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Photosynthetic properties of the calcifying Phaeophyceae *Padina pavonica* (L.) Thivy (Dictyotales) under high – irradiance stress

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IN MEMORY OF MY BELOVED

GREAT – GRANDMOTHER

MATHILDE FÖLSNER!

1 Einleitung

Der Begriff Makrophyten fasst Makroalgen und Seegräser zusammen und umschreibt Pflanzen, die mit freiem Auge sichtbar und bestimmbar sind. Neben Phytoplankton stellen sie wichtige Primärproduzenten, insbesondere in Küstenregionen, dar und stehen als Nahrungsquelle an der Basis des Nahrungsnetzes für zahlreiche Tierarten (Häder & Figueroa 1997, Häder et al. 1998). Zusätzlich bieten sie Schutz und Versteckmöglichkeiten für viele Arten und Kinderstuben für deren Nachkommenschaft (Pudwill 2010). Makroalgen werden industriell zur Nahrungsproduktion genutzt und ihre Stoffwechselprodukte sowohl für pharmazeutische, biologische als auch kosmetische Zwecke verwendet.

Im Gegensatz zu Phytoplankton sind Makroalgen mit dem Substrat verankert und dadurch etlichen im Meer vorherrschenden und miteinander agierenden Stressfaktoren ausgesetzt (Nahrung, Licht, Temperatur, Austrocknung, Fraß). Als photoautotrophe Organismen sind Makrophyten auf Licht als primäre Energieressource angewiesen (Ragni & D´Alcala 2004). Unterliegt die Lichtstärke (Lichtintensität) jedoch enormen tageszeitlichen und saisonalen Fluktuationen, kann dies besonders an sonnenexponierten Standorten zu Stress führen. Im Gegensatz zu terrestrischen Lebensräumen verändert sich die Lichtqualität im aquatischen Millieu mit der Einstrahlungstiefe. Die Wassersäule und andere lichtabsorbierende Komponenten führen hier zu einer spektralen Veränderung des eintreffenden Lichtes, das mit der Tiefe durch Attenuation (= Abschwächung) verringert wird (Häder & Figueroa 1997, Franklin & Forster 1997, Karsten et al. 2001). Obwohl die Wassersäule auch als UV-Filter fungiert, können sichtbares Licht und UV-Strahlung in beachtliche Tiefen vordringen (Karentz 1989, Bischof et al. 1998). Dabei wird Letzteres als ausschlaggebender Faktor in der Tiefenzonierung von Makrophyten angesehen (Maegawa et al. 1993, Häder et al 1996, Häder & Figueroa 1997, Franklin & Forster 1997).

Um in einer heterogenen Umwelt wie dieser zu überleben, die von zahlreichen schwankenden physikalischen als auch chemischen Parametern bestimmt ist, haben Makrophyten im Laufe der Evolution zahlreiche Anpassungsstrategien entwickelt. Diese gewährleisten sowohl eine verbesserte Photosyntheseleistung als auch eine schnellere Reaktion auf schlagartige Veränderungen bestimmter Stressparameter wie Schwankungen der Lichtintensität. Wenn eine Pflanze überschüssiges Licht aufnimmt (= absorbiert), kann es zur Lichtübersättigung und somit zur Hemmung der Photosynthese kommen, auch bekannt als Photoinhibition (Osmond 1994). Dabei kann die Photosyntheseaktivität dramatisch abfallen. Photoinhibition ist art- als auch tiefenabhängig (La Franklin 2006), wobei das Ausmaß der Hemmung sowohl von der Intensität, der Wellelänge sowie der

Dauer der Lichtaussetzung bestimmt wird (Nultsch 1987). Man unterscheidet dynamische Photoinhibition und chronische Photoinhibition (Osmond 1994). Erstere ist ein reversibler Prozess, wenn oxidativer Stress einsetzt (Osmond 1994), und ist mit einem Schutzmechanismus verbunden, über den überschüssiges Licht wieder als Hitze abgegeben werden kann (cf. Demmig et al. 1988, Krause & Weis 1991, Demmig-Adams & Adams III 1992). Im Gegensatz dazu ist chronische Photoinhibition meistens mit irreversiblen Schäden des Photosystems II verbunden, verursacht durch starke Lichtintensitäten oder UV-Strahlung (Krause & Weis 1991, Osmond 1994, Long et al. 1994, Adir et al. 2003). Dadurch können die unter anderem für die Photosynthese wichtigen Enzyme und Proteine inaktiviert und im schlimmsten Fall ernsthaft beschädigt werden (Vincent & Neale 2000). Chronische Photoinhibition kann somit einen enormen Einfluss auf photosynthetische Prozesse und Primärproduktion mariner Organismen ausüben (cf. Franklin & Forster 1997, Häder et al. 1998, Aguilera et al. 1999, Bischof et al. 1999, Karentz & Bosch 2001). Hinsichtlich des Anstiegs von schädlicher UV-B Strahlung und deren möglichen Vordringens in beträchtliche Tiefen kann die Tiefenzonierung etlicher Algengruppen nicht vorhersehbaren drastischen Änderungen unterliegen (Bischof et al. 1999).

Exzessives Licht initiiert in Pflanzen unterschiedlichste Schutzmechanismen, um inhibitorische Prozesse zu minimieren und im besten Fall auch zu vermeiden. Spezielle sauerstoffhaltige Pigmente, die sogenannten Xanthophylle, spielen in einem lichtabhängigen Schutzmechanismus in höheren Pflanzen sowie in Braun- und Grünalgen eine prominente Rolle (= Xanthophyll-Zyklus; Demmig-Adams & Adams III 1996a, Demmig-Adams & Adams III 1996b, Yamamoto et al. 1999, Müller et al. 2001). Diese speziellen Karotinoide fungieren als Lichtsammelpigmente und dienen zudem als Schutz vor potentiell schadhafter Lichtenergie (Demmig-Adams & Adams III 1992, Demmig-Adams & Adams III 1996a). Sobald die Lichtintensität steigt und über dem Sättigungspunkt liegt, wird der pH-Wert im Innenraum (Lumen) der Thylakoidmembran geringer (Thylakoid = allseitig geschlossene Vesikel zum Aufbau eines Protonengradienten). Der pH-Wert fungiert hier als Sensor und löst eine schnelle reversible Umwandlung von Violaxanthin über Antheraxanthin in Zeaxanthin aus (Demmig-Adams & Adam III 1992, Demmig-Adams & Adams III 1996a, Demmig-Adams & Adams III 1996b). Insbesondere Zeaxanthin besitzt aufgrund seiner molekularen Struktur die Fähigkeit, überschüssig aufgenommenes Licht wieder in Form von Wärme abzustrahlen (= thermische Dissipation), auch bekannt als nicht-photochemisches Quenching (qN) (Demmig-Adams & Adams III 1996a, Young et al. 1997).

Je nach photosynthetischer Kapazität der Pflanze regt das eintreffende Licht Chlorophyll an: Diese Energie wird für photochemische Prozesse genutzt (= photochemisches Quenching) oder wie eben erwähnt ein überschüssiger Teil in Wärme umgewandelt. Zudem geht ein geringer Anteil der Energie in Form von Fluoreszenz verloren. Diese drei Arten der Nutzung von aufgenommener Anregungsenergie stehen miteinander in Konkurrenz. Die Erhöhung des einen resultiert in den Abfall der anderen beiden (Müller et al. 2001, Maxwell & Johnson 2002, Baker 2008).

Fluoreszenzmessungen, die auf den sogenannten Kautsky Effekt (Kautsky et al. 1960) beruhen, bieten eine gute Alternative, um qualitative und quantitative Aussagen über Änderungen des allgemeinen Gesundheitszustandes einer Pflanze in Stresssituationen zu treffen (Rohácek & Bartáck 1999). Dies schließt Veränderungen der Photosyntheseleistung, photoinhibitorische Prozesse und Art der Nutzung von aufgenommener Anregungsenergie mit ein.

In der gegenwärtigen Studie habe ich mich auf die Photosyntheseeigenschaften der kalzifizierenden Braunalge (Phaeophyceae) *Padina pavonica* fokussiert. Diese Makroalge stellt einerseits aufgrund ihrer ausgedehnten vertikalen Tiefenverbreitung von sonnenexponierten Felsküsten bis zu Tiefen von 30m, anderseits wegen ihrer für Braunalgen untypischen extrazellulären Kalkbildung einen hervorragenden Organismus für Studien über Stark- und Schwachlichtakklimatisierungsprozesse dar. Trotz ihrer weiten Verbreitung von tropischen bis hin zu temperaten Gebieten existieren nur wenige Publikationen über die Photosyntheseeigenschaften und Anpassungsfähigkeit dieser Makroalge. Gemäß der Intensität und den spektralen Eigenschaften des vorhandenen Umgebungslichts in den entsprechenden Tiefenzonen werden schließlich unterschiedliche Ansprüche an die Leistung des photosynthetischen Apparates der Algen gestellt. Diese Anpassungsfähigkeit ist entscheidend, um sich in Habitaten mit verschiedenen vorherrschenden Lichtklimaten behaupten zu können. Demnach ist die vertikale Makroalgenzonierung zum großen Teil von den umgebenden Lichtbedingung und deren Akklimatisierungspotential abhängig (Markager & Sand-Jensen1992, Hanelt 1998, Aguilera 1999, Karsten et al. 2001, Johansson & Snoailjs 2002). Folglich kann man annehmen, dass Arten aus tieferen oder beschatteten Habitaten sich langsamer an höhere Lichtintensitäten akklimatisieren als jene der Starklichtzonen, da sie sich durch eine geringere Photosyntheseaktivität, ein früheres Einsetzen von photoinhibitorischen Schäden und weniger effektive Schutzmechanismen auszeichnen. Dieses Phänomen wurde auch schon innerhalb einer Art, die sowohl an sonnenexponierten als auch schattigen Standorten vorkommt, beobachtet. Außerdem weisen Individuen aus tieferen Schichten meistens einen erhöhten Gehalt an Lichtsammelpigmenten auf, um das noch rare vorhandene Licht so

effizient wie möglich zu nutzen (Dring 1982). Aus diesem Grund haben wir angenommen, dass Individuen von *P. pavonica* aus seichteren und starklichtdominierten Habitaten einen erheblicheren Gehalt an Schutzpigmenten aufweisen, schnellere und effizientere Schutzmechanismen besitzen sowie eine höhere Photosynthesekapazität aufzeigen und zudem auch resistenter gegenüber Starklicht sind, was wiederum mit einem späteren Einsetzen von photoinhibitorischen Schäden einhergeht. Im Gegensatz dazu sollten Arten aus tieferen Schichten einen höheren Gehalt an Lichtsammelpigmenten für effizientere Lichtnutzung haben und aufgrund ihrer angenommenen stärkeren Empfindlichkeit gegenüber Starklicht früher Photoinhibition zeigen, als ihre Artgenossen aus seichten Gebieten. Weiters wird vermutet, dass Exemplare aus tieferen Zonen langsamer und weniger effektiv auf kurzzeitigen oder auch länger andauernden Starklichtstress reagieren, resultierend in einer Abnahme der Photosyntheseleistung und früheren Einsetzen von inhibitorischen Prozessen.

Um diese Hypothesen zu prüfen, wurden Thalli (Thallus = Habitus der Alge) von seichten Standorten bis zu 30m Tiefe in den Monaten September (2007) und Mai (2008) gesammelt, um Vergleiche der Photosyntheseeigenschaften zwischen *Padinas* aus unterschiedlichen Tiefenzonen zu ziehen. Zusätzlich wurde auch ein saisonaler Vergleich zwischen Frühling und Herbst gezogen. Für diese Studie wurden Fluoreszenz-Messungen sowohl im Feld als auch im Labor, und Pigmentanalysen mittels HPLC (High Performance Liquid Chromatography) durchgeführt.

2 Literaturverzeichnis

- Adir N, Zer H, Shochat S, Ohad I (2003) Photoinhibition- a historical perspective Photosynth. Res. 76: 343-370
- Aguilera J, Karsten U, Lippert H, Vögele B, Philipp E, Hanelt D, Wiencke C (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the arctic. Mar. Ecol. Prog. Ser. 191: 109-119
- Apprill AM, Lesser MP (2003) Effects of ultraviolet radiation on *Laminaria saccharina* in relation to depth and tidal height in the Gulf of Maine. Mar. Ecol. Prog. Ser. 256: 75-85
- Baker N (2008) Chlorophyll fluorescence: A probe of photosynthesis in vivo. Annu. Rev. Plant. Biol. 59: 89-113
- Bischof K, Hanelt D, Tüg H, Karsten U, Brouwer PEM, Wiencke C (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway) Polar Biol. 20: 388-395
- Bischof K, D Hanelt, Wiencke C (1999) Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV-radiation. Plant Biol. 1: 435-444
- Demmig-Adams B, Winter K, Krüger A, Czygan FC (1988) Zeaxanthinand the heat dissipation of excess light energy in *Nerium oleander* exposed to a combination of high light and water stress. Plant Physiol. 87: 17-24
- Demmig-Adams B, Adams III WW (1992) Photoprotection and other responses of plants to high light stress Annu. Rev. Plant Physiol. 43 (1): 599-626
- Demmig-Adams B, Adams III WW (1996a) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci. 1 (1): 21-26
- Demmig-Adams B, Adams III WW (1996b) Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. Planta 198 (3): 460-470
- Dring MJ (1982) The biology of marine plants. Edward Arnold London pp. 43-63
- Franklin LA, Forster R (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. Eur. J. Phycol. 32: 207-232
- Häder DP, Herrmann H, Santas R (1996) Effects of solar radiation and solar radiation deprived of UV-B and total UV on photosynthetic oxygen production and pulse amplitude modulated fluorescence in the brown alga *Padina pavonica*. FEMS Microbiol. Ecol. 19 (1): 53-61

- Häder DP, Figueroa F (1997) Photoecophysiology of marine macroalgae. Photochem. Photobiol. Sci. 66 (1): 1-14
- Häder DP, Kumar HD, Smith RC, Worrest RC (1998) Effects on aquatic ecosystems. J. Photochem. Photobiol. B: Biol. 46: 53-68
- Hanelt D (1998) Capability of dynamic photoinhibition in arctic macroalgae is related to their depth distribution. Mar. Biol. 131: 36-369
- Johansson G, Snoeijs (2002) Macroalgal photosynthetic responses to light in relation to thallus morphology and depth zonation. Mar. Prog. Ser. 244: 63-72
- Karentz D Report on studies related to the ecological implications of ozone depletion on the Antarctic environment. Antarctic J. US. 24: 175-176
- Karentz D, Bosch I (2001) Influence of Ozone-Related Increases in Ultraviolet Radiation on Antarctic Marine Organisms. Amer. Zool. 41: 3-16
- Karsten U, Bischof K, Wiencke C (2001) Photosynthetic performance of arctic macroalgae after transplantation from deep to shallow waters. Oceolog. 127: 11-20
- Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: The basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 313-49
- Kautsky H, Appel W, Amann H (1960) Chlorophyll Fluoreszenz und Kohlensäureassimilation. Die Fluoreszenzkurve und die Photochemie der Pflanze. Biochem. 322: 277-293
- La Franklin G, Lovelock CE, Larkum AWD (2006) Photoinhibition of photosynthesis on a coral reef. Plant Cell Enviro. 19 (7): 825-836
- Long SP, Humphries S, Falkowski PG (1994) Photoinhibition of photosynthesis in nature. Ann. Rev. Plant Biol. 45 (1): 633-662
- Maegawa M, Kunieda M, Kida W (1993) The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths. Jap. J. Phycol. 41: 207-214
- Markager S, Sand-Jensen K (1992) Light requirements and depth zonation of marine macroalgae. Mar. Ecol. Prog. Ser. 88: 83-92
- Maxwell K, Johnson G (2002) Chlorophyll fluorescence- a practical guide. J. Exp. Bot. 51(345): 659-668
- Müller P, Li X, Niyogi K (2001) Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125: 1558-1566
- Nultsch W, Pfau J, Materna-Weide M (1987) Fluence and wavelength dependence of photoinhibition in the brown alga *Dictyota dichotoma.* Mar. Ecol. Prog. Ser. 41: 93-97

- Osmond CB (1994) What is photoinhibition? Some insights from comparisons of shade and sun plants: In: Baker NR, Bowyer JR (eds) Photoinhibition of photosynthesis from molecular mechanisms to the field. Bios. Sci. Publ. Oxford, pp. 1-14
- Pudwill Robert (2010) Untersuchungen von Makroalgen und Seegraswiesen am Roten Meer bei El Quiseir/Ägypten. http://umweltplanung.pr-naturetours.de/Download-Dateien/Marine_Makrophyten.pdf
- Ragni M, D´Alcala MR (2004) Light as an information carrier underwater. J. Plankton Res. 26 (4): 433-443
- Rohácek K, Barták M (1999) Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. Photosynth. 37 (3): 339-363
- Vincent WF, Neale PJ (2000) Mechanisms of damage to aquatic organisms. In: de Mora D, Demers S, Vernet M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, pp. 149-176
- Yamamoto HY, Bugos RC, Hieber AD (1999) Biochemistry and molecular biology of the xanthophyll cycle. In: Frank HA, Young AJK, Britton G, Cogdell RJ (eds) The Photochemistry of Carotenoids. Dordrecht, The Netherlands: Kluwer Academic Publishers pp. 293-303
- Young A, Phillip D, Ruban A, Horton P, Frank H (1997) The xanthophyll cycle and carotenoid-mediated dissipation of excess excitation energy in photosynthesis. Pure Appl. Chem. 69 (10): 2125-2130

3 Photosynthetic characteristics of the calcifying Phaeophyceae *Padina pavonica* (L.) Thivy (Dictyotales) along an irradiance gradient

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3.1 Abstract

We studied photosynthetic properties of the common calcifying Phaeophyceae *Padina pavonica* comparing spatial as well as seasonal courses of photosynthetic acclimation patterns according to its depth distribution and natural prevailing light conditions. The study was conducted in the bay of Calvi (Corsica, France) at two seasons (spring, autumn). Both, *in situ* measurements and laboratory analyses took place using chlorophyll fluorescence techniques. Additionally, photosynthetic pigments were analyzed by high performance liquid chromatography. Generally, an increased photosynthetic efficiency and higher total pigment content per unit thallus area were observed in autumn. Due to acclimation processes, different degrees of photoinhibition in *Padinas* were recorded, respectively to their depth habitats. Fluorescence parameters like the absolute electron transport rates (ETR_{max}), the photochemical quenching (qP) and the thermal dissipation as heat (qN) yielded higher values in occupied sun exposed specimens in both, *in situ* and laboratory measurements. Unexpectedly, the initial slope of Rapid Light Curves (α) and the dark yield (F_v/F_m) were comparable at all depth habitats, which might be related to specific absorption- and reflection properties of the calcifying thalli at prevailing irradiances by compensating different irradiance supply. Specimens of high-irradiance habitats showed significantly higher amounts of light-harvesting pigments and photo-protective pigments per unit surface area than deeper growing ones. We assume that high-irradiance exposed thalli require higher amounts of photosynthetic pigments since their thalli are fleshier and more calcified to drive light-absorption more efficient. Contrarily, deeper growing ones improve their absorption properties through thinner and less calcifying appearance. *P. pavonica* seems to be well acclimated and responds adequate to its different environmental ambient irradiance regimes, which include high to intermediate irradiance quantities.

Keywords: *Padina pavonica*, photosynthesis, chlorophyll fluorescence, pigment, light gradient, season

3.2 Introduction

Macrophytes are important primary producers in marine ecosystems along the coastlines and a basis of the oceanic food web, even with a significant minor biomass production than phytoplankton (Mann 1973, Häder et al. 1998). For these photosynthetic organisms, solar radiation is the primary energy source and information carrier (Ragni & D´Alcala 2004), at the same time excess irradiance emersion might also be quite harmful. Recent studies on the effects of ultraviolet-radiation to aquatic organisms (cf. Maegawa et al. 1993, Dring et al. 1996a, Franklin & Forster 1997, Bischof et al. 1998a, Bischof et al. 1998b, Häder et al. 1998, Hoyer et al. 2001, Häder et al. 2003, Roleda et al. 2004) has also led to an increased interest on the potential damage because of excess photosynthetic active radiation (PAR) (Hanelt et al. 1992, Franklin et al. 1992, Hanelt et al. 1993, Hanelt et al. 1995, Aguilera et al. 1999). The penetration of solar visible as well as UV-radiation to greater depths is believed to play a key role in determining macroalgae depth distribution (Bischof et al. 1998a, Hanelt 1998, Bischof et al. 1999). The water column, acting as a remarkable ecological UV-protection, and light-absorbing components cause a spectral shift of the incident light, which also gets diminished with depth due to attenuation (Franklin & Forester 1997, Häder & Figueroa 1997, Karsten et al. 2001).

