universität wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„Photosynthetic characteristics of the macroalga *Padina pavonica* along a light gradient“

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2019/ Vienna, 2019

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 190 482 445
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Lehramtsstudium UF Bewegung und Sport UF Biologie und Umweltkunde
Betreut von / Supervisor:	Ao. Univ.-Prof. Mag. Dr. Michael Schagerl

Abstract

It has long been acknowledged that photosynthetic characteristics of macroalgae change in response to the varying light environment. High irradiance causes raised electron transport rates and increased amounts of photoprotective pigments in algae. On the contrary, under low light conditions, light-harvesting efficiency is higher. These adaptations provide optimized conditions for photosynthesis in the respective habitat. We focused on photosynthetic properties of *Padina pavonica*, which belongs to the extremely rare group of calcifying brown algae. The taxon is very common on rocky shores of the Mediterranean Sea and also in the tropics, which implies good acclimation strategies. The study was conducted along the rocky shores of Rovinj (Croatia) in the early summer of 2018. Photosynthetic measurements were carried out along vertical transects by pulse amplified modulated fluorescence and showed enhanced photosynthetic capacity (relative electron transport rates (ETR), absolute ETR, ETR_{max}, I_k) in high light specimens. Interestingly, pigment contents per thallus area and organic weight were also increased in high light *Padina* indicating package effect. The initial slope (α), and maximum dark fluorescence yield (F_v/F_m), however, did not show a specific trend along the light gradient.

Keywords: photosynthesis, *Padina pavonica*, acclimation, photoinhibition, light, environmental conditions

Acknowledgement

First of all, I want to thank my advisor Ao. Univ-Prof. Mag. Dr. Michael Schagerl from the University of Vienna for realizing this project and supporting me throughout the process of researching and writing this thesis.

I would also like to thank the staff of the marine biological institute Ruđer Bošković, who greatly contributed to the project by providing lab facilities and their good coorperation.

Finally, I would like to express my profound gratitude to my colleagues, especially Daniel Harder, for the pleasant collaboration.

Table of contents

1 Introduction

Sessile organisms are exposed to the environment with no chance of escaping unfavorable conditions. One of the challenges, which sessile algae are facing, are a highly variable light climate especially near the water surface (Graham, Graham, & Wilcox, 2008; Hanelt, Wiencke, & Bischof, 2003). Macroalgae may react to these conditions through their specific life history (Garcia-Sanchez et al., 2014; Menendez & Comin, 1989; Menendez, Hernandez, & Comin, 2002). The underwater light climate is changed by absorption and scattering of dissolved substances and particles. Moreover, pure water itself causes shifts in spectral properties by absorbing light significantly in the red region of the spectrum (Kirk, 2011). The decrease of light intensity along the water column is referred to as attenuation and is often used for characterization of biological significant zones. Moreover, exposition and morphology of coastal areas are responsible for local irradiance conditions. Shaded areas appear in shallow water layers on inclined substrates, in caves or canopy dominated sites (Ott, 1996).

Especially in the intertidal zone, sessile organisms are exposed to highly varying irradiance, temperature, and salinity (Schagerl & Möstl, 2011). The changing conditions are very pronounced in shallow areas, so macroalgae of the upper littoral have to cope with much more varying day-night fluctuations than specimens growing in deeper areas. These adaptations are reflected in the photosynthetic characteristics and include enhanced light absorption properties in shaded areas and photoprotective mechanisms in high light environments (Hanelt et al., 2003). A major problem of algae exposed to excess light is the production of reactive singlet oxygen, which is formed when the excited chlorophyll is transferred to a stable triple state because of electron congestion of subsequent enzymatic reactions (Pospisil, 2012). Carotenoids are able to prevent damage by quenching the triplet states of chlorophyll (Frank & Cogdell, 1996). Another way to cope with excess light is reducing the photosynthetic capacity due to photoinhibition (Franklin, Osmond, & Larkum, 2003; Larkum, 2003). Photoinhibition is induced via excitation pressure, which builds up in photosystem II (PSII) caused by increased photon absorption delivery (Larkum, 2003). Mechanisms to protect PSII from photodamage include heat dissipation, also referred to as non-photochemical quenching (NPQ) via the xanthophyll cycle (Harker et al., 2010; Young & Frank, 1996) and additional photoprotective pigments such as ß-carotene (Franklin & Forster, 1997; Graham et al., 2008). Xanthophylls involved in the xanthophyll cycle of Phaeophyceae are violaxanthin, antheraxanthin and zeaxanthin (Harker et al., 2010), the latter being responsible for heat dissipation of excess energy

(Demmig, Winter, Krüger, & Czygan, 1988). The reversible conversion from the light-harvesting pigment violaxanthin (formed during dark periods) to zeaxanthin (high light) only takes a few minutes (Demmig-Adams & Adams, 1996). Thermal excitation energy dissipation through the xanthophyll cycle is considered to be the main photoprotection mechanism (Gilmore, Itoh, & Govindjee, 2000).
Furthermore, excess light exposure can promote rearrangement of chloroplasts in the so-called high intensity arrangement leading to increased self-shading of chloroplasts and transmission of light through the thallus (Hanelt et al., 2003). Chloroplast movement in response to light exposure is very common in marine brown algae (Nultsch & Pfau, 1979).

Absorption of ultraviolet (UV)-B light (290-320nm) has damaging effects on organisms most notably involving DNS and proteins, if protective mechanisms are lacking (Lüning, 1985). In many coastal regions, the water column itself provides UV-protection to subtidal benthic macroalgae (Franklin & Forster, 1997). Additionally, exposed algae synthesize aromatic amino acids, beta-carotene and phenols as UV-sunscreen (Abdala-Diaz, Cabello-Pasini, Perez-Rodriguez, Conde Alvarez, & Figueroa, 2006; Franklin & Forster, 1997; Graham et al., 2008).

Whereas photoacclimation of macroalgae exposed to high irradiance is reflected in the ability to minimize the formation of singlet oxygen by an increase of protective photopigment concentration and in chloroplast rearrangement, low light adapted algae optimize their photosynthetic capacities by low onsets of saturation and high photosynthetic efficiencies (Ott, 1996). To ensure optimal adaptation to chronic low light availability, low light macroalgae have slower growing and respiration rates to store greater amounts of carbon. Another characteristic adaptation to low light is increased pigment content (Lüning, 1985). However, according to the cost-benefit ratio of increasing pigment content relative to light availability, the opposite could also be plausible (Raven, 1984). This is explained by the fact, that the effectiveness of light collection is lessened by packaging of pigments within chloroplasts also referred to as the package effect (Kirk, 2011).

Previously, photosynthetic measurements were performed under laboratory conditions, which implied transfer of organisms from their habitats into an artificial environment. Nowadays, chlorophyll fluorescence is commonly used to determine photosynthetic properties *in situ*. (Häder et al., 1996; Schreiber & Bilger, 1993; Schreiber, 2004). This method is very practical, because reliable measurements of samples can be conducted

rapidly and are basically noninvasive (Beer & Axelsson, 2004; Maxwell & Johnson, 2000). The theory behind the highly sophisticated fluorescence technique is based on temporary changes of chlorophyll fluorescence of intact PSII. Absorbed photons are either utilized for photosynthesis, chlorophyll fluorescence and heat dissipation (Maxwell & Johnson, 2000; Reece et al., 2009). If photons are absorbed in excess, energy is either dissipated as fluorescence or thermal radiation during photoinhibition (Graham et al., 2008), because PSII electron acceptors are saturated (Hanelt et al., 2003). For light acclimation measurements, the Pulse-Amplitude-Modulation (PAM) fluorescence techniques measures first actual fluorescence (F_t) followed by a saturation flash causing maximum fluorescence ($F_{m'}$) of illuminated sample. $F_{m'}$- $F_t/F_{m'}$ is a proxy for photochemical quenching (Genty, Briantais, & Baker, 1989). This noninvasive method enables repeated measurements under natural light conditions thus providing essential information of the actual state of photosynthetic processes (Beer & Axelsson, 2004; Ralph & Gademann, 2005).
Light-thriving algae including *P. pavonica* are dominating the benthic community of the well-lit upper infralittoral, forming large populations on rocks. Photophilous algae may also reach depths up to 30 to 50 meters, if the water is clear (Simboura et al., 2018). *P. pavonica* belonging to the family Dictyotaceae is a perennial species (Carter, 1927). Other species of Dictyotaceae are widely distributed in warmer seas and quite common in the Mediterranean Sea (Oltmanns, 1922; Silberfeld et al., 2013). Characteristic features of *P. pavonica* are stalked fan-shaped fronds, which are often inrolled at the apical margin (Benita, Dubinsky, & Iluz, 2018; Carter, 1927; Fritsch, 1945; Geraldino, Liao, & Boo, 2005). The stalks are connected to the rhizome, which possess tufts of rhizoids attached to the substrate. The thalli develop from cylindrical shoots with an apical cell, which undergoes longitudinal division. The cells at the front edge then become meristematic, which leads to further cell divisions and finally to the formation of the inrolled fan shape (Fritsch, 1945; Oltmanns, 1922). The fronds are usually visible from spring to summer (Airoldi, 2000), whereas during unfavorable seasons only rhizoids, germlings or filamentous thalli are visible (Uddin et al., 2015). Both surfaces of the thallus have rows of hairs, which form concentric zones. The hairs are more notable on the upper side facing the incoming light and are strongly developed when exposed to increased irradiance (Oltmanns, 1922). Like the majority of brown algae, *Padina* is an isomorph-haplodiplont (Carter, 1927; Mable & Otto, 1998). The reproductive organs, both sexual and asexual, are situated on either side of the hair lines mostly on the dorsal surface of the fan. The sexual reproductive organs, Oogonia and Antheridia, mostly develop on separate individuals,

although monoecious specimens may also occur (Fritsch, 1945; Gómez Garreta, Rull Lluch, Barceló Martí, & Ribera Siguan, 2007). Tetrasporophytes seem to be more abundant than gametophytes (Carter, 1927).

