Pigment variations in *Emiliania huxleyi* (CCMP370) as a response to changes in light intensity or quality

José L. Garrido[1*], Christophe Brunet[2] Francisco Rodríguez[3]

[1]Instituto de Investigaciones Marinas (CSIC), Vigo, Spain.

[2] Stazione Zoologica Anton Dohrn, Naples, Italy.

[3] Instituto Español de Oceanografia (IEO), Vigo, Spain.

*For correspondence. Instituto de Investigaciones Marinas (CSIC). Av. Eduardo Cabello, 6. 36208 Vigo (Spain). E-mail garrido@iim.csic.es. Tel. +34986231930; Fax +34986292762

Running title: *Light-induced pigment changes in* Emiliania huxleyi

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

Originality-Significance Statement.

We show, by the first time, the drastic pigment variation by either the interconversion of different pigments with the same chromophore (fucoxanthin xantophylls and chlorophylls *c*) or the *ex novo* synthesis of protective carotenoids (diadinoxanthin and diatoxanthin) that the bloom forming coccolithophore of global importance *E. huxleyi* experiences as a response to different light qualities or intensities, and their combinations.

Our work reveals the importance that fucoxanthin derivatives (of outstanding global ocean relevance) can have in photoacclimation to the natural light fields, and relates these changes with the success of *Emiliania huxleyi* to thrive in changing light environments (well lit upper water layers, downwelling/upwelling conditions in open ocean and coastal areas). It also stresses the need for both high resolution pigment methods (seldom employed in the literature) and proper characterization of light sources in laboratory experiments.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

Summary

Many studies on photoacclimation examine the pigment responses to changes in light intensity, but variations in light climate in the aquatic environment are also related to changes in spectral composition. We have employed an HPLC method with improved resolution towards chlorophyll *c* and fucoxanthin-related xanthophylls to examine the pigment composition of *Emiliania huxleyi* CCMP 370 under different light intensities and spectral qualities.

To maintain its photosynthetic performance, *E. huxleyi* CCMP370 promotes drastic pigment changes that can be either the interconversion of pigments in pools with the same basic chromophoric structure (Fucoxanthin type or chlorophyll *c* type), or the *ex novo* synthesis (Diatoxanthin). These changes are linked either to variations in light quality (Fucoxanthin related xanthophylls) or in light intensity (chlorophyll c_3/Monovinyl chlorophyll c_3, Diadinoxanthin/Diatoxanthin, β,ε–carotene/ β,β-carotene). Fucoxanthin and 19'-hexanoyloxyfucoxanthin proportions were highly dependent on spectral conditions. Whereas Fucoxanthin dominated in green and red light, 19'-hexanoyloxyfucoxanthin prevailed under blue spectral conditions.

Our results suggest that the huge pigment diversity enhanced the photoacclimative capacities of *E. huxleyi* to efficiently perform under changing light environments. The ubiquity and success in the global ocean as well as the capacity of *E. huxleyi* to form large surface blooms might be associated to the plasticity described here.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

Introduction

On a vertical scale, the light intensity decreases exponentially in the aquatic environments and the light spectrum undergoes profound variations with depth. Indeed, in the visible part of the spectrum, the red color disappears quickly while blue or green are the dominant colors at depth (see Brunet *et al.*, 2014). Variability in the underwater light field is mainly determined by the optics of the aquatic medium (Kirk, 2011). This means that composition of the water in different ocean and coastal areas strongly constrains the properties of light (intensity and spectral quality) available to photosynthesizers. The light environment differs notably between low-productive oceanic environments (enriched in blue-green wavelengths that go deeper in the water column) and highly productive coastal and estuarine waters (with blue waveband attenuated and shallower photic layers relatively enriched in the green region) (Kirk, 2011).

To thrive in such variable and complex light environment, microalgae have evolved a diversity of adaptative and acclimative mechanisms for optimizing the photosynthetic rate. During their short life time in the water column, microalgae have to finely balance light harvesting and photoprotective capacity. The kinetics of regulative or acclimative responses are coupled to relevant natural time scales of variation in light (from seconds to days), as for instance induced by the passage of clouds or vertical motion in the water column or related to the natural diel cycle of light (Brunet *et al.*, 2011). On a larger temporal scale, microalgae genetically evolve in response to the environmental constraints of the ecological niche where they grow leading to the development of specific photoadaptative traits (Falkowski and Chen, 2003).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

Photoacclimation responses in algae involve many processes that can act as photoprotective (e.g., state transitions, thermal dissipation, xanthophyll cycle activation, different content of carotenoids within pigment protein complexes, etc.) or to increase the light harvesting capability. They induce structural changes in the photosynthetic apparatus (aiming to modify the size of photosystem (PS) II antenna and/or the number of reaction centers (RCs)) and the optimization of light and dark photosynthetic reactions. These qualitative and quantitative changes are reversible under variable irradiance (Falkowski and Chen, 2003), while the light history of the cells allows them to photoacclimate by differential gene expression of light harvesting complex (Falkowski and Chen, 2003).

One of the main targets of the intracellular changes in response to light variations regards the pigment-protein complexes forming the antenna of the photosystems (Falkowski & Chen, 2003; Brunet *et al*., 2011). Many information are available on the effect of light intensity on the pigment pool variations across a broad range of microalgal species (Schofield *et al.*, 1990; van Leeuwe & Stefels, 1998; Brunet *et al.*, 2011), however few studies have described the influence of spectral changes in photosynthetic pigments of eukaryotic microalgae (Mouget *et al.*, 2004; Schellenberger Costa *et al.*, 2013; Brunet *et al.*, 2014). Most of these studies used diatoms as a model, while little information is available on other taxa (e.g., Schlüter *et al.*, 2000).

The haptophyte *Emiliania huxleyi* (Lohman) has a global distribution and can form massive blooms of up to 250,000 km^2, predominantly in sub-boreal latitudes of the Northern Hemisphere (Tyrrel and Merico, 2004). The proliferations of *E. huxleyi* create significant interest due to their influence on the carbon cycle, the production of dimethylsulfoniopropionate (a precursor of dimethylsulphide) and their potential role in global climate (Poulton *et al.*, 2007). *E. huxleyi* blooms are typically observed in

shallow mixed layers suggesting its tolerance to high irradiances and competitive advantage over other species (Nanninga and Tyrrell, 1996).

E. huxleyi represents an ideal candidate to (i) investigate the role of the physiological and genotypic diversity of a species on its ecological success in aquatic environments, and (ii) to study the dynamics and advantages of a complex pigment pool for coping with variable light conditions and increase the tolerance to high irradiance regime.

The genetic delineation using mitochondrial markers (*cox1b*-ATP4, Hagino *et al.*, 2011) separates *E. huxleyi* in at least two clades that could reflect environmental preferences (*I*: warm-water strains from temperate/tropical areas and *II*: cool-water from subartic latitudes). In addition, five morphotypes (A, B, B/C, C, and R) based on coccolith ornamentation have been described (Young & Westbroek, 1991). These morphotypes cannot be assigned to every strain (due to the occurrence of naked cells or malformed coccoliths), and they appear randomly distributed across molecular clades *I* and *II*. The changes in pigment composition in variable light regimes have been compared in calcifying *vs* non-calcifying strains (Leonardos and Harris, 2006), without significant differences among them.

Pigment analyses in this species represent a formidable challenge given its complex xanthophyll and chl *c* composition (Zapata *et al.*, 2004). Among the various pigments sharing the fucoxanthin (F) chromophore (i.e. with similar absorption features) that have been characterized in *E. huxleyi* (Egeland *et al.*, 2000; Airs & Llewellyn, 2006), the most abundant, besides F itself, are 19'-butanoyloxyfucoxanthin (BF), 19'-hexanoyloxy-4-ketofucoxanthin (HKf) and 19'-hexanoyloxyfucoxanthin (HF), (Stolte *et al.*, 2000; Zapata *et al.*, 2004). Far less is known for chlorophylls *c*, which in *E. huxleyi* are represented by a diverse array of compounds (Zapata *et al.*, 2004): chl c_3, MV chl c_3

(Álvarez *et al.*, 2012), chl c_2, chlorophyll c_2 monogalactosyldiacylglyceride ester (chl c_2-MGDG [18:4/14:0]) (Garrido *et al.*, 2000) and traces of divinyl protochlorophyllide *a* (MgDVP). Different proportions of F and HF between morphotypes have been reported (e.g. HF:chl *a* ratio 11 times higher in morphotypes B/C than in morphotype A; Cook *et al.*, 2011), and HKf seems restricted to morphotypes A and R (Van Lenning *et al.*, 2004; Zapata *et al.*, 2004; Cook *et al.*, 2011).

The interconversion of fucoxanthin related xanthophylls following changes in light intensity has been suggested to be controlled by a light harvesting efficiency regulation (van Leeuwe & Stefels, 1998; Alami *et al.*, 2012). The light-mediated transformation of fucoxanthins in *E. huxleyi*, namely F into HF when the irradiance increases, has been previously documented (Schlüter *et al.*, 2000; Stolte *et al.*, 2000; Lefebvre *et al.*, 2010). Schlüter *et al.* (2000) found that the relative proportion of HF:F was larger in blue *vs* green light, but as the absorption spectra of F derivatives is almost identical (Egeland, 2011), the role of the shift between F and HF in chromatic adaptation has been dismissed. However, the transformations between individual chls *c* associated with light intensity are still little known (Lefebvre *et al.*, 2010).

While a number of studies have addressed the light-dependent responses in *E. huxleyi* (Nanninga & Tyrrell, 1996; Suggett *et al.*, 2007; van de Poll *et al.*, 2007; Ragni *et al.*, 2008; Lefebvre *et al.*, 2010; Mckew *et al.*, 2013a,b), a high resolution analysis of pigment pattern dynamics related to spectral acclimation is still not reported in the literature. This is an important issue for the pigment application in chemotaxonomic and physiological studies (Stolte *et al.*, 2000; Van Lenning *et al.*, 2004; Zapata *et al.*, 2004; Cook *et al.*, 2011).