Being sessile, macroalgae are exposed to drastic varying irradiance conditions and are therefore also affected by various other stress factors. Hence, marine macrophytes developed diverse adaptation- and acclimation strategies operating on different time-scales. On one hand photosynthesis effectiveness needs to be improved, on the other hand a fast reaction to pronounced daily as well as seasonal changes of irradiance must be guaranteed. Excessively absorbed irradiance may lead to photoinhibition, which is mainly limiting at the level of photosystem II (PSII) (Krieger-Liszkay et al. 2000, Adir et al. 2003). In the worst case, excess light energy causes photo-oxidative damage of the photosynthetic tissues as a byproduct of photosynthesis (Müller et al. 2001). Adjustment processes of PS II are essential to modulate the photosynthetic electron flow to the demand of ATP and NADPH (Markager & Sand-Jensen 1992, Porcar-Castell et al. 2008) thereby minimizing photo-oxidative damage, when photosynthesis is saturated and photoinhibition occurs. Photoinhibition can be described as the light-dependent decline of photosynthetic effectiveness taking place when plants absorb more photons than can actually be used for photosynthetic processes (Long et al. 1994, Osmond 1994). It is postulated that different light qualities generate changes in pigment synthesis (Algarra et al. 1991, Figueroa et al. 1995), whereas blue light supports the pigment accumulation (Figueroa et al. 1995). The dimension of photoinhibition is depth- and species-specific (La Franklin et al. 2006), highly related to the environmental conditions and therefore impacted by quantity, wavelength and

duration of prevailing irradiances (Agel et al. 1987, Nultsch et al.1987, Häder et al. 1996a, Häder et al. 1996b) The terms dynamic and chronic photoinhibition were established by Osmond (1994) and have been achieved in photoinhibition terminology. When excitation pressure on PSII increases, plants undergo dynamic photoinhibition, which is related with the heat dissipation of the excessive absorbed photons as photo-protective mechanism in order to avoid irreversible damage (Demmig et al. 1988, Demmig-Adams & Adams III 1992, Häder & Figueroa 1997). Contrary, chronic photoinhibition, mostly occurring in shade-grown plants, is caused by photo-damage of PS II reaction centers due to harmful irradiance intensities followed by a decrease of photosynthetic electron transport and a non-reversible damage of the photosynthetic apparatus with corresponding degradation of the D1 protein (Krause & Weis 1991, Osmond 1994, Long et al. 1994, Adir et al. 2003).

Avoiding overexcitation is an essential mechanism for plants, which allows their survival (Asada 1999). Some carotenoids (Car) are involved in a reversible light-dependent pigment conversion, called the xanthophyll-cycle (XP-cycle). It can be found in higher plants as well as in various algae groups, especially in green algae and chromalveolates, and is accomplished by a specific group of oxygenated Car, so-called xanthophylls (XP), which are mostly bound to light-harvesting complex (LHC) proteins (cf. Demmig-Adams & Adams III 1996a, Demmig-Adams & Adams III 1996b, Yamamoto et al. 1999, Müller et al. 2001). Studies of the XP-cycle already have revealed its importance in the fast recovery from photoinhibition as a short response to varying light supply (e.g: Demmig-Adams & Adams III 1996a, Demmig-Adams & Adams III 1996b, Niyogi et al. 1997, Niyogi et al. 2004). Macroalgae, containing a lager XP pool size, have often been demonstrated to tolerate higher irradiances and longer duration of irradiance emersion (e.g.: Harker et al. 1999). XPs are found within the PSII antenna complexes and have a prominent function in the process of non-photochemical quenching of fluorescence signals (qN; Goss et al. 2008). Due to several authors (e.g.: Krause & Weis 1991, Müller et al. 2001, Baker 2008, Goss et al. 2008) qN can be divided in three components depending on the different relaxation kinetics as followed: the energy-dependent quenching (qE), the transition state quenching (qT) and last but not least the photoinhibitory quenching (qI). Excess irradiance leads to overexcitation of the chlorophyll (Chl) reaction centre, resulting in an interruption of the electron flow and subsequent chloroplast lumen acidification related with the formation of the proton motive force, namely qE (Young et al. 1997, Müller et al. 2001, Niyogi et al. 2004). Besides qE, two other components of qN can play a dominant role under certain circumstances, namely qI which gets prominent under excess irradiance resulting from photoinhibition, and the transition state quenching qT which is caused by phosphorylation of the light harvesting complexes under low-light exposure (Baker 2008). To sum up, qE

can be seen as a protection for PSII against excessive short-time irradiance exposure, whilst XPs acts as a further photo-protective mechanism if continuously exposed to high irradiances (Müller et al. 2001). Usually, a decrease of lumen pH stimulates the rapid de-epoxidation of violaxanthin (VX) via the intermediate antheraxanthin (AX) to zeaxanthin (ZX) (Yamamoto 1979, Demmig-Adams & Adams III 1996a). ZX has the ability to dissipate the excess absorbed light energy as heat, which is reflected in an elevated qN, and in a reduction of the yield and maximal photosynthesis rates (Gilmore et al. 1995, Demmig-Adams & Adams III 1996a). Through the pigment conversion into ZX, two more conjugated double-bounds are established in order to enhance the energy and life span of the excited states (Young et al. 1997).

The exact roles of ZX and AX in the qN pathway are still under debate (Demmig-Adams & Adams III 1996a, Young et al. 1997). Two models were proposed, namely a direct Car-Chl interaction and an indirect quenching process, which is based on the Car-mediated alterations in the structure and organisation of the LHC (Young et al. 1997). Due to Goss et al. (2008), both qN-effective and -ineffective ZX can be established: qN-effective ZX needs to be synthesized in the presence of a proton gradient in either light or dark, otherwise qN-ineffective ZX gets formed. Another factor controlling qN is the pH-value of the thylakoid stroma, which must be neutral to slightly basic to initiate qN-effective ZX production (Goss et al. 2008). Furthermore changes of the LHC macrostructure involving the right rebinding and conformation of ZX in its binding pocket have to take place during ZX synthesis to guarantee qN (Goss et al. 2008).

Another fluorescence quenching mechanism known as the photochemical quenching (qP) is indicating photosynthetic processes. The direct competition of these processes (qN, qP and Chl-*a* fluorescence) for excitation energy assures that a drop in the rate of one process will result in an increased rate of the challenging pathways (Baker 2008). Therefore measurements of Chl-*a* fluorescence emission (Krause & Weis 1991, Maxwell & Johnson 2000, Kramer et al. 2004, Baker 2008) provide a good alternative to draw conclusions about changes in the photosynthesis efficiency.

Fluorescence measurements are based on the so-called Kautsky effect (Kautsky et al. 1960): To estimate a non-stressed reference point the tissues get dark acclimated, which allows the PSII reaction centers to relax. A modulated weak measuring beam is switched on for obtaining the parameter F_0, where all reaction centers are open (dark-acclimated fluorescence minimum, when primary quinone acceptor of PS II (Q_A) is fully oxidized). When a saturated light pulse is given, all photosynthetic reaction centers become fully saturated and fluorescence reaches a maximum value F_m (= dark-acclimated fluorescence maximum, the primary quinone acceptor of PSII (Q_A) is fully reduced). The difference

between F_m and F_0 represents the variable fluorescence F_v. If then exposed to light, fluorescence drops down to the level F_t after a short and strong increase. The parameters F_0' and F_m' are the analogous minimal and maximal fluorescence, measured when the tissues are exposed to ambient light. Fluorescence yield is lowered by use of the excitation energy for qP and qN. The ratio of F_v/F_m can be used to determine the maximum quantum efficiency of PSII (Maxwell and Johnson 2000), in contrast to $(F_m' - F_t)/F_m' = \Phi PSII$ (Genty et al. 1989), which can be defined as operation efficiency yield (ΦPSII). In accordance to Logan et al. (2007) F_v/F_m can be used as a robust fluorescence parameter and low values indicates that the plant has received stress. The ΦPSII represents an estimation of the actually achieved PSII efficiency of the thalli under prevailing irradiance conditions at a given point of time (Baker 2008). Finally, F_v'/F_m' ratio is an equivalent to the F_v/F_m ratio measured in the illuminated state and stand for the maximum efficiency of PSII at a given photon flux density (Lichtenthaler & Burkart 1999). A detailed explanation of Chl-*a* fluorescence measurements is given in numerous publications (e.g.: Schreiber 1986, Krause & Weis 1991, Beer et al. 2000, Maxwell & Johnson 2000, Kramer et al. 2004, Schreiber et al. 2004, Logan et al. 2007, Baker 2008, Porcar-Castell et al. 2008).

In addition to dynamic photoinhibition and the XP-cycle several other mechanisms including alterations in the size of photosynthetic units (PSU) (Malkin & Fork 1981, Malkin et al. 1981), alterations in the cross section for light absorption (Kroon 1994, Lichtenthaler & Burkart 1999), changes in the abundance and composition of pigments in accordance to the quality of ambient light (Algarra et al. 1991, Apprill & Lesser 2003), chloroplast movement (Müller et al. 2001) and the presence of Car (Dring 1982, Hanelt & Nultsch 1991, Demmig-Adams & Adams III 1996a, Bischof et al. 1999) have been proposed to have a protective function against damage of the photosynthetic tissues. Generally, Car such as β-Car possess a prominent function as energy quenchers of excited triplet Chl and singlet oxygen within the reaction centers (Mimuro & Katch 1991, Demmig-Adams et al. 1996, Franklin & Forster 1997), whereas secondary Car, situated outside the chloroplast, often act as screening pigments (Franklin & Forster 1997, Cockell & Knowland 1999). The production of UV-absorbing mycosporine-like amino acids (MAAs) can also act as protection against UV radiation (Dring et al. 1996b, Häder et al. 1998, Karsten & West 2000, Apprill & Lesser 2003), which concentration continuously decreases with depth (Hoyer et al. 2001).

For the present study, we focused on light acclimation of the calcifying Phaeophyceae *Padina pavonica,* which is a common seaweed at sun-exposed rocky shore regions down to 30m depth. Its extracellular carbonate layers are rather untypical among brown algae. Because of its expanded vertical distribution, *Padina* demonstrates an excellent study

organism for intermediate to high-light acclimation processes in response to varying irradiance supply and despite its wide occurrence from oligotrophic tropical to temperate regions this species remains largely understudied.

So far, it was of great interest if a correlation between photoinhibition effects and *Padina* specimen zonation does exist and the question has arisen, if *Padinas* from different depths show typical low- and high-light acclimation according to their light supply. We investigated if specimens near the surface exposed to high-irradiance, contain more photo-protective XPs than LH-pigments, and we believed that the reverse case will occur for organisms, which are growing in shaded areas. We assumed that total pigment content is generally higher in shade-specimens. Among other physical (nutrients uptake, temperature, salinity tolerance, desiccation) and biological factors (grazing, inter- and intra-specific competition), the vertical macroalgal zonation is proposed to be structured by the ambient light conditions and related photosynthetic properties, such as compensation and saturating points and pigment composition (Lüning 1981, Markager & Sand-Jensen 1992). We expected that deeper growing *Padinas* are more sensitive against high radiation due to lower compensation irradiances and to an acclimation to low-light levels at greater depths whereas high-light specimens probably show faster and more efficient photosynthetic protection mechanisms. The deeper the specimens are growing, the slower and less effectively they may react to short-time exposure to strong irradiances.

With the aid of fluorescence techniques and HPLC pigment analysis, applied in the field as well as in defined lab experiments, we investigated on the spatial and the seasonal differences (spring and autumn) of photosynthetic performance in *P. pavonica*.

3.3 Material & Methods

Investigations were carried out in September 2007 (autumn) and May 2008 (spring) in the Mediterranean Sea near Calvi (42°34´N, 8°45´O, Corsica, France) at the bay of the STARESO (station de recherchés sous – marines et oceanographique). Solar radiation was recorded using a data logger (Skye data hog 1) in five minutes intervals. Incident irradiance of the appropriate depths (Iz) for harvested thalli were determined with the light guide of the Diving PAM. Light percentage of surface radiation (%) was calculated for each thallus and finally three light classes (LCT) were defined (LCT 1: < 11.5 %, LCT 2: 14.5 – 21.5 %, LCT 3: > 24.5 % of surface irradiance; LCT = Light Class irrespective of the season).

Sampling and *in situ* fluorescence measurements were conducted once a day at 11 am by Scuba Diving. Ten thalli per day were selected by random sampling. Samples and measurements were taken at varying depths from 0 – 30 m. Rapid Light Curves (RLC´s) were recorded by a submersible pulse-amplitude modulation fluorometer (DIVING PAM; Walz, Effeltrich, Germany) applying the method of Schreiber et al. (1986, Schreiber 2004) to get insight into the actual photosynthetic performance under given attached irradiance supply: A dark leaf clip, attached to the fiber optic of the fluorometer was used to fix the thalli during measurements. First, the actual ΦPSII was determined, followed by exposure to eight steps (10 s each) of increasing artificial actinic light from the internal light source of the Diving PAM. ΦPSII was estimated by the equation (Genty et al. 1989): $\Phi PSII = (F_m' - F_t)/ F_m'$. F_t is the fluorescence steady state level at ambient irradiance, followed by a single (0.8 s) saturating light pulse generating the maximal fluorescence F_m'. ETR_{max} and α were estimated using the MS Excel Solver applying the following equation by Platt et al. (1980): $ETR = ETR_{max} \times [1-\exp(-\alpha \times PAR/ ETR_{max})] \times \exp(-\beta \times PAR/ ETR_{max})$. The absolute ETR was estimated by following formula: $ETR_{absolute} = \Phi PSII * PAR * AF * 0.5$. PAR (photosynthetic active radiation; photon flux density; µmol quants m^{-2} s^{-1}) is the given irradiance, 0.5 is a factor considering two photons are needed for the transport of one electron and β is the drop of the RLCs. AF is the absorption factor, calculated by the formular: AF = A/100. Thallus absorption (A) was determined for each thallus by considering reflection (R) and transmission (T): A = 100 - R % - T %. R was measured over the whole range of visible light (400-700nm) with the aid of a Spectroradiometer equipped with a reflection probe (Ocean Optic: USB4000 Miniature Fiber Optic Spectrometer; bifurcated optic fiber: Reflection/Back scattering Probes P400-2-UV-VIS) and T was estimated with the light sensor of the Diving PAM with sunlight as 100 % incident irradiance.

After in situ measurements, samples were transported in black bags to exclude external irradiance for dark acclimation for further analysis in the laboratory, where the initial

measures F_v/F_m, F_m and F_0 and were determined after 15 minutes dark incubation to guarantee a fully oxidized electron transport chain (duration of light pulse 0.7s). Afterwards samples were exposed to three light intensities (80, 850, 2900 µmol photons s^{-1} m^{-2}; duration of light pulse 0.7s; each intensity for one minute). At each intensity step F_v'/F_m', ΦPSII, qN and qP were calculated from the initial parameters as followed: $qN = (F_m - F_m')/(F_m - F_0')$; $qP = (F_m' - F_t)/(F_m' - F_0')$; $F_v'/F_m' = (F_m' - F_0')/ F_m'$; $\Phi PSII = (F_m' - F_t)/ F_m'$ (Genty et al. 1989).

High Performance Liquid Chromatography (HPLC) was used for pigment quantification. Disks of a defined area of each thallus apex were cut out with a punch. The samples were immediately frozen and stored in liquid nitrogen at – 80°C until pigment extraction. For extraction issues were ground in 90% acetone (homogenizer Polytron PT 1600 E) under dim light and then stored for 10 h in a refrigerator (4°C). After centrifugation (15 min), the supernatant was separated and analysed (HPLC system Hitachi EliteLaChrom, Diode Array Detector Hitachi L-2455, column thermostat L-2300 with temperature of 35°C, column Superspher RP – 18, 100 LIChrocart, precolumn LIChrocart rP-18 endcapped) according to a modified protocol of Wright et al. (1991). Peaks were detected at 440 nm and identified by comparing retention times and spectral data with those of authentic standards (DHI Bioproducts). Analyzed pigments were subdivided into three categories: the XP pool size (µg cm $^{-2}$; sum of VX, AX, ZX), the light-harvesting pigments (LH -pigments; µg cm $^{-2}$; sum of Chl-*a*, Chl-c, Fucoxanthin (FX)) and the remaining pigments (µg cm $^{-2}$; sum of β-Car, Diadinoxanthin (DdX), Diatoxanthin (DtX), Neoxanthin (NX)).

Statistical analyses were performed using the SPSS 15.0. All data were tested for homogeneity of variance (Levene-test) and normal distribution (Kolmogorov-Smirnov test). If both criteria were met, GLM procedures repeated measurements (2 factors: light class and season; for F_v/F_m, ΦPSII, qN, qP) or GLM univariate (2 factors: light class and season; for pigments) with Schéffe post-hoc tests were calculated; else we conducted non parametric comparisons (Kruskal Wallis H-test and Mann Withney U-test; for ETR_{max} and α) at a 95% significance level. Mean values and its standard deviations as well as medians (*) are listed in Table 1 and Table 2.

3.4 Results

***In situ* measurements**

Field measurements of *Padina pavonica* showed characteristic light acclimation according to the respective LCT (Fig. 1a). The absolute ETR_{max} (µmol electrons cm^{-2} s^{-1}) definitely increased with enhanced irradiance availability and differed significantly between LCT1 and LCT2 as well and between LCT1 and LCT3 (LCT1: n = 94; LCT2 n = 74 LCT3: n = 75; LCT1 – LCT2: p = 0.000, LCT2 – LCT3: p = 0.106, LCT1 – LCT3: p = 0.000). *Padina* specimens of sun exposed locations reached visibly higher values than thalli of shaded habitats. Moreover, May measurements yielded lower absolute ETR_{max} values compared to September ones (Table 2; May: n = 85; September: n = 148; p = 0.000). Unexpectedly, α values (Fig. 1b) were comparable in all LCTs and yielded around 0.15. (LCT1: n = 94; LCT2 n =: 74 LCT3: n = 75; p = 0.787), though a significant difference between the seasons with slightly higher values in September could be observed (Table 2; May: n = 85; September: n = 148; p = 0.000).

Laboratory measurements

Both qN and qP tended to increase with enhanced light supply (Fig. 2). Clear and significant differences could be proven for qP within the LCT1 and LCT3 as well as between LCT2 and LCT3 (LCT1: n = 78; LCT2 n =: 79 LCT3: n = 70; LCT2 – LCT3: p = 0.002, LCT1 – LCT3: p = 0.000, LCT1 – LCT2: p = 0.050). For qN LCT1 highly varied from LCT2 and from LCT3 (LCT1: n = 78; LCT2 n =: 79 LCT3: n = 70; LCT2 – LCT3: p = 0.421, LCT1 – LCT3: p = 0.000, LCT1 – LCT2: p = 0.010). Exposure to stepwise increasing irradiances (80, 850, 2900 µmol photons m^{-2} s^{-1}) induced a rapid decline in qP corresponding to a fast increase in qN with highly significant differences (Fig. 2; p = 0.000). In addition, noteworthy differences of qN and qP between the two seasons could be obtained. qN indicated slightly higher values in May (Table 2; May: n = 60; September: n = 157; p = 0.002), in contrast to qP, where higher values were recorded in September (Table 2; May: n = 60; September: n = 157; p = 0.000). F_v/F_m values were approximately 0.6 irrespective of the LCT with a p = 0.859 (Table 1; LCT1: n = 78; LCT2 n =: 79 LCT3: n = 70). Also within the seasons no significant differences were found for F_v/F_m (Table 2; May: n = 60; September: n = 157; p = 0.050). F_v'/F_m' revealed a strong decline with increasing irradiance, which resulted in highly significant differences (Table 1; p = 0.000). Neither between the seasons (Table 2; May: n = 60; September: n = 157; p = 0.050) nor between the LCTs (Table 1; LCT1: n = 78; LCT2 n =: 79 LCT3: n = 70; p = 0.997) differences were recorded. Statistical analysis showed a significant decrease of ΦPSII with stepwise increasing irradiances (p = 0.000) and LCT3 differed from LCT1 and LCT2 (Table 1; LCT1: n = 78; LCT2 n =: 79 LCT3: n = 70; LCT2 – LCT3: p = 0.043, LCT1 – LCT3: p = 0.000, LCT1 –

LCT2: p = 0.060), with higher values for shallow water thalli. ΦPSII did not differ between the seasons (Table 2; May: n = 60; September: n = 157; p = 0.051).

