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Comparison of photosynthetic responses in diploid and haploid life-cycle phases of *Emiliania huxleyi* (Prymnesiophyceae)

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ABSTRACT: *Emiliania huxleyi* is a ubiquitous coccolithophore, capable of forming large blooms. This species presents a digenetic heteromorphic life cycle, the non-motile diploid phase typically bearing coccoliths and the flagellated haploid phase being non-calcified. Oxygen production rates at different irradiances of both phases were studied in mid-exponential and transitional growth phases in cultures grown under identical conditions. There were no significant differences in basic photosynthetic parameters ($\alpha^{\text{chl } a}$, maximum light utilization coefficient; $P^{\text{chl } a}_{\text{max}}$, the light-saturated maximal rate of photosynthesis; E_k, the light-saturation parameter) between the 2 life-cycle phases; however, whereas the diploid phase did not exhibit photoinhibition at irradiances up to 1000 µmol photons m^{-2} s^{-1}, photoinhibition was recorded in the haploid phase above 400 to 500 µmol photons m^{-2} s^{-1} and photosynthetic rate decreased to ca. 75% of P_{max} at 1400 µmol photons m^{-2} s^{-1}. The 2 phases cultured under identical (non-saturating in terms of light) conditions did not present any significant differences in pigment content. These results are discussed in an ecological context. The lack of photoinhibition could confer a competitive advantage on the diploid stage, notably in a turbulent environment. For the haploid stage, the occurrence of photoinhibition may indicate niche separation (spatial and/or temporal) relative to the diploid phase.

KEY WORDS: *Emiliania huxleyi* · Coccolithophore · Life cycle · Haplo-diploid · Photosynthesis · Pigments

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INTRODUCTION

Emiliania huxleyi (Lohmann) Hay et Mohler is numerically the most abundant coccolithophore in the modern ocean and is capable of forming vast blooms in the open sea. These blooms, which are often clearly visible in satellite images, may have a surface area >100 000 km^2 (Brown & Yoder 1994) with cell concentrations up to 10 000 cells ml^{-1} and concentrations of detached coccoliths up to 300 000 ind. ml^{-1} (Holligan et al. 1993). This species has been intensely studied in the contexts of biogeochemistry (especially relating to the global carbon and sulphur cycles), cellular carbon physiology, biomineralization, and plankton ecology (see review by Paasche 2001 and references therein).

This interest has focussed on the coccolith-bearing stage in the life cycle of this species. Klaveness (1972) reported that *Emiliania huxleyi* presents a heteromorphic life cycle with the coccolith-bearing non-motile ('C-cell') stage alternating with non-calcified non-motile ('N-cell') and non-calcifying (but organic scale-bearing) flagellated ('S-cell') stages, all stages being capable of independent asexual reproduction. Green et al. (1996) demonstrated that this cycle is digenetic, the C-cell and N-cell stages typically being diploid relative to the haploid motile S-cell stage. Despite the immense interest in this species and the fact that this life-cycle was elucidated over 30 yr ago, very little is currently known about the distribution, physiology or ecological role of the motile haploid stage of *E. huxleyi*.

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Blooms of the coccolith-bearing stage of *E. huxleyi* typically occur in highly stratified water with a mixed layer depth always ≤30 m, and lack of photoinhibition in the diploid life cycle phase at light intensities up to at least 1000 µmol photons m^{-2} s^{-1} has been suggested to contribute to the dominance of this species in high irradiance surface waters (Nanninga & Tyrrel 1996). Recent evidence suggests that all coccolithophores exhibit a dimorphic haplo-diploid life cycle (Houdan et al. 2004). Among the hypotheses for the maintenance of a haplo-diploid cycle is the possibility that each phase occupies a distinct ecological niche (see review by Valéro et al. 1992), and some evidence for niche separation of known coccolithophore life-cycle associations is available from distribution studies in the natural environment (e.g. Cros 2002).

Differences in the physiological capacities of alternate coccolithophore life-cycle phases may provide evidence for each phase being adapted to a different niche. In order to test for this, photosynthetic responses and pigment composition of the 2 phases (diploid non-motile coccolith-bearing and haploid motile stages) of *Emiliania huxleyi* cultured under identical conditions were compared in this study.

