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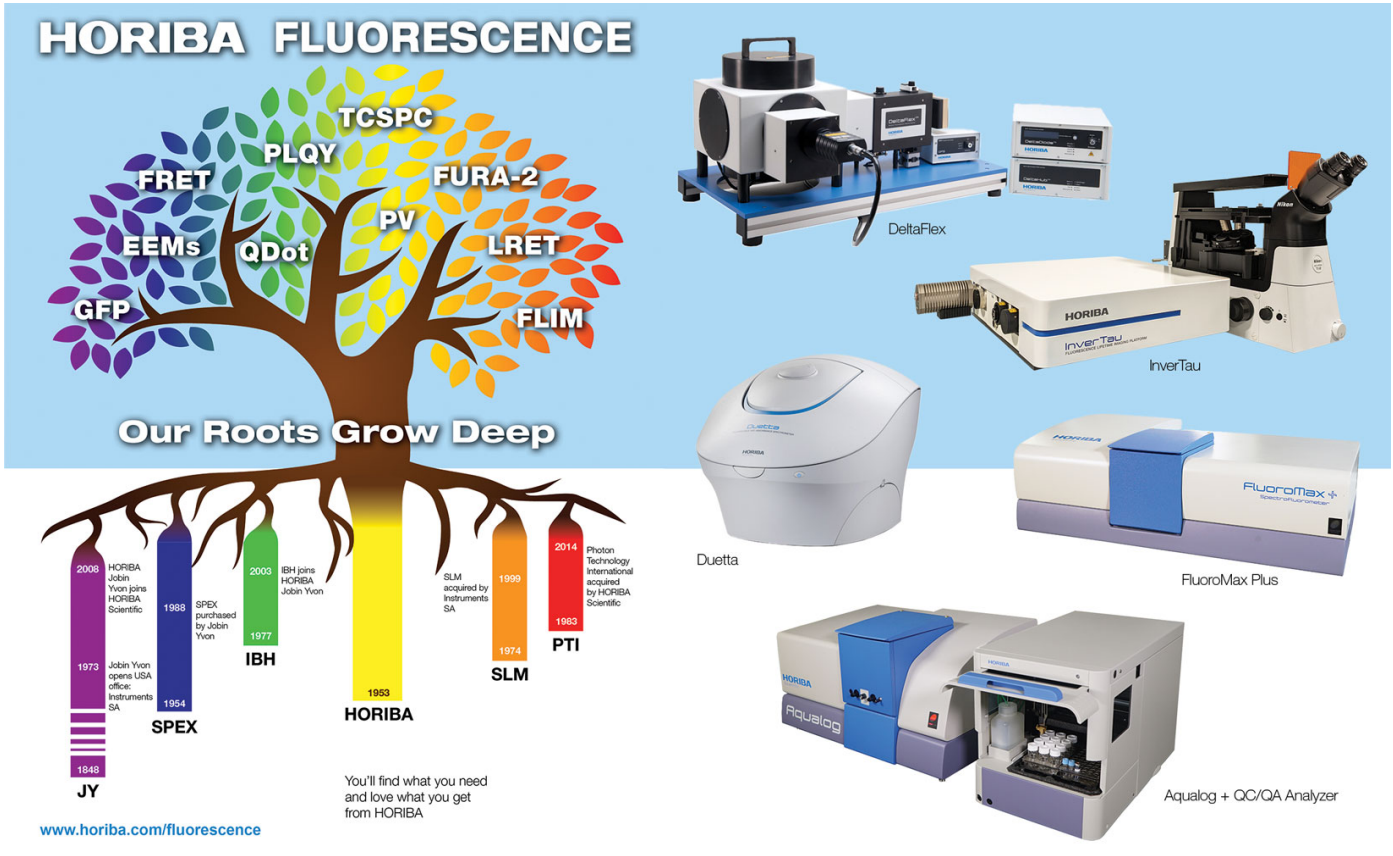

Methods and Applications in Fluorescence

PAPER

Combining *Nitellopsis obtusa* autofluorescence intensity and F680/F750 ratio to discriminate responses to environmental stressors

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Abstract

Detection of autofluorescence parameters is a useful approach to gain insight into the physiological state of plants and algae, but the effect of reabsorption hinders unambiguous interpretation of *in vivo* data. The exceptional morphological features of *Nitellopsis obtusa* made it possible to measure autofluorescence spectra along single internodal cells and estimate relative changes in autofluorescence intensity in selected spectral regions at room temperatures, avoiding the problems associated with thick or optically dense samples. The response of algal cells to controlled white light and DCMU herbicide was analyzed by monitoring changes in peak FL intensity at 680 nm and in F680/F750 ratio. Determining the association between the selected spectral FL parameters revealed an exponential relationship, which provides a quantitative description of photoinduced changes. The ability to discern the effect of DCMU not only in the autofluorescence spectra of dark-adapted cells, but also in the case of light-adapted cells, and even after certain doses of excess light, suggests that the proposed autofluorescence analysis of *N. obtusa* may be useful for detecting external stressors in the field.

