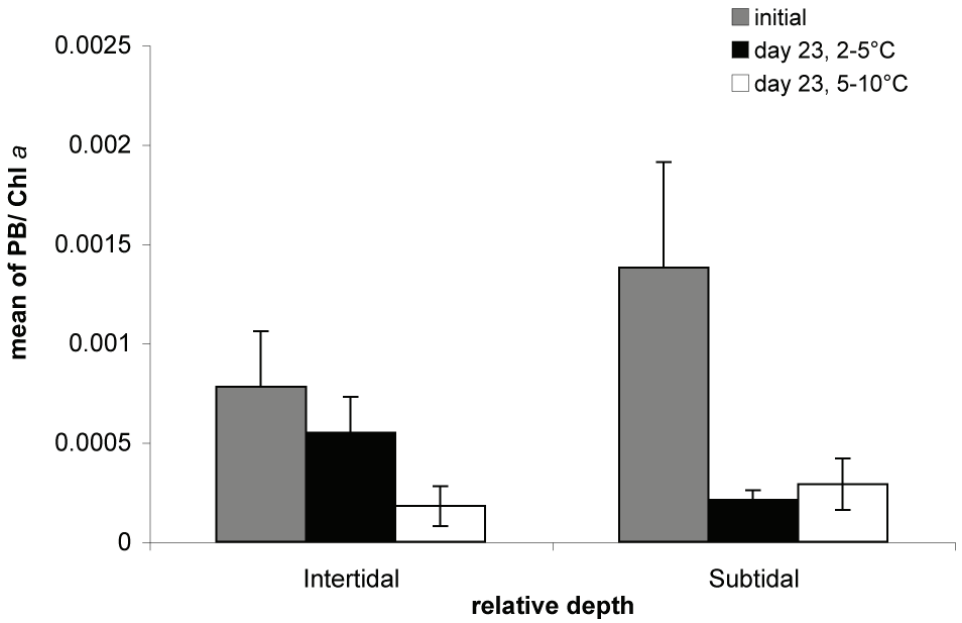


Fig. 3 Ratio of phycobiliproteins to chlorophyll a content in *Palmaria decipiens* from two different shore levels. Bars represent initial values (grey) and final values after exposure for 23 days at two different temperatures (2-5°C, black and 5-10°C, white). Values are means of triplicate measurements with standard error bars. No statistical significant differences were observed (p>0.1).

The results for D1 protein concentration are shown as the mean percentage of the initial value for both temperature treatments after 23 days of exposure (see figure 4). For intertidal specimens of *P. decipiens* no significant differences were found as values remained almost constant during exposure at both temperatures. In subtidal specimens, no differences between initial D1 concentration and values measured in specimens kept at 5-10°C were found (p>0.1). In specimens exposed to lower temperatures, the protein concentration decreased significantly to about 68% of the initial value (p=0.0384, one-way analysis with Tukey HSD post-hoc test).

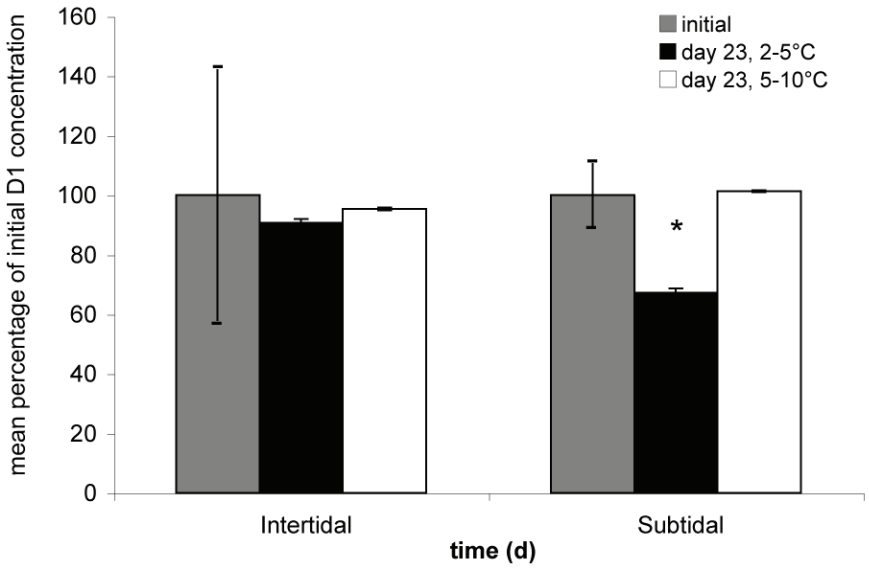


Fig. 4 Changes in D1 protein concentration of *Palmaria decipiens* from two different shore levels, given as the percentage of the initial value. Bars represent initial values (grey) and final values after exposure for 23 days at two different temperatures (2-5°C, black and 5-10°C, white). Values are means of triplicate measurements with standard error bars. Asterisk represents significant difference within the depth.

Changes in fatty acid (FA) composition are presented as mean ratio of saturated (i.e. no double bond) to unsaturated fatty acids (i.e. monounsaturated with 1 double bond and polyunsaturated with 2 or more double bonds) (see figure 5a). A detailed list of all fatty acids is shown in table 2 for subtidal specimens and in table 3 for intertidal specimens. Overall, 32 different FA were detected in this study, with 14:0, 15:0, 16:0, 18:0 and 20:0 as saturated FA. In total, the 16:0 fatty acid was the most abundant saturated FA and 20:5(n-3) the most abundant unsaturated FA. During the exposure of subtidal specimens, amounts of total lipid content remained at around 3.2 µg/ mg dry weight (see table 2). Within the initial samples of subtidal specimens of *P. decipiens*, the 16:0 FA was the most abundant saturated FA. During the exposure of 23 days, this pattern did not change at 5-10°C. At 2-5°C the most abundant FA after the exposure was 18:0. Highest amounts of unsaturated FA were detected for 20:5(n-3) with 0.501 µg/mg dry weight, followed by 18:1(n-9) with 0.466µg/ mg dry weight, 18:2(n-6) with 0.064 µg/ mg dry weight and 18:1(n-7) with 0.057 µg/ mg dry weight (see table 2). Apart from 20:5(n-3) all amounts increased during the exposure, but nevertheless did not reach values as high as 20:5(n-3).

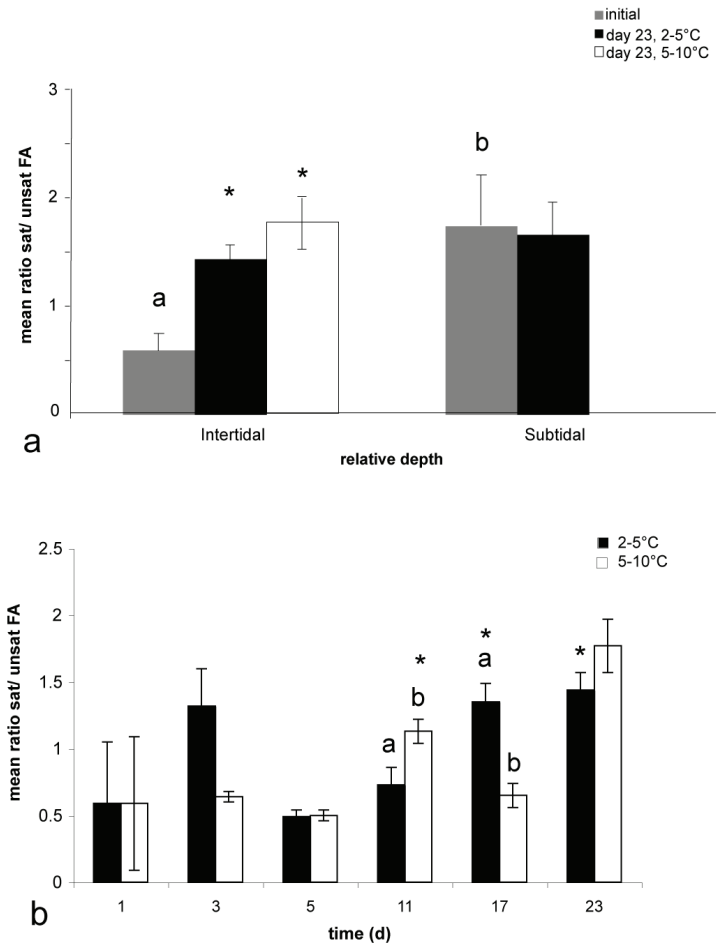


Fig. 5 a: Fatty acid composition of *Palmaria decipiens* from two different shore levels, presented as the ratio of saturated to unsaturated fatty acids. Asterisks show significant differences in comparison to initial values within the respective depth, different letters indicate significant differences between initial values of both shore levels. Note that due to sample limitation no values for subtidal specimens exposed to 5-10°C after 23 days are available **b:** Fatty acid composition of intertidal *P. decipiens* versus exposure time in days. In both cases, bars represent initial values (grey) and final values after exposure for 23 days at two different temperatures (2-5°C, black and 5-10°C, white). Values are means of triplicate measurements with standard error bars. Bars marked with asterisks represent significant different FA composition within a temperature treatment to the respective initial ratio, different letters mark significant differences between temperature treatments on the respective sampling days.

In intertidal specimens a decrease in total lipid content was found during the exposure in both temperature treatments: initial values of 7.9 µg/ mg dry weight decreased to values of 3.3 µg/ mg dry weight and 3.0 µg/ mg dry weight, respectively (see table 3). In intertidal specimens a significantly increased amount of saturated fatty acids in comparison to the initial value was found in both temperature treatments (Tukey HSD posthoc with $p < 0.05$), especially regarding 16:0 fatty acid

where initial values of 1.794 µg/ mg dry weight increased to amounts of 18.764 µg/ mg dry weight and 18:0 with intial values of 0.167 µg/ mg dry weight which increased to values of 1.753 µg/ mg dry weight (see table 2). Values increased in the cold treatment from an initial ratio of 0.59 to 1.44, and to even slightly higher ratios in the warm treatment to 1.77 by the end of the exposure. Initial ratios of saturated to unsaturated FA differed significantly between specimens of the two shore levels (Tukey HSD posthoc with $p<0.05$, see fig. 5a). Intertidal specimens prevailed had a higher amount of unsaturated FA. When regarding the ratios of saturated to unsaturated FA, specimens of both shore levels exhibited elevated amounts of PUFAs during the exposure (see table 2 and 3).