With exception of *Padina* (Okazaki, Pentecost, Tanaka, & Miyata, 1986) and *Newhousia imbricata* discovered in Hawaii (Kraft, Saunders, Abbott, & Haroun, 2004), brown algae are not known to be calcifying. Calcification of brown algae occurs extracellular and aragonite is often deposited as concentric bands on thalli surface, like in *Padina* (Lüning, 1985). The fronds of *Padina* appear whitish because of lime incrustations on the upper side of the algae (Fritsch, 1945). The cause for $CaCO_3$ precipitation is still under debate, but it can be assumed that the carbonate layer benefits the algae in their environment (Benita et al., 2018). For example, it is suggested that the calcifying layer of the algae serves as a protection against excess light (Bürger, 2010), and prevents animal feeding (Littler & Littler, 1980).

Although *P. pavonica* is very common, there are still very few studies dealing with the photosynthetic properties. We studied the taxon since it is found along a wide irradiance gradient near the surface to around 10% of surface radiation (Haberleitner, 2010). We assumed that individuals from high light areas show greater photosynthetic capacity than their shaded counterparts. Contrarily, specimens subjected to low light availability should be more sensitive to photoinhibition, if exposed to high irradiance. Furthermore, the relationship of pigment content and adaptation to high and low light habitats was studied. It can be assumed that specimens from low light habitats have increased light-harvesting pigment content, than those from high light exposed sites. On the other hand, algae from high light zones probably have elevated protective pigments in order to avoid chronic damage. However, when keeping the carbonate cover in mind, specimens from high light areas might also show low light acclimation due to increased reflection and absorption, which makes this taxon very interesting for investigating photosynthetic properties.

2 Material and Methods

The study was conducted in Rovinj, Croatia, in the north-eastern part of the Adriatic Sea over a period of three weeks in June 2018. Sampling took place near the rocky shore at the west coast of Rovinj (45°04'58.4"N 13°38'12.1"E). The northern part of the temperate warm Adriatic Sea is shallow and increases its depth towards the south (Gacic, Poulain, Zore-Armanda, & Barale, 2001). Laboratory measurements were carried out at the marine biological station “Ruđer Bošković” and the University of Vienna.

Luminous flux (lux) was recorded in one-minute intervals using submersible data loggers (HOBO Pendant Temperature/Light 64K Data Logger) attached to a lead weight. The data loggers were placed at each sampling spot before sampling. A reference logger was mounted on top of a flagpole to measure flux above water surface. Percentage of incoming luminous flux were then calculated and four light classes were defined (LC1 > 25%, LC2 ≤ 25% to >10%, LC3 ≤ 10% to >5 %, LC4 ≤ 5% of incoming flux; LC = light class).

Photosynthetic characteristics were measured with the submersible PAM fluorometer Diving-PAM-II (Walz, Germany). Each morning, thalli were harvested by free-diving at different water depths, reaching from 0.1 to 11.3 m. Immediately before performing measurements, fronds were kept submersed and shaded for exactly 3 min by a dark leaf clip (DIVING-LC). The maximum variable fluorescence F_v was calculated from minimum (F_0) and maximum fluorescence (F_m) as $F_v = F_0\text{-}F_m$. F_0 is the fluorescence before a saturation pulse (PSII reaction centers open), and F_m is the maximal fluorescence during the pulse (all PSII reaction centers closed). The maximal quantum yield F_v/F_m (Kitajima & Butler, 1975) of PSII can then be determined, which is a measure of the overall photosynthetic performance (Hanelt, 1992; Hanelt & Nultsch, 1995). Afterwards, the actual acclimation state was measured by exposing the thalli to increasing actinic light steps using rapid light curve (RLC) measurements. The effective quantum yield (Y(II)) of chlorophyll fluorescence $(F_{m'}\text{-}F')/F_{m'}$ (Genty et al., 1989) and relative electron transport rate (rETR) were measured using saturation-pulse technique as described by Schreiber (2004). RLCs were conducted with nine increasing light steps from 13 to 220 µmol m^{-2} s^{-2}; saturating pulse intensity was 4000 µmol m^{-2} s^{-2} with 0.6s duration. Y(II) were used to calculate rETR through PSII using $rETR = PAR \cdot AF \cdot P_{PS2}/P_{PS1} \cdot Y(II)$, where PAR is the incoming quantum flux density of photosynthetically active radiation, P_{PS2}/P_{PS1} is the light absorption capacity (= 0.5) and the AF representing the sample absorbance (0.84 is the default value used for green leaves; Heinz Walz GmbH, 2018). The integrated software WinControl-3 estimated characteristic photosynthetic parameters of RLCs by curve fitting procedures using the equation of Jassby & Platt (1976): $rETR = ETR_{max} \cdot \tanh(\alpha \cdot PAR/ETR_{max})$, where α is the initial slope of the RLC. The onset of saturation I_k is then calculated as $I_k = rETR_{max}/\alpha$. $rETR_{max}$ is a parameter of the capacity of PSII to absorb light energy, while the initial slope (α) of RLC is a parameter of light harvesting efficiency of photosynthesis. In order to obtain absolute aETR, the actual absorption of the respective frond needs to be estimated (AF_{true}), which represents the fraction of absorbed light (Beer, Björk, & Beardall, 2014). Therefore, the absorption of each frond was calculated

(absorption = 100 – reflectance [%] – transmittance [%]) and true absorption factor (AF_{true} = absorption/100) was then computed. For further information on the methods of transmission and reflection measurements see Harder (2019). To calculate the absolute aETR following equation was used $aETR = PAR \cdot AF_{true} \cdot P_{PS2}/P_{PS1} \cdot Y(II)$ (Beer & Axelsson, 2004).

After measurements in the field, specimens were transported to the laboratory in ice-boxes. Thalli were then cut into halves and fresh-weights (FW) of both halves were measured (scale Mettler MT5). One part of the thallus was used for reflection measurements and for transmission measurements via a Spectroradiometer (Ocean Optics – USB4000). Then it was immediately frozen and stored at -80°C until pigment analyses (see below). The remaining part was used for dry weight (DW) and organic weight (OW) measurements. The thalli where placed in a drying chamber (90°C) for 12h to measure DW. Afterwards, they were treated with 20% of acetic acid (CH_3COOH) for 10 min. to dissolve the carbonate layer. After rinsing with distilled water, redrying and reweighing, OW was measured. The carbonate content was then determined by subtracting OW from DW.
For pigment extraction, thalli were homogenized with 90% acetone using a dispersing device (Polytron PT 1600 E) and then stored in the refrigerator (4°C) for 12 h. After centrifugation on the following day, the supernatant was analyzed and pigments were quantified by High-Performance Liquid Chromatography (HPLC system Hitachi Elite LaChrom, Diode Array Detector Hitachi L-2455, column thermostat L-2300 with temperature of 35°C, column Superspher RP — 18, 100 LI Chrocart, precolumn LI Chrocart rP-18 endcapped) according to a modified protocol of Wright et al. (1991). Peaks were quantified at 440 nm and identified through comparing retention times and spectral data with those of authentic standards (DHI Bioproducts). For peak analyses, calibration, and peak area integration, we used EZ Chrom Elite Client Version 3.2. Relative pigment concentrations were calculated per unit thallus area (cm^2) and OW (mg). For detailed interpretation, pigments were divided into four groups. The xanthophyll cycle pool (violaxanthin, antheraxanthin, zeaxanthin), light-harvesting pool (fucoxanthin, chl a, chl c), protective UV-absorbing-Pool (β-carotene) and total pigments. We calculated pigment concentrations per OW and per unit thallus area and found exactly the same pattern. For detailed analysis of results, pigments per unit thallus area were considered to facilitate comparisons with other studies and literature (Lüning, 1990, Dring, 1986, Colombo-Pallotta, García-Mendoza, & Ladah, 2006).

Statistical analysis was conducted using Microsoft Excel (Office 365 ProPlus) and SigmaPlot 12.5 (Systat Software, San Jose, CA, USA). Shapiro-Wilk test and Kolmogorov-Smirnov test was used for testing the normal distribution, respectively. Variance homogeneity of data was tested using Levene's test. One-Way ANOVA was conducted if more than two factors were normally distributed and homogeneity of variance was met. If the data did not follow a normal distribution or variance homogeneity, nonparametric tests were performed (Kruskal-Wallis). Post-hoc tests were carried out using Dunn's test, and Tukey-Kramer test, respectively.

