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DIPLOMARBEIT

Titel der Diplomarbeit

Optical properties in relation to the carbonate layer and morphological studies of the brown alga *Padina pavonica* (L.) Thivy

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When one tugs at a single thing in nature,
he finds it attached to the rest of the world
~ John Muir ~

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Vorwort

Padina pavonica ist eine im Mittelmeer häufig anzutreffende Braunalge, die durch ihren typisch blatt-trichterförmigen Habitus selbst von Laien leicht erkannt werden kann. *Padina pavonica* lagert Kalk ab, dennoch dürfte ihr Beitrag zu den imposanten Kalkriffen, die in Millionen von Jahren entstanden sind, eher gering gewesen sein. Tatsächlich bildeten neben „gesteinsbildenden" Korallen hauptsächlich Muscheln, Schnecken und Schwämme, sowie marine Mikroorganismen und makroskopische Grünalgen den biogenen Kalkstein. Sie formten durch Ablagerung und Ausscheidung über Jahrmillionen massive Gebirge (z.B.: Kalkalpen, Leithagebirge) und prägen heute noch ganze Küstenlandschaften. Die Bewunderung ist groß, wenn man sich vor Augen führt, dass mikroskopisch kleine Algen in der Lage sind, Hunderte von Metern mächtiger Schichten aus Kalk zu bilden, wie zum Beispiel die weißen Klippen in Südengland. Sehr alte und eindrucksvolle Formationen (Stromatolithen), die heute in Westaustralien in einer kleinen Bucht namens Shark Bay zu bewundern sind, wurden von kalkbildenden Cyanobakterien erbaut. Natürlich brauchte es dazu sehr viel Zeit und die richtigen Bedingungen, trotzdem beweist es augenscheinlich die enorme Leistungsfähigkeit der Algen.

In dieser Aufzählung dürfen natürlich Korallenriffe nicht unerwähnt bleiben. Algen führen im Gegensatz zu den Korallen meist ein Schattendasein, wenn es um Riffbildung geht, obwohl diese Organismengruppe nicht unwesentlich zum Bestehen spezifischer Korallen und zur Farbenpracht der Riffe beiträgt. Solche mit diesen Korallen in Symbiose lebenden Algen, sogenannte Zooxanthellen, versorgen ihren Wirt mit wichtigen Assimilationsprodukten (z.B.: Zucker, Aminosäuren) und unterstützen ihn in der Kalkproduktion, als Gegenleistung erhalten sie ausreichend Nährstoffe und Kohlendioxid. In rezenten Korallenriffen spielen Kalkrotalgen eine ökologisch wichtige Rolle, da sie durch Zementierung das ohnehin empfindliche Ökosystem stabilisieren und so das Vorhandensein der hohen Artenvielfalt ermöglichen. Dennoch sind sie aufgrund ihrer hohen Resistenz gegenüber Umweltveränderungen nicht zu unterschätzende Konkurrenten, da sie die Korallen mehr und mehr verdrängen.

Der Vorgang, bei dem sich Kalk niederschlägt, wird „Kalzifikation" genannt und ist bei allen vorangestellten Beispielen einschließlich *Padina pavonica* zu finden. Der Kalk

selbst taucht in unterschiedlicher Größe und Form auf, die chemische Grundformel ist dennoch fast immer die gleiche ($CaCO_3$).

Braunalgen sind üblicherweise nicht kalzifizierend, *Padina* bildet hier eine Ausnahme. Sie erreicht vor allem in den Tropen eine beachtliche Individuenanzahl, verglichen mit der Grünalge *Halimeda*, zum Beispiel, ist ihr Beitrag als Kalkproduzent jedoch gering. *Padina* ist mit momentan 37 Arten auf der ganzen Welt vertreten. Wie kommt es zu so einer Verbreitung bzw. wie sind die Anpassungen hier vonstatten gegangen? Gibt es eine unterschiedliche Kalkproduktion bei Arten in warmen bzw. kühlen Gewässern? Und wieso hat sich eine einzige Art (*P. arborescens*) innerhalb der kalkbildenden *Padina*-Gruppe entwickelt, die keinen Kalk ausscheidet? Oder stellt sie eine ursprünglichere Art dar? Diese Aufzählungen sollen nur einen kleinen Einblick über die vielen Fragen geben, die noch offen sind und auf Klärung warten.

1 Einleitung

1.1 Allgemeines

Padina pavonica (L.) Thivy gehört zu der Klasse der Braunalgen (Phaeophyceae) und kommt auf der ganzen Welt in warm temperierten Gewässern vor. Ihr Verbreitungsgebiet zieht sich von den tropischen Meeren über das Mittelmeer bis hin zu kühleren Gewässern in Südengland (Lüning 1985, Guiry 2010), wo sie ihre nördliche Grenze erreicht. Sie wächst bevorzugt an lichtexponierten Fels- und Sandküsten des Sublitorals (Lüning 1985) und heftet sich mit ihren rhizomähnlichen Haftorganen (Fritsch 1945) an steiniges Substrat. Das Sublitoral ist jener Bereich, der 0.3 m bis maximal 20 m des vertikalen Wasserkörpers abdeckt (Riedl 1964b, 1966). Einzelne Individuen finden auch auf anderen Algen oder Seegras geeignete Stellen zum Aufwachsen. *P. pavonica* tritt in kleinerer Form häufiger im oberen und mittleren Sublitoral auf, wobei größere Individuen vereinzelt unter 20 m zu beobachten sind. *P. pavonica* besitzt einen diplohaplontischen Lebenszyklus (van den Hoek et al. 1993). Dies bedeutet, dass zu Beginn ein diploider (2n) Sporophyt als Folge einer Reduktionsteilung Tetrasporen (n) ausbildet, aus denen eigenständige haploide (n) Gametophyten heranwachsen. Diese bilden männliche und weibliche Gameten auf ein- und derselben Alge (monözisch), die nach der Fusionierung die diploide Zygote bilden. Aus der Zygote entwickelt sich wieder ein Sporophyt, womit sich der Kreis schließt.

Die zwei Generationswechsel unterscheiden sich morphologisch nicht voneinander, sie sind isomorph, und dadurch ist eine Identifizierung nur bei Vorhandensein von Gameten bzw. Sporen möglich (Garreta et al. 2007). Der Habitus von *Padina* ähnelt einem Fächer oder Trichter mit konzentrischen weißen Bändern, die durch Ablagerung von Kalk entstehen und je nach Art mehr oder weniger vorhanden sein können. Zur Artbestimmung kann der Bestimmungsschlüssel von Wynne & de Clerck ('key to the species of Padina known for the Western Atlantic', 1999) herangezogen werden. Folgende Merkmale lassen auf die Art *P. pavonica* schließen: Die Bildung mehrerer Reihen von Haaren mit sporenbildenden Organen auf beiden Seiten, 3 Zellschichten (3-4 im basalen Bereich), Sporangien mit Indusium (Membran), sowie eine starke Verkalkung an der Oberseite und eine spärliche an der Unterseite. Diese Verkalkung geht auf eine extrazelluläre Ablagerung von Kalziumkarbonat ($CaCO_3$) zurück und bis vor kurzem galt *Padina* noch als einzige kalzifizierende Braunalgengattung.

1.2 Kalzifikation

Kalzifikation ist jener Prozess, bei dem Kalk in Form von Kalziumkarbonat ($CaCO_3$) mit einem geringen Anteil an Magnesiumkarbonat ($MgCO_3$) in die Zelle (intrazellulär), zwischen den Zellen (interzellulär) oder außerhalb der Zelle (extrazellulär) abgelagert wird. Das Kalziumkarbonat ist dabei immer die dominierende Komponente und kommt in der Natur am häufigsten in zwei polymorphen Kristallisationsformen vor: Calcit und Aragonit. Letzteres ist das löslichere Mineral und enthält verhältnismäßig mehr Magnesium (Mg) als der stabilere Calcit, der aus diesem Grund von vielen Rifforganismen (z.B.: kalkbildenden Korallen) gebildet wird. Unter den „Kalkalgen" gibt es Arten, die nur Calcit, nur Aragonit oder auch beide Formen bilden können. In der Klasse der Braunalgen sind derzeit zwei kalkbildende Gattungen vertreten: die Gattung *Padina* und die vor kurzem erst auf Hawaii entdeckte monospezifische Gattung *Newhousia imbricata* (gen. et sp. nov.) (Kraft et al. 2004). Erstere scheidet Aragonit ausschließlich extrazellulär aus, die neu beschriebene Art lagert Kalziumkarbonat, welches hauptsächlich aus Aragonit mit einem geringen Anteil an Calcit besteht, sowohl extra- als auch intrazellulär ein (Kraft et al. 2004). Die meisten Arten von *Padina* lagern den aragonitischen Kalk in einer Abfolge weißer Bänder auf ihren fächerförmigen Thalli ab (Borowitzka et al. 1974; Okazaki et al. 1986).

Das Phänomen der Kalzifikation taucht nicht nur in marinen Organismen auf, sondern auch bei aquatischen und terrestrischen Pflanzen. Einige Autoren (Borowitzka 1974 - 1984; McConnaughey 1991 - 1998) haben sich mit diesem Thema befasst und sowohl den chemischen Ablauf als auch den physiologischen Hintergrund genauer untersucht. Die Autoren konnten einen Zusammenhang zwischen Kalkabscheidung und der Photosynthese feststellen, wobei sich die zwei Prozesse gegenseitig fördern (für weitere Details siehe McConnaughey 1998).

Neben der Reaktion auf die Photosynthese und die damit verbundene Erhöhung des pH-Wertes kann die Kalzifikation auch eine strukturelle Verteidigung gegenüber Herbivoren darstellen. Laut Hay et al. (1994) können sich Algen durch Kalziumkarbonat-Ablagerungen mit zusätzlichen Sekundärstoffwechselprodukten synergetisch bzw. additiv gegenüber Herbivoren verteidigen. Im Laufe der Zeit konnten sich die Organismen anpassen und sich eine neue Nische zu Nutze machen. Kalkrotalgen, die vorwiegend in Schattenbereichen von Felsen oder Höhlen leben und als die einzigen photoautotrophen Organismen bis in hunderte Meter Tiefe vorkommen, haben Strategien entwickelt, um das wenige Licht nutzen zu können. Die typischen Rotalgenpigmente sind Phycoerythrin und Phycocyanin. Sie absorbieren vorwiegend grünes und blaues Licht, also jenen Bereich im Lichtspektrum, der in große Tiefen vordringt. Das langsame Wachstum von Rotalgen spiegelt das geringe Lichtangebot wider, die krustenförmige Wuchsform schützt nicht nur vor Tierfraß, sondern stellt eine ideale Oberfläche dar, um Licht effizient für die Photosynthese auffangen zu können. Die Kalzifizierung scheint diese Lebensweise zu unterstützen.

1.3 Reflexion, Transmission, Absorption

Licht sorgt aufgrund seiner spektralen Zusammensetzung für viele Möglichkeiten zur Absorption und Nutzung. Pigmente fangen Licht aus bestimmten Wellenlängenbereichen auf und ermöglichen damit letztendlich die Umwandlung dieser Energie in Biomasse. Unterschiedliche Anpassungen/Strategien helfen dem Organismus, mit variabler Lichtversorgung zurechtzukommen. *P. pavonica* weiß sowohl im Stark- als auch im Schwachlicht mit den jeweiligen Lichtverhältnissen sehr gut umzugehen. Da zu viel Licht der Alge schaden kann, versucht sie mit strukturellen Anpassungen wie Tahllusverzweigungen (Littler & Littler 1980) dem hohen

Lichtangebot entgegen zu wirken. Eine weitere Möglichkeit besteht, die am Thallus aufliegende Kalkschicht als eine Art Sonnenschutz zu nutzen. Das Karbonat fällt an der Oberfläche des Thallus aus, vermindert damit die Lichtdurchlässigkeit (Transmission) des Thallus, erhöht gleichzeitig die Reflexion und bewirkt, dass weniger Licht im Inneren aufgefangen wird (Absorption).