Table 2: Fatty acid content (µg/mg dry weight) of subtidal specimens of *Palmaria decipiens* exposed to two different temperatures (2-5°C and 5-10°C) at ambient light conditions prevailing at Potter Cove between January and February 2008. Values of FA are given in (µg) and are means of three replicates with standard deviation (± SD). Total lipid (TL) is given in (µg/ mg dry weight). n.d. = not detected. Due to sample limitation, no FA data is available for specimens exposed to 5-10°C.

Fatty acid	Initial	Day 23 (2-5°C)
14:0	0.420 ± 0.05	0.274 ± 0.11
15:0	0.024 ± 0	0.009 ± 0.01
16:0	1.170 ± 0.02	1.227 ± 0.37
16:1(n-7)	n.d.	0.030 ± 0
16:2(n-4)	n.d.	n.d.
16:3(n-4)	n.d.	n.d.
16:4(n-1)	n.d.	0.006 ± 0.01
18:0	0.466 ± 0.18	0.326 ± 0.4
18:1(n-9)	0.279 ± 0.02	0.384 ± 0.39
18:1(n-7)	0.057 ± 0.02	0.140 ± 0.11
18:2(n-6)	0.064 ± 0.01	0.185 ± 0.28
18:3(n-6)	n.d.	n.d.
18:3(n-3)	n.d.	0.011 ± 0.02
18:4(n-3)	n.d.	n.d.
20:0	0.017 ± 0.01	0.024 ± 0.04
20:1(n-9)	0.076 ± 0	0.041 ± 0.02
20:1(n-7)	n.d.	n.d.
20:3(n-6)	n.d.	n.d.
20:4(n-6)	0.016 ± 0	0.010 ± 0.02
20:4(n-3)	n.d.	n.d.
20:5(n-3)	0.501 ± 0.32	0.485 ± 0.41
22:1(n-9)	0.092 ± 0.01	0.051 ± 0.01
22:1(n-7)	n.d.	0.058 ± 0.09
22:5(n-3)	n.d.	n.d.
22:6(n-3)	n.d.	n.d.
TL (µg/mg)	3.2 ± 0.21	3.33 ± 1.46

A second analysis on intertidal specimens was performed in order to test for the course of fatty acid composition over time. Therefore, samples additionally collected on day 3, 5, 11 and 17 were analysed, indicating an acceleration of fatty acid

turnover after day 11 of exposure (see figure 5b). A significant temperature effect was detected on day 11 ($p=0.0241$) and day 17 ($p=0.0192$, ANOVA with subsequent Tukey HSD posthoc test). Within the 2-5°C temperature treatment, FA composition differed significantly from the initial value on days 17 ($p=0.0107$) and 23 ($p=0.0152$), in the warmer treatment on day 11 ($p=0.00243$).

Discussion

In the study presented the endemic Antarctic macroalga *P. decipiens* exhibited a flexible photosynthetic response to experimental treatments confirming the high degree of physiological adaptation to the range of environmental variables at their growth site and indicating that changes in fatty acid composition may play an important role in the acclimation process. The experiment was designed to expose specimens to a more natural range of environmental parameters in the field (Wiencke et al. 2008) underlining the ecological relevance of findings in contrast to laboratory experiments, which tend to exceed the typical range of abiotic factors. Although one might be surprised that 5-10°C water temperatures can be reached in Antarctica, Abele et al. (1999) have shown temperatures up to 8°C in tide pools on King George Island and even temperatures as high as 18°C were measured in the area of Gerlach Strait (Gustavo Ferreyra, personal communication). However, we are very well aware that a complete picture, including the whole variety of abiotic as well as biotic parameters occurring *in situ*, could not be drawn by our experimental setup. Nevertheless, as we focus on physiological responses to changing environmental factors the design seems suitable to address our questions.

Despite minor variances and only few consistent differences in photosynthetic responses were observed related to the experimental temperature treatments applied, the importance of light/temperature interactions is indicated by our data: On average, maximum quantum yield as well as ETRmax were exhibiting slightly higher values by the end of exposure to higher temperatures. This might be indicative for a compensation of photoinhibition by elevated temperatures, as previously demonstrated for *Laminaria* species and subantarctic green algae, respectively (Bruhn and Gerard 1996; Rautenberger and Bischof 2006). Due to a temperature-mediated elevated activity of photosynthetic secondary reactions, electrons might be

459 drained off more efficiently from the electron transport chain, thus reducing the
460 likelihood for photoinhibition. Due to the primary effect of temperature on enzymatic
461 reactions, ETRmax i.e. the photosynthetic capacity seems to be slightly more
462 affected as photosynthetic efficiency (see figures 1 and 2). However, this interaction
463 is only effective within the general temperature tolerance range of the respective
464 species. Thus, there is a strong light/temperature interaction within eurythermal
465 macroalgal taxa, like *Ulva* (Rautenberger and Bischof, 2006) or *Laminaria* (Bruhn
466 and Gerard, 1996) and only slight responses being observed for stenothermal
467 species like *P. decipiens* (this study; Wiencke et al. 1993; Bischoff-Bäsmann and
468 Wiencke 1996).
469 Initial values for ETRmax do also display the differential degree of acclimation to the
470 respective radiation conditions at the respective shore levels, as a general trend in
471 algal ecophysiology along a depth gradient (Hanelt et al. 1997a; Wiencke and
472 Clayton, 2002,). Despite their occurrence in the intertidal, Antarctic macroalgae are
473 generally considered as being shade adapted (Kirst and Wiencke, 1995), however, at
474 the given radiation conditions at the Antarctic Peninsula, intertidal macroalgae can be
475 exposed to irradiances as high as 1500 µmol photons m^{-2} s^{-1} under low-tide
476 conditions. Thus, Antarctic intertidal algae may be even stronger characterised by
477 their flexible response towards changing radiation conditions, which may also include
478 their ability for dynamic photoinhibition (Hanelt et al. 1994; 1997a). However, these
479 former studies neglected the interactive influence of temperature.
480 On the level of photosynthetic pigments, no significant temperature effect was found.
481 The considerable reduction in the total amount of pigments and also the ratio of
482 phycobilliproteins to chlorophyll a thus represents a response to altered radiation
483 conditions. *Palmaria decipiens* is known to adjust its photosynthetic apparatus by
484 changing pigment ratios and overall content to seasonal changes in radiation
485 conditions (Lüder et al. 2001). In line with the pronounced differences in maximal
486 photosynthetic rates related to the respective growth site of specimens, also the
487 content and ratio of photosynthetic pigments differed largely between species from
488 different shore levels. In subtidal *P. decipiens*, the ratio of pycobiliproteins to
489 chlorophyll a was much higher than in intertidal specimens. By increasing the amount
490 of antennae pigments such a phycobiliproteins, light harvesting becomes more
491 efficient at greater depths, whereas, *vice versa*, excessive light absorption is avoided
492 by lower pigment concentrations in high light environments near the water surface.

493 Furthermore, phycobiliproteins are known to degrade rapidly under exposure to high
494 light and UV-radiation, while other pigment types are less susceptible (Tevini 1994;
495 Gómez et al. 2004). However, the reduction in pigment concentration and
496 composition observed in our study has to be considered as an acclimation
497 mechanism rather than a result of photodamage as photosynthetic performance was
498 hardly impaired (see figures 1, 2 and 3).
499 The maintained integrity of photosynthetic functions is also mirrored by only minor
500 variations in D1 protein content. However, one particular treatment indeed resulted in
501 a pronounced loss of D1: the exposure of subtidal specimens at low temperatures. It
502 has to be considered that even under the protective shield of the black gauze,
503 experimental irradiance exceeded *in situ* conditions nine times, which may result in
504 an increased defragmentation of the protein (Aro et al. 1993; Osmond, 1994).
505 However, whether there is an interactive effect of temperature involved in this
506 marked of degradation is doubtful as the low temperature treatment is rather close to
507 *in situ* temperature conditions. In contrast, in the higher temperature treatment,
508 pronounced D1 fragmentation might have been counterbalanced by faster
509 resynthesis of degraded protein or facilitated reintegration by an increased fluidity of
510 thylakoid membranes.
511 Our results indicate that an important factor in acclimation to changing light and
512 temperature conditions is the fatty acid composition. Previously, no studies have
513 addressed changes in fatty acid composition in short-term experiments as an
514 acclimation mechanism in Antarctic macroalgae. Nevertheless, it has been shown by
515 Leu et al. (2006) that light has an effect on algal fatty acid composition: The course of
516 phytoplankton blooms from the peak to the early breakdown stage is characterised
517 by a high light induced decrease of polyunsaturated FA as well as an increase of
518 monounsaturated fatty acids and a higher amount of the saturate 18:0 (Leu et al.
519 2006). This increase of 18:0 fatty acid in phytoplankton was also reported by Fahl
520 and Kattner (1993) for Antarctic waters and was related to the poor quality of the
521 particular organic matter. In combination with the findings of Leu et al. (2006), an
522 increase of 18:0 FA may point to phytoplankton in a poor physiological condition at
523 the end of a bloom. In our study, an increase of 18:0, 18:1 and 18:2 occurred in
524 subtidal and intertidal specimens in both temperature treatments. This result taken
525 together with decreasing ETRmax values as well as a significantly decreases amount

