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CHLOROPHYLL *A* FLUORESCENCE AND ABSORPTION IN TWO *CHLAMYDOMONAS* SPECIES

FLUOROSCENCJA I ABSORPCJE CHLOROFILU *A* DWÓCH GATUNKÓW *CHLAMYDOMONAS*

Abstract: Phytoplankton densities in lakes and oceans are often measured via *in vivo* chlorophyll *a* (Chl *a*) fluorescence. This quick and non-invasive method has large advantages over traditional sampling and extraction methods. Here we hypothesize that measurements of *in vivo* fluorescence might overestimate the actual Chl *a* concentration when algal cells contain relatively high concentrations of Chl *a* degradation products, as a result of reaching the stationary phase in growth or living in a stress-full environment. Therefore the *in vivo* and *in vitro* fluorescence of Chl *a* was measured in two species of *Chlamydomonas* and compared with total Chl *a* content. Regular sampling over the full range of their growth curves was obtained. *Chlamydomonas reinhardtii* was selected as a species living in neutral, non-stressed environments and *Chlamydomonas acidophila* inhabits very acidic (pH 2.0–3.4), stress-full environments. Scattering of fluorescence during *in vivo* measurements resulted in an on average 25-fold lower Chl *a* concentration compared with *in vitro* measurements in both species. Cells of *C. reinhardtii* scattered approx. 1.5-fold more of the *in vivo* fluorescence than *C. acidophila*. The cellular Chl *a* content increased during the first fortnight period in both *Chlamydomonas* species. After reaching its maximum, the cellular Chl *a* content decreased with time in both species. This decrease was not accompanied by an increase of Chl *a* degradation products. The percentage of Chl *a* degradation products to total Chl *a* concentration was not significantly different between *C. acidophila* and *C. reinhardtii*; both species containing approximately 16 % of Chl *a* degradation products to total Chl *a*. Only 73–80 % of the concentration of Chl *a* measured by the *in vitro* fluorometric method was recovered in the HPLC. Therefore, despite the settings of the fluorometer, fluorescence possibly overestimated the Chl *a* concentration. In conclusion we find that external low pH or stationary growth does not result in increased concentrations of degradation products of Chl *a*. In addition, the extrapolation from the *in situ* detection of Chl *a* fluorescence with multiparameter sensors to concentrations of Chl *a* must be performed with great care as the use is subject to species-specific scattering of the fluorescence signal.

Keywords: *Chlamydomonas reinhardtii*, *Chlamydomonas acidophila*, freshwater ecology, chlorophyll *a* fluorescence, chlorophyll *a* absorption, *in vivo* measurements, *in vitro* measurements, growth, degradations products, multiparameter probe, HPLC

During the last decades, new methods have been introduced that detect *in situ* chlorophyll *a* (Chl *a*) fluorescence in order to estimate the phytoplankton population density and distribution over the water column [1]. Such measurements are non-

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-invasive, fast, labour extensive and do not produce toxic waste, and are therefore very popular. Changes of *in vivo* Chl *a* fluorescence over time in freshwater has been recognized to be a good way to identify the factors controlling phytoplankton growth [2]. By using the fluorescence of Chl *a*, only the phytoplankton and not the bacteria or suspended matter is detected.

However, dependent on the growth phase or stress condition of an algal cell, Chl *a* can be converted to its degradation products chlorophyllide *a* and pheophytin *a* [3–4]. Besides growth phase and pH, numerous other variables can influence the degradation of Chl *a*, such as the presence of predators, oxygen concentration and microbial processing [5]. Under metal contaminated conditions, the exchange of magnesium by zinc is possible, changing the molecular characteristics of Chl *a*, but still enabling its use in light-harvesting [6]. The degradation products of Chl *a* have other absorption characteristics than Chl *a* and also another *in situ* fluorescence, but do not necessarily hamper or change light harvesting processes. It has been suggested that Chl *a* fluorescence in stress-full environments is higher than in other water bodies and that this enhanced fluorescence overestimates the Chl *a* concentration and consequently phytoplankton biomass present in the lake.

In a study to resolve the importance of the concentration of degradation products of Chl *a* compared to total Chl *a* concentration, Stich and Brinker [7] compared with uncorrected versus corrected Chl *a* concentrations taken from six different lakes in south-western Germany over a two-year period. The uncorrected Chl *a* concentration consisted of the sum of Chl *a* and its degradations products, whereas corrected ones consisted of Chl *a* concentrations only (the correction was performed via acidification of the extract that transforms all Chl *a* into pheophytin *a*). They concluded that uncorrected values were more reliable than corrected values, the method to determine uncorrected values was less timeconsuming and values measured on different types of spectrophotometers were comparable [7]. Their study emphasized that in lakes, the concentration of Chl *a* degradation products to the total Chl *a* concentration are usually insignificant.

