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Removing the light history signal from normalized variable fluorescence (F_v/F_m) measurements on marine phytoplankton

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Abstract

Variable fluorescence normalized to maximal fluorescence, F_v/F_m, determined by Fast Repetition Rate Fluorometry (FRRF) is being increasingly used to compare photosynthetic electron transport capacity in natural phytoplankton communities. Interpreting results of such studies is, however, complicated by the fact that both nutrient status and light history (photoinhibition under in situ conditions) are known to influence F_v/F_m. Thus, the value of F_v/F_m measurements in the field would be greatly enhanced if the light history signal could be separated from other influences. Here, both field and laboratory studies demonstrate that dark treatment (30 min–4 h) before FRRF measurement is not sufficient to remove a light history signal in F_v/F_m. The signal could, however, be essentially eliminated by incubation of samples in low light prior to F_v/F_m determination. For the study conditions tested, the most effective treatment for removal of the signal was 4 h at 50 μmol m^{-2} s^{-1}. However, the effectiveness of the light treatment in removing the light history was influenced by temperature. Therefore, no universal protocol for eliminating the light history signal can be developed, but recommendations are given for developing site-specific approaches for separating the light history signal from other factors influencing F_v/F_m. Carrying out light incubations before determining F_v/F_m not only provides the possibility for eliminating a light history signal in the measurements but the difference between F_v/F_m measured after light and dark incubations appears also to be a potentially useful indicator of the degree of photoinhibition experienced by phytoplankton under natural conditions.

Fast Repetition Rate Fluorometry (FRRF) in general has, over the last two decades, proven itself to be a valuable tool for the study of photosynthetic systems and processes in phytoplankton (e.g., Baker 2008; Smyth et al. 2004; Suggett et al. 2003). Variable fluorescence normalized to maximal fluorescence (F_v/F_m), in particular, has shown itself as a potentially valuable indicator of both the degree of photoinhibition (e.g., Heraud and Beardall 2000; Lesser and Farrell 2004; Samuelsson and Richardson 1982; Vassiliev et al. 1994) and the degree of nutrient limitation (Beardall et al. 2001 and references therein) experienced by phytoplankton. Although species and other differences (Kruskopf and Flynn 2005; Suggett et al. 2009) preclude interpreting F_v/F_m as an absolute indicator of the degree of nutrient limitation in natural populations, it does appear to be a universal response that F_v/F_m decreases in response to nutrient limitation. Thus, provided the photoinhibition and nutrient impacts on F_v/F_m can be separated, F_v/F_m is a potentially useful tool for interrogating natural phytoplankton populations with regard to their nutritional status as low values can be interpreted as suggesting nutrient stress.

It can also be noted here that other parameters derived from FRRF (i.e., absorption cross-section of PSII photochemistry, σ_{PSII}) are also expected to respond to nutrient stress. However, σ_{PSII} has been demonstrated to be more directly influenced by cell size than F_v/F_m (Suggett et al. 2009). As field sampling will often take place on populations with different

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size distributions, F_v/F_m becomes the most likely parameter determined through FRRF to have the potential to interrogate natural phytoplankton populations with respect to their nutritional status and to identify hydrographically or otherwise segregated populations in situ (e.g. Guidi et al. 2012; Richardson et al. 2014; Rynearson et al. 2013).

Variable fluorescence normalized to maximum fluorescence, F_v/F_m is determined following exposure of samples to darkness and is assumed to represent the maximum quantum efficiency of PSII photochemistry, i.e., the potential photochemical efficiency of open RCIIs (Baker 2008). That samples have been exposed to darkness before determination of F_v/F_m does not, however, necessarily imply that incident light conditions at the time of sample collection do not influence the resulting F_v/F_m determination.

Indeed, Vassiliev et al. (1994) observed a decrease in F_v/F_m in dark-adapted phytoplankton samples taken from environments where the light intensity was > ~ 800 μmol m^{-2} s^{-1} compared with samples from lower light intensities, and on a 2008 cruise studying the spring bloom in the North Atlantic, we identified a clear effect of light at the time of collection in F_v/F_m determinations made on samples taken from the surface layer, where the F_v/F_m ratios decreased significantly with increasing light intensity (Fig. 1) despite a 30-min dark adaptation of the samples before measurement.

The value of F_v/F_m as a diagnostic tool for identifying nutrient depletion (*see* Beardall et al. 2001) becomes greatly diminished in field studies when its measurement is influenced by the light climate (time of day or depth) of the sampling. Even under light-controlled laboratory conditions, its value as a comparative tool will be limited when different species are being examined as individual species have different responses to light climate. Thus, it is surprising that attempts to interpret F_v/F_m data from natural environments have not given greater consideration to the potential influence of light climate on the interpretation of the results and/or the possibility of eliminating the light history signal in F_v/F_m measurements, thereby simplifying the interpretation of identified differences between populations.