526 of D1 protein may also point to a poor physiological condition of *Palmaria decipiens*
527 in this case.
528 An adjustment of fatty acids due to changing experimental temperatures was
529 apparent in intertidal as well as in subtidal specimens exposed at 5-10°C. In these
530 cases, the amount of saturated fatty acids increased, which would result in more rigid
531 membranes compared to the fluidity at 2-5°C. This could be considered as an
532 acclimatory response to elevated temperatures compared to *in situ* conditions. Thus,
533 by changing the fatty acid composition in respect to changing temperatures, the
534 membrane fluidity may be maintained. The respective degree of
535 saturation/desaturation of membranes is a crucial parameter to maintain optimum
536 membrane fluidity (Harwood and Guschina, 2009). Although initial total lipid content
537 was equal in specimens from the two shore levels, the pronounced differences in
538 fatty acid composition in initial values of subtidal and intertidal specimens may be
539 explained by the different temperature ranges algae are exposed to at their specific
540 growth site. The subtidal is a very stable environment with only minor variations in
541 temperatures ranging between -1.8°C and +2°C (Wiencke and Clayton, 2002;
542 Wiencke et al. 2008). In contrast, in the intertidal temperatures may vary largely due
543 to tidal changes and atmospheric forcings. During low tide, organisms may be
544 exposed to deep subzero temperatures and may be submerged again shortly after by
545 the water column with the incoming flood. In order to keep membranes fluid even
546 under extreme temperature conditions in the intertidal, a high amount of unsaturated
547 fatty acids has to be maintained (Phleger 1991; Nelson et al. 2002). Nelson et al.
548 (2002) showed that in macroalgae from the Northeastern Pacific lipid content varies
549 seasonally and that environmental temperature determines fatty acid composition.
550 Our data supports this finding with respect to short-term temperature responses. *De*
551 *novo* synthesis of fatty acids starts with acetyl CoA, which is elongated to 14:0 and
552 16:0 and then desaturated and further elongated by various elongases and
553 desaturases in different steps (Harwood and Guschina, 2009, and references
554 therein). Thus, future studies should focus on how the latter reactions are triggered
555 and modified by environmental conditions. According to Graeve et al. (2002) polar
556 macroalgae are rich in polyunsaturated fatty acids (PUFA) which are constituents of
557 the phospholipids. Generally, biological activity is related to the essential PUFAs, and
558 especially phospholipids are considerably important for the functionality of
559 photosynthetic membranes (Sanina et al. 2004; Aro et al. 2005). It is essential to all

560 organisms to maintain membranes operative, especially those containing the
561 photosynthetic machinery, in a wide range of changing environments. In our study,
562 no changes in fatty acid composition was found in subtidal *P. decipiens* exposed to
563 temperatures that are similar or moderately increased compared to the *in situ*
564 conditions. The ratio of saturated to unsaturated FA remained the same, although
565 irradiance did strongly increase compared to the respective growth site. For a re-
566 integration of *de novo* synthesized D1 protein into the thylakoid membrane of PS II,
567 degraded protein is transferred from the grana to the stroma site of the chloroplast by
568 lateral diffusion (Aro et al. 2005) and newly synthesized protein needs to be
569 transferred back to the grana site. This process is dependent of membrane fluidity. If
570 in our results, D1 concentration decreases due to slowed down *de novo* synthesis or
571 to impaired re-integration of D1 into the membrane or just because D1 turnover is
572 unbalanced under excessive light intensities, is still debatable. However, it is clearly
573 shown that under the latter conditions D1 degradation was favoured and the efficient
574 reintegration might have been precluded, as here fatty acid composition was not
575 adjusted. It is thus assumed that in the presented study fatty acid composition is
576 solely triggered by temperature signals.
577 Overall our results clearly demonstrate the high flexibility of an endemic Antarctic red
578 alga to respond to changes in abiotic conditions and that fatty acid composition plays
579 a key role even in short term responses. Future studies should focus in more detail
580 which mechanisms and signal transduction pathways govern the regulation of fatty
581 acid composition. This would certainly strengthen our understanding of physiological
582 acclimation mechanisms to polar conditions.
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584
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593

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Table 3: Fatty acid (FA) content (µg/ mg dry weight) of intertidal specimens of *Palmaria decipiens* exposed to two different temperatures (2-5°C (**I**) and 5-10°C (**II**) at ambient light conditions prevailed at Potter Cove between January and February 2008. Total lipid (TL) is given in (µg/ mg dry weight).

Fatty acid	Initial	Day 3 (I)	Day 5 (I)	Day 11 (I)	Day 17 (I)	Day 23 (I)	Day 3 (II)	Day 5 (II)	Day 11 (II)	Day 17 (II)	Day 23 (II)
14:0	0.678 ± 0.12	0.151 ± 0.26	0.437 ± 0.34	0.352 ± 0.04	0.837 ± 0.08	5.966 ± 3.03	0.495 ± 0.12	0.358 ± 0.17	0.642 ± 0.29	0.395 ± 0.04	0.355 ± 0
15:0	0.017 ± 0	0.057 ± 0.04	0.034 ± 0.04	0.014 ± 0	0.062 ± 0.01	0.182 ± 0.01	0.021 ± 0.01	0.016 ± 0.01	0.038 ± 0.03	0.015	0.015 ± 0
16:0	1.794 ± 0.28	3.002 ± 2.9	1.556 ± 1.16	1.193 ± 0.08	6.061 ± 1.58	18.746 ± 0.18	1.448 ± 0.29	0.99 ± 0.42	3.373 ± 2.71	1.347 ± 0.14	1.163 ± 0.12
16:1 (n-7)	0.012 ± 0.01	0.215 ± 0.01	0.126 ± 0.22	0.024 ± 0.01	0.208 ± 0.01	0.351 ± 0.02	0.016 ± 0.01	0.014 ± 0.01	0.101 ± 0.12	0.021 ± 0.01	0.035 ± 0
16:2 (n-4)	0.005 ± 0.01	0.003 ± 0	0.009 ± 0.02	0.001 ± 0	0.013 ± 0.01	n.d.	0.005 ± 0	n.d.	0.008 ± 0.01	0.004 ± 0	n.d.
16:3 (n-4)	0.006 ± 0	0.068 ± 0.09	0.025 ± 0.03	0.005 ± 0	0.077 ± 0.01	0.017 ± 0	0.016 ± 0.01	0.007 ± 0	0.033 ± 0.05	0.003 ± 0	0.001 ± 0
16:4 (n-1)	n.d.	0.054 ± 0.08	0.01 ± 0.01	0.005 ± 0	0.156 ± 0	n.d.	0.009 ± 0	0.005 ± 0	0.065 ± 0.1	0.007 ± 0.01	n.d.
18:0	0.167 ± 0	3.219 ± 4.82	0.263 ± 0.35	0.064 ± 0.01	7.351 ± 2.84	1.753 ± 0.14	0.113 ± 0.07	0.089 ± 0.01	3.085 ± 3.78	0.295 ± 0.32	0.133 ± 0.25
18:1 (n-9)	0.341 ± 0.05	1.221 ± 1.67	0.609 ± 0.75	0.211 ± 0.01	2.873 ± 0.59	3.593 ± 0.11	0.236 ± 0.08	0.181 ± 0.08	1.33 ± 1.54	0.215 ± 0.07	0.220 ± 0.13
18:1 (n-7)	0.091 ± 0.05	0.403 ± 0.57	0.134 ± 0.14	0.95 ± 0.01	0.980 ± 0.23	1.564 ± 0.07	0.081 ± 0.02	0.065 ± 0.03	0.49 ± 0.51	0.098 ± 0.02	0.107 ± 0.01
18:2 (n-6)	0.058 ± 0.02	0.571 ± 0.86	0.135 ± 0.17	0.033 ± 0.01	1.484 ± 0.27	0.867 ± 0.08	0.044 ± 0	0.031 ± 0.01	0.638 ± 0.85	0.034 ± 0.02	0.059 ± 0.1
18:3 (n-6)	n.d.	0.047 ± 0.06	0.022 ± 0.01	0.019 ± 0	0.076 ± 0.01	n.d.	0.017 ± 0	0.013 ± 0	0.044 ± 0.04	0.036 ± 0.02	n.d.
18:3 (n-3)	0.014 ± 0.01	0.046 ± 0.06	0.015 ± 0.01	0.005 ± 0.01	0.143 ±0.04	0.044 ± 0.01	0.014 ± 0	0.009 ± 0	0.055 ± 0.08	0.088 ± 0.07	0.003 ± 0.01
18:4 (n-3)	0.022 ± 0.04	0.022 ± 0.02	0.049 ± 0.01	0.017 ± 0.01	0.055 ± 0.03	0.026 ± 0	0.022 ± 0	0.022 ± 0	0.027 ± 0.02	0.092 ± 0.1	n.d.
20:0	0.008 ± 0	0.130 ± 0.20	0.021 ± 0.02	0.003 ± 0	0.153 ± 0.24	0.255 ± 0.02	0.009 ± 0.01	0.008 ± 0	0.010 ± 0.02	0.039 ± 0.06	0.013 ± 0.02
20:1 (n-9)	0.096 ± 0.01	0.079 ± 0.03	0.102 ± 0.09	0.049 ± 0	0.164 ± 0	0.49 ± 0.03	0.076 ± 0.01	0.06 ± 0	0.128 ± 0.09	0.051 ± 0.03	0.035 ± 0.01
20:1 (n-7)	n.d.	0.008 ± 0.01	0.013 ± 0.02	0.002 ± 0	0.026 ± 0	n.d.	0.004 ± 0.01	n.d.	0.045 ± 0.06	0.015 ± 0.02	n.d.
20:2 (n-6)	n.d.	0.009 ± 0.01	0.010 ± 0.01	0.003 ± 0	0.021 ± 0	n.d.	0.008 ± 0.01	0.008 ± 0	0.011 ± 0.01	0.017 ± 0.01	n.d.
20:3 (n-6)	n.d.	n.d.	0.015 ± 0	0.006 ± 0	0.055 ± 0.04	0.033 ± 0	0.007 ± 0.01	0.007 ± 0	0.016 ± 0.01	0.027 ± 0.03	n.d.
20:4 (n-6)	0.035 ± 0.02	0.038 ± 0.05	0.049 ± 0	0.018 ± 0.01	0.085 ± 0.01	0.396 ± 0.01	0.042 ± 0.01	0.030 ± 0.02	0.072 ± 0.07	0.085 ± 0.09	0.016 ± 0.01
20:4 (n-3)	0.044 ± 0.02	0.020 ± 0.02	0.039 ± 0.01	0.018 ± 0.01	0.042 ± 0.01	0.1 ± 0	0.022 ± 0.01	0.015 ± 0.01	0.024 ± 0.03	0.016 ± 0	0.01 ± 0.02
20:5 (n-3)	4.368 ± 2.10	1.322 ± 0.87	2.539 ± 0.99	1.383 ± 0.69	2.193 ± 0.52	10.577 ± 0.18	2.473 ± 1.01	2.236 ± 1.5	1.769 ± 0.66	2.171 ± 0.87	0.605 ± 0.01
22:1 (n-11)	n.d.	0.051 ± 0.05	0.070 ± 0.03	0.073 ± 0	0.203 ± 0.03	n.d.	0.119 ± 0.02	0.096 ± 0.01	0.150 ± 0.1	0.069 ± 0.02	n.d.
22:1 (n-9)	0.143 ± 0.02	0.122 ± 0.11	0.161 ± 0.01	0.103 ± 0.06	0.783 ± 0.07	1.014 ± 0.01	0.118 ± 0.16	0.129 ± 0.01	0.311 ± 0.54	0.119 ± 0.11	0.062 ± 0.01
22:1 (n-7)	0.01 ± 0	0.129 ± 0.20	0.014 ± 0.01	0.007 ± 0.01	0.084 ± 0.04	0.291 ± 0.03	0.036 ± 0.03	0.015 ± 0	0.038 ± 0.07	0.016 ± 0.01	0.021 ± 0.03
22:5 (n-3)	n.d.	n.d.	0.024 ± 0.01	0.012	0.047 ± 0	n.d.	0.015 ± 0	0.018 ± 0.02	0.027 ± 0.03	0.019 ± 0.01	n.d.
22:6 (n-3)	n.d.	0.468 ± 0.54	0.198 ± 0.07	0.156 ± 0.02	1.274 ± 0.11	n.d.	0.176 ± 0.04	0.138 ± 0.05	0.675 ± 0.67	0.181 ± 0.05	n.d.
TL (µg/ mg)	7.9 ± 2.29	11.32 ± 13 07	7.44 ± 4.42	3.96 ± 0.64	25.92 ± 6.39	3.3 ± 0.58	5.27 ± 1.42	4.35 ± 2.34	16.63 ± 12.29	5.32 ± 1.08	3.0 ± 0.70