Concentrations of Chl *a* and its degradation products can be measured using different methods, see eg [8–9]. Basically, two methods can be distinguished, a photometric and a fluorimetric determination after the extraction of the pigments in an alcohol. The *in situ* Chl *a* concentration can also be detected by these two methods, but scattering and re-absorption characteristics of particles prohibit quantification. Large effort has been made into the establishment of a reproducible and accurate determination of Chl *a* concentrations. In numerous studies, measuring devices, extraction solvents and procedures have been compared (eg [10–12]). From these comparisons, no method has been considered superior, as the efficiency of the extraction method and solvent varies with, for example, the composition of the phytoplankton community. When properly extracted, a fluorometric method with certain lamp and filter settings allows a fast and more reliable determination of Chl *a* in presence of Chlorophyll *b* (Chl *b*) and pheopigments than the photometric methods described thus far [13]. However, the method will not discriminate chlorophyllide *a* from Chl *a* because the two pigments have identical spectral properties, and only chromatographic analysis (HPLC) can separate between these two.

With the increasing use of *in situ* fluorescence probes it is of general interest to know to what extend measurements of the *in situ* fluorescence of Chl *a* equal those of the extracted concentration and, to know to what extend the *in situ* fluorescence signal is influenced by Chl *a* degradation products. Therefore, we compared the Chl *a* absorption and fluorescence of two *Chlamydomonas* species over their growth curve. To possibly detect the influence of external pH on the accumulation of Chl *a* degradation products we selected the acidophilic species *Chlamydomonas acidophila* and the mesophile *Chlamydomonas reinhardtii*.

Chlamydomonas species can be found in a wide variety of habitats worldwide [14]. *Chlamydomonas acidophila* is a dominant phytoplankton species in very acidic lakes [15–[16]. Although the pH of these lakes is extremely low, the internal pH of *C. acidophila* is neutral [17–18]. The maintenance of the large pH gradient over the plasma membrane is considered a stressful condition, likely enhancing metabolic costs [17]. *Chlamydomonas reinhardtii* is a well-described species abundant in neutral, eutrophic fresh water lakes and soils [19–23]. As a consequence of the neutral pH of its environment, *C. reinhardtii* has only a minor pH gradient over its plasma membrane.

Methods

Cultures

Growth experiments were started with 7 day-old pre-cultures of *Chlamydomonas reinhardtii* Dangeard (Algal collection Göttingen, SAG 11-32b) and *Chlamydomonas acidophila* Negoro (SAG 2045).The cell density at the beginning of the experiment were between 10,000 and 80,000 cells cm^{-3}. *C. reinhardtii* was grown in Woods Hole Medium at pH 7.0 [24].

C. acidophila was grown in 111 Medium at pH 2.65 [25]. The 111 Medium reflects the chemical composition of the water from Lake 111; an acidic, metal-rich lake in Lusatia, Germany. Cultures were maintained at $20 \pm 1\ ^{o}C$ at a light: dark cycle of 16:8 h. Incident light irradiance was 80 µmol PAR $m^{-2}\ s^{-1}$. Experiments were performed in triplicate. Every day or every second day about 80 cm^3 was aseptically removed from each culture for measurements.

Determination of cell density

For cell density, samples were fixed with 1 % Lugol solution and counted on an automatic particle counter (Casy 1, Model TT, Schärfe, Reutingen, Germany). Growth rates (µ) were calculated over the first 4 days assuming exponential growth.

Fluorometry

For *in vitro* determination of Chl *a* fluorescence, algal culture was filtered on Whatman GF/F filters, immediately rinsed with 0.1 M ammonium acetate [3] and extracted in 90 % ethanol by vigorous shaking for 5 minutes. The extract was then measured on a fluorometer (Turner TD-700, F4T41/2B2 lamp, ex: 436 nm (FS10), em:

680 nm (FS10), GAT Bremerhaven, Germany), which was calibrated with a commercially obtained Chl *a* standard (Sigma, Germany).

In vivo Chl *a* fluorescence was determined directly on life culture suspensions, eventually diluted with culture medium to avoid changes in the sensitivity settings of the fluorometer.