The purpose of this study was to examine how widespread a light history effect may be on determinations of F_v/F_m on natural populations and to investigate possible treatments for reducing or eliminating the signal relating to light history.

Materials and procedures

Field studies

We realized that a light history signal in F_v/F_m was affecting our measurements of F_v/F_m even at the comparatively low light intensities experienced in the North Atlantic (Fig. 1) while sampling at the onset of the spring bloom with the RV *KNORR* (Woods Hole Oceanographic Institution) from 2-20 May 2008 (NAB 2008 project http://www.bco-dmo.org/project/2098). On that cruise, triplicate sub-samples were collected for F_v/F_m determination from Niskin bottles sampling at selected depths

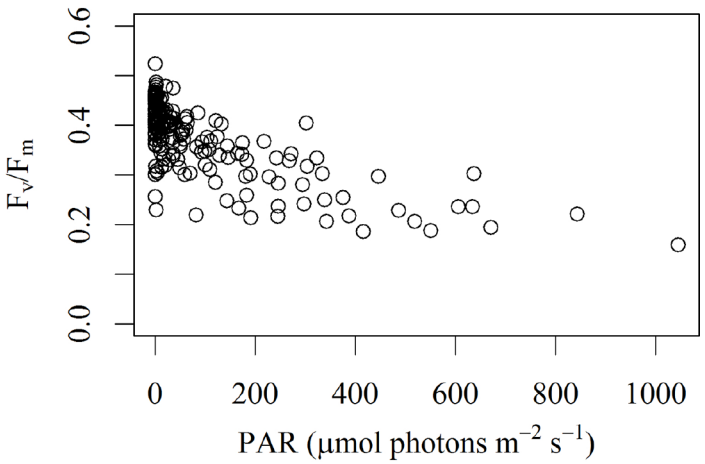

Fig. 1. F_v/F_m determined on natural samples collected at various depths in the water column in the North Atlantic during the period 2-20 May 2008 plotted against the light intensity at the time and depth of sample collection. Samples were dark incubated for 30 min before F_v/F_m determination.

in the euphotic zone. These samples were kept in dark bottles covered with aluminum foil to prevent light exposure and incubated for ≥ 30 min in an insulated box. F_v/F_m was measured within the dark chamber of a FAST*tracka* II fluorometer (Chelsea Instruments Group Ltd.) mounted and secured in the lab, using FAST*tracka* II firmware. A single turnover protocol with 30 sequences per acquisition, each including 100 saturation and 50 relaxation flashlets, was utilized. The sequence interval was set to 100 ms; the PMT eht (extra high tension) and LED light source (excitation peak of 470 nm) were optimized for each sample. F_v/F_m was calculated from saturation phase fits following (Kolber et al. 1998).

The developed procedure for removal of the light history signal (see below) was tested in the field on a cruise in the Sargasso Sea on RV *DANA* (Technical University of Denmark) in March-April 2014. In this case, two sub-samples were taken from Niskin bottles from 10 m. Two measurements were made on each sub-sample, thus yielding 4 measurements per study depth. The collected samples were stored either in 250 mL brown glass bottles in ambient laboratory light (approximately in situ temperature) for a minimum of 30 min before measurement or in 1 L clear glass Millipore Bluecap bottles exposed to cool white fluorescent light (approx. 50 μmol m^{-2} s^{-1}). The same instrument and overall procedure was used for determining F_v/F_m as on the *KNORR* cruise. The software used was, however, upgraded to FASTpro Version 2.5.3. A single turnover protocol with 12 sequences per acquisition, each including 100 saturation flashlets, was utilized. The sequence interval was set to 100 ms.

In both field studies, the average PAR recorded by the rosette-mounted light sensor at the depth and during the time of the sample collection (rosette lowered with CTD) in the Seabird CTD "bottle cast" file was taken to represent in situ light condition of the sample.

Laboratory studies

Cultures of four different marine phytoplankton species were obtained from the Scandinavian Culture Collection for Algae and Protozoa (SCCAP) housed at the University of Copenhagen. These included two diatoms: *Chaetoceros socialis* (K-0550) and *Attheya longicornis* (K-1530), a chlorophyte: *Brachiomonas cf. submarina* (K-0582) and a dinoflagellate: *Heterocapsa triquetra* (K-0447). These were maintained in culture medium L1 (http://www.sccap.dk/media/marine/2.asp) at 15°C and day/night regulated illumination of ~100 and 0 μmol m^{-2} s^{-1} for 16 h and 8 h, respectively.