MATERIALS AND METHODS

Strain and culture conditions. The original (non-axenic) culture of *Emiliania huxleyi* employed in this study (strain AC472 from the Algobank Culture Collection, Caen, France, isolated from a sample collected west of New Zealand in October 1998) was clonal, 1 cell in the coccolith-bearing phase being isolated from a plankton sample with a micropipette. This strain has been identified as *E. huxleyi* type R (see Paasche 2001, Young et al. 2003). Following phase change in culture (i.e. haploid S-cells formed in a diploid C-cell culture), pure cultures of each phase (i.e. only 1 life-cycle phase present) were established by isolating 1 cell of each phase from the mixed-phase culture using the same method. The stock cultures of each life-cycle phase were maintained in sterile single-use polystyrene culture flasks (Iwaki) in filter-sterilized enriched seawater K/2 (-Tris) medium (Keller et al. 1987) at 17°C with daylight fluorescent tubes providing an irradiance level of 90 µmol photons m^{-2} s^{-1} measured with a 4π quantum photometer (LiCor) with a photoperiod of 16 h light:8 h dark. For the experiments, batch cultures were grown in triplicate under the same culture conditions. Growth was monitored daily both by cell counting using a Malassez haematocyter and by fluorimetric measurement of *in vivo* chlorophyll *a* (chl *a*) (Turner Designs). Specific growth rates (µ) were calculated using the equation $\mu = \ln(C/C_{-1})/t$, where C is the fluorescence reading on the day of the measurement of photosynthetic activity, C_{-1} the fluorescence reading of the day before, and t the time in days between the 2 readings ($t = 1$).

Measurements of photosynthetic activity. Experiments were conducted with triplicate pure cultures of each (diploid and haploid) phase of *Emiliania huxleyi*. For each life-cycle phase, analyses were conducted on cultures at 2 different growth rates, ca. 0.75 d^{-1} (= mid exponential growth phase) and ca. 0.35 d^{-1} (= transitional growth phase, between exponential and stationary phases) (Table 1). The maximal growth rates obtained in our laboratory for both phases of this strain were ca. 1 d^{-1} when light intensity was higher than used in our experiments (Houdan 2003). The macronutrient concentrations used in our experimental cultures were non-limiting (authors' unpubl. results). The photosynthesis versus irradiance (*P–E*) measurements were conducted 8 h into the light period (i.e. the middle of light period). Immediately prior to measurement of photosynthetic activity, cultures were concentrated by gentle centrifugation (5 min at 164 × *g*, 17°C) in order to obtain a sufficient concentration to produce a signal response with the oxygen electrode. Microscopic observations were conducted in order to visually confirm the integrity of cells after centrifugation. Gross photosynthetic capacity and dark respiration were assessed by measuring oxygen evolution with a Clarke-type oxygen electrode (Hansatech) in a 2 ml chamber according to Dubinsky et al. (1987). The samples were maintained for 5 min in the dark and then progressively exposed to 12 different irradiances ranging from 0 to 1500 µmol photons m^{-2} s^{-1} (3 min for each step). To determine the quantity of chl *a*, 5 ml of culture were filtered onto a GF/C filter (Whatman) in duplicate and stored frozen at –80°C. Chl *a* was subsequently extracted by grinding the filters in 4 ml of 90% acetone (Aminot & Chaussepied 1983). Six ml of 90% acetone were added, and samples were stored in the dark at 4°C for 6 h. This was followed by centrifugation (15 min at 164 × *g*), and chl *a* was quantified in the supernatant by fluorimetry (Turner Designs) according to Welschmeyer (1994).

Determination of pigment content. The pigment content of 1 pure culture of each phase was determined in triplicate in mid-exponential phase in cultures grown under identical conditions to those described above. The cells were harvested by filtration onto Whatman GF/C filters and stored frozen at –80°C until analysis. Pigment extraction and HPLC quantification was conducted following Van Lenning et al. (2003). The concentration of all pigments was normalized to that of chl *a*, giving ratios in ng:ng.

Calculation of photosynthetic parameters. *P–E* curves were fitted for cultures of both life-cycle phases

by non-linear parametric estimation according to the model of Platt et al. (1980):

$$P^{chl\,a} = P_s^{chl\,a}\left[1-\exp\left(-\frac{\alpha^{chl\,a}E}{P_s^{chl\,a}}\right)\right]\exp\left(-\frac{\beta^{chl\,a}E}{P_s^{chl\,a}}\right)$$

where according to Sakshaug et al. (1997), $P^{chl\,a}$ is the photosynthetic rate, $\alpha^{chl\,a}$ is the maximum light-utilization coefficient, $\beta^{chl\,a}$ is the photoinhibition parameter, and $P_s^{chl\,a}$ is the potential maximum photosynthetic rate if there was no photoinhibition.