The Biology of an abundant Antarctic rhodophyte, *Palmaria decipiens*: An overview of recent advances

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The Biology of an Antarctic rhodophyte, *Palmaria decipiens*: an overview of recent advances

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Abstract

Palmaria decipiens represents one of the dominant rhodophyte species in Antarctic coastal ecosystems. Due to its high abundance in the intertidal and upper subtidal it plays a key role in ecosystem structure and function, providing habitat, food and shelter for a multitude of associated organisms. The physiology, reproductive strategy and life cycle of *P. decipiens* is considered as being strongly adapted to the Antarctic environment, which is characterized by permanent low water temperatures and a strong seasonality in light climate. Considering its obvious ecological significance and adaptive strategies *P. decipiens* was frequently studied as a typical representative of an endemic Antarctic macroalga. Here we provide an overview on the recent literature summarizing the present knowledge gained on the alga, during the last 25 years. This review focuses on the species life cycle and physiological responses, such as temperature requirements, photosynthetic characteristics, pigment content and protective mechanisms with regard to enhanced UV-radiation. The ecology of *P. decipiens* is reviewed focussing on grazing activity and abundance patterns. As hitherto most studies on *P. decipiens* were conducted at King George Island off the Western Antarctic Peninsula this overview serves as a summary of baseline data from an ecosystem particularly prone to environmental change.

Key Words

Coastal ecology, King George Island, physiology, Palmariales, red algae

Introduction

Antarctic coastal zones represent habitats in one of the most challenging environments on Earth. Being exposed to very low water temperatures, long periods of darkness, strong winds and sea-ice formation, benthic communities have evolved various adaptation and acclimation mechanisms to cope with these particular abiotic settings. Macroalgae play a key role in shallow benthic ecosystems, providing food, shelter and habitat for a variety of associated organisms such as fish, amphipods or mollusks as well as being an important source of nutrients when decomposing (Nedzarek & Rakusa-Suscezewski 2004). An example for the importance of

seaweeds in the Antarctic is indicated by the littoral biomass of 74.000 tons wet weight produced per year in Admiralty Bay on King George Island (Isla 25 de Mayo), South Shetland Islands (Zielinski 1990, Oliveira *et al.* 2009). One of the dominating species in terms of biomass is the rhodophyte *Palmaria decipiens* endemic to the Antarctic region and several sub-Antarctic islands (Kerguelen Islands, Macquarie Island, Campbell Island; Wiencke & Clayton 2002). This algal species may be regarded as a key species in shallow benthic communities, which structures the habitat and is of high nutritional value for a variety of associated organisms such as limpets (e.g. *Nacella concinna*) or amphipods (e.g. *Gondogeneia antarctica*) and fish (e.g. *Notothenia neglecta*; Iken *et al.* 1997, Amsler *et al.* 2009).

In the context of global climate change, the Antarctic and especially the western Antarctic Peninsula will undergo rapid and drastic changes regarding rising air and water temperatures with subsequent glacier retreat and increasing fresh water input, as well as changes in rainfall, air pressure declines or increasing wind speeds (recently reviewed by Turner *et al.* 2005). Therefore, intense research activities are ongoing to clarify the state-of-the art and to try to predict future scenarios for Antarctic coastal ecosystem function, in which benthic primary producers play an essential role.

So far, most of the research on the physiological adapation mechanisms in Antarctic macroalgae has been conducted in the area of King George Island, South Shetland Islands (62°S, 58°W). A detailed overview on the structure and function of the Potter Cove ecosystem at King George Island and the possible impacts of global and anthropogenic changes on the system is contained in the synopsis by Wiencke *et al.* (2008). In this review we present the existing knowledge on the abundant Antarctic rhodophyte *P. decipiens* in the context of basic physiological adaptation towards the abiotic environment and potential responses to environmental change.

A) Taxonomy and morphology

Within the taxonomic system, *Palmaria decipiens* is classified in the phylum Rhodophyta and the class Florideophyceae. It belongs to the order Palmariales and the genus *Palmaria* which comprises nine species (http://www.algaebase.org).

First descriptions of *Palmaria decipiens* appeared in the end of the 19th and early 20th century as species of various genera (*Rhodymenia*, *Leptosarca*, *Gracilaria*, *Leptosomia*). In 1987 Ricker integrated all so far described species that mainly

differed in color and shape as morphotypes of the species *P. decipiens* (Ricker 1987 and references therein). This review focusses on research conducted after the integration of the respective species as *P. decipiens*.

P. decipiens is characterized by a thallus up to 70 cm long, with reddish to purple color and a slippery glossy surface. It is a pseudoperennial macroalga with its major growth period in late winter and spring (i.e. October to December). The first year frond is simple, i.e. an unbranched, lanceolate blade with a width of 8-15 cm arising from the holdfast. Older plants proliferate from the base of previous year's blade. Generally, blades are attenuated to a terete, narrow stipe from a minute, discoid holdfast. A characteristic feature is the two to three cell layer thick medulla with nearly isodiametric cells and cruciately divided tetrasporangia embedded in a cortex (Ricker 1987, Wiencke & Clayton 2002).

B) Life cycle and Reproduction

The life cycle and reproduction of Palmariales has been unresolved until the discovery of the microscopic female gametophyte and the lack of carposporophytes in *Palmaria palmata*, the northern congener species of *P. decipiens*, by van der Meer & Todd (1980). Seven years later, Ricker (1987) observed the development of the female gametophyte of *P. decipiens* for the first time. Half of the tetraspores of the alga germinate into spherical, irregularly shaped cell crusts, the female gametophytes bearing colourless, sessile carpogonia, which develop trichogynes shortly after settlement of tetraspores. Male gametophytes are similar in morphology to the tetrasporophytes. Spermatangia have, however, not been observed so far (Wiencke & Clayton 2002). As growth and development of males is much slower than of females, fertilization of females is only possible by mature males of the previous season, indicating a life span for Palmariales of several years (van der Meer & Todd 1980 for *P. palmata*, Wiencke 1990). The fertilized female will be overgrown by sporophytes, which will become mature and releases tetraspores after undergoing meisosis (van der Meer & Todd 1980).

Interestingly, seasonal growth is supposed to be triggered by daylength in *P. decipiens* (Wiencke 1990, Wiencke *et al.* 2009). The optimum growth period of the alga is coinciding with increasing light intensities in spring, which is regarded to be an adaptation to the strong seasonality in the light climate in Antarctica. This is classified

as “season anticipation” strategy (*sensu* Kain 1989; Wiencke 1990, Wiencke *et al.* 2009).

C) Geographic distribution and Habitat

Palmaria decipiens is one of the at least 37 rhodophyte species endemic to Antarctica (Wiencke & Clayton 2002, Hommersand *et al.* 2009). It occurs in the whole Antarctic region from the 77°S in the Ross Sea to a few sub-Antarctic islands (detailed information in Ricker 1987). It inhabits lower intertidal zones as well as depths down to 30 m (Wiencke & Clayton 2002). The northern distribution limit is most probably determined by the 4°C winter isotherm as growth rates are optimal at temperatures ≤ 5°C and almost zero at 10°C (Müller *et al.* 2009).