In this study, we exposed *E. huxleyi* (clone CCMP370, non-calcifying, clade *II* "cool waters" *sensu* Hagino *et al.*, (2011)), to different light intensities and spectral light conditions with the aim of investigating the pigment behavior of this species. Our study consisted in two different experiments, shifting cells from a pre-acclimation condition to different light intensities and different spectral conditions (white, blue, red and green) to study the concomitant changes in pigment composition. The different light conditions applied during our study try to simulate different kind of water masses and/or depth of the water column. Together with the pigment composition, we report the photosynthetic dynamics measured by Pulse Amplitude Modulated (PAM) fluorescence.

Results

Experiment #1: Effect of different spectral lights

E. huxleyi (CCMP 370) cultures grown under white LED medium light (130 μmol photons $m^{-2}s^{-1}$) were shifted to eight different light conditions, consisting in four light colors -blue, white, green and red - under high light (HL) or low light (LL) (Fig. 1).Irradiance spectra of the light emitted by the white LED source and of the white LED light transmitted after passing through the blue, green and red filters are shown in Fig. 2. After six days from the shift, cell concentration in HL varied a little, ranging from $7.8x10^6$ to $8.8x10^6$ cells ml^{-1} (Table 1). On the contrary, under LL significant differences were found after Tukey´s test for pair-wise comparisons ($p<0.05$), between red light (2-3 fold lower densities) and all the other spectral conditions.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

HPLC analyses confirmed the presence of the expected chl *c* compounds (chl c_3, MV chl c_3, chl c_2, chl c_2-MGDG [14:0/18:4] and traces of MgDVP) and F derivatives (F, HKf and HF). In addition, a minor carotenoid with similar spectra to HF eluted between F and HKf, tentatively identified as pentanoyloxy-fucoxanthin (PF), as described by Airs & Llewellyn (2006). Representative chromatograms illustrating the behavior of the whole set of pigments at each light condition are shown in Fig. 3.

The chl *a* content per cell was always higher at LL compared to HL (Table 1). Both in HL or LL treatments, chl *a* concentration was maximal under red light decreasing then under green, white and blue (Table 1).

The cellular content of total chl *c* was tightly correlated to chl *a* concentration (r^2= 0.97, $p < 0.0001$, n=24; Fig. 4A), revealing that total chl *c* content was affected by the same light signaling than chl *a*. The diverse chlorophyll *c* compounds followed similar trends being correlated to chl *a* ($p<0.0001$, n=24), at the exception of MV chl c_3 ($p>0.05$; n=24). This pigment showed a different behavior than chl c_3, chl c_2 or chl c_2-MGDG, acting as a "high light pigment". Indeed, the MV chl c_3 : chl *a* ratio was higher under HL than under LL (Fig. 5, except under red light) in contrast to all the other accessory chl *c* : chl *a* ratios (Fig. 5). Moreover, MV chl c_3 was inversely correlated to chl c_3 (r^2= 0.90, $p < 0.0001$, n=24).

Under red light, all the accessory chl *c* : chl *a* ratios showed an opposite trend compared to white, blue or green lights **(**Fig. 5**)**, except for the chl c_2-MGDG which showed similar values under HL and LL. Tukey's test for differences between means pointed out the statistical significance for most of these trends (excepting chl c_2-MGDG), under red light.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

The sum of fucoxanthin pigments content per cell (ΣFs) was strongly correlated with chl *a* (r^2= 0.99, p <0.0001, n=24; Fig. 4B), with the ratio ΣFs:chl *a* strikingly constant over a broad range of light intensities and spectral qualities (the ratios differ less than 16%; Table 1). However, the ratio of individual xanthophylls with fucoxanthin chromophore to chl *a* showed a great variability among the different spectral lights (Fig. 6). Interestingly, F and HF were inversely correlated (r^2= 0.89, p <0.0001, n=24). Blue light maximized the proportion of HF in the fucoxanthin pool (68.2%) (major compound also under white light), whereas under green or red light F became the dominant compound (39.6% and 64.4%, respectively). Tukey's test highlighted the statistical significance for these trends in HF and F mean values under blue and green light.

HKf did not exhibit a clear trend among the different light spectral conditions (range of variability 14.4%-31.2% of total fucoxanthins in HL and LL). PF was a minor pigment and behaved similarly to HF (maximum in the blue and minimum in red light), especially at HL.

The de-epoxidation state of the xanthophyll cycle (DES) reached higher values at HL compared to LL (Table 1). Under HL, the highest DES was measured for white and blue lights (no significant differences between them and both significantly higher than green and red; Table 1). At LL, low DES values under all light qualities were found (Table 1). The PSII operating efficiency, F'_v/F'_m, decreased at HL compared to LL, and did not present any variations among the four spectral lights (Table 1).

Experiment #2: downwelling and upwelling simulation experiment

The aim of this experiment was to investigate the acclimation processes developed by *E. huxleyi* experiencing simulated upwelling or downwelling events as it can occur in productive coastal waters (enriched in green wavelengths; Kirk, 2011) or in oligotrophic open ocean ("blue waters"). For this purpose, *E. huxleyi* were acclimated to LL (blue or green) and then shifted to white HL in a simulation of an upwelling event, or acclimated to white HL and subsequently shifted to blue or green LL, imitating a downwelling episode.

The Σchls *c* : chl *a* ratio remained nearly constant in upwelling (they decreased by 8% in oceanic blue light acclimated cells waters and increased by 1% in coastal green light acclimated cells), while a large increase (~46%) was revealed in downwelling under both spectral conditions. The ratio of individual chls *c* to chl *a* showed the same trends in downwelling (Fig. 7A,C) and upwelling treatments **(**Fig. 7B,D**)** independently of oceanic (blue) or coastal (green) light conditions. In the downwelling treatment, a large increase of chl c_2 (blue: + 95%, green: + 106% relative to the initial ratios), and chl c_3 (+ 75% and + 81%) was observed. In contrast, chl c_3 decreased under upwelling treatment (blue: -22%, green: -11%). The chl c_2-MGDG : chl *a* ratio varied a little (10-22%) with the same trend than chl c_2 and chl c_3. As reported from the experiment #1, chl c_3 and MV chl c_3 showed inverse trends in LL and HL. However, these pigments were inversely correlated in the case of downwelling (r^2= 0.64 in "blue" and r^2= 0.68 in "green" waters, p <0.0001, n=15), whereas thatcorrelation was not significant in upwelling treatments (r^2< 0.3). At the end of the upwelling experiment, the extent of increase of the MV chl c_3 : chl *a* ratio was higher (blue: 57%, and green: 136%) than the extent of its decrease in downwelling (~34%). Significant differences in chls *c* trends between the start and the end of the experiments were indicated by Tukey's test (Fig. 7), especially for chl c_2, chl c_3 and MV chl c_3 in downwelling conditions.

In "coastal" waters (green light experiment, time 4 in Fig. 8A or time 0 in Fig. 8B), F was the most important compound in the fucoxanthin family (~ 60% of ΣFs). The F : chl *a* ratio increased gradually with time after downwelling event (+197%, Fig. 8A), i.e. from high white light to low green light, while it decreased in cells experiencing an upwelling event (Fig. 8B). In the latter, i.e. from low green light to white light, HF became dominant in the fucoxanthin family.

In contrast, cells acclimated in high white light and shifted towards low blue light ("oceanic" waters, downwelling, Fig. 8 C) strongly increased the HF : chl *a* ratio over time (64% increase, accounting for a 71% of ΣFs). After 4 days of downwelling experiment, the HF : chl *a* and F : chl *a* ratios (Fig. 8 A,C) were similar to their initial values revealed during the upwelling experiments (Fig. 8 B,D). The dominance of HF was maintained during the upwelling conditions from low blue to high white light, although in a lesser extent.

Interestingly, F and HF were inversely and significantly correlated in both downwelling and upwelling experiments ($p < 0.0001$, n=15), and significant differences between means of both pigments at the onset and the end of these experiments were observed (Fig. 8). Also, HF was inversely correlated to HKf only in upwelling experiments, (blue light: $r^2 = 0.96$, $p<0.0001$, n=15; green light: $r^2 = 0.47$, $p<0.0005$, n=15). The minor fucoxanthin derivative PF did not show relevant variability during the experimental treatments (Fig. 8A,B,C,D).

The ratio diadinoxanthin + diatoxanthin (Dt+Dd) : chl *a* revealed that cells strongly modulated the XC pigment pool, with a huge synthesis of these pigments during upwelling treatment and *vice versa*, a sharp decrease when cells experienced low light (Table 2). No difference was noticed between oceanic or coastal light conditions. The

XC was activated by upwelling event; the maximal value of DES was reached at a certain point within the first 24 hours, being observed in our first measurements, one day after the shift (0.59 for oceanic acclimated cells, 0.55 for coastal acclimated cells). After 4 days, the DES of the oceanic waters acclimated cells (that had stabilized above 0.5 in days 1, 2 and 3) decreased significantly to 0.4, while it remained at around 0.5 in coastal acclimated cells (no significant differences for days 2, 3 and 4). The downwelling treatment rapidly led to a decrease of the DES (< 0.07). While Dt ratio to chl *a* (and hence DES) abruptly changed the first day after the light shift and then stabilized, the changes in F-relatedpigments were continuous and gradual.

The PSII operating efficiency, F'_v/F'_m, varied in an opposite way between upwelling and downwelling conditions (Table 2). During upwelling, it decreased from > 0.6 to ~0.3 after one day, and recovered a little to ~ 0.4. During downwelling, F'_v/F'_m increased from ~ 0.4 to ~ 0.6 after two days, identifying a change of acclimation state of the cells between upwelling and downwelling simulations.

Discussion

Light-induced pigment variations in E. huxleyi

E. huxleyi CCMP 370 seems to maintain nearly stoichiometric relations among similar chromophores in the major pigment-protein complexes. Hypothesizing that the pigments here analyzed are bound to protein in pigment-protein complexes, the interconversion between individual molecules by changing substituents in the periphery of the chromophores (ethyl by vinyl in chl c_3 forms, addition of an acyloxy chain or a

carbonyl function in fucoxanthins), would modify their possibilities for binding to the protein scaffold in the complexes (Croce and van Amerongen, 2014). This could induce fine adjustment in light harvesting or excitation energy transfer to maintain an optimal photosynthetic capacity (as shown by F_v'/F_m', Table 1). These changes in individual pigments could be linked to changes in the abundance and composition of proteins in the light-harvesting antenna of PSII involved in the trade-off between light absorption and photoprotection (McKew *et al*. 2013a). However, this remains a hypothesis until LHC with different pigment composition, from cells cultivated under distinct light intensities and qualities, are isolated and analyzed.