Thalli contained Chl-*a* and Chl-c, FX and VX as major pigments, followed by β-Car, NX, AX, ZX, DdX and DtX. HPLC analyses of pigment extracts revealed also traces of Chl-b, Lutein (L) and Dinoxanthin (DnX), which were probably originating from epiphytes. For all three pigment categories a higher total pigment content (µg cm^{-2}) was observed in September (Table 2; May: n = 108; September: n = 158; p = 0.000). High-irradiance supply caused elevated pigment contents (µg cm^{-2}) in *Padina*. Unexpectedly, plants of high-irradiance locations showed increased LH-pigments (Fig.3). Also XP-cycle involved pigments (Fig. 4) as well as remaining pigments (Fig. 5) yielded higher values in sun exposed specimens. Significant differences were proven between all LCTs, except between LCT2 and LCT3 (LCT1: n= 102; LCT2 n =: 79 LCT3: n = 85; XP-pool size: LCT2 – LCT3: p = 0.416; LCT1 – LCT2: p = 0.000, LCT1 – LCT3: p = 0.000; LH-pigments: LCT2 – LCT3: p = 0.786, LCT1 – LCT2: p = 0.040, LCT1 – LCT3: p = 0.004; remaining pigments: LCT2 – LCT3: p = 0.324, LCT1 – LCT2: p = 0.040, LCT1 – LCT3: p = 0.000).

3.5 Discussion

Shaded and deeper growing macroalgae are often reported to be more sensitive against excess irradiance than sun exposed specimens, which is characterized by a higher predisposition to photoinhibition and faster photo-damage of PSII (Markager and Sand-Jensen 1992, Hanelt et al. 1993, Häder et al. 1996a, Häder et al. 1996c, Hanelt et al. 1997a, Bischof et al. 1998a, Bischof et al. 1998b, Aguilera 1999, Johansson & Snoeiljs 2002). Differences in pigment composition and photosynthetic properties may also come about within a species, when the algae mature at different sites of irradiances (Dring 1982, Emsinger et al. 2005, Walter et al. 2005). Due to the fact that *Padina pavonica* apparently favours CO_2 over HCO_3^- and shows higher photosynthesis in air than in water, *Padina* can be considered as an original light-alga and consequently may profit from exposure (Einav et al. 1995). This might partly explain their higher observed occurrence in sun exposed habitats. *P. pavonica*, which is known for its wide vertical distribution (Einav et al. 1995, Häder et al 1996a, Franklin and Forster 1997, Häder and Figueroa 1997), appears to be well adjusted to various depths, where different environmental conditions are predominant.

With the aid of fluorescence measurements in the field, different degrees of photoinhibition in *Padina* thalli were recorded. Exposure to high irradiance usually leads to alterations in initial slope α and the bulge of RLC (Henley et al. 1991, Hanelt et al. 1995). Low-light species normally show a lower ETR_{max} at low intensities accompanied with a steep slope (higher values for α), in contrast to high-light species, which can utilize higher intensities with a higher ETR_{max} and a flat slope (Ott 1996, Walters et al. 2005). Indeed, thalli exposed to high-irradiance levels showed higher ETR_{max} values than the algae sampled from low-light habitats (Fig. 1a); however the initial slopes (Fig. 1b) remained the same in all LCTs. Johannson and Snoeils (2002) stated that α combines both morphological and physiological features of a plant. The α of 32 investigated macroalga species (19 rhodophytes and 13 phaeophytes) even more reflected the morphology than the physiology (Johannson & Snoeils 2002). Also, the F_v/F_m ratio (Table 1) showed similar values in all LCTs. Normally, F_v/F_m values = 0,82 are found in unstressed vascular plants, although brown algae show typically lower values (Franklin & Forster 1997), which is in accordance to our measurements (around 0.6). Due to Malkin et al. (1981) similar F_v/F_m values might indicate an equal absorption cross section of PSII. Higher ETR_{max} values for high-irradiance species but no differences of α were also proven by other authors (Silva et al. 1998, Ensminger et al. 2005), who suggested that alterations in the acclimation potential based on α might not be demonstrated with the aid of fluorescence techniques. ΦPSII (Table 1) and qP (Fig 2a) showed lower values in low-light species, which goes along with the results of the absolute ETR_{max}.

Due to Häder et al. (1996a) *Padina pavonica* (Saronikos Gulf, near Korinth, Greece) growing in deeper habitats showed faster photoinhibition than thalli from rocky pools. The authors hypothesized that *Padinas* growing at different sites of ambient irradiance regimes may belong to different ecological strains due to their different morphological appearance (shallow water specimens were thicker and showed higher calcification than deeper growing thalli; Häder et al. 1996a, Häder & Figueroa 1997). This might cause specific absorption- as well as reflection properties at prevailing irradiances. Also other macroalgae such as *Halimeda tuna* ((J. Ellis & Solander) J.V. Lamouroux) and *Dictyota dichotoma* ((Hudson) J.V. Lamouroux) provided different morphology due to their respective habitat (Häder & Figueroa 1997). Species with a wide vertical distribution are believed to develop phenotypical and niche-specific differentiation (Lynch and Gabriel 1987, Ensminger et al. 2005). Results of this study partly approve these assumptions. Under laboratory conditions, a slower decline of qP, F_v'/F_m' and ΦPSII as well as faster enhancement of qN with stepwise increasing high fluency photon rates in sun exposed thalli pointed out that thalli of light-poor habitats are less adjusted for sudden enhanced irradiances, which is indicated by a lower photosynthetic efficiency, an earlier start of photoinhibition and finally slower reacting of the XP-cycle and its corresponding dissipation as heat. Both quenching mechanisms showed a trend to enlarge within the LCTs (Fig. 2). The decline of qP and the simultaneous increase of qN with stepwise enhanced irradiance exposure emphasized the antagonism relationship of those quenching pathways and the rapid switch to heat dissipation as photo-protective response to increased irradiance stress.

Further points of consideration were pigment contents. Unexpectedly, the analyses revealed a higher total pigment content in sun exposed plants (Table 1). It was already documented by Dring (1982) that low-light algae possess a higher LH-pigment and higher total pigment content than sun exposed specimens due to the fact, that they need them to catch the rarely available photons. In contrast, shallow-water thalli should have an increased pool of photo-protective pigments because they have to cope with excess irradiance. Indeed, the sun exposed algae produced more photo-protective pigments (Fig. 4, 5) which go along with the higher qN values of high-light specimens. This indicates an enhanced XP-cycle activity in sun exposed habitats. However, an increase of LH-pigments was also detectable in high-light *Padinas* (Fig. 3). This untypical behaviour of *Padina* to enlarge the LH-pigment pool with enhanced irradiance supply might be explained by their different thallus morphology and its absorption- and reflection properties compared to low-light specimens. In this manner, macroalgae can be considered as optical intricate systems showing different light absorption- and scattering properties in accordance to their morphology (Mercado et al.

1996). It is often mentioned that thallus anatomy and morphology (e.g.: thallus form, thickness, area, cell size, shape, carbonate layer) under variable light supply generates alterations in the absorption properties regardless of Chl-*a* amounts, which subsequently influences the light-harvesting properties and photo-acclimation strategies of algae (Lüning & Dring 1985, Frost-Christensen & Sand-Jensen 1992, Markager 1993). Older and thicker thalli remain to be less sensitive against high-irradiances as a consequence of more protective tissues (Johansson & Snoeijs 2002). They are believed to have reduced light absorption properties because of low Chl-*a* amounts thereby preventing high Chl-*a* density and insufficient photon absorption, which serves as a possible explanation of their lower growth rate and high-irradiance acclimation of thick thalli and the dominance of thin thalli in deeper or shaded habitats. (Markager & Sand-Jensen 1992, Agusti et al. 1994, Enríquez et al. 1994, Enríquez et al. 1996). The authors (Agusti et al. 1994, Enríquez et al. 1994, Enríquez et al. 1996) stated that marine macroalgae possess a diverse repertoire of light absorption properties, reflected in an interaction of thallus thickness and pigment content. Besides, Ramus (1981) suggested that low-light acclimation is provided by an enhanced Chl-*a* content, which in turn is accompanied with an enhancement of the absorption factor, though other studies (Frost-Christensen & Sand-Jensen 1992) assumed that after a certain degree of thickness enhancement, absorption probably becomes almost independent of pigment content. Also, Gomez et al. (1997) did not found a significant increase of Chl-*a* with depth, except in *Himantothallus grandifolius* ((A.Gepp &E.S. Gepp) Zinova). *Gigartina skottsbergii* (Setchell & N.L.Gardner) and *Kallymenia antarctica* (Hariot) of shallower locations even revealed a higher Chl-*a* content than those of deeper habitats, while for *Desmarestia anceps* (Montagne) and *Palmaria decipiens* ((Reinisch) R.W.Ricker) no changes in Chl-*a* content were recorded. Some studies documented that shade-adapted specimens with a thinner appearance have other optical qualities to utilize photons more efficient (Lüning 1990, Binzer & Sand Jensen 2002) avoiding the self-shading of pigments themselves, which is called the package-effect (Littler 1980). The package effect could lead to drastically lower efficiency of photon collection (Mercado et al. 1996). In regard to *Padina,* higher LH-pigment content in shallow water habitats can be probably explained by their different optical absorption properties in accordance to their thallus thickness and carbonate layers. So far, shallow water specimens might improve their photon collection by a greater LH-pigment content because of their thicker calcifying thalli, protecting them against wave action and harmful excessive PAR/UV radiation. In contrast, deeper growing specimens might enhance their absorption capacity by their thinner and less calcifying tissues; they also save energy costs for pigment production, and avoid the package effect.

Pigment composition can change within a few days when plants are transferred to different irradiances (Dring 1982). In accordance to Dring (1981) alterations in pigment composition with greater depths are mainly acclimation processes to low-irradiance, and consequently not to the spectral composition of underwater light climate. Transplantation experiments might give more insight into *Padinas* acclimation strategies which allow them to settle down into different depths.

Nultsch and Pfau postulated in 1979, that chloroplast movement from low irradiance/high absorption position to a high irradiance/low absorption position is widely common in Phaeophyceaeta, , but needs several hours to complete (Nultsch and Pfau 1979, Hanelt & Nultsch 1991).The blue light-induced chloroplast movement is a further effective photo-protective mechanism during inhibition phase which causes a decrease in absorption cross section (Hanelt and Nultsch 1991, Bischof et al. 1999). It would be of great interest if this phenomenon plays also a certain role in *Padina*.

It should be also mentioned here, that morphological plasticity can also be considered as a response to grazing pressure. Dimorphism in *Padina* species as protection against grazing has already been observed (Allender & Kraft 1983, Lewis et al. 1987).

Marine macroalgae have also to deal with seasonal alterations including varying light supply, fluctuations in temperature and nutrients availability. In this regard, a further aim of this study was to find out if differences between spring and autumn *Padinas* exist. ETR_{max} differed with higher values in the autumn (Table 2), α and qP (Table 2) were also significantly higher in autumn, hence photoinhibition occurred earlier in spring. These results indicate an increased photosynthetic capacity in September compared to May. Markager & Sand-Jensen (1992) reported that ETR_{max} values are normally higher for photosynthesis processes than those values for maturing thalli. Consequently we assumed that *Padina* may use the energy for photosynthesis activity in autumn which resulted in a higher ETR_{max} and consequently higher qP, contrary to spring, where the energy is needed for growth which goes along with higher energy costs. An additional explanation could be that *Padinas* are thicker and more calcified in September. The LH-pigments (Table 2) were significantly higher in September which is an evidence for enhanced light-capturing during autumn. The photo-protective pigments (Table 2) yielded also significantly higher values in September, whereby qN was slightly higher in spring. In the case of *Laminaria saccharina* (Linnaeus), spring populations showed more photo-protective pigments, which was interpreted as protection of their tissues against rapidly increasing irradiances during spring time, whereas the winter populations evolved their antenna complexes to capture a maximum of photons (Gevaert et al. 2002). As photosynthesis and related processes involve enzymatic steps (Hanelt 1996), one possible explanation for less photo-protective pigments

in spring could be the lower sea water temperature during spring (September: ~ 23°C, May: ~ 14.5°C), which slowed down the XP-cycle and other enzymatic reactions. This phenomenon could also be responsible for the later settlement in deeper habitats and showed lower abundance than in September due to the fact that macroalgae have the ability to acclimate to temperature alterations to a different degree, as shown in some antarctic macroalgae (Thomas & Wiencke 1991)

According to Hanelt et al. (2003) the seasonal variability of photosynthetic performance in macroalgae is strongly linked to their life strategy. The seasonal variations of photosynthetic characteristics in various alga groups were investigated by Bischof et al. (2002) and different responses were given according to their life strategies, age, morphological and physiological characteristics, and vertical distribution. Saroussi & Beer (2007) found, that seasonal changes in irradiance and temperature can strongly influence the absorption factor and the number of reaction centers per thallus area. A further point of view could be the reproductive stage and age of a thallus, which also plays an important role in the photosynthetic performance of macroalgae and their capability to acclimate to sudden irradiance changes (Hanelt et al. 1997b, Roleda et al. 2004, Varela et al. 2006). However, further experiments must be carried out to address this question explicitly.

P. pavonica seems to obtain optimal photosynthetic performances over a broad range of irradiances. Primarily, *P. pavonica* can be seen as a high-light species, which also has ability to acclimate to low irradiances. According to Ensminger et al. (2005), photosynthetic acclimation to different irradiance regimes provokes alterations in photosynthetic performance as well as in photosynthetic pigments at different sites of growth, which is seemly the case here and therefore allows this taxon to respond adequately to the prevailing irradiance regimes.

3.6 References

- Adir N, Zer H, Shochat S, Ohad I (2003) Photoinhibition-a historical perspective Photosynth. Res. 76: 343-370
- Agel G, Nultsch W, Rhiel E (1987) Photoinhibition and its wavelength dependence in the cyanobaterium *Anabaena variabilis.* Arch. Microbiol. 147 (4): 370-374
- Aguilera J, Karsten U, Lippert H, Vögele B, Philipp E, Hanelt D, Wiencke C (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the arctic. Mar. Ecol. Prog. Ser. 191: 109-119
- Agusti S, Enriquez S, Frost-Christensen H, Sand-Jensen K, Duarte C (1994) Light harvesting among photosynthetic organisms. Funct. Ecol. 8 (2): 273-279
- Algarra P, de la Villa G, Niell J (1991) Effects of light quality and irradiance level interactions on short -term pigment response of the red alga *Corallina elongate*. Mar. Ecol. Prog. Ser. 74: 27-32
- Allender BM, Kraft GT (1983) The marine algae of Lord Howe Island (New South Wales): the Dictyotales and Cutleriales (Phaeophyta). Brunonia 6: 73-130
- Asada K (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Ann. Rev. Plant Physiol. Plant Mol. Biol. 50: 601-639
- Apprill AM, Lesser MP (2003) Effects of ultraviolet radiation on *Laminaria saccharina* in relation to depth and tidal height in the Gulf of Maine. MEPS 256: 75-85
- Baker N (2008) Chlorophyll fluorescence: A probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59: 89-113
- Binzer T, Sand-Jensen K (2002) Production in aquatic macrophyte communities: A theoretical and empirical study of the influence of spatial light distribution. Limnol. Oceanogr. 47 (6): 1742-1750
- Bischof K, Hanelt D., Wiencke C. (1998a) UV-radiation can affect depth-zonation of antarctic macroalgae. Mar. Biol. 131: 597-605
- Bischof K, Hanelt D, Tüg H, Karsten U. (1998b) Acclimation of brown algal photosynthesis to ultraviolet radiation in arctic coastal waters (Spitsbergen, Norway). Polar Biol. 20: 388-395
- Bischof K, D Hanelt, Wiencke C (1999) Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. Plant Biol. 1: 435-444
- Bischof K, Hanelt D, Aguilera J, Karsten U, Vögele B, Sawall T, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an arctic

fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. Mar. Biol. 140 (6): 1097-1106

- Cockell CS, Knowland J (1999) Ultraviolet radiation screening compounds. Biol. Rev. 74: 311-345
- Demmig B, Winter K, Krüger A, Czygan FC (1988) Zeaxanthin and the heat dissipation of excess light energy in *Nerium oleander* exposed to a combination of high light and water stress. Plant Physiol. 87: 17-24
- Demmig-Adams B, Adams III WW (1992) Photoprotection and other responses of plants to high light stress Annu. Rev. Plant Physiol. 43 (1): 599-626
- Demmig-Adams B, Adams III WW (1994) Capacity for energy dissipation in the pigment bed in leaves with different xanthophyll cycle pools. Aust. J. Plant Physiol. 22: 249-260
- Demmig-Adams B, Adams III WW (1996a) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci. 1 (1): 21-26
- Demmig-Adams B, Adams III WW (1996b) Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. Planta 198 (3): 460- 470
- Demmig-Adams B, Gilmore A, Adams III WW (1996) In vivo function of carotenoids in higher plants. FASEB J. 10: 403-412
- Dring MJ (1981) Chromatic adaptation of photosynthesis in benthic marine algae: An examination of its ecological significance using a theoretical model. Limnol. Oceanogr. 26 (2): 271-284
- Dring MJ (1982) The biology of marine plants. Edward Arnold. London. pp. 43-63
- Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K (1996a) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta) Mar. Biol. 126: 183-191
- Dring MJ, Wagner A, Boeskov J, Lüning AK (1996b) Sensitivity of intertidal and subtidal red algae to UVA and UVB radiation, as monitored by chlorophyll fluorescence measurements: influence of collection depth and season and length of irradiation. Eur. J. Phycol. 31: 293-302
- Einav R, Breckle S, Beer S (1995) Ecophysiological adaptation strategies of some intertidal marine macroalgae of the Israeli Mediterranean coast. Mar. Ecol. Prog. Ser. 125: 219-228
- Enríquez S, Agusti S, Duarte C (1994) Light absorption by marine macrophytes. Oecolog. 98: 121-129

- Enríquez S, Duarte CM, Sand-Jensen K, Nielson SL (1996) Broad-scale comparison of photosynthetic rates across phototrophic organisms. Oecolog. 108: 197-206
- Ensminger I, Hagen C, Braune W (2001) Strategies providing success in a variable habitat II: Ecophysiology of Photosynthesis of *Cladophora glomerata*. Plant Cell Environ. 23 (10): 1129-1136
- Ensminger I, Foerster J, Hagen C, Braune W (2005) Plasticity and acclimation to light reflected in temporal and spatial changes of small-scale macroalgal distribution in a stream. J. Exp. Bot. 56 (418): 2047-2058
- Figueroa F, Aguilera J, Niell F (1995) Red and blue light regulation of growth and photosynthetic metabolism in *Porplivra umbilicalis.* (Bangiales, Rhodophyta). Eur. J. Physiol. 30: 11-18
- Franklin LA, Levavasseur G, Osmond CB, Henley WJ, Ramus J (1992) Two components of onset and recovery during photoinhibition of *Ulva rotundata*. Planta 186: 399-408
- Franklin LA, Forster R (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. Eur. J. Phycol. 32: 207-232
- Frost-Christensen H, Sand-Jensen K. (1992) The quantum efficiency of photosynthesis in macroalgae and submerged angiosperms. Oecolog. 91 (3): 377-384
- Genty B, Briantais J, Baker N (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochem. Biophys. Acta 990: 87-92
- Gevaert F, Creach A, Davoult D, Holl AC, Seuront L, Lemoine Y (2002) Photo-inhibition and seasonal photosynthetic performance of the seaweed *Laminaria saccharina* during a simulated tidal cycle: chlorophyll fluorescence measurements and pigment analysis. Plant Cell Environ. 25: 859-872
- Gilmore A, Hazlett T, Govindjee (1995) Xanthophyll cycle-dependent quenching of photosystem II chlorophyll a fluorescence: Formation of a quenching complex with a short fluorescence lifetime. Proc. Natl. Acad. Sci. 92: 2273-2277
- Gómez I, Weykam G, Klöser H, Wiencke C (1997) Photosynthetic light requirements, metabolic carbon balance and zonation of sublittoral macroalgae from King George Island (Antarctica). Mar. Ecol. Prog. Ser. 148: 281-293