3 Results

F_v/F_m mean values ranged between 0.65 and 0.54 and showed highest values in LC4. LC3 showed highest stress reactions reaching the F_v/F_m mean value of 0.54, which is similar to LC2 indicating the mean value of 0.56. Interestingly, the mean value of LC1 was fairly high reaching 0.62, plus no significant difference could be was proven between LC4 and LC1 (Fig. 1).

RLCs showed characteristic shapes: rETR was highest in *P. pavonica* specimens acclimated to high light and decreased with less light availability (Fig. 2). aETR showed exactly the same pattern (Fig. 3). Fronds growing under high light conditions, showed higher effective quantum yields with increasing light steps, than their shaded counterparts. Interestingly, *Padinas* from LC4 had highest values of Y(II) in the beginning and decreased the most during light-treatment (Fig. 4). ETR_{max} increased significantly with enhanced light availability ($p=<0.001$; LC1: n=38, LC2: n=45, LC3: n=33, LC4: n=41; Fig. 5). The onset of saturation I_k showed highly significant differences between LC4 and all other LCs ($p=<0.05$; LC1: n=38, LC2: n=45, LC3: n=33, LC4: n=41; Fig. 6). The initial slope α did not show a clear trend among light classes, however, highly significant results among all groups could be proven ($p=<0.001$; LC1: n=38, LC2: n=45, LC3: n=33, LC4: n=41; Fig. 7). LC4 showed the highest α mean value, followed by LC1, LC2 and LC3. Compared to high light specimens, we found a 30% decrease of total pigment concentration per leaf-area (Table 1) and OW (Table 2) in low light specimens. Fucoxanthin, chlorophylls (a and c), β-carotene and antheraxanthin per leaf-area decreased constantly with decreased light, while violaxanthin and zeaxanthin concentration values were slightly variable along the light gradient (Table 1). Chlorophyll a content was highest among all pigments and also had highest amounts in LC1. Mean pigment ratios per unit chlorophyll a showed a certain pattern in different light classes. Chlorophyll c to

chlorophyll a ratio stayed relatively unchanged, while fucoxanthin to chlorophyll a ratio increased by 15% along the light gradient. In contrast to the above trend, the ratio of light protecting pigments decreased slightly with less light availability (Table 1). Pigment concentrations per organic dry weight (Table 2) generally showed the same pattern as in Table 1, but with more pronounced differences.

The light-harvesting pool per leaf-area consisting of fucoxanthin, chlorophyll a and c tended to decrease gradually with lower light (Fig. 8). No significant differences between light classes were obtained when considering the unit pigments per leaf-area. Photoprotective pigments (violaxanthin, antheraxanthin and zeaxanthin) per leaf-area decreased significantly with decreasing light ($p=<0.001$; LC1: n=114, LC2: n=205, LC3: n=105, LC4: n=132; Fig. 9). The UV-protecting pigment ß-carotene showed results comparable to the xanthophyll cycle pigments. The relative pigment content showed overall significant differences ($p=<0.001$; LC1: n=38, LC2: n=69, LC3: n=35, LC4: n=44; Fig. 10) of ß-carotene decreasing with less light availability. Significant differences were proven between LC1 and LC2, LC1 and LC3, LC1 and LC4, LC2 and LC3 and besides LC2 and LC4.

4 Discussion

Beside resistance against slight water movement, *P. pavonica* is well acclimatized to high light conditions. This is reflected by high density appearance of this organism in shallow areas (Bürger, Clifford, & Schagerl, 2017). We found a slightly higher species distribution in deeper areas, which can be explained by decreased anthropogenic influences in contrast to the shallow zones (Purker, Siedler, & Smelhausova, 2018). This points to the fact, that *P. pavonica* is well represented along the vertical depth gradient, which can be attributed to its acclimation characteristics.

F_v/F_m is seen as an indicator of overall photosynthetic performance (Franklin & Forster, 1997; Machalek, Davison, & Falkowski, 1996). Unstressed green algae and green plants usually show an F_v/F_m value of 0.83, while this value is lowered in brown and red algae (Hanelt, 2017). In the study of Haberleitner (2010), F_v/F_m measurements showed a value of about 0.6 for *P. pavonica*. In our study, shaded *P. pavonica* ecotypes showed a slightly increased F_v/F_m (0.65) compared to specimens growing at intermediate light supply (LC2 and LC3). Interestingly, *P. pavonica* growing at high light conditions (LC1) again showed higher F_v/F_m than their counterparts. A significant influence of differences in the carbonate layer however can be excluded, because no correlation between reflectance and absorbance

to $CaCO_3$ cover was found (Purker et al., 2018). These results are in accordance to Bürger (2010), who was not able to detect significant differences in carbonate contents per unit area and dry mass of specimens along a depth gradient in early summer. Differences were only found in autumn, which imply that differences in carbonate cover become more pronounced during the main growing season.

High F_v/F_m as obtained from LC1, is obviously a result of a successful acclimation to high light (Walters, 2005). Here, morphological differences might play a key role. *P. pavonica* of shallow sites appear to have thicker fronds (Bürger et al., 2017), which might indicate a package effect to minimize photoinhibition. This was also the case in *Codium fragile* exposed to high irradiances, which showed no signs of photoinhibition because of its thick morphology (Arnold & Murray, 1980). For this reason, high F_v/F_m may rather be explained by thicker thalli in high light ecotypes, which limits photoinhibition (Raven, 2011).

High light adapted organisms are assumed to show high ETRs under high light conditions, while organisms adapted to dim light conditions have steeper α, because of their ability to use low light more efficiently (Ott, 1996; Walters, 2005). The photosynthetic characteristics of *P. pavonica* specimens from high and low light habitats differed markedly. We found high light acclimated algae linked to increased rETR, aETR, and ETR_{max}. Moreover, a higher I_k and slower decline of Y(II) at higher irradiances, were obtained in contrast to dark-acclimated algae. A decline of RLCs at high irradiances is not necessarily linked to photoinhibition, but rather to down-regulation of PSII as time is too short for photodamage to arise (Ralph & Gademann, 2005; White & Critchley, 1999). Long-lasting damage to the photosynthetic apparatus is prevented by down-regulation of photosynthetic efficiency (Franklin & Forster, 1997). Although there is no evidence of photodamage, a more pronounced down-regulation of PSII in algae inhabiting greater depths can be assumed from our data. Consequently, our result underlines the fact that *P. pavonica* ecotypes are well acclimated to both various light climates and different depths.

Unexpectedly, there was no clear trend of α between LC1 to LC3, although algae originating from LC4 showed highest α values as expected. Already several other studies showed unexpected patterns of α, or even no changes, after algae were exposed to different irradiances (Ensminger, Foerster, Hagen, & Braune, 2005; Gómez, Weykam, Klöser, & Wiencke, 1997; Haberleitner, 2010; Silva, Santos, Serôdio, & Melo, 1998; Weykam, Gómez, Wiencke, Iken, & Klöser, 1996). Even an opposite pattern was observed in red algae *Gelidium sesquipedale* by Silva et al. (1998). The authors found a slight decrease of

α with depth when it was achieved by the fluorescence method, whereas the oxygen measurements showed the expected pattern. Henley (1993, as cited in Silva et al., 1998, p. 298) suggested that more data points in the region of limiting irradiance are needed to deliver reliable results. Further, at high light levels, Calvin-cycle limitations may not affect PSII activity (values of α), because other protective mechanisms serve as electron sinks (e.g. water-water cycle; Asada, 2000). Therefore, chlorophyll fluorescence method, might not be able to detect changes in acclimation (Ensminger et al., 2005). This may explain, high α values in specimens exposed to high irradiance in our study. Further, morphology of the thallus has impact on α (Johansson & Snoeijs, 2002). The study of Weykam et al. (1996) compared various macroalgae, and it turned out that α of some low light macroalgae was particularly high, in contrast to species inhabiting the upper sublittoral. However, filamentous and foliose algae inhabiting upper sublittoral and intertidal, such as *Urospora penicilliformis* showed both high α and P_{max} values thus suggesting that this species probably is able to cope with both low and high light supply, while most deep water species are able to only utilize low light more efficiently.