The maximum photosynthetic rate ($P_{max}^{chl\,a}$) and the light-saturation parameter (E_k) are defined as follows (Platt et al. 1980):

$$P_{max}^{chl\,a} = P_s^{chl\,a}\left(\frac{\alpha^{chl\,a}}{\alpha^{chl\,a}+\beta^{chl\,a}}\right)\left(\frac{\beta^{chl\,a}}{\alpha^{chl\,a}+\beta^{chl\,a}}\right)^{\beta^{chl\,a}/\alpha^{chl\,a}}$$

$$E_k = \frac{P_{max}^{chl\,a}}{\alpha^{chl\,a}}$$

E_k in this study corresponds to the irradiance at which photosynthesis was optimal, whereas E_{sat}, employed notably by Nanninga & Tyrrel (1996), is the irradiance at which the maximal rate of photosynthesis is first attained.

Due to the absence of statistically significant photoinhibition in the diploid phase culture, an alternative model was employed which removes this potential source of bias. *P–E* curves for the diploid phase culture were fitted by non-linear parametric estimation according to the model of Webb et al. (1974):

$$P^{chl\,a} = P_{max}^{chl\,a}\left[1-\exp\left(\frac{\alpha^{chl\,a}E}{P_{max}^{chl\,a}}\right)\right]$$

In practise, both models produced very similar curves for the diploid phase cultures. Four *P–E* response curves (each from measurements on triplicate cultures) were obtained for *Emiliania huxleyi* (see Fig. 1), 2 for cultures of the diploid stage (2N) at growth rates of 0.78 and 0.34 d^{-1} and 2 for cultures of the haploid stage (N) at growth rates of 0.74 and 0.37 d^{-1} (Table 1). With these curves, 3 characteristics of photosynthesis were calculated: $\alpha^{chl\,a}$ the maximum light-utilization coefficient, $P_{max}^{chl\,a}$ the light-saturated maximal rate of photosynthesis, and E_k the light-saturation parameter.

Statistical analyses. All statistical analyses and fitting were performed with Sigma Stat 2.0 software. Two-way ANOVAs were performed to test the difference between the means for culture variables (μ, chl *a* concentration per cell, pigment content per cell) using the life-cycle stage (2N or N) and the physiological state (growth rate) or pigment type (for pigment content) as factors. When significant differences were observed, means were ordered using the post-hoc Student-Newman-Keuls test. The Student's paired *t*-test was used to test for significant differences in each of the photosynthetic parameters between different experimental conditions (Scherrer 1984).

RESULTS

For each phase the high and low growth rates were significantly different ($p < 0.05$), but between phases there was no statistically significant difference between either high growth rates or low growth rates ($p < 0.05$; Fig. 1). At equivalent growth rates there was no significant difference between values of chl *a* content per cell, but for each phase it increased when growth was no longer exponential (Table 1).

There was no statistically significant difference in the values for either the maximum light-utilization coefficient [$\alpha^{chl\,a} \approx 4.4$ μmol O_2 mg chl a^{-1} h^{-1} (μmol photons m^{-2} $s)^{-1}$] or the light-saturation parameter (E_k ca. 90 μmol photons m^{-2} s^{-1}) between the 2 growth rates of each phase or between the 2 phases (Fig. 2). For all cultures the maximal rate of photosynthesis ($P_{max}^{chl\,a}$) was attained at irradiances (E_{sat}) of between 300 and 400 μmol photons m^{-2} s^{-1} (Fig. 1), and even though the values of $P_{max}^{chl\,a}$ varied, there was no significant difference between either life-cycle phase or growth rate.

In the diploid coccolith-bearing phase, in cultures at both high and low growth rates, there was no significant photoinhibition ($p > 0.05$) at light intensities up to at least 1000 μmol photons m^{-2} s^{-1}. By contrast, the motile non-calcifying haploid phase exhibited statistically significant photoinhibition at light intensities greater than 400 μmol photons m^{-2} s^{-1} ($\beta^{chl\,a} \approx 0.012 \pm 0.005$, Fig. 1).