Each culture was examined individually for a light history signal in measurements of variable fluorescence. Culture material was first diluted with L1 media to a degree where chlorophyll concentrations resembled natural field concentrations. Before determination of F_v/F_m (employing the same instrument and protocol as those used on the *KNORR* cruise), samples of this diluted culture material were incubated in 60 mL plastic (NUNC) culture bottles for 2 h at 14 different light intensities in a temperature-controlled (15°C) incubator. Samples were continuously rotated during incubation to maintain phytoplankton in suspension.

Subsequent investigations of the effect of various light/dark treatments and temperature on the presence of a light history signal in F_v/F_m determinations were carried out using the same incubation procedures using either *Attheya longicornis* or *Chaetoceros socialis* as the test organism. Possible "bottle effects" impacting F_v/F_m were investigated by incubating *A. longicornis* in NUNC bottles under the standard culture conditions (100 μmol m^{-2} s^{-1}, 15°C) and taking samples for determination of F_v/F_m at 10 different time intervals between 0 and 270 min. The possible influence of temperature on the effectiveness of the developed light treatment to remove the light history signal was tested for by incubating *C. socialis* at 14 different light intensities (15°C) and subsequently comparing the effectiveness of the treatment at 5 and 25°C.

Finally, the method developed to remove the light history signal in laboratory cultures was tested on natural samples. In the first of these tests, surface water samples collected from Langelinie Pier in Copenhagen during the period 25–28 Mar 2011 were incubated at 14 different light intensities as described above. During the post-incubation period and before determination of F_v/F_m, these were subjected either to darkness (30 min or 2 h) or light (2 h at 100 or 4 h at 50 μmol m^{-2} s^{-1}). In the second test of natural communities, samples were taken directly from in situ conditions (10 m and not incubated) in the Sargasso Sea and held either in darkness or light (4 h, ~50 μmol m^{-2} s^{-1}; further detail given under "Field studies").

Data analysis

Analyses were done in the free and open source statistical software R version 3.1.0 (R Core Team 2014). For samples from each incubation light intensity, the mean F_v/F_m was calculated, and the effect of increasing incubation light intensity was investigated by regressing the mean F_v/F_m values on light intensity using ordinary least square estimation. The specific light intensity where a light history signal became apparent was determined as the intercept between the linear regression of the decreasing curve and the horizontal line where no light history was detected. The differences in the effect treatment on removal of the light history were quantified by calculating the percentage change in F_v/F_m from PAR = 0 to PAR = 300 based on the regression slopes for each experiment.

Assessment

The light history signal (i.e., observed reduction in F_v/F_m for samples taken from or incubated at high light relative to F_v/F_m recorded for samples collected/incubated at low light intensities) identified in the North Atlantic sampling carried out in May 2008 was also found in all species tested in the laboratory (Fig. 2). For all species, no significant differences in F_v/F_m were noted at the lowest incubation light intensities, whereas a light history effect on F_v/F_m appeared at light intensities of between ~ 60 and 90 μmol m^{-2} s^{-1}, depending on species. The maximum F_v/F_m recorded for the dinoflagellate, *H. triquetra*, was lower than for the other species at all light intensities. There also appeared to be differences between species with respect to the magnitude of the reduction of F_v/F_m with increasing light intensity of incubation with the rate of reduction being slowest for *C. socialis* and greatest for *H. triquetra*. Increasing the length of the dark treatment between incubation and measurement of F_v/F_m from 30 min to 2 and 4 h had no influence on the light history signal.

The decrease in recorded F_v/F_m with increasing incubation light intensity suggests that the phenomenon is likely a result of photoinhibition. Indeed, Samuelsson and Richardson (1982) used a reduction in variable fluorescence to demonstrate that the onset of photoinhibition occurs in the dinoflagellate, *Amphidinium carterae*, at a light intensity somewhere between 15 and 80 μmol m^{-2} s^{-1}. The response patterns of the different phytoplankton groups studied also fit well the known responses of the different groups to light availability. The dinoflagellate appeared to be more sensitive to high light than the diatoms and chlorophyte, and it has earlier been shown that the growth of dinoflagellates tends, on average, to saturate at lower light intensities than that of diatoms or chlorophytes (Richardson et al. 1983).