In order to survive environments characterized by low light, sessile algae developed many strategies including smaller xanthophyll cycle pools, thinner thalli, increased photosynthetic unit sizes, and restricted PSII repair cycles (Runcie, Gurgel, & Mcdermid, 2008) and increased pigment content (Kirk, 2011; Lüning, 1985; Machalek et al., 1996; Valenzuela-Espinoza, Millán-Núñez, Trees, Santamaría-Del-Ángel, & Núñez-Cebrero, 2007; Wu, Jiang, Liu, & Deng, 2015). In contrast to what was expected, we found *Padina* exposed to high irradiances provided with higher total pigment contents. Considering the light-harvesting pool, we found chlorophyll a to be the dominating pigment in algae inhabiting high light environments, followed by the typical accessory pigments of brown algae fucoxanthin and chlorophyll c, which absorb light in the blue and green regions of the spectrum (Enríquez, Agustí, & Duarte, 1994). Other studies revealed comparable results (Haberleitner, 2010; Raven, 1984; Rodrigues, Dos Santos, Yoneshigue-Valentin, Strbac, & Hall, 2000). Raven (1984) suggested that chlorophyll content is adjusted to all irradiances and decreases with low light availability, because an increase in photosynthetic pigments in an environment with only few photons would not be profitable for the algae. This hypothesis seems to be applicable also to the deep-water brown algae *Laminaria abyssalis,* which also had decreased pigment contents with diminishing light (Rodrigues et al., 2000). This apparent contradiction might be explained by the morphological structure. (Heriyanto, Dita, Shioi, Limantara, & Brotosudarmo, 2017). The morphology of *Padina*

sp. is characterized by multilayered, flattened and translucent thalli, so light can penetrate to lower cell layers easily (Hay, 1986). As mentioned above, shade adapted algae tend to be morphologically thin (Lüning, 1990). Indeed, *P. pavonica* occurring in greater depths seem to have thinner thalli (Bürger et al., 2017). In order to maximize photon absorption rates, self-shading of pigments, also referred to as the package effect has to be minimized (Raven, 1984). When photosynthetic pigments are partitioned into packages rather than being evenly dispersed the light-harvesting, efficiency is lowered (Kirk, 2011). For this reason, a large package effect can also help to avoid photoinhibition, especially if the algae have a large optical thickness (Raven, 2011).

Some strategies of algae to optimize their light-harvesting systems, may be counterintuitive. Perhaps, algae which overproduce pigments may try to reach broader absorption peaks of the light-harvesting pigments. In this way fewer types of pigments are needed to cover the whole PAR spectrum underwater (Ramus, 1978, as cited in Larkum, 2003, p.287; Raven, 2011). Large packaging leading algae to act as a black-body absorber while at the same time giving protection against photoinhibition may explain higher pigment contents of *P. pavonica* growing in high light environments. Further, because light is absorbed by other plant material as well (e.g. proteins), light absorption cannot be traced back to light-harvesting pigments alone. There is evidence that absorption is enhanced through chlorophyll content, but that it is further increased through thickness of thalli. In multicellular algae scattering is of greater importance for efficient absorption, than increasing pigment concentration, because the optical path is increased and the chances that a photon will be absorbed is higher. Therefore, increasing thalli thickness may lead to increasing absorptance (Enríquez et al., 1994). Absorption of incident photons without increase of relative pigment content may thus be explained by the calcium carbonate precipitation of algae, which also lead to more scattering (Lüning, 1990). This theory could apply to *P. pavonica* as it is a calcifying macroalgae. However, the light-harvesting efficiency may better be explained by relative absorption per unit biomass rather than absolute, because it reflects the carbon turnover. As a matter of fact, thicker algae absorb less light on biomass basis, than thinner macrophytes with resembling chlorophyll a content. So the amount of relative photosynthetic components is higher in thin algae than in their thicker counterparts (Enríquez et al., 1994). Another study reports, that the correlation between photosynthetic efficiency and increased chlorophyll a content with depth is not obligatory. The reason for this can result from the low ratio of photosynthetic

to non-photosynthetic cells, which can be attributed to the thallus structure for example in the red alga *Gigartina skottsbergii* (Gómez et al., 1997).

In accordance to other studies and literature, the photoprotective pigment pool is higher in high light adapted specimens (Colombo-Pallotta et al., 2006; Demmig, Winter, Krüger, & Czygan, 1987; Graham et al., 2008; Kirk, 2011). Also, a close relationship between the formation of carotenoids in the xanthophyll cycle and NPQ was found (Demmig et al., 1987; Gilmore & Yamamoto, 1992). As a consequence, the non-radiative energy dissipation or non-photochemical quenching (NPQ) in shallow growing algae is higher, than in deeper specimens.

The results indicate that the PSII antenna size between light classes seem to be similar, since chlorophyll c/chlorophyll a ratio is relatively unchanged and fucoxanthin/chlorophyll a ratio only increase about 15% from LC1 to LC4. Nevertheless, there is a trend of an increase in the fucoxanthin to chlorophyll ratio with depth, which suggest more efficient light harvesting in low light areas (Kirk, 2011). A higher fucoxanthin to chlorophyll a ratio in deeper growing organisms was also found in the study of Smith & Melis (1987). The authors suggested that an increased absorption of the blue-green wavelength is needed at greater depth, as the blue-green light climate increases with depth. As expected, in high light, concentration of carotenoids increased relative to chlorophyll a. Therefore, this study confirms that *P. pavonica* invests in photoprotection mostly in high light exposed ecotypes. The study of Colombo-Pallotta et al., (2006) investigating pigment composition and photosynthetic performance of *M. pyrifera* goes along with our results.

5 Conclusion

P. pavonica shows differential light acclimation characteristics to a wide range of light climates. Our hypothesis, that light-harvesting pigment contents are increased in ecotypes growing in low light habitats could not be confirmed. However, higher photoprotective pigments in thalli from high light zones could be determined. Further, higher photosynthetic capacity under high light conditions could be indicated in sun exposed individuals. A more enhanced down-regulation of PSII in algae inhabiting greater depths can be witnessed from our data. This indicates that algae growing in the shade are more susceptible to high light stress than specimens growing under high irradiances, which goes along with our other hypothesis. Finally, high light adapted ecotypes did not show evidence of low light acclimation, despite their carbonate layer.

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Index

Figures

Tables

Attachment

Figures

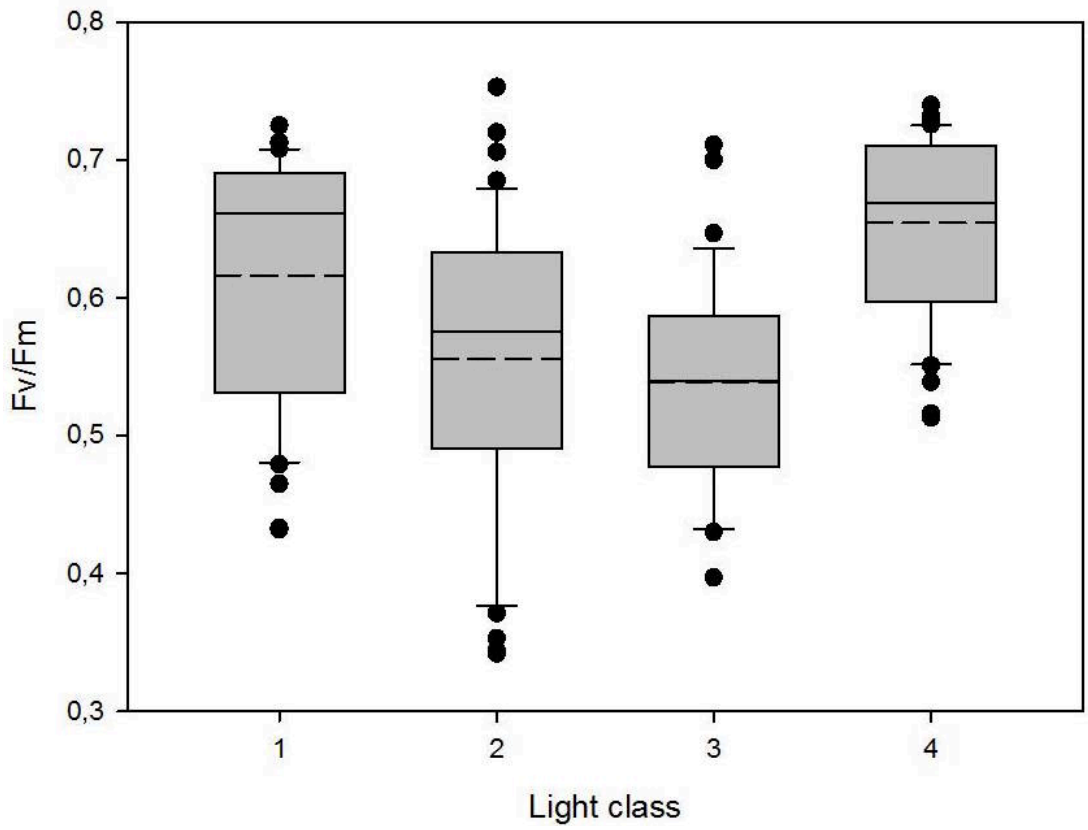

Figure 1: Box plots of F_v/F_m versus light class with median (solid line), mean (slashed line), standard deviation and statistical outliners (black dots). Number of samples in each light class: LC1: n=38, LC2: n=45, LC3: n=33, LC4: n=41. Letters represent significant differences at the level of $p<0.05$ (a=LC1, b=LC2, c=LC3, d=LC4).