1.4 Morphologie

Die Morphologie der Zelle und des Thallus bezogen auf die Photosyntheseleistung und andere physiologische Eigenschaften wurden von diversen Autoren bereits dokumentiert (Raven et al. 1979, Littler and Littler 1980, Littler and Arnold 1982, Lüning & Dring 1985, Johansson & Snoeijs 2002). In manchen Algen ähnelt sich der Habitus sehr stark, obwohl sie nicht näher miteinander verwandt sind. Dies lässt sich auf das Konzept der ökologischen Nische zurückführen. Einige Autoren haben aus diesem Grund, dem Beispiel von Littler & Littler (1980) folgend, verschiedene Arten entsprechend ihrer ähnlichen morphologischen Struktur in funktionelle Formgruppen zusammengefasst und mit Eigenschaften wie Primärproduktion (Littler and Arnold 1982), Resistenz gegenüber Herbivorie (Littler & Littler 1983), Anpassung an Substratverhältnisse und Umweltstörungen (Littler and Littler 1984), sowie anderen evolutionären Strategien verglichen. Es zeigte sich sowohl ein bemerkenswerter Trend innerhalb der Gruppen, als auch eine deutliche Linie zwischen den Gruppen, wie etwa das Beispiel von Littler & Arnold (1982) anhand der höheren Produktivität von blattartigen dünnen Thalli gegenüber inkrustierenden Algen zeigte. Dieses Modell konnte sich jedoch nicht durchsetzen, da die Einteilung in die 8 Formgruppen bei vielen Algenarten zu Problemen führte und es als Alternative leicht zu messende Variablen gibt, zum Beispiel die Thallusdicke. Das Trockengewicht, die Thallusfläche und die Thallusdicke gelten als adäquate Parameter, um sich auf die Photosynthese und Absorption zu beziehen (Lüning & Dring 1985, Enríquez et al. 1994, Han et al. 2003). Dabei kommt es bei der Photosyntheserate nicht nur auf die morphologischen Aspekte, sondern auch auf die Dichte und Zusammensetzung der Pigmente an (Lüning & Dring 1985).

1.5 Ziele

Die vorliegende Arbeit untersucht die Beziehung zwischen optischen Eigenschaften der Thalli, der Lichtversorgung und der Kalkablagerung in *P. pavonica*. Unter der Annahme, dass die Photosynthese mit der Kalzifikation gekoppelt ist, erwarteten wir einen deutlichen Anstieg des Kalkgehalts in den Thalli, die einer höheren Lichteinstrahlung ausgesetzt sind. Diese erhöhte Kalkablagerung sollte zu einer höheren Reflexion führen. Außerdem werden erstmals Reflexionsspektren zwischen kalzifizierenden und dekalzifizierenden *Padina* Thalli verglichen. Durch eine weitere Untersuchung der Thallusdicke und des Zellvolumens von *P. pavonica* erwarteten wir uns zusätzliche Informationen über die Beziehung von Morphologie und Absorptionseigenschaften.

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2 **Summary**

The common Phaeophyceae *Padina pavonica* was selected to shed light on relations between calcification and optical properties which may act as key factors for occurrence and photosynthetic performance. We sampled specimens from locations with different light supply in the Bay of Calvi (Corse, France) in spring and autumn. Carbonate layer per dry mass and per thallus area, respectively, differed significantly between seasons, so did the thallus water content. Seasonal differences in absorption, reflection and transmission depending on changes of incoming irradiance at the respective growth site could also be observed. With consistent absorption, reflection showed a significant increase at elevated irradiances, contrarily transmission decreased. Reflectance spectra standardized to the maximum reflectance were compared between calcified and de-calcified thalli resulting in carbonate smoothing the spectral reflectance. In autumn, positive relations between reflection and carbonate and negative correlations between absorption and carbonate content were found. Nevertheless, thalli with increased carbonate layers showed also increased reflection, which probably enables the frequent occurrence of *P. pavonica* at high irradiance areas. In a further investigation we studied morphological changes on a macroscopic and microscopic scale, respectively, in dependence to different irradiance supply. Thallus thickness, cell volume per thallus and cell volume per cell layer were compared between seasons and irradiance supply, respectively. Each thallus mainly consists of three cell layers: the upper surface, the middle and the lower surface cell layer. Thalli in spring were small and thick and contained significantly larger cells than specimens collected in September. Additionally, thalli from shallow areas showed significantly greater thallus thickness than specimens from deeper areas, but averaged cell volumes per thallus did not change. In contrast, each cell layer differed significantly in terms of the cell volume with increasing irradiance supply. Growth rates and thallus areas of *P. pavonica* in spring were comparable in the port and on the shore at different irradiance supply. These results assume that not only irradiance but also other factors influence the growth of either thallus or cells.

3 Optical properties of *Padina pavonica* (L.) Thivy in relation to its carbonate layer

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3.1 Abstract

This study is proposed to shed light on relations between calcification and optical properties which may act as key factors for occurrence and photosynthetic performance of the Phaeophyceae *Padina pavonica*. We sampled specimens from locations with different light supply in the Bay of Calvi (Corse, France) in spring and autumn and compared carbonate layers with optical properties. Carbonate layer per dry mass and per thallus area, respectively, differed significantly between seasons, so did the thallus water content. Seasonal differences in absorption, reflection and transmission depending on changes of incoming irradiance at the respective growth site could also be observed. With consistent absorption, reflection showed a significant increase at elevated irradiances, contrarily transmission decreased. Reflectance spectra standardized to the maximum reflectance were compared between calcified and de-calcified thalli resulting in carbonate smoothing the spectral reflectance. In autumn, positive relations between reflection and carbonate and negative correlations between absorption and carbonate content were found. Nevertheless, thalli with increased carbonate layers showed also increased reflection, which probably enables the frequent occurrence of *P. pavonica* at high irradiance areas.

Keywords: *Padina pavonica*, calcium carbonate, reflection, absorption, irradiance

3.2 Introduction

Phaeophyceae are not calcifying, but there exist a few exceptions with the genus *Padina* being one of them. To date, 37 species (Guiry 2010) of this genus are taxonomically accepted based on morphological traits like occurrence or absence of hair lines, their adjacency to sporangial sori and the degree of calcification (Wynne & de Clerck 1999). *Padina pavonica* (L.) Thivy is commonly found on rocky shores and easily can be recognized by its extracellular concentric carbonate layers, which are responsible for the whitish appearance. It is worldwide found in oligotrophic, irradiance exposed sublittorals of tropical and temperate shore regions (Lüning 1985, Guiry 2010). The isomorphic diplohaplontic life cycle was already described by Carter (1927). After spore settlement and germination, a dark coloured 'central nodule' with filamentous rhizoids starts to develop (Carter 1927). Gametophytes and sporophytes of *P. pavonica* have the same appearance, however, as in other phaeophycean genera, sporophytes are more frequently found (Doust & Doust 1990). *P. pavonica* is known as 'seasonal annual' (Airoldi 2000) with a 'perennial rhizome' (Fritsch 1945) and visible thalli are abundant from spring to summer with an extension to autumn in some areas (Einav 1995, Sala & Boudouresque 1997, Piazzi et al. 2002). Some specimens also occur during the winter months either on cliffs in the western Mediterranean Sea or at rocky shores in the warmer eastern Mediterranean Sea (Piazzi et al. 2002, Turna et al. 2002).

Padina pavonica possesses a biologically induced extracellular calcification which results in whitish precipitations. These carbonate deposits, predominantly needle forming aragonite, are arranged in concentric bands on the thallus surface (Borowitzka et al. 1974; Okazaki et al. 1986) with interspaces where reproductive structures like tetrasporangia can develop. Formerly, *Padina pavonica* was described with one carbonate layer on the sun-facing surface only (Fritsch 1945; Okazaki et al. 1986), nowadays it is accepted that the whole thallus is calcifying (Wynne & de Clerck 1999). The sun facing side with the enrolled margins represents the 'upper' surface (Fritsch 1945, Bitter 1899) and the opposite side the 'lower' surface. Carter (1927) called them 'dorsal' and 'ventral', respectively.

For the marine environment, calcification processes were investigated in detail for the chlorophyte *Halimeda*. It turned out, that calcification is based on pH changes caused by photosynthesis (Borowitzka & Larkum 1976, de Beer & Larkum 2001). Chemical

reactions lead to acidic and alkaline zones on the thallus surface and this in turn provides optimal microenvironments to promote calcification (McConnaughey 1998). The pH-ranges between 8.2 and 8.4 support carbon uptake via bicarbonate in an optimal way and keep the exchange system stabilised. Higher pH leads to a dramatic decline in photosynthetic rates and an increase in carbonate precipitation (Sand-Jensen & Gordon 1984), whereas in acidic waters carbonates start to dissolve. Therefore calcifying organisms are very sensitive against pH shifts. As photosynthesis commonly induces alkaline conditions around the plants, the question arises, why calcification is not always taking place on water plants. It is assumed that with the exception of calcifying organisms, organic substances are released to prevent active carbonate nucleation (Borowitzka & Larkum 1976, Borowitzka 1982). Interestingly, precipitation processes were also observed in the dark, which were explained by non-metabolic physical processes (Borowitzka & Larkum 1976, Borowitzka 1982).

In *Padina*, extracellular calcification is arranged in concentric bands comparable to Charophyceae where it is attributed to different pH-based areas (McConnaughey 1998). Protons from carbonate deposition sites, i.e. the alkaline bands, are transported and finally released at the non-calcifying areas of the thallus, the acid bands, for bicarbonate conversion. McConnaughey & Whelan (1997) assumed that protons generated through calcification mechanisms may support nutrient uptake. This is an advantage especially in nutrient-depleted waters where calcareous organisms often predominate over non-calcifying organisms. Moreover, carbonate deposits function as skeleton and act as a protection measure against grazers (McConnaughey & Whelan 1997).

To date, only scarce information about optical properties of photosynthetic organisms is available. For some macroalgae absorption (A) measurements neglecting reflection (R) were conducted (Markager & Sand-Jensen 1992), in other studies carbonate layers were removed chemically before measurements (Enríquez et al. 1994). R spectra were measured in both pigmented and bleached corals and some macroalgae, respectively. Such data were further used for calibration to detect coral reefs by remote sensing (Myers et al. 1999, Hochberg et al. 2003; Hochberg et al. 2004). There exist some studies of *Padina*, in which photosynthetic performances were measured. However, R which plays an important role was not considered until now (Enríquez et al. 1994, Markager & Sand-Jensen 1992). This study aims to shed light on optical properties linked to carbonate precipitation. We expected significantly higher amounts of

carbonate deposits on thalli exposed to high irradiances, because photosynthesis induces carbonate precipitation. Assuming that thalli of *P. pavonica* growing in high irradiance areas show higher R due to increased calcification, and transmission (T) decreases in high irradiance areas due to reduced water contents, A remains the same. Additionally, we compared reflectance (R_T) spectra between calcified and de-calcified thalli of *P. pavonica* based on the maximum reflectance.