1. Introduction

Characean macroalgae, phylogenetically closely related to land plants (Leliaert *et al* 2012), provide a suitable model system for studying various physiological processes in response to external stressors (Eremin *et al* 2013, Kisnieriene *et al* 2019). Exploration and discovery of many general principles of plant cell structure and physiology was first done on characean internodal cells before their confirmation in higher plants (Foissner and Wasteneys 2014). The advantage of using single cell systems in studies based on autofluorescence measurements lays in the ability to monitor physiological activity under strictly controlled conditions that cannot be ensured in multicellular plants composed of individual cells whose responses may be highly variable.

Nitellopsis obtusa algae are particularly well suited for photosynthesis research at the single cell level due to the unique morphological characteristics of their internodal cells, such as cylindrical shape, extensive length and diameter (Beilby and Casanova 2014). The chloroplasts within these cells are organized in helical files and, unlike in other autotrophs (Wada 2013, Foissner and Wasteneys 2014), are unable to rearrange in response to excessive light (Foissner and Wasteneys 2014). Moreover, the preference of clear, low-nutrient water shows the potential of these macroalgae as useful indicators of oligo- to mesotrophic habitats (Foissner and Wasteneys 2014).

In the natural environment, plants are exposed to a wide range of adverse conditions on a continuous basis. It is known that sudden changes in ambient factors, such as light, induce their acclimatization due to altered physiological processes, including photosynthesis (Yavari *et al* 2021). Photosynthesis is performed by adaptive and sensitive photosystems that respond to various external stressors, such as excess light

(Demmig-Adams and Adams 1992), altered temperature (Kalaji *et al* 2011, Mathur *et al* 2014), and pollutants (Suresh Kumar *et al* 2014). Photochemical reactions in photosystem II (PSII) are particularly susceptible to stress-induced inhibition (Murata *et al* 2007). These reactions are directly linked to other processes that dissipate absorbed light energy: fluorescence (FL) from excited chlorophyll *a* molecules and its non-photochemical quenching (NPQ) (Baker 2008).

The assessment of the activity of PSII can be accomplished via various fluorescence-based techniques, which have proven to be a suitable tool for monitoring the photosynthetic performance and physiological state of terrestrial vegetation (Kalaji *et al* 2011), aquatic plants (Haynes *et al* 2000), and microalgae (Kalnaitytė-Vengelienė *et al* 2024). In agricultural applications, the use of pulse amplitude modulation (PAM) fluorimeters is widespread to determine a number of photosynthesis parameters, such as the maximum quantum efficiencies of PSII photochemistry and NPQ, but their interpretation is often complex and sometimes controversial (Schreiber 2004). In addition, dark adaptation of the samples is necessary. Spectrally-resolved methods provide additional information and are particularly suitable for remote sensing (Mohammed *et al* 2019), but lack specificity because different environmental factors induce indistinguishable responses in the recorded fluorescence (Pedros *et al* 2008).

The autofluorescence spectra of Characean algae measured at room temperature consist of a spectral band with a red peak around 680–690 nm and another band in the far-red region visible as a shoulder around 730-740 nm (Gruszecki *et al* 2006). Conventionally, the emission intensities recorded around the first peak correspond mainly to the performance of PSII, and the second spectral band is associated with the activities of both PSI and PSII, with the contribution of PSI increasing at longer wavelengths (Franck *et al* 2002). The changes in the ratio of FL intensities at these spectral regions reflect the effects of environmental stressors induced on photosynthetic performance, as shown in several studies on higher plants (Lichtenthaler 1988, da Silva *et al* 2012) and microalgae (Eullaffroy and Vernet 2003). Natural and artificial stressors with well-known action mechanisms affecting particular elements of electron transfer between the two photosystems are commonly applied in photosynthesis research (Powles 1984, Draber *et al* 1991, Dayan *et al* 2010). Among them, the herbicide 3-(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU, diuron) is often used to validate fluorescence-based techniques (Konishi *et al* 2009, Herritt *et al* 2020). Real-time measurements of changes in the physiological state of photosynthetic activity, reflecting the cellular response mechanism, can serve to determine the physiological signature of the applied external stimulus. However, in most experiments carried out in higher plants *in vivo*, the influence of reabsorption was significant and required the use of an appropriate corrective approach to interpret the FL data (Agati *et al* 1993, Gitelson *et al* 1998, Cordón and Lagorio 2006).

This study aims to highlight single macroalgal cells as a sensitive model system for distinguishing processes induced by external stressors such as light and a selected herbicide DCMU at room temperature using a combination of changes in peak FL intensity at 680 nm and in the F680/F750 intensity ratio. The morphological features of *Nitellopsis obtusa* allowed us to measure autofluorescence spectra and evaluate relative intensities in selected spectral regions, avoiding the problems associated with thick or optically dense specimens, since chloroplasts are organized in a single helical layer within the algal cell. To better utilize the relationship between autofluorescence parameters and physiological states and processes, as well as to test the specificity of non-invasive optical methods, appropriate methodological strategies for statistical analysis and data evaluation were applied.