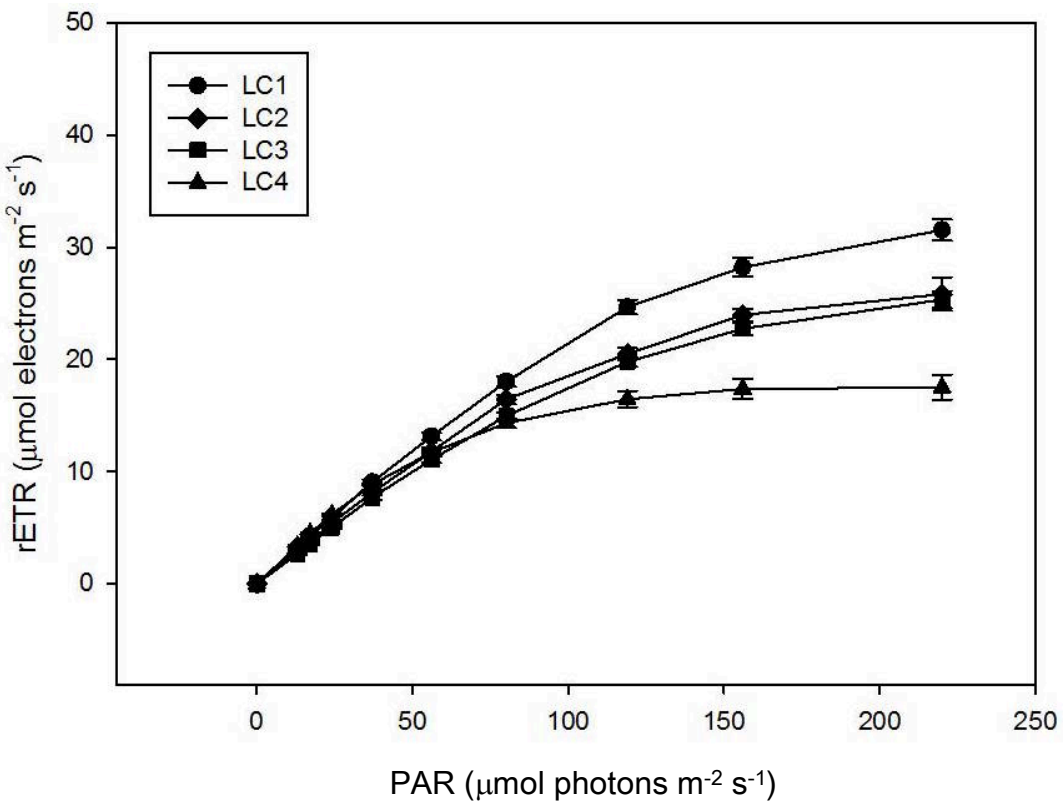

Figure 2: Means and standard errors of rETR (µmol electrons cm^{-2} s^{-1}) plotted against PAR (µmol photons $cm^{-2}s^{-1}$) of various light classes. Number of samples in each light class: LC1: n=38, LC2: n=45, LC3: n=33, LC4: n=41.

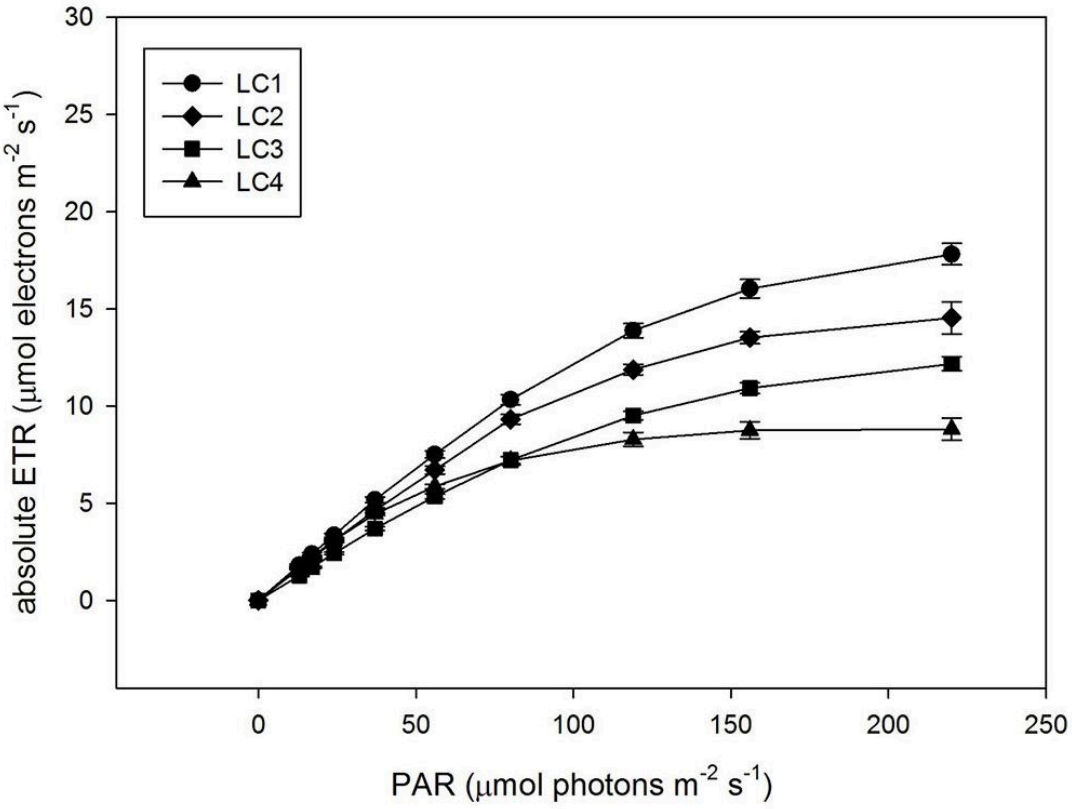

Figure 3: Means and standard errors of absolute ETR (µmol electrons cm^{-2} s^{-1}) plotted against PAR (µmol photons cm^{-2} s^{-1}) of various light classes. Number of samples in each light class: LC1: n=38, LC2: n=45, LC3: n=33, LC4: n=41.

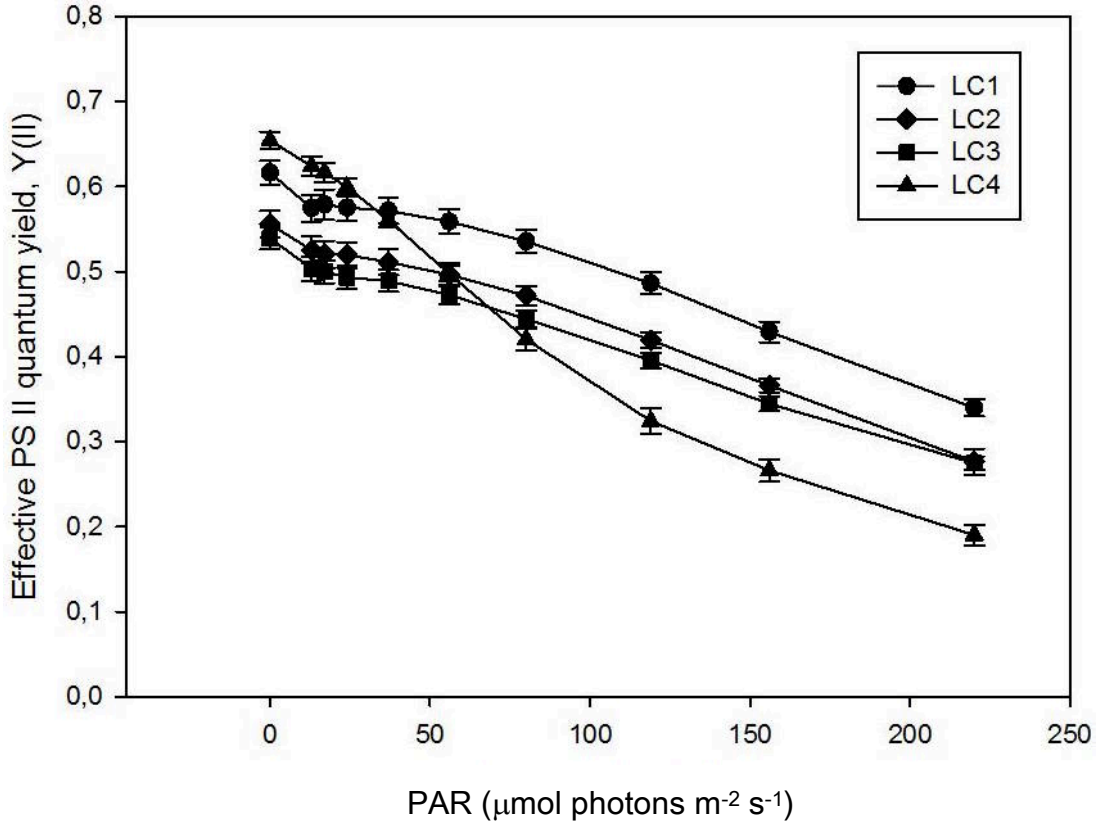

Figure 4: Means and standard errors of Y(II) plotted against PAR (µmol photons cm^{-2} s^{-1}). Number of samples in each light class: LC1: n=38, LC2: n=45, LC3: n=33, LC4: n=41.