Emiliania huxleyi contained the following pigments: chl *a*, divinyl and monovinyl chlorophyll c_3 (DV, MV chl c_3), chlorophyllid *a* (chlide *a*), mg-divinyl-phytoporphyrin (MgDVP), chlorophyll c_2 (chl c_2), fucoxanthin (Fx), 19′-butanoyloxyfucoxanthin (BFx), 4-keto-19′-hexanoyloxyfucoxanthin (4-KetoHFx), 19′-hexanoyloxyfucoxanthin (HFx), diadinoxanthin (Ddx), diatinoxanthin (Dtx) and an unknown type of zeaxanthin (Unk-2).

The relative concentrations of the principal pigments in each life-cycle phase are shown in Table 2. For all of these pigments, there was no significant difference in relative concentration between the diploid and haploid phases ($p > 0.05$).

DISCUSSION

The photosynthetic characteristics E_k and $P_{max}^{chl\,a}$ of *Emiliania huxleyi* vary with irradiance (E_{sat}) in much the same way as those of other phytoplankton (Nielsen

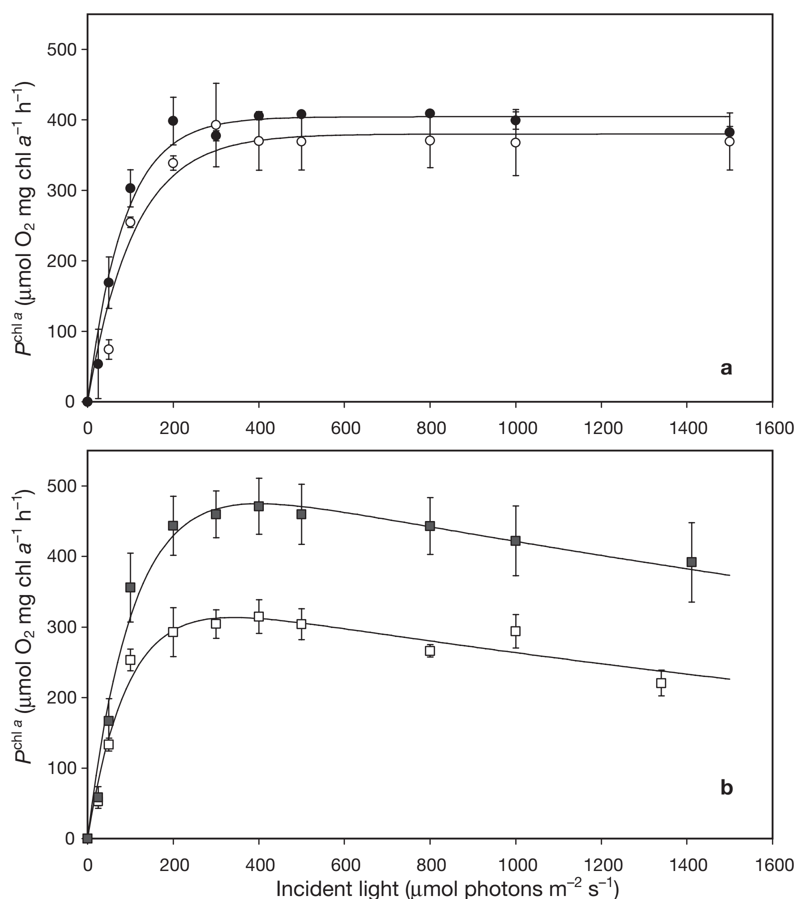

Fig. 1. *Emiliania huxleyi.* Photosynthesis–irradiance response curves (mean ± SE) of the (a) diploid and (b) haploid stages at 2 different growth rates, around 0.70 (open symbols) and 0.35 d^{-1} (solid symbols). Also shown are the associated fitted *P–E* models for the haploid (Platt et al. 1980) and diploid (Webb et al. 1974) stages (Student's paired *t*-test: $p < 0.001$, $35 < n < 30$, $0.82 < r^2 < 0.95$)