PSII repair involves partial disassembly of PSII holocomplexes, degradation of the damaged subunit (most notably the D1 subunit) by specific proteases, incorporation of a newly synthesized subunit into the sub-complex, and reassembly of the holocomplex (Nixon et al. 2010; Nath et al. 2013). At the biochemical level, several of these processes require energy in the form of, e.g., ATP. We hypothesized, therefore, that if the source of the light history signal in F_v/F_m recorded here is a result of photoinhibition, that energy accumulation via photosynthesis during low light exposure of the samples might permit faster repair of the photosynthetic system, and that

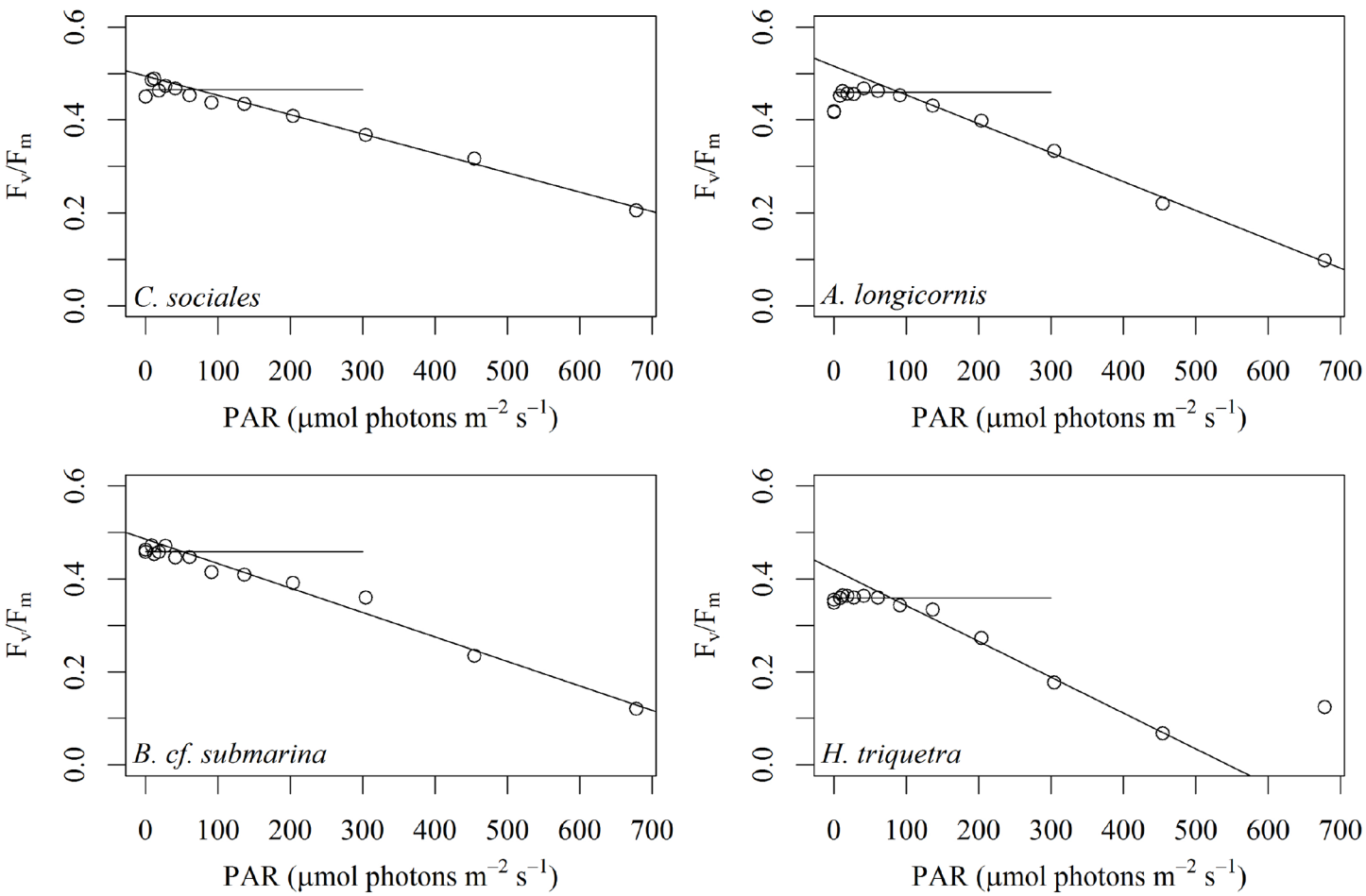

Fig. 2. F_v/F_m determined on four different species of phytoplankton following 2 h incubation to 14 different light intensities. Samples were dark incubated for 30 min before F_v/F_m determination.

this might potentially lead to the elimination of the light history signal.

This, indeed, turned out to be the case. Fig. 3 illustrates the F_v/F_m recorded in *A. longicornis* with post-incubation treatments of darkness (30 min and 2 h), 50 and 100 µmol m^{-2} s^{-1} (each for both 2 and 4 h), respectively. The post-incubation treatment (light or dark) greatly influenced the subsequent determinations of F_v/F_m for the samples, with the light-treated samples exhibiting a considerably higher F_v/F_m than those of the dark-treated. A post-incubation treatment of 200 µmol m^{-2} s^{-1} (2 h) was also tested, but this treatment yielded results that did not differ from those obtained after the dark post-incubation treatments suggesting that, at the higher light intensity, photo damage proceeds faster than PSII repair.