Although a former study on a different strain of *E. huxleyi* (CCMP1516) did not detect Dt under 1000 μmol photons $m^{-2}s^{-1}$, the increase of Dt in the present study is the common response of chromophytes experiencing HL (Brunet *et al.*, 2011). The high XC pigment pool (Dt and Dd) found in our study agrees with the HL-adapted state and reveals that this species is able to finely acclimate to high light, and vice-versa. This is confirmed by the high value of the de-epoxidation state when cells experience high light and the reversibility of F_v'/F_m' under changing light climate.

Although a general increase in chls (both *a* and *c*s) is expected under red light since only chls (especially chl *a*) can capture light above 600 nm (cells perceive both HL or LL red radiation as LL, as evidenced by the chl *a* content per cell), the proportion of chl c_3 pigments in the light harvesting complexes of *E. huxleyi* mainly depends on the light intensity: Chl c_3 seems a LL pigment while MV chl c_3 is more synthesized under HL condition. The subtle structural difference between chl c_3 and MV chl c_3 (a double bond) can induce drastic changes in properties that affect light harvesting and energy transfer. On one side, while MV chl c_3 absorbs at a bit shorter wavelengths, the divinylic structure of chl *c* extends the chromophore into the red region of the spectrum

(Egeland, 2011), improving red light harvesting. On the other, the change in a peripheral substituent of the chl *c* porphyrin macrocycle affects the positioning (distance and orientation) of pigments in the protein complex (Melkozernov and Blankenship, 2006) and modify their fluorescence emission spectra (Garrido *et al.*, 1995) that overlaps to a different degree with the Q_y band of chl *a*, resulting in different efficiencies in the excitation energy transference by the Förster resonance mechanism (Falkowski and Raven, 2007).

The substitution of fucoxanthin for its acyloxy-derivatives as light increases has been related to a less efficient energy transfer from the antennae to the reaction centers (Alami *et al.*, 2012). Our results point out that the structural changes in fucoxanthin pigments can affect both their efficiency in energy transfer and their capacities in harvesting light of different spectral qualities. Modifications in side substituents could affect the conformation of the protein and the binding sites, modifying their spatial arrangement and the types and extents of amino acid-chromophore interactions that can induce spectral shifts and hence result in marked spectral inhomogeneity *in vivo* (Scholes *et al.*, 2011). These structural properties could underlie that HF acts as a specialized blue light harvester, explaining its dominance in open ocean waters (Schlüter *et al.*, 2000; Not *et al.*, 2008; Liu *et al.*, 2009).

The interconversions within fucoxanthins and chl *c* pigments are probably also related to the adjustment of the efficiency of excitation energy transfer towards the PSII core of the photosynthetic apparatus. Suggett *et al.* (2007) showed that different strains of *E. huxleyi* can exhibit distinct strategies of photosystem II (PSII) acclimation, relative to changes in cellular content of chl *a* and PSII size and effective cross section of PSII (ρ_{PSII}), i.e. the effective antennae size for light harvesting. ρ_{PSII} is determined by the

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

transfer efficiency (Φ_T) of energy from accessory carotenoid and chlorophylls to the core antenna chl *a* (Mauzerall and Greenbaum, 1989).

A striking finding is that the balance F/HF depends much more on the light quality than on light intensity and that HF predominates at LL condition. This result contradicts previous reports on the predominance of HF at high light in *E. huxleyi* (Leonardos and Harris, 2006; Lefebvre *et al.*, 2010; Mckew *et al.*, 2013) and other prymnesiophyceans or pelagophyceans (van Leeuwe & Stefels, 1998; Stolte *et al.*, 2000; Leonardos & Harris, 2006; Alami *et al.*, 2012). This discrepancy can be attributed to spectral differences between lamps, as the white light part of our experiment #1 was conducted using white LED lamps, whose emission spectrum is enriched in blue light relative to fluorescent lamps (Piasecki *et al.*, 2010) and the results with different light colors in the same experiment confirmed that sensing of enriched blue environment by *E. huxleyi* triggered the dominance of HF (even under low light). This suggests the need of a reexamination of the trends of fucoxanthin-related pigments in different species under strict control of light quality, and raise doubts on the significance of pigment ratios derived from laboratory cultures to evaluate natural phytoplankton populations (Higgins *et al.*, 2011).

The chl *a* quota, ranging between 97 fg cell^{-1} (blue HL) and 205 fg cell^{-1}(red LL) are low relative to the range found in many studies on *E. huxleyi* (126-310 fg cell^{-1}; Thompson & Calvert, 1995; Harris *et al.*, 2005; Leonardos & Harris, 2006; Lefebvre *et al.*, 2010). This discrepancy might be related to strain variability and light source differences, but also to analytical reasons, as many HPLC methods do not separate chlc_2-MGDG from chl *a*. Indeed, many studies do not report chl c_2-MGDG at all (Stolte *et al.*, 2000; Harris *et al.*, 2005; Leonardos and Harris, 2006; Cook *et al.*, 2011; Mckew *et al.*, 2013) and probably overestimate chl *a* due to this co-elution.

Discrepancies in other pigments (mainly between chlc_3 and MV chlc_3 and HKf and HF) seem also related to coelution problems in HPLC. For example, Van Lenning *et al.* (2004) reported the presence of these pigments in *E. huxleyi* CCMP 1516 that were not found in the same clone in a recent study (Mckew *et al.,* 2013a).

The different mechanisms work together for coping with light in the sea

Reports on how eukaryotic microalgae acclimate to marine light radiation are scarce, except for Chlorophyta (e.g. Humbeck *et al.*, 1988). Growth and photosynthesis in different light qualities have been investigated in several algal groups like chlorophytes, diatoms, prymnesiophytes and dinoflagellates (Faust *et al.*, 1982; Wilhelm *et al.*, 1985; Schofield *et al.*, 1990; Mouget *et al.*, 2004; Schellenberger Costa *et al.*, 2013; Brunet *et al.*, 2014). Among chl *c*-containing phytoplankton, diatoms have been the most studied group, with contradictory results about the existence of chromatic acclimation (reviewed by Mouget *et al.*, 2004). Brunet *et al.* (2014) reported a spectral radiation photoprotective mechanism mediated by blue and red light in a diatom (*Pseudo-nitzschia multistriata*). Their results demonstrated that red radiation was required to allow the increase in the XC pool size, but the extent of NPQ was determined by blue fluence rate. In our survey, XC pool increased in blue spectral conditions without red light (Table 1), suggesting that such phenomenon, as described by Brunet *et al.* (2014), does not occur in *E. huxleyi*. This suggests that the light signaling mechanisms from photoreception to the downstream biochemical and physiological events do function differently between these two species. The different responses might be mediated by the different taxa or ecophysiological state.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

At depth, oceanic waters become dominated by blue-green or blue light (Kirk, 2011). Both our results and those of Schlüter *et al.* (2000) support the hypothesis that HF and F are respectively best suited to blue and green light environments. The dominance of HF in the haptophyte *E. huxleyi* under blue light can be related with the fact that HF is the major carotenoid in open ocean waters (Not *et al.*, 2008; Viviani *et al.*, 2011) that in turn has been explained by molecular results supporting the dominance of haptophytes in the ocean (Liu *et al.*, 2009). The abundance of F in productive coastal waters is associated with a larger fraction of diatoms in the nano- and microplankton, but the contribution of haptophytes (whose xanthophyll pool enriches in F under green light) could be also important in these environments.

The ability of *E. huxleyi* to acclimate to simultaneous changes in light quality and intensity seems to elapse at time scales compatible with upwelling-downwelling phenomena that promote such changes in the marine environments. Average vertical velocities of around 10 m day^{-1} have been described in upwelling areas (Walsh *et al.*, 1974; McClean-Padman and Padman, 1991), and values of 12-18 m day^{-1} are typical in frontal zones and mesoscale gyres (Vélez-Belchí and Tintoré, 2001; Barth *et al.*, 2004). As the deep chlorophyll maximum (DCM) is typically located between 40 and 120 m, the average times for the gradual change from low-light to high-light (and *vice versa*) in the ocean are thus of several days, which is coherent with the time scales of pigment responses to combined light quality and intensity changes observed in our experiments (3 days).

Experimental procedures

Emiliania huxleyi CCMP 370 (from National Center of Marine Algae and Microbiota, Bigelow, USA) was grown in L1 medium (Guillard and Hargraves, 1993), on a 12:12 light:dark (L:D) cycle at 15 ºC and 130 µmol photons $m^{-2}s^{-1}$. A home-made lighting device was placed inside a constant temperature (15 ºC) chamber. The lighting device (Fig. 1) consisted in eight rows of 5 cool white LED lamps each (Dayron 1W, color temperature 7000 K), separated by thin black wood boards. On each row, methacrylate shelves sliding along metal rods can be placed at different heights to achieve different incident irradiance values. Additional perforated shelves are used as a rack to fix the position of the tubes. The height of each shelf is fixed with metallic clamps. Blue, green or red light was achieved by placing filter rectangles (cut out from light filter sheets: 172 Lagoon Blue, 124 Dark Green and 182 Light Red; Lee Filters, Andover, Hampshire, UK) in the corresponding lamp row (Fig. 1). Irradiance spectra of the light emitted by the LED source and of light transmitted by the blue, green and red filters were measured using a Flame Spectrometer (Ocean Optics, Dunedin, FL, USA) while light intensities were measured inside the tubes or flasks with a submersible Spherical Micro Quantum Sensor (Walz, Effeltrich, Germany). Cultures were maintained under a 12:12 L:D cycle. During the light period, an additional oscillating fan was used to renovate the air between the lamps and methacrylate shelves

Experimental setup

Experiment 1 ("different spectral light qualities"). E. huxleyi pre-acclimated at 130 µmol photons $m^{-2}s^{-1}$ with white light-emitting diodes (LED's) were placed in triplicate (7 ml cultures in 30 ml tubes) at initial densities of $1x10^6$ cells ml^{-1} in two light intensities (low light (LL): 18 µmol photons $m^{-2}s^{-1}$ and high light (HL): 425 µmol

photons $m^{-2}s^{-1}$), each of them provided with four different spectral conditions (blue, green, red and white). Three tubes of each of the eight treatments were taken for HPLC pigment analyses and Pulse Amplitude Modulated Fluorescence (PAM) after 6 days from the experiment start.. Cell counts were performed using a Multisizer III counter (Beckman Coulter). The content of different pigments per cell was calculated combining HPLC pigment data with cell counts.