3.3 Material and Methods

Study site

Field studies were carried out in September 2007 and in April/May 2008 in the Mediterranean Sea at the marine biology station Stareso (Station de Recherches Sous-marines et Oceanographiques) located north-east of Corsica in the Bay of Calvi (42°35'63" N, 8°44'62" E). The site is dominated by a rugged coastline consisting of granites followed by a sandy bottom with seagrass patches. The sheltered east side of the Bay with its rocky substrate offers an ideal habitat for *Padina pavonica* resulting in a high abundance of this taxon.

Sampling and field measurements

Sampling was conducted by scuba diving between 1 and 30 m depth; ten thalli were measured per day. Incoming irradiance was recorded with a data logger (Skye Data Hog2 equipped with a photosynthetically active radiation PAR SKP 210 sensor) fixed to a stationary position on the shore. Submersible data loggers were used for recording temperature and illuminance (lux) at different depths (Onset computers, Hobo UA-002-08). We used a conversion factor of 0.0139 to covert lux into µmol photons m^{-2} s^{-1} PAR. From obtained data, the coefficient of attenuation $K_d = 1/z * \ln (I_0/I_z)$ with depth (z), surface irradiance (I_0) and irradiance at depth z (I_z) was calculated.

Laboratory measurements

Each thallus was photographed using a digital camera (Nikon Coolpix 4500) for thallus area measurements applying the software program "Image J". Wet mass (WM) of each thallus was estimated using a fine scale; dry mass (DM) was recorded after drying specimens for 16 hours at 95° C. Water content of the thalli (% WC per WM) was calculated as (WM - DM)/WM x 100. After DM determination, the carbonate layer was dissolved with 1 M HCl and thalli were then dried again and reweighed. The percentage of carbonate was calculated from dry mass (DM) and dry mass without carbonate (DMC) with (DM – DMC)/DM x 100.

In this study, total R is used as averaged reflected irradiance regardless of the wavelength, R_T means the light reflected at specific wavelengths along the PAR range. Total A values are calculated from T and R and stands for the light absorbed by the thallus. Total T is defined as irradiance passing through the thallus. T was measured with the light guide of the Diving PAM by exposing thalli against sunlight. First,

intensity (μmol photons m^{-2} s^{-1}) of incident irradiance I_0 was measured and afterwards the transmitted irradiance I_T through the calcified thalli by clamping it in front of the sensor: $T = (I_T/I_0) \times 100$. R_T measurements (% of incoming irradiance) were obtained with a spectroradiometer (Ocean Optic: USB4000 Miniature Fibre Optic Spectrometer) equipped with a bifurcated optical fibre (Reflection/Backscattering Probes P400-2-UV-VIS). The fibre optics was vertically positioned into a black container filled with sea water; thalli were placed at 1 cm distance from the submerged fibre optics. R was calibrated against a standard (WS-1-SS White Standard with Stainless-steel Housing), R_T spectra were recorded at wavelengths between 400 and 700 nm with 0.2 nm resolution. R_T spectra were averaged to R for absorption calculations: $A = 100 - R - T$. For a comparison of reflectance between calcified and non-calcified specimens, values were divided through the R minimum at 670 nm.

Statistics

Satistical analyses were performed using SPSS 14.0 (Statistical Package for the Social Sciences). If the criteria were met, a group comparison within LCs for each season (LCA for April/May and LCS for September) was conducted by applying one-way analysis of variance (ANOVA) including a post hoc test after Scheffé to find significant differences between groups. Otherwise the non-parametric Kruskal-Wallis test was performed. Sampling locations were grouped according to prevailing irradiances into three light classes (LC) grouped according to comparable sampling numbers and an even distribution. LC 1 ranged from $> 0 < 12$ % incoming irradiance, LC 2 from ≤ 12 to 20 % and LC 3 comprises sites ≥ 20 % irradiance. In total, 200 thalli were collected in September 2007 ($n_{LCS1} = 66$; $n_{LCS2} = 68$ and $n_{LCS3} = 66$) and 158 thalli in April/May 2008 ($n_{LCA1} = 48$, $n_{LCA2} = 56$; $n_{LCA3} = 54$). Spearman rank correlation and linear regression were performed to show positive or negative relationships between optical properties and carbonate layers.

3.4 Results

Thalli of *Padina pavonica* started to develop in April and grew until autumn when the harsh winter surf started. Thalli in spring were considerably smaller compared to specimens at the end of the growth season. Independent of season, smaller thalli seemed to be more abundant in shallow waters, densely covering the substratum whereas larger thalli appeared only scattered in deeper waters. Light intensities expressed as a percentage of incoming irradiance were highly related to depth and did not change significantly between spring and autumn (data not shown), nor did the attenuation coefficient, K_d. In April/May a median K_d of 0.25 m^{-1} was calculated, in September the median of K_d amounted 0.21 m^{-1}.

In general, thalli in April averaged a significantly lower carbonate content than in September based on both DM and area, respectively (Table 1, Fig 1). No significant variations in carbonate per area occurred between LCAs, but within LCSs a significant increase could be detected (Fig 1). Contrarily, carbonate based on DM showed no significant relation to irradiance within LCAs and LCSs, respectively (Table 1). A general season comparison between optical properties revealed significantly lower R and higher T in spring, contrary to consistent A in both seasons (Table 1). In April/May, *P. pavonica* tended to a higher R in LCA3 (Table 1), however, no significant differences could be calculated with our data set (post hoc test after Scheffé: $p_a = 0.066$; Table 1). In autumn, R significantly increased with increasing irradiance supply (Table 1) with lower values in LCS1, whilst LCS 2 and 3 were grouped together (post hoc: $p_{a,b} < 0.05$; Table 1). Relations between R and DM based on carbonate content showed a negative trend in spring, which however was not significant (Spearman ρ: r = - 0.101; p > 0.05, n = 151). Also, no significant correlations between A and carbonate could be detected (Spearman ρ: r = -0.115, p > 0.05, n = 151). In autumn, a significant positive correlation between R and carbonate could be found (Spearman ρ: r = 0.221, p = 0.002, n = 199) basically deriving from relationships within LCS2 (r = 0.287, p < 0.05, n = 66; Fig 2). Additionally, autumn thalli showed a significant negative correlation between A and carbonate content (Spearman ρ: r = -0.150, p < 0.05, n = 200). As demonstrated in Fig 3B, R of calcified specimens showed significantly higher values than de-calcified thalli (Mann-Whitney-test: p = 0.000, n =

200; Fig 3 B). In general, R_T was lower at short wavelengths and increased at around 550 nm; at 670 nm a sharp drop was recorded (Fig 3 A, B).

Spring thalli showed a mean of about 84 %, autumn thalli contained 76 % WC per WM as an average (Table 1). WCs remained constant for LCA, but within LCS, WCs showed a significant increase towards elevated light supply (Table 1, ANOVA: $p < 0.01$).

3.5 Discussion

Algae living on sublittoral rocks have to cope with high irradiance, low nutrient availability, grazing pressure, high currents and areal competition for which several adaptations have been established to outcompete other species. These ecological, physiological and morphological adaptations are known as photoprotective pigments acting against photooxidation (Rowan 1989), chemical reactions facilitating nutrient uptake (McConnaughey & Whelan 1997) and structural defences against grazers (Padilla 1989). *Padina pavonica* responds to these stress factors with calcification which also affects the optical properties of the thallus. In spring, young exemplars showed similar R due to comparable carbonate amounts in all LCs (Table 1, Fig 1) but with time differences became detectable. R significantly increased with higher irradiance supply in autumn (Table 1, Fig 3 B), which can be attributed to the increased carbonate content (Fig 1). Those thalli showed a slight but significant positive relation between R and carbonate (Fig 2). According to these results and to the negative correlations in spring, we assume that the amount of aragonite is not the only important factor, but also structural characteristics of crystals have to be considered for R. The results of Borowitzka et al. (1974) point in such a direction, as these authors mentioned that younger parts of the thallus are covered by needle-like aragonite crystals. Contrarily, older parts show small and irregular oriented crystals due to the loss of the needle-like shape. This phenomenon can also be assumed for older thalli as aragonite crystals change their shape with time. Compared to *P. pavonica*, Wefer (1980) observed lower carbonate contents in *P. sanctae-crucis* (38 % of DM). He described the subtropical relative as dominant carbonate producer on rocky substrates, almost absent in sandy areas, which fits well to our observations. In contrast to the findings of Fritsch (1945), calcification took place on both thallus sides with the sun-exposed surface being more calcified than the opposite one. This result is in accordance to Wynne & de Clerck (1999). Also Okazaki et al. (1986) documented *P. pavonica*, from the United Kingdom, with only one calcified thallus surface which could explain the drastically diminished content of < 19 % carbonate per unit DM. We found significantly lower carbonate contents in spring (Table 1, Fig 1), which could be explained by enhanced carbonate solution at lower water temperatures and by the fact that young specimens just started to precipitate carbonate. De Beer & Larkum (2001) mentioned a possible

dissolution of carbonate at low light intensities due to a pH-driven decalcification because of respiration activities. Carbonate patterns of different thallus areas still remain unclear: Borowitzka et al. (1974) alluded lower carbonate precipitation at the basal region which is the oldest part. Here, most probably low photosynthesis but increased respiration occurs. In contrast, Okazaki et al. (1986) mentioned *Padina* reaching slightly higher amounts of carbonate in older parts of the thalli. For the green genus *Halimeda*, Borowitzka & Larkum (1977) suggested that specimens grow until a maximum size is reached, but become more calcified with age. For *Halimeda* higher calcification rates were observed in younger thalli (Borowitzka & Larkum 1977). They found that calcification is only initiated when the chloroplasts reach functional maturity which indicates a certain relation between calcification and photosynthesis. Also Okazaki et al. (1986) described an initial calcification at the enrolled margin of *P. pavonica* with cells containing chloroplasts.

Due to the fact that *P. pavonica* lacks MAAs (mycrosporine-like amino acids) and other UV-absorbing compounds (Karsten et al. 1998), calcium carbonate might act as sunscreen to protect the sensitive photosynthetic apparatus against excess light and ultraviolet (UV) radiation. Our study did not include reflectance spectra out of the PAR range which might explain the low relations between optical properties and carbonate content (Fig 2). Besides chlorophylls *a* and *c*, *P. pavonica* contains various carotenoids with being fucoxanthin as the major accessory pigment responsible for the brownish colour. Fucoxanthin preferably absorbs at around 450 nm, chlorophyll *a* shows maxima at around 430 and 665 nm, and chlorophyll *c* at 450, 585 and 630 nm, respectively (Wright et al. 1991). Some of these A maxima are clearly related to reflection minima in our reflectance spectra (e.g., drop at around 670 nm, Fig 3). This result indicates that not only the surface is important for R characteristics, but also deeper layers and substances within the organism. For brown pigmented corals, Myers et al. (1999) and Hochberg et al. (2004) reported three reflectance peaks at 570, 600 and 650 nm, which were also observed in our study, but in a less distinctive way. Obtained R_T spectra are comparable to intact coral surfaces (Enríquez et al. 2005); they showed a drop at around 670 nm and elevated reflection between 560 and 600 nm (Fig 3 A, B) . The depression at 670 nm of both calcified and de-calcified thalli is caused by chlorophyll *a* absorption (Gitelson 1992, Jeffrey 1997). The drop between 400 and 500 nm (Fig 3 A) represents

predominantly chlorophyll *c* and fucoxanthin. A comparison between calcified and decalcified thalli revealed, that the carbonate layer is smoothing the R_T curve (Fig 3 A).