2. Materials and methods

2.1. Plant material

Nitellopsis obtusa (N.A. Desvaux) J. Groves algae (figure 1) were collected from Lake Stanka (Lithuania) during autumn months. The culture was maintained at room temperature (21 $\pm$ 1 °C) in glass aquaria filled with tap water and kept under controlled lighting conditions (9.5 $\pm$ 0.2 μmol photons m^{-2} s^{-1}) with a light/dark photoregime of 12/12 h.

2.2. Experimental procedures and spectroscopic measurements

Prior to the measurements, internodal cells around 6–8 cm in length and up to 1 mm in diameter were separated from neighboring cells. Afterwards the cells were placed in artificial pond water (APW) containing 0.1 mM KCl, 1.0 mM NaCl, 0.1 mM $CaCl_2$ (pH around 7) and kept under selected conditions: darkness for 12 h (a); maintenance light (9.5 $\pm$ 0.2 μmol photons m^{-2} s^{-1}) for 12 h (b); darkness for 12 h and subsequent illumination with intense white LED light (LED line, Poland; 829 μmol photons m^{-2} s^{-1}) for 15 min (c) and 30 min (d).

Separated internodal cells selected for DCMU treatment were placed in APW supplemented with 100 μM DCMU and kept under the illumination conditions stated above. All chemicals used were purchased from Sigma-Aldrich (USA).

For spectroscopic measurements, an optical system with an extreme solarization resistant bifurcated fiber consisting of seven 230 μm fibers (Ocean Insight, USA) was used (figure 2). The arm containing the central fiber was connected to a low intensity (0.13 mW) 405 nm LED excitation source (Ocean Optics, USA) to excite the algal cell directly from below. The six surrounding fibers were used to collect the FL signal into another arm, allowing the emitted light to pass

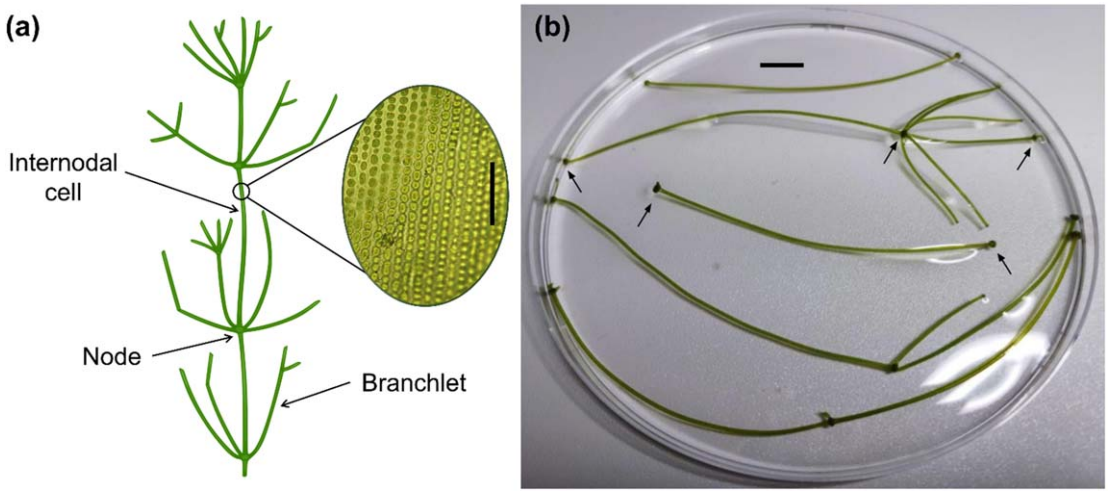

Figure 1. Illustration of the apical part of *N. obtusa* (a) with a micrograph showing the chloroplast layer within an internodal cell. A photograph of separated algal cells is shown (b) with black arrows indicating nodes connecting the internodal cells. The bars are 40 μm for the micrograph in (a) and 1 cm in (b). The chloroplast layer in (a) was imaged using an Eclipse 80i microscope (Nikon, Japan) through the 40 × /0.75 objective Plan Fluor, DIC M/N2 (Nikon, Japan). A Digital Sight DS-SMc color camera (Nikon, Japan) was used to capture the image.

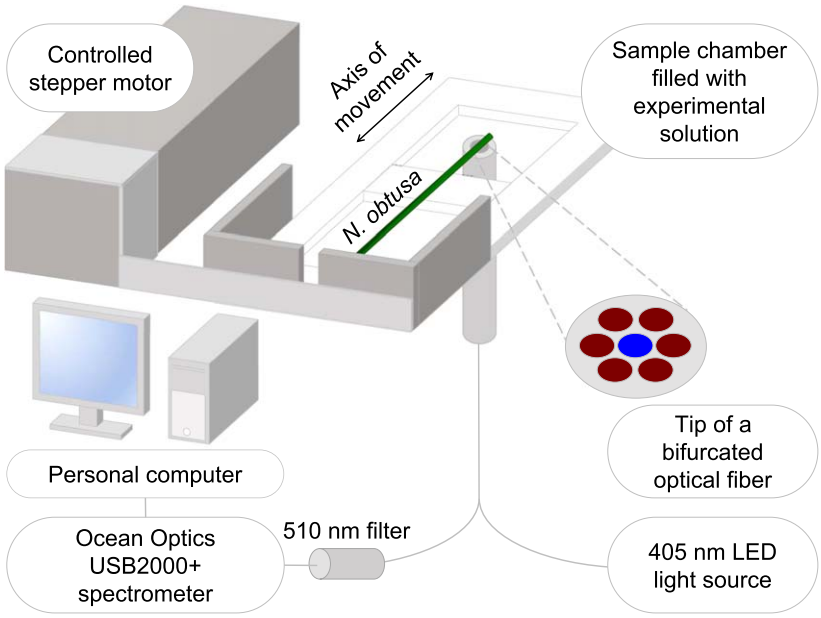

Figure 2. Experimental arrangement for measuring autofluorescence spectra along *N. obtusa* internodal cells.