Experiment 2 ("downwelling and upwelling simulation experiment").

E. huxleyi cultures were acclimated to LL (18 μmol photons $m^{-2}s^{-1}$) in green spectral light (15 tubes), and white HL (15 tubes) conditions (425 μmol photons $m^{-2}s^{-1}$) during four days. After this period, initial samples (day 0) were collected (3 tubes from green LL and 3 tubes from white HL). Then, 12 tubes from green LL cultures were shifted to white HL (upwelling simulation in coastal waters), whereas 12 tubes in white HL were shifted to green LL conditions (downwelling simulation in coastal waters). Samples for HPLC were taken before the shift (day 0) under white HL, green LL, and daily during four days after the shift. The same experimental setup with blue light was used to simulate upwelling and downwelling in open ocean waters.

Pigment analyses

Samples (5 mL) of *E. huxleyi* CCMP 370 cultures at each tested condition were filtered under reduced pressure onto 25 mm diameter glass fibre filters (Whatman GF/F), making sure that vacuum was not higher than 200 mm Hg. Filters were immediately

placed in a freezer at -80 ºC and kept frozen until extraction (within two days after sampling).

Frozen filters of *E. huxleyi* samples were extracted under low light in polytetrafluoroethylene (PTFE)-lined screw capped tubes with 5 ml 90% acetone using a stainless steel spatula for filter grinding. The tubes were placed in an ultrasonic bath with ice and water and sonicated for 5 min. Tubes were subsequently centrifuged for 5 min at 3500 x *g* at 4ºC (Heraeus Biofugue-Stratos) to remove cell and filter debris. Extracts were filtered through 25 mm diameter syringe filters (MFS HP020, 25 mm, and 0.20 μm pore size, hydrophilic PTFE) previous to be injected in the HPLC system.

Pigments were separated using a Waters Alliance HPLC System (Waters Corporation) consisting of a 2695 separations module, a Waters 996 diode-array detector (1.2 nm optical resolution) and a Waters 474 scanning fluorescence detector. Pigment separation was performed using the HPLC method of Zapata *et al.*, (2000), with a reformulated mobile phase A (Garrido and Roy, 2015). The column was a C8 Waters Symmetry (150 × 4.6 mm, 3.5 μm particle size, 100 Å pore size). Eluent A was methanol: acetonitrile: 0.025 M aqueous pyridine (50:25:25 by vol.). Eluent B was methanol:acetonitrile:acetone (20:60:20 by vol.). The elution gradient was as follows: (time (min): %B) t0: 0%, t22: 40%, t28: 95%, t37: 95%, t40: 0%. Flow rate was 1.0 ml min^{-1}, and column temperature was fixed at 25°C. Solvents were HPLC grade (Panreac), pyridine was reagent grade (Merck). Pigments were identified by co-chromatography with authentic standards obtained from SCOR reference cultures (Jeffrey *et al.*, 1997) and diode-array spectroscopy (see Zapata *et al.*, 2000). After checking for peak purity, spectral information was compared with a library of chlorophyll and carotenoid spectra from pigments prepared from phytoplankton cultures (Egeland, 2011)

HPLC calibration was performed using pigment standards isolated from microalgal cultures (see Zapata *et al.*, 2000), or supplied by DHI (Denmark). Molar extinction coefficients (ε ; L $mol^{-1}cm^{-1}$), derived from the specific coefficients provided by Egeland (2011), were used for pigment quantification. MGDG-chl c_2 was quantified by using the molar extinction coefficient of chl c_2. For fucoxanthin related compounds the molar extinction coefficient for fucoxanthin was used, following the recommendations of Egeland (2011). Thus pigment to chl *a* ratios are expressed on a molar base (mol mol^{-1}).

Chlorophyll a Fluorescence Yield

Photochemical efficiency of photosystem (PS) II was estimated by pulse amplitude modulated (PAM) fluorescence using a Water-PAM fluorometer (Heinz Walz, Effeltrich, Germany). Measurements were done in the culture chamber. F'_0 and F'_m are defined as the steady-state PSII fluorescence level and the maximum PSII fluorescence level measured on light-acclimated cells, respectively. F'_m was recorded after a saturating pulse of bright red light (655 nm, 3500 µmol photons m^{-2} s^{-1}, lasting 0.2 s), causing a complete reduction of the PSII acceptor pool. The Fv′ / Fm′, the PSII operating efficiency, was therefore estimated with Fv′ = Fm′ − F′.

Statistical analysis

Statistical analyses to determine the effects of different light intensities or qualities on pigments were performed by comparing analysis of variance (ANOVA). Homogeneity of variances was tested using Levene test. Whenever the analysis of variance assumption was violated, data were rank-transformed to create a 'uniform' distribution.

Homogeneous groups were established *a posteriori* using the Tukey test for pair-wise comparisons among groups. These analyses were performed using the STATISTICA 7.0 software (Tulsa, OK, USA). Linear regression analysis between variables were performed using an online tool (Vassarstats, 2015) for statistical computation

Acknowledgments

The technical help of Javier Tamame, Noelia Sanz, Lourdes Nieto and María José Pazó is gratefully acknowledged. The authors wish to thank Professor Gabriel Rosón (Department of Applied Physics, Faculty of Marine Science, University of Vigo) for advice and helpful ideas and discussion on vertical displacement of marine water masses and Dr JMF Babarro for his help with statistical analysis This work is a contribution of Microalgas Nocivas, IEO, Unidad Asociada al IIM (CSIC) and was supported by Xunta de Galicia through project Incite09 402 253PR. All authors disclose any potential sources of conflict of interest.

References

Airs, R.L. and Llewellyn, C. A. (2006) Improved detection and characterization of fucoxanthin-type carotenoids: Novel pigments in *Emiliania huxleyi* (Prymnesiophyceae). *J. Phycol.* **42**: 391–399.

Alami, M., Lazar, D., and Green, B.R. (2012) The harmful alga *Aureococcus anophagefferens* utilizes 19'- butanoyloxyfucoxanthin as well as xanthophyll cycle carotenoids in acclimating to higher light intensities. *Biochim. Biophys. Acta - Bioenerg.* **1817**: 1557–1564.

Álvarez, S., Zapata, M., Garrido, J.L., and Vaz, B. (2012) Characterization of [8-ethyl]-chlorophyll *c*3 from *Emiliania huxleyi*. *Chem. Commun.* **48**: 5500.

Barth, J. a., Hebert, D., Dale, A.C., and Ullman, D.S. (2004) Direct Observations of Along-Isopycnal Upwelling and Diapycnal Velocity at a Shelfbreak Front. *J. Phys. Oceanogr.* **34**: 543–565.

Brunet, C., Chandrasekaran, R., Barra, L., Giovagnetti, V., Corato, F., and Ruban, A. V. (2014) Spectral radiation dependent photoprotective mechanism in the diatom *Pseudo-nitzschia multistriata*. *PLoS One* **9.**:

Brunet, C., Johnsen, G., Lavaud, J., and Roy, S. (2011) Pigments and photoacclimation processes. In, Roy,S., Llewellyn,C.A., Egeland,E.S., and Johnsen,G. (eds), *Phytoplankton Pigments: Characterization, Chemotaxonomy and Applications in Oceanography*. Cambridge University Press, Cambridge (U.K.), pp. 445–471.

Cook, S.S., Whittock, L., Wright, S.W., and Hallegraeff, G.M. (2011) Photosynthetic pigment and genetic differences between two southern ocean morphotypes of *Emiliania huxleyi* (Haptophyta). *J. Phycol.* **47**: 615–626.

Croce, R. and van Amerongen, H. (2014) Natural strategies for photosynthetic light harvesting. *Nat. Chem. Biol.* **10**: 492–501.

Egeland, E.S. (2011) Data sheets aiding identification of phytoplankton carotenoids and chlorophylls. In, Roy,S., Llewellyn,C.A., Egeland,E.S., and Johnsen,G. (eds), *Phytoplankton Pigments: Characterization, Chemotaxonomy and Applications in Oceanography*. Cambridge University Press, Cambridge (U.K.), pp. 665–822.

Egeland, E.S., Garrido, J.L., Zapata, M., Maestro, M.A., and Liaaen-Jensen, S. (2000) Algal carotenoids. Part 64. Structure and chemistry of 4-keto-19′-hexanoyloxyfucoxanthin with a novel carotenoid end group. *J. Chem. Soc. Perkin Trans. 1* 1223–1230.

Falkowski, P.G. and Chen, Y.-B. (2003) Photoacclimation of light harvesting systems in eukaryotic algae. In, Green,B.R. and Parsons,W.W. (eds), *Light harvesting Antennas in Photosynthesis*. Kluwer Academic Publishers, Dordrecht, pp. 423–447.

Falkowski, P.G. and Raven, J.A. (2007) Aquatic Photosynthesis (second edition). Princeton University Press, Princeton.

Faust, M.A., Sager, J.C., and Meeson, B.W. (1982) Response of *Prorocentrum Mariae-Lebouriae* (Dinophyceae) to light of different spectral qualities and irradiances: growth and pigmentation. *J. Phycol.* **18**: 349–356.

Garrido, J.L., Otero, J., Maestro, M.A., and Zapata, M. (2000) The main nonpolar chlorophyll c from *Emiliania huxleyi* (Prymnesiophyceae) is a chlorophyll c2-monogalactosyldiacylglyceride ester: A mass spectrometry study. *J. Phycol.* **36**: 497–505.

Garrido, J.L. and Roy, S. (2015) The Use of HPLC for the Characterization of Phytoplankton Pigments. In, Stengel,D.B. and Connan,S. (eds), *Natural Products From Marine Algae: Methods and Protocols*. New York, pp. 241–252.

Garrido, J.L., Zapata, M., and Muniz, S. (1995) Spectral characterization of new chlorophyll-*c* pigments isolated from *Emiliania huxleyi* (Prymnesiophyceae) by high-performance liquid-chromatography. *J. Phycol.* **31**: 761–768.

Guillard, R.R.L. and Hargraves, P.E. (1993) *Stichochrysis immobilis* is a diatom, not a chrysophyte. *Phycologia* **32**: 234–236.