Table 1. Characteristics of haploid (N) vs. diploid (2N) phases of *Emiliania huxleyi* cultures: growth rate (µ), the ratio of chl *a* concentration over cell concentration (chl *a*/cell). Mean ± SE is given (n = 3 except where specified). Results from 2-way ANOVAs showed significant differences between the means ($p < 0.05$). Means not sharing a common superscript are significantly different ($p < 0.05$, Student-Newman-Keuls test)

	µ (d^{-1})	chl *a*/cell (µg l^{-1})
N	0.74 ± 0.01[a]	1.22 ± 0.02[a]
N	0.37 ± 0.02[b]	1.42 ± 0.07[b]
2N	0.78 ± 0.01[a]	1.17 ± 0.11[a]
2N	0.34* ± 0.03[b]	1.34 ± 0.03[b]

*n = 2

1997), increasing with the increase in the irradiance of acclimatization for $P^{chl\ a}_{max}$, for example. Within species, photosynthetic parameters are acclimation dependent, i.e. they vary with environmental factors such as nutrients, dissolved organic carbon, temperature and daily irradiance (Nielsen 1997). Comparing the range of values of photosynthetic parameters for a species in the literature may provide some idea of the reliability of each set of results, but direct comparisons between the values reported in different studies can only be made when culture conditions are very similar. In balanced growth, phytoplankton tend to acclimate to light conditions by matching E_k to the incident irradiance level by varying the values of $\alpha^{chl\ a}$ and $P^{chl\ a}_{max}$ (Sakshaug et al. 1997, McIntyre et al. 2002). The fact that E_k was similar to the culture irradiance level in all of our experiments indicates that the cultures were well acclimated to the light conditions. Under broadly similar conditions of temperature, irradiance/photoperiod, nutrients and specific growth rate, our results for the diploid C-cell stage of *E. huxleyi* are broadly comparable with those of Nielsen (1997). $P^{chl\ a}_{max}$ and E_k were lower in our study (396 compared to 435 µmol O_2 mg chl a^{-1} h^{-1}, and 108 compared to 153 µmol photons m^{-2} s^{-1}, respectively) and $\alpha^{chl\ a}$ was higher (3.8 compared to 2.84 µmol O_2 mg chl a^{-1} h^{-1} [µmol photons m^{-2} s]$^{-1}$). Van Bleijswijk et al. (1994) reported that there are no significant differences in light-saturation kinetics between *E. huxleyi* types A and B (2 morphotypes of this species, Young & Westbroek 1991), and Paasche (1999) came to the same conclusion when comparing clones from different latitudes. In both cases these authors were referring to analyses conducted exclusively on diploid stage cultures. Our results for E_k and E_{sat} suggest that light-saturation kinetics do not vary between the diploid and haploid life-cycle phases of the same strain. At present, it is not possible to generalize our results for the haploid stage to other strains due to the lack of comparative studies.

The functional role that the presence of coccoliths plays in the ecology of coccolithophores has been a subject of speculation and debate for many years (see review by Young 1994). One of the earliest hypotheses was that coccoliths might act as protective screens against the harmful effects of high light intensities (Berge 1962, Braarud et al. 1952). If coccoliths served such a function, then 2 possible effects might be observed (Nanninga & Tyrrel 1996): (1) the irradiance at which $P^{chl\ a}_{max}$ is attained (E_{sat}) would be higher in cal-