Climatic and topographic limits

Studies by Klöser *et al.* (1993, 1996) using video recording in the Potter Cove area on King George Island (South Shetland Islands) have shown that *Palmaria decipiens* is a very common species on hard bottom substrate down to 30m depths (Wiencke & Clayton 2002). Hard substrate may be found directly underneath the glacier foot on the western side of the bay, on the mouth of the bay, Barton Peninsula and the intertidal platform further outside the bay with its characteristically rising monolith M Rock. In these areas, *P. decipiens* may form dense stocks, especially after community disruption through ice scouring and subsequent exclusion of competing species (Zielinski 1990, Klöser *et al.* 1993).

D) Physiological responses to environmental conditions

Temperature

Antarctic water temperatures are very stable throughout the year and even surface temperatures do not vary strongly during the seasons. This temperature constancy is set by the particular oceanographical situation of Antarctica: surrounded by the circumpolar current driven by strong westerly winds, water masses within the polar front are almost isolated from neighbouring oceans (Rintoul *et al.* 2001). For instance, at the area of King George Island (62°14′S, 58°40′W), water temperatures range seasonally between -1.8 and +2°C (Klöser *et al.* 1993, Wiencke *et al.* 1994,

Wiencke 1996). In recent years, however, an increase of about 1.5°C has been documented in winter (Schloss *et al.* 2008).

Most endemic Antarctic seaweeds are strongly cold adapted with growth temperatures between 0-10°C and with an optimum at ≤ 5°C (e.g. Wiencke & tom Dieck 1989). Their upper survival temperature usually is within the range of 7-18°C (Wiencke *et al.* 1994, Bischoff-Bäsmann & Wiencke 1996). Generally, Antarctic species such as *Palmaria decipiens* exhibit the lowest temperature requirements for growth and survival worldwide (Wiencke *et al.* 1994). These adaptations can be interrelated with their evolutionary history in combination with the long cold-water history of Antarctica that began with the glaciation of East Antarctica 38 My ago and which reached West Antarctica at least 9 My ago (Zacher *et al.* 2009 and references therein). Overall, water temperature is considered to be the determining factor for macroalgal geographic distribution (Lüning 1990, Wiencke *et al.* 2007). The northern distribution limits of Antarctic macroalgae are often determined by winter water temperatures that allow growth and reproduction of the most sensitive early life stages (Wiencke & tom Dieck 1989). The northernmost location at which *P. decipiens* occurs, is Macquarie Island (54°S, 158°E), where water temperatures of 5 to 8°C coincide well with the maximum growth temperature of this species (Wiencke & tom Dieck 1989).

First studies regarding temperature effects and demands of Antarctic macroalgal species, including *P. decipiens*, were carried out on Signy Island in 1977 by Drew. It was demonstrated that photosynthetic rates, measured as µg C m^{-2} h^{-1}, increased in contrast to the temperature-growth pattern towards a maximum at temperatures between 11 and 18°C, with a subsequent sharp decline with further increasing temperatures. Maximum carbon fixation rates of 6-8 µg C m^{-2} h^{-1} were recorded for *P. decipiens* at the temperature optimum of photosynthesis (Drew 1977). Drew argued that this temperature-photosynthesis pattern does not reflect an adaptation towards the low temperatures in the environment. On the other hand, the fact that Antarctic species exhibit similar carbon fixation and photosynthetic rates as temperate algae at higher temperatures points to a considerable low temperature adaptation (Thomas & Wiencke 1991). These latter authors also documented differences in photosynthetic rates with respect to thallus age. Young *P. decipiens* thalli had rates of oxygen production almost twice as high in comparison to older plants and the same applies for rates of dark inorganic carbon fixation. In contrast,

light independent carbon fixation rates are higher in older tissues (Thomas & Wiencke 1991).

Interestingly, studies performed on male gametophytes, monitoring growth rates for several weeks showed that optimal photosynthesis is performed at 15°C which is considerably above optimum growth temperature and even much higher than Antarctic water temperatures (Wiencke & tom Dieck 1989, 1990, Wiencke 1990, Wiencke *et al.* 1993, Eggert & Wiencke 2000). Nevertheless, especially the intertidal specimens of *P. decipiens* may endure and survive periods of freezing events and far subzero temperatures during low tide conditions.

More recently, studies on the interactive effects of temperature and increasing irradiance have been performed to get a more complete picture of the influence of environmental factors on the physiology of *P. decipiens* (Becker *et al.* 2009, 2010). These studies revealed that photosynthetic performance of subtidal *Palmaria* specimens was stronger inhibited at 0°C and high light conditions in comparison to 8°C and high light intensities (Becker *et al.* 2009). This impairment is thought to be due to slowed down enzymatic reactions at lower temperatures, whereas high light intensities consequently may result in an increase in the generation of reactive oxygen species. Additionally, low temperature might alter membrane fluidity, which subsequently influences turnover rates of proteins, such as the reaction centre D1 protein localised in photosystem II (Aro *et al.* 1993, 2005, Becker *et al.* 2010). Furthermore, mesocosm experiments applying elevated temperatures at ambient light intensities showed that specimens from two shore levels (intertidal and subtidal) exhibited decreasing maximum quantum yields of photosynthesis, with the intertidal specimens exposed at 5-10°C being most affected (Becker *et al.* 2010). Ambient atmospheric light intensities in these mesocosm experiments meant an overall PAR (photosynthetically active radiation, 400-700 nm) intensity increase as well as a change in light spectrum compared to *in situ* conditions for specimens from both shore levels. Hence, in that study it was concluded that even optimum photosynthetic temperatures of 5-10°C can not compensate for high light stress (Becker *et al.* 2010).

Salinity

The distribution of macroalgae was investigated along gradients of the physio-chemical conditions occurring within Potter Cove on King George Island, South Shetland Islands, Antarctica (Klöser *et al.* 1993). Abiotic parameters such as light, temperature, salinity and sediment input at two different sites were correlated to the

inhabiting macroalgal species and thus, characteristic community structures were detected (Klöser *et al.* 1993). One of the stations was in direct proximity to a glacier, while the other one was closer towards the mouth of the bay. Therefore, the latter one was less affected by ice scouring or changing salinities (Klöser *et al.* 1993). However, measurable effects of salinity changes on macroalgal community structure were absent. Although it had been reported previously that high densities of *Palmaria decipiens* might indicate enhanced freshwater input, this finding could not be confirmed in the study in Potter Cove (Klöser *et al.* 1993 and references therein). *P. decipiens* seems to be very abundant not only in coastal areas located in close proximity to Fourcade glacier and especially in new ice free areas (María Liliana Quartino, pers. comm. 2008), but also in the intertidal zone with pronounced salinity changes and subtidal zones with full marine salinity (Klöser *et al.* 1993, Quartino *et al.* 2005). Thus, this euryhaline species may be regarded to be opportunistic in terms of settlement and may outcompete other common dominant species. Particularly in newly open areas following glacier retreat, which are characterized by intense ice scouring, *P. decipiens* can build dense stocks (Klöser *et al.* 1993, S. Becker personal observation).

Generally, an increase in freshwater input due to glacier run-off is expected to cause decreasing salinities in coastal areas under climate change scenarios. In a short-term experiment by Becker (previously unpublished data) the effects of changing salinities on *P. decipiens* from different shore levels were investigated. Specimens from the intertidal as well as the subtidal were exposed to decreasing salinities ranging from 28 PSU to 5 practical salinity unit (PSU) for five days at light intensities of 53 µmol photons m^{-2} s^{-1}. Photosynthetic performance was measured via chlorophyll fluorescence. Photosynthetic efficiency was nearly unaffected by changing salinities except for salinities of 5 PSU, at which values did decrease significantly (Figure 1a and 2a). Similar responses were observed for specimens from both shore levels. In intertidal specimens (Figure 1a and b) a greater tolerance towards low salinities is likely: as algae may become fully emerged during low tide. If this coincides with rain fall, specimens are exposed to strongly reduced salinites. But also subtidal algae inhabiting the hard substrate in close proximity to glaciers experience fresh water input during melting events in summer seasons. Since stratification is rare in Potter Cove (Klöser *et al.* 1993) due to vertical mixing by strong winds, which are common

in these high latitudes, a broader tolerance limit is essential to cope with changing salinity conditions.