- Goss R, Opitz C, Lepetit B, Willhelm C (2008) The synthesis of NPQ-effective zeaxanthin depends on the presence of a transmembrane proton gradient and a slightly basic stromal side of the thylakoid membrane. Planta 228: 999-1009
- Häder DP, Herrmann H, Santas R (1996a) Effects of solar radiation and solar radiation deprived of UV-B and total UV on photosynthetic oxygen production and pulse amplitude modulated fluorescence in the brown alga *Padina pavonica*. FEMS Microbiol. Ecol. 19: 53 -61
- Häder DP, Lebert M, Mercado J, Aguilera J, Salles S, Flores-Moya A, Jimenez C (1996b) Photosynthetic oxygen production and PAM fluorescence in the brown alga *Padina pavonica* measured in the field under solar radiation. Mar. Biol. 127: 61-66
- Häder DP, Porst M, Herrmann H, Schäfer J, Santas R (1996c) Photoinhibition in the mediterranean green alga *Halimeda tuna* Ellis et Sol measured in situ. Photochem. Photobiol. Sci. 64 (3): 428-434
- Häder DP, Figueroa F (1997) Photoecophysiology of marine macroalgae. Photochem. Photobiol. Sci. 66 (1): 1-14
- Häder DP, Kumar H, Smith R, Worrest R (1998) Effects on aquatic ecosystems. J. Photochem. Photobiol. B: Biol. 46: 53-68
- Häder DP, Lebert M, Helbling W (2001) Photosynthetic performance of marine macroalgae measured in Patagonia on site. Photochem. Photobiol. Sci. 8: 145-151
- Häder DP, Lebert M, Sinha R, Barbieri E, Helbling E (2002) Role of protective and repair mechanisms in the inhibition of photosynthesis in marine macroalgae. Photochem Photobiol Sci. 1: 809-814
- Häder DP (2003) Aquatic ecosystems: effects of solar ultraviolet radiation and interactions with other climatic change factors. Photochem. Photobiol. Sci. 2: 39-50
- Hanelt D, Nultsch W (1991) The role of chromatophore arrangement in protecting the chromatophores of the brown alga *Dictyota dichotoma* against photodamage. J. Plant Physiol. 138: 470-475
- Hanelt D (1992) Photoinhibition of photosynthesis in marine macrophytes of the South China Sea. Mar. Ecol. Prog. Ser. 82: 199 -206
- Hanelt D, Huppertz K, Nultsch W (1992) Photoinhibition of photosynthesis and its recovery in red algae. Bot. Acta 105: 278-284
- Hanelt D, Huppertz K, Nultsch W (1993) Daily course of photosynthesis and photoinhibition in marine macroalgae investigated in the laboratory and field. Mar. Ecol. Prog. Ser. 97: 31-37

- Hanelt D, Uhrmacher S, Nultsch W (1995) The effect of photoinhibition in marine macrophytes of the South China Sea. Bot. Act. 108: 99-107
- Hanelt D (1996) Photoinhibition of photosynthesis in marine macroalgae. Sci. Mar. 60 (1): 243-248
- Hanelt D, Melchersmann B, Wiencke C, Nultsch W (1997a) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar. Ecol. Prog. Ser. 149: 255-266
- Hanelt D, Wiencke C, Karsten U, Nultsch W (1997b) Photoinhibition and recovery after high light stress in different developmental and life history stages of *Laminaria saccharina* (Phaeophyta). J. Phycol. 33: 387-395
- Hanelt D (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. Mar. Biol. 131: 361-396
- Hanelt D, Wiencke C, Bischof K (2003) Photosynthesis in marine macroalgae. In: Larkum AWD, Douglas SE, Raven JA (eds) Photosynthesis in algae. Advances in photosynthesis and respiration. Kluwer Acadamic Publishers, Netherlands, 14: 413-435
- Harker M, Berkaloff C, Lemoine Y, Britton G, Young A, Duval J, Rmiki N, Rousseau B (1999) Effects of high light and desiccation on the operation of the xanthophyll cycle in two marine brown algae. Eur. J. Phycol. 34: 35-42
- Henley W, Levavasseur G, Franklin LA, Lindley ST, Ramus J, Osmond CB (1991) Diurnal responses of photosynthesis and fluorescence in *Ulva rotundata* acclimated to sun and shade in outdoor culture. Mar. Ecol. Prog. Ser. 75 (1): 19-28
- Hoyer K, Karten U, Sawall T, Wiencke C (2001) Photoprotective substances in antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages Mar. Ecol. Prog. Ser. 211: 117-129
- Johansson G, Snoeijs (2002) Macroalgal photosynthetic responses to light in relation to thallus morphology and depth zonation. Mar. Prog. Ser. 244: 63-72
- Karsten U, West JA (2000) Living in the intertidal zone-seasonal effects on heterosides and sun-screen compounds in the red alga *Bangia atropurpurea* (Bangiales). J. Exp. Mar. Biol. Ecol. 254: 221-234
- Karsten U, Bischof K, Wiencke C (2001) Photosynthetic performance of arctic macroalgae after transplantation from deep to shallow water. Oceolog. 127: 11-20
- Kautsky H, Appel W, Amann H (1960) Chlorophyll Fluoreszenz und Kohlensaureassimilation. Die Fluoreszenzkurve und die Photochemie der Pflanze. Biochem. 322: 277-293

- Kiirikki M (1996) Mechanisms affecting macroalgal zonation in the northern Baltic Sea. Eur. J. Phycol. 31: 225-232
- Kramer DM, Johnson G, Kiirats O, Edwards GE (2004) New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. Photosynth. Res. 79: 209-218
- Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: The basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 313-349
- Krieger-Liszkay A, Kienzler K, Johnson GN (2000) Inhibition of electron transport at the cytochrome b_6f complex protects photosystem II from photoinhibition. FEBS 486: 191-194
- Kroon B (1994) Variability of photosystem II quantum yield and related processes in *Chlorella pyrenoidosa* (Chlorophyta) acclimated to an oscillating light regime simulating a mixed photic zone. J. Phycol. 30: 84-852
- La Franklin G, Lovelock CE, Larkum AWD (2006) Photoinhibition of photosynthesis on a coral reef. Plant Cell Enviro. 19(7): 825-836
- Lewis SM, Norris JN, Searles RB (1987) The regulation of morphological plasticity in tropical reef algae by herbivory. Ecol. 68 (3): 636-641
- Lichtenthaler HK, Burkart S (1999) Photosynthesis and high light stress. Bulg. J. Plant. Physiol. 25 (3-4): 3-16
- Littler MM (1980) Morphological form and photosynthetic performances of marine macroalgae: tests of a functional/form hypothesis. Bot. Mar. 22: 161-165
- Littler M, Arnold K (1982) Primary productivity of marine macroalgal functional-form groups from southwestern north America. J. Phycol. 18: 307-311
- Logan BA, Adams III WW, Demmig-Adams B (2007) Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. Funct. Plant Biol. 34: 853-859
- Long SP, Humphries S, Falkowski PG (1994) Photoinhibition of photosynthesis in nature. Ann. Rev. Plant Biol. 45 (1): 633-662
- Longstaff BJ, Kileda T, Runcie JW, Cheshire A, Dennison WC, Hurd C, Kana T, Raven JA, Larkum AWD (2002) An in situ of photosynthetic oxygen exchange and electron transport rate in the marine macroalga *Ulva lactuca* (Chlorophyta). Photosynth. Res. 74: 281-293
- Lüning K (1981) Light. In: Lobban CS, Wynne MJ (eds) The biology of seaweeds. Blackwell Scientific Puplishers, Oxford pp 326-355

- Lüning K, Dring MJ (1985) Action spectra and spectral quantum yield of photosynthesis in marine macroalgae with thin and thick thalli. Mar. Biol. 87: 119-129
- Lüning K (1990) Seaweeds: Their Environment, biogeography and ecophysiology. John Wiley & Sons, New York. pp. 287-307
- Lynch M, Gabriel W (1987) Environmental Tolerance. American Nat. 129 (2): 283-303
- Maegawa M, Kunieda M, Kida W (1993) The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths. Jap. J. Phycol. 41: 207-214
- Malkin S, Fork DC (1981) Photosynthetic units of sun and shade plants. Plant Physiol. 67: 580-583
- Malkin S, Armond PA, Mooney HA, Fork DC (1981) Photosystem II photosynthetic unit sizes from fluorescence induction in leaves. Correlation to photosynthetic capacity. Plant Physiol. 67: 570-579
- Mann KH (1973) Seaweeds: Their productivity and strategy for growth. Sci. 182: 975-981
- Markager S, Sand-Jensen K (1992) Light requirements and depth zonation of marine macroalge. Mar. Ecol. Prog. Ser. 88: 83-92
- Markager S (1993) Light absorption and quantum yield for growth in five species of marine macroalgae. J. Phycol. 29: 54-63
- Maxwell K, Johnson G (2002) Chlorophyll fluorescence- a practical guide. J. Exp. Bot. 51(345): 659-668
- Mercado J, Jiménez C, Niell F, Figueroa F (1996) Comparison of methods for measuring light absorption by algae and their application to the estimation of the package effect. Sci. Mar. 60 (1): 39-45
- Mimuro M, Katch T (1991) Carotenoids in photosynthesis: Absorption, transfer and dissipation of light energy. Pure & Appl. Chem. 63 (1): 123-130
- Müller P, Li X, Niyogi K (2001) Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125: 1558-1566
- Niyogi KK, Björkman O, Grossman AR (1997) The roles of specific xanthophylls in photoprotection. Proc. Natl. Acad. Sci. 94: 14162-14167
- Niyogi KK, Li XP, Rosenberg V, Jung HS (2004) Is PsbS the site of non-photochemical quenching in photosynthesis? J. Exp. Bot. 56 (411): 375-382
- Nultsch W, Pfau J (1979) Occurrence and biological role of light-induced chromatophore displacements in seaweeds. Mar. Biol. 51 (1): 77-82

- Nultsch W, Pfau J, Materna-Weide M (1987) Fluence and wavelength dependence of photoinhibition in the brown alga *Dictyota dichotoma.* Mar. Ecol. Prog. Ser. 41: 93-97
- Oltmanns F (1992) Über die Kultur und Lebensbedingungen der Meeresalgen. Jahrb. Wiss. Bot. 23: 349-440
- Osmond CB (1994) What is photoinhibition? Some insights from comparisons of shade and sun plants: In: Baker NR, Bowyer JR (eds) Photoinhibition of photosynthesis from molecular mechanisms to the field. Bios. Sci. Publ. Oxford, pp. 1-14
- Ott J (1996) Meereskunde. UTB für Wissenschaft pp.121-123
- Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J. Mar. Res. 38: 687-701
- Porcar-Castell A, Pfündel E, Korhonen J, Juurola E (2008) A new monitoring PAM fluorometer (MONI-PAM) to study the short- and long-term acclimation of photosystem II in field conditions. Photosynth. Res. 96: 173-179
- Ragni M, D´Alcala MR (2004) Light as an information carrier underwater. J. Plankton Res. 26 (4): 433-443
- Ramus J (1981) The capture and transduction of light energy. In: Lobban CS, Wynne MJ (eds) The biology of seaweeds. University of California Press, Berkeley, pp 458-492
- Roleda M.Y, Hanelt D, Kräbs G, Wiencke C (2004) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. Phycol. 43 (5): 603-613
- Saroussi S, Beer S (2007) Acclimations of macroalgae as reflected in photosynthetic parameters derived from PAM fluorometry, and possible implications for abundance patterns. Mar. Ecol. 28: 377-383
- Schofield O, Evens T, Millie D (1998) Photosystem II quantum yields and xanthophyll-cycle pigments of the macroalga *Sargassum natans* (Phaeophyceae): Responses under natural sunlight. J. Phycol. 34: 104-112
- Schreiber U, Schliwa Q, Bilger U (1986). Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorimeter. Photosynth. Res. 10: 51-62
- Schreiber U (2004) Pulse-Amplitude-Modulation (PAM) fluorometry and saturation pulse method: an overview. chlorophyll *a* fluorescence: A signature of photosynthesis, ed. GC Papageorgiou, Govindjee, Dordrecht, The Netherlands: Springer pp. 279-319.

- Silva J, Santos R, Serodio J, Melo RA. 1998 Light response curve for *Gelidium sesquipedale* from different depths, determined by two methods: O_2- evolution and chlorophyll fluorescence. J. App. Phycol. 10: 295-301
- Thomas DN, Wiencke C (1991) Photosynthesis, dark respiration and light independent carbon fixation of endemic Antarctic macroalgae. Polar Biol. 11: 329-337
- Walters RG (2005) Towards an understanding of photosynthetic acclimation. J. Exp. Bot. 56: 435-447
- Wright SW, Jeffrey SW, Mantoura RFC, Liewellyn CA, Bjornland T, Repeta D, Welschmeyer N (1991) Improved HPLC method for analysis of chlorophylls and carotenoids from marine phytoplankton. Mar. Ecol. Prog. Ser. 77: 183 -196
- Varela DA, Santelices B, Correa JA, Arroyo MK (2006) Spatial and temporal variation of photosynthesis in intertidal *Mazzaella laminarioides* (Bory) Fredericq (Rhodophyta, Gigartinales) J. Appl. Phycol. 18: 827-838
- Verhoeven A, Adams III W, Demmig-Adams B, Croce R, Bassi R (1999) Xanthophyll cycle pigment localization and dynamics during exposure to low temperatures and light stress in *Vinca major*. Plant Physiol. 120: 727-737
- Yamamoto HY (1979) Biochemistry of the violaxanthin cycle. Pure Appl. Chem. 51: 639-648
- Yamamoto HY, Bugos RC, Hieber AD (1999) Biochemistry and molecular biology of the xanthophyll cycle. In: Frank HA, Young AJK, Britton G, Cogdell RJ (eds) The Photochemistry of Carotenoids. Dordrecht, The Netherlands: Kluwer Academic Publishers pp. 293-303
- Young A, Phillip D, Ruban A, Horton P, Frank H (1997) The xanthophyll cycle and carotenoid-mediated dissipation of excess excitation energy in photosynthesis. Pure Appl. Chem. 69 (10): 2125-2130

3.7 Figure and Table Legends

Fig. 1 Box-blots with mean values (−) and its standard deviations and outliers (●) **a:** Changes of the absolute ETR_{max} versus light class (LCT1, LCT2, LCT3) expressed in µmol electrons cm^{-2} s^{-1}. The significant differences ($p < 0.05$) within LCTs are marked with the letters a for LCT1, b for LCT2 and c for LCT3. **b**: The initial slope α versus the light class (LCT1, LCT2 and LCT3). No significant differences were recorded.

Fig. 2 Box-blots with mean values (−), standard deviations and outliers (●) **a:** qN of increasing irradiance steps (repeated measurements: 80 PAR, 850 PAR, 2900 PAR) versus light class (LCT1, LCT2, LCT3). The significant differences ($p < 0.05$) within the LCT are marked with the letters a for LCT1, b for LCT2 and c for LCT3. Within increasing irradiance steps a significance level of $p = 0.000$ could be proven **b:** qP of increasing irradiance steps (repeated measurements: 80 PAR, 850 PAR, 2900 PAR) versus light class (LCT1, LCT2, LCT3). The significant differences ($p < 0.05$) within the LCTs are marked with the letters a for LCT1, b for LCT2 and c for LCT3. Within increasing irradiance steps a significance level of $p = 0.000$ could be proven.

Fig. 3 Box-blots with mean values (−), standard deviations and outliers (●). XP-pool size (VX, AX, ZX) versus light class (LCT1, LCT2 and LCT3) expressed in µg cm^{-2}. The significant differences ($p < 0.05$) within the LCTs are marked with letters a for LCT1, b for LCT2 and c for LCT3, respectively.

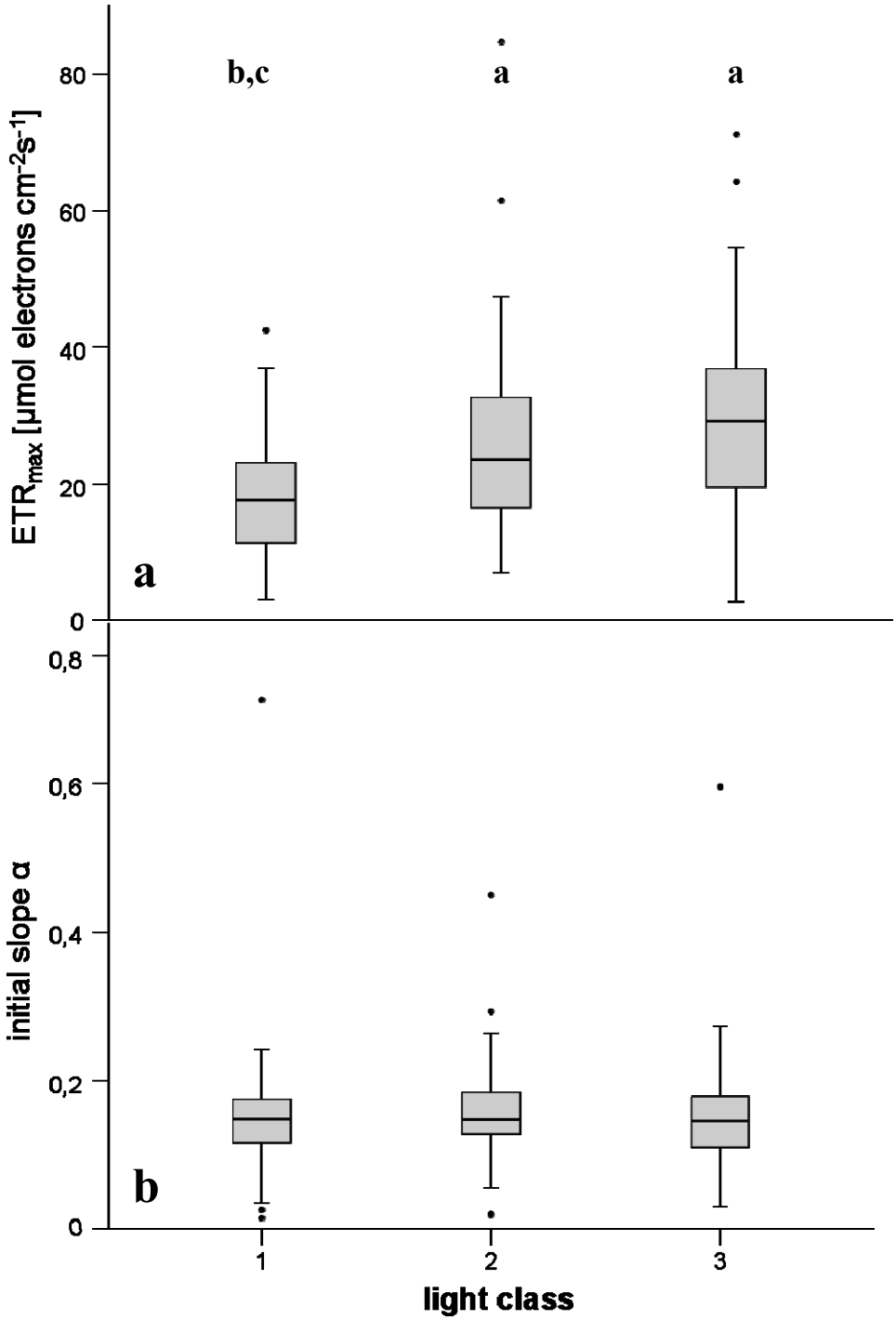
Fig. 4 Box-blots with mean values (−), standard deviations and outliers (●)**.** LH-pigments (Chl-*a*, Chl-c FX) versus light class (LCT1, LCT2, LCT3) expressed in µg cm^{-2}. The significant differences ($p < 0.05$) within the LCTs are marked with letters a for LCT1, b for LCT2 and c for LCT3.