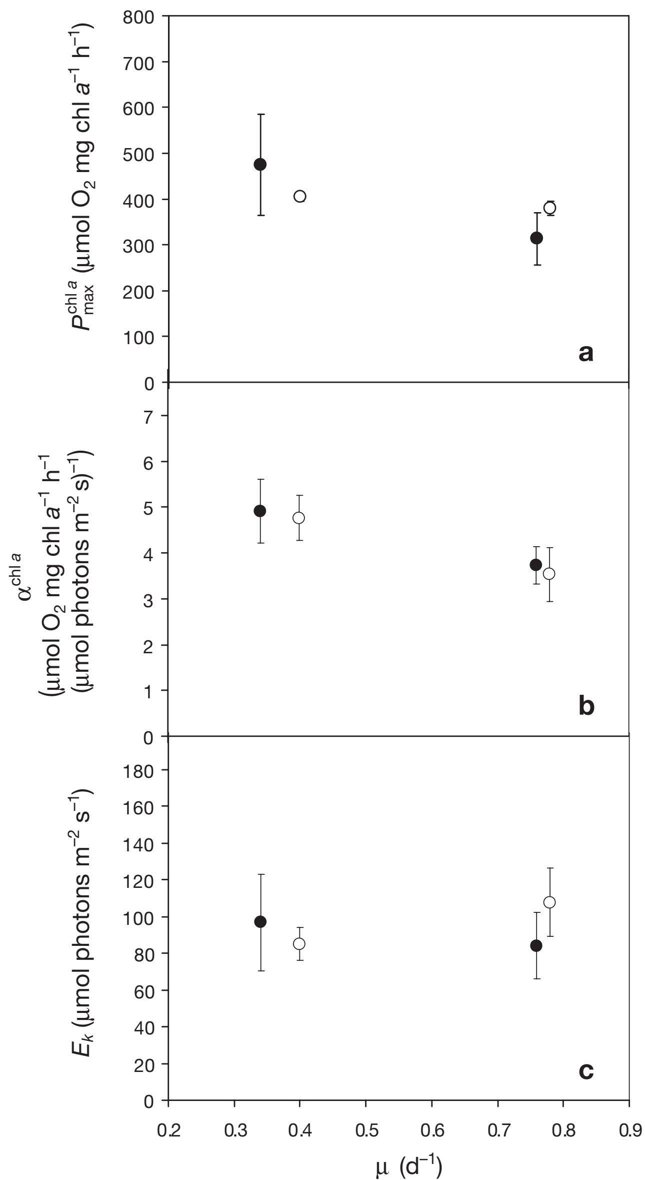

Fig. 2. *Emiliania huxleyi.* Photosynthetic parameters (mean ± SE) for the haploid (solid circles) and diploid (open circles) stages: (a) $P^{chl\,a}_{max}$ is the maximal photosynthetic rate, (b) $\alpha^{chl\,a}$ the maximum light utilization coefficient and (c) E_k the light-saturation parameter

cified cells, and (2) photoinhibition would be absent on exposure to high light intensities in calcified cells, but not in non-calcified cells. The fact that E_{sat} was similar in calcified and non-calcified cells was previously demonstrated using diploid coccolith-bearing and decalcified C-cell cultures and diploid non-calcifying N-cell cultures of *Emiliania huxleyi* by Nanninga & Tyrrel (1996). Our results are not quantitatively comparable to those just cited due to differences in culture conditions, but the conclusion is the same, i.e. that the E_{sat} values for calcified and non-calcified cells cultured under identical (non-saturating in terms of light) conditions are similar, and therefore that coccoliths do not act as protective light screens. However, the second possible effect cited by Nanninga & Tyrrel (1996) was observed in our study, i.e. photoinhibition was exhibited by the haploid non-calcified phase, but not by the diploid calcified cells. Nanninga & Tyrrel (1996), using calcified and decalcified C-cells, and non-calcified N-cells, demonstrated that the presence or the absence of coccoliths did not have an influence on the lack of photoinhibition of the diploid phase of *E. huxleyi*. It can be concluded, therefore, that the presence of photoinhibition in the haploid non-calcified phase of *E. huxleyi* in our study was not linked to the absence of coccoliths.

The remarkable lack of photoinhibition in the diploid phase of cultured *Emiliania huxleyi* after short-term exposure to irradiances of up to 1500 µmol photons m^{-2} s^{-1}, which is equivalent to the maximum downwelling irradiance normally encountered in the field, has been reported by several authors (see compilation by Nanninga & Tyrrel 1996) and is confirmed here. This has been suggested to contribute to the dominance of this species in surface waters of the ocean when mixed layer depths are shallow (Nanninga & Tyrrel 1996). Very high in-water scalar light intensities (>900 µmol photons m^{-2} s^{-1}) have been measured in surface waters during blooms of this species (see Nanninga & Tyrrel 1996), and high irradiances, up to 800 µmol photons m^{-2} s^{-1}, have little if any inhibitory effect on the growth of the diploid phase of *E. huxleyi* in culture (Brand & Guillard 1981). The vast majority of laboratory experiments conducted to date have evidently used cultures acclimated to non-saturating light conditions, and hence a more conservative interpretation would be that the diploid phase adapted to relatively low (non-saturating) light is able to maintain its photosynthetic rate upon short-term exposure to extremely high light conditions, such as, for example, could be experienced by a non-motile cell in turbulent conditions. Balch et al. (1992), however, did not observe photoinhibition in *E. huxleyi* (diploid phase) acclimated to an irradiance level of 1160 µmol photons m^{-2} s^{-1}. This strongly suggests that the capacity to avoid photoinhibition is an inherent characteristic of the coccolith-bearing phase of this species, which would, therefore, provide a competitive advantage in surface layers of stratified water columns, as well as in mixed water columns.