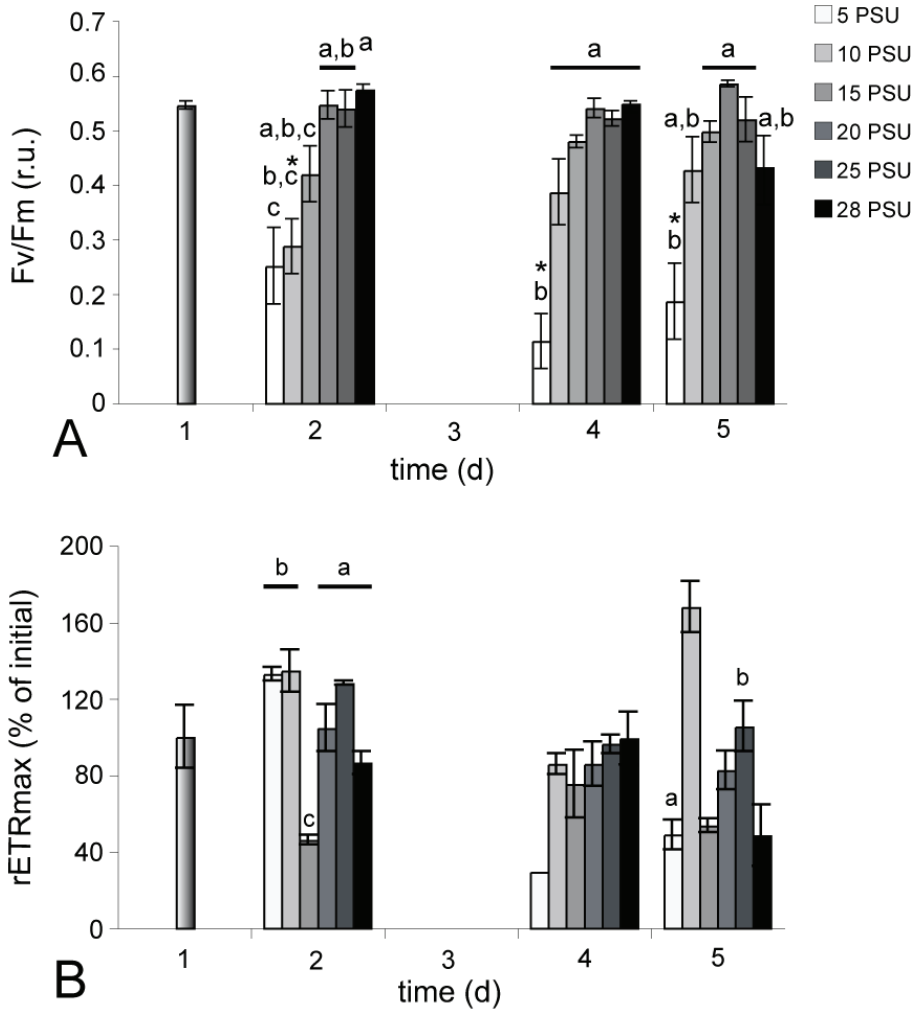

Figure 1 **a:** Photosynthetic efficiency (Fv/Fm) in relative units (r.u.) of intertidal specimens of *Palmaria decipiens* exposed to six different salinities at 2°C and 50 µmol photons m^{-2} s^{-1} for five days. Values are means of triplicates with standard error bars. Asterisks mark significant differences within a salinity treatment in comparison to initial values, different letters mark significant salinity effects (Tukey-Kramer post-hoc test with $p < 0.05$). **b:** Photosynthetic efficiency (Fv/Fm) of subtidal specimens of *Palmaria decipiens*. Conditions as stated in **a**.

Regarding the effects of increased fresh water input on the photosynthetic capacity expressed as the relative maximal electron transport rate (rETRmax) a similar pattern as for Fv/Fm values was found. rETRmax values of intertidal specimens decreased during the exposure to 60% (see figure 2a and b). Again, the strongest impact was found at salinities of 5 PSU. However, photosynthetic capacity of subtidal specimens

was less affected. rETRmax values decreased over time by only 20% (see figure 2b). However, algae exposed to 5 PSU died after the second day of the treatment. Nevertheless, these experiments indicate a strong tolerance towards salinity changes of the alga. We regard *P. decipiens* to be a pioneer species (María Liliana Quartino, pers. comm. 2008) and is often found in disturbed zones, a high tolerance towards abiotic changes might be evolutionary advantageous.

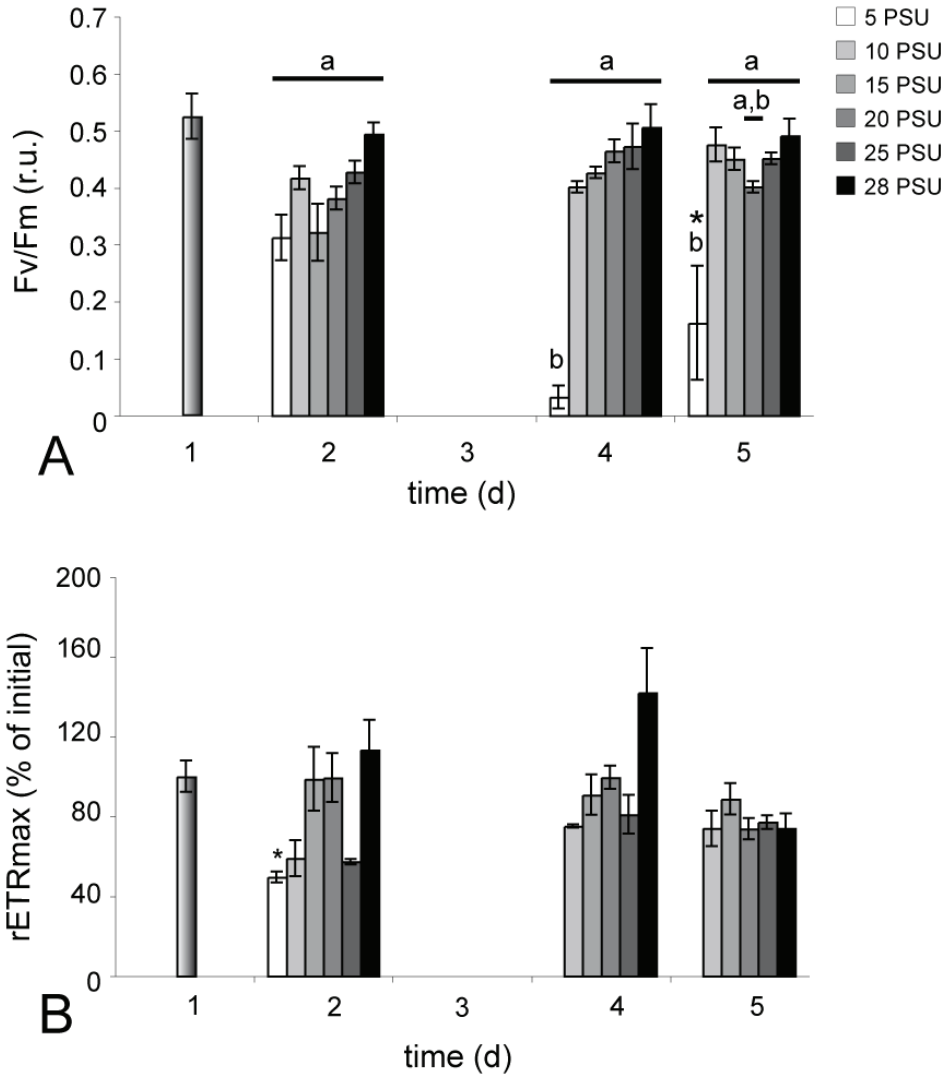

Figure 2 **a:** Relative maximum electron transporte rate (rETRmax) of intertidal specimens of *Palmaria decipiens*, given as percent of initial value, exposed to six different salinities at 2°C and 50 µmol photons m^{-2} s^{-1} for five days. Values are means of triplicates with standard error bars. Asterisks mark significant differences within a salinity treatment in comparison to initial values, different letters mark significant salinity effects (Tukey-Kramer post-hoc test with $p<0.05$). **b:** rETRmax of subtidal specimens of *Palmaria decipiens*. Conditions as stated in **a**.

In this context, internal osmolytes may play an important role. It has been shown by Karsten *et al.* (1990) that *P. decipiens* exhibits traces of ß-dimethylsulphoniopropionate (DMSP). This low molecular weight compound is known to act as a protectant under low temperatures. For instance, under externally increased salinities the concentration of internal DMSP is also enhanced (Karsten *et al.* 1990, Kirst and Wiencke 1995). Usually, increased amounts are observed at increased light intensities, low temperatures, cell age and population densities. Nevertheless, detailed knowledge about the role of osmolytes still is lacking for *P. decipiens* and still remains an open field of research.

Photosynthesis and pigments

Most of the experiments carried out on *Palmaria decipiens* so far have also considered photosynthetic performance as a marker for physiological fitness of the alga and its ability to cope with the particular Antarctic conditions as well as with a changing environment (e.g. Drew 1977, Thomas & Wiencke 1991, Wiencke *et al.* 1993, Eggert & Wiencke 2000, Becker *et al.* 2009, 2010). In a study performed by Weykam & Wiencke (1996) photosynthetic performance of *P. decipiens* was observed throughout an annual cycle under fluctuating light intensities mimicking Antarctic daylengths. Photosynthetic performance measured via oxygen production is maximal during late winter/ early summer conditions (November/December), which is shortly after the exhibition of maximal growth rates in late winter (October/ November; Weykam & Wiencke 1996). As already concluded by Thomas & Wiencke (1991), the early growth in late winter prepares the alga for an optimal use of favourable light conditions in early summer with freshly grown photosynthetic tissue. Generally, photosynthesis versus irradiance curves (PI-curves) recorded during spring (October) and winter (July) conditions showed a higher photosynthetic capacitiy (Pmax) in spring (Weykam & Wiencke 1996). Photosynthetic and metabolic demands are generally very low for Antarctic endemic species. Especially *P. decipiens* is able to grow under very low light conditions, and growth has been suggested to be triggered by daylengths (Wiencke 1990, 1996, Lüder *et al.* 2001a). In extreme, photosynthetic pigments may be totally degraded during winter conditions, as has been studied by Lüder *et al.* (2001a, 2002): in an experiment simulating six months of darkness it was shown that by the end of the dark period, *P. decipiens* has lost its ability to perform photosynthesis (Lüder *et al.* 2002). Within 24 hours of re-illumination, pigments are newly synthesized and within a couple of days,

photosynthetic performance reaches comparable values to pre-darkness conditions and growth is induced. The first pigment to be accumulated after light exposure is chlorophyll *a*, followed by the phycobiliproteins shortly afterwards (Lüder *et al.* 2002). These proteins play an important role for shade adapted plants and algae to increase light harvesting efficiency in that range of the light spectra which is hardly exploited by chlorophylls. Hence, especially deep growing rhodophytes or those living in regions that are light limited as Antarctica, usually exhibit high amounts of phycobilisomes, bearing the phycobiliproteins. Interestingly, Lüder *et al.* (2001b) discovered a separation of phycobilisomes into two different size bands by using gel electrophoresis. Furthermore, different ways of degradation throughout the dark period could be detected. These authors could show, that especially the lower subunit disappears completely in darkness and suggested that different physiological functions of the subunits might be an advantage for *P. decipiens* in the acclimation process to seasonal light conditions (Lüder *et al.* 2001b). By modulating its pigment content, *P. decipiens* is able to adapt quickly to the respective conditions in Antarctica. The alga possesses maximal pigment concentrations during favourable spring light conditions and decreases pigment content during summer to avoid photodamage through excessive light intensities (Lüder *et al.* 2001a, b, 2002).
In accordance with these findings, Becker *et al.* (2010) could show that specimens from different shore levels exhibit particularly different chlorophyll *a* to phycobiliprotein ratios. Subtidal specimens have a higher amount of the antennae pigments phycoerythrin and phycocyanin and are thus, well adapted to the lower light intensities compared to those found in the intertidal zone. This indicates that *Palmaria decipiens* specimens may react flexibly to their given habitat and reveal physiological plasticity.