There is a slightly negative slope (Table 1) for all of the regression lines describing the results for samples measured after the different post-incubation light treatments, suggesting that the light history signal may not be entirely removed by the treatment. Comparing the different treatments, however,

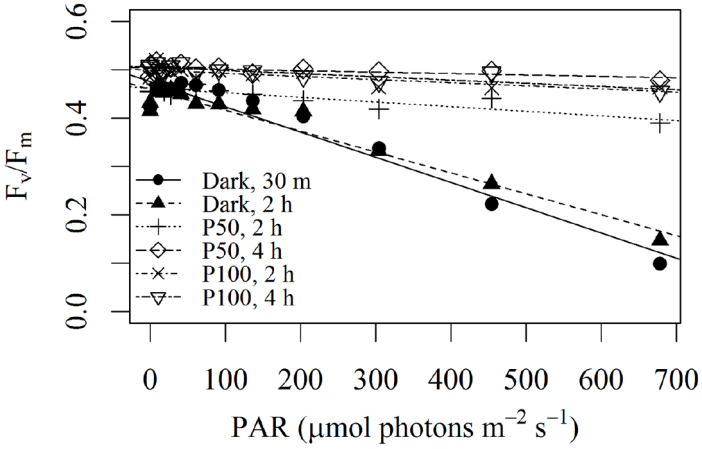

Fig. 3. Comparison of F_v/F_m determined after dark and light treatments on *Attheya longicornis*. Measurements are corrected for a possible "bottle effect" (see text) by increasing the values obtained after 2-h incubations by 1.2% and those after 4 h by 2.4%. P denotes photon flux density in µmol m^{-2} s^{-1}.

Table 1. Slopes of the regression lines for the different post-incubation treatments shown in Fig. 3, average F_v/F_m at incubation light intensities of 0 and 300 μmol m^{-2} s^{-1}, as well as the absolute and percentage differences between F_v/F_m following post-incubation for samples incubated at these two light intensities.

Treatment	Slope	F_v/F_m (PAR = 0)	F_v/F_m (PAR = 300)	F_v/F_m (Δ)	F_v/F_m (%Δ)
Dark, 30 m	–5.18E-04	0.476	0.319	–0.157	–33.0%
Dark, 2 h	–4.33E-04	0.460	0.330	–0.130	–28.3%
P50, 2 h	–9.59E-05	0.462	0.434	–0.028	–6.1%
P50, 4 h	–2.98E-05	0.504	0.495	–0.009	–1.8%
P100, 2 h	–6.44E-05	0.499	0.480	–0.019	–3.8%
P100, 4 h	–6.67E-05	0.506	0.486	–0.020	–4.0%

it can be seen that the differences between F_v/F_m measured on dark incubated samples (i.e., not experiencing in situ photoinhibition) and those incubated at 300 μmol m^{-2} s^{-1} (i.e., typical for many marine study sites and where a clear reduction in F_v/F_m is seen in samples that were post-incubation treated in darkness) were only a few percent for most of the low-light treatments. Thus, for the purposes of most biological oceanographic studies, we argue that a post-collection/incubation low light treatment can be considered to eliminate the residual effects of incubation light history on F_v/F_m measurements. With the post-incubation light treatment of 4 h at 50 μmol m^{-2} s^{-1}, the difference between F_v/F_m measured on dark incubated samples and those incubated at 300 μmol m^{-2} s^{-1} was less than 2%. As a result, we identify this treatment as being the most effective under our study conditions.

Low-light treatment also removed the light history signal in natural samples subjected to incubation at different light intensities (Fig. 4). A similar response in F_v/F_m determined after light/dark treatment was observed on natural phytoplankton taken directly from in situ conditions (Sargasso Sea samples from 10 m: Fig. 5).

Thus, we conclude that for the conditions tested here a period of 2–4 h incubation in light (50 or 100 μmol m^{-2} s^{-1}) is sufficient to essentially eliminate a signal in F_v/F_m emanating from exposure to previous light climate. An incubation of 2–4 h is, however, quite long. Therefore, we tested for potential influence of time (i.e., "bottle effects") on the subsequent determinations of F_v/F_m. Indeed, we found a significant (P = 0.03) decline in F_v/F_m over time (t; 0–270 min). This decrease was, however, relatively constant from time zero (a = $–9.80 \times 10^{-5}$t) and relatively small (approx. 0.6% h^{-1}). Thus, it can, in theory, be corrected for as we have done in the data plotted in Figs. 3-5. We believe a bottle effect may be common as comparison of F_v/F_m determined on discrete samples incubated in darkness collected on the *KNORR* cruise in 2008 with profiles of F_v/F_m obtained in situ during nighttime (when phytoplankton would not be expected to be expressing signs of photoinhibition) generally showed the F_v/F_m determinations on discrete samples to be slightly lower than the F_v/F_m determined via in situ profiling.