Our results indicate that this lack of photoinhibition is exhibited only in the diploid phase within the life cycle of *Emiliania huxleyi*. Under our culture conditions, the haploid motile non-calcifying (S-cell) phase of *E. huxleyi* exhibited a photoinhibition response similar to that of the majority of microalgal species tested to date (Kirk 1994), photoinhibition being initiated at

Table 2. Chl *a*-normalized pigment content for the haploid and diploid stages of *Emiliania huxleyi* (cultures at mid-exponential phase). Two-way ANOVAs with interactions (stage and pigment) showed no significant differences between the 2 stages

Pigment	Diploid stage	Haploid stage
Divinyl chlorophyll c_3 (DV-chl c_3)	0.191 ± 0.023	0.196 ± 0.006
Monovinyl chlorophyll c_3 (MV-chl c_3)	0.060 ± 0.013	0.065 ± 0.014
Chlorophyllid *a* (chlide *a*)	0.007 ± 0.013	0.000 ± 0.000
Mg-divinyl-phytoporphyrin (MgDVP)	0.017 ± 0.006	0.019 ± 0.005
Chlorophyll c_2 (chl c_2)	0.280 ± 0.019	0.332 ± 0.005
Fucoxanthin (Fx)	0.067 ± 0.027	0.203 ± 0.072
19'-butanoyloxyfucoxanthin (BFx)	0.003 ± 0.00	0.005 ± 0.00
4-keto-19'-hexanoyloxyfucoxanthin (4-KetoHFx)	0.052 ± 0.008	0.082 ± 0.015
19'-hexanoyloxyfucoxanthin (HFx)	1.017 ± 0.050	0.862 ± 0.094
Diadinoxanthin (Ddx)	0.000 ± 0.000	0.009 ± 0.015
Diatoxanthin (Dtx)	0.028 ± 0.010	0.018 ± 0.004
Unknown type zeaxanthin (Unk-2)	0.282 ± 0.382	0.298 ± 0.167

400 to 500 µmol photons m^{-2} s^{-1} and the photosynthetic rate decreasing to ca. 75% of $P^{\mathrm{chl}\ a}_{\max}$ at 1400 µmol photons m^{-2} s^{-1}. It should be stressed that, given our experimental conditions, the interpretation is limited to stating that the haploid S-cell phase acclimated to (probably) non-saturating light is not capable of short-term adaptation to avoid photoinhibition at high irradiance, in contrast to the diploid phase.

This indicates that the remarkable variability in the capacity to integrate short-term exposure to high irradiances demonstrated here for *Emiliania huxleyi* is related to other factors, potentially to differences in photosynthetic pigment physiology between the diploid and haploid phases. Accessory pigments may contribute to light harvesting or to photoprotective dissipation ('quenching') of excitation energy. An important short-term (on the scale of minutes) modulator of energy flow to the photosynthetic reaction centres involves an enzymatic de-epoxidation reaction of specific xanthophyll pigments, in the case of chromophytes the conversion of diadinoxanthin to diatoxanthin, the latter being the quenching form (McIntyre et al. 2002). In addition, β-carotene may serve as an energy quencher, although it is not regulated in the same manner as the xanthophyll cycle (McIntyre et al. 2002). Our preliminary results, which — for the first time — compare the pigment composition of the 2 phases within the haplo-diploid life cycle of coccolithophores, indicate that there are no fundamental differences in pigment content between the 2 phases cultured under identical conditions. β-carotene was not detected in either phase of this strain of *E. huxleyi* grown and harvested at non-limiting irradiance in our experiment, but has been reported to be produced in trace amounts in this species (e.g. Garrido & Zapata 1998). Future laboratory studies should test whether similar physiological responses are observed in the 2 phases of *E. huxleyi* cultivated under a range of light intensities, during short-term exposure to very high light intensities, and if so, whether these photoprotective carotenoids are differentially expressed. Differences in the photosynthetic physiology of these 2 phases may also be a result of differential structural packaging of pigments. If this were the case, photoinhibition responses could differ even if overall pigment composition was similar. No obvious differences in chloroplast structure were detected in the only study that has compared the ultrastructure of the 2 phases of *E. huxleyi* (Klaveness 1972), but a focussed biophysical approach (*in vivo* fluorescence, light absorption) would be required to confirm this fact.