During the *Padina* growth season T decreased from spring to autumn which could be explained by reduced WCs (Table 1). Accordingly, these increased WCs correspond well to the findings of Bürger & Schagerl (2010), who observed larger cells in spring.

No direct herbivore interactions were observed during our study, although visible feeding marks could be detected for some individuals. Calcification is believed to act as protective cover to fend off mechanical attacks by herbivores (Johansen 1981; Steneck 1982) also combined with thallus form (Littler & Litter 1980; Littler et al. 1983). However, there exist some studies in which calcification as a protection measure seems to be of minor importance. Some non-calcified algae were studied to be more resistant to tissue loss because calcification makes the algae more fragile and easier to break (Padilla 1989). Besides, chemical compounds as defenders should also be regarded in *Padina* species (Steinberg & Paul 1990) which could be promoted by calcification (Hay et al. 1994). Hereu (2006) studied grazing on *P. pavonica* by sea urchins and herbivorous fish in the Mediterranean Sea. He observed a significant negative relation of algal height and weight, respectively, to the presence of fish or sea urchins and revealed a moderate impact if both occurred together.

In this study, we focused mainly on the relation between calcification and optical properties. Carbonate precipitation on the thallus surface increases reflection which protects the organism against excess irradiance. Commonly, algae are not able to escape from environmental changes but have to cope with biotic and abiotic stressors. In this way, calcification seems to represent an adequate adaptation to distract herbivores from grazing and to avoid harmful irradiation from damaging essential cell compounds. This evolutionary adaptation has been evolved during long periods but today anthropogenic impacts change the environment in such a very short time that especially sessile organisms are not able to escape or develop appropriate adaptation mechanisms.

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3.7 Table and Figure Legends

Table 1 Mean values (± standard deviation SD) and median values (± interquartil range) marked with an asterisk (*) from all individuals. Carbonate content ($CaCO_3$), optical properties (R, A, T) and water content (WC), given in % were grouped into three light classes (LC1-3) and two seasons (April/May and September). Significant differences between LC1 and LC3 and between the seasons are expressed with a 5 % probability (p). Bold numbers were tested with t-test between seasons or ANOVA between LCs, others with the Kruskal-Wallis-test and Mann-Whitney-test, respectively. Post hoc tests were conducted after Scheffé. Letters within a parameter indicate significant differences.

Fig 1 Comparison of area based calcium carbonate ($CaCO_3$ mg cm^{-2}) between LCA 1 and 3 (grey boxes) and LCS 1 and 3 (white boxes). Significant differences between LCs of April/May and September were calculated with ANOVA and the post-hoc test after Scheffé. Letters within a parameter indicate significant differences.

Fig 2 Relationships between absorption (A) and reflection (R), respectively, and carbonate based on dry mass (DM) in LC2 in September, using linear regression.

Fig 3 Reflectance spectra (R_T) of calcified and de-calcified thalli in September. (A) Mean values of log reflectance from 400 to 700 nm standardised to 670 nm from thalli before (bold dotted line) and after acidification (bold solid line). (B) Mean reflectance in percent of thalli with (calcified) and without $CaCO_3$ (de-calcified) of LC 1 – 3 in September.

Table 1 Mean values (± standard deviation SD) and median values (± interquartil range) marked with an asterisk (*) from all individuals. Carbonate content ($CaCO_3$), optical properties (R, A, T) and water content (WC), given in % were grouped into three light classes (LC1-3) and two seasons (April/May and September). Significant differences between LC1 and LC3 and between the seasons are expressed with a 5 % probability (p). Bold numbers were tested with t-test between seasons or ANOVA between LCs, others with the Kruskal-Wallis-test and Mann-Whitney-test, respectively. Post hoc tests were conducted after Scheffé. Letters within a parameter indicate significant differences.

		April/May 2008			**September 2007**			
	LC	mean ±SD median*	n	p (LC)	mean ±SD median*	n	p (LC)	p (Apr/Sept)
$CaCO_3$ per DM	1	69.78 ± 11.85*	48		73.33 ± 9.62*	66		0.012
	2	69.14 ± 10.88*	56	0.105	73.91 ± 12.28*	68	0.694	0.000
	3	66.67 ± 17.43*	54		75.00 ± 6.93*	66		0.000
R	1	23.55 ± 11.41 [a]	45		24.54 ± 6.99 [a]	66		**0.605**
	2	23.62 ± 10.88 [a]	54	**0.026**	28.09 ± 7.40 [b]	68	**0.000**	**0.011**
	3	29.25 ± 13.81 [a]	52		29.53 ± 6.24 [b]	66		0.343
A	1	34.37 ± 12.83	45		39.12 ± 8.29	66		**0.032**
	2	39.16 ± 13.05	54	**0.205**	39.89 ± 8.48	68	**0.530**	**0.725**
	3	37.90 ± 14.72	52		40.79 ± 8.74	66		0.474
T	1	42.08 ± 8.06 [a]	45		36.34 ± 5.63 [a]	66		0.001
	2	37.22 ± 8.00 [b]	54	**0.000**	32.02 ± 5.79 [b]	68	**0.000**	**0.000**
	3	32.85 ±8.95 [c]	52		29.68 ± 6.41 [b]	66		0.044
WC per WM	1	84.40 ± 3.68	48		72.20 ± 1.11 [a]	66		0.000
	2	84.43 ± 3.51	56	**0.721**	75.52 ± 0.88 [a,b]	68	**0.004**	0.000
	3	83.94 ± 3.34	54		76.63 ± 0.87 [b]	66		0.000

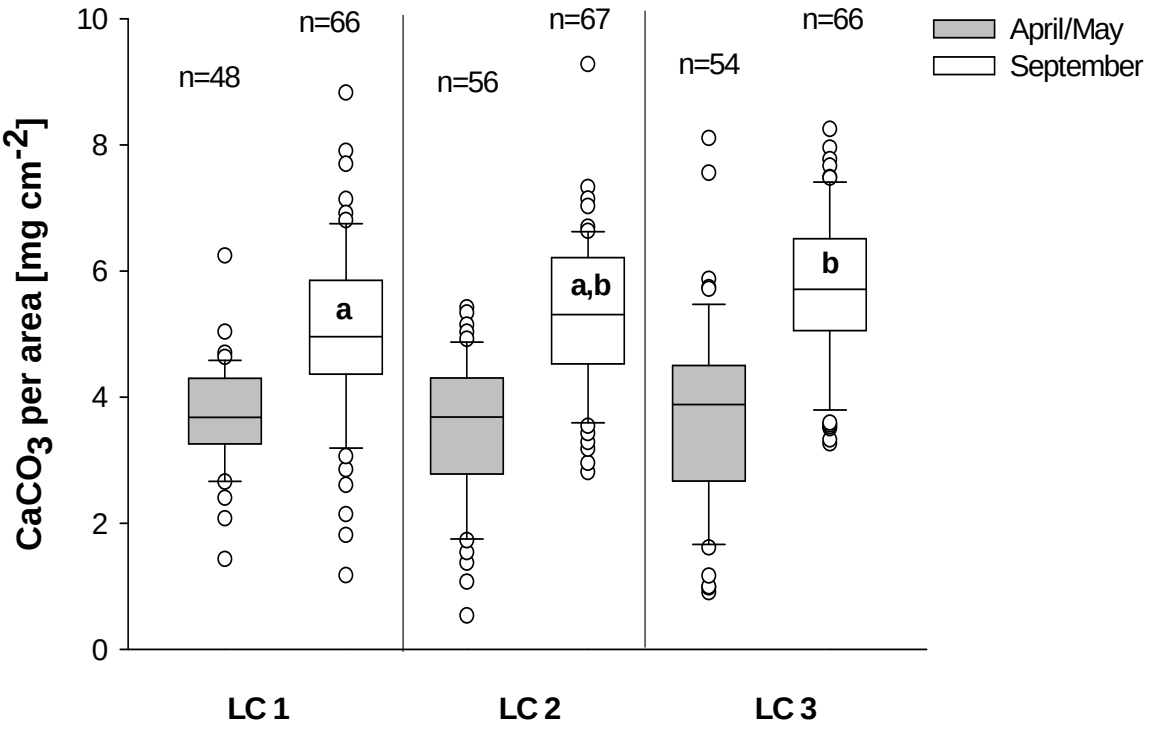

Fig 1 Comparison of area based calcium carbonate (CaCO3 mg cm^{-2}) between LCA 1 and 3 (grey boxes) and LCS 1 and 3 (white boxes). Significant differences between LCs of April/May and September were calculated with ANOVA and the post-hoc test after Scheffé. Letters within a parameter indicate significant differences.

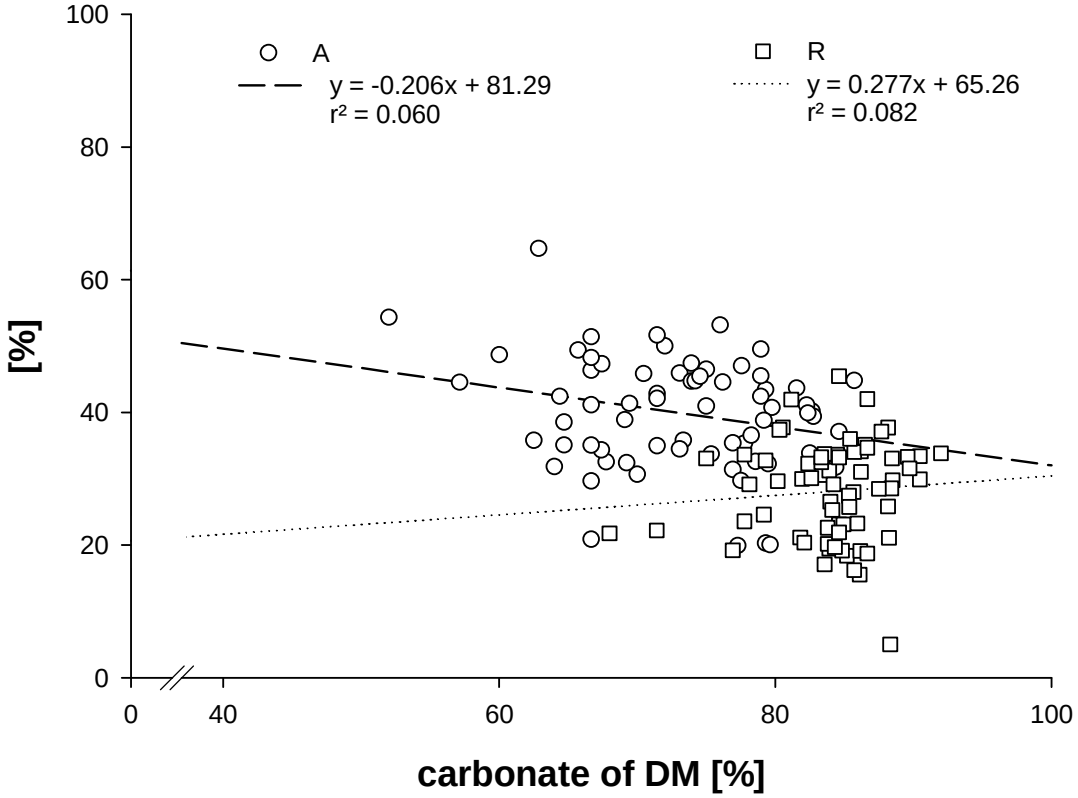

Fig 2 Relationships between absorption (A) and reflection (R), respectively, and carbonate based on dry mass (DM) in LC2 in September, using linear regression.