Hagino, K., Bendif, E.M., Young, J.R., Kogame, K., Probert, I., Takano, Y., et al. (2011) New evidence for morphological and genetic variation in the cosmopolitan coccolithophore *Emiliania huxleyi* (prymnesiophyceae) from the cox1b-atp4 genes. *J. Phycol.* **47**: 1164–1176.

Harris, G.N., Scanlan, D.J., and Geider, R.J. (2005) Acclimation of *Emiliania huxleyi* (Prymnesiophyceae) to photon flux density. *J. Phycol.* **41**: 851–862.

Higgins, H.W., Wright, S.W., and Schlüter, L. (2011) Quantitative interpretation of chemotaxonomic pigment data. In, Roy,S., Llewellyn,C.A., Egeland,E.S., and Johnsen,G. (eds), *Phytoplankton Pigments: Characterization, Chemotaxonomy and Applications in Oceanography*. Cambridge (U.K.), pp. 257–313.

Humbeck, K., Hoffmann, B., and Senger, H. (1988) Influence of energy flux and quality of light on the molecular organization of the photosynthetic apparatus in *Scenedesmus*. *Planta* **173**: 205–212.

Jeffrey, S.W., Mantoura, R.F.C., and Wright, S.W. eds. (1997) Phytoplankton pigments in oceanography Unesco publishing, Paris.

Kirk, J.T.O. (2011) Light and Photosynthesis in Aquatic Ecosystems Third. Cambridge University Press, Cambridge (U.K.).

van Leeuwe, M.A. and Stefels, J. (1998) Effects of Iron and Light Stress on the Biochemical Composition of antarctic *Phaeocystis* sp. (Prymnesiophyceae). II. pigment composition. *J. Phycol.* **503**: 496–503.

Lefebvre, S.C., Harris, G., Webster, R., Leonardos, N., Geider, R.J., Raines, C. a., et al. (2010) Characterization and expression analysis of the LHCF gene family in *Emiliania huxleyi* (haptophyta) reveals differential responses to light and co2. *J. Phycol.* **46**: 123–134.

Van Lenning, K., Probert, I., Latasa, M., Estrada, M., and Young, J.R. (2004) Pigment diversity of coccolithophores in relation to taxonomy, phylogeny and ecological preferences. In, Thierstein,H.R. and Young,J.R. (eds), *Coccolithophores: From Molecular Processes to Global Impact*. Springer Verlag, Berlin, pp. 51–73.

Leonardos, N. and Harris, G.N. (2006) Comparative effects of light on pigments of two strains of *Emiliania huxleyi* (Haptophyta). *J. Phycol.* **42**: 1217–1224.

Liu, H., Probert, I., Uitz, J., Claustre, H., Aris-Brosou, S., Frada, M., et al. (2009) Extreme diversity in noncalcifying haptophytes explains a major pigment paradox in open oceans. *Proc. Natl. Acad. Sci. U. S. A.* **106**: 12803–12808.

Mauzerall, D. and Greenbaum, N.L. (1989) The absolute size of a photosynthetic unit. *Biochim. Biophys. Acta* **974**: 119–140.

McClean-Padman, J. and Padman, L. (1991) Summer upwelling on the Sydney inner continental shelf: The relative roles of local wind forcing and mesoscale eddy encroachment. *Cont. Shelf Res.* **11**: 321–345.

Mckew, B. a., Davey, P., Finch, S.J., Hopkins, J., Lefebvre, S.C., Metodiev, M. V., et al. (2013) The trade-off between the light-harvesting and photoprotective functions of fucoxanthin-chlorophyll proteins dominates light acclimation in *Emiliania huxleyi* (clone CCMP 1516). *New Phytol.* **200**: 74–85.

Melkozernov, A.N. and Blankenship, R.E. (2006) Photosynthetic Functions of Chlorophylls. In, Grimm,B., Porra,R.J., Rüdiger,W., and Scheer,H. (eds), *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications*. Springer, Dordrecht, pp. 397–412.

Mouget, J.L., Rosa, P., and Tremblin, G. (2004) Acclimation of *Haslea ostrearia* to light of different spectral qualities–Confirmation of chromatic adaptation in diatoms. *J. Photochem. Photobiol. B Biol.* **75**: 1–11.

Nanninga, H.J. and Tyrrell, T. (1996) Importance of light for the formation of algal blooms by *Emiliania huxleyi*. *Mar. Ecol. Prog. Ser.* **136**: 195–203.

Not, F., Latasa, M., Scharek, R., Viprey, M., Karleskind, P., Balagué, V., et al. (2008) Protistan assemblages across the Indian Ocean, with a specific emphasis on the picoeukaryotes. *Deep. Res. Part I Oceanogr. Res. Pap.* **55**: 1456–1473.

Piasecki, T., Breadmore, M.C., and Macka, M. (2010) White LEDs as broad spectrum light sources for spectrophotometry: Demonstration in the visible spectrum range in a diode-array spectrophotometric detector. *Electrophoresis* **31**: 3737–3744.

van de Poll, W.H., Visser, R.J.W., and Buma, A.G.J. (2007) Acclimation to a dynamic irradiance regime changes excessive irradiance sensitivity of *Emiliania huxleyi* and *Thalassiosira weissflogii*. *Limnol. Oceanogr.* **52**: 1430–1438.

Poulton, A.J., Adey, T.R., Balch, W.M., and Holligan, P.M. (2007) Relating coccolithophore calcification rates to phytoplankton community dynamics: Regional differences and implications for carbon export. *Deep. Res. Part II Top. Stud. Oceanogr.* **54**: 538–557.

Ragni, M., Airs, R.L., Leonardos, N., and Geider, R.J. (2008) Photoinhibition of PSII in *Emiliania huxleyi* (Haptophyta) under high light stress: The roles of photoacclimation, photoprotection, and photorepair. *J. Phycol.* **44**: 670–683.

Schellenberger Costa, B., Jungandreas, A., Jakob, T., Weisheit, W., Mittag, M., and Wilhelm, C. (2013) Blue light is essential for high light acclimation and photoprotection in the diatom *Phaeodactylum tricornutum*. *J. Exp. Bot.* **64**: 483–493.

Schlüter, L., Møhlenberg, F., Havskum, H., and Larsen, S. (2000) The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: Testing the influence of light and nutrients on pigment/chlorophyll a ratios. *Mar. Ecol. Prog. Ser.* **192**: 49–63.

Schofield, O., Bidigare, R., and Prézelin, B. (1990) Spectral photosynthesis, quantum yield and blue-green light enhancement of productivity rates in the diatom *Chaetoceros gracile* and the prymnesiophyte *Emiliania huxleyi* . *Mar. Ecol. Prog. Ser.* **64**: 175–186.

Scholes, G., Fleming, G., Olaya-Castro, A. and van Grondelle, R. (2011) Lessons from nature about solar light harvesting. *Nat. Publ. Gr.* **3**: 763–774.

Stolte, W., Kraay, G.W., Noordeloos, A. M., and Riegman, R. (2000) Genetic and physiological variation in pigment composition of *Emiliania huxleyi* (Prymnesiophyceae) and the potential use of its pigment ratios as a quantitative physiological marker. *J. Phycol.* **36**: 529–539.

Suggett, D.J., Le Floc'H, E., Harris, G.N., Leonardos, N., and Geider, R.J. (2007) Different strategies of photoacclimation by two strains of *Emiliania huxleyi* (Haptophyta). *J. Phycol.* **43**: 1209–1222.

Thompson, P. A. and Calvert, S.E. (1995) Carbon isotope fractionation by *Emiliania huxleyi*. *Limnol. Oceanogr.* **40**: 673–679.

Tyrrel, T. and Merico, A. (2004) *Emiliania huxleyi*: bloom observations and the conditions that induce them. In Thierstein, H.R. and Young J.R. (eds) *Coccolithophores: From Molecular Processes to Global Impact*. Springer Verlag, Berlin.

Vélez-Belchí, P. and Tintoré, J. (2001) Vertical velocities at an ocean front . *Sci. Mar.* **65**: 291–300.

Viviani, D.A., Björkman, K.M., Karl, D.M., and Church, M.J. (2011) Plankton metabolism in surface waters of the tropical and subtropical Pacific Ocean. *Aquat. Microb. Ecol.* **62**: 1–12.

Walsh, J.I., Kelley, J.C., Whitledge, T.E., and Huntsman, S. a (1974) Spin-up of the Baja California. **19.**:

Wilhelm, C., Krámer, P., and Aloysius Wild (1985) Effect of different light qualities on the ultrastructure, thylakoid membrane composition and assimilation metabolism of *Chlorella fusca*. *Physiol. Plant.* **64**: 359–364.

Young, J.R. and Westbroek, P. (1991) Genotypic variation in the coccolithophorid species *Emiliania huxleyi*. *Mar. Micropaleontol.* **18**: 5–23.

Zapata, M., Jeffrey, S.W., Wright, S.W., Rodríguez, F., Garrido, J.L., and Clementson, L. (2004) Photosynthetic pigments in 37 species (65 strains) of Haptophyta: Implications for oceanography and chemotaxonomy. *Mar. Ecol. Prog. Ser.* **270**: 83–102.

Zapata, M., Rodríguez, F., and Garrido, J.L. (2000) Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C8 column and pyridine containing mobile phases. *Mar. Ecol. Prog. Ser.* **195**: 29–45.

Figure legends

Fig. 1. Lighting device employed for the setting of different light intensities and spectral qualities.

Fig. 2. Spectral properties of the white LEDs and the light from the LEDs after passing through the blue, green and red filters.

Fig. 3. Representative HPLC pigment profiles of *E. huxleyi* under the eight light conditions assayed. Peak identification: 1 chlc_3, 2 MVchlc_3, 3 chlc_2, 4 F, 5 PF, 6 HKf, 7 HF, 8 Dd, 9 Dt, 10 chlc_2-MGDG, 11 chl *a*, 12 β,ε-car and 13 β,β-car

Fig. 4. Chls *c* and fucoxanthin concentrations *vs* chl *a* (attomol per cell). A) Concentration of total chl *cs*, chlc_3, chlc_2, chlc_2-MGDG, MVchlc_3 vs chl *a* concentration. B) Concentration of 3, 4, 5, 6, 7, 8, 9, 10, 11, HKf, HF, and PF vs chl *a* concentration

Fig. 5. Chl *c* : chl *a* ratios under the eight light conditions (experiment #1, four spectral lights at two light intensities). Data represents mean (n=3) and error bars correspond to the SD. Different letters indicate significant differences ($p < 0.05$), after analysis of variance (ANOVA), Tukey's post hoc test.**Fig. 6.** Fucoxanthin related xanthophylls : chl *a* ratios under the eight light conditions (experiment #1, four spectral lights at two light intensities). Data represents mean (n=3) and error bars correspond to the SD. Different letters indicate significant differences ($p < 0.05$), after analysis of variance (ANOVA), Tukey's post hoc test.