The haploid S-cells could play a role in the biology of *Emiliania huxleyi* beyond that of serving as gametes (Paasche 2001). The results of this study provide some indications as to the possible ecology of the motile haploid phase of *E. huxleyi*. Flagellates, which typically dominate in the middle part of the seasonal succession of phytoplankton in summer, when the water column is stratified (see Margalef 1978), might intuitively be thought to be better adapted to high light conditions. However, Richardson et al. (1983) concluded that on average diatoms have a higher light-saturation irradiance (E_{sat}) for growth than dinoflagellates. Direct comparison of E_{sat} values is difficult, since this parameter depends on a number of factors related to culture conditions, but indirect support is provided by the conclusion that it is the non-motile phase of *E. huxleyi* which seems to be better adapted to short-term exposure to high light due to the capacity to avoid photoinhibition. An alternative hypothesis is that highly motile (i.e. flagellated) phytoplankton cells are able to avoid photoinhibition by actively migrating away from harmfully high irradiances. This behavioural response would preclude the need for flagellates to have evolved specific physiological mechanisms for preventing photoinjury. Being apparently less capable of supporting extreme variations in light intensities, the haploid phase of *E. huxleyi* may be present earlier or later in the year, when fluctuations in irradiance are less pronounced, or concurrently with the diploid phase but lower in the water column, possibly employing a vertical migration strategy to optimise the exploitation of light and nutrients. In this context, however, it is interesting to note that, in our experience, the motile phase of *E. huxleyi* exhibits neither positive nor negative

phototactic responses under a range of culture irradiance levels, whereas the majority of motile coccolithophores maintained in our culture collection actively migrate towards light (Houdan 2003). Non-flagellated phytoplankton cells may have some degree of control over their position in the water column through osmolyte-related vacuolar regulation of buoyancy (McKay et al. 2000) or possibly by excessive production of coccoliths to enhance sinking (Young 1994), for example, but these processes are unlikely to be as efficient as active swimming on short time-scales. In terms of nutrient physiology, the non-motile phase of *E. huxleyi* is highly competitive under conditions of nutrient stress (particularly phosphorus stress, Riegman et al. 2000) such as those characteristically encountered in the high-light surface waters of a stratified water column. Since flagella are absent in this phase, the evolution of a system for protection against the harmful effects of high irradiance represents an essential complementary aspect of the highly successful ecological strategy of this phase of the life cycle.

The results of this study demonstrate an important physiological difference between diploid and haploid phases in the life cycle of *Emiliania huxleyi*, and we therefore stress the opinion of Paasche (2001) that it is important to state clearly which cell form was used in a given investigation. Further physiological comparisons of coccolithophore life-cycle phases are essential for developing an understanding of the total ecological strategy of these important microalgae, and for *E. huxleyi* there is a clear need for quantification of the spatial and temporal distribution of the non-calcifying haploid stage in the field. This is complicated by the fact that positive identification of S-cells in plankton samples is next to impossible using routine light microscopy and is very difficult (and time-consuming) using electron microscopy. Development of innovative techniques involving molecular genetics will probably be required before data on the distribution and abundance of this phase can be gathered routinely.

CONCLUSIONS

P–E curves have been derived for both the diploid coccolith-bearing and haploid non-calcifying phases in the life cycle of *Emiliania huxleyi* cultured under identical conditions. Basic photosynthetic responses were similar, but whereas the diploid phase did not exhibit photoinhibition at irradiances up to at least 1000 µmol photons m^{-2} s^{-1} in accordance with previous studies, photoinhibition in the haploid phase was recorded at and above light intensities of 400 to 500 µmol photons m^{-2} s^{-1}. This remarkable variability may have important implications for the overall ecology of the species. The dimorphic life cycle of *E. huxleyi*, with each phase capable of independent asexual propagation and easy to culture, and each exhibiting a different response to high-light exposure, clearly makes this species a very promising model for the study of the biochemical and structural bases for adaptive avoidance of the effects of photoinhibition.

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