through an in-line filter holder containing a 510 nm long-pass emission filter. The filter was connected to a spectrometer USB2000 (Ocean Optics, USA) equipped with the Sony ILX511 2048-element CCD array via a single 400 μm fiber.

To ensure registration of autofluorescence spectra along single cells of *N. obtusa* in 1 mm intervals, the optical fiber system was paired with a stepper motor (Changzhou Jkongmotor, China) controlled by an Arduino Uno R3 microcontroller board. During these measurements, an internodal cell was placed in a custom-made Plexiglas chamber filled with the experimental solution (APW or APW supplemented with 100 μM DCMU). Since the central area of the chamber was used to immobilize the cell, autofluorescence from the central part of the cell (2–3 cm) was not registered. All experiments were carried out at room temperature (21 ± 1 °C).

To confirm the reproducibility of the values of the selected FL parameters, a set of experiments was conducted in which registration of autofluorescence spectra was repeated twice along the internodal cells, maintaining a time interval of 1 h between the measurements.

To evaluate the possible effect of reabsorption on the autofluorescence spectra, additional spectroscopic measurements were performed on internodal cells stacked on top of each other. In these experiments, the

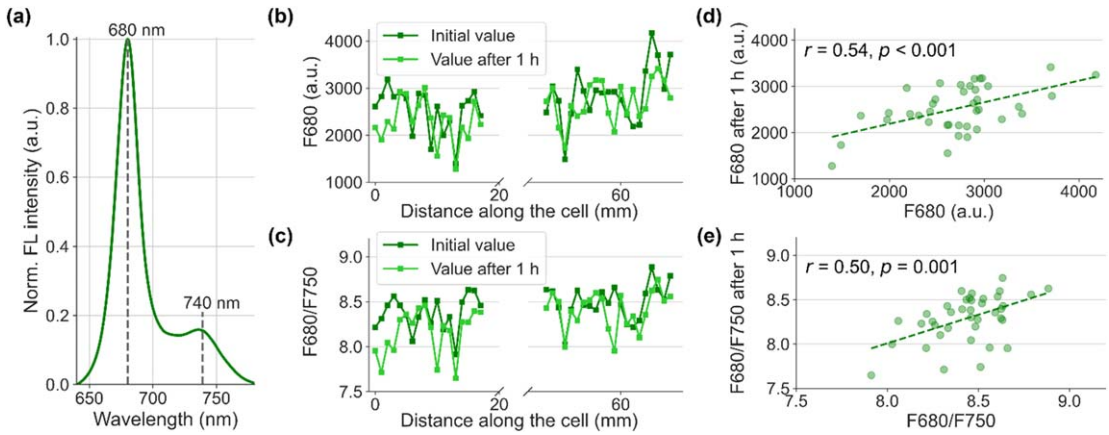

Figure 3. A typical *N. obtusa* autofluorescence spectrum corrected for the detector spectral sensitivity ($\lambda_{ex} = 405$ nm, (a)) and representative data of spatial and time-related changes of F680 (b) and the F680/F750 ratio (c) recorded along an internodal cell adapted to maintenance light. The correlation between FL parameters recorded at the same places in the cell with an interval of 1 h is presented in (d-e).

nodes of the selected algal cells were cut off to ensure that the internodes lost turgor and could be stacked uniformly on top of each other. In this way, chloroplast layers of de-turgorized cells were brought into close proximity, mimicking the arrangement of chloroplasts in the leaves of higher plants. The autofluorescence spectrum was first recorded in a sample containing one internodal cell, and then each time after uniform placement of an additional algal cell on top of the previous one.

2.3. Data analysis

The recorded autofluorescence spectra were processed using OriginPro 2018 software (OriginLab, USA). To reduce noise and automate further data analysis, the spectra were filtered with an FFT filter function (15-point window was selected, cut-off frequency close to 0.01 Hz), ensuring that the peak FL intensity value at 680 nm decreased less than 5%. Correction of the spectra was achieved via division by the detector spectral sensitivity function using Python (version 3.9.12). A representative spectrum before and after filtering and subsequent correction is shown in figure S1.