HPLC analysis

For total pigment analysis on HPLC, cultures were filtered as described above, immediately frozen in liquid nitrogen and stored at –80 °C till later analysis. Extraction with 90 % acetone was performed in dark and cold conditions using a Mini-Beadbeater (Biospec Products, Bartlesville, OK, USA). HPLC analysis was done following Gilmore and Yamamoto [26] using an ODS1-column (non-end capped, lightly carbon-coated, Spherimage-80, 5 µm, Knauer, Berlin, Germany). Absorbance was recorded at λ = 440 nm, 470 nm, 647 nm and 665 nm with a multiwavelength UV/VIS detector (PHD 601, GAT, Bremerhaven, Germany), but concentrations were only calculated by using the signal at λ = 440 nm. The system was calibrated using a commercial standard of Chl *a* and Chl *b* (Sigma, Germany).

Multiparameter probe

Fluorescence of Chl *a* from cell suspensions of life algal cultures with various cell density was measured with a multiparameter probe (Ocean Seven 316, with an integrated Seapoint chlorophyll fluorometer, Idronaut, Italy). The Seapoint chlorophyll fluorometer (SCF) uses modulated blue LED lamps and a blue excitation filter to excite chlorophyll *a*. The fluorescent light emitted by the chlorophyll *a* passes through a red emission filter and is detected by a silicon photodiode. More specific, the fluorometer had the following filters: ex: 470 nm CWL (30 nm FWHM), em: 685 nm CWL (30 nm FWHM). These settings suggest the detection of *Chl b* and pheopigments besides Chl *a* [13]. *In vivo* fluorescence of the same suspensions was simultaneously measured on the calibrated fluorometer as described above.

Calculations were performed in Excel software (Microsoft Office Excel 2003). Tests for significance were performed using SPSS software (version 15.0 for Windows, Chicago IL).

Results

Growth curves

Despite the difference in pH of the medium, no significant differences in growth rates between the two *Chlamydomonas* species were detected (Fig. 1). The exponential growth rates over the first 4 days were calculated as 0.52 ± 0.14 d^{-1} for *C. reinhardtii* and 0.39 ± 0.15 d^{-1} for *C. acidophila* (mean ± SE). No lag-phase in growth was detected in any of the cultures and exponential growth started immediately after inoculation. In both *Chlamydomonas* cultures, the stationary phase was reached after approximately

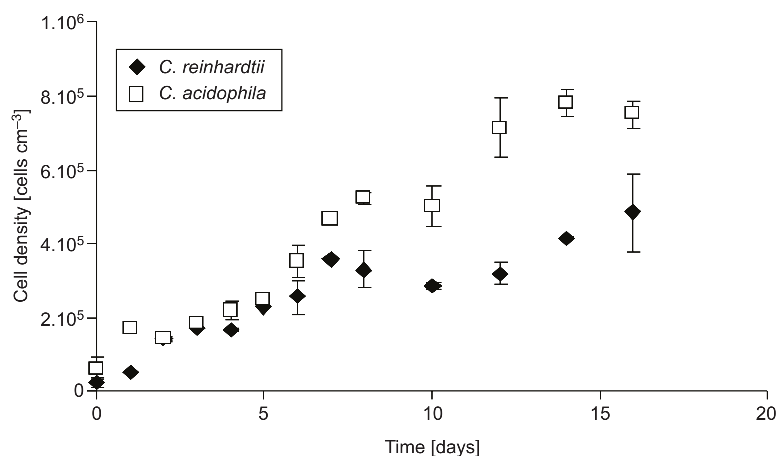

Fig. 1. Cell densities in [cells cm^{-3}] of *C. reinhardtii* and *C. acidophila* over time (in days) in batch culture (Mean ± SE of 3 replicates)

7–8 days. Increases in densities after the onset of the stationary phase were likely the result of evaporation or non-exponential growth resulting from increased CO_2 availability to the algal cells due to increased surface to volume ratio in the Erlenmeyer flasks. After reaching the stationary phase, *C. acidophila* cultures were denser than cultures of *C. reinhardtii*, this difference being nearly 2-fold.

In vivo Chl *a* fluorescence

The *in vivo* fluorescence increased over time, similar to increases in cell density for both Chlamydomonas species (Fig. 2). The increase in Chl *a* fluorescence was faster in