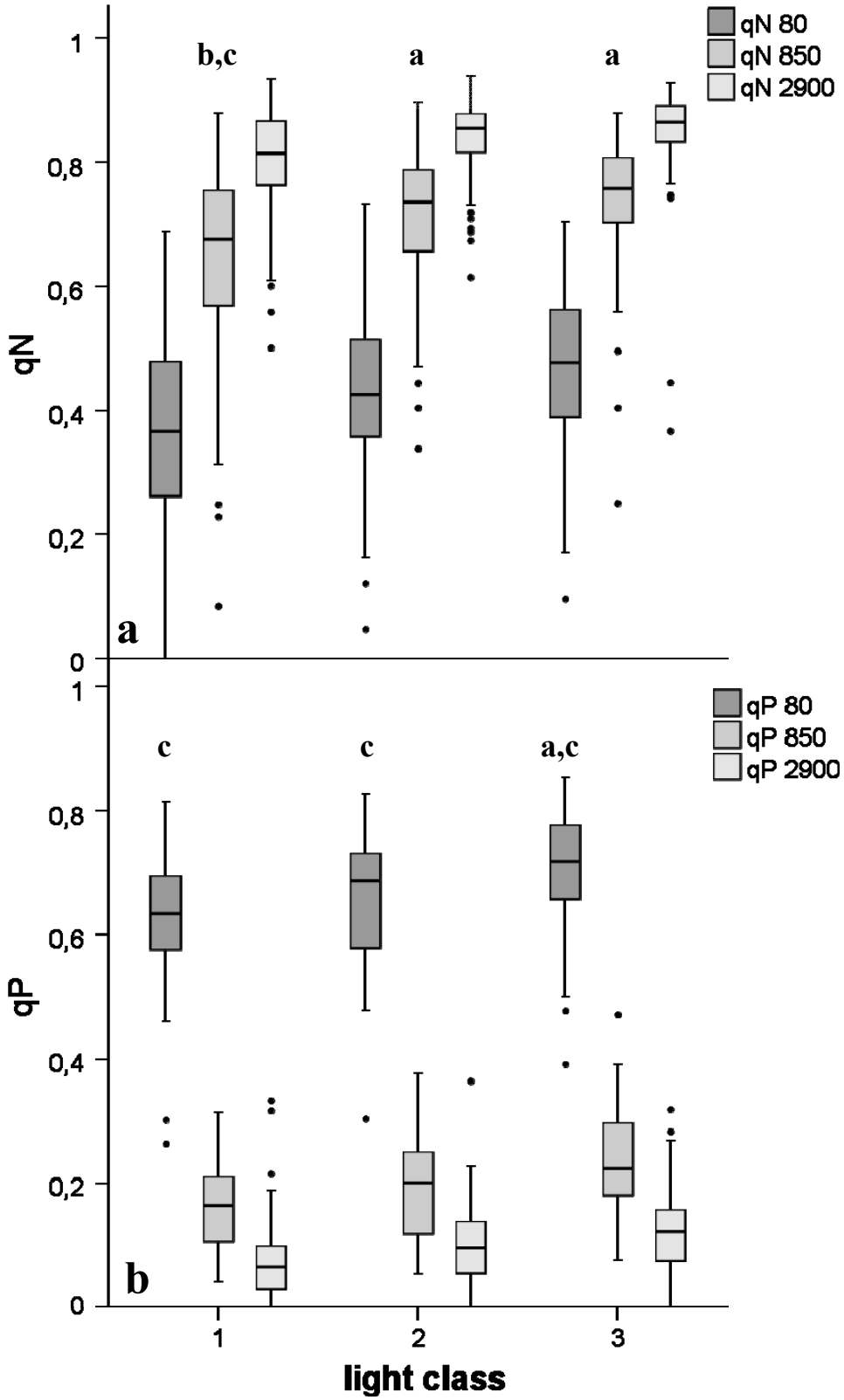
Fig. 5 Box-blots with mean values (−), standard deviations and outliers (●). Remaining pigments (β -Car, DdX, DtX, NX) versus light class (LCT1, LCT2, LCT3) expressed in µg cm^{-2}. The significant differences ($p < 0.05$) within the LCTs are marked with letters a for LCT1, b for LCT2 and c for LCT3.

Table 1. Mean values with standard deviations and medians (marked with *) of the measured parameters qP, qN, F_v/F_m, F_v'/F_m', ΦPSII and total pigment content (µg cm^{-2}) of LCTs 1-3.

Table 2. Mean values with standard deviations and medians (marked with *) of the measured parameters ETR_{max} (absolute electron transport rate; µmol cm^{-2} s^{-1}), α, qP, qN, F_v/F_m, $F_{v'}/F_{m'}$, ΦPSII, XP-pool size (µg cm^{-2}), LH-pigments (µg cm^{-2}), remaining pigments (µg cm^{-2}) and total pigment content (µg cm^{-2}) of seasons.

3.8 Figures and Tables




qN 80
qN 850
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b,c
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qN
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qP 80
qP 850
qP 2900
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c
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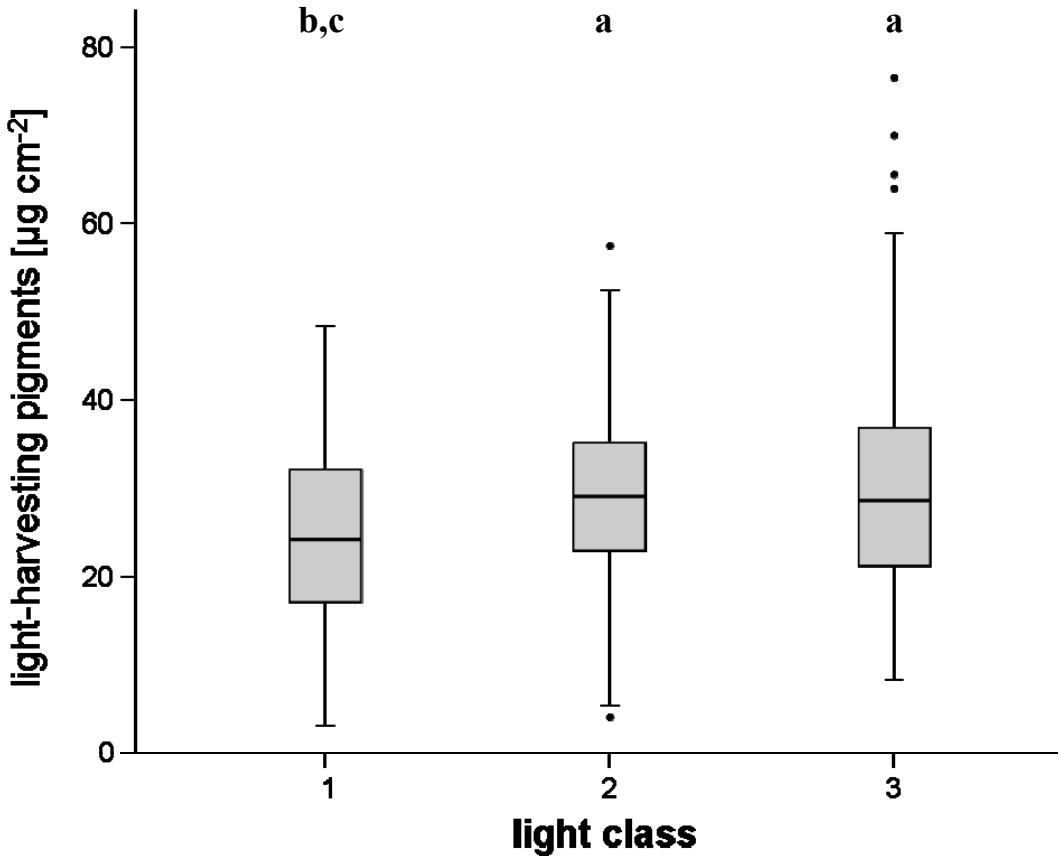
b
1
2
3
light class


b,c
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80
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20
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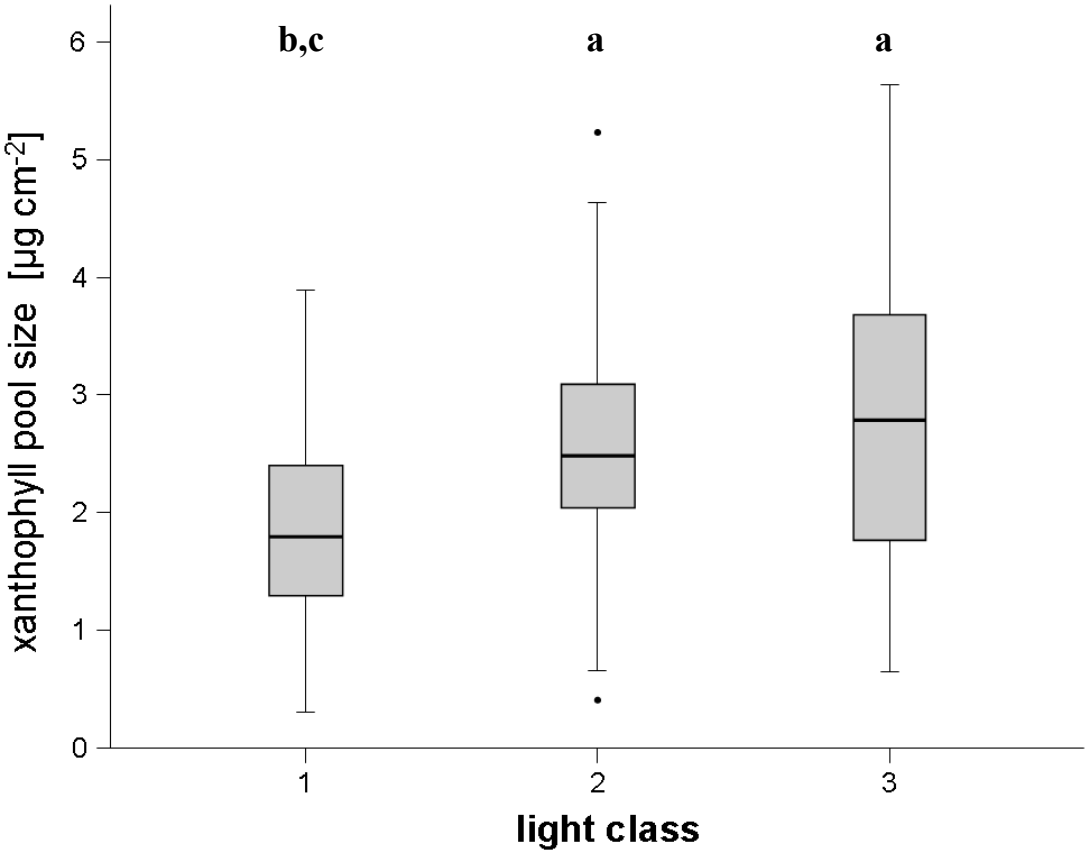
light-harvesting pigments [µg cm-2]
1
2
3
light class


b,c
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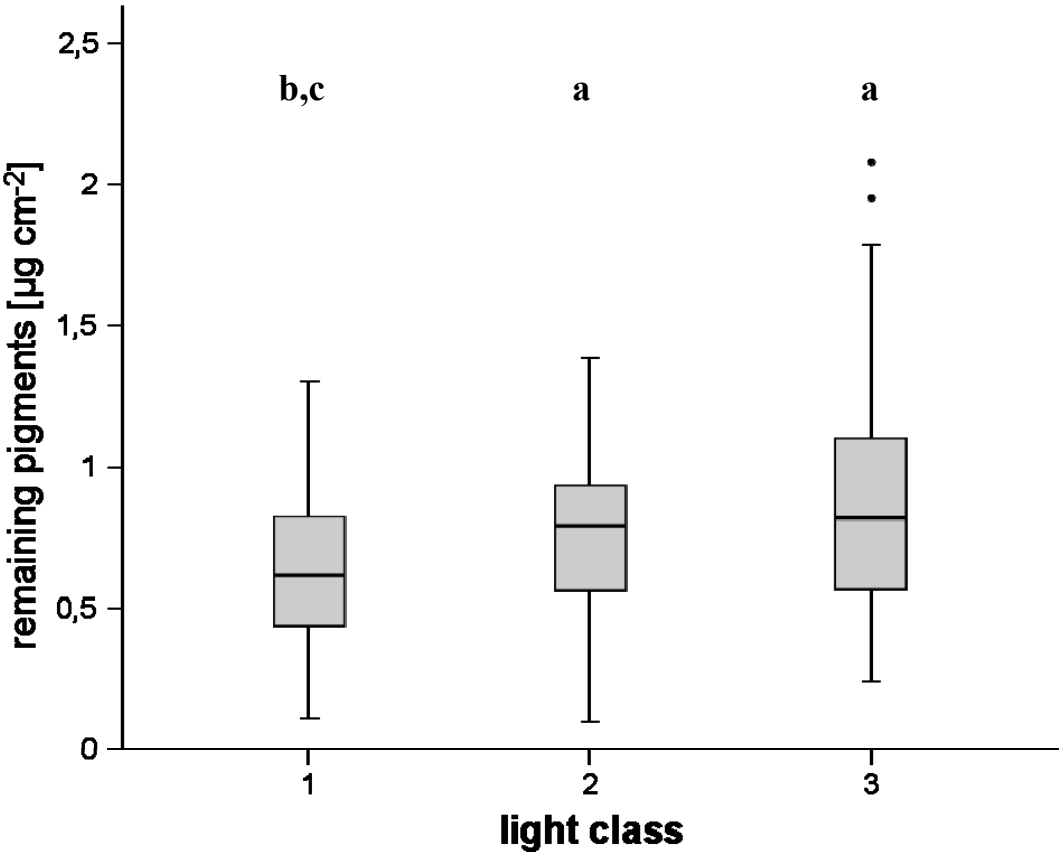
a
xanthophyll pool size [µg cm-2]
0
1
2
3
4
5
6
1
2
3
light class


b,c
a
a
2,5
2
1,5
1
0,5
0
remaining pigments [µg cm-2]
1
2
3
light class

variable	LCT1	LCT2	LCT3
F_v/F_m	0.61 +/- 0.07	0.60 +/- 0.05	0.60 +/- 0.07
F_v'/F_m' 80	0.51 +/- 0.07	0.51 +/- 0.05	0.51 +/- 0.06
F_v'/F_m' 850	0.35 +/- 0.09	0.35 +/- 0.06	0.36 +/- 0.07
F_v'/F_m' 2900	0.25 +/- 0.07	0.26 +/- 0.07	0.26 +/- 0.07
ΦPSII 80	0.33 +/- 0.07	0.34 +/- 0.06	0.36 +/- 0.07
ΦPSII 850	0.06 +/- 0.03	0.07 +/- 0.03	0.08 +/- 0.03
ΦPSII 1800	0.02 +/- 0.02	0.03 +/- 0.02	0.03 +/- 0.02
total pigment content	27.59 +/- 10.90	32.54 +/- 10.94	33.98 +/- 15.11

variable	May	September
absolute ETR_{max}*	15.45	25.17
absolute α_{max}*	0.13	0.15
qP	0.30 +/- 0.23	0.33 +/- 0.27
qN	0.69 +/- 0.19	0.63 +/- 0.22
Fv/Fm	0.58 +/- 0.09	0.61 +/- 0.05
Fv´/Fm´	0.37 +/- 0.12	0.37 +/- 0.13
ΦPSII´	0.13 +/- 0.04	0.15 +/- 0.04
XP-pool size	2.09 +/- 0.93	2.55 +/- 1.05
remaining pigments	0.60 +/- 0.29	0.84 +/- 0.31
LH-pigments	23.37 +/- 10.60	31.16 +/- 11.38
total pigment content	26.06 +/- 11.65	34.55 +/- 12.61

4 Photosynthetic properties of the calcifying Phaeophyceae *Padina pavonica* (L.) Thivy (Dictyotales) before and after temporary high – irradiance supply

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4.1 Abstract

In a survey conducted in the bay of Calvi (Corsica, France) at two seasons (spring, autumn), photosynthetic performance of the common Phaeophyceae *Padina pavonica* in response to temporary high irradiance supply of 500 µmol photons m^{-2} s^{-1} for 4 h was studied. Measurements were done with the aid of pulse amplified modulated fluorescence techniques to search for possible differences in photo-acclimation in accordance to the natural irradiance environment of *Padina*. Differences in acclimation properties of the plants growing in various habitats were recorded after temporary high photon flux exposure. Whereas the initial dark yield (F_v/F_m) of non-treated specimens from different depth habitats yielded comparable values, F_v/F_m values were significant diminished after treatment, especially in thalli originating from low-irradiance locations. Alterations in F_v/F_m were rather caused by the reduced F_m levels than by increased F_0, which implies that the lower F_v/F_m ratios are likely due to activation of photo-protective mechanisms than by photo-damage. The ability to dissipate excessive irradiances via heat (non-photochemical quenching qN) was minimized after temporary irradiance treatment, whereby thalli originating from shallow sun exposed locations experienced the lowest reduction, possibly in accordance to elevated xanthophyll-cycle activity. On the other hand, lower qN activity can also be seen as an acclimation to temporal high irradiance. In contrast, no signficant impact on photochemical quenching (qP) could be observed. We found, that deeper growing *Padina pavonica* are more sensitive and consequently more impacted under temporary high irradiance exposure than shallow water specimens. *Padinas* morphological plasticity and physiological flexibility probably plays a key role for its extended occurrence over a broad range of light supply.

Keywords: *Padina pavonica*, photosynthetic performance, temporary high irradiance exposure, acclimation, chlorophyll fluorescence, PAM

4.2 Introduction

Algae attract immense attention, primarily as photoautotrophic primary producers at the base of many food webs (Häder & Figueroa 1997) and for economic purposes (e.g., for food additives, cosmetics, drugs). Interestingly, recent investigations clearly indicated a great impact of global warming and ultraviolet (UV)-B radiation on aquatic systems and its inhabitants, mainly on macrophytes on account of being sessil (e.g.: Häder et al. 1996a, Häder et al. 1998, Aguilera et al. 1999, Bischof et al. 1999, Wiencke et al. 2000, Karsten et al. 2001, Roleda et al. 2004, Roleda et al. 2006). Excessive UV-B radiation (wavelength 290 – 400nm) can lead to the inactivation, or for the worst case to damage of proteins, enzymes and nucleic acids and consequently has a great impact on the efficiency of photosynthetic processes and productivity of marine organisms (Franklin & Forster 1997, Aguilera et al. 1999, Bischof et al. 1999, Karentz & Bosch 2001, Vincent & Neale 2000, Apprill & Lesser 2003). The harmful effects of increased UV-B radiation resulting from the decrease of stratospheric ozone and its possible penetration to considerable depths will probably change the depth zonation of several algal taxa (Bischof et al. 1999), which will be of great ecological and economical relevance for the future.

Especially shallow water species have to cope with extreme ranges of daily altering irradiance levels, temperature and salinity and therefore they constantly have to adjust their photosynthetic apparatus and metabolism (Walters et al. 2005). This process called acclimation is a key to survive in this harsh and extremely varying environment; it is necessary to content with other species for light and nutrients (Walters et al. 2005). Towards lowest irradiance levels, the light compensation point is believed to influence the depth zonation of macroalgae (Markager & Sand-Jensen 1992). Nevertheless, also excess irradiance might be a key factor for vertical algal distribution. Here, photosynthetic organisms are often confronted with more photons than they can actually deal with, which might cause serious damage of the organisms (Long et al. 1994, Osmond 1994), if no protective mechanisms are developed.

In many cases, a reversible depression of the maximum photosynthesis rate can be observed at highest irradiance levels, which is called photoinhibition (Osmond 1994). Such dynamic inhibition processes can be seen as a response to physiological stress under high-irradiance condition, mainly impacting on photosystem II (PSII) (Krieger-Liszkay et al. 2000, Adir et al. 2003). Contrarily, chronic photoinhibition resulting in a non-reversible damage is caused by e.g., oxygen radicals or UV-B radiation. UV-B mainly affects the reaction centres and can provoke the degradation of D1 protein (Osmond 1994, Krause & Weis 1991, Long et al. 1994, Adir et al. 2003). Photoinhibition can be estimated either as a decline of photosynthetic oxygen production (Hanelt 1992, Häder & Schärfer 1994) or as a decrease in

F_v/F_m during pulse amplitude-modulated (PAM) fluorescence measurements (cf. Schreiber et al. 1986, Demmig et al. 1987, Krause & Weis 1991, Hanelt 1992, Lichtenthaler & Burkart 1999).

Plants evolved a diverse repertoire of short- and long-term adjustment strategies to protect themselves avoiding overexcitation and consequently photo-oxidative damage of the PSII to guarantee optimal photosynthesis efficiency (Müller et al. 2001). Actually, carotenoids (Car) provide the ability to protect photoautotrophic organisms in several ways, including the quenching of triplet chlorophyll (Chl) and singlet oxygen (Demmig-Adams et al. 1990, Britton 1995, Demmig-Adams & Adams III 1996, Demmig-Adams et al. 1996a, Shimidzu et al. 1996, Franklin & Forster 1997)). Especially oxygenated xanthophylls (XP) are involved in energy dissipation and enable a potential photo-protective function, playing a key role in the so-called xanthophyll-cycle (XP-Cycle); simplified, the XP-cycle is an inter-conversion of violaxanthin (VX) via antheraxanthin (AX) to zeaxanthin (ZX) regulated by acidification of the thylakoid lumen (Demmig-Adams et al. 1990, Demmig-Adams & Adams III 1996, Demmig- Adams et al. 1996a). ZX is known to dissipate excessive energy into heat (qN) to accommodate the photosynthetic apparatus to the predominant irradiance regimes (Demmig-Adams et al. 1990, Demmig-Adams & Adams III 1994, Demmig-Adams & Adams III 1996, Demmig- Adams et al. 1996a).