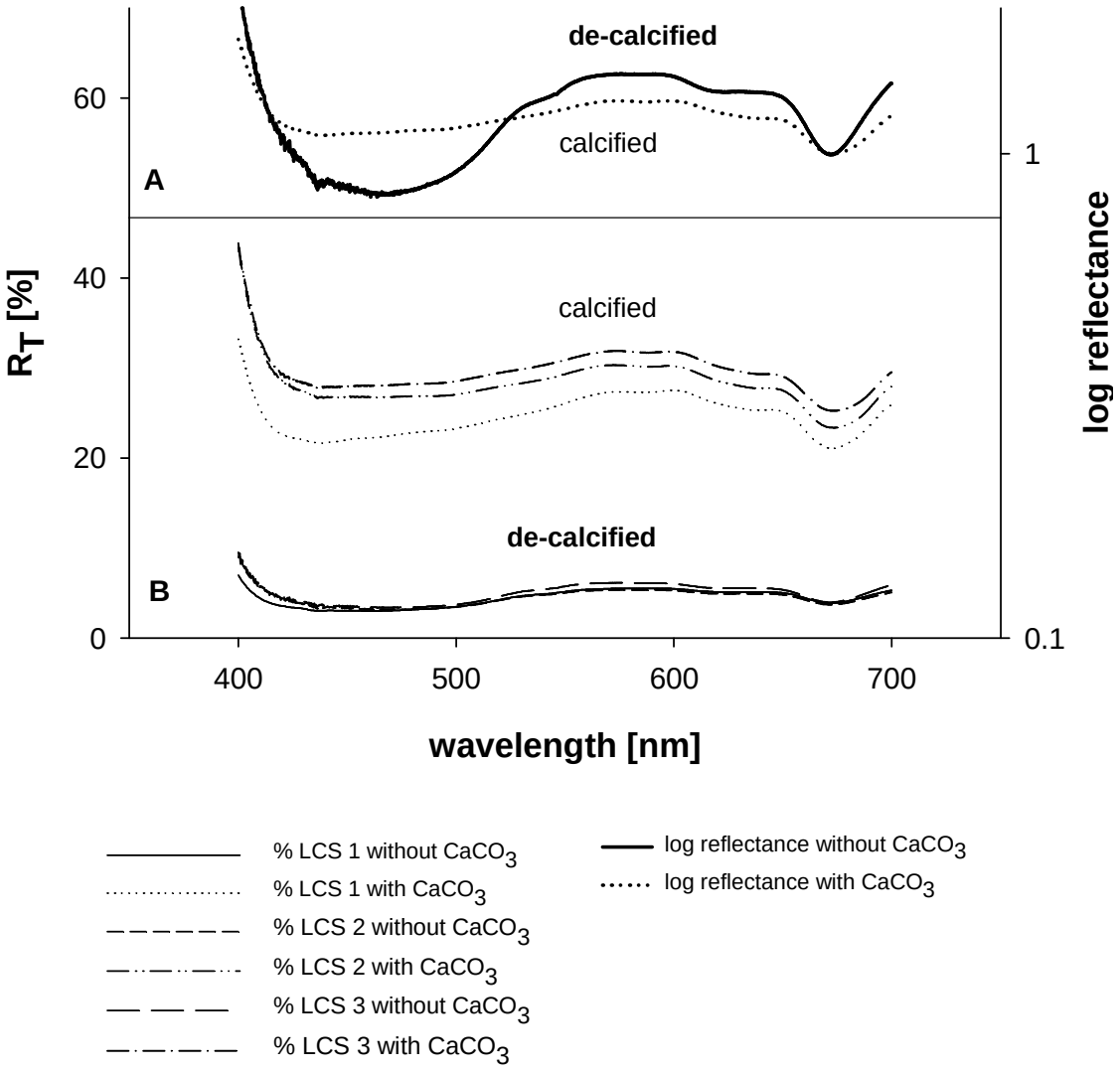

Fig 3 Reflectance spectra (R_T) of calcified and de-calcified thalli in September. (A) Mean values of log reflectance from 400 to 700 nm standardised to 670 nm from thalli before (bold dotted line) and after acidification (bold solid line). (B) Mean reflectance in percent of thalli with (calcified) and without $CaCO_3$ (de-calcified) of LC 1 – 3 in September.

4 Morphological studies of the brown alga *Padina pavonica* (L.) Thivy

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4.1 Abstract

The common Phaeophyceae *Padina pavonica* was selected to study morphological changes on a macroscopic and microscopic scale, respectively, in dependence to different irradiance supply. The study was conducted in the Bay of Calvi in western Corsica, where specimens were collected at different depths in spring and autumn. Thallus thickness, cell volume per thallus and cell volume per cell layer were compared between seasons and irradiance supply, respectively. Each thallus mainly consists of three cell layers: the upper surface, the middle and the lower surface cell layer. Thalli in spring were small and thick and contained significantly larger cells than specimens collected in September. Additionally, thalli from shallow areas showed significantly greater thallus thickness than specimens from deeper areas, but averaged cell volumes per thallus did not change. In contrast, each cell layer differed significantly in terms of the cell volume with increasing irradiance supply. Growth rates and thallus areas of *P. pavonica* in spring were comparable in the port and on the shore at different irradiance supply. These results assume that not only irradiance but also other factors influence the growth of either thallus or cells.

Keywords: *Padina pavonica*, morphology, thallus thickness, cell volume, growth rate

4.2 Introduction

Padina pavonica (L.) Thivy (Phaeophyceae, Dictyotales) is a worldwide distributed species (Guiry 2010) of rocky shores of the sublittoral in tropical and temperate regions (Lüning 1985). *P. pavonica* shows an isomorphic diplohaplontic life cycle, which is common for Dictyotales (Garreta et al. 2007). Gametophytes are generally rare in Dictyotales (Doust & Doust 1990, van den Hoek et al. 1993), which has also been proven for the genus *Padina* (Carter 1927, Thornber 2006) and specifically for *P. pavonica* (Garreta et al. 2007). The fan-shaped thalli (Carter 1927) with an enrolled margin are easily to recognize and arise from a prostrate perennial rhizome (Fritsch 1945). Thalli usually consist of three cell layers, sometimes four layers can be found in the basal regions (Wynne & de Clerck 1999). Growth takes place at the marginal zone with longitudinal divisions of the apical cells (Fritsch 1945). *P. pavonica* possesses a biologically induced extracellular calcification through photosynthetic CO_2-assimilation forming concentric zones (Bürger & Schagerl 2010). Calcified bands (Fritsch 1945, Borowitzka et al. 1974, Okazaki et al. 1986) are alternating with carbonate-free spaces where reproductive structures can develop. In this area, hairs prevent small particles to settle down (Carter 1927). The 'upper' surface facing the sun appears heavier calcified than the 'lower' surface (Wynne & de Clerck 1999, Bürger & Schagerl 2010).

Not only light intensity and quality (Markager & Sand-Jensen 1992) act as key factors for algal distribution, but also water movements, salinity, substrate inclination (Piazzi et al. 2002) and nutrient supply (Hay 1986, Creed et al. 1997). Additionally, biological interactions like space competition, algae-herbivore-interactions, grazing pressure (Hereu 2006) and physiological limitations e.g. sensitivity against desiccation (Einav et al. 1995) have an effect on distribution and abundance of algae. Interactive effects of grazing by limpets were studied on algal assemblages in mid- and low-shore areas (Benedetti-Cecchi et al. 2000). Hereu (2006) observed a biomass reducing effect on *Padina pavonica* by sea urchins and herbivorous fishes in the Mediterranean Sea.

Thallus and cell morphology in relation to photosynthetic performance and other physiological characteristics have been studied in green, brown and red algae by several authors (Raven et al. 1979, Littler & Littler 1980, Littler & Arnold 1982, Lüning & Dring 1985, Johansson & Snoeijs 2002). Littler & Littler (1980) developed the so-called functional-form model (FFM), where morphological similar macroalgae were

grouped together and examined for related physiology and ecology. In this model, *P. pavonica* belongs to the Thick Leathery-Group (de los Santos et al. 2009). In the meantime, this model has been adopted in many studies. Padilla & Allen (2000) addressed the positive and negative aspects of diverse hypotheses in relation to functional algal groups. Very recently, a study compared the FFM with a power scaling approach based on the surface to biomass ratio leading to similar results and recommended the use of a scaling factor instead of morphological groupings (de los Santos et al. 2009).

Besides dry mass and thallus area, also thallus thickness is a key variable to explain photosynthetic performance and absorption properties (Lüning & Dring 1985, Enríquez et al. 1994, Han et al. 2003). Algae show variable photosynthetic rates with regard to thallus thickness and pigment composition, respectively. King & Schramm (1976) investigated higher photosynthetic rates for sheet-like or filamentous thalli than for coarsely branched species indicating that the so-called package effect plays a major role. Additionally, Lüning & Dring (1985) analyzed photosynthesis action spectra of green and brown algae and found that thickness was more likely influencing photosynthesis than did pigment composition. The basic parts of Ulvaceae are usually thicker with reduced pigment contents and lower photosynthetic rates; however, based on chlorophyll *a*, photosynthesis is comparable to other thallus areas (Han et al. 2003). Raven et al. (1979) confirmed the hypothesis that 'giant' celled algae behave like shade plants and small-celled algae prefer either sunny or shaded habitats. It is mentioned that large cells facilitate transports over long distances by lowering the numbers of barriers through cell walls. Contrarily, a canopy-forming small-celled *Laminaria* shows a 'phloem-type translocation'. Interestingly, specimens of this genus showed lower photosynthetic rates in growing regions than in mature parts (Raven et al. 1979).

Researches are scarce to prove the relation between morphology and photosynthetic performances in macroalgae. In this study, we concentrated on morphological traits of *Padina pavonica* including thallus thickness and cell volume in dependence of irradiance supply and season. Additionally, we considered the thallus area and growth rate which might have an influence on outcompeting other taxa.

4.3 Material and Methods

Study site

Field studies were conducted in September 2007 and in April/May 2008 in the Mediterranean Sea at Stareso (Station de Recherches Sous-marines et Oceanographiques) located in the Bay of Calvi (42°35'63" N, 8°44'62" E), Corsica. The region is dominated by a rocky coastline which extends to the sandy water bottom covered with seagrass patches (*Posidonia oceanica*). The sun-exposed hard substrate and the protection against high wave exposure in the east seem to facilitate high abundances of *Padina pavonica*. In the end of September, more frequent wave disturbances caused by stormy weather lead to a massive loss of *Padina* specimens in shallow waters.

Field and laboratory measurements

Five thalli per day were randomly collected in April/May (n = 85) between 1 and 20 meters depth by scuba diving and more than twice the number in September (n = 200) between 1 and 30 meters depth. Due to morphometric measurements, we had to collect thalli with a minimum surface area of about 5 cm^2. Underwater irradiance was measured with waterproof temperature/light data loggers (Onset: Hobo UA-002_08; software program: Hoboware Pro) at specific depths; one more data logger (Skye Data Hog2 equipped with a PhAR sensor; software program: SkyeLynx) was fixed to a stationary position on the shore. We classified *in situ* irradiances into three light classes (Haberleitner & Schagerl 2010): light class 1 (LC1) ranged from > 0 <12 % incoming irradiance, light class 2 (LC2) from ≤ 12 to 20 % and light class 3 (LC3) ≥ 20 %. For a seasonal comparison, light classes were grouped together for April/May (LCA) and September (LCS). In the laboratory, each thallus was photographed with a digital camera (Nikon Coolpix 4500) and with the aid of the software program “Image J” the respective thallus area was estimated. Part of the thallus was conserved in 4 % formaldehyde for morphological analysis in Vienna. Each thallus was treated with 1 M HCl to dissolve the calcareous layer and cut transversely and longitudinally (about 70µm thin) within the first two centimetres of the margin with a sledge microtome. The thin sections were photographed under a compound microscope (Polyvar, Weitfeld Photomikroskop, Reichert-Jung) and cell length (l), cell width (w) and cell height (h), shown in Fig 3A & C, were measured with the software program cell^F (analySIS;

Olympus soft imaging solutions). With the respective measurements, cell volumes for each cell layer (VL) were calculated. Additionally, we averaged VL to cell volume per thallus (VT) for a comparison with thallus thickness.