The FL parameters evaluated were the peak FL intensity at 680 nm (F680) and the F680/F750 ratio determined from the intensities recorded at 680 nm and 750 nm. Methods of statistical analysis were applied in the programming language Python, except for principal component analysis (PCA), which was carried out in the programming language R (version 4.2.1). All data are expressed as median ± median absolute deviation (MAD). The number of cells is denoted by k, and n is the total number of the autofluorescence spectra recorded. The Shapiro-Wilk test was used to confirm the normality of data distributions. For assessment of reproducibility, the significance of changes in FL parameters over time was determined using the non-parametric Mann–Whitney test, and correlation coefficients were calculated between two values of both FL parameters obtained at the same places of the cell with an interval of 1 h. Pearson's correlation coefficient (r) was used for normally distributed data, otherwise Spearman's correlation coefficient (r_s) was applied. The significance of changes in FL parameters induced by illumination, DCMU, or the combination of these factors was determined using the non-parametric Kruskal-Wallis test, followed by Dunn's test. In all cases, the significance level was set to $p = 0.05$. To evaluate whether changes in autofluorescence spectra induced by selected environmental factors can be adequately described by two parameters, PCA was performed.

3. Results

3.1. Spatial and temporal variation of *N. obtusa* autofluorescence

The autofluorescence spectra of *N. obtusa* internodal cells had a main peak at 680 nm and a shoulder in the far-red region at about 740 nm (figure 3(a)). The parameters F680 and F680/F750 being measured along a single internodal cell at 1 mm intervals displayed variations as shown in figures 3(b)–(c). However, repeated registration along the same internodal cell showed that the pattern of variations did not change significantly over 1 h ($p = 0.236$ for F680; $p = 0.712$ for the F680/F750 ratio, $k = 5$, $n = 219$) and that the values of the two selected parameters were correlated (figure 3(d)–(e), table 1).

The comparison of autofluorescence spectra of different intensities recorded from two random cells revealed slight differences around the far-red shoulder but not at the short-wavelength side of the main FL peak, indicating that the reabsorption effect was not significant (figure S2). However, the presence of reabsorption was detectable, when autofluorescence was recorded from multiple chloroplast layers in the case

Table 1. Median values of FL parameters: F680 (left) and the F680/F750 ratio (right) recorded twice with an interval of 1 h in the same spots of *N. obtusa* internodal cells exposed to maintenance light, and the corresponding correlation coefficients (r_s) ($k = 5, n = 219$).

	F680	r_s	p	F680/F750	r_s	p
Initial value	2301 ± 476			8.64 ± 0.18		
Value after 1 h	2427 ± 523	0.84	<0.001	8.66 ± 0.16	0.74	<0.001

Table 2. Median values of *N. obtusa* FL parameters, F680 and the F680/F750 ratio, under selected treatment conditions.

Treatment conditions	F680 (a.u.)	F680/F750
Maintenance light ($k = 10, n = 406$)	2189 ± 354	8.33 ± 0.37
Darkness ($k = 16, n = 598$)	4285 ± 654	9.04 ± 0.17
Maintenance light + 100 μM DCMU ($k = 5, n = 180$)	3263 ± 465	7.67 ± 0.22
Darkness + 100 μM DCMU ($k = 15, n = 579$)	5081 ± 656	7.94 ± 0.19
Darkness + 15 min illumination ($k = 5, n = 178$)	1051 ± 201	7.21 ± 0.18
Darkness + 30 min illumination ($k = 5, n = 193$)	495 ± 56	6.10 ± 0.20
Darkness + 100 μM DCMU + 15 min illumination ($k = 6, n = 216$)	1512 ± 184	6.61 ± 0.26
Darkness + 100 μM DCMU + 30 min illumination ($k = 6, n = 212$)	663 ± 126	6.35 ± 0.36

of stacked cells (figure S3). Moreover, the degree of spectral differences was also not related to the peak intensity values of the analyzed spectra. The distribution of the selected FL parameters, F680 and the F680/F750 ratio, for individual algal cells kept under maintenance light for 12 h is shown in figure S4. Notably, the range of variability in the pooled data was driven primarily by marked differences between some individual cells rather than large differences between parameters measured within a single cell. Compared to algae cells kept in darkness, autofluorescence of cells adapted to maintenance light had decreased values of F680 and the F680/F750 ratio. The median values of these FL parameters measured under all applied treatment conditions are presented in table 2.

3.2. Effects of DCMU and illumination conditions on *N. obtusa* autofluorescence

The impact of 100 μM DCMU on the autofluorescence spectra of *N. obtusa* cells that were kept in darkness to avoid additional light-induced effects is shown in figure 4(a). Treatment with the herbicide led to a significant increase in F680 (figure 4(a), table 2, table S1) and a significant decrease in the F680/F750 ratio (table 2, table S2). The relative changes induced by DCMU in different spectral regions were identified by dividing the median autofluorescence spectrum of herbicide-treated cells by the corresponding spectrum of untreated cells maintained in darkness (figure 4(b)). Two spectral regions with lower and higher relative increases in FL intensity were detected, starting from 650 nm to 680 nm and from about 700 nm to 775 nm, respectively. Similar differences in the FL parameters persisted between DCMU-treated and untreated cells in the groups of cells adapted to maintenance light (table 2).