With the aid of Chl-*a* fluorescence measurements the quenching mechanisms can be determined. Kautsky and his colleagues (1960) firstly discovered that alterations of excitation energy affect the yield of Chl-*a* fluorescence. Chl-*a* fluorescence techniques facilitate the measurement of various photosynthetic parameters and give quantitative as well as qualitative information (Róhacek & Bártack 1999) on photosynthetic processes based on the fact that Chl-*a* fluorescence is almost exclusively emitted from PSII (Häder et al. 1996c, Krieger-Liszkay et al. 2000, Adir et al. 2003).

Absorption of photons generates excitation of Chl-*a* molecules from the ground state to an excited state, of which the excitation energy can either be dissipated as heat (qN), used as energy for photochemistry processes (qP) or the *Chl-a* molecule might turn back to the ground state via Chl-*a* fluorescence (Maxwell & Johnson 2000, Müller et al. 2001, Baker 2008). These three mechanisms complement each other (Maxwell & Johnson 2000). An increase in one of these pathways leads to a decrease of the other two. In order to determine a non-excitated reference point the thalli first have to be dark acclimated. First, the initial (minimum) fluorescence (F_0) is measured after a weak measuring light is given assuming that all reaction centres are open and the primary quinone acceptor of PSII (Q_A) is maximally oxidized. After application of a saturating light impulse, the maximal fluorescence is reached (F_m; all reaction centres are closed and Q_A is fully reduced). The so-

called variable fluorescence (F_v) is calculated by subtracting F_0 from F_m. With these initial parameters, the F_v/F_m ratio, - defined as the dark yield or maximal operation efficiency of PSII - can be calculated, which is often considered as a parameter for the plant´s health. F_v/F_m is a widely used parameter for investigations on their health condition (Logan et al. 2007). F_v/F_m provides information about the maximal possible electron transfer of PSII and the degree of photoinhibition can also be estimated (Beer et al. 2000). A decline of F_v/F_m is often observed, when plants experienced excessive irradiance exposure (Häder et al. 1996c, Baker 2008). This so-called dynamic photoinhibition can also be seen as a reversible physiological process, protecting the plant from photo-damage caused by excess photon flux rates (Häder et al. 1996c). F_t is the steady state fluorescence at ambient light at time t. Light acclimation of a previously dark acclimated thallus results in a decline of F_m, termed $F_m´$ and in a decrease or enhancement of F_0, known as $F_0´$. The last measured parameter is $F_0´$ after darkening and immediate application of a far-red light to excite only PSI to guarantee full relaxation of PSII within seconds (Logan et al. 2007). Quenching pathways such as qN and qP lead to reduced or increased $F_0´$ values and/or reduced $F_m´$ values, therefore the fluorescence yield is lowered (Beer et al. 2000). A detailed description of Chl-*a* fluorescence measurements is given in several publications (Schreiber 1986, Bolhar-Nordenkampf et al.1989, Kooten & Snel 1990, Krause & Weis 1991, Beer et al. 2000, Maxwell & Johnson 2000, Dreuw et al. 2003, Force et al. 2003, Kramer et al. 2004, Schreiber et al. 2004, Logan et al. 2007, Baker 2008, Demmig-Adams et al. 2008, Porcar-Castell et al. 2008).

It is assumed that the sensitivity against high-irradiance is highly connected to the vertical patterns of photosynthetic organisms (Maegawa et al. 1993, Häder et al 1996a, Häder & Figueroa 1997, Franklin & Forster 1997) and that the ranges to adjust are reflecting the plants underwater light supply to assure optimal photosynthetic performance (Hanelt et al. 1997, Bischof et al. 1998). Generally, sun-acclimated tissues exhibit a higher photosynthetic capacity, a larger XP pool size and consequently a faster and more effective qN (Demmig-Adams & Adams III 1992, Demmig-Adams & Adams III 1994).

For our comparative physiological study the brown alga *Padina pavonica* has been in focus because of its extended vertical occurrence and its untypical extracellular calcification among Phaeophyceae. Up to now, only a few studies on the photosynthetic performance of *Padina pavonica* (Häder et al. 1996a, Häder et al. 1996b, Häder & Figueroa 1997) have been conducted, although this species is very common and can be found on oligotrophic tropical to temperate rocky shore regions. The alga usually populates shallow sun-exposed regions, though it also occurs in depths down to around 30m, yet not as abundant as in shallow water habitats. Results of a further study (Haberleitner & Schagerl 2010) proposed

that *P. pavonica* show optimal photosynthetic efficiencies at different irradiance levels due to a combination of acclimation mechanisms such as XP-cycle, varying absorption and reflection caused by thallus thickness and carbonate layers. According to Häder et al. (1996b) *Padina pavonica* is able to acclimate quickly to enhanced photon flux rates by minimizing the light-harvesting pigments. The question came up whether *Padina* specimens originating from different depths react in a similar manner to temporary high-irradiance exposure.

We collected *Padina* specimens from different sites of growth and exposed them to temporary high irradiance. Especially shallower sun exposed alga species are impacted by fluctuations in solar radiation and temperatures in their natural habitats. We expected that specimens growing in such habitats would enhance their physiological performance in terms of an improved photosynthetic efficiency and increased photo-protection. Because of *Padinas* habitat-specific resistance to excessive radiation, we supposed that the facility to dissipate excessive absorbed photons as heat (qN) should be most pronounced in shallow sun-exposed algae due to a more efficient XP-cycle. Furthermore we assumed that the capability to drive effective photochemistry (qP) after high temporal irradiance treatment is also more manifested in *Padinas* of sun-exposed habitats. Contrarily, we expected an earlier photoinhibition for specimens of light-poor locations when exposed to temporary high irradiances. We already compared several fluorescence parameters of thalli between the two seasons in another study (Haberleitner & Schagerl 2010) and we could show, that irradiance-treated thalli showed no relevant differences between the seasons. We therefore focused in this contribution on the comparison *Padinas* along a light gradient and did not consider seasonal comparisons.

4.3 Material & Methods

The bay of the STARESO ("station de recherchés sous – marines et oceanographique") nearby Calvi (Corsica, France,42°34´N, 8°45´O) in the Mediterranean Sea was chosen to examine responses of *Padina pavonica* to temporary high irradiance supply. The study was performed during two seasons in September 2007 and May 2008. Six to ten thalli were collected by random sampling every day at 11 am via Scuba Diving; shallow water habitats down to 30m were considered. Solar radiation was measured with a data logger (Skye data hog 1) in 5 min intervals. Incident photon flux rates of the respective depths (Iz) for collected thalli were recorded with the light guide of the Diving PAM. For each thallus the light supply was calculated as a percentage of surface radiation (%). Three light classes (LCT) were defined (LCT 1: < 11.5 %, LCT 2: 14.5 – 21.5 %, LCT 3: > 24.5 % of surface irradiance; LCT = Total Light Class irrespective of the season).

A PAM fluorometer (FMS2, Hansatech) was used to measure possible alterations in the photosynthetic performance before and after temporary high irradiances in an aquarium. First, the initial parameters F_0 and F_m were determined and F_v/F_m calculated (intensity of saturation light pulse 85 relative units; duration of light pulse 0.7s) using an automatic running script after 15 minutes dark incubation to assure a fully oxidized electron transport chain. Then the script included an exposure to three stepwise increasing short-term irradiance intensities provided by the internal light source (80, 850, 1800 µmol photons s^{-1} m^{-2}; each for one minute). At each step $F_v´/F_m´$, qN and qP were estimated based on the initial parameters as followed: $qN = (F_m – F_m´)/(F_m – F_0´)$; $qP = (F_m´ - F_t)/(F_m´ - F_0´)$; $F_v´/F_m´ = (F_m´ - F_0´)/ F_m´$ and $F_v´ = F_m´ - F_0´$. Immediately after measurements, thalli were transferred into a flow-through aquarium (water temperature ~23°C, permanent water exchange during experiment), affixed to a grid to guarantee exact exposure towards the light source (halogen spotlight 500W) and irradiated with 500 µmol photons m^{-2} s^{-1} for 4 h (= temporary irradiance treatment). Thereafter the samples were once more dark acclimated for 15 min again and the whole script was repeated.

The software package SPSS 15.0 was used for statistical analyses. Homogeneity of variance (Levene-test) and normal distribution (Kolmogorov-Smirnov Test) was tested for all data and criteria were met. Mean values and their standard deviations were calculated from 117 thalli (LCT1: n = 62; LCT2: n = 29; LCT3: n = 26; May: n = 59; September: n = 58). Criteria were met and GLM repeated measurements (for $F_v´/F_m´$, $F_0´$, $F_m´$, qN, qP; repeated measurements = irradiance steps 80, 850, 1800 PAR; 2 factors = light class, before/after temporary irradiance exposure) or GLM univariate (F_v/F_m, F_0, F_m; 2 factors =light class, before/after temporary irradiance exposure) were used, respectively. Post-hoc tests (Schéffe-test) using a level of significance of < 0.05 was used to identify significant differences.

In the following chapters, samples measured before temporary irradiance treatment, were called non-treated and those after irradiance exposure were termed irradiance-treated.

4.4 Results

Differences of non-treated thalli between the light classes (LCTs)

F_v/F_m ratio of non-treated thalli yielded around 0.6 in all LCTs and the statistical analysis showed no significant differences between them (Fig. 1). The comparable F_v/F_m ratio originated from similar F_0 and F_m levels in all three LCTs (Table 1).

Within the sudden stepwise increasing irradiances (short-term exposure to 80, 850, 1800 PAR), F_v'/F_m' (Table 1) was significantly reduced ($p = 0.000$). F_v'/F_m' values were also comparable in all LCTs due to similar F_0' and F_m' levels (Table 1). F_0' (Table 1) differed significantly between the irradiance levels 80 PAR and 850 PAR and between 80 PAR and 1800 PAR, but not between 850 PAR and 1800 PAR (80 PAR – 850 PAR: $p = 0.000$, 80 PAR – 1800 PAR $p = 0.000$). For F_m' (Table 1) highly significant differences were found in-between all irradiance levels ($p = 0.000$). Both, F_0' and F_m' were reduced during exposure to stepwise increasing short-term irradiances. qN (Fig. 2) increased rapidly ($p = 0.000$) with stepwise increasing irradiance supply. The inverse case occurred for qP (Fig. 3), where a rapid decrease ($p = 0.000$) could be observed. qN (Fig. 2) showed significant higher values in LCT1 than in LCT2 (LCT1 – LCT2: $p = 0.017$), yet qP (Fig. 3) did not vary between the LCTs.

Differences of irradiance-treated thalli between the light classes (LCTs)

Different responses to temporary high irradiance supply were detected within the LCTs for most parameters. Interestingly, the F_v/F_m ratio (Fig. 1) of irradiance-treated thalli was drastically reduced and strong significant differences within the LCT1 and LCT2 as well as between the LCT1 and LCT3 were noted (LCT1 – LCT2: $p = 0.000$, LCT1 – LCT3: $p = 0.000$). The lowest reduction occurred in LCT3. The reduction of F_v/F_m is mainly based on the decrease in the F_m levels (Table 1) due to the fact that the F_0 values exhibited no significant differences between the LCTs again. Generally, after temporary irradiance exposure significantly higher values for F_m were found in LCT3 compared to LCT1 (LCT1 – LCT3: $p = 0.008$).

Again, highly significant differences were found in-between all irradiance levels for F_v'/F_m' ($p = 0.000$). In addition, F_v'/F_m' (Table 1) differed significantly between the LCT1 and LCT2 and between LCT1 and LCT3 (LCT1 – LCT2: $p = 0.017$, LCT1 – LCT3: $p = 0.000$); LCT3 showed the highest values. No significant differences in F_0' levels (Table 1) between LCTs were recorded after temporary irradiance treatment, whilst F_0' values (Table 1) were reduced during stepwise increasing irradiance steps and showed differences between the irradiance levels 80 PAR and 850 PAR and between 80 PAR and 1800 PAR (80 PAR – 850 PAR: $p = 0.000$, 80 PAR – 1800 PAR $p = 0.000$). F_m' (Table 1) also experienced a decrease during stepwise increasing irradiances and exhibited higher values at the irradiance level 80

PAR than at the other two irradiance levels (p = 0.000). Shallow water specimens yielded slightly higher, although insignificant F_m' values (Table 1) than that ones of deeper habitats. qN (Fig. 2) increased rapidly (p = 0.000) with stepwise increasing irradiance supply. Contrarily, a rapid decrease (p = 0.000) could be observed for qP (Fig. 3). According to the LCTs qN (Fig. 2) showed highly significant differences between LCT1 and LCT2 as well as between LCT1 and LCT3 (LCT1 – LCT2: p = 0.001, LCT1 – LCT3: p = 0.000) after temporary irradiance treatment, whereby sun-exposed specimens (LCT3) yielded higher values than deeper growing algae. No significant differences could be proven for qP (Fig. 3) between the LCTs.

Comparison between non-treated and irradiance-treated thalli

Significant differences were proven for F_v/F_m (Fig. 1; p = 0.000). While values in LCT3 yielded the highest values and experienced only a minimal reduction (~ 4.9%), F_v/F_m values of deep growing specimens (LCT1) were reduced by approximately 27%. The reduction of thalli in LCT2 was between these two extremes and yielded around 15%. F_0 of irradiance-treated thalli yielded slightly lower values than non-treated thalli, however no significant differences could be proven for them (Table 1). F_m values (Table 1) definitely decreased after temporary irradiance treatment (p = 0.000; Table 1). F_m values of thalli growing in deeper habitats experienced a loss of 40% whereas a decrease of 10 % was observed for shallow water specimens.

Besides, F_v'/F_m' (Table 1) also showed significant differences between non-treated and irradiance-treated thalli (p = 0.000) for stepwise increasing irradiances. For LCT3, the lowest decrease was observed; thalli belonging to LCT1 experienced the highest reduction. Thalli exposed to 80 PAR from LCT3 experienced only a marginal decrease, whilst F_v'/F_m' values of deeper growing specimens were reduced of about 26%. At 1800 PAR F_v'/F_m' ratio was reduced about 31% in LCT1 and about 3% in LCT3. Between non-treated and irradiance-treated thalli no noteworthy differences could be recorded for F_0' (Table 1). F_m' (Table 1) differed significantly between non-treated and irradiance-treated thalli (p = 0.020), whereas irradiance-treated thalli showed lower values. In general, qN (Fig. 2) was significantly higher before temporary irradiance treatment than afterwards (p = 0.000). For fast irradiance increase responses, qN differences between non-treated and irradiance-treated thalli within the LCTs appeared as follows: in LCT3 a decrease of around 13% at an irradiance level of 80 PAR and around 4% at 1800 PAR was remarkable, whereas in LCT1 a reduction of 24% at a irradiance level of 80 PAR and 22% at 1800 PAR was detected. Results of 850 PAR were in-between the ranges of 80 and 1800 PAR. No relevant impact on qP (Fig. 3) by temporary irradiance treatment was found, however qP appeared to increase slightly with increasing irradiance supply (within the LCTs) at the irradiance level

80 PAR. In general, qP showed a broad range between all thalli and was approximating to the initial values, which they yielded before temporary irradiance treatment, especially at the irradiance levels 850 PAR and 1800 PAR.

4.5 Discussion

In general, species-specific sensitivity of macroalgae to ambient irradiance conditions is believed to fit well with the vertical zonation of submerged algae, whereby the physiological potential to acclimate to various underwater irradiance conditions seems to be the major determining factor (Markager and Sand-Jensen 1992, Hanelt 1998, Aguilera 1999, Karsten et al. 2001, Johansson & Snoeiljs 2002).

As our results clearly demonstrated, photosynthetic efficiency of *Padina pavonica* varied over spatial scales in the field and responded adequately to its ambient underwater light climate (Haberleitner & Schagerl 2010). Already Häder et al. (1996a) demonstrated that *Padina´s* morphology and carbonate layer varied according to their natural depth habitat and that the thalli showed acclimation characteristics in accordance to their site of growth. In accordance, our study also proved that deeper growing *Padinas* underwent faster photoinhibition than sun-exposed individuals. Interestingly, *P. pavonica* is not the only macroalga with a vertical distribution. The green alga *Halimeda tuna* ((J. Ellis & Solander) J.V. Lamouroux) and the brown alga *Dictyota dichotoma* ((Hudson) J.V. Lamouroux) are also well known for their vertical distribution and they showed different photosynthetic properties and morphological appearance depending on their natural depth habitat. (Häder et al. 1996c, Häder & Figuera 1997, Beach et al. 2003). Besides, individuals of *Laminaria saccharina* (Linnaeus) and *Sphacelaria plumosa* (Lyngbye) also exhibited a different degree of photo-acclimation in different depths in accordance to their predominant light environment (Hanelt 1998). Moreover other species of the genus *Padina* are reported to exhibit a high tolerance and a fast recovery among highest irradiance levels (e.g: *Padina boryana* (Thivy), Hanelt et al. 1994). In accordance with those authors, results of the current study confirm the assumption that *P. pavonica* features a typical light- to shade-type photo-acclimation over depth.

Our physiological measurements revealed different patterns after temporary high irradiance treatment. Sun- and deeper growing thalli differed in their photo-protective response and in their photosynthetic capacity. We discovered a general significant reduction of most parameters after thalli experienced temporary high irradiance. After temporary irradiance treatment F_v/F_m (Fig. 1) decreased differently according to the specimen's site of growth in their natural environment. While specimens of all LCTs indicated comparable F_v/F_m values before temporary irradiance treatment, suggesting that the PSII was not impaired, different initial rates of photoinhibition were found afterwards with deeper growing specimens much more affected than shallow water plants. F_v/F_m was obviously reduced by the additional temporary irradiance stress and photoinhibition occurred earlier for deeper settled thalli. Their variable degree of photo-acclimation of their photosynthetic apparatus to temporary

irradiance stress may probably be based on their different absorption-and reflection patterns due to their variable carbonate layers, respectively to their natural depth habitat (Häder et al. 1996a). Alterations in F_v/F_m can result from changes in either F_0 or F_m level (Schofield et al. 1998). The activation of photo-protective mechanisms is usually reflected in a relative stable or decreasing F_0 level, whereas an increase of F_0 is related with a damage of D1-protein and consequently of PSII (Schofield et al. 1998). In the case of *P. pavonica* the temporary irradiance treatment affected the F_m level (Table 1) rather than the F_0 level, which emphasizes *Padinas* general high tolerance and strong resistance against high-irradiance stress. The relative stability in F_0 within the LCTs proposed that the reaction centres were not damaged, whereby the slight but insignificant reduction after temporary irradiance exposure might be an evidence for damage. In contrast to F_v/F_m, F_v'/F_m' (Table 1) is comparable with the operation quantum yield of PSII (Beer et al. 2000). According to Demmig et al. (1996b), qN and F_v'/F_m' show an inverse relationship in plants experiencing environmental stress; a decrease in F_v'/F_m' can be used to measure the extent of thermal heat loss. In the case of this study, no differences between the LCTs were noticed before temporary irradiance treatment, however thalli of LCT3 experienced the lowest reduction afterwards, supporting the assumption that shallow water specimens are better adjusted and therefore react faster and more efficient to temporary high-photon flux stress than deeper growing specimens. The fast decline of F_v'/F_m' with stepwise increasing short-term irradiances, which is a typical incident for dynamic photoinhibition (Ensminger et al. 2005), was highly related to rising thermal energy loss (qN) most probably regulated by the XPs. Photo-inactivation might be a further reason for an irradiance induced suppression of F_v'/F_m' (Oxborough 2004). Häder et al. (1996b) also showed an inverse relationship between qN and F_m' for *P. pavonica*, which is obviously also here the case. Contrarily to F_m', F_0' was only slightly diminished during stepwise increasing short-term irradiances (Table 1).

The XP-cycle is a key mechanism to prevent plants from potential photo-damage through the associated heat loss of the excess absorbed photons (qN) (Schofield et al. 1998). Dependent on the different relaxation kinetics of qN, three components can be distinguished, namely the energy-dependent quenching qE, the photoinhibitory quenching qI and last but not least the transition state quenching qT (Krause & Weis 1991, Horton et al. 1995, Müller et al. 2001, Baker 2008, Goss et al. 2008): qE is believed to be the major component under moderate to excess irradiance exposure activated by intra-thylakoid acidification and protecting from irradiance-induced damage. qI, relaxing over a longer

time-scale is seen as an indicator for photoinhibition and becomes important under excessive high irradiance. Nevertheless, qT is dominating under low-irradiance levels.