Photoprotection

Low values for initial saturation of photosynthesis (Ik) coupled with a high initial slope of the PI-curve (alpha) are typical for shade-adapted plants (Wiencke 1993, Weykam *et al.* 1996, Weykam & Wiencke 1996, Eggert & Wiencke 2000, Becker unpublished data). Although being considered as strongly shade-adapted (e.g. Wiencke 1990, Weykam & Wiencke 1996), *Palmaria decipiens* is able to cope with high light intensities up to 1500 µmol photons m^{-2} s^{-1} through down regulation of photosynthetic performance via dynamic photoinhibition (Hanelt *et al.* 1994). Thus, the alga may

withstand even stressful light conditions that might occur during low tide. However, recovery is slower than for example in the phaeophyte *Adenocystis utricularis* (Hanelt *et al.* 1994, Hanelt *et al.* 1997). Dynamic photoinhibition functions as a photoprotective mechanism by which excessive light energy is dissipated as harmless heat (Hanelt *et al.* 1994, and references therein). Photosynthetic variables (fluorescence and oxygen production) measured at highest light intensities around noon decreased to a minimum and recovered with decreasing irradiances until solar irradiances became sub-saturating. In their study, Hanelt and co-workers (1994) were able to detect a diurnal pattern in dynamic photoinhibition in *P. decipiens*, which was more pronounced in intertidal specimens than in those from the subtidal.

Lipid content and fatty acid composition

Hitherto, there is only scant information on lipid content and fatty acid composition (FA) of *Palmaria decipiens*. A baseline study has been undertaken by Graeve *et al.* (2002), in which lipid composition was analysed for a number of Antarctic macroalgae. In their study they analyzed fatty acid contents of Polar macroalgae and found high amounts of 20:5(n-3), which characterises marine organisms and also indicates a phylogenetical old lineage (Graeve *et al.* 2002). In general, the most abundant fatty acids of Antarctic rhodophytes including *P. decipiens* were 16:0, 20:5(n-3), 16:1(n-7) and 20:4(n-6), with an extremely high amount of polyunsaturated fatty acids (Graeve *et al.* 2002).

A recent study by Becker *et al.* (2010) showed the ability of the alga to adjust fatty acid composition with changing environmental factors such as temperature. It was investigated if changes in lipid content and/or fatty acid composition occur under changing light and temperature levels. In total, 32 different fatty acids present in *P. decipiens* were analyzed, with 16:0 as the most abundant. However, during the exposure to increased light intensities (in comparison to in situ light intensities) and two different temperatures over three weeks, 18:0 became the most abundant FA. For unsaturated FA Becker *et al.* (2010) found an overall increase in concentrations in specimens from the subtidal. In specimens originating from the intertidal zone, an overall decrease in FA content was observed, with an increase of saturated FA over time. The authors argued that this adjustment of FA might indicate that *P. decipiens* can react to changing temperatures by modulating the FA content and thus, may maintain membranes fluid at the given environmental conditions (Becker *et al.* 2010).

Effects of ultraviolet (UV, 280-400 nm) radiation

Ozone depletion has been first observed in 1985 by Farman *et al.* over Antarctica and is severely affecting life in both Polar regions. Especially the Atlantic sector of the Antarctic continent between 70°S and 80°S is influenced by increasing UV-irradiances as a result of ozone depletion. Therefore, a lot of effort has been undertaken to understand physiological and biochemical processes to adapt to changes in UV-radiation as well as possible strategies of Polar organisms to cope with a changing radiation environment.

UV-protective mechanisms

A variety of organisms contain mycosporine-like amino acids (MAAs), low-molecular-weight water-soluble molecules that absorb in the range of 310 to 365 nm and are therefore regarded as UV-protecting metabolites (Oren & Gunde-Cimerman 2007).

MAAs are synthetised by cyanobacteria and some diatoms and in particular red macroalgae. Generally, Antarctic red algal species were found to exhibit higher amounts in MAAs than Arctic species, which may be due to the different radiation climates in the two regions (Hoyer *et al.* 2002). Studies by Hoyer *et al.* (2002) have shown that *Palmaria decipiens* differentially accumulates MAAs in a flexible response to the respective light regime. Within their experiments, *P. decipiens* was exposed to different radiation treatments, comprising PAR only, PAR+UVA (ultraviolet radiation A, 320-400 nm) and PAR+UVA+UVB (ultraviolet radiation B, 280-320 nm). Under the conditions applied, *P. decipiens* exhibited an increase in total MAA content, with the highest concentrations in samples treated with the full radiation spectrum (Hoyer *et al.* 2002). Up to seven different MAAs were induced in *P. decipiens*, particularly shinorine, porphyra-334, palythine and asterina-330 as well as traces of usujirene. The same MAAs were found in the northern congener species *Palmaria palmata*. The increase in concentrations was most pronounced in porphyra-334 (for structural details see Dunlap & Shick 1998, Hoyer *et al.* 2002).

Overall, the content of MAAs in *P. decipiens* is flexibly induced in response to changing irradiances. It is also depth-related, as shallow water specimens contain higher amounts of MAAs than subtidal specimens (Hoyer *et al.* 2002). Concentrations of MAAs differ even among the different tissues of one individual organism. It could be shown that marginal tissues have a four times higher amount of MAAs than basal tissues. According to Hoyer *et al.* (2001) this may be due to higher

growth activities in basal tissues and thus, differences in cell wall thickness. Additionally, Post & Larkum (1993) could show previously that tissue concentration of MAAs in *P. decipiens* varies according to the season, with highest concentrations in summer. Based on their studies and by comparison of different Antarctic species, Hoyer *et al.* (2001, 2002) proposed a classification into different physiological groups: (a) species generally lacking MAAs and show no induction mechanism, this being mostly subtidal species, (b) species always maintaining a certain amount of MAAs in varying concentrations depending on the environmental conditions and (c) species always containing high concentrations of MAAs in regard to their high light environment, this being species of intertidal and supralitoral zones. Regarding this classification scheme and in accordance with the above mentioned studies, *Palmaria decipiens* belongs into group (b) (Hoyer *et al.* 2001, 2002).

Physiology, ultrastructure and gene expression under UV-exposure

Experiments performed by Michler *et al.* (2002) revealed the negative influence of UV radiation on photosynthetic performance and on growth rates of *Palmaria decipiens*. It was detected that *P. decipiens* grew significantly less under the full light spectra in comparison to PAR only. Although photosynthetic parameters such as Fv/Fm and rETRmax differed only slightly, highest values were measured in treatments without UV radiation (Michler *et al.* 2002).

In short term, unifactorial UV experiments, Poppe *et al.* (2002, 2003) investigated ultrastructural changes in *P. decipiens* occurring during exposure. They showed that the ultrastructure of chloroplasts as well as mitochondria changed significantly after exposure to UV-radiation for 6 to 8 hours. The thylakoids disrupted and formed translucent vesicles carrying stroma and phycobilisomes inside (Poppe *et al.* 2002). During continued exposure, these changes disappeared again and cells expressed a similar ultrastructure as control specimens. Another study by Poppe *et al.* (2003) confirmed that these ultrastructural changes are correlated to exposure time and that the damage in the chloroplast fine structure becomes apparent in widened thylakoids and the development of vesicle-like formations. Persisting exposure led to thylakoids disruption and formation of inside-out vesicles. In mitochondria, UV-exposure led to swollen and mostly to tubuli of the sacculi type, whereas nuclei, golgi bodies and the endoplasmatic reticulum generally remained unaffected (Poppe *et al.* 2003). Overall, these changes were reversible even under continued exposure.

Regarding photosynthetic performance and (stress-)reactions, an important factor is the functioning and turn-over rate of the D1-protein, which is located in photosystem II (PSII; e.g. Aro *et al.* 1993, 2005). Expression of the psbA gene which encodes for the D1-protein and the rbcL gene, encoding for the large subunit of RubisCO which is the key enzyme of carbon dioxide fixation, was studied in detail by Poppe *et al.* (2003). *P. decipiens* was exposed to varying UV-radiations that either mimicked normal Antarctic conditions or higher UV-intensities that might occur during midday in austral summer under ozone depleted conditions (Poppe *et al.* 2003). It was found that under moderate UV-intensities both transcripts increased during 16h UV-exposure and remained high even in darkness, with the most pronounced increase during the first two hours of exposure. During longer exposure to higher intensities, transcripts increased constantly until the end of exposure, whereas controls exposed to PAR (photosynthetically active radiation, 400-700 nm) only increased during the first three days and then declined. Transcripts for RubisCO were at a lower level than those of D1 and showed a maximum at the end of exposure (Poppe *et al.* 2003). Poppe *et al.* (2003) argued that this increase might be due to an increased requirement for D1 and RubisCO, as light-dark fluctuations had been observed previously.

E) Ecological parameters

Biomass and abundance

Palmaria decipiens grows predominantly epilithic but also sometimes epiphytic and usually occurs in the lower eulittoral and upper sublittoral down to 15-30 m. The individual recordings down to several hundred meters depths by Zaneveld (1966) in the Ross Sea need confirmation. In the eulittoral the alga can be the dominant canopy species, whereas in the sublittoral it is a common understorey species below the large brown macroalgal stands (Wulff *et al.* 2009). On King George Island the upper sublittoral is dominated by the phaeophyte *Desmarestia menziesii* and *P. decipiens* is commonly competing with *Iridaea cordata* and *Curdiea racovitzae* at the same littoral fringe (DeLaca & Lipps 1976, Zielinski 1981, Heywood & Whitaker 1984, Quartino *et al.* 2001). In a detailed study investigating biomass and abundance of macroalgae on King George Island, carried out at an inner site of Potter Cove, *P. decipiens* was recorded to be one of the most abundant species in terms of density

on the rocky surfaces but not the most dominant one in terms of biomass (Quartino *et al.* 2001). Individual size of the specimens ranged from small germlings up to large plants with blades of 60 cm. The mean dry biomass found was from 5.57 to 20.81 g m^{-2}, which is comparable to the biomass estimate found for the competing species *Iridaea cordata* (Quartino *et al.* 2002).