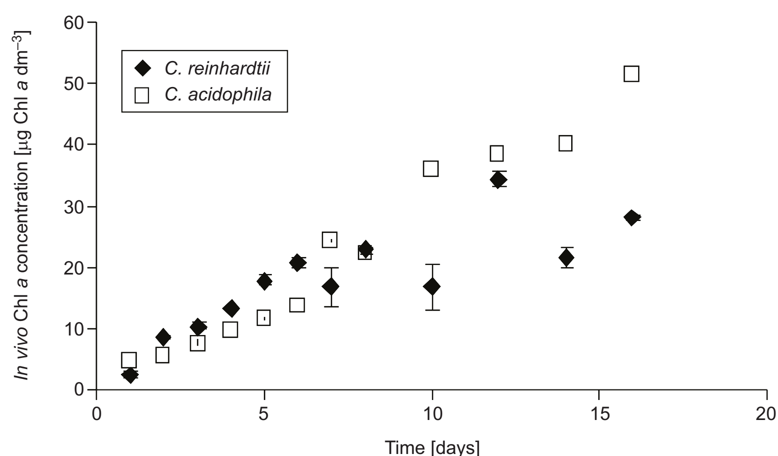

Fig. 2. *In vivo* fluorescence of Chl *a* in [µg Chl *a* dm^{-3}] in cultures of *C. reinhardtii* and *C. acidophila* over time (in days) in batch cultures (Mean ± SE of 3 replicates)

cultures of *C. reinhardtii* than in those of *C. acidophila* (3.5 and 1.9 μg Chl *a* dm^{-3} d^{-1} for *C. reinhardtii* and *C. acidophila*, respectively). Although the differences of Chl *a* fluorescence in both species reached in the stationary phase was smaller than the difference in cell density (Fig. 1), comparable to differences in cell density, *in vivo* fluorescence of Chl *a* was greater in cultures of *C. acidophila* than in cultures of *C. reinhardtii* (Fig. 2).

Cellular Chl *a* content

The cellular Chl *a* content of both *Chlamydomonas* species increased over time (Fig. 3). After reaching the highest value, the cellular Chl *a* content decreased again in *C. reinhardtii*, whereas it remained more or less constant in *C. acidophila* (Fig. 3). The results show a faster increase of cellular Chl *a* content in *C. reinhardtii* during the first 10 days of culturing, but equal cellular Chl *a* contents in both species after 16 days. The maximum cellular Chl *a* content in *C. reinhardtii* was 2.8 pg $cell^{-1}$ and in *C. acidophila* only 1.4 pg $cell^{-1}$ (Fig. 3).

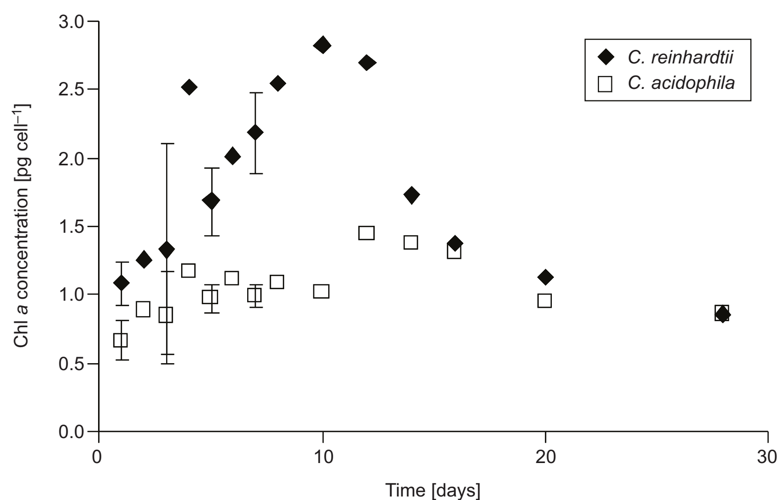

Fig. 3. Cellular chlorophyll *a* content in [pg Chl *a* $cell^{-1}$] over time (in days) in batch cultures of *C. reinhardtii* and *C. acidophila* (Mean ± SE of 2 replicates)

In vitro and *in vivo* fluorescence of Chl *a*

There was a linear relationship between the *in vitro* fluorescence and the *in vivo* fluorescence of Chl *a* in both species (Fig. 4a, b). In both species the measurement of the *in vivo* Chl *a* fluorescence was lower than that of the *in vitro* fluorescence**.** On average, the *in vivo* Chl *a* concentration was about a 23-fold lower than the *in vitro* concentration. For *C. reinhardtii* the *in vivo* Chl *a* fluorescence was 31-fold lower than the *in vitro* fluorescence (Fig. 4a) and for *C. acidophila* this was only 15-fold (Fig. 4b).