In our study, the ability to dissipate the excessive excitation energy was minimized after the temporary irradiance treatment, and strong distinctions between thalli originating from different irradiance regimes in their natural environment appeared (Fig. 1). Shallow water specimens reacted more efficiently to temporary irradiance stress as reflected in the heat dissipation of a greater fraction of absorbed energy (increased qN, Fig 2) than shaded ones. The lower qN can also be interpreted as an acclimation to high irradiance; therefore there is no need to dissipate a lot of energy. With stepwise increasing short-term irradiance exposure, differences between the LCTs became stronger. From our results, it might be concluded that qE dominates before temporary high irradiance treatment, while qI and subsequently reversible photoinhibition gained in importance afterwards. Ruban and Horton (1999) reported that an increase of qE correlates with an increased ZX-pool. Additional proof for changing acclimation processes at different growth irradiance conditions was provided by a further study (Haberleitner & Schagerl 2010), which showed a higher accumulation of VAZ (sum of VX, AX, ZX) in shallow water specimens than in deeper growing thalli supporting the assumption, that sun-acclimated thalli (LCT3) might drive qE and subsequently qN more sufficient. According to Harker et al. (1999) effectiveness of the qN process can depend on the pool size as well as on the performance of the XP-cycle. Furthermore, the dissipation of excess photons is seen as an important factor influencing the vertical range of algae. It is worth to mention that the XP-cycle has a further photo-protective role in addition to dissipation as heat of excessive energy, namely as supporters of the thylakoid membrane lipids including photo-protectors and stabilizers (Sarry et al. 1994, Tardy & Harvaux 1996). The higher VAZ content of shallow water specimens (Haberleitner & Schagerl 2010) indicates a more effective xanthophyll interconversion, consequently a minimized level of inhibition and therefore enables a higher chance to survive at longer duration of excess irradiances. As stated before, it is also proposed that a decline of F_0 level is linked to the photo-protective XP-cycle. Such a decline as response to irradiance exposure was proven for *Ulva rotundata* (Bilding; Henley et al. 1992) and *Dictyota dichotoma* (Uhrmacher et al. 1995) and was explained by a greater heat loss as photo-protection. In contrast, an only slow accumulation of ZX may result in increased F_0 levels at high irradiances (Franklin et al. 1992). Franklin et al. (1992) have demonstrated that the photo-protective decline and an increase of the F_0 caused by photo-damage can also go on simultaneously; consequently it is not trivial to distinguish between these two factors. Already Demmig-Adams (1990) reported that a slow conversion rate of VX to ZX is typical for shade-adapted plants. These arguments led to our conclusion, that *Padina* might

possesses a general high accumulation rate of ZX due to the almost constant F_0 level after temporary irradiance exposure. Deeper growing *Padinas* seem to have a lower XP-cycle activity because irradiance changes do not occur in such a high manner.

In contrast to qN, qP indicates the number of open reaction centres. Alterations in qP originate from the closure of reaction centres caused by (over)saturation of photosynthesis by high fluency rates (Maxwell & Johnson 2000). The facility to drive photochemistry (qP) was nearly the same before and after temporary irradiance exposure as documented in Fig. 3. Nevertheless, a slight, but insignificant increase in the irradiance level of 80 PAR and little differences between the LCTs at this low irradiance level were noticeable, probably caused by a rapid switch to effective energy dissipation (qN) to protect the plants from excessive irradiance-induced damage. As expected, qP was diminished during stepwise increasing irradiance, also resulting mainly from the activation of photo-protective mechanisms, such as the XP-cycle associated qN.

Combining all arguments it seems that shallow water specimens possess a higher acclimation potential for photo-protection and for effective photosynthesis under temporary high photon flux rates than the deeper growing ones. Apparently, *Padinas* have developed a certain adjustment potential to certain photon-flux rates, which allows them to settle down to considerable depths. In the case of polar algae it was reported that the ability to deal with temporary irradiance stress is genetically fixed (Hanelt et al. 1997). Previous studies (Lynch & Gabriel 1987, Ensminger et al. 2005) demonstrated that the ability of algae to acclimate their photosynthetic performance to various light regimes was more manifested in algae with an extended vertical distribution. Moreover, their fitness was improved by their phenotypical plasticity and ecotypical differentiation to compete successful in a spatial heterogeneous environment, requiring a higher extent of specialisation (Ensminger et al. 2005). Phenotyical plasticity, such as alterations in the photosynthetic acclimation potential to diverse irradiance regimes is reflected in a discernible photosynthetic efficiency, according to the algae´s site of growth (Ensminger et al. 2005). The variability of environmental factors requires various dynamic photosynthetic acclimation mechanisms to resist biotic and abiotic stress, operating on different time-scales and varying in-between high-irradiance and low-irradiance growth (Walters et al. 2005). Several studies of seagrass depth-acclimation processes displayed the magnitude of depth-associated alterations in leaf metabolism and growth strategies in accordance to their natural depth habitat and its predominant irradiance regime (e.g.: Buia et al. 1992, Alcoverro et al. 2001, Oleson et al. 2002).

Obviously, *Padina pavonica* possesses the capability to adjust its photosynthetic apparatus in response to its natural underwater light climate. Different habituated specimens can be

considered as low- or high-light algae, whereby morphological plasticity and physiological flexibility respectively to their depth habitat may play a certain role in regard to *Padina´s* different morphological appearance. This alga appears to have a competitive advantage among other species under altering irradiance conditions in the field which is probably the main explanation for their spatial and world-wide distribution.

4.6 References

- Adir N, Zer H, Shochat S, Ohad I (2003) Photoinhibition- a historical perspective Photosynth. Res. 76: 343-370
- Aguilera J, Karsten U, Lippert H, Vögele B, Philipp E, Hanelt D, Wiencke C (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the arctic. Mar. Ecol. Prog. Ser. 191: 109-119
- Alcoverro T, Cerbian E, Ballesteros E (2001) The photosynthetic capacity of the seagrass Posidonia ocenaica: influence of nitrogen and light. J. Exp. Mar. Biol. Ecol. 261: 107-120
- Algarra P, de la Vina G, Niell, J (1991) Effects of light quality and irradiance level interactions on short-term pigment response of the red alga *Corallina elongata*. Mar. Ecol. Prog. Ser. 74: 27-32
- Apprill AM, Lesser MP (2003) Effects of ultraviolet radiation on *Laminaria saccharina* in relation to depth and tidal height in the Gulf of Maine. Mar. Ecol. Prog. Ser. 256: 75-85
- Baker N (2008) Chlorophyll Fluorescence: A probe of photosynthesis in vivo. Annu. Rev. Plant. Biol. 59: 89-113
- Beach K, Walters L, Vroom P, Smith C, Coyer J, Hunter C (2003) Variability in the ecophysiology of *Halimeda spp.* (Chlorophyta, Bryopsidales) in the Conch Reef, Florida Keys, USA J. Phycol. 39 (4): 633-643
- Beer S, Larsson C, Poryan O, Axelsson L (2000) Photosynthetic rates of *Ulva* (Chlorophyta) measured by pulse amplitude modulated (PAM) fluorometry. Eur. J. Phycol. 35: 69-74
- Bischof K, Hanelt D., Wiencke C. (1998) UV-radiation can affect depth-zonation of antarctic macroalgae. Mar. Biol. 131: 597-605
- Bischof K, D Hanelt, Wiencke C (1999) Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. Plant Biol. 1: 435-444
- Bolhar-Nordenkampf HR, Long SP, Baker NR, Öquist G, Schreiber U, Lechner EG (1989) Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: A review of current instrumentation. Funct. Ecol. 3 (4): 497-514
- Britton G (1995) Structure and properties of carotenoids in relation to function. FASEB J. 9: 1551-1558
- Buia MC, Zupo V, Mazzella L (1992) Primary production and growth dynamics in *Posidonia ocenaica*. PSZN I: Mar. Ecol. 13: 2-16

- Demmig B, Winter K, Kruger A, Czygan FC (1987) Photoinhibition and zeaxanthin formation in intact leaves: A possible role of the xanthophyll cycle in the dissipation of excess light energy. Plant Physiol. 84 (2): 218.224
- Demmig-Adams (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochem. Biophys. Acta .1020: 1-24
- Demmig-Adams B, Adams III WW (1994) Capacity for energy dissipation in the pigment bed in leaves with different xanthophyll cycle pools. Aust. J. Plant Physiol. 22: 249-260
- Demmig-Adams B, Adams III WW (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis Trends Plant Sci. 1 (1): 21-26
- Demmig-Adams B, Gilmore A, Adams III WW (1996a) In vivo function of carotenoids in higher plants. FASEB J. 10: 403-412
- Demmig-Adams B, Adams III WW (1996b) Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. Planta 198: 460-470
- Demmig-Adams B, Adams III WW, Barker DH, Logan BA, Bowling DR, Verhoeven AS (2008) Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. Physiol. Plant 98 (2): 253-264
- Dreuw A, Fleming GR, Head-Gordon M (2003) Chlorophyll fluorescence quenching by xanthophylls. Phys. Chem. Chem. Phys. 5 (15): 3247-3256
- Ensminger I, Foerster J, Hagen C, Braune W (2005) Plasticity and acclimation to light reflected in temporal and spatial changes of small- scale macroalgal distribution in a stream. J. Exp. Bot. 56 (418): 2047-2058
- Force L, Critchley C, van Rensen JJS (2003) New fluorescence parameters for monitoring photosynthesis in plants. Photosynth. Res. 78 (1): 17-33
- Franklin LA, Forster R (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. Eur. J. Phycol. 32: 207- 232
- Franklin LA, Levavasseur G, Osmond CB, Henley W, Ramus J (1992) Two components of onset and recovery during photoinhibition of *Ulva Rotundata*. Planta 186 (3): 399-408
- Genty B, Wonders J, Baker NR (1990) Non-photochemical quenching of F_0 in leaves is emission wavelength dependent: consequences for quenching analysis and its interpretation. Photosynth. Res. 26 (2): 133-139

- Goss R, Opitz C, Lepetit B, Willhelm C (2008) The synthesis of NPQ-effective zeaxanthin depends on the presence of a transmembrane proton gradient and a slightly basic stromal side of the thylakoid membrane. Planta 228: 999-1009
- Haberleitner E, Schagerl M (2010) Photosynthetic characteristics of the calcifying Phaeophyceae *Padina pavonica* (L.) Thivy (Dictyotales) along a light gradient. diploma thesis pp. 8-32
- Häder DP, Schärfer J (1994) In-situ measurement of photosynthetic oxygen production in the water column. Envirom. Monitoring Assessm. 32: 259-268
- Häder DP, Herrmann H, Santas R (1996a) Effects of solar radiation and solar radiation deprived of UV-B and total UV on photosynthetic oxygen production and pulse amplitude modulated fluorescence in the brown alga *Padina pavonica*. FEMS Microbiol. Ecol. 19 (1): 53-61
- Häder DP, Lebert M, Mercado J, Aguilera J, Salles S, Flores-Moya A, Jimenez C (1996b) Photosynthetic oxygen production and PAM fluorescence in the brown alga *Padina pavonica* measured in the field under solar radiation. Mar. Biol. 127: 61-66
- Häder DP, Porst M, Herrmann H, Schäfer J, Santas R (1996c) Photoinhibition in the mediterranean green alga *Halimeda tuna* Ellis et Sol measured in situ. Photochem. Photobiol. Sci. 64 (3): 428-434
- Häder DP, Figueroa F (1997) Photoecophysiology of marine macroalgae. Photochem. Photobiol. Sci. 66 (1): 1-14
- Häder DP, Kumar H, Smith R, Worrest R (1998) Effects on aquatic ecosystems. J. Photochem. Photobiol. B: Biol. 46: 53-68
- Hanelt D (1992) Photoinhibition of photosynthesis in marine macrophytes of the South China Sea. Mar. Ecol. Prog. Ser. 82: 199-206
- Hanelt D (1994) Tidal dependence of photoinhibition in marine macrophytes of the south China Sea. Bot. Acta 44: 67-77
- Hanelt D, Melchersmann B, Wiencke C, Nultsch W (1997) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar. Ecol. Prog. Ser. 149: 255-266
- Hanelt D (1998) Capability of dynamic photoinhibition in arctic macroalgae is related to their depth distribution. Mar. Biol. 131: 361-369
- Harker M, Berkaloff C, Lemoine Y, Britton G, Young A, Duval J, Rmiki N, Rousseau B (1999) Effects of high light and desiccation on the operation of the xanthophyll cycle in two marine brown algae. Eur. J. Phycol. 34: 35-42

- Henley WJ, Lindley ST, Levavasseur G, Osmond CB, Ramus J (1992) Photosynthetic response of *Ulva rotundata* to light and temperature during emersion on an intertidal sand flat. Oecol. 89: 516-523
- Horton P, Ruban AV, Walters RG (1996) Regulation of light harvesting in green plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 47: 655-684
- Hoyer K., Karten U., Sawall T., Wiencke C. (2001) Photoprotective substances in antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages Mar. Ecol. Prog. Ser. 211: 117-129
- Johansson G, Snoeijs (2002) Macroalgal photosynthetic responses to light in relation to thallus morphology and depth zonation. Mar. Prog. Ser. 244: 63-72
- Kautsky H, Appel W, Amann H (1960) Chlorophyll Fluoreszenz und Kohlensäureassimilation. Die Fluoreszenzkurve und die Photochemie der Pflanze. Biochem. 322: 277-293
- Karentz D, Bosch I (2001) Influence of Ozone-Related Increases in Ultraviolet Radiation on Antarctic Marine Organisms. Amer. Zool. 41: 3-16
- Karsten U, West JA, (2000) Living in the intertidal zone-seasonal effects of heterosides and sun-screen compounds in the red alga *Bangia atropurpurea* (Bangiales) J. Exp. Mar. Biol. Ecol. 254: 221-234
- Karsten U, Bischof K, Wiencke C (2001) Photosynthetic performance of arctic macroalgae after transplantation from deep to shallow waters. Oceolog. 127 (1): 11-20
- Kooten O, Snel JFH (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth. Res. 25 (3): 147-150
- Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Ann. Rev. Plant. Physiol. Plant. Mol. Biol. 42: 313-249
- Kramer DM, Johnson G, Kiirats O, Edwards GE (2004) New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. Photosynth. Res. 79 (2): 209-218
- Krieger-Liszkay A, Kienzler K, Johnson GN (2000) Inhibition of electron transport at the cytochrome b_6f complex protects photosystem II from photoinhibition. FEBS 486: 191-194
- Lichtenthaler HK, Burkart S (1999) Photosynthesis and high light stress. Bulg. J. Plant. Physiol. 25 (3-4): 3-16
- Logan BA, Adams III WW, Demmig-Adams B (2007) Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. Funct. Plant. Bio. 34: 835-859

- Long SP, Humphries S, Falkowski PG (1994) Photoinhibition of photosynthesis in nature. Ann. Rev. Plant Biol. 45 (1): 633-662
- Lynch M, Gabriel W (1987) Environmental Tolerance. Americ. Nat. 129 (2): 283-303
- Maegawa M, Kunieda M, Kida W (1993) The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths. Jap. J. Phycol. 41: 207-214
- Markager S, Sand-Jensen K (1992) Light requirements and depth zonation of marine macroalge. Mar. Ecol. Prog. Ser. 88: 83-92
- Maxwell K, Johnson G (2002) Chlorophyll fluorescence- a practical guide. J. Exp. Bot. 51 (345): 659- 668
- Müller P, Li X, Niyogi K (2001) Non-photochemical quenching. A response to excess light energy. Plant. Physiol. 125: 1558-1566
- Oleson B, Enríquez S, Duarte CM, Sand-Jensen K (2002) Depth-acclimation of photosynthesis, morphology and demography of *Posidonia ocenica* and *Cymodocea nodosa* in the Spanish Mediterranean Sea. Mar. Ecol. Prog. Ser. 236: 89-97
- Osmond CB (1994) What is photoinhibition? Some insights from comparisons of shade and sun plants: In: Baker NR, Bowyer JR (eds) Photoinhibition of photosynthesis from molecular mechanisms to the field. Bios. Sci. Publ. Oxford, pp. 1 -14
- Oxborough K (2004) Imaging of chlorophyll a fluorescence: theoretical and practical aspects of an emerging technique for the monitoring of photosynthetic performance. J. Exp. Bot. 55 (400): 1195-1205
- Porcar-Castell A, Pfündel E, Korhonen J, Juurola E (2008) A new monitoring PAM fluorometer (MONI-PAM) to study the short- and long-term acclimation of photosystem II in field conditions. Photosynth. Res. 96: 173-179
- Rohácek K, Barták M (1999) Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. Photosynth. 37 (3): 339-363
- Roleda M.Y, Hanelt D, Kräbs G, Wiencke C (2004) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. Phycol. 43 (5): 603-613
- Roleda MY, Hanelt D, Wiencke C (2006) Growth and DNA damage in young Laminaria sporophytes exposed to ultraviolet radiation: implication for depth zonation of kelps on Helgoland (North Sea) Mar. Biol. 148 (6): 1201-121

- Ruban AV & Horton P (1999) The xanthophyll cycle modulates the kinetics of non photochemical energy dissipation in isolated light-harvesting complexes, intact chloroplast and leaves of spinach. Plant Physiol. 119: 531-542
- Schofield O, Evens T, Millie D (1998) Photosystem II quantum yields and xanthophyll-cycle pigments of the macroalga *Sargassum natans* (Phaeophyceae): Responses under natural sunlight. J. Phycol. 34: 104-112
- Schreiber U, Schliwa Q, Bilger U (1986). Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorimeter. Photosynth. Res. 10: 51-62
- Schreiber U (2004) Pulse-Amplitude-Modulation (PAM) fluorometry and saturation pulse method: an overview. chlorophyll a fluorescence: A signature of photosynthesis, ed. GC Papageorgiou, Govindjee, Dordrecht, The Netherlands: Springer pp. 279-319.
- Sarry JE, Montillet JL, Sauvaire Y, Havaux M (1994) The protective function of the xanthophyll cycle in photosynthesis, FEBS Lett. 353: 147-150
- Shimidzu N, Goto M, Miki W (1996) Carotenoids as singlet oxygen quenchers in marine organisms. Fish Sci. 62: 134-137
- Tardy F, Havaux M (1996) Photosynthesis, chlorophyll fluorescence, light-harvesting system and photoinhibition resistance of a zeaxanthin accumulating mutant of *Arabidopsis thaliana*. J. Photochem. Photobiol. 34: 87-94
- Uhrmacher S, Hanelt D, Nultsch W (1995) Zeaxanthin content and the degree of photoinhibition are linearly correlated in the brown alga *Dictyota dichotoma*. Mar. Biol. 123: 159-165
- Walters RG (2005) Towards an understanding of photosynthetic acclimation. J. Exp. Bot. 56: 435-447
- Wiencke C, Gómez I, Pakker H, Flores-Moya A, Altamirano M, Hanelt D, Bischof K, Figueroa FL (2000) Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. Mar. Ecol. Prog. Ser. 197: 217-229
- Vincent WF, Neale PJ (2000) Mechanisms of damage to aquatic organisms. In: de Mora D, Demers S, Vernet M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, pp. 149-176

4.7 Figure and Table Legends

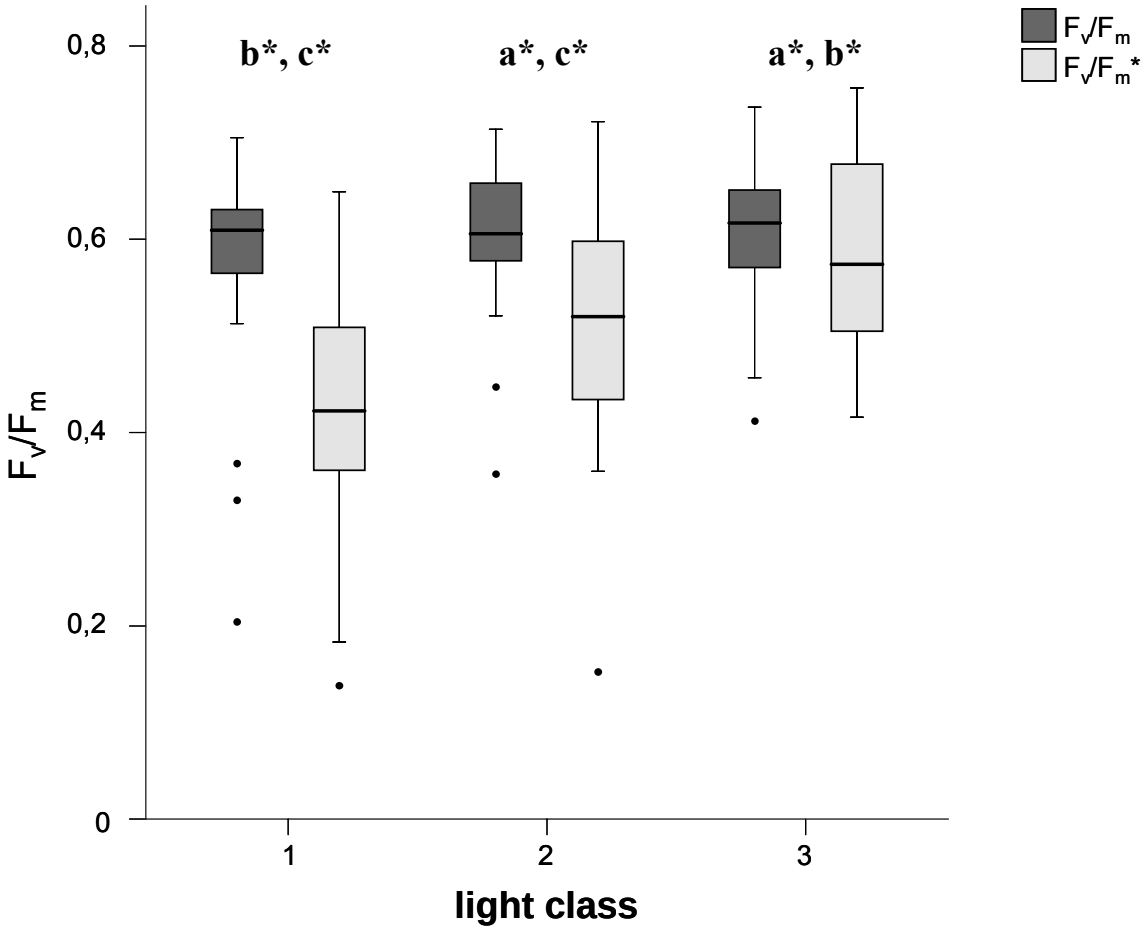
Fig. 1: F_v/F_m against LCTs: box-blots with their means (−), standard deviations and outliers (●). F_v/F_m after temporary irradiance supply is marked with a star (*). Significant differences between the LCTs after temporary high-irradiance stress are marked with the letters a* (LCT1), b* (LCT2), c* (LCT3). No significant differences were proven for non-treated thalli within the LCTs. Significant differences were recorded between non- and irradiance-treated thalli with a $p = 0.000$.