Growth rates were determined at 5 m and 14 m depth *in situ* in spring (port: 5 m, n = 13; shoreline: 5 m, n = 20; 14 m, n = 15). Each thallus was marked with two holes at a defined distance from the thallus margin. After two weeks, thalli were collected and the distance between the holes and the margin was remeasured.

Statistical analyses were performed with SPSS 14.0 and figures were made with SigmaPlot 11. Depending on normal distribution (Kolmogorov-Smirnov-test) and homogeneity of variances (Levene-test), group comparisons were conducted either parametrically, applying Student tests (t-test) or analysis of variances (ANOVA) including a post hoc test after Scheffé, or non-parametrically with Kruskal-Wallis-tests. Linear regression analysis was calculated for correlation between cell volume (VT) and thallus thickness.

4.4 Results

Padina pavonica appeared first in April in shallow areas of the port and on the shore, and reached a remarkable abundance within one month, with various thallus sizes indicating a different start of growth.

An increase of thallus thickness, ranging from 87.2 to 148.4 µm in April/May and from 55.9 to 123.6 µm in September, could be observed in both seasons with elevating light supply (Fig 1). Applying a two-way ANOVA, significant differences were found between LCs ($p = 0.020$) and seasons ($p = 0.001$) but no interactions between LC x season could be detected ($p = 0.765$). Averaged VT was higher in April/May than in September but remained constant within LCA and LCS (ANOVA: LCA: $p = 0.099$, LCS: $p = 0.134$; Table 1). Averaged cell length and width remained constant within LCs in both seasons, however, averaged cell height showed a noticeable decrease in LCA1 compared to LCA 2 and 3 (Table 1). Thallus thickness did not increase the number of cell layers but showed a high correlation with VT (Fig 2).

Thalli mainly consisted of three cell layers in the apical region with the 'inrolled' layer located at the upper thallus surface (US), the middle layer (ML) and the 'not inrolled' layer at the lower surface (LS). In April/May cells from ML were clearly larger than those from US and LS (Fig 3A); LS cells represented the smallest cells in both seasons (data not shown). Taking a closer look, VL varied significantly within LCA and LCS with the exception of LS cells (Table 2). US and ML cells from LCA1 were significantly smaller than cells from LCA 2 or 3, whereas cells from US and LS were significantly larger in LCS2 and ML cells were significantly larger in both LCS 2 and 3, compared to LCS1 (Table 2). On a macroscopic scale, thallus areas showed significantly smaller thalli in April/May compared to those in September (ANOVA: $p = 0.004$; Table 1). No significant differences of thallus areas could be detected between LCA 2 and 3 specimens collected from the shore and port (two-way ANOVA; $p = 0.079$; Fig 4).

Growth rates were calculated from thalli in spring. Distances between the holes remained constant; growth took place only at the margins. Maximum growth rates were obtained in the port with about 0.67 mm day^{-1}, however no significant differences could be calculated between thalli collected from different locations (ANOVA: $p = 0.562$, Table 3).

4.5 Discussion

In this study, we investigated morphological traits of *Padina* at irradiance conditions, which are comparable in spring and autumn. As optical properties of *Padina* change with seasons (Bürger & Schagerl 2010; Haberleitner & Schagerl 2010), morphological characteristics and components like pigments could play a decisive role. *Padina pavonica* consists of multilayered thalli which are known to be flattened and translucent to facilitate light penetration through the thallus (Hay 1986). The calcium carbonate layer at the thallus surface increases the reflection rate to prevent damage of the photosynthetic apparatus (Bürger & Schagerl 2010). In contrast to other authors using thallus thickness as an interspecific macroalgal parameter (Littler & Arnold 1982, Agustí 1994, Enríquez et al. 1994 & 1995), we used this parameter to show adequate acclimation towards specimens from areas experiencing different irradiances. In general, algae with thin thalli seem to have an advantage in growth, reproduction and photosynthesis (King & Schramm 1976, Littler & Arnold 1982, Lüning & Dring 1985, Enríquez et al. 1995). However, thick algae with low productivity rates consist of a robust thallus matrix yielding a positive effect on resistance against physical stress and desiccation (Littler & Littler 1980). In our study, thalli were considerably thicker at the beginning of the growing season than in September (Fig 1) and thin thalli are located in deeper waters with low light supply. Interestingly, absorption neither did significantly change with season nor with decreasing irradiance (Bürger & Schagerl 2010), which might be explained by the alignment of chloroplasts, which is parallel to the thallus surface in algae from low light areas and vertical in algae from high light areas (Hanelt et al. 2003). These photo-oriented movements allow chloroplasts to avoid self-shading, when light is limited and to protect cell damage at excessive light exposure (Hanelt et al 2003). Low absorption properties were obtained from thick LCA1 thalli which were caused by decreased pigment contents (Bürger & Schagerl 2010, Haberleitner & Schagerl 2010). Accordingly, other studies described a decline in light absorption per unit biomass with increasing tissue thickness in regard to chlorophyll *a* concentration, which is called the package effect (Raven 1984, Agustí 1991, Agustí et al. 1994, Enríquez et al. 1994, Finkel and Irwin 2000). As the thallus thickness is higher at the beginning of the growing season (Table 1) and light harvesting pigments per unit area are low (Haberleitner & Schagerl 2010), an increase of the pigments over the seasons

probably compensates the changes of morphology resulting in comparable light absorption properties in spring and autumn.

Increased thallus thickness in spring is caused by larger cells containing more water (Bürger & Schagerl 2010). The development of young thalli is characterised by cell elongation and broadening of the thallus as a consequence of longitudinal divisions of apical cells. Cells from the LS in the apical region elongate slower than cells from the other two layers resulting in an enrolled margin of the thallus (Fritsch 1945). Increased VT in spring did not show enhanced absorption properties, but increased transmission rates due to lower pigment contents (Haberleitner & Schagerl 2010). Additionally, changing irradiance supply did not alter VT (Table 1). This fits well with the statement of Raven & Kübler (2002) that absorption in large cells is reduced due to the package effect which is also strongly linked to light harvesting pigment concentrations (Finkel & Irwin 2000).

Regardless of thallus size, *P. pavonica* consists of three cell layers close to the marginal zone (Fig 3 A, B, C). In April/May cells of the LS and US cell layers appear in a similar size and shape (Fig 3 A) making it difficult to distinguish the layers. In September, the LS layer comprises apparently smaller cells surrounded by thicker cell walls (Table 2, Fig 3 B & C). The US cells evolving from dividing ML cells are separated by a thin cell wall from each other. The margin preferentially rolls up to the irradiance exposed thallus surface (Bitter 1899). As mentioned before, thalli are considerably thinner at low irradiances, but VT did not show significant differences of all LCs due to high variances of VL (Table 1). Cell width is similar in all LCs (Table 1), which can be explained by rapid longitudinal cell divisions at the very beginning of the margin (Fritsch 1945). Contrarily, cell height and length are more variable leading to significant lower cell heights and by trend higher lengths in LC1 compared to LC2 and LC3 in both seasons (Table 1).

Depending on the season, cell length highly differs between cell layers. In general, cells of the ML are considerably larger (Table 2, Fig 3 A) than US or LS cells indicating a cell division zone. Sometimes, a fourth cell layer may develop from the ML cells (Fritsch 1945). The smallest cells belong to the ‘not inrolled’ cell layer from LS (Table 2, Fig 3 C) where irradiance is low. The microscopic observations showed decreased chloroplast numbers or even no plastids in cells of the ML (Fig 3 A, B, C) suggesting that this layer is photosynthetically inactive. Interestingly, VL differs considerably

between the three cell layers (Table 2, Fig 3), which indicates a correlation between cell volume and irradiance on a microscopic scale. According to the high irradiance exposure, cells are larger on the upper thallus surface than on the shaded LS (Table 2). VL from US and ML cells increases significantly between LCA1 and LCA 2 and 3 (Table 2) and cells from US and LS show considerably higher VL in LCS2 than in other LCS.

Besides irradiance, growth is influenced by temperature, nutrient availability and density patterns (Creed et al. 1997). The protected port areas experience higher temperature and enriched nutrient supply resulted in visibly lower abundance of *Padina* - probably *Padina* was outcompeted by fast-growing filamentous algae. However, *P. pavonica* specimens from different locations were comparable in size in the nutrient-rich port compared to the wave exposed coastline (Fig 4). This indicates that besides irradiance and nutrient availability, other factors come into play for growth and dispersal (Hay 1986, Creed et al. 1997). In areas with high irradiances thalli might be size-regulated by higher population densities whereas single specimens in areas with low irradiance are able to grow larger ('intraspecific competition' hypothesis after Creed et al. 1997).

P. pavonica starts to grow in spring which eventually results in increased biomass and coverage during summer and autumn (Einav et al. 1995, Sala & Boudouresque 1997, Airoldi 2000, Piazzi et al. 2002). September thalli were significantly larger in our study (Table 1) indicating enhanced growth during summer when the water temperature rises. We assume that reduced wave action in deeper areas might also be responsible for the persistence of larger thalli. Before *Padina* gets mechanically disrupted from harsh winter surf, it starts reproducing during late summer and early autumn (Airoldi 2000, Wolcott 2007). In the Bay of Calvi, smaller thalli of *P. pavonica* grew more frequently in shallow waters where wave and light exposure are strong (Bürger et al. 2006). Large individuals can be found scattered down to 30 meter depths. Different observations were made from other studies which described *P. pavonica* either as less abundant (Airoldi 2000; Balata & Piazzi 2008) which could be explained by environmental impact, predatory or space competition, or as frequently found subtidal algae with a wide vertical distribution (Einav et al. 1995). Previous studies showed that a tropical relative, *P. sanctae-crucis*, grows much faster (0.81 mm per day) in 1.5 m depth (Wefer 1980). Higher, however, not significant growth rates are examined in 5 m depth in the

port area (Table 3) with similar irradiance exposure, less wave exposure and higher nutrient supply contrary to the shore. Unexpectedly, growth rate was lowest in 5 m depth at the sun exposed rocky coast contributing to the hypothesis that these thalli invest more energy on reproduction than on growth as it is already known for Ulvaceae (Han et al. 2003). Due to comparable growth rates and thallus areas in markedly different irradiance and nutrient exposed locations (port versus shore), other biotic and abiotic factors have to be considered affecting *Padina pavonica* which raises interesting topics for further studies with e.g. laboratory aquarium experiments to confine these factors.

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4.6 References

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4.7 Table and Figure Legends

Table 1 Thallus and cell measurements. Thallus area (cm^2), cell measurements and calculated cell volume (μm^3) are listed for three light classes (LC) in April/May and September. Averages (± standard deviation SD) or medians (± interquartile range; marked with *) were calculated from all individuals investigated. Significant differences between light classes (p LCA and p LCS) or seasons (p A/S) are expressed with a 5 % probability. The bold numbers were tested with ANOVA between light classes and with t-test between seasons. Post hoc tests are conducted after Scheffé and same letters within a parameter label same sub groups.