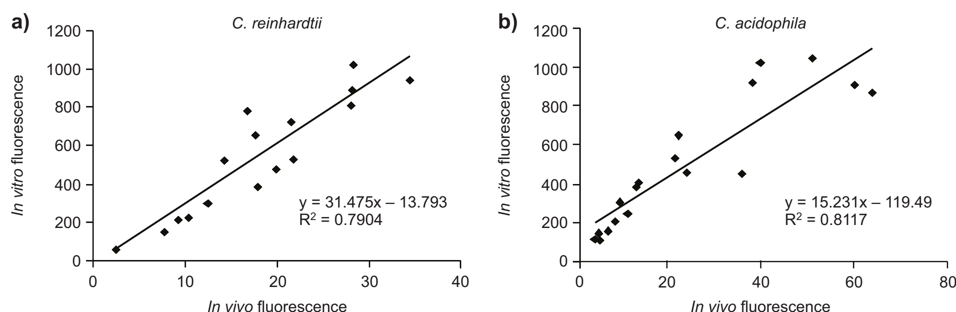

Fig. 4. The *in vitro* Chl *a* fluorescence in relation to the *in vivo* fluorescence in *C. reinhardtii* (a) and *C. acidophila* (b)

These results therefore show that *C. reinhardtii* scatters approximately 1.5-fold more of the emitted light than *C. acidophila*.

Comparison between fluorescence and absorption

The concentration of Chl *a* obtained via HPLC was linearly related to the concentration of Chl *a* obtained via fluorescence in both Chlamydomonas species (Fig. 5a, b). The slopes of the curves reveal that only 73–80 % of the concentration of Chl *a* measured via fluorescence was recovered when measuring via absorption in the HPLC (Fig 5a, b; Table 1). The recovery increased to 86–91 % when the concentration of degradation products of Chl *a* were included in the analysis of this linear relationship (Table 1). These degradation products mainly consisted of chlorophyllides *a* as hardly any pheophytin a was detected (results not shown).

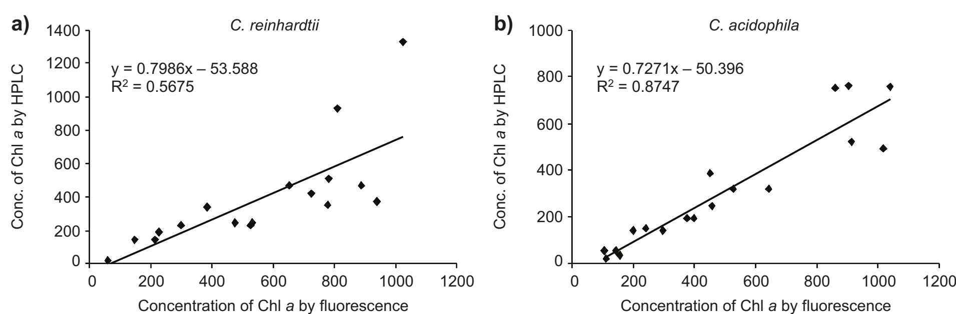

Fig. 5. The concentration of Chl *a* as measured by HPLC in relation to the concentration of Chl *a* as measured by fluorescence in *C. reinhardtii* (a) and *C. acidophila* (b). The fluorescence on both axis is given in [μg Chl *a* dm^{-3}]

The recovery of measurements of the Chl *a* concentration in the HPLC was always higher in *C. reinhardtii* than in *C. acidophila*, suggesting that either the measurement of total pigment composition via HPLC was more problematical in *C. acidophila* or that

the Chl *a* molecules in *C. acidophila* were more fluorescent (Table 1). These differences were, however, smaller than 5 % and were therefore considered not important.

Table 1

The square of the correlation coefficient (R^2) and the slope of linear regressions through the concentration of Chl *a* or Chl *a* + degradation products as measured by HPLC against Chl *a* fluorescence as measured by fluorometer

		R^2	Slope
C. reinhardtii	Chl *a* concentration	0.57	0.80
	Chl *a* + degrad. prod. concentration	0.60	0.91
C. acidophila	Chl *a* concentration	0.87	0.73
	Chl *a* + degrad. prod. concentration	0.92	0.86

Concentration of degradations products of Chl *a*

When analysing the percentage of Chl *a* degradation products to total Chl *a* concentration in both *Chlamydomonas* species, it was observed that there was no significant difference between *C. reinhardtii* and *C. acidophila* (Paired T-test, t = –2.1, df = 13, p = 0.056; Fig. 6). On average, the percentage of Chl *a* degradation products to total Chl *a* concentration was slightly higher in *C. acidophila* compared with *C. reinhardtii*, but this difference was not significant (ie 5.6 %). In addition, the percentage of Chl *a* degradation products in relation to the concentration of Chl *a* did not change over growth in *C. reinhardtii* (ANOVA, F = 1.13, df = 1,12, p = 0.31) and also not in *C. acidophila* (ANOVA, F = 3.97, df = 1.12, p = 0.07; Fig. 6).