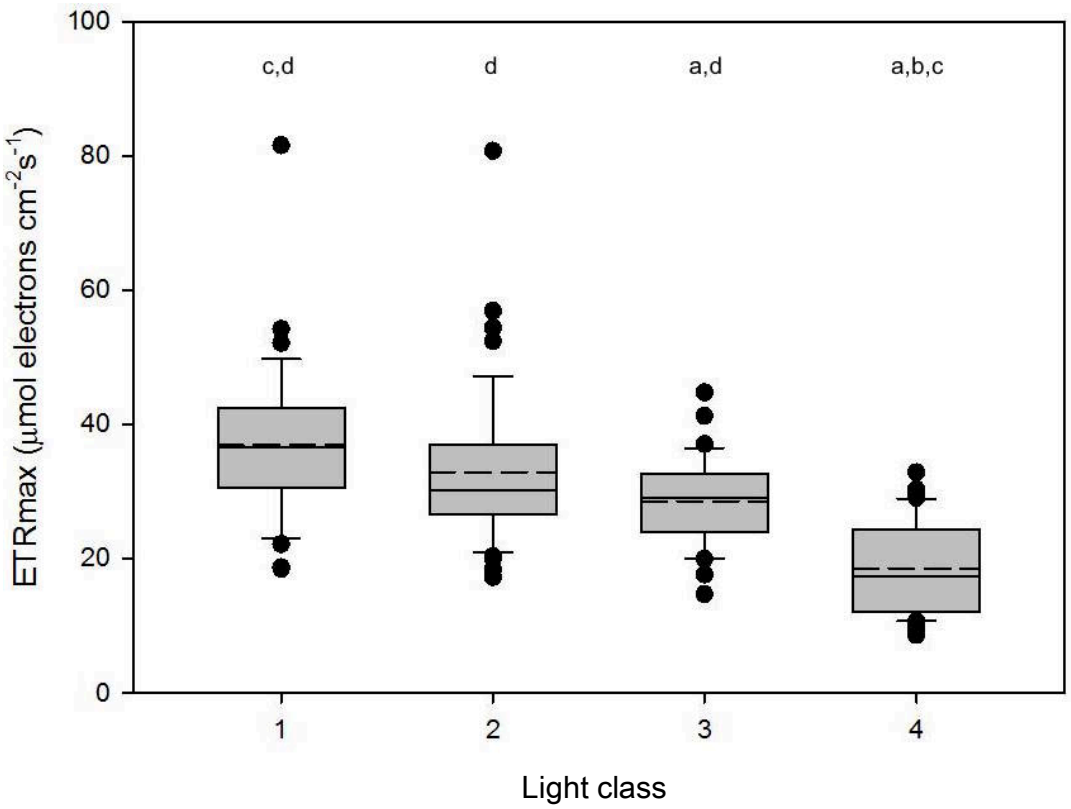

Figure 5: Box plots of ETRmax (µmol electrons cm^{-2} s^{-1}) versus light class with median (solid line), mean (slashed line), standard deviation and statistical outliners (black dots). Number of samples in each light class: LC1: n=38, LC2: n=45, LC3: n=33, LC4: n=41. Letters represent significant differences at the level of $p<0.05$ (a=LC1, b=LC2, c=LC3, d=LC4).

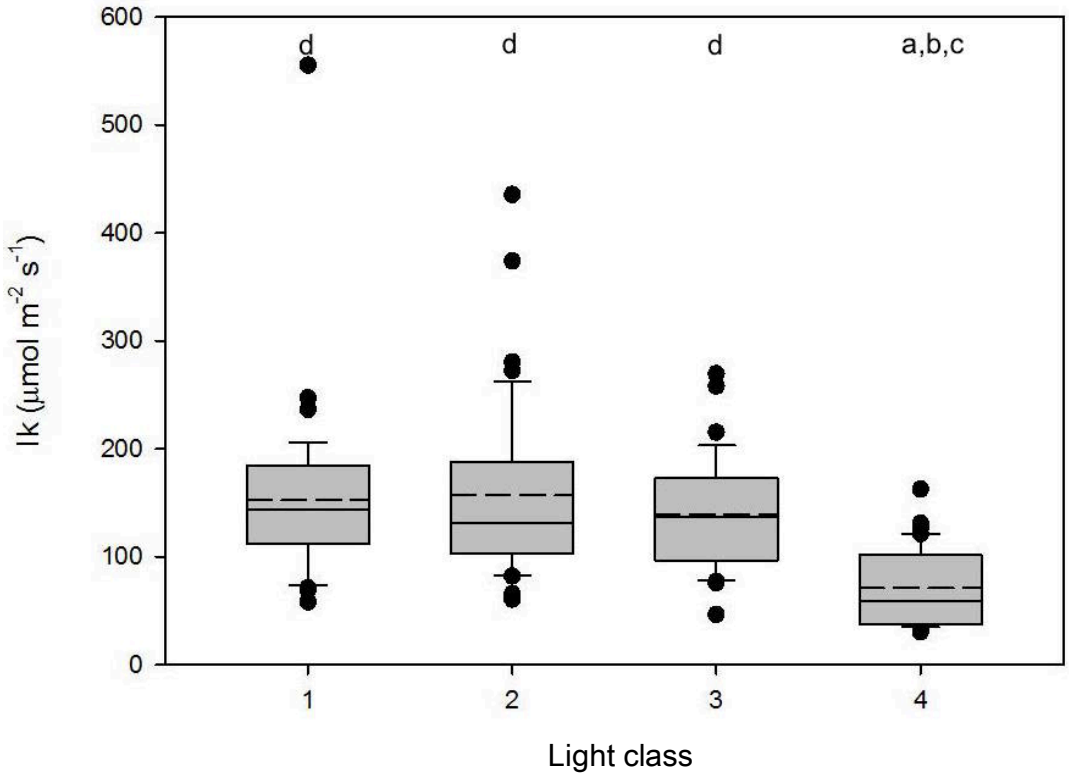

Figure 6: Box plots of I_k versus light class with median (solid line), mean (slashed line), standard deviation and statistical outliners (black dots). Number of samples in each light class: LC1: n=38, LC2: n=45, LC3: n=33, LC4: n=41. Letters represent significant differences at the level of $p<0.05$ (a=LC1, b=LC2, c=LC3, d=LC4).

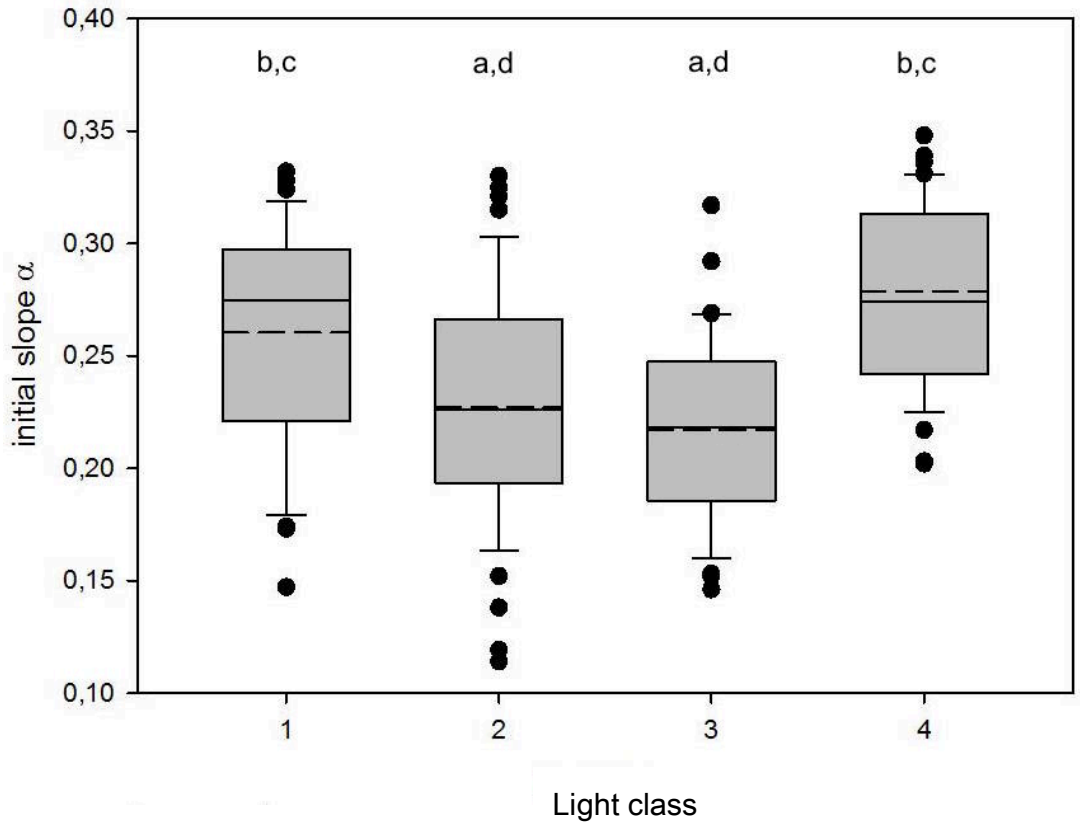

Figure 7: Box plots of initial slope versus light class with median (solid line), mean (slashed line), standard deviation and statistical outliners (black dots).Number of samples in each light class: LC1: n=38, LC2: n=45, LC3: n=33, LC4: n=41. Letters represent significant differences at the level of $p<0.05$ (a=LC1, b=LC2, c=LC3, d=LC4).