Fig. 7. Distribution of chl *c* : chl *a* ratios (chl c_3, MV chl c_3, chl c_2, chl c_2-MGDG) over time (in days, 0 corresponds to the day before the shift). A) downwelling and green light, B) upwelling and green light, C) downwelling and blue light, D) upwelling and blue light. Data represents mean (n=3) and Error bars corresponds to the SD. Results of the Tukey test for pair-wise comparisons are included in the plots. Distinct letters or numbers indicate significant differences ($p < 0.05$) between experimental groups.

Fig. 8. Distribution of fucoxanthins: chl *a* ratios over time (in days, 0 corresponds to the day before the shift). A) downwelling and green light, B) upwelling and green light, C) downwelling and blue light, D) upwelling and blue light. Data represents mean (n=3) and error bars corresponds to the SD. Results of the Tukey test for pair-wise comparisons are included in the plots. Distinct letters or numbers indicate significant differences ($p < 0.05$) between experimental groups.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

Table 1. Pigment amount (pmol cell^{-1}), accessory pigments : chl *a* ratios, DES (de-epoxidation state) and F'_v/F'_m in experiment #1. HL = High light, LL = Low light (*µmol photons $m^{-2}s^{-1}$). Total chls *c* = sum of chl c_2, c_3, MVc_3, c_2-MGDG ; (**total fucoxanthins = sum of BF, F, HF, HKf, PF). Different letters indicate significant differences ($p < 0.05$), after analysis of variance (ANOVA), Tukey's post hoc test.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

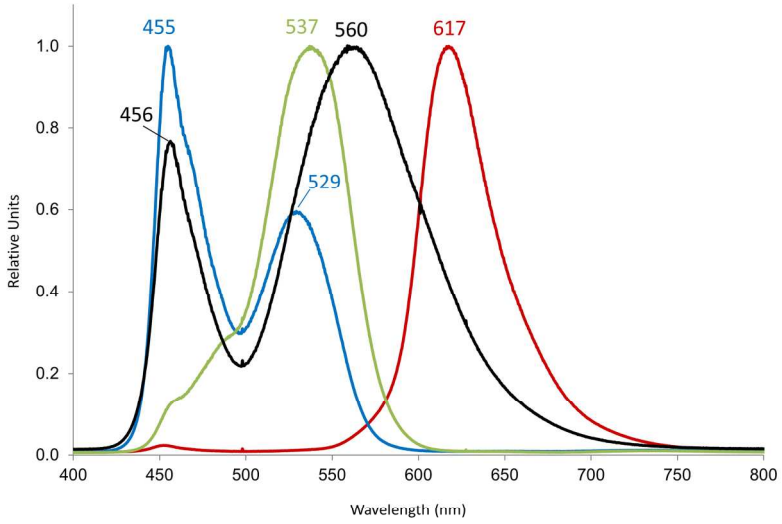
		HL				LL			
Pigments **Light intensity***		**Blue 409±37**	**White 426±60**	**Green 404±47**	**Red 458±32**	**Blue 16±2**	**White 16**	**Green 16±1**	**Red 16±1**
Chl c_3	amol cell^{-1}	7.24±0.75(a)	8.37±0.13(a,b)	12.46±0.61(b)	23.92±0.75(c)	18.70±0.76(d)	20.63±1.00(d)	24.12±0.22(c)	24.53±1.10(c)
	ratio to chl *a*	0.066±0.001(a)	.069±0.002(a,b)	0.086±0.001(b)	0.121±0.001(c)	0.109±0.002(d)	0.117±0.001(c,d)	0.120±0.001(c)	0.107±0.001(d)
MV chl c_3	amol cell^{-1}	7.59±1.19(a)	8.74±0.21(a,b)	9.45±0.18(b)	4.65±0.37(c)	6.86±0.35(a)	6.84±0.32(a)	7.73±0.37(a)	10.79±0.28 (b)
	ratio to chl *a*	0.069±0.005(a)	0.072±0.001(a)	0.065±0.002(a)	0.023±0.001(b)	0.040±0.001(c)	0.039±0.002(b,c)	0.038±0.001(b,c)	0.047±0.003(a,c)
chl c_2	amol cell^{-1}	10.13±1.46(a)	11.22±0.07(a)	16.96±1.51(b)	30.60±1.94(c)	23.01±0.71(d)	22.39±1.93(d)	25.03±0.46(d)	25.74±0.79(d)
	ratio to chl *a*	0.093±0.004(a)	0.093±0.002(a)	0.117±0.008(b)	0.155±0.005(c)	0.134±0.005(d)	0.127±0.005(b,d)	0.124±0.002(b,d)	0.112±0.001(b)
chlc_2-MGDG	amol cell^{-1}	6.64±0.75(a)	7.65±0.24(a)	10.47±0.79(b)	15.56±0.60(c)	13.19±0.70(d)	13.58±0.88(d)	16.37±0.22(c)	18.58±1.15(c)
	ratio to chl *a*	0.061±0.002(a)	0.063±0.003(a)	0.072±0.003(b)	0.079±0.002(c)	0.077±0.002(b,c)	0.077±0.002(b,c)	0.081±0.001(c)	0.081±0.002(c)
F	amol cell^{-1}	7.12±0.43(a)	16.74±1.24(b)	53.28±2.32(c)	124.45±3.95(d)	10.20±.77(a)	18.27±1.81(b)	76.69±4.73(c,d)	143.57±6.98(d)
	ratio to chl *a*	0.066±0.003(a)	0.138±0.006(b)	0.366±0.002(b,d)	0.629±0.010(c)	0.060±0.004(a)	0.104±0.013(b)	0.381±0.016(d)	0.626±0.004(c)
HKf	amol cell^{-1}	19.61±1.93(a)	32.92±2.16(b)	41.94±0.92(c)	40.94±1.14(c)	24.28±0.38(a,b)	35.53±1.04(b)	48.50±1.76(c,d)	58.73±1.76(d)
	ratio to chl *a*	0.180±0.004(a)	0.272±0.011(b)	0.289±0.006(b)	0.207±0.005(c)	0.142±0.002(d)	0.202±0.010(c)	0.241±0.004(e)	0.256±0.005(b,e)
HF	amol cell^{-1}	67.03±6.82(a)	52.65±0.83(b)	35.42±1.35(c)	24.18±0.67(c)	127.29±4.80(d)	115.58±7.05(e)	70.31±1.51(a)	29.63±0.57(c)
	ratio to chl *a*	0.615±0.004(a)	0.436±0.013(b)	0.244±0.006(c)	0.122±0.002(d)	0.743±0.007(e)	0.655±0.013(f)	0.349±0.013(g)	0.129±0.005(d)
Dd	amol cell^{-1}	41.36±5.03(a)	41.91±0.58(a)	43.95±1.59(a)	48.54±3.99(a)	44.23±3.89(a)	33.99±2.46(a,b)	34.71±.44(a,b)	35.57±1.19(a,b)
	ratio to chl *a*	0.379±0.010(a)	0.347±0.007(a,b)	0.302±0.006(b)	0.245±0.011(c)	0.258±0.021(c)	0.193±0.006(d)	0.172±0.001(d,e)	0.155±0.003(e)
Dt	amol cell^{-1}	60.50±3.31(a)	71.52±4.07(b)	49.25±3.26(c)	25.07±0.84(d)	0.47±0.03(e)	1.33±0.86(e)	0.58±0.04(e)	0.96±0.03(e)
	ratio to chl *a*	0.558±0.050(a)	0.592±0.027(a)	0.339±0.018(a)	0.127±0.001(a,b)	0.003±0.000(b)	0.008±0.005(b)	0.003±0.000(b)	0.004±0.000(b)
βε-car	amol cell^{-1}	0.61±0.52(a)	0.65±0.54(a)	1.69±0.08(a,b)	1.27±0.80(a)	8.87±0.32(b)	7.00±0.82(b)	10.23±0.54(b,c)	11.90±0.52(c)
	ratio to chl *a*	0.006±0.006(a)	0.005±0.005(a)	0.012±0.001(a)	0.007±0.004(a)	0.050±0.001(b)	0.041±0.005(b)	0.051±0.002(b)	0.052±0.002(b)
ββ-car	amol cell^{-1}	13.84±1.72(a)	15.17±1.07(a)	14.13±0.65(a)	14.84±1.37(a)	9.37±0.83(b)	10.20±0.74(b)	9.64±0.65(b)	10.17±0.43(b)
	ratio to chl *a*	0.127±0.004(a)	0.126±0.006(a)	0.097±0.002(b)	0.075±0.005(c)	0.053±0.002(d)	0.060±0.005(d)	0.048±0.002(e)	0.044±0.001(e)
Tot Chls *c*	ratio to chl *a*	0.289±0.008(a)	0.298±0.007(a)	0.339±0.007(a,d)	0.378±0.006(b)	0.360±0.001(c)	0.360±0.006(c,d)	0.364±0.003(b,c)	0.348±0.003(d)
Tot Fxs**	ratio to chl *a*	0.901±0.011(a)	0.885±0.005(a)	0.926±0.011(a,b)	0.977±0.010(b)	0.981±0.002(b)	0.999±0.010(c)	1.005±0.008(c)	1.053±0.008(c)
Chl *a*	amol cell^{-1}	109.07±11.06(a)	120.80±3.73(a,b)	145.42±6.07(b)	197.85±6.73(c)	171.41±5.41(d)	176.27±7.80(d)	201.44±3.87(c,e)	229.15±9.84(e)
Cells ml^{-1}		8.6×10^6 $\pm2.25\times10^5$(a)	7.95×10^6 $\pm2.18\times10^5$(a,b)	8.45×10^6 $\pm3.73\times10^5$(a)	8.70×10^6 $\pm1.48\times10^5$(a)	6.91×10^6 $\pm2.05\times10^5$(b)	5.58×10^6 $\pm3.27\times10^5$(b,c)	4.48×10^6 $\pm3.36\times10^5$(b,c)	2.73×10^6 $\pm9.35\times10^4$(c)
DES		0.59±0.02(a)	0.62±0.01(a)	0.53±0.02(b)	0.35±0.01(c)	0.03±0.02(d)	0.01±0.00(d)	0.02±0.00(d)	0.03±0.00(d)
F'_v/F'_m		0.47±0.03(a)	0.48±0.07(a)	0.52±0.01(a,b)	0.46±0.01(a)	0.68±0.02(b)	0.65±0.01(b)	0.65±0.02(b)	0.68±0.00(b)

Table 2. F'_v/F'_m, ratios of xanthophyll cycle pigments to chl *a* and DES values (average and S.D.) in experiment # 2. Different letters indicate significant differences ($p < 0.05$), after analysis of variance (ANOVA), Tukey's post hoc test.