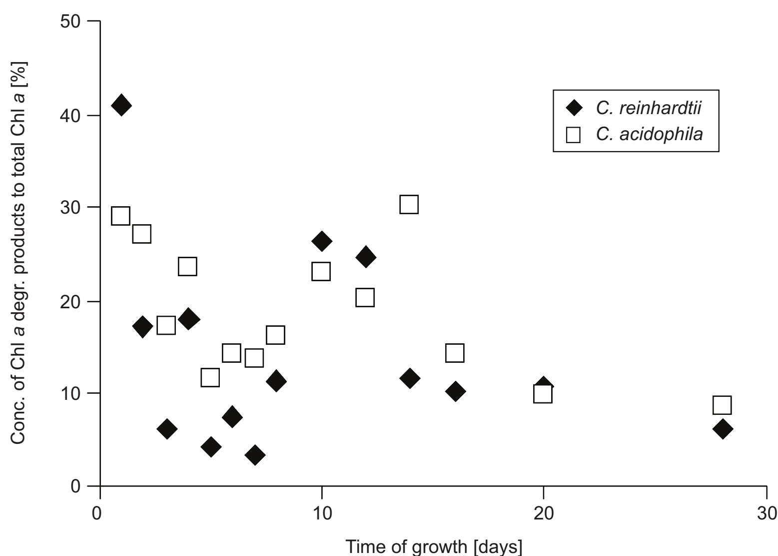

Fig. 6. The percentage of Chl *a* degradation products to total Chl *a* concentration in *C. reinhardtii* and *C. acidophila* over the time of growth

Multiparameter probe

The measurements of Chl *a* fluorescence made by the multiparameter probe were linearly related to the measurements of Chl *a* fluorescence gathered by the fluorometer (Fig. 7). The measurements reveal that the same signal of the multiparameter probe (here in mV) resulted in a higher fluorescence of Chl *a* in the fluorometer in *C. acidophila* than in *C. reinhardtii* (Fig. 7). The difference in fluorescence between the two *Chlamydomonas* species was approximately 1.5-fold.

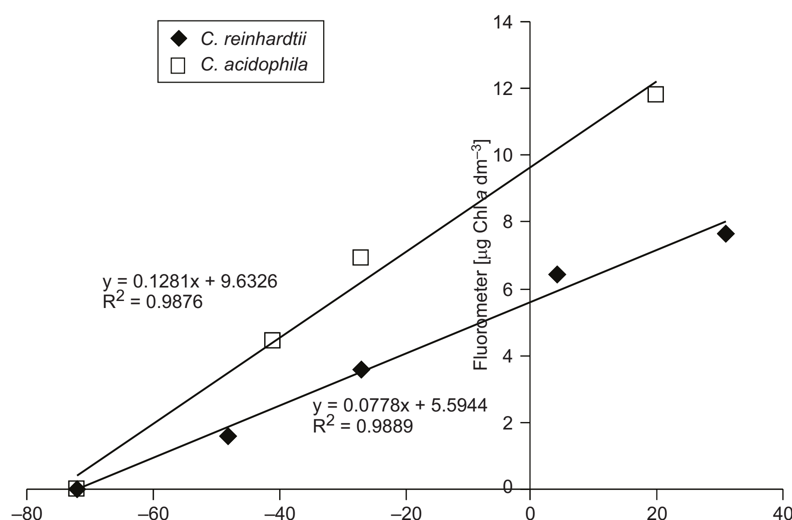

Fig. 7. *In vivo* chlorophyll a fluorescence of *C. reinhardtii* and *C. acidophila* measured with a fluorometer in relation to measurement with a multiparameter sensor

Discussion

In this study we compared the chlorophyll a fluorescence and absorption in an acidophilic and a mesophilic *Chlamydomonas* species over their growth curve. We hypothesized that stress conditions such as the stationary phase in growth or a low external pH increases the cellular concentration of Chl *a* degradation products and consequently enhances fluorescence of Chl *a* compared with its absorption.

In contrast to expectations, no increase in the concentration of Chl *a* degradation products was found in either *Chlamydomonas* species over the course of time of growth in batch culture. Several studies suggest that stressful conditions, such as nutrient limitation, high oxygen concentrations, or the presence of predators will enhance the conversion of Chl *a* to its degradation products, chlorophyllide *a* and pheophytin *a* [3–5]. During batch growth, light or nutrients start limiting growth and upon entering the stationary phase, stress increases by some growth limiting condition. In support to our findings, recent experiments with *C. acidophila* showed [27] no enhanced

concentration of pheophytin a to Chl *a* + pheophytin *a* in P-limited cells compared with P-saturated ones.