The selected illumination conditions had a significant impact on dark-adapted internodal *N. obtusa* cells (table 2, table S1, table S2). Illumination with white LED light resulted in a decrease in both selected FL parameters (figure 5(a), table 2), and the reductions after exposure for 30 min were significantly greater than after 15 min (tables S1, S2). The relative decrease in intensity was greater in the FL spectral region up to 700 nm than in the far-red region from 700 nm (figure 5(c)).

Internodal cells kept in darkness and treated with 100 μM DCMU also showed a decrease in F680 and the F680/F750 ratio values after illumination with white LED light (figure 5(b), table 2). Variations of parameters F680 and F680/F750 being measured along single internodal cells after different illuminations are shown in figure S5(a) and those in the presence of the herbicide - in figure S5(b). The decrease in FL intensity, but not in the F680/F750 ratio value, was significantly greater in DCMU-treated cells after 30 min than after 15 min (table S1, table S2). Differences in the ratio, however, were significant for separating groups of treated and untreated cells after 15 min of illumination, but not after 30 min of illumination. As shown in figure 5(c), the decrease in FL intensity in the relative spectra of cells after both illuminations was greater in the FL spectral region up to 700 nm compared to the far-red region from 700 nm, in consistence with the results obtained after illumination of cells untreated with DCMU. After 30 min of illumination, the effect of DCMU treatment on relative changes in FL intensity appeared to be masked by photoinduced spectral changes.

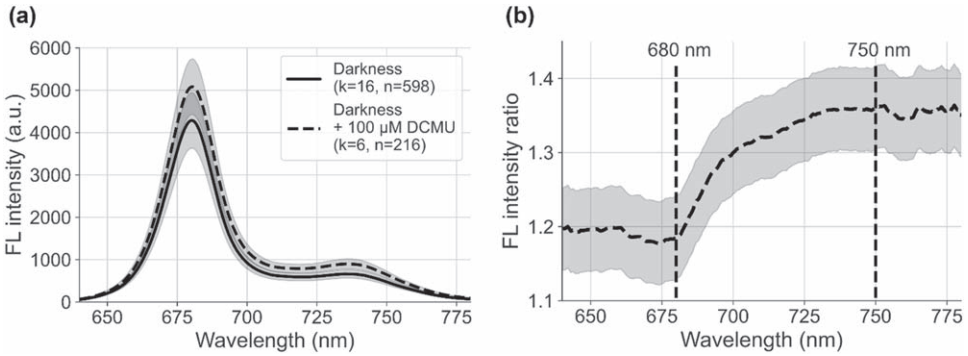

Figure 4. Effect of DCMU on *N. obtusa* autofluorescence spectra (a) and the relative spectrum (b) displaying DCMU-induced changes after 12 h treatment in darkness. Solid and dashed curves represent medians with transparent MAD values, vertical lines mark the spectral positions selected for FL estimation.

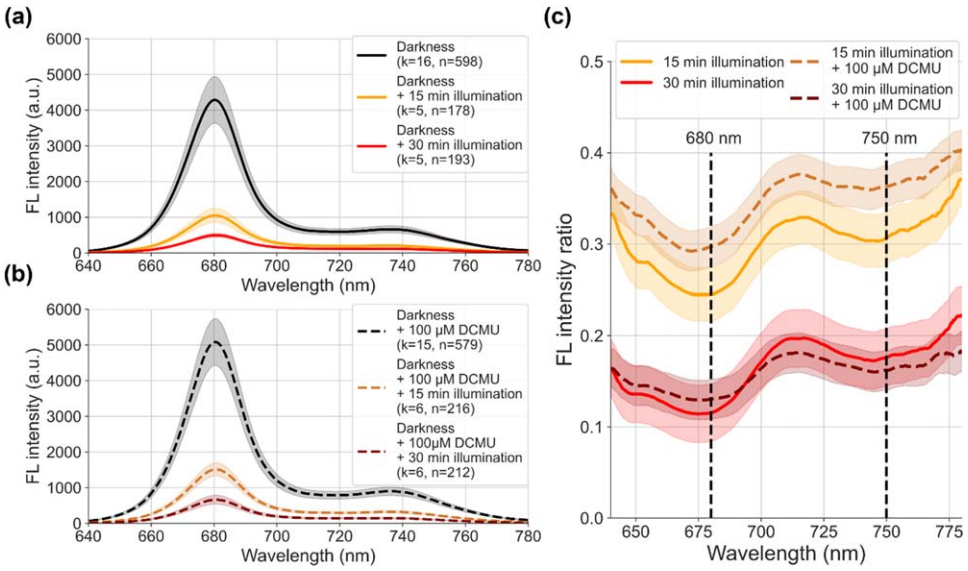

Figure 5. Median *N. obtusa* autofluorescence spectra before and after illumination with white LED light without (a) and with DCMU (b), and relative spectra displaying illumination-induced changes (c). Solid and dashed lines represent medians with transparent MAD values, except for black dashed lines in (c).