Table 2 Averaged cell volumes (VL) of upper surface (US), middle (M) and lower surface (SL) cell layer are shown within LCA and LCS. Significant differences between light classes (p LCA and p LCS) were tested with one-way ANOVA (bold).

Table 3 Mean values with standard deviation (± SD) of growth rates (mm d^{-1}) in April/May are shown for specimens of *Padina pavonica* in shallow and deeper areas on shoreline and in shallow water of the port area. Minimum and maximum temperatures (°C) of the adequate locations are also listed. Significances are expressed with a 5 % probability (p).

Fig 1 Thallus thickness (µm) is shown from LC1 to LC3 in two seasons. Significances were tested between LCs within April/May (ANOVA: p = 0.000) with small letters representing the sub groups of the post-hoc-test after Scheffé and within September (ANOVA: p = 0.000) with large letters. Number of samples is present on the top of each box.

Fig 2 Linear regression analysis showing the high and significant correlation ($y = 0.018x + 9.369$; $r^2 = 0.656$; $p = 0.000$) between cell volume (VT) and thallus thickness in *P. pavonica* (n = 282).

Fig 3 Thin sections of *Padina pavonica* thalli (bars = 50 µm) consisting of three cell layers: ‘not inrolled’ on the lower surface (LS), middle, and ‘inrolled’ on the upper

surface (US). Double arrows give example measurements of cell height (h), width (w) and length (l). (A) Transverse sections from spring. Cells of the middle cell layer are visibly larger containing no or less pigments. The superficial ‘not inrolled’ and ‘inrolled’ cells are difficult to distinguish but show visibly pigment contents. (B) Transverse section from thalli in autumn. Pigments are located at the margin of the ‘not inrolled’ cells on LS. Cells on US are dividing into ‘inrolled’ and middle cells. (C) Longitudinal section from September thalli with pigments located in the superficial cells.

Fig 4 Thallus area [cm^2] of *P. pavonica* (ln transformed) in two light classes (LC2 and LC3) between specimens on shore and in the port area.

Table 1 Thallus and cell measurements. Thallus area (cm^2), cell measurements and calculated cell volume (μm^3) are listed for three light classes (LC) in April/May and September. Averages (± standard deviation SD) were calculated from all individuals investigated. Significant differences between light classes (p LCA and p LCS) or seasons (p A/S) are expressed with a 5 % probability. The bold numbers were tested with ANOVA between light classes and with t-test between seasons. Post hoc tests are conducted after Scheffé and same letters within a parameter label same sub groups.

		April/May			**September**			
	LC	mean ± SD	n	p LCA	mean ± SD	n	p LCS	p (A/S)
ln thallus area	total	2.59 ± 0.43	154		2.74 ± 0.50	193		**0.004**
ln cell volume per thallus	1	11.35 ± 0.20	22		10.86 ± 0.39	65		
	2	11.43 ± 0.20	33	**0.099**	10.98 ± 0.36	67	**0.134**	
	3	11.47 ± 0.20	30		10.86 ± 0.39	65		
cell length per thallus	1	97.50 ± 8.61	22		84.11 ± 13.58	65		
	2	92.97 ± 11.05	33	**0.142**	83.67 ± 13.27	67	**0.652**	
	3	92.45 ± 8.82	30		82.04 ± 13.69	65		
cell height per thallus	1	31.26 ± 3.67[a]	22		23.75 ± 3.78[a]	65		
	2	35.54 ± 5.04[b]	33	**0.001**	25.84 ± 3.56[b]	67	**0.007**	
	3	36.11 ± 4.80[b]	30		25.05 ± 3.93[a,b]	65		
cell width per thallus	1	28.16 ± 2.72	22		27.01 ± 4.05	65		
	2	28.37 ± 2.12	33	**0.324**	27.89 ± 3.86	67	**0.580**	
	3	29.13 ± 2.71	30		26.28 ± 3.64	65		

Table 2 Averaged cell volumes (VL) of upper surface (US), middle (ML) and lower surface (SL) cell layer are shown within LCA and LCS. Significant differences between light classes (p LCA and p LCS) were tested with a one-way ANOVA (bold).

ln VL		**April/May**			**September**		
cell layer	LC	mean ± SD	n	p LCA	mean ± SD	n	p LCS
US	1	11.29 ± 0.35[a]	220		10.86 ± 0.47[a]	496	
	2	11.35 ± 0.37[a,b]	330	**0.009**	10.95 ± 0.42[b]	445	**0.001**
	3	11.39 ± 0.35[b]	300		10.83 ± 0.49[a]	362	
ML	1	11.48 ± 0.42[a]	220		10.88 ± 0.50[a]	492	
	2	11.64 ± 0.40[b]	330	**0.000**	11.05 ± 0.46[b]	445	**0.000**
	3	11.71 ± 0.40[b]	300		10.97 ± 0.54[b]	362	
LS	1	11.03 ± 0.35	220		10.55 ± 0.51[a]	502	
	2	11.04 ± 0.34	330	**0.782**	10.64 ± 0.45[b]	443	**0.006**
	3	11.05 ± 0.33	300		10.55 ± 0.46[a]	362	

Table 3 Mean values with standard deviation (± SD) of growth rates (mm d^{-1}) in April/May are shown for specimens of *Padina pavonica* in shallow and deeper areas on shoreline and in shallow water of the port area. Minimum and maximum temperatures (°C) of the adequate locations are also listed. Significances are expressed with a 5 % probability (p).

depth [m]	growth rate [mm d^{-1}]	region	Temperature Min [°C]	Temperature Max [°C]	n	p
5	0.47 ± 0.13	port	15.95	19.09	13	
5	0.42 ± 0.16	shore	15.95	18.71	20	0.562
14	0.46 ± 0.16	shore	14.80	16.52	15	
mean	0.45 ± 0.15				48	

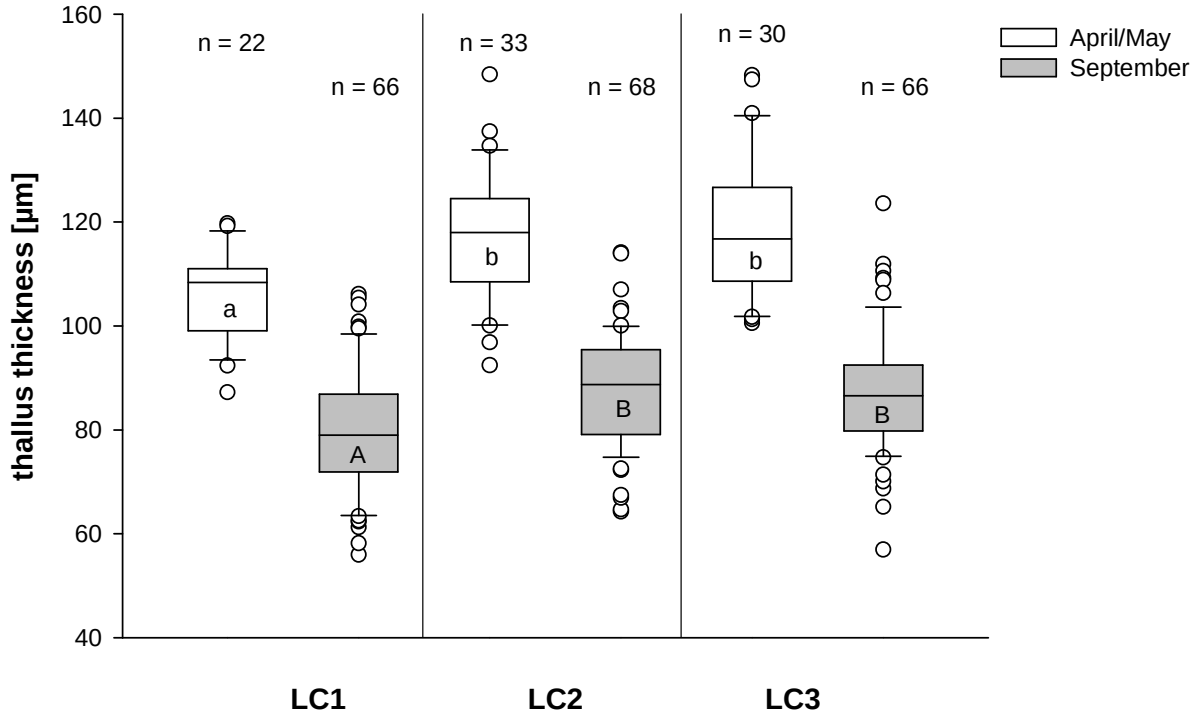

Fig 1 Thallus thickness (µm) is shown from LC1 to LC3 in two seasons. Significances were tested between LCs within April/May (ANOVA: p = 0.000) with small letters representing the sub groups of the post-hoc-test after Scheffé and within September (ANOVA: p = 0.000) with large letters. Number of samples is present on the top of each box.

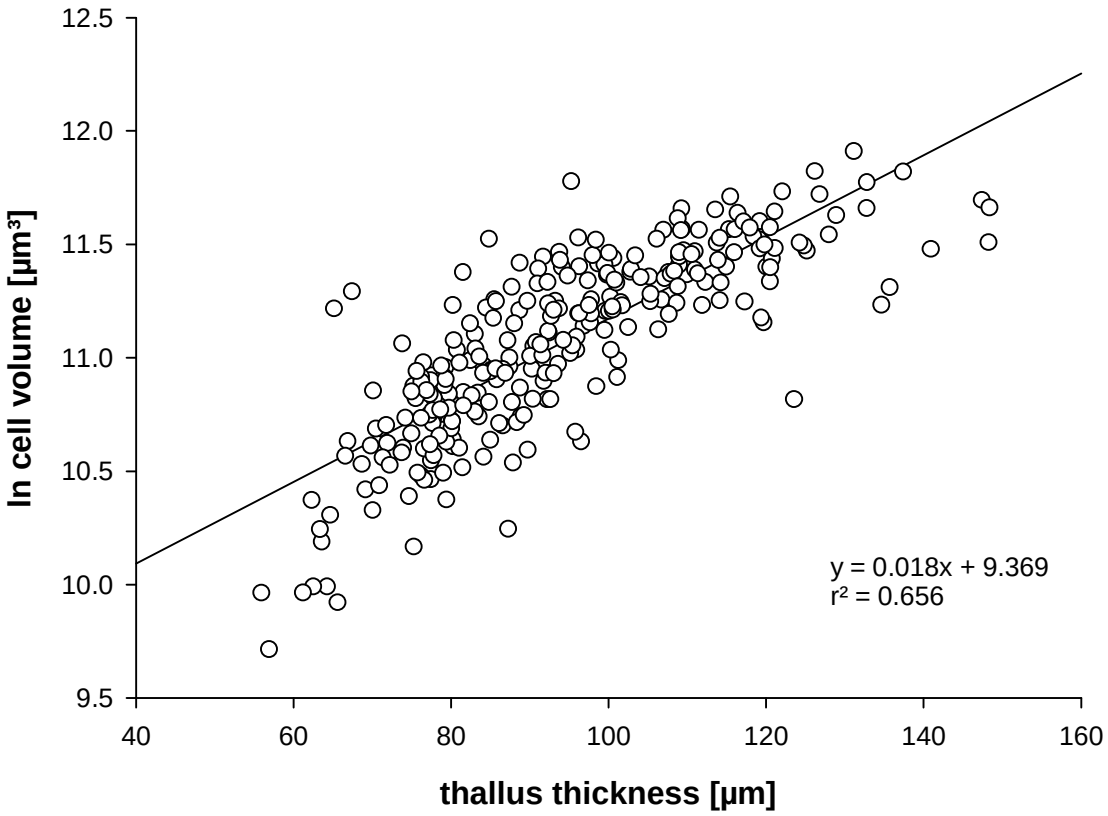

Fig 2 Linear regression analysis showing the high and significant correlation (y = 0.018x + 9.369; r^2 = 0.656; p = 0.000) between cell volume (VT) and thallus thickness in *P. pavonica* (n = 282).