Temperature of the post-incubation treatment was also shown to significantly affect the performance of the light

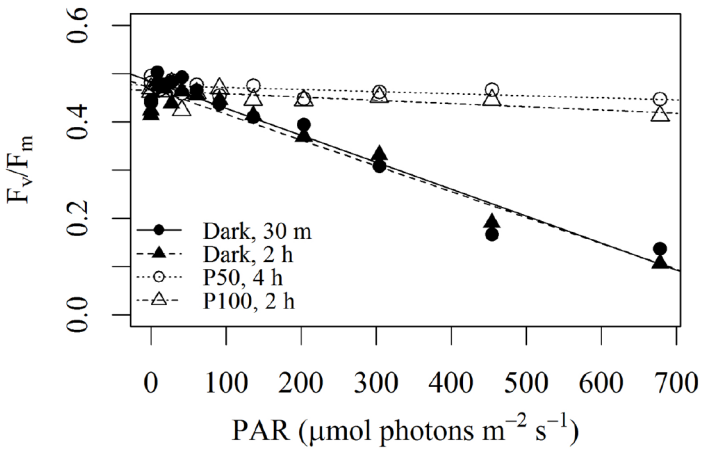

Fig. 4. Comparison of F_v/F_m determined after dark and light treatments on natural phytoplankton samples collected from surface water at Langelinie Pier in the Copenhagen harbor. Measurements are corrected for a possible "bottle effect" (see text) by increasing the values obtained after 2 h incubations by 1.2% and those after 4 h by 2.4%.

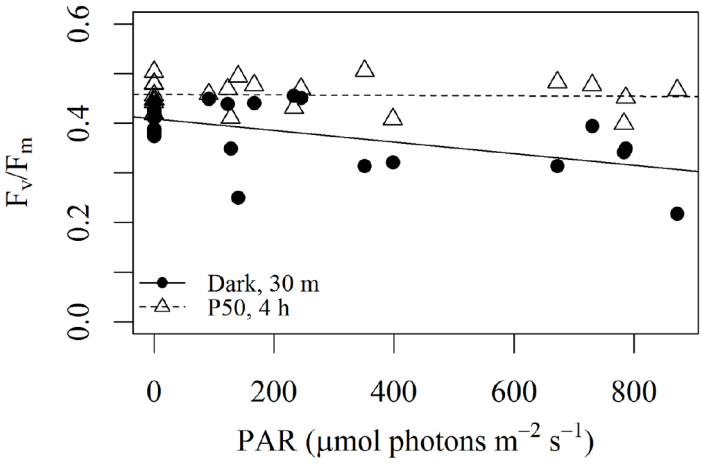

Fig. 5. F_v/F_m recorded after 30 m dark incubation and after 4 h light incubation at ~50 μmol m^{-2} s^{-1} plotted as a function of the in situ light intensity at the depth (10 m) and time of sample collection for samples collected in the Sargasso Sea in April 2014. Values for F_v/F_m have been corrected for a bottle effect: determinations made at 30 min dark incubation have been increased by 0.3%; determinations made after 4 h light incubation have been increased by 2.4% (see text).

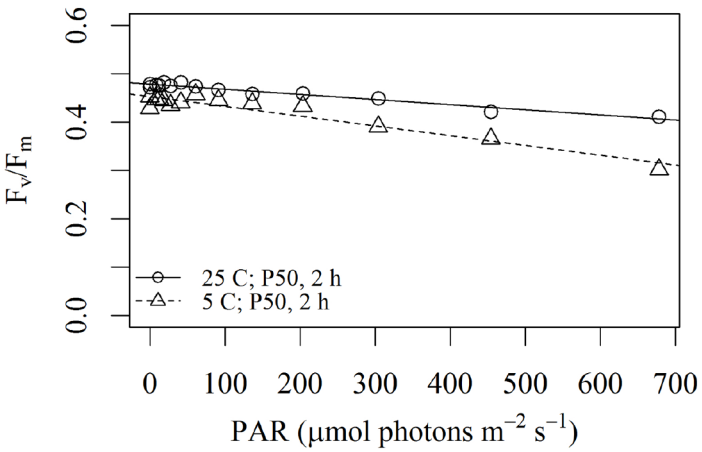

Fig. 6. Influence of temperature on F_v/F_m measured on samples of *Chaetoceros socialis* after post-incubation light treatment of 2 h at 50 μmol m^{-2} s^{-1}. The light intensity of incubation is given on the x-axis. O = post-incubation treatment at 25°C; Δ = post-incubation treatment at 5°C.

treatment in removing the light history signal (Fig. 6). After a post-incubation treatment of 2 h at 50 μmol m^{-2} s^{-1} at 25°C, the F_v/F_m determined for samples incubated at 300 μmol m^{-2} s^{-1} or higher was 85-98% of the F_v/F_m recorded for samples incubated at lower light intensities. With the same post-incubation light treatment carried out at 5°C, the F_v/F_m for samples incubated at 300 μmol m^{-2} s^{-1} or higher was only 66-91% of that recorded for the low light incubated samples.