Later studies corroborated the presence of *P. decipiens* along the rocky surfaces of Potter Cove and surrounding areas (Quartino *et al.* 2005, Quartino & Boraso de Zaixso 2008). The recorded frequency (percentage of sample units where the species was present) in the whole area was, however, relatively low (19.37%). This fact denotes that this species is only present at particular sites and depths. *P. decipiens* can grow on severely to extremely exposed sites, especially on weakly inclined, not too steep slopes. The species is obviously able to tolerate the steady movements of surge waves but not sudden breakers battering against steep rock faces. In such situations the species is principally growing in crevices on the vertical face of M Rock outside Potter Cove (Klöser *et al.* 1996). In a new classification system with regard to abiotic conditions of the respective macroalgal habitat, Quartino *et al.* (2005) proposed *P. decipiens* to an indicator species for group B. Species of this group prefer boulder substrate at intermediate depths of 6-15 m with low temperatures down to subzero degrees. Regarding radiation and salinity, species belonging to group B settle in intermediate PAR ranges as well as at intermediate to low salinities (>33.5 PSU) with intermediate nitrogen concentrations, which are generally not limiting in Antarctic waters (Quartino *et al.* 2005).

In Antarctic coastal waters, ice formation and ice scouring belong to the most structuring factors of macroalgal communities (Quartino *et al.* 2005, Wulff *et al.* 2009 and references therein). In general, assemblages and succession processes are always very distinct and highly dependent on a variety of abiotic and biotic forcings; thus, at each moment each patch is in its very own succession state. Floating ice is considered to have the most disturbing impact in intertidal zones and is the main reason for the patchiness of communities (Quartino *et al.* 2005). Interestingly, after ice abrasion, *P. decipiens* increases in biomass and may form characteristic and dense fringes, as reported for Marion Cove on King George Island (Chung *et al.* 1994, Quartino *et al.* 2005). A similar phenomenon was observed after an unusually strong winter in 2007 in Potter Cove, which neighbours Marion Cove. After sea-ice break up, the intertidal platform of Penon Uño was almost pure rock

(macroscopically), but a *P. decipiens* cover developed within days (S. Becker, personal observation).

Climate warming has especially resulted in glacial retreat along the western Antarctic Peninsula (Braun & Gossmann 2002). Consequently, new ice-free areas have become available for benthic colonization coincidencing with an alteration of the characteristics of the water column. Thus, increased sediment input and salinity changes, and changing ice disturbance patterns are expected in coastal areas. A notorious example of this situation is observed at Potter Cove, where benthic macroalgal communities have been studied and monitored regularly over the last 18 years. In 1993-1994, the sublittoral vegetation was documented by subaquatic video transects, providing information of the vertical macroalgal distribution (Klöser *et al.* 1996). The soft bottom habitats of the inner cove were completely devoid of macroalgae and only some individuals were found on few boulders and stones of moraine deposits, which occurred in front of the glacier cliffs (Klöser *et al.* 1996). Over the last years, an apparent melting of the Fourcade glacier has exposed new ice-free areas, providing substrate for macroalgal colonization. In one of the most turbid new ice –free areas, located close to the glacier, *Palmaria decipiens* was the only conspicuous species colonizing hard substrata. In this atypical situation *P. decipiens* appeared growing under very turbid conditions and low light conditions caused by sediment input.

Grazing and habitat

As *Palmaria decipiens* is usually found in dense stands in shallow subtidal macroalgal communities (Amsler *et al.* 1995) and often dominates intertidal zones, it has been hypothesized that this rhodophyte plays a vital role in coastal ecosystem function forming the base of the food web. Intense research regarding grazer-alga interactions in Antarctica has been recently reviewed by Amsler *et al.* (2009). *P. decipiens* is directly consumed by fish e.g. *Notothenia coriiceps* and starfish *Odentaster validus*, an extremely abundant consumer and key stone predator in these waters (Iken *et al.* 1997, Amsler *et al.* 2005). Besides direct consumption by fish, *P. decipiens* has also been reported to be grazed by one of the most abundant amphipods (*Gondogeneia antarctic*a) along the western Antarctic Peninsula. Curriously, *Prostebbingia graciliaris*, another abundant amphipod, finds *P. decipiens* to be unpalatable (Huang *et al.* 2006, Amsler *et al.* 2009, Aumack *et al.* 2010). *P. decipiens* is the only non-filamentous macroalga tested so far that is consumed by *G.*

antarctica, however, thallus palatability has been investigated slightly less intense for amphipods as it has been for fish and starfish. There is evidence that at least one amphipod species feeds on this particular rhodophyte, but nonetheless only few amphipods are directly associated with the alga in nature, at least during daylight where most of the sampling is conducted (Huang *et al.* 2007). It is an important question for future research, if this pattern changes during night (Charles D. Amsler, pers. comm. 2009).

Macroalgae may exhibit chemical defenses that may be induced as a protective mechanism to prevent from excessive grazing activity (Amsler 2008). Although many macroalgae found in western Antarctic waters are known to produce secondary metabolites that aid in their defense against grazing, previous testing on *P. decipiens* indicated that it is palatable to the species mentioned above (Amsler *et al.* 2005, 2009, Huang *et al.* 2006, Aumack *et al.* 2010).

P. decipiens is the exclusive host of *Elachista antarctica*, which is a filamentous phaeophyte only found growing within, and emerging out of the thallus of *P. decipiens* (Wiencke & Clayton 2002, Peters *et al.* 2005). It is surprising that *E. antarctica* is present only on a palatable species of macroalgae considering that the standing biomass of other unpalatable species is very high. In order to confirm the palatability of *P. decipiens*, and to understand the relationship between *E. antarctica* and *P. decipiens*, feeding assays were conducted with four amphipods commonly associated with *P. decipiens*: *Prostebbingia gracilis*, *Gondogeneia antarctica*, *Oradarea bidentata* and *Paraphimedia integricauda.* Feeding assays were conducted to determine consumption rates (mgAlgae mgAmphipod^{-1} h^{-1}) for all four amphipod species in three different trials. In feeding assays, one amphipod was isolated with either *P. decipiens* or *E. antarctica* for specific periods of time. Preference experiments in which an individual amphipod was offered a feeding choice between *P. decipiens* and *E. antarctica* were also conducted using all four amphipod species. *G. antarctica* consumed both species but grazed *P. decipiens* at a faster rate than the epiphyte, whereas *P. gracilis*, *O. bidentata* and *P. integricauda* fed on the epiphyte *E. antarctica* at much faster rates. *P. gracilis* consumed the epiphyte at a significantly faster rate than the basiophyte in all three trials. *O. bidentata* consumed the epiphyte at a significantly greater rate in two of the three trials and *P. integricauda* showed significantly higher feeding of the epiphyte in one of the three trials. *G. antarctica* consumed both host and epiphyte but grazing was significantly

higher on *P. decipiens* in one of the no choice trials and one preference trial, and never showed significantly higher grazing on the epiphyte.

Studies by Campana (unpublished data) have shown that palatability of *P. decipiens* is not affected by different radiation treatments after five hours of exposure (Gabriela Laura Campana, pers. comm. 2009). In her study, the alga was exposed to different radiation climates consisting of PAR alone , PAR+UVA and PAR+UVA+UVB and apparently no differences in the palatability could be dected after exposure to UV radiation (Campana *et al.* 2008 and references therein, Campana *et al.* 2009). However, in recruitment experiments under different radiation treatments, red algal recruitment (including *P. decipiens*) was negatively affected under UV-exposure (Zacher *et al.* 2007). Nevertheless, grazing reduces biomass and density significantly and is thus one of the major biotic factors in ecosystem structuring. In a two-factorial experiment dealing with the combination of UV-radiation and grazing effects, interestingly, Zacher & Campana (2008) found that subtidal specimens of *P. decipiens* were only growing in the presence of grazers, and especially *P. decipiens* revealed increased densities, while UV-radiation overall significantly reduced algal densities regardless the shore level.

Outlook and future perspectives

Although a lot of effort has been undertaken to study *Palmaria decipiens* as one important representation of the Antarctic macroalgal flora, still interesting research questions remain unanswered. As it has been shown, that *P. decipiens* is able to cope with extensive periods of darkness by reducing its pigment content, it is still not clear how released carbon and nitrogen components will be translocated and processed. Weykam *et al.* (1997) found that survival in darkness is achieved by consuming floridean starch reservoirs. Additionally, in that study it was shown that the first newly synthesized carbon is used for growth with high priority, and subsequently new pigments are synthetized. The molecular mechanisms behind this pattern remain yet unclear. As a matter of fact, genetic mechanisms and how gene expression is triggered by environmental factors should be addressed in future research.

On the level of community structure and grazing influence, changing grazing patterns under changing environmental conditions such as increased UV-radiation, rising water turbidity, fresh water input or elevated temperatures need to be addressed with

respect to the rapidly ongoing climate change on the western Antarctic Peninsula. To what extent UV-radiation influences palatability by changing macroalgal flavour for potential grazers should be addressed in future investigations, also it needs to be taken into account that grazing might occur during the night or in darkness, so that data available to date might be under- or overestimating grazing rates and grazer influence. Increased input of turbid meltwater will undoubtedly lead to low light conditions in the sublittoral. An important question to be answered is how the lower depth limit of *Palmaria decipiens* can be affected by these changes and how this affects the function of coastal Antarctic ecosystems.

Even nowadays Antarctic research is one the most challenging fields of investigation, which is also highly dependent on logistic support, and hence, research activities are somewhat limited to accessible locations. Nevertheless, it should be of high interest to investigate the key organisms in Antarctic ecosystems in order to understand basic physiological adaptations and predict ecosystem responses in an era of climate change.

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