In our study, the maximum cellular Chl *a* content of *C. acidophila* (1.4 pg $cell^{-1}$) was approximately 2-fold lower than that of *C. reinhardtii* (2.8 pg $cell^{-1}$), although the acidophilic species can reach a Chl *a* content similar to that of *C. reinhardtii*, ie 2.2–2.5 pg $cell^{-1}$ [28–29]. Obviously, some nutrient in the 111 Medium inhibited the Chl *a* synthesis. Studying the elemental ratios in the medium provokes that Chl *a* synthesis is most likely inhibited by a limitation of nitrogen, provided as ammonium [25]. The molar N to P ratio in 111 Medium is 3.7, whereas this is 20 in, for example, Woods Hole medium [24]. A molar N : P ratio below 16 would suggest N-limiting conditions according to the Redfield ratio [30]. An N-limitation can explain the low cellular Chl *a* content in *C. acidophila* as it will decrease the Chl *a* content in algae [31].

Between the results gathered from the two *Chlamydomonas* species only a minor difference was found, this being a slightly higher fluorescence of Chl *a* compared with its absorption measured *via* HPLC in *C. acidophila* than in *C. reinhardtii*. Assuming a higher fluorescence of Chl *a* degradations products than from Chl *a* itself, the results suggests that *C. acidophila* contains more degradation products than *C. reinhardtii*. The latter was found although the difference was not significant (Fig. 6), and therefore our results support this hypothesis. The enhanced concentrations of Chl *a* degradation products (especially in the form of chlorophyllide *a*) in *C. acidophila* could both be the result of the low external pH in the growth medium, but could also have resulted from the N-limitation. In contrast, Meyns et al [32] obtained a lower Chl *a* concentration of 10 to15 % in HPLC than in absorption measurements. They assumed that the presence of degradations products provided part of the explanation for this difference, but could not fully explain their observations. Possibly, the lower Chl *a* concentration estimation by HPLC is a general problem related to the attachment and detachment of Chl *a* molecules to the column?

There are several well-known degradation products of Chl *a*: pheophytin *a*, chlorophyllide *a* and pheophorbide *a* [8, 33]. In addition, a red pigment accumulated during growth in a Chl *b*-less mutant of *C. reinhardtii* that was considered a breakdown product of Chl *a* [34]. This red pigment had a larger similarity to carotenoids than chlorophylls in its absorption characteristics and was water soluble. In contrast to their experiments under aerobic conditions [34], under anaerobiosis pyropheophorbide *a* was the main Chl *a* degradation product [22]. Pyropheophorbide *a* is a pigment more similar to pheophytins in its absorption characteristics. The difference in Chl degradation in response to oxygen concentration can explain the presence of degradation products in field samples and might explain the accumulation of pheopigments in stationary phase grown algal cultures, when cell densities are very high and oxygen gradients within the culture can be significant.

A full description of Chl *a* degradation to other products such as chlorophyllide *a* is provided by Mantile et al [33]. In the HPLC measurements, we detected mainly chlorophyllide *a* and hardly any pheophytin *a*. This is a logic result of the fact that pheophytin *a* does not absorb much light at λ = 440 nm, the wavelength of our Chl *a* detection in the HPLC. In contrast, the absorption spectrum of chlorophyllide *a* is

largely identical to that of Chl *a* and both pigments absorb well at λ = 440 nm [8]. The absorption spectra of pheophytin *a* and pheophorbide *a* are also alike and are called pheopigments. Welschmeyer [13] described a fluorometric method with a certain lamp and filter settings that allows for a reliable measurement of Chl *a*, without interference of Chl *b* and pheopigments. However, the fluorescence method will not discriminate chlorophyllide *a* and Chl *a* because they have nearly identical absorption spectra. Most likely, the enhance concentrations of chlorophyllide *a* in our fluorescence measurements resulted in an overestimation of the 'true' Chl *a* concentration by minimal 10 and maximal 27 %.

The percentage of Chl *a* degradation products to total Chl *a* concentration ranged between 3 and 40 %, the average value being only 16 % (Fig. 6). These results support the study from Stich and Brinker [7] who emphasized that in lakes, the concentration of Chl *a* degradation products to the total Chl *a* concentration are usually insignificant. Unfortunately, in their investigations only a comparison between acidified and non-acidified extracts was made, without any information on the possible concentrations of Chl *a* degradation products [7].