	Downwelling					Upwelling				
	White HL	GREEN LL				GREEN LL	White HL			
	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
F'v/F'm	0.414(a)	0.623(a,b,c)	0.650(b)	0.641(b)	0.627(c)	0.649(a)	0.306(b)	0.406(c)	0.434(c)	0.432(c)
	±0.039	±0.003	±0.017	±0.007	±0.006	±0.014	±0.017	±0.028	±0.011	±0.017
Dd/chl*a*	0.539(a)	0.397(a,b)	0.237(b,c)	0.205(b,c)	0.198(c)	0.179(a)	0.275(b)	0.411(c)	0.512(d)	0.385(c)
	±0.076	±0.021	±0.001	±0.006	±0.007	±0.002	±0.006	±0.015	±0.015	±0.024
Dt/chl*a*	0.442(a)	0.031(b)	0.016(c)	0.010(d)	0.008(e)	0.008(a)	0.334(b)	0.356(b)	0.346(b)	0.394(b)
	±0.113	±0.001	±0.000	±0.001	±0.001	±0.000	±0.017	±0.033	±0.01	±0.083
Dd+Dt/chl*a*	0.981(a)	0.428(a,b)	0.253(b,c)	0.215(c)	0.206(c)	0.187(a)	0.609(a,b)	0.767(b)	0.858(b)	0.780(b)
	±0.038	±0.022	±0.002	±0.007	±0.008	±0.002	±0.014	±0.023	±0.020	±0.085
DES	0.448(a)	0.072(b)	0.062(c)	0.047(d)	0.039(e)	0.044(a)	0.548(b)	0.463(c)	0.404(c)	0.502(b,c)
	±0.096	±0.004	±0.001	±0.003	±0.004	±0.003	±0.017	±0.031	±0.009	±0.056
	White HL	BLUE LL				BLUE LL	White HL			
	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
F'v/F'm	0.429(a)	0.596(a)	0.638(b)	0.632(b)	0.567(a)	0.694(a)	0.348(b)	0.433(b)	0.425(b)	0.426(b)
	±0.044	±0.012	±0.006	±0.007	±0.077	±0.024	±0.01	±0.051	±0.045	±0.026
Dd/chl*a*	0.551(a)	0.470(b)	0.283(c)	0.252(c)	0.218(d)	0.213(a)	0.263(b)	0.345(c)	0.366(c)	0.379(c)
	±0.023	±0.002	±0.011	±0.006	±0.001	±0.019	±0.015	±0.013	±0.02	±0.008
Dt/chl*a*	0.479(a)	0.035(a,b)	0.015(b,c)	0.015(b,c)	0.012(c)	0.010(a)	0.379(b,c)	0.509(c)	0.437(b,c)	0.280(a,b)
	±0.02	±0.002	±0.003	±0.006	±0.001	±0.003	±0.043	±0.025	±0.08	±0.031
Dd+Dt/chl*a*	1.030(a)	0.505(b)	0.298(c)	0.268(d)	0.230(e)	0.223(a)	0.642(b)	0.854(c)	0.802(c)	0.658(b)
	±0.043	±0.004	±0.013	±0.001	±0.001	±0.022	±0.032	±0.034	±0.067	±0.036
DES	0.465(a)	0.068(b)	0.050(b)	0.037(b)	0.052(b)	0.045(a)	0.590(b)	0.596(b)	0.541(b)	0.424(c)
	±0.044	±0.003	±0.008	±0.023	±0.007	±0.009	±0.04	±0.03	±0.055	±0.024

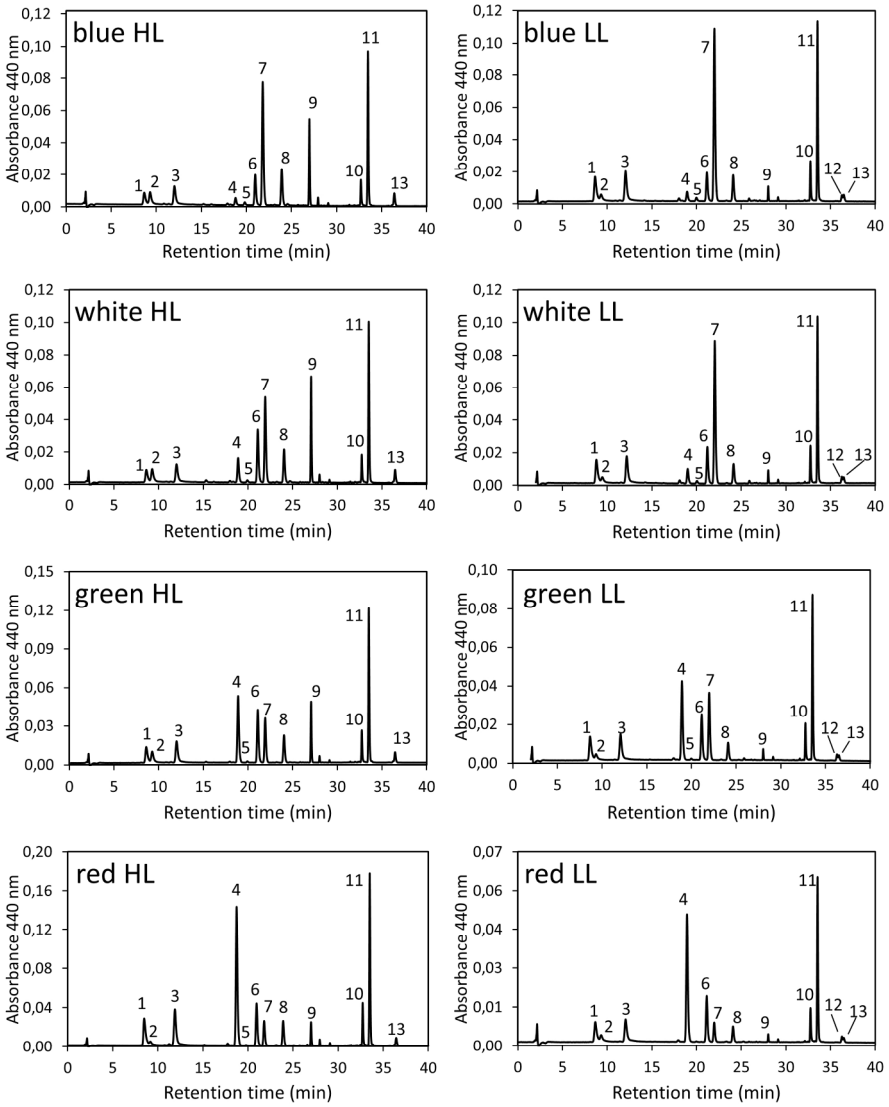
Fig. 1. Lighting device employed for the setting of different light intensities and spectral qualities.
154x140mm (300 x 300 DPI)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373



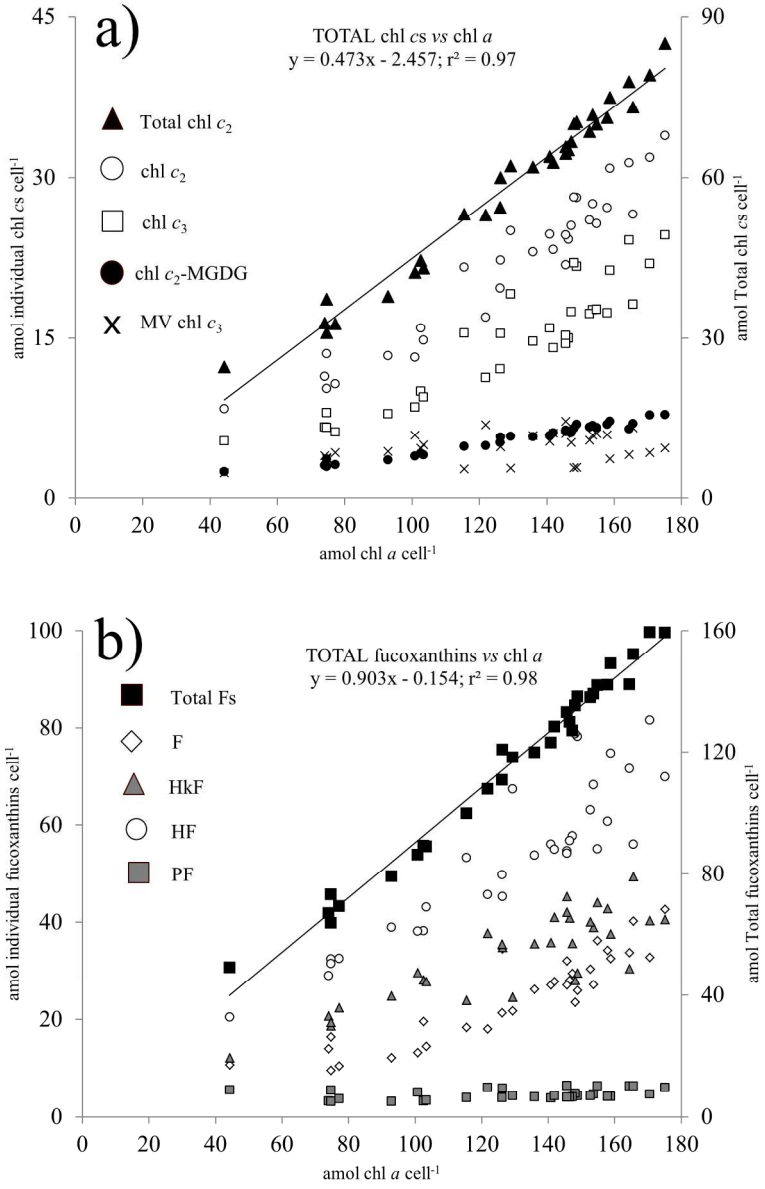
Spectral properties of the white LEDs and the light from the LEDs after passing through the blue, green and red filters
190x142mm (300 x 300 DPI)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373