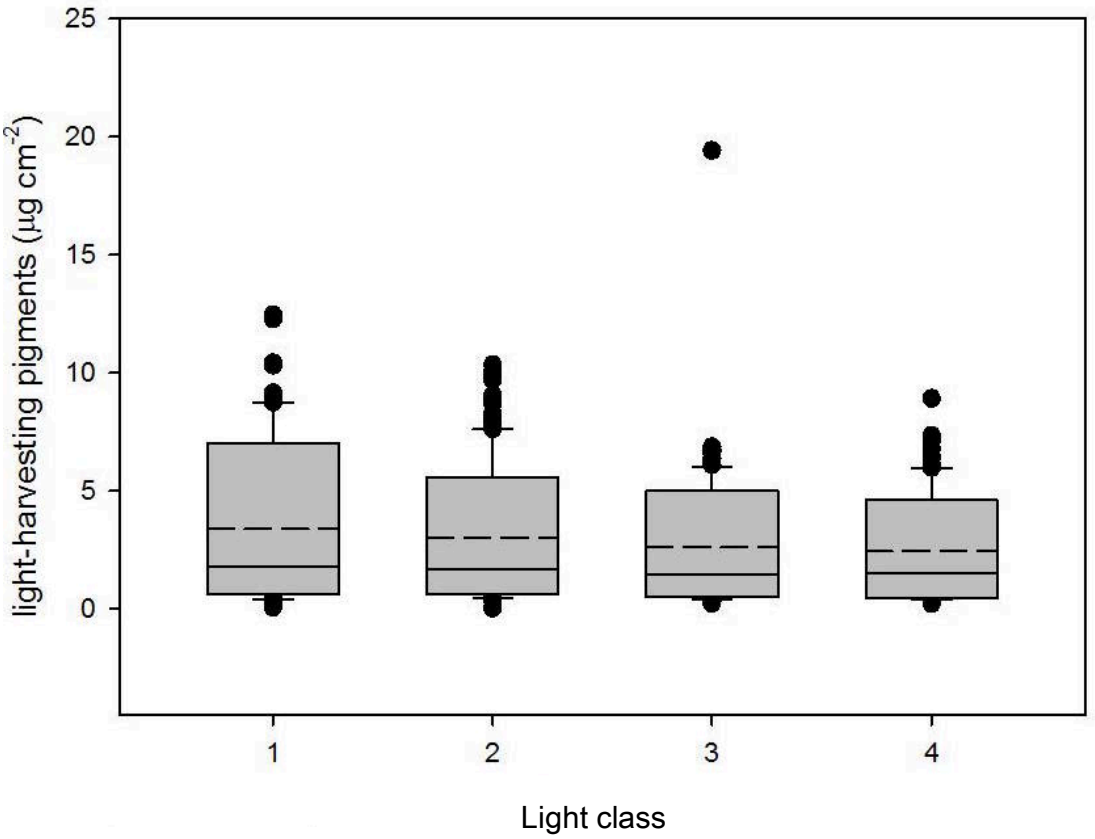

Figure 8: Box plots of relative light-harvesting pool versus light class with median (solid line), mean (slashed line), standard deviation and statistical outliners (black dots). Number of samples in each light class: LC1: n=114, LC2: n=205, LC3: n=105, LC4: n=132. Letters represent significant differences at the level of $p<0.05$ (a=LC1, b=LC2, c=LC3, d=LC4).

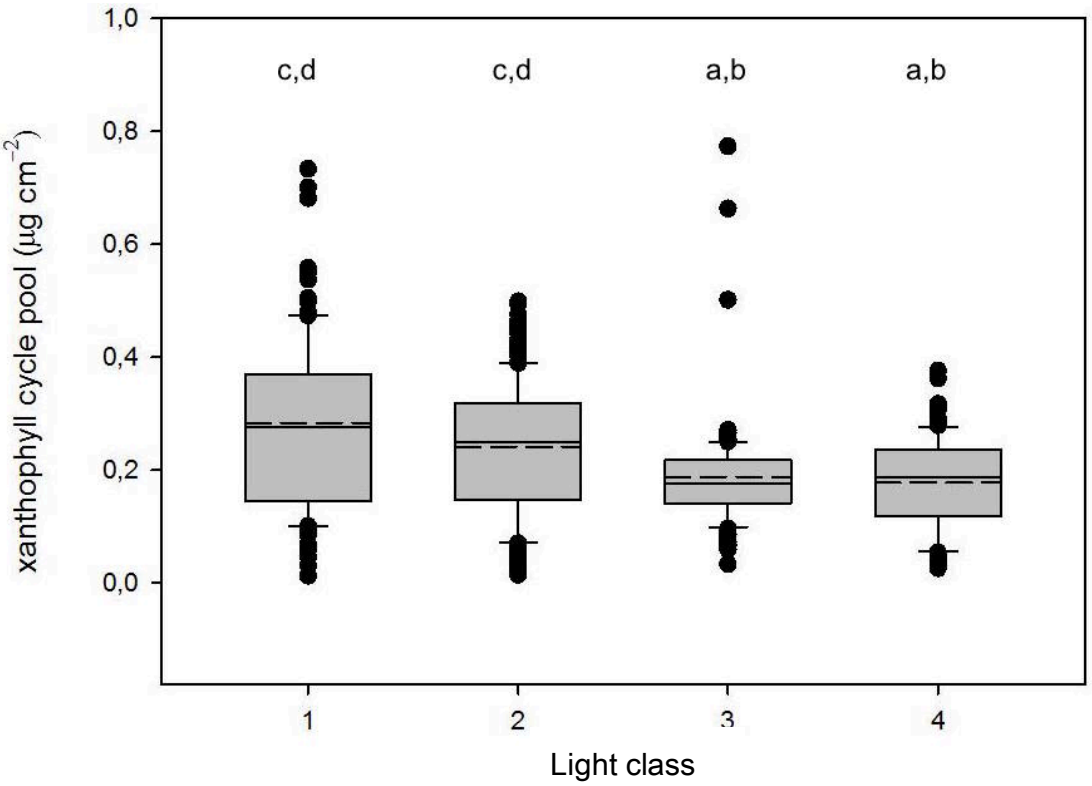

Figure 9: Box plots of relative xanthophyll cycle pool versus light class with median (solid line), mean (slashed line), standard deviation and statistical outliners (black dots). Number of samples in each light class: LC1: n=114, LC2: n=205, LC3: n=105, LC4: n=132. Letters represent significant differences at the level of $p<0.05$ (a=LC1, b=LC2, c=LC3, d=LC4).

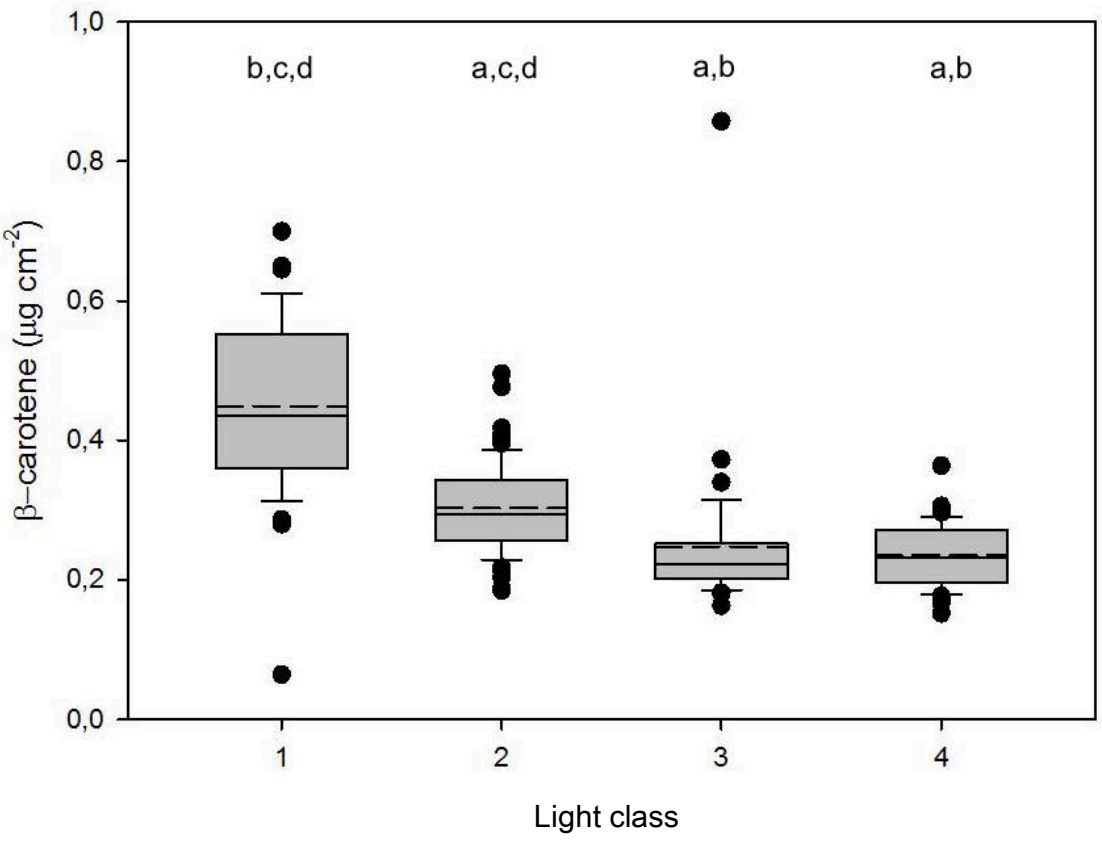

Figure 10: Box plots of ß-carotene versus light class with median (solid line), mean (slashed line), standard deviation and statistical outliners (black dots). Number of samples in each light class: LC1: n=38, LC2: n=69, LC3: n=35, LC4: n=44. Letters represent significant differences at the level of $p<0.05$ (a=LC1, b=LC2, c=LC3, d=LC4).