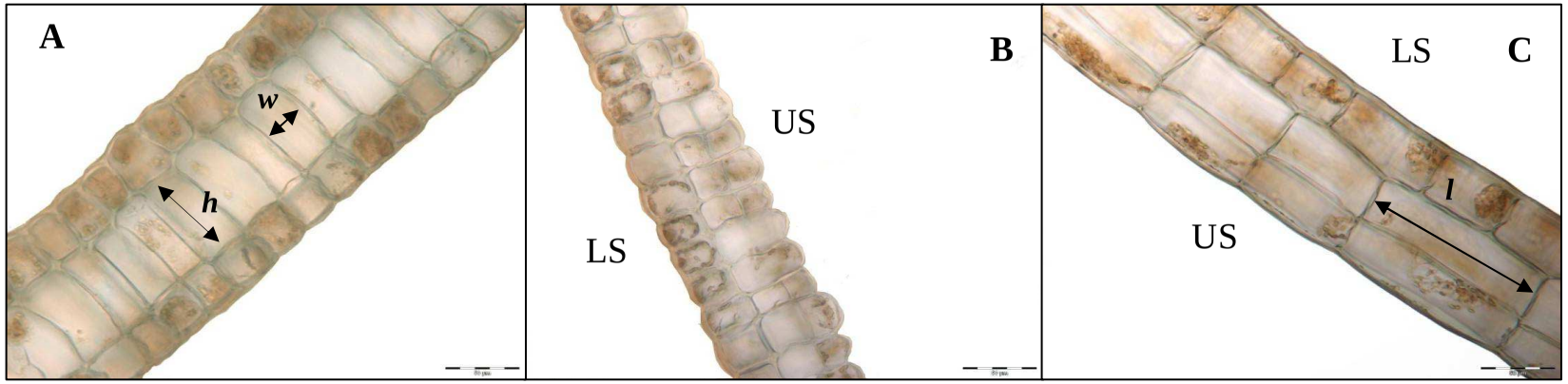

Fig 3 Thin sections of *Padina pavonica* thalli (bars = 50 µm) consisting of three cell layers: ‘not inrolled’ on the lower surface (LS), middle and ‘inrolled’ on the upper surface (US). Double arrows give example measurements of cell height (h), width (w) and length (l). (A) Transverse sections from spring. Cells of the middle cell layer are visibly larger containing no or less pigments. The superficial ‘not inrolled’ and ‘inrolled’ cells are difficult to distinguish but show visibly pigment contents. (B) Transverse section from thalli in autumn. Pigments are located at the margin of the ‘not inrolled’ cells on LS. Cells on US are dividing into ‘inrolled’ and middle cells. (C) Longitudinal section from September thalli with pigments located in the superficial cells.

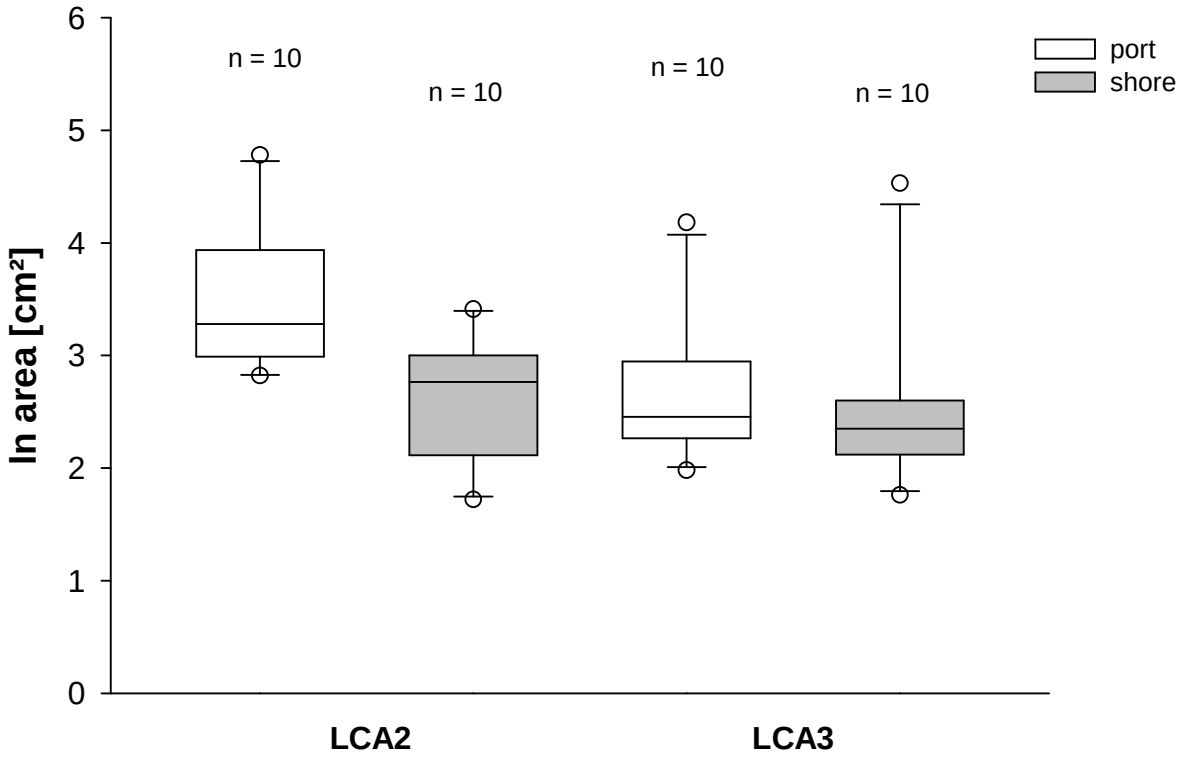

Fig 4 Thallus area [cm^2] of *P. pavonica* (ln transformed) in two light classes (LC2 and LC3) between specimens on shore and in the port area.

5 Zusammenfassung

Padina pavonica kommt vorwiegend an steinigen Küsten in der gemäßigten Zone vor und ist durch einen fächerförmigen Thallus mit konzentrisch weißen Kalkbändern aus Calcit ($CaCO_3$) auf der Thallusoberfläche leicht identifizierbar. Die Kalkausscheidung macht diese Gattung innerhalb der Klasse der Braunalgen (Phaeophyceae) zu einer Seltenheit. Aus diesem Grund und aufgrund ihres häufigen und weit verbreiteten Auftretens stellt *P. pavonica* eine optimale Alge zur Erforschung der Kalzifikation und ihrer optischen Eigenschaften dar. Im Herbst 2007 und im Frühjahr 2008 wurden Individuen aus unterschiedlichen Tiefen in der Bucht von Calvi (Korsika, Frankreich) gesammelt und in 3 Lichtklassen eingeteilt. Sowohl die Kalkschicht pro Trockengewicht und pro Thallusfläche als auch der Wassergehalt des Thallus zeigen deutliche saisonale Schwankungen. Innerhalb der jeweiligen Saison konnten in Bezug auf die Lichtklassen lediglich im Herbst Unterschiede aufgezeigt werden. Während die Absorption sich nicht wesentlich verändert, weder zwischen den Jahreszeiten noch zwischen den unterschiedlichen Lichtverhältnissen, nimmt die Reflexion im Herbst deutlich zu. Die Transmission verringert sich im Herbst sowie mit zunehmender Lichteinstrahlung. Dies bestätigt die positive Beziehung zwischen Kalk und Reflexion und lässt die Interpretation zu, dass Kalk eine Art Schutzschicht gegen übermäßige Strahlung darstellt. Vergleiche von standardisierten Reflexionsspektren zwischen verkalkten und entkalkten Thalli resultierten in einer weniger ausgeprägten Spektrenkurve in den verkalkten Thalli. Außerdem deutet eine schwach negative Tendenz zwischen Absorption und Kalkgehalt auf den Zusammenhang zwischen Photosynthese und Kalzifikation hin.

Im zweiten Teil der Arbeit wurden die morphologischen Unterschiede in Abhängigkeit vom vorhandenen Licht in makro- und mikroskopischer Hinsicht untersucht. Dazu wurden sowohl die Thallusdicke, das Zellvolumen pro Thallus als auch Zellvolumina der Zellschichten zwischen den Jahreszeiten und den Lichtbedingungen verglichen. Jeder Thallus besteht am Rand hauptsächlich aus drei Zellschichten. Im Frühjahr wurden verglichen mit September kleinere und dickere Thalli mit größeren Zellen gemessen. Zusätzlich zeigten Thalli aus seichtem Wasser eine deutlich höhere Thallusdicke als jene aus tieferen Gebieten, obwohl sich das Zellvolumen pro Thallus nicht veränderte. Bei näherer Betrachtung unterschieden sich jedoch die Zellvolumina

pro Zellschicht mit ansteigendem Licht signifikant voneinander. Dies ist einerseits auf eine Zellteilungsphase in den größeren Zellen der mittleren Zellschicht zurückzuführen, als auch auf eine Zellvergrößerung durch erhöhte Bestrahlung in der oberen Zellschichte. Die kleinsten Zellen befinden sich auf der lichtabgewandten Seite, wobei der Unterschied relativ gering ist.

Bei einem weiteren Experiment im Frühjahr wurden Hafenexemplare mit Küstenexemplaren aus unterschiedlichen Tiefen verglichen. Trotz unterschiedlicher Lichtverhältnisse und der Annahme, dass der Nährstoffgehalt zwischen Hafen- und Küstenbereich variiert, veränderten sich die Wachstumsrate und die Thallusfläche nicht signifikant. Da das Licht als abhängiger Faktor ausgeschlossen werden kann, sind weitere Studien notwendig, um die abiotischen und biotischen Faktoren zu bestimmen, die ein gleichmäßiges Wachstum in seichten als auch in tiefen Bereichen begünstigen.

6 Lebenslauf

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Berufserfahrung

2001	Aushilfe im Tierpark Rosegg, Kärnten
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2008 – 2010	Aushilfe in der Wienbibliothek im Rathaus, Wien

2010 Aushilfe in der Frauenabteilung der Stadt Wien (MA57)

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2003 Meeresbiologische Exkursion, Malediven, Indopazifik

2004 Volontariat, Arbeit mit Fledermäusen, Barro Colorado Island, Panama

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http://www.reefcourse.com/d_report2005.php

2006 Meeresbiologischer Kurs am Alfred-Wegener Institut (AWI), Biologische Anstalt Helgoland, Deutschland

2007 Meeresbiologische Exkursion, Bodega Bay, Bodega Marine Laboratories, Kalifornien

schriftliche Arbeit: Bürger K., Hannak J., Kompatscher S., Oberlechner M., Ühlein M (2007) Distribution of Californian crabs on the rocky shore and high intertidal rocky fringes (HIRF) of Bodega Bay. Department of Marine Biology, University of Vienna, unpublished

Weitere Qualifikationen

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