Discussion

It is well known that normalized variable fluorescence, F_v/F_m, reflects the electron transport capacity in PSII, and thus, the physiological state of photosynthetic organisms and that both photo-damage and nutrient depletion are among the processes that can result in a lowering of F_v/F_m (e.g., Vassiliev et al 1994; Beardall et al. 2001). The fact that both photo-damage and nutrient stress can lower F_v/F_m makes the interpretation of F_v/F_m data from natural samples difficult. The purpose of this study was to explore the possibility for separating the photo-damage and other physiological effects on F_v/F_m measured in natural samples. The study shows that the photo-damage effect can largely be eliminated by low light treatment of samples before determination of F_v/F_m. Thus, the remaining F_v/F_m signal can be expected to mirror other physiological influences, including nutritional state, on the populations being sampled.

Taxonomic and other differences between samples are known to influence F_v/F_m (Kruskopf and Flynn 2005; Suggett et al. 2009). Therefore, even after removal of a light history signal, it will not be possible to use F_v/F_m to determine the absolute level of nutrient stress in mixed natural populations. Nevertheless, the universal response of decreasing F_v/F_m in response to increasing nutrient stress does imply that low values of F_v/F_m after correction for possible photo-damage influence in natural phytoplankton populations would suggest the possibility of nutrient limitation of the population. This, then, may give the opportunity to identify differences between communities that can be important in understanding ecosystem performance and guide sampling strategy.

Indeed, Richardson et al. (2014) used elevated F_v/F_m values obtained in measurements of phytoplankton taken from the sub-surface chlorophyll peak in the Sargasso Sea as evidence that the phytoplankton community in that layer was more nutrient replete than those communities found higher in the water column. In addition, elevated F_v/F_m at specific sites in the sub-surface chlorophyll peak noted in that study led to the hypothesis that vertical transport of nutrients was occurring at or near these sites. Subsequent analyses provided support for this hypothesis. In that particular case, the sub-surface phytoplankton peak was found at depths of >100 m, i.e., at extremely low light intensities. Thus, it could be assumed that photoinhibition was not influencing the F_v/F_m signal. If, however, F_v/F_m is to be useful as a more general diagnostic tool in the interrogation of plankton communities collected from environments experiencing a fluctuating light environment, it is necessary to develop methods to separate the light history (photoinhibition) signal from that deriving from other physiological processes.

The determination of F_v/F_m requires by definition that the samples upon which the measurements are being made are dark adapted. In practice, most protocols for measuring on discrete samples, therefore, include a period of at least 15 min in darkness before measurements being made. Here, it is demonstrated that a 30-min dark incubation before sampling does not eliminate the light history signal from the resultant F_v/F_m measurement. On the other hand, when the samples are incubated at 50 or 100 μmol m^{-2} s^{-1} for 2–4 h, the light history signal is essentially removed. F_v/F_m decreases slightly over time with increasing incubation time (approx. 0.6% h^{-1}), but this decrease is demonstrated to start at time zero, and thus, will also affect measurements made after dark incubation. The decrease was constant over time in the study carried out here and can, therefore, be corrected for (as done here). Alternatively, when the incubations to be compared are all of the same duration, this apparent "bottle effect" can be assumed to apply equally to all samples made under given conditions and ignored.

The light history signal observed both in the laboratory and field data presented here appears to derive from exposure to high light, and therefore, is believed to represent a signal derived from photoinhibition experienced during the incubation or time of collection. Photoinhibition is the reduction of photosynthetic rate in response to prolonged or pronounced excess light absorption and refers to the reduction of photosynthetic capacity independent of changes in pigment concentration. Photoinhibition is, therefore, due to net photo-damage of PSII (Murata et al. 2007). A well-known consequence of photoinhibition is damage of the D1 reaction center protein of PSII. Recovery from photoinhibition requires synthesis of new D1

protein, and this is part of a carefully orchestrated PSII repair cycle in which the exchange of the damaged D1 protein takes place starting by disassembly of the PSII holo-complex, degradation of damaged D1 protein by specific proteases, synthesis of new D1protein via translation followed reassembly of the holo-complex, and the activation of the reaction center (Nixon et al. 2010). This process clearly demands metabolic energy for both degradation of the damaged D1 protein and biosynthesis of new D1 protein (Nixon et al. 2010).