3.3. The combination of FL intensity and the F680/F750 ratio for discrimination of light- and DCMU-induced effects

The correlation between F680 and the F680/F750 ratio extracted from the entire data set containing all spectra recorded in internodal cells kept under selected illumination conditions was significant regardless of the DCMU treatment ($r_s = 0.88$, $p < 0.001$ without and $r_s = 0.83$, $p < 0.001$ with DCMU treatment). To approximate these dependencies and describe changes in selected FL parameters caused by illumination, exponential functions were used ($R^2 = 0.89$ without and $R^2 = 0.83$ with DCMU treatment) (figure 6). Treatment with DCMU affected the autofluorescence properties of cells in darkness, but not the general tendency of light-induced changes in FL parameters.

Visualization of the spectral data in PCA space (figure 7) revealed that the first two principal components explain 99.9% of the variance. The first component (PC1) separated the autofluorescence spectra of algae according to the illumination conditions. Distinction of the spectra by the second component (PC2), associated with the DCMU treatment, was successful, with the exception of the spectra recorded in cells from the groups illuminated with white LED light for a longer time (30 min).

4. Discussion

In studies of autotrophs, light-induced effects are unavoidable, therefore, they must be evaluated and distinguished before assessing the influence of other environmental factors on physiological processes. Illumination of *N. obtusa* cells resulted in the expected autofluorescence quenching throughout the whole spectral range (figure 5(a)), which is commonly

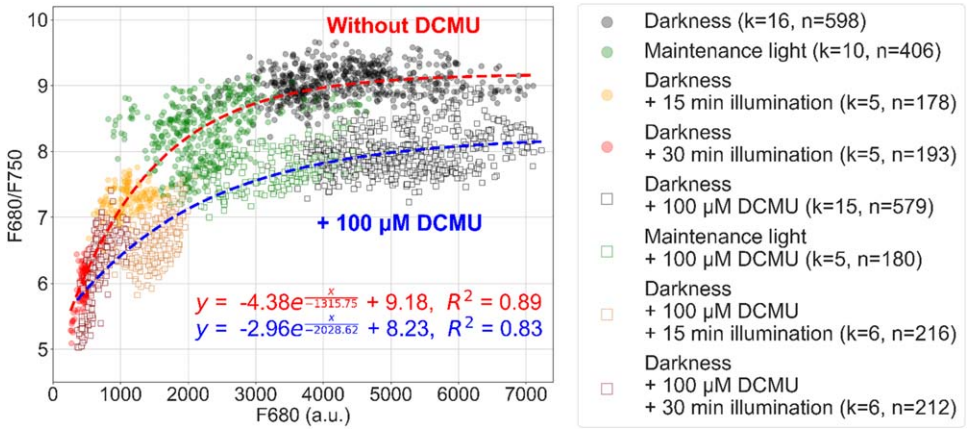

Figure 6. The relationship between *N. obtusa* F680 and the F680/F750 ratio with and without DCMU treatment. Dashed lines show exponential approximations, where x is the F680 value and y is the F680/F750 ratio. R^2– determination coefficient.

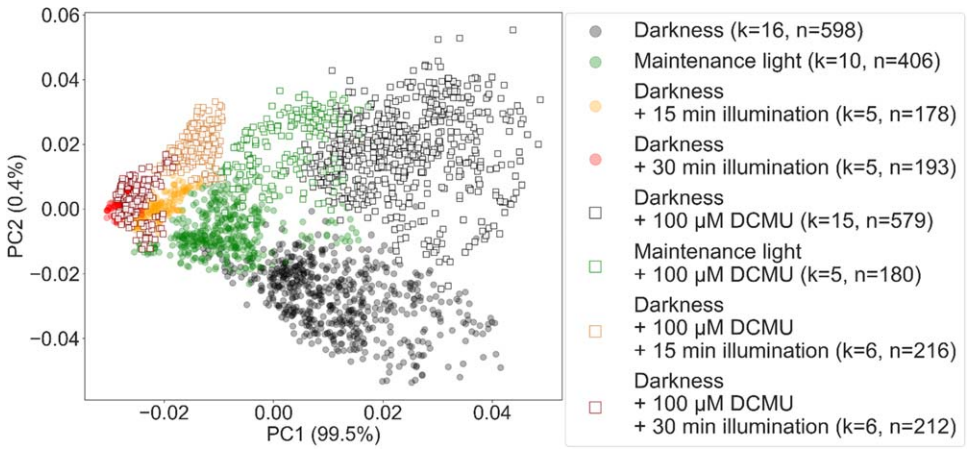

Figure 7. The score plot of PCA analysis for autofluorescence of *N. obtusa* cells recorded after different treatment conditions. Percentages next to the names of the axes indicate the variance explained by the component.

interpreted as a structural reorganization of the antenna complexes in plants leading to favored emission from PSI (Krause and Weis 1984, Johnson *et al* 2011). In our study, a relatively greater decrease in FL intensity was photoinduced in the spectral region around 680 nm (figure 5(c)) associated with PSII compared to the far-red spectral region where the impact of PSI is more pronounced (Franck *et al* 2002, Rizzo *et al* 2014). Distinct responses of the photosystems to illumination with white light were indicated by lower values of the F680/F750 ratio, which confirms the increased contribution of emission from PSI. Moreover, it is known that intense illumination causes photoinhibition of PSII, which reduces its efficiency and leads to excess energy dissipation (Agati 1998, Murata *et al* 2007).