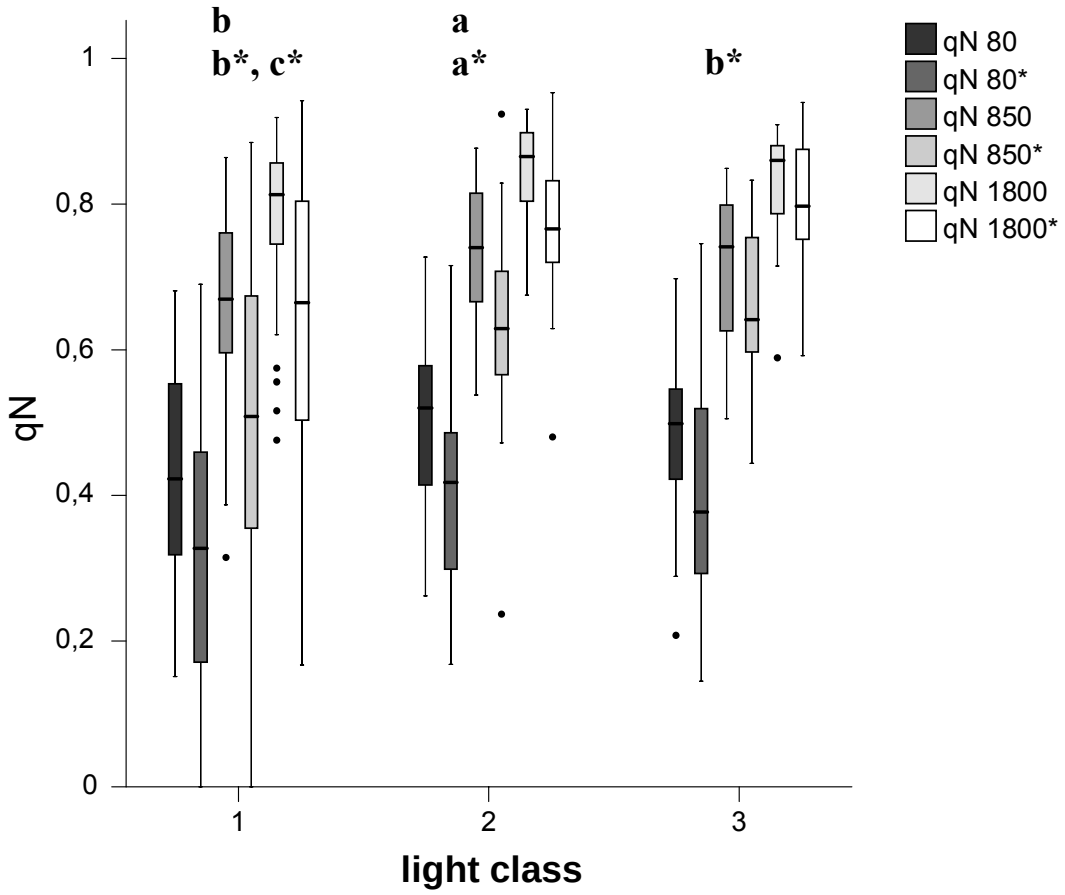
Fig. 2: qN against LCTs: box-blots with their means (−), standard deviations and outliers (●); 80, 850, 1800 PAR = stepwise increasing irradiance levels; qN after temporary irradiance emersion is marked with a star (*). Significant differences between the LCTs are marked with the letters a (LCT1), b (LCT2), c (LCT3) before temporary irradiance exposure and with the letters a* (LCT1), b* (LCT2), c* (LCT3) afterwards. Significant differences were recorded between non- and irradiance-treated thalli with a $p = 0.000$ and within the stepwise increasing irradiance levels ($p = 0.000$)

Fig. 3: qP against LCTs: box-blots with their means (−), standard deviations and outliers (●); 80, 850, 1800 PAR = stepwise increasing irradiance levels; qP after temporary irradiance exposure is marked with a star (*). No significant differences were recorded, except within the stepwise increasing irradiance levels ($p = 0.000$).

Table 1: Means with their standard deviations of the measured parameters F_v'/F_m', F_0, F_0', F_m, F_m' for each LCT. All parameters after 4h irradiance exposure are marked with stars (*).

4.8 Figures and Tables

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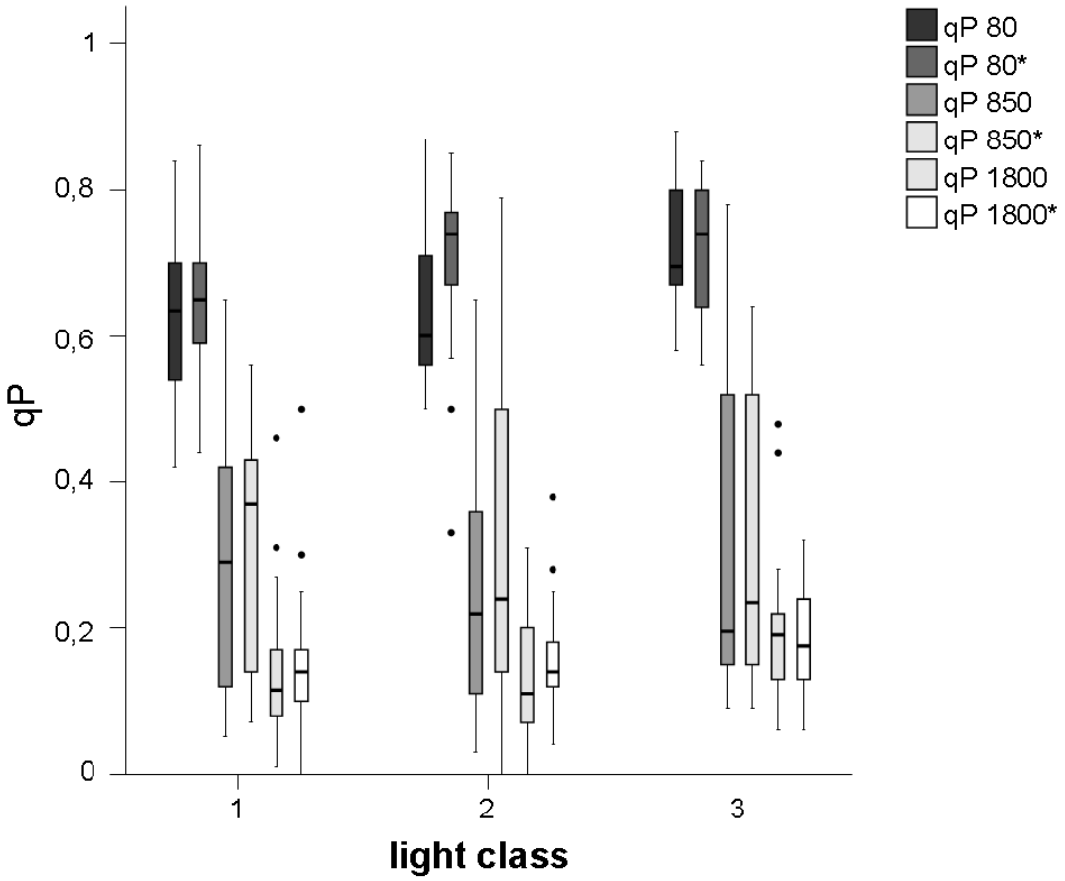
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variable	LCT1	LCT2	LCT3
F_m'/F_v' 80	0.50 +/- 0.08	0.50 +/- 0.08	0.51 +/- 0.07
F_m'/F_v' 80*	0.37 +/- 0.09	0.45 +/- 0.11	0.51 +/- 0.09
F_m'/F_v' 850	0.37 +/- 0.09	0.40 +/- 0.14	0.40 +/- 0.08
F_m'/F_v' 850*	0.28 +/- 0.08	0.34 +/- 0.10	0.39 +/- 0.08
F_m'/F_v' 1800	0.29 +/- 0.09	0.27 +/- 0.07	0.29 +/- 0.05
F_m'/F_v' 1800*	0.21 +/- 0.07	0.26 +/- 0.07	0.27 +/- 0.07
F_0	238.33 +/- 98.16	236.97 +/- 100.91	225.81 +/- 84.06
F_0*	198.56 +/- 73.48	202.86 +/- 96.65	207.88 +/- 87.96
F_m	604.82 +/- 268.17	616.07 +/- 288.49	599.46 +/- 266.24
F_m*	362.90 +/- 177.92	462.28 +/- 293.31	537.62 +/- 279.65
F_0' 80	223.92 +/- 118.73	202.00 +/- 102.79	195.15 +/- 92.35
F_0' 80*	185.26 +/- 68.29	179.48 +/- 100.37	179.90 +/- 87.38
F_0' 850	213.69 +/- 131.10	171.04 +/- 103.10	172.04 +/- 85.32
F_0' 850*	184.39 +/- 77.55	172.79 +/- 95.35	168.69 +/- 79.40
F_0' 1800	214.89 +/- 128.51	180.93 +/- 102.79	168.00 +/- 85.22
F_0' 1800*	188.52 +/- 72.54	167.21 +/- 90.86	165.77 +/- 79.55
F_m' 80	451.71 +/- 236.62	401.79 +/- 210.82	410.15 +/- 209.22
F_m' 80*	292.11 +/- 105.24	346.28 +/- 225.58	388.35 +/- 222.68
F_m' 850	351.37 +/- 202.99	292.79 +/- 158.18	290.12 +/- 150.44
F_m' 850*	256.37 +/- 89.49	271.10 +/- 162.33	283.81 +/- 145.40
F_m' 1800	298.50 +/- 172.46	243.52 +/- 131.35	238.58 +/- 96.00
F_m' 1800*	235.76 +/- 82.75	226.17 +/- 121.56	231.23 +/- 113.06

5 Zusammenfassung

Wir untersuchten die Photosyntheseeigenschaften der kalzifizierenden Phaeophyceae *Padina pavonica* aus unterschiedlichen Tiefenhabitaten (0 – 30 m), um einen räumlichen und saisonalen Vergleich bezüglich ihres Akklimatisierungspotentials an unterschiedliche Lichtverhältnisse zu ziehen. Die Studie wurde in der Bucht von Calvi (Korsika, Frankreich) mit Hilfe von PAM Fluoreszenz-Messtechniken sowohl *in situ* als auch im Labor durchgeführt. Weiters wurden Pigmente mittels HPLC (High Performance Liquid Chromatographie) analyisert.

Grundsätzlich zeigten Thalli im Feld unterschiedliche Photosyntheseleistung je nach Tiefenhabitat und vorherrschendem Lichtklima. Höhere absolute maximale Elektronentransportraten (ETR_{max}) der Lichtkurven (Rapid Light Curves = RLC) wurden für Exemplare aus Licht exponierten Standorten gemessen. Im Gegensatz dazu war der maximale Anfangsanstieg α der Thalli in allen Tiefenhabitaten vergleichbar. Dieses Ergebnis beruht möglicherweise auf die unterschiedlichen Absorptions- und Reflektionseigenschaften infolge der tiefenabhängig ausgebildeten Kalkschichten und der Thallusdicken.

In Labormessungen wurden die Thalli ansteigenden kurzzeitigen Lichtintensitäten sowie temporärem (4 Stunden) Starklichtstress ausgesetzt, um mögliche Gegensätze in ihrer Fähigkeit, sich variablen Lichtbedingungen anzupassen, zu erörtern. Thalli aus Starklichtzonen wiesen gegenüber Individuen aus lichtarmen Habitaten eine effizientere Nutzung von photochemischer Energie (qP) sowie eine effizientere Abgabe von überschüssiger Anregungsenergie in Form von Wärme (qN) auf. Zudem ist der Gehalt an Lichtsammel- und Schutzpigmenten pro Thallusfläche in Exemplaren aus sonnenexponierten Habitaten höher. Wir nehmen an, dass *Padinas* aus Starklichtzonen aufgrund ihrer fleischigeren und stärker kalzifizierenden Thalli einen höheren Gehalt an photosynthetischen Pigmenten benötigen, um effizient Licht-Absorption zu betreiben. Im Gegensatz dazu steigern tiefer situierte Thalli ihre Absorptionseigenschaften durch ihre dünnere und weniger kalzifizierende Gestalt. *Padina pavonica* scheint optimal an die verschiedenen vorherrschenden Lichtverhältnisse in ihrer heterogenen Umwelt angepasst zu sein, wobei tiefer liegende und beschattete Individuen empfindlicher auf plötzlich einsetzenden Starklichtstress antworten.

Unterschiede in der Fähigkeit sich zu akklimatisieren wurden nach länger andauernder Starklichtaussetzung in einem Aquarium deutlicher. Der „Dark Yield" (F_v/F_m), der als Indikator für Starklichtstress verwendet wird, zeigte ungeachtet ihres natürlichen Lichthabitates für alle Thalli vor der Starklichtbestrahlung ähnliche Werte, während nach der Bestrahlung eindeutige Unterschiede zum Vorschein kamen (signifikante Reduktion).

Insbesondere Thalli aus tieferen Zonen wurden erheblich in ihrer Photosyntheseleistung gehemmt. Änderungen im „Dark Yield“ wurden vielmehr von der maximalen Fluoreszenz (F_m) als von der minimalen Fluoreszenz (F_0) beeinträchtigt, höchstwahrscheinlich durch die Aktivierung von Schutzmechanismen und nicht durch Schädigung des Photosystems II bedingt. Auch die Anlage, exzessive Strahlung als Wärme abzustrahlen (qN), war in Individuen aus Starklicht-Zonen am stärksten ausgeprägt, eventuell aufgrund eines effizienteren Xanthophyll-Zyklus. Im Falle des Aquariumexperiments wurde kein nennenswerter Einfluss auf die Nutzung der photochemischen Energie (qP) beobachtet, wahrscheinlich wegen des schnellen Wechsels zu thermaler Dissipation. Zusammenfassend kann man sagen, dass *Padina pavonica* Schwachlicht- beziehungsweise Starklichtcharakter entsprechend ihres natürlichen Lichthabitats aufweist. Thalli aus tieferen Zonen sind anfälliger gegenüber kurzzeitiger, sowie länger andauernder Strahlungseinwirkung, wobei morphologische Plastizität, physiologische Flexibilität sowie nischentypische Differenzierung möglicherweise eine wichtige Rolle spielen.

6 Summary

We studied photosynthetic properties of the common calcifying Phaeophyceae *Padina pavonica* and compared spatial and seasonal patterns of photosynthetic acclimation according to its depth habitat and natural prevailing light conditions. Moreover, we investigated the acclimation potential in response to sudden increased irradiance levels as well as to temporary irradiance supply for 4 hours in an aquarium (500µmol photons m^{-2} s^{1}). The study was conducted in the bay of Calvi (Corsica, France) at two seasons (spring 2008, autumn 2007). Both, *in situ* measurements and laboratory analyses were done by menas of PAM chlorophyll fluorescence techniques. Additionally, pigment analyses were conducted by high performance liquid chromatography (HPLC). Generally, higher photosynthetic efficiency and increased total pigment content per unit thallus area were observed in autumn. Due to acclimation, different degrees of photoinhibition were recorded under a range of irradiances. Maximum absolute electron transport rates (ETRmax) of *in situ* Rapid Light Curves (RLCs) measurements yielded higher values in occupied sun exposed than in light-poor habitats. Unexpectedly, the maximal initial slope α of the RLC was comparable at all irradiance levels, which might be related to different specific absorption- and reflection properties of thalli depending on their depth habitats.

During laboratory measurements, shallow water thalli showed increased photochemical quenching (qP) and also dissipated energy as heat (qN) more efficient compared to specimens from light-poor habitats. Furthermore, plants of high-irradiance habitats showed significantly higher amounts of photosynthetic pigments as well as photo-protective pigments per unit surface area than deeper growing ones. We assume that high-irradiance specimens need higher amounts of photosynthetic pigments because of their fleshier and more calcifying thalli to drive light-absorption more efficient. Contrarily, deeper growing ones improve their absorption properties through thinner and less calcifying appearance. *P. pavonica* seems to be well acclimated and responds adequately to different ambient irradiance environments. Shade exposed specimens respond more sensitive to sudden irradiance stress than individuals of shallow waters.

Possible differences in response to temporary irradiance exposure were also investigated. The acclimation potential varied much clearer after temporary high photon flux treatment within the different habituated thalli. The dark yield (F_v/F_m), which is a well established indicator for plants experiencing excessive stress, yielded fairly the same values before temporary irradiance treatment irrespective from the depth habitat, whilst F_v/F_m values were significant diminished afterwards; especially deeper habituated thalli were drastic inhibited.

The alterations of the F_v/F_m ratio were affected rather by the F_m levels than by the F_0, which led to the assumption that the lower F_v/F_m ratio is reached due to activation of photo-protective mechanisms and not through photo-damage. Also the ability to dissipate excessive photon flux as heat (qN) was minimized after temporary high-irradiance, whereby shallower water thalli experienced the lowest reduction, probably in accordance to an increased xanthophyll-cycle pool. In the case of the aquarium experiment, no reliable impact on the photochemical quenching (qP) could be observed, except at low-irradiance levels, probably caused by a rapid switch to thermal dissipation for photo-protection.

To sum up, deeper habituated *Padina pavonica* are more sensitive and consequently more impacted under temporary duration to high irradiances than shallow water specimens, whereby morphological plasticity and physiological flexibility within the different habituated individuals probably play a key role in their acclimation potential to different irradiances regimes in a heterogeneous and harsh environment.

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Berufserfahrung im Bereich der Biologie

- Laborassistent (Department für Meeresbiologie/Limnologie)
  Untersuchungen zum Auftreten von Cyanotoxinen in den Sodaseen Nakuru und Bogoria (Afrika, Kenia)
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2005 & 2006 Ägypten (Dahab) (2 Wochen/Jahr)

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2006 Türkei (Fethyie) (5 Wochen)

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- Chemisches Grundpraktikum
- Physikalisches Grundpraktikum und bioakustisches Praktikum
- Ökophysiologie der Algen
- Primärproduktion in Ozeanen
- Molekularbiologische Methoden der Phylogenie
- Aquatische mikrobielle Ökologie
- Pflanzenphysiologische Übungen
- Tierphysiologische Übungen
- Vertebratenpraktikum
- Meeresbiologische Laborarbeiten
- Elektronenmikroskopie (TEM, REM)

Kenntnisse im Bereich der Biologie

- High performance Liquid Chromatography (HPLC)
- DAPI-Färbung, Epifluoreszenzmikroskopie
- TEM, REM
- Fluoreszenzmessungen mittels Pulse Amplitude Modulation (PAM)
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Zusätzliche Kenntnisse

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