Exposure to light at a level below that which apparently causes photoinhibition may provide the energy necessary for the cell to repair the photo-damage experienced during the earlier high light exposure and, thus, lead to elimination of the light history signal and other workers (Shelly et al. 2003) have also demonstrated that light treatment is necessary for recovery from photo-damage caused by UV to occur. Lowering the temperature to 5°C during the regenerating light-treatment slows down metabolic processes possibly explaining the temperature sensitivity demonstrated here in the effectiveness of the light treatment in eliminating the light history signal. Again, a temperature effect on the repair process following photoinhibition has also been reported by other workers (Li et al. 2012; Helbling et al. 2013).

The light intensity at which the light history signal appears differs in the different datasets collected in this study. For the species cultured in the laboratory under day/night regulated light condition of 100/0 μmol m^{-2} s^{-1}, the reduction in F_v/F_m observed in the "high light" incubated samples appeared in samples incubated between ~60 and 90 μmol m^{-2} s^{-1}, depending on the species being examined. In the field data collected at the onset of the spring bloom in the North Atlantic, the light signal is recorded at least from ~100 μmol m^{-2} s^{-1}, whereas for surface samples from the Sargasso Sea, it first appears at light intensities above ~300 μmol m^{-2} s^{-1}.

Average incident light experienced by phytoplankton in surface waters of the Sargasso Sea would be higher than that experienced by phytoplankton in surface waters of the North Atlantic at the time of the spring bloom. We, therefore, can expect the photosystems of phytoplankton in the Sargasso Sea to be better adapted to deal with exposure to high light than phytoplankton of the North Atlantic and, as a result, to first experience photoinhibition at higher light intensities than phytoplankton in the North Atlantic. Similarly, the different species studied in the laboratory can be expected to have different genotypic responses to light intensity. Thus, we suggest that the light intensity at which the onset of the light history signal in F_v/F_m, i.e., when a significant difference in F_v/F_m determined after light and dark incubations is noted, indicates the light intensity at which the population experiences photoinhibition and may be a useful diagnostic tool for assessing the degree of photoinhibition being experienced by a population under in situ conditions.

Initially, it may seem surprising that the cultured species appeared by this diagnostic to experience photoinhibition at the light intensity at which they were cultured. We note, however, that, under culturing conditions, the phytoplankton were held in denser concentrations and in larger vessels. Thus, the actual light climate experienced by the individual phytoplankton was likely lower under culturing conditions than under the experimental incubations despite the fact that light conditions appeared nominally to be the same. It is also possible that some or all of the species were experiencing photoinhibition under the culturing conditions. Earlier studies (Samuelsson and Richardson 1982) have demonstrated photoinhibition in a dinoflagellate when held under similar conditions.

Comments and recommendations

This study indicates that the light treatment used to eliminate the light history signal in F_v/F_m must be below the level causing photoinhibition in the population and that the light level initiating photoinhibition in natural populations varies. In addition, temperature was clearly shown here to influence the length of the light incubation period required to eliminate the light history signal from F_v/F_m. For these reasons, it is not possible to develop a universal protocol for the elimination of this light signal. The study does, however, provide general guidelines for how this can be achieved: Prior to determination of F_v/F_m, samples should be exposed to light of an intensity below that which is believed to cause photoinhibition in the population. The light intensity where photoinhibition is experienced can be approximated either by determining the light intensity when a light signal in F_v/F_m is registered (see Fig. 2) or by identifying the light intensity at which a significant difference in F_v/F_m is recorded between measurements on samples incubated in light and dark. This study did not determine the minimum incubation light intensity required to remove the light history signal. Presumably, however, the incubation period required to repair photo-damage will increase at very low light intensities. We suggest, therefore, that the light climate chosen for post-collection treatment should be approximately half that of the level where the onset of photoinhibition is believed to occur.

The length of the low light incubation treatment required to remove the light history signal has been demonstrated here to be a function of temperature and can be expected to be longest under cold conditions. In an effort to mimic natural conditions as closely as possible, post-collection light incubations should be carried out as closely to in situ temperatures as possible and the length of incubation time adjusted to account for temperature variability. We found here that a 4 h incubation at 50 μmol m^{-2} s^{-1} was sufficient to remove the light history signal at temperatures of 15° and above (surface temperatures in the Sargasso Sea were > 20°). When applied at lower temperatures, we recommend that controls are carried out to ensure that the light history signal is eliminated. If not, the length (or, perhaps in some cases, light intensity) of incubation could be increased.

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