Tables

Table 1: Pigment concentration per leaf-area, ratio of mean chl c, fucoxanthin and xanthophyll pool to mean chl a from different light classes. Mean values are in µg pigment cm^{-2} ± SD (n=38 for LC1, n= 69 for LC2, n= 35 for LC3, n= 44 for LC4); LC=Lichtclass, SD = standard deviation, chl a=chlorophyll a, chl c = chlorophyll c, fuc=fucoxanthin, β-caro= β-carotene, vio=violaxanthin, ant=antheraxanthin, zea=zeaxanthin, ΣXP = xanthophyll cycle pigment pool (vio, ant, zea).

Lichtclass	chl a	chl c	fuc	β-caro	vio	ant	zea	chl c: chl a	fuc: chl a	ΣXP: chl a	Total pigment content
LC1	7.81±2.16	0.54±0.22	1.80±0.55	0.45±0.13	0.40±0.14	0.28±0.09	0.16±0.12	0.07	0.23	0.04	11.44±3.4
LC2	6.82±1.62	0.54±0.15	1.67±0.45	0.30±0.06	0.33±0.09	0.26±0.06	0.08±0.04	0.08	0.24	0.03	10±2.47
LC3	5.81±2.51	0.46±0.20	1.55±0.65	0.25±0.11	0.19±0.09	0.23±0.10	0.15±0.08	0.08	0.27	0.03	8.63±3.75
LC4	5.44±1.18	0.43±0.09	1.49±0.31	0.23±0.05	0.21±0.05	0.22±0.05	0.10±0.06	0.08	0.27	0.03	8.12±1.79

Note: Mean values are in µg pigment cm^{-2} ± SD (n=38 for LC1, n= 69 for LC2, n= 35 for LC3, n= 44 for LC4); LC=Lichtclass, SD = standard deviation, chl a=chlorophyll a, chl c = chlorophyll c, fuc=fucoxanthin, β-caro= β-carotene, vio=violaxanthin, ant=antheraxanthin, zea=zeaxanthin, ΣXP = xanthophyll cycle pigment pool (vio, ant, zea)

Table 2: Pigment concentration per organic weight, ratio of mean chl c, fucoxanthin and xanthophyll pool to mean chl a from different light classes. Mean values are in µg pigment mg^{-1}±SD (n=38 for LC1, n= 69 for LC2, n= 35 for LC3, n= 44 for LC4); LC=Licht class, SD = standard deviation, chl a=chlorophyll a, chl c = chlorophyll c, fuc=fucoxanthin, β-caro= β-carotene, vio=violaxanthin, ant=antheraxanthin, zea=zeaxanthin, ΣXP = xanthophyll cycle pigment pool (vio, ant, zea).

Lichtclass	chl a	chl c	fuc	β-caro	vio	ant	zea	chl c: chl a	fuc: chl a	ΣXP: chl a	Total pigment content
LC1	1.05±0.35	0.07±0.02	0.24±0.08	0.06±0.02	0.05±0.02	0.04±0.01	0.02±0.01	0.07	0.23	0.04	1.52±0.12
LC2	0.82±0.31	0.07±0.02	0.21±0.07	0.04±0.01	0.04±0.01	0.03±0.01	0.01±0.01	0.08	0.25	0.03	1.21±0.11
LC3	0.68±0.19	0.05±0.02	0.18±0.05	0.03±0.01	0.02±0.01	0.03±0.01	0.02±0.01	0.08	0.27	0.03	1.01±0.07
LC4	0.65±0.31	0.05±0.01	0.19±0.05	0.03±0.01	0.03±0.01	0.03±0.01	0.01±0.01	0.08	0.29	0.03	0.99±0.11

Note: Mean values are in µg pigment mg^{-1} ± SD (n=38 for LC1, n= 69 for LC2, n= 35 for LC3, n= 44 for LC4); LC=Licht class, SD = standard deviation, chl a=chlorophyll a, chl c = chlorophyll c, fuc=fucoxanthin, β-caro= β-carotene, vio=violaxanthin, ant=antheraxanthin, zea=zeaxanthin, ΣXP = xanthophyll cycle pigment pool (vio, ant, zea)

Zusammenfassung

Die Photosynthese ist für Algen ein lebensnotwendiger Prozess, welcher mit Hilfe anorganischer Stoffe Lichtenergie in organische Verbindungen umwandelt und diese anschließend für den Aufbau von Biomasse oder Reservestoffe verwendet. Im Gegensatz zu Landpflanzen erhalten aquatische Algen nicht das komplette Sonnenlichtspektrum, da das Medium Wasser und die darin aufgelösten Substanzen und Partikel, Teile des Lichts absorbieren beziehungsweise streuen (Graham et al., 2008; Ott, 1996). Die Strahlungsenergie wird daher stark vermindert. Die Abnahme der Lichtintensität entlang der Wassersäule nennt sich in der Fachsprache „Attenuation". (Ott, 1996). In tieferen Gewässern ist aus diesem Grund eine abnehmende Photosyntheseleistung der Algen zu beobachten (Graham et al., 2008). Auf der anderen Seite sind Algen in der oberen Wasserschicht, dem Eulitoral, Starklicht und stark schwankenden Lichtverhältnissen ausgesetzt, welche eine Inhibition des Photosyntheseapparates nach sich ziehen kann. Die sogenannte Photoinhibition führt zu einer Abnahme der Photosyntheseaktivität (Hanelt et al., 2003). Um besser mit den genannten Problemen umzugehen, besitzen Algen Anpassungsmechanismen (Raven & Geider, 2003). So haben Algen Schutzmechanismen entwickelt, um überschüssige Lichtenergie über Carotinoide in Form von Wärme abzubauen. Dieser Prozess wird durch einen sinkenden pH-Wert im Lumen der Thylakoide eingeleitet, welcher durch Aufnahme von starkem Licht ausgelöst wird (Demmig-Adams & Adams, 1996). Außerdem sind unterschiedliche Lichtsammelpigmente, welche verschiedene Absorptionsmaxima des Wellenlichts aufweisen können, eine Anpassung an die Attenuation des Lichts in Gewässern (Ott, 1996).

Die Forschungsarbeit wurde in Rovinj (Kroatien) im Frühsommer 2018 durchgeführt. Der Zeitraum der Forschungstätigkeit im Feld betrug drei Wochen, wobei mehrere Apnoetauchgänge im Mittelmeer zur Untersuchung getätigt wurden. In dieser Studie untersuchten wir die Braunalge *Padina pavonica,* welche zur Gruppe der kalzifizierenden Algen gehört (Okazaki et al., 1986).
P. pavonica kommt entlang eines breiten Lichtgradienten vor, der sich bis etwa 10% der Oberflächenstrahlung erstreckt (Haberleitner, 2010). Wir nahmen an, dass Ökotypen aus stark belichteten Bereichen eine größere Photosyntheseaktivität aufweisen, als die im Schatten lebenden Exemplare. Im Gegensatz dazu sollten Individuen aus schwach belichteten Bereichen empfindlicher gegen Photoinhibition sein, wenn diese einer hohen Lichtintensität ausgesetzt werden. Eine weitere Hypothese besagt, dass Proben, welche ans Schwachlicht angepasst sind, erhöhte Lichtsammelpigmente aufweisen. Auf der anderen

Seite sollten Algen aus Starklichtzonen erhöhte Schutzpigmente aufweisen, um chronische Schäden zu vermeiden. Zieht man jedoch die Karbonatschicht der Alge in Betracht und die daraus resultierende erhöhte Reflexion und Absorption, können Organismen auch aus stark belichteten Standorten, Schwachlicht-Akklimatisierung aufweisen.
Photosynthesemessungen, welche entlang eines vertikalen Transekts mit Puls-Amplitudenmodulation (Diving PAM-II, Walz, Germany) durchgeführt wurden, zeigten erhöhte photosynthetische Kapazitäten (relative ETR, absolute ETR, ETR_{max}, I_k) in Starklichtalgen. Die anfängliche Steigung (α) der Lichtkurven (Rapid light curve (RLC)) und F_v/F_m (Maximale PSII Effizienz) zeigten jedoch kein spezifisches Muster entlang des Lichtgradienten. Mit Hilfe der Hochleistungsflüssigkeitschromatographie (HPLC) wurden die Pigmente analysiert. Unerwarteterweise war die Menge der Gesamtpigmente pro Thallusfläche und organischem Gewicht bei *Padinas* aus Starklichtzonen erhöht, was auf den Packungseffekt hinweist. Aus den Ergebnissen kann man schließen, dass *P. pavonica* auf unterschiedliche Lichtbedingungen mit verschiedenen Anpassungen reagiert. Starklichtalgen zeigten trotz vorhandener Kalkschicht keine Schwachlicht-Akklimatisierung, was auf eine fehlende Korrelation zwischen Absorption bzw. Reflexion und Kalkauflage zurückzuführen ist.

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