The use of *in vivo* fluorescence measurements via multiparameter probes has become eg [1, 35] and it is therefore of importance to what extend measurements of the *in situ* fluorescence of Chl *a* equal those of the extracted concentration and to know to what extend the *in situ* fluorescence signal is influenced by Chl *a* degradation products. In Jeffrey et al [8] contour plots of Chl *a* are provided recorded in the Pacific Ocean from measurements with a calibrated multiparameter probe and with HPLC, showing remarkably similar contours (Fig. 4.14, page 150). In contrast, our data show already 1.5-fold differences in scattering and re-absorption characteristics between two species of *Chlamydomonas*, expecting much larger differences in measurements on natural phytoplankton. The advantages of using a multiparameter probe in measuring the *in vivo* Chl *a* concentration (ie non-invasive, fast, labour extensive and not producing any toxic waste) largely compensate for the disadvantages (ie calibration difficulty). Based on our results we emphasize to take care for interpreting the *in vivo* Chl *a* concentration into the Chl *a* quantification as the *in vivo* fluorescence will be influenced by concentrations of eg chlorophyllide *a*, Chl *b*, species specific scattering and re-absorption of emitted light.

Acknowledgements

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FLUOROSCENCJA I ABSORPCJE CHLOROFILU *A* DWÓCH GATUNKÓW *CHLAMYDOMONAS*

Abstrakt: Zagęszczenia fitoplanktonu w jeziorach i oceanach często jest mierzone *in vivo* za pomocą fluorescencji chlorofilu *a* (Chl *a*). Ta szybka i nieinwazyjna metoda ma dużą przewagę nad tradycyjnymi metodami pobierania próbek i ekstrakcji. W tej pracy badamy hipotezę, że pomiary *in vivo* fluorescencji rzeczywistego stężenia Chl *a* mogą prowadzić do zawyżonych ocen stężeń, jeśli komórki glonów zawierają stosunkowo duże stężenia produktów rozpadu Chl *a* w wyniku osiągnięcia stanu stacjonarnego wzrostu lub w wyniku przebywania w środowisku zawierającym wiele czynników stresowych. Zmierzono fluorescencję Chl *a in vivo* i *in vitro* dla dwóch gatunków *Chlamydomonas* i porównano z całkowitą zawartością Chl *a*. Uzyskano próbki w pełnym zakresie ich krzywej wzrostu. *Chlamydomonas reinhardtii* został wybrany jako gatunek żyjący w warunkach naturalnych, nie stresowych, a *Chlamydomonas acidophila* zamieszkuje stresogenne, bardzo kwaśne środowisko (pH 2,0–3,4). Rozpraszanie fluorescencji w czasie pomiarów *in vivo* wskazywało na średnio 25-krotnie mniejsze stężenie Chl *a* w porównaniu z pomiarami *in vitro* dla obu gatunków. W warunkach *in vivo* komórki *C. reinhardtii* rozpraszały ok. 1,5-krotnie silniej niż *C. acidophila*. W okresie pierwszych dwóch tygodni eksperymentu zawartość Chl *a* w komórkach rosła u obu gatunków *Chlamydomonas*. Po osiągnięciu maksimum zawartość Chl *a* zmniejszała się z czasem u obu gatunków. Stosunki zawartości produktów rozpadu Chl *a* do całkowitej zawartości Chl *a* nie różniły się statystycznie istotnie pomiędzy *C. acidophila* i *C. reinhardtii*. Oba gatunki zawierały około 16 % produktów degradacji Chl *a* w stosunku do całkowitego jego stężenia. Tylko 73–80 % stężenia Chl *a* mierzonego metodą fluorymetryczną *in vitro* zostało oznaczone za pomocą HPLC. Dlatego też, niezależnie od ustawienia fluorymetru, metoda fluorescencyjna prawdopodobnie zawyża stężenie Chl *a*. W rezultacie okazuje się, że niskie zewnętrzne pH lub stacjonarna równowaga wzrostu nie powodują zwiększenie stężenia produktów rozkładu Chl *a*. Ponadto ekstrapolacje fluorescencyjnego wykrywania Chl *a in situ* za pomocą czujnika wieloparametrowego do stężenia Chl *a* muszą być wykonane z dużą starannością ze względu na zależność rozpraszania fluorescencyjnego od rodzaju badanego gatunku.

Słowa kluczowe: *Chlamydomonas reinhardtii, Chlamydomonas acidophila*, ekologia wód słodkich, fluorescencja chlorofilu *a*, absorpcja chlorofilu *a*, pomiary *in vivo*, pomiary *in vitro*, wzrost, produkty rozkładu, sondy wieloparametrowe, HPLC