To validate the suitability of the selected FL parameters for detecting impaired photosynthetic performance in *N. obtusa* under different illumination conditions in combination with an external stressor, the herbicide DCMU with a well-established mechanism of action was used. This compound interacts with the D1 protein of PSII and blocks the electron transfer to plastoquinone (PQ) and, consequently, to PSI (Lavergne 1982). Interestingly, the inhibitory activity of DCMU led to an enhanced autofluorescence of algae with a relatively larger increase in the far-red region of the FL spectra (figure 4), which was reflected in a decrease in the F680/F750 ratio in cells kept in the dark or under maintenance light (table 2). However, the combined treatment with DCMU and excess light on *N. obtusa* cells caused a dose-dependent quenching of autofluorescence, and the decrease in FL intensity was larger in the spectral region of the main peak activity (figure 5(c)). In the presence of DCMU, the FL reduction was smaller, and the effect was still discernible at least at the lower white light dose applied; however, changes in parameters were consistent with those observed in algae not treated with herbicide, indicating a predominant effect of light compared to DCMU.

The variation in F680 values and the F680/F750 ratio being detected along internodal cells of *N. obtusa* under maintenance light (figures 3(b)–(c)) could be

related to pH banding, a phenomenon observed in characean algae (Beilby and Bisson 2012). Under constant illumination, alternating acidic and alkaline bands emerge along the cell, with an enhanced photosynthetic quantum yield in the acidic areas (Bulychev *et al* 2001, Krupenina *et al* 2008). Suppression of the banding phenomenon is expected in the dark assuming that it is dependent on photosynthetic light reactions and photo-induced electron transport (Bulychev and Krupenina 2008a). Nevertheless, studies of *Chara corallina* have shown that local illumination at a low intensity (190 μmol photons m^{-2} s^{-1}) can induce formation of alkaline bands where the photosynthetic quantum yield is reduced (Dodonova and Bulychev 2011). Therefore, it is possible that variations in the FL parameters, which were detected along both dark-adapted algae and DCMU-treated algae, were caused by the violet light excitation used in our setup. Local illumination may also induce the formation of an unidentified cytoplasmic messenger that activates ion channel transport (Beilby and Bisson 2012, Quade *et al* 2022), suggesting its possible contribution to the banding phenomenon. Thus, while the banding results in natural physiological variability of the measured FL parameters, the alterations along the internodal cell did not change significantly over the measurement time and do not impair applicability of the selected FL parameters to discriminate the effect of external stressors in *N. obtusa* internodal cells.

Using exponential models, a quantitative description of light-induced changes in the FL parameters is given for the first time. Any of the selected FL parameters gave statistically significant results for distinguishing the autofluorescence spectra of *N. obtusa* cells recorded after different illumination without DCMU (figure 6). Similarly, each of the parameters allowed us to separate DCMU-treated and untreated cells kept in darkness, or under maintenance light. However, when additional white illumination was considered, both FL parameters were required for the effective discrimination. For example, the F680/F750 ratio, but not F680, differed in DCMU-treated and untreated cells after 15 min illumination. Additionally, in cells treated with DCMU, the effect of white light illumination at the higher dose differed from that induced by the lower dose by a decrease in F680, but not in the F680/F750 ratio (figure 6, table S2). Notably, algal cells exposed to white LED light for 30 min were indistinguishable from DCMU-treated cells, indicating that the higher applied light dose induced photoinhibition of PSII. This process is known to inactivate the reaction center of the D1 protein (Long *et al* 1994), resulting in a blockade of electron transfer to PQ—the same effect that is caused by DCMU.

Visualization of spectral data in PCA space confirmed a clear separation between the autofluorescence signals recorded in algal cells kept under the selected conditions, with the exception of the spectra recorded after a higher dose of illumination. The fact that most of the variability in the data was explained by two principal components supports the use of two FL parameters (e.g., F680 and the F680/F750 ratio) to distinguish the effects of selected environmental stressors. Comparing our proposed FL parameters has an advantage over PCA, because it provides sufficient accuracy, but does not require additional data preprocessing steps such as centering and scaling, and the FL intensity only needs to be analyzed at the selected wavelengths.

5. Conclusions

In the present study, we showed that changes in *N. obtusa* autofluorescence parameters, particularly F680 values and the F680/F750 ratio measured at room temperature, can be used for effective detection of stress-inducing factors affecting photosynthetic activity. The exceptional morphological characteristics of the internodal macroalgae cells allowed us to avoid reabsorption, which affects both the shape and intensity of the main FL peak and is a common problem in FL measurements of plants *in vivo*. The suitability of the selected spectral parameters to detect and distinguish the effects of ambient factors such as changes in temperature remains to be investigated. However, the ability to distinguish the effect of the herbicide DCMU not only on dark-adapted cells, but also in the case of light-adapted cells and even after certain doses of excess illumination suggests that the proposed analysis of FL parameters may be useful for integration into remote sensing devices identifying external stressors of *N. obtusa* or other algae cells in the field.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Declarations

The authors have no competing interests to declare that are relevant to the content of this article.

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