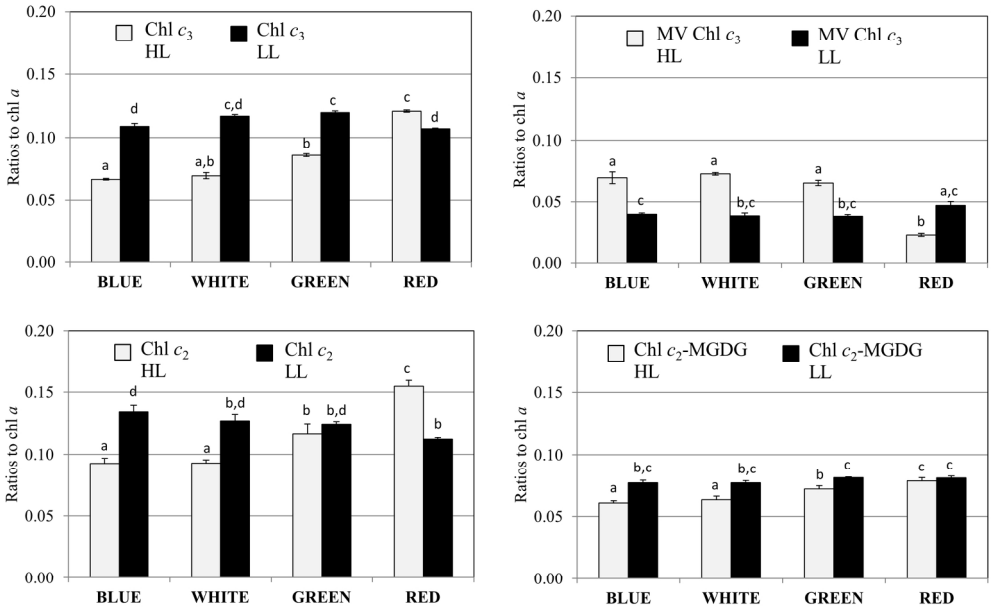
Representative HPLC pigment profiles of *E. huxleyi* under the eight light conditions assayed. Peak identification: 1 chlc3, 2 MVchlc3, 3 chlc2, 4 F, 5 PF, 6 HKf, 7 HF, 8 Dd, 9 Dt, 10 chlc2-MGDG, 11 chl a, 12β,ε-car and 13 β,β-car
254x338mm (300 x 300 DPI)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373



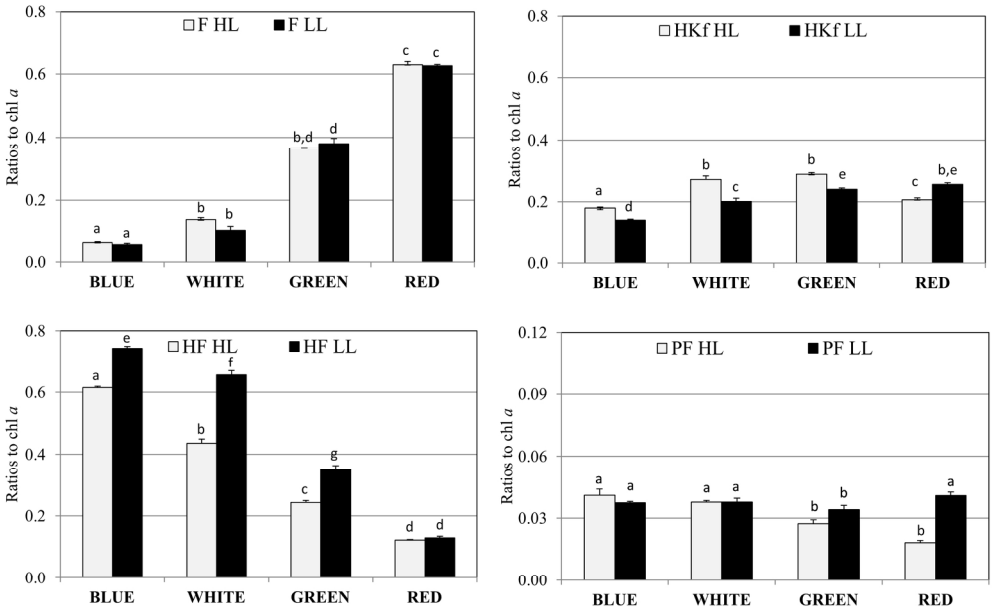
Chls c and fucoxanthin concentrations vs chl a (attomol per cell). A) Concentration of total chl cs, chlc2, chlc3, chlc2-MGDG, MVchlc3 vs chl a concentration; B) Concentration of total Fs, F, HKf, HF, and PF vs chl a concentration
254x338mm (300 x 300 DPI)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373

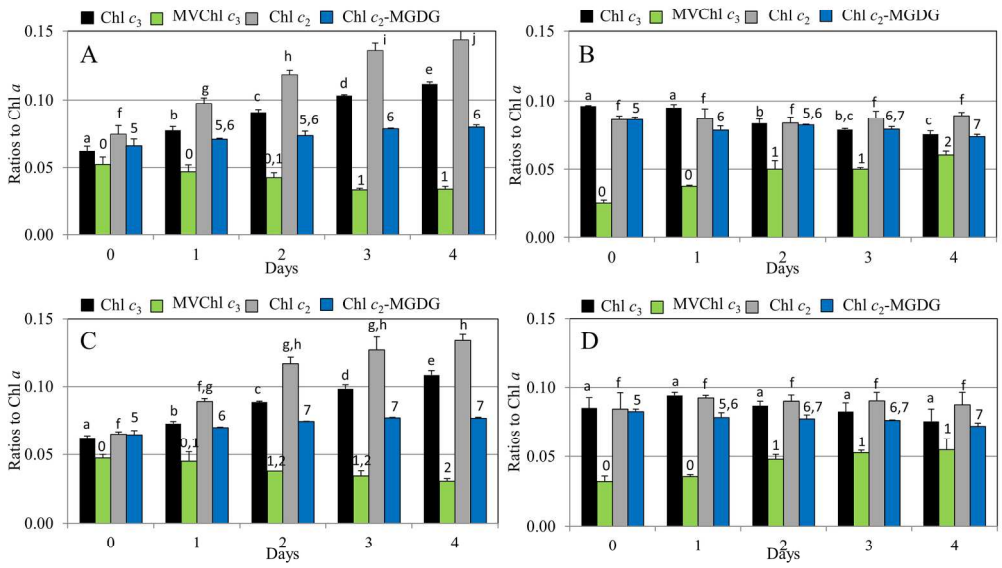


Chl c : chl a ratios under the eight light conditions (experiment #1, four spectral lights at two light intensities). Data represents mean (n=3) and error bars correspond to the SD. Different letters indicate significant differences ($p < 0.05$), after analysis of variance (ANOVA), Tukey's post hoc test.
190x142mm (300 x 300 DPI)

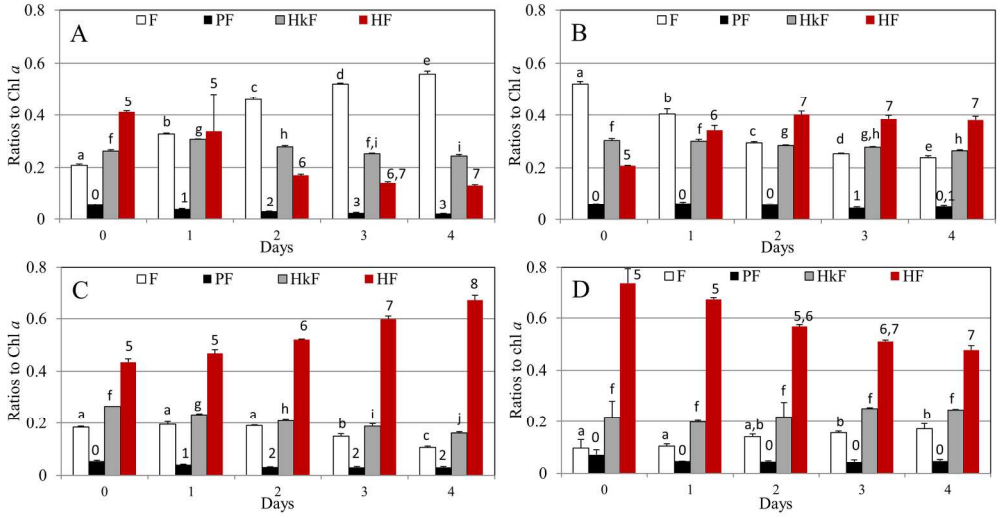
This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13373



Fucoxanthin related xanthophylls : chl a ratios under the eight light conditions (experiment #1, four spectral lights at two light intensities). Data represents mean (n=3) and error bars correspond to the SD. Different letters indicate significant differences ($p < 0.05$), after analysis of variance (ANOVA), Tukey's post hoc test.
190x142mm (300 x 300 DPI)



Distribution of chl c : chl a ratios (chl c3, MV chl c3, chl c2, chl c2-MGDG) over time (in days, 0 corresponds to the day before the shift). A) downwelling and green light, B) upwelling and green light, C) downwelling and blue light, D) upwelling and blue light. Data represents mean (n=3) and Error bars corresponds to the SD. Results of the Tukey test for pair-wise comparisons are included in the plots. Distinct letters or numbers indicate significant differences ($p < 0.05$) between experimental groups.
190x142mm (300 x 300 DPI)



Distribution of fucoxanthins: chl a ratios over time (in days, 0 corresponds to the day before the shift). A) downwelling and green light, B) upwelling and green light, C) downwelling and blue light, D) upwelling and blue light. Data represents mean (n=3) and error bars corresponds to the SD. Results of the Tukey test for pair-wise comparisons are included in the plots. Distinct letters or numbers indicate significant differences ($p < 0.05$) between experimental groups.
190x142mm (300 x 300 DPI)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology