



Physiological responses to light regime of a Mediterranean lagoon strain of *Chaetoceros tenuissimus* and a collection strain of *Chaetoceros calcitrans*

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Abstract

Diatoms play a fundamental ecological role as primary producers in aquatic food webs. Among these, a little number of solitary species of the genus *Chaetoceros* are of great interest as live food for aquaculture, including major taxa like *Chaetoceros calcitrans* which have been well studied in terms of growth and essential lipid content. In contrast, the globally distributed species *Chaetoceros tenuissimus* has not been investigated as a potential live prey for aquaculture. For a preliminary analysis of the effect of culture conditions on growth and biomass content of a new strain of *C. tenuissimus* (CT16ED, hereafter CTEN) isolated from a Mediterranean lagoon, we first considered the light regime, comparing cultivation under a light–dark (L:D) cycle (12:12 h L:D photoperiod) and under continuous light, which conditions were also applied to a *C. calcitrans* strain, CCAP1085/3 (CCAL). Both strains had a similar growth rate under continuous light, but growth rate was lower in CTEN than in CCAL under the L:D cycle. Photosynthetic pigments content was higher under the L:D cycle than under continuous light in both strains, and higher pigment content was found in CCAL than in CTEN, indicating different photoacclimation. The lipid content of CTEN did not change markedly with photoperiod or growth phase, unlike CCAL. Both strains had high proportions of essential lipids, particularly n-3 polyunsaturated fatty acids, including EPA and DHA, but these varied less in CTEN than in CCAL. CTEN was richer in DHA (22:6n-3) with a more balanced DHA:EPA ratio. From these results, a potential interest of CTEN for use in aquaculture is discussed, with regard to its nutritional quality (essential lipid content), its growth, and its high-light niche offering possibilities for cultivation in outdoor systems in the Mediterranean area.

Keywords Bacillariophyceae · *Chaetoceros tenuissimus* · Photoacclimation · Essential lipids · Nutritional quality · Aquaculture

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Introduction

Diatoms have a fundamental ecological role as primary producers contributing largely to aquatic food webs in marine ecosystems (Rines and Hargraves 1988; Armbrust 2009). With more than 200 species, the genus *Chaetoceros* Ehrenberg is highly diverse and is one of the most important genera of diatoms (Rines and Hargraves 1988; Shevchenko et al. 2006; Lee and Lee 2011). Species of *Chaetoceros* are observed in seas and oceans worldwide (Luca et al. 2019) with very different sizes, the majority forming colonies (as cell chains or aggregates) and a few others living as solitary cells (Rines and Hargraves 1988; Lee and Lee 2011).

Chaetoceros isolates used in the aquaculture industry have generally been selected from species with solitary cells, as these present advantageous characteristics (e.g., small size, high growth rate) for their use as a food resource

for farmed animals (Ragg et al. 2010; Miller et al. 2014). Many strains identified as *Chaetoceros calcitrans*, *Chaetoceros simplex* var. *calcitrans* or *Chaetoceros calcitrans* forma *pumilus*, which have sometimes been unclearly classified in literature, may not be phylogenetically close species (Grzebyk et al. 2022). They have proven to be excellent for feeding the larval stages of shellfish in aquaculture due to their little size, rapid growth and substantial production of essential nutrients (Volkman et al. 1989; Miller et al. 2014). Beside these well-studied species, another small solitary species, *Chaetoceros tenuissimus* Meunier (1914), has not yet been investigated for potential use in aquaculture through the analysis of its nutritional quality, particularly in terms of carotenoid pigment or lipid content, although it is considered a cosmopolitan species observed in brackish and coastal environments around the world (Tomaru et al. 2018; Grzebyk et al. 2022; Pastorino et al. 2022) and sometimes forms very dense blooms with up to 10^7 cells L^{-1} (Rines and Hargraves 1988; Tomaru et al. 2018).

In order to consider the use of microalgae in aquaculture, in particular diatoms, an essential first step is to investigate their growth and their nutritional quality. These are strongly influenced by the culture conditions, including light intensity (Zhang et al. 2017), temperature (Converti et al. 2009), salinity (Minggat et al. 2021) as well as the stoichiometry and concentrations of inorganic nutrients (Mortensen et al. 1988; Roessler 1990; Reitan et al. 1994; Larson and Rees 1996). Light conditions are one of the major factors affecting the physiology of microalgae and their photosynthetic activity. The photoperiod is an important parameter determining the amount of photon energy that is supplied for the photosynthetic process (Falkowski et al. 1985; Grobbelaar 2009) and, therefore, it directly influences the growth of microalgae and their production of interesting biochemical compounds such as pigments and lipids (Amini Khoeyi et al. 2012; Lim and Zaleha 2013; Fakhri et al. 2015). In the literature, two light regimes are often used: the 12:12 h light:dark (L:D) photoperiod and continuous light (i.e., 24:00 h L:D). Generally, continuous light is particularly appreciated in aquaculture, as several authors have been able to show high CO_2 fixation rates, high growth rates and maximum biomass production under these conditions (Beigbeder and Lavoie 2022). However, others have demonstrated a lower production of essential lipids under continuous light compared to photoperiods including a dark phase (Amini Khoeyi et al. 2012; Lim and Zaleha 2013). In addition, Krzemińska et al. (2014) demonstrated that the effect of the photoperiod on the growth rates of green microalgae appeared to be species-dependent.

For aquaculture purposes, microalgae are widely used for their ability to produce large amounts of essential nutrients (Hemaiswarya et al. 2011; Khozin-Goldberg et al. 2011). Among the most sought after are polyunsaturated fatty acids (PUFA), especially long-chain (LC) n-3 PUFA

(Khozin-Goldberg et al. 2011) including EPA (20:5n-3; eicosapentaenoic acid) and DHA (22:6n-3; docosahexaenoic acid). These essential lipids allow the proper ontogenetic development of the larval stages of many bivalve and fish species (Brown 2002; Ragg et al. 2010). For example, it is well established that these LC n-3 PUFA play an important role in the functioning of organs such as the brain or the eyes of fish (Tocher 2003). Because most of these essential lipids are not synthesized by aquaculture livestock species (Miller et al. 2014), the supply of microalgae as a direct or indirect source of food is therefore necessary for the proper development of these organisms (Brown 2002; Tocher 2003; Ragg et al. 2010). In aquaculture, diatoms are widely used for their ability to produce large amounts of high quality lipids (Miller et al. 2014). Within diatoms, high content of EPA has been found in the genus *Chaetoceros*, especially in *C. simplex* var. *calcitrans* (Volkman et al. 1989; Brown 2002).

Furthermore, many studies have shown that mixed cultures of microalgae are particularly beneficial and profitable for hatchery-rearing of various species (González-Araya et al. 2011, 2012; Gomes et al. 2021). Thus, the study of the biochemical composition of various strains of *Chaetoceros* can be of great interest to improve the diet of aquaculture species. Moreover, the study of new strains is needed to boost their potential for industrial applications (Jusoh et al. 2020). Uncontrolled variations of salinity, light and temperature often jeopardize the reliability of cultures for aquaculture purposes, hence, there is a strong interest to study more robust species or strains naturally exposed to variable environmental conditions (Eryalçın et al. 2015; Hotos and Avramidou 2021). Specifically, lagoon environments are extremely unstable and subjected to constant and intense modifications in salinity, temperature, irradiance and nutrient load, so that they sustain a high diversity (specific, genetic...) of their biota (Pérez-Ruzafa et al. 2019). Species found in such harsh systems could potentially show interesting features for aquaculture applications, due to their adaptive capacities to constantly changing environment and hence higher resistance towards variations in the culture system (Tzovenis et al. 2009; Hotos and Avramidou 2021 and references herein). In fact, it has already been demonstrated that, even within the same species, estuarine and lagoon strains may show peculiar features and higher resistance capacities towards change in comparison to their pelagic counterparts (Fisher and Schwarzenbach 1978). Moreover, while microalgae culture in controlled conditions, like photobioreactors, is widely used and very efficient, it is also highly expensive and requires specific expertise (Fernández et al. 2021). So, there is a growing interest towards the isolation of algal species suitable for cost-effective large-scale cultures and towards outdoor culture systems, like open raceway ponds, since these can allow to reduce costs

of production and develop a more sustainable exploitation, by exploiting natural energy (Borowitzka 1997; Banerjee et al. 2011; Fernández et al. 2021). Nevertheless, these systems are also subjected to uncontrolled variations of environmental parameters and high seasonal variability. Closed outdoor bioreactors exploiting natural sunlight represent a promising design option for large-scale algae cultivation, effectively balancing energy costs reduction while preventing exposure to contamination and predatory organisms (Borowitzka 1997; Touloupakis et al. 2022). In this context, the interest towards microalgae strains issued from natural environment and locally isolated seems a logical option in the optic of selecting species more adapted to local conditions and variable environment, with the bonus of avoiding the use of exotic species (Tzovenis et al. 2009; Banerjee et al. 2011; Guo et al. 2015; Pérez et al. 2017; Cheregi et al. 2019), which could be a potential ecological threat if accidentally released in the natural environment.

A strain of *C. tenuissimus* (CT16ED) was isolated from a French Mediterranean lagoon (Corsica Island) and subsequently characterized with genetic analyses and a detailed morphological description (Grzebyk et al. 2022). Solitary cylindrical cells were 3–6 µm in diameter and 3 to 12 µm in length, and bore thin setae 25–30 µm in length. During the exponential growth phase in culture, the strain forms cell chains, these extending from 2 cells to sometimes around 20 cells. In order to assess the interest of this CT16ED strain for use in aquaculture, an ecophysiological and biochemical study was conducted. Experimental cultures were carried out under two photoperiods (24:00 L:D and 12:12 L:D) in order to measure growth, photosynthetic activity, and the content in photosynthetic pigments and lipids. The same study was carried out in parallel with a strain of *C. calcitrans* (CCAP 1085/3), which is commonly used in aquaculture and whose ecophysiological characteristics and biochemical composition have already been documented. The information obtained was used to interpret the ecophysiological and biochemical responses of the two strains in relation to their different geographical and ecological origins. The implications for the interest of the strain CT16ED in aquaculture and its conditions of use were also discussed.

Material and methods

Strains and culture methods

Chaetoceros tenuissimus CT16ED (hereafter called CTEN) was isolated from a Mediterranean lagoon (Diana lagoon 42°07'55"N 09°32'12"E, Corsica Island, France) during May 2016 (Grzebyk et al. 2022). *Chaetoceros calcitrans* CCAP 1085/3 (hereafter called CCAL), was obtained from the Culture Collection of Algae and Protozoa (Oban, UK).

Culture medium was made with natural seawater, sterilized with UV at 365 nm (Bio UV TTPE HO lamp), 0.2 µm filtered and autoclaved at 121 °C for 20 min. Then, salinity was adjusted to 25 with distilled water and enriched with Cell-Hi F2P nutrient powder (Varicon Aqua, Worcester, UK) with 0.4 g L⁻¹ and 40 mg L⁻¹ of Na₂SiO₃·5H₂O solution at final Si concentration of 188 µmol L⁻¹, corresponding to 4 times and ~1.8 times, respectively, to the concentrations of the F/2 medium (Guillard 1973).

The two strains of *Chaetoceros* were maintained as unialgal stock cultures in 100-mL Erlenmeyer flasks, incubated at 18 ± 1 °C under 40 µmol photons m⁻² s⁻¹ (as monitored with a Spherical Micro Quantum Sensor US-SQS/L Walz), and with a 18:06 h light:dark (L:D) photoperiod. Cultures were not axenic but bacterial contamination was considered negligible based on regular observations under the inverted optical microscope.

Prior to all experiments, initial stock cultures were used to prepare cultures for the inoculation of experimental bottles, through the following procedure. Stock cultures were sequentially volume scaled-up with an initial transfer from 100-mL Erlenmeyer flasks to 250-mL Erlenmeyer flasks (with a 50mL inoculum volume) and grown in the same conditions as described above for one week. Growth was monitored through cell counts as described later and each 250-mL Erlenmeyer flask was transferred, at late logarithmic growth phase into 1-L borosilicate Duran bottles (200 mL culture volume; Cell-Hi F2P at 0.4 g L⁻¹; Si at 40 mg L⁻¹; vitamins) and incubated for one week prior to experiments. These 1-L bottles were incubated under a 24:00 h L:D photoperiod (i.e., continuous light) at 18 ± 1 °C and used as stock cultures for the inoculation of the experimental bottles.

Experiment for setting the inoculum concentration

A preliminary study was performed to find an adequate inoculum concentration in order to assess the range of growth response of both CTEN and CCAL strains before further experiments. Cultures were carried out in the growth medium as described above in one-liter borosilicate Duran bottles under a 24:00 L:D photoperiod (i.e., continuous light, at 189 ± 30 µmol photons m⁻² s⁻¹ delivered by white (Cool white F36 W/240 Lux Line OSRAM) and pink (F36 W/840 T8 pink SYLVANIA; wavelength 400–525 nm and 600–700 nm) fluorescent lamps in equal proportion) at 18 ± 1 °C. For CTEN, inoculum concentrations of 10 × 10³, 50 × 10³, 100 × 10³ and 300 × 10³ cells mL⁻¹ were tested, without replicates. For CCAL, inoculum densities of 10 × 10³, 50 × 10³ and 100 × 10³ cells mL⁻¹ were tested, without replicates. Cultures were monitored during 9 to 15 days in the same conditions as stock culture (e.g., 24:00 L:D; 18 ± 1 °C; Cell-Hi F2P at 0.4 g L⁻¹; Si at 40 mg L⁻¹; vitamins). Each

culture was sampled daily for monitoring growth by cell count, as described later.

Photoperiod experiments

The cultures were carried out in triplicate per species for two photoperiods, 24:00 L:D and 12:12 L:D. The 12 bottles (2 strains \times 2 photoperiods \times triplicates) were inoculated at $1.1 \pm 0.1 \times 10^5$ cells mL⁻¹, incubated at 18 ± 1 °C under 189 ± 30 μ mol photons m⁻² s⁻¹ delivered by white (Cool white F36 W/240 Lux Line, OSRAM) and pink (F36 W/840 T8 pink, SYLVANIA) fluorescent lamps in equal proportions. Non-axenic cultures were regularly checked for bacterial contamination under an inverted microscope. A bacterial contamination appeared only in CTEN replicates under 12:12 L:D test, but this was only identified via lipid analyses at the end of the experiment, while, based on microscopy observations, it was considered negligible. CTEN and CCAL cultures were monitored over 9 days for both photoperiod treatments. Cultures were continuously aerated with 1% CO₂ in air. pH was measured each day (7.7 ± 0.7 ; Hanna Instruments HI 991300). Samples were taken from each bottle at the same time of light cycle, i.e., corresponding to the beginning of the light phase (within the first hour after turning on light) for cultures under the 12:12 L:D photoperiod: i) every day for growth and size measurements; ii) every other day for in vivo measurements of chlorophyll *a* fluorescence and photosynthetic activity; and iii) only on day 4 for lipid and pigment contents.

Analytical methods

Growth measurements

For growth monitoring during experiments, samples (10 mL) were taken daily for cell counts. Cell counts were performed under an inverted optical microscope with a Nageotte counting cell for samples with low cell concentrations, below 6×10^5 cells mL⁻¹, and with a Malassez counting cell when samples reached higher cell concentrations. A minimum of 400 cells were counted in each sample for an accuracy of $\pm 10\%$ (Lund et al. 1958). The growth rate (*k*) defined as the number of division per day (div day⁻¹) was calculated according to Guillard's formula (Guillard 1973; Eq. 1):

$$k = \frac{\log N_2 - \log N_1}{(t_2 - t_1) \times \log 2} \quad (1)$$

where N_1 and N_2 are the cell concentration on the days of the start (t_1) and end (t_2) of the considered growth period, respectively.

Chaetoceros cells have a round cylindrical shape and because of setae, they usually settle longitudinally. The

longitudinal cell section area measurement can be used as an approximation of cell size. To determine cell section area, photographs were taken each day for all cultures (except at day 8 under the 12:12 L:D photoperiod) with an inverted optical microscope (Leica MC HD 170). Area measurements were made with the image processing freeware (ImageJ, NIH) on a minimum of 15 cells per sample.

Chlorophyll *a* and in vivo cellular fluorescence monitoring during growth

To describe the temporal evolution of chl *a* biomass over the experimental period, in vivo chl *a* fluorescence was measured every two days through pulse amplitude modulated (PAM) fluorometry (Schreiber et al. 1995; Garrido et al. 2013), using a Phyto-PAM ED fluorometer and its dedicated Phyto-Win software (Heinz Walz GmbH, Germany; Schreiber et al. 1986). Although the factory calibration parameter was established from and for green algae, to allow the estimation of chl *a* biomass (which can be expressed in equivalent μ g L⁻¹ of chl *a*), it is assumed that the applied calibration factor can be used with other phytoplankton groups (including diatoms). Because all measurements were carried out under the same conditions and at the same time of the light cycle, this relative method can be considered sufficiently precise and reliable to monitor the temporal evolution of the chl *a* biomass in the cultures. The Phyto-PAM ED fluorometer was used to estimate photosynthetic activity by measuring photosystem II (PSII) fluorescence. For each culture, an aliquot (8 mL) was incubated in darkness for 30 min at the growth temperature, for fully reoxidizing the electron acceptors of PSII (Bolhar-Nordenkamp et al. 1989), before performing the measurement. The maximum quantum yield of PSII (F_v/F_m) is considered as an indicator of the cell physiological status (Maxwell and Johnson 2000) and was calculated after cell excitation with a saturating pulse (4000μ mol photons m⁻² s⁻¹, F_m) over a weak blue light (1μ mol photons m⁻² s⁻¹; (Genty et al. 1989); Eq. 2).

$$F_v/F_m = (F_m - F_0)/F_m \quad (2)$$

where F_v is the variable fluorescence, F_0 and F_m are the minimum and the maximum fluorescence of the sample after incubation in the dark (F_0) and light saturation pulse (F_m).

Pigment and lipid content during exponential growth

Pigments and lipids quantifications were carried out for the two photoperiods and for all cultures on day 4 around the middle of exponential growth phase. For pigment analyses, a 10 mL aliquot of each culture was filtered on a GF/F filter (diameter 25 mm, porosity 0.7 μ m) and stored at -80 °C until analysis. Analyses were performed at the Villefranche

Sea Institute (CNRS IMEV, SAPIGH, France). Filters were extracted (2 h) in 3 mL of 100% methanol, disrupted by sonication and clarified by filtration (GF/F Whatman). Analysis of pigment composition was performed by HPLC as described by Ras et al. (2008).

For lipid analyses, a 100 mL aliquot of algal culture was filtered on a 47-mm diameter GF/F filter (Whatman), followed immediately by passing 10 mL of boiling distilled water through the filter in order to inhibit lipase activity and prevent lipid degradation (González-Fernández et al. 2020). Filters were then placed in 6 mL of $\text{CHCl}_3/\text{MeOH}$ mix (2:1, v:v) under N_2 flux for storage until processing lipid analyses. Analyses were performed at the Lipidocean core facility (UMR CNRS 6539 LEMAR, France). Fractionation of neutral and polar lipids was made by solid phase extraction, and quantification of fatty acids in both fractions was realized by GC-FID as described by González-Fernández et al. (2020). Total fatty acid content was calculated as the sum of polar and neutral lipid fractions. For both lipid fractions, the fatty acids were classified into four groups: i) saturated fatty acids (SFA); ii) monounsaturated fatty acids (MUFA); iii) polyunsaturated fatty acids (PUFA); and iv) branched fatty acids (BRFA). In addition, the sum of some PUFA in the total content was calculated focusing on two essential PUFA subgroups: n-3 PUFA (i.e., 16:3n-3; 16:4n-3; 18:4n-3; 18:5n-3; 20:4n-3; 20:5n-3; 22:5n-3; 22:6n-3) and n-6 PUFA (i.e., 18:2n-6; 18:3n-6; 20:3n-6; 20:4n-6; 22:4n-6; 22:5n-6). Within the first sub-group, two essential LC n-3 PUFA were also focused on because of their high nutritional value for cultivated shellfish species: EPA (20:5n-3) and DHA (22:6n-3).

Statistical analyses

All statistical tests were performed with R statistical software (RStudio Team 2021). Since our data did not meet the assumptions for the application of a parametric statistical test (normality and homogeneity of variances between groups were assessed through Shapiro–Wilk’s and Levene’s tests, respectively), statistical tests for mean comparisons were carried out by one factor non-parametric Kruskal–Wallis tests ($\alpha = 0.05$) in order to identify differences between photoperiods within species (growth rate, lipid and pigment contents), and between days within photoperiod and species (cell section area, chl *a*, F_v/F_m).

Results

Experiment for setting the inoculum concentration

Under continuous light, all cultures of both strains, whatever the inoculum concentration, grew without a lag phase and

with a similar range of growth rates between 1.1 and 1.6 div day^{-1} during the exponential growth phase (Fig. 1A and B; Table S1). Through days 4–7, the CTEN cultures reached maximum cell concentrations between 4.2×10^6 cells mL^{-1} and 4.6×10^6 cells mL^{-1} (Fig. 1A; Table S1). The CCAL cultures grew further until the last day of the experiment (day 14), reaching maximum concentrations around 3×10^7 cells mL^{-1} (Fig. 1A; Table S1). As a result, the inoculum concentration of 100×10^3 cells mL^{-1} was chosen as the most suitable to allow observation of the exponential growth phase of the two strains over a fairly long period.

Photoperiod experiments

Cell growth and morphology

The maximum densities obtained after 8 days in CCAL cultures were between 7 and 8 times higher than those of CTEN cultures, depending on photoperiod treatments (Fig. 2A; Table S2). In the 24:00 L:D treatment, the

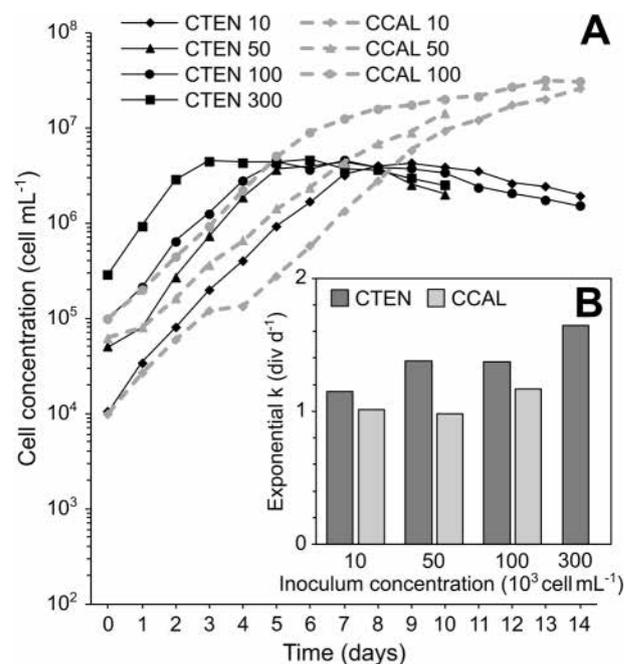


Fig. 1 Preliminary experiment for setting the inoculum concentration under a 24:00 L:D photoperiod. **A:** Growth curves based on cell concentration (cells mL^{-1}) for strain CTEN (full black lines) and strain CCAL (dot grey lines) cultures with inoculum concentrations of 10×10^3 (diamond); 50×10^3 (triangle); 100×10^3 (full circle) and 300×10^3 (square) cells mL^{-1} . **B:** Exponential growth rate (k ; div day^{-1}) in CTEN (dark grey) and CCAL (light grey) cultures depending on inoculum concentration (10×10^3 to 300×10^3 cells mL^{-1}); the periods considered for calculation are the following: day 0 – day 7 for CTEN 10, day 1 – day 5 for CTEN 50; day 0 – day 4 for CTEN 100; day 0 – day 2 for CTEN 300; day 0 – day 8 for CCAL 10; day 1 – day 6 for CCAL 50 and day 1 – day 5 for CCAL 100.

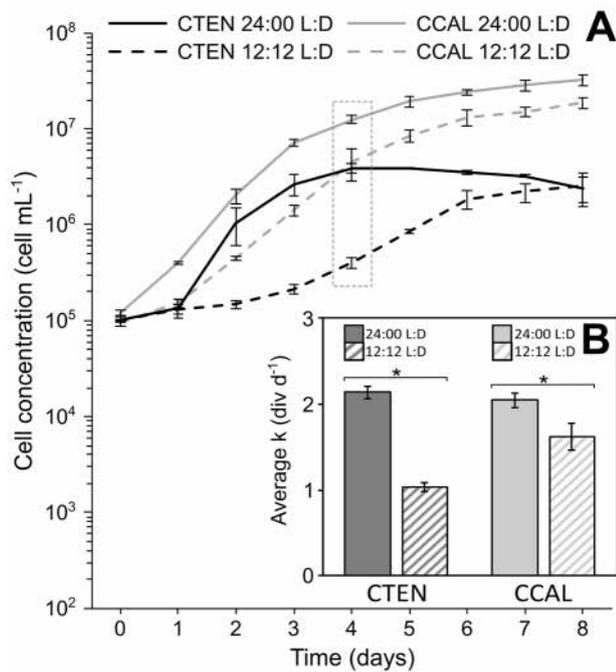


Fig. 2 Cell growth of CTEN and CCAL depending on photoperiod treatment. **A:** Evolution of cell concentration (cells mL⁻¹; mean ± SD; n = 3) for CTEN (black lines) and CCAL (grey lines) cultures under 24:00 L:D (full lines) and 12:12 L:D (dot lines) photoperiod treatments. **B:** Average growth rate (k; div day⁻¹) for CTEN (dark grey) and CCAL (light grey) at each photoperiod; the periods considered for calculation were the following: day 1 – day 3 for CTEN 24:00, day 0 – day 2 for CCAL 24:00, day 3 – day 6 for CTEN 12:12 and day 1 – day 4 for CCAL 12:12. Error bars represent the standard deviation between replicate cultures (n = 3). Statistical comparisons (Kruskal–Wallis test) were made between photoperiods (*; p-value < 0.05) within species. The box with grey dot lines on day 4 indicates the samples taken for the biochemical analyses of pigment and lipid contents

maximal densities were reached on day 5 for CTEN (3.9×10^6 cells mL⁻¹) and on day 8 for CCAL (33×10^6 cells mL⁻¹). During exponential growth phase, highest growth rates were similar for a short period, with $k = 2.1 \pm 0.1$ div day⁻¹ in the CTEN cultures (from day 1 to day 3 after one-day lag phase) and $k = 2.0 \pm 0.1$ div day⁻¹ in the CCAL cultures (between day 0 and day 2) (Fig. 2B; Table S2). The CTEN cultures quickly reached a maximum density plateau on day 4 whereas CCAL continued to grow at a lower growth rate until day 8. Under the 12:12 L:D photoperiod, both CTEN and CCAL kept growing until day 8 (i.e., did not reach the stationary phase), reaching 2.6×10^6 cells mL⁻¹ and 19×10^6 cells mL⁻¹, respectively (Fig. 2A; Table S2). Exponential growth rates under 12:12 L:D photoperiod were significantly lower (p-value < 0.05) than under continuous light for both species, with CTEN showing lower values (1.0 ± 0.1 div day⁻¹) compared to CCAL (1.6 ± 0.2 div day⁻¹).

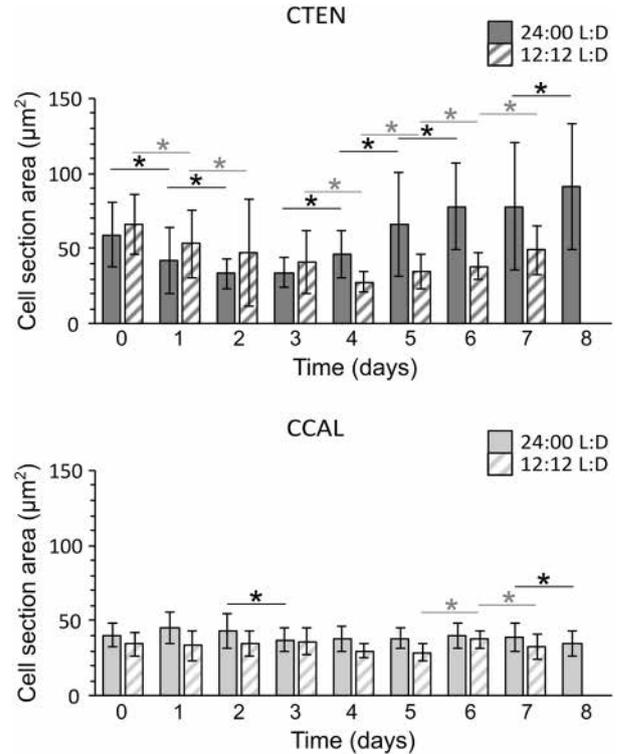


Fig. 3 Variation in cell section area during photoperiod experiments (µm²; mean ± SD) of CTEN (dark grey; top figure) and CCAL (light grey; bottom figure) cultures under 24:00 L:D photoperiod (full bar) or 12:12 L:D photoperiod (hatched bar). Error bars represent the standard deviation between replicate cultures (n = 3). Significant differences (black or grey * for continuous light and for 12:12 L:D photoperiod, respectively; p-value < 0.05, Kruskal–Wallis test) for CTEN and CCAL within photoperiods are expressed for time n compared to time n-1

The CTEN cultures showed morphological changes during cultures, with transiently the formation of short cell chains (mostly with two to four cells) during exponential growth, whereas CCAL cultures contained only solitary cells (Fig. S1). From micrograph analyses, in CTEN cultures under both photoperiods, large variations in cell section area (i.e., cell size) were observed during growth (Fig. 3; Table S2). Under the 24:00 L:D photoperiod, a significant decrease in cell size occurred between day 0 (59 ± 21 µm²) and day 2 and stabilized until day 3 (average section area ~ 34 µm²). It was followed by a significant gradual increase from day 4 during the late growth period and then the stationary phase (up to 91 ± 42 µm² on day 8), which increase corresponded to a change in shape from square to rectangular (Fig. S1). Under the 12:12 L:D photoperiod, the initial gradual decrease in cell section area during early growth until day 4 (halved to 28 ± 6.5 µm²) was followed by a gradual increase during the end of exponential growth phase, then the late growth period until day 7 (up to 49

$\pm 16 \mu\text{m}^2$). In CCAL cultures under both photoperiods, the shape of cell section remained square (Fig. S1) and minor variations of cell section areas occurred throughout growth, and overall, with values slightly lower under the 12:12 L:D photoperiod (mean range between 28.8 and 37.6 μm^2) than under continuous light (mean range between 34.7 and 45.0 μm^2) (Fig. 3; Table S2).

Chlorophylls, carotenoid pigments and photosynthetic activity

In general, taking into account the different sampling interval, the evolution of chl *a* biomass followed that of the cell concentration over time for the two strains (Fig. S2, Table S2).

In HPLC analyzes performed on the fourth day of culture, all CCAL samples contained chl *a* without any degradation products. In contrast, all CTEN samples contained a significant proportion of chlorophyllide *a* (chl *a*); data not shown): considering that chl *a* resulted from the degradation of chl *a* during the extraction process (see discussion for explanations), it was converted back to allow comparisons of chl *a* cellular content. In both strains, cellular chl *a* content was significantly higher under the 12:12 L:D photoperiod than under continuous light, with a larger gap measured for CTEN (Table 1). Under each photoperiod respectively, CTEN had lower cell chl *a* content than CCAL and, indeed, the content of CTEN under 12:12 L:D

increased to reach a value similar to that of CCAL under continuous light.

The cellular content of chlorophyll $c_1 + c_2$ (chl *c*) was slightly higher in CTEN than in CCAL under both light treatments (Table 1); while this content was similar in CTEN in both light treatments, it was increased by 33% in CCAL under the 12:12 L:D cycle compared to continuous light. Regarding carotenoid pigments, the cellular content of xanthophylls was higher in CCAL cells than in CTEN cells by approximately 3.5 times and 1.7 times in moles under continuous light and under the L:D cycle, respectively (Table 1). In CCAL, the cellular contents of light-harvesting fucoxanthin (F) and photoprotective diadinoxanthin (Dd) were similar under both photoperiods (variations of a few %), and only the β -carotene content increased by 35% in the 12:12 L:D treatment compared to continuous light. In CTEN, cell content of fucoxanthin and diadinoxanthin doubled in the 12:12 L:D treatment compared to continuous light, while the content of β -carotene only increased by 22%. Diatoxanthin (Dt) was not detected or was only measured at a very low concentration in the culture samples (Table S4), and it represented only a little part (4.1%) of the content of xanthophyll pigments in CTEN cells under the 12:12 L:D photoperiod (Table 1). Small amounts of zeaxanthin (Z) were also detected in CTEN cultures, constituting a trace amount in cells grown under continuous light, but representing a cellular content close to that of Dt under the 12:12 L:D cycle (Table 1, Table S4). Between cells grown under continuous light and under the 12:12 L:D photoperiod, the F:chl *a* and

Table 1 Cellular pigment molar content (mean \pm SD; $n = 3$), and average molar ratios (mean \pm SD; $n = 3$), in CTEN and CCAL cultures under the two photoperiod treatments (24:00 L:D; 12:12 L:D) on day 4

	Pigments	CTEN		CCAL	
		24:00 L:D	12:12 L:D	24:00 L:D	12:12 L:D
Moles ($\times 10^{-15}$) per cell	chl <i>a</i>	0.373 \pm 0.154	0.573 \pm 0.034*	0.575 \pm 0.062	0.697 \pm 0.055*
	chl <i>c</i>	0.196 \pm 0.068	0.186 \pm 0.014	0.126 \pm 0.014	0.168 \pm 0.016*
	Xanthophylls ⁽¹⁾	0.143 \pm 0.056	0.312 \pm 0.019*	0.499 \pm 0.056	0.517 \pm 0.042
	F	0.126 \pm 0.051	0.253 \pm 0.017*	0.437 \pm 0.051	0.448 \pm 0.036
	Dd	0.014 \pm 0.005	0.032 \pm 0.001*	0.061 \pm 0.005	0.069 \pm 0.005
	Dt	< LD ⁽²⁾	0.015 \pm 0.002	0.001 \pm 0.000	< LD ⁽²⁾
	Z	0.002 \pm 0.000	0.012 \pm 0.001*	< LD ⁽²⁾	< LD ⁽²⁾
	β -carotene	0.016 \pm 0.001	0.020 \pm 0.001*	0.027 \pm 0.006	0.035 \pm 0.002*
	Molar ratios	chl <i>c</i> :chl <i>a</i>	0.568 \pm 0.245	0.324 \pm 0.007*	0.219 \pm 0.001
F:chl <i>a</i>	0.397 \pm 0.223	0.443 \pm 0.029	0.760 \pm 0.014	0.642 \pm 0.006*	
(Dd + Dt):chl <i>a</i>	0.046 \pm 0.030	0.082 \pm 0.001*	0.108 \pm 0.004	0.099 \pm 0.001*	
F:Dd	9.127 \pm 1.499	7.865 \pm 0.613	7.205 \pm 0.320	6.488 \pm 0.051*	
F:(Dd + Dt)	9.127 \pm 1.499	5.410 \pm 0.442*	7.051 \pm 0.376	6.488 \pm 0.051*	

⁽¹⁾ Sum of pigments fucoxanthin, diadinoxanthin and diatoxanthin, and zeaxanthin

⁽²⁾ Below the detection limit

Statistical comparisons (Kruskal–Wallis test) of the content of each pigment were carried out between the two photoperiod treatments for each strain (*; p -value < 0.05). Cellular pigment contents in pg cell^{-1} are reported in Table S3

the F:Dd molar ratios decreased significantly but moderately in CCAL, while their mean variations in CTEN were larger in magnitude but not significant (Table 1). In CCAL (Dd + Dt):chl *a* ratios were significantly lower under L:D photoperiod than continuous light, while they showed an opposite trend in CTEN (Table 1).

In the monitoring of photosynthetic activity, CTEN and CCAL cultures showed different patterns in variations of F_v/F_m values along growth (Fig. 4, Table S2). In CTEN cultures, under the 24:00 L:D photoperiod, a significant increase of F_v/F_m was observed between inoculation and day 2 when it reached the highest value (0.62 ± 0.02) after exponential growth, followed by successive significant decreases until day 8 (0.21 ± 0.04) along late growth and during stationary phase (Fig. 4, Table S2). Under the 12:12 L:D photoperiod, the average F_v/F_m increased slowly from 0.41 ± 0.02 on inoculation day during early slow growth, then reached highest values at ~ 0.61 during exponential growth (between days 4 and 6), and started decreasing during late growth (Fig. 4, Table S2). In CCAL cultures under both photoperiods, average F_v/F_m values varied between 0.56 and 0.71, overall increasing slightly during growth (Fig. 4, Table S2).

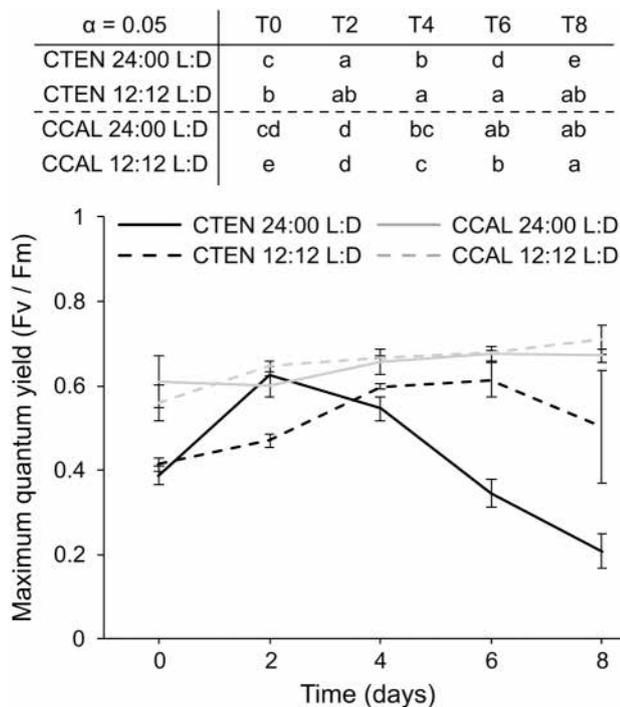


Fig. 4 Variation of maximum quantum yield (F_v/F_m) during the photoperiod experiments (mean \pm SD) for CTEN (black line) and CCAL (grey line) cultures under 24:00 L:D (full lines) or 12:12 L:D (dot lines) photoperiod. Error bars represent the standard deviation ($n = 3$). Statistical comparisons (Kruskal–Wallis test) were made between times and significant differences (p -value < 0.05) are reported in the table at the top and represented by different letters

Lipid content

With CTEN, total fatty acid (TFA) content was similar under both light regimes. However, the relative distribution between neutral lipids (NL) and polar lipids (PL) varied in opposite way (Table 2, Table S4). The NL content was higher under continuous light representing a higher proportion of storage lipids (NL:TFA ratio at 60%), whereas under the 12:12 L:D regime, the PL content was higher and storage lipids accounted for 39% (Table 2). Within PL, MUFA and total n-3 PUFA cellular contents were two-fold lower under continuous light than under 12:12 L:D photoperiod (Table 2). Under continuous light, the MUFA proportion tended to be lower than under the 12:12 h L:D photoperiod (24% vs. 31%), while the SAFA and PUFA proportions were slightly higher (+ 2.5% and + 7%, respectively; Table 2). In PL, most individual FAs varied according to light regime (Table 3). Only 18:3n-6 was significantly higher in proportion under continuous light than under the 12:12 L:D photoperiod, as well as 14:0 and 16:1n-7, 16:3n-4 and 22:6n-3 to a lesser extent. Conversely, polar 16:0, 18:0 and 18:1n-7 were proportionally higher under the 12:12 L:D photoperiod.

Similarly to PL, no significant change in the content of the main FA classes was observed in NL according to light regime, possibly because of the large standard deviation of CTEN FA contents under 24:00 L:D photoperiod (Table 2). However, the percentages of the FA classes, especially SAFA and MUFA were strongly modulated by the light regime. SAFA percentage was lower under continuous light (36% vs. 55% under 12:12 L:D photoperiod) while MUFA percentage was higher (36% vs. 22% under 12:12 L:D; Table 2). The lower SAFA relative content under the 24:00 L:D photoperiod reflected mostly the lower percentages of 16:0 and 18:0 under this regime (Table 3). At the opposite, the percentages of 14:0, 16:1n-7 and 20:5n-3 were much higher under 24:00 L:D than 12:12 L:D photoperiod (Table 3).

In CCAL, total FA (TFA) was significantly higher under continuous light than under the 12:12 L:D cycle, mostly due to the significantly higher content of NL (Table 2). Within NL, the contents of main FA classes (SAFA, MUFA, PUFA) were significantly higher under the 24:00 L:D photoperiod. However, when expressed in percentage, SAFA and PUFA were similar under the two light regimes, and only MUFA was significantly higher under continuous light. Change in proportion of MUFA between the two light regimes, decreasing from 42% under continuous light to 32% under the light cycle, appeared inversely compensated by the change of proportion of BRFA (increasing from 2% under continuous light to 10% under the light cycle). Within PL, only SAFA and total n-6 PUFA contents were significantly different between the two light

Table 2 Cellular lipid content (pg cell⁻¹ and percentage; mean ± SD; n = 3) in CTEN and CCAL cultures under the two photoperiod treatments (24:00 L:D; 12:12 L:D) on day 4

		pg cell ⁻¹				Percentage of total FA			
		CTEN		CCAL		CTEN		CCAL	
		24:00 L:D	12:12 L:D	24:00 L:D	12:12 L:D	24:00 L:D	12:12 L:D	24:00 L:D	12:12 L:D
Polar Lipids	Total FA (TFA)	2.45 ± 1.13	2.60 ± 0.29	4.92 ± 1.08	2.08 ± 0.30*				
	Total PL	0.92 ± 0.18	1.60 ± 0.22	1.99 ± 0.14	1.82 ± 0.27				
	BRFA	0.02 ± 0.01	0.07 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	1.6 ± 0.2	4.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1*
	SAFA	0.26 ± 0.06	0.40 ± 0.06	0.46 ± 0.02	0.37 ± 0.07*	27.7 ± 0.5	25.2 ± 1.0	23.1 ± 1.2	20.1 ± 0.5*
	MUFA	0.22 ± 0.06	0.49 ± 0.08	0.42 ± 0.04	0.39 ± 0.09	23.7 ± 1.7	30.9 ± 1.1	21.3 ± 0.5	21.4 ± 1.7
	PUFA	0.44 ± 0.06	0.63 ± 0.08	1.10 ± 0.10	1.05 ± 0.12	46.9 ± 2.4	39.7 ± 0.6	55.3 ± 1.3	58.0 ± 2.1
	n-3 PUFA ⁽¹⁾	0.22 ± 0.02	0.35 ± 0.04	0.53 ± 0.08	0.48 ± 0.04	23.4 ± 2.4	22.3 ± 1.0	26.3 ± 2.2	26.9 ± 3.4
	20:5n-3 (EPA)	0.15 ± 0.01	0.29 ± 0.04	0.44 ± 0.08	0.40 ± 0.03	15.9 ± 4.0	18.3 ± 0.5	22.1 ± 2.2	22.2 ± 3.2
	22:6n-3 (DHA)	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01*	5.8 ± 1.6	3.1 ± 0.5	2.5 ± 0.2	1.9 ± 0.2*
	n-6 PUFA ⁽²⁾	0.03 ± 0.00	0.03 ± 0.01	0.16 ± 0.03	0.02 ± 0.01*	3.1 ± 0.1	1.6 ± 0.2	8.2 ± 1.8	1.4 ± 0.2*
Neutral Lipids	Total NL	1.53 ± 0.95	1.00 ± 0.07	2.92 ± 1.22	0.26 ± 0.03*				
	BRFA	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.02 ± 0.01*	1.6 ± 0.4	5.0 ± 0.5	1.9 ± 0.8	9.9 ± 0.9*
	SAFA	0.53 ± 0.28	0.55 ± 0.06	1.22 ± 0.56	0.11 ± 0.02*	35.9 ± 3.6	54.8 ± 3.1	41.4 ± 1.5	42.0 ± 2.3
	MUFA	0.54 ± 0.32	0.22 ± 0.00	1.22 ± 0.50	0.08 ± 0.01*	35.7 ± 1.3	22.0 ± 1.7	41.7 ± 0.6	31.7 ± 0.5*
	PUFA	0.44 ± 0.33	0.18 ± 0.02	0.44 ± 0.18	0.04 ± 0.00*	26.8 ± 5.3	18.3 ± 1.9	15.0 ± 0.2	16.3 ± 1.8
	n-3 PUFA ⁽¹⁾	0.26 ± 0.21	0.09 ± 0.02	0.22 ± 0.10	0.02 ± 0.00*	15.6 ± 4.3	8.7 ± 2.0	7.4 ± 0.5	8.2 ± 1.8
	20:5n-3 (EPA)	0.23 ± 0.19	0.06 ± 0.01	0.18 ± 0.08	0.01 ± 0.00*	13.4 ± 4.5	5.9 ± 0.9	6.1 ± 0.4	4.5 ± 1.2
	22:6n-3 (DHA)	0.02 ± 0.01	0.01 ± 0.02	0.01 ± 0.00	0.00 ± 0.00*	0.9 ± 0.1	1.1 ± 1.3	0.3 ± 0.1	0.9 ± 0.3*
	n-6 PUFA ⁽²⁾	0.02 ± 0.01	0.01 ± 0.00	0.08 ± 0.05	0.00 ± 0.00*	1.2 ± 0.1	0.8 ± 0.2	2.6 ± 0.5	0.5 ± 0.4*

⁽¹⁾ Sum of lipids 16:3n-3; 16:4n-3; 18:4n-3; 18:5n-3; 20:4n-3; 20:5n-3 (EPA); 22:5n-3; 22:6n-3 (DHA)

⁽²⁾ Sum of lipids 18:2n-6; 18:3n-6; 20:3n-6; 20:4n-6; 22:4n-6; 22:5n-6

Statistical comparisons (Kruskal–Wallis test) were made between photoperiod treatments (*; p-value < 0.05) within strains

regimes, with notably a significantly higher content and proportion of n-6 PUFA under continuous light (0.16 pg cell⁻¹ accounting for 8.2% of PL) (Table 2). In PL, the percentages of most individual FA were different between the two light regimes. For the two major n-3 PUFA, the 20:5n-3 did not vary proportionally between the two light regimes, while the 22:6n-3 was significantly higher under continuous light (Tables 2 and 3). Percentages of 16 PUFA (16:2n-4, 16:3n-3, 16:3n-4 and 16:4n-1) were significantly lower under the 24:00 photoperiod (Table 3). At the opposite, 18:3n-6 and 20:4n-6 showed significantly higher values under continuous light compared to 12:12 L:D photoperiod, passing from 0.5% to 2.6% and 4.7%, respectively (Table 3). Within polar SAFA, only 16:0 and 22:0 were in significantly higher proportion under continuous light (Table 3). Other significant changes of individual FA concerned minor FA.

For both species, PL were dominated by PUFA (55–58% for CCAL and 47–40% for CTEN under 24:00 L:D and 12:12 L:D

photoperiods, respectively) whereas PUFA were in much lower proportion in NL (15–16% for CCAL and 27–18% for CTEN under continuous light and 12:12 L:D cycle, respectively).

Examining more precisely the cellular contents in n-3 PUFA, in CTEN, EPA and DHA were present in equivalent amounts under both light regimes. EPA (0.35–0.37 pg cell⁻¹; 14–15% of total lipids; Fig. 5, Tables 2 and 3) was mainly in the form of PL under the 12:12 L:D photoperiod and in the form of NL under continuous light (Fig. 5, Tables 2 and 3). DHA was found in much lower quantity (0.06–0.07 pg cell⁻¹; 2.4–2.7% of total lipids) and was mostly in the form of PL (Fig. 5, Tables 2 and 3).

In CCAL, EPA and DHA were found in higher amount in cells under 24:00 L:D regime. EPA (0.62 pg cell⁻¹) than under the 12:12 L:D photoperiod (0.41 pg cell⁻¹; Fig. 5, Tables 2 and 3), but for both regimes mostly under the form of PL (71% and 97% of total EPA, respectively). DHA was also in higher amount under continuous light (0.06 pg cell⁻¹) than under 12:12 L:D photoperiod (0.04

Table 3 Cellular lipid content expressed as percentage of total FA in CTEN and CCAL cultures under the two photoperiod treatments (24:00 L:D; 12:12 L:D) on day 4

	Polar Lipids							
	CTEN		CCAL		CTEN		CCAL	
	24:00 L:D	12:12 L:D	24:00 L:D	12:12 L:D	24:00 L:D	12:12 L:D	24:00 L:D	12:12 L:D
iso15:0	0.9 ± 0.2	1.7 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.3	0.2 ± 0.2	2.3 ± 0.4*
iso16:0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.1	0.6 ± 0.2	0.4 ± 0.2	3.7 ± 0.3*
iso17:0	0.7 ± 0.1	2.6 ± 0.1	0.2 ± 0.0	0.4 ± 0.0*	0.5 ± 0.2	4.0 ± 0.3	1.3 ± 0.4	3.9 ± 0.3*
14:0	18.5 ± 0.1	11.5 ± 0.9	12.1 ± 0.7	12.1 ± 0.3	19.6 ± 1.8	11.5 ± 1.1	15.3 ± 0.3	11.1 ± 0.4*
15:0	0.4 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.9 ± 0.0	0.7 ± 0.0*
16:0	8.0 ± 0.4	10.2 ± 0.6	9.9 ± 0.6	6.9 ± 0.2*	13.7 ± 4.3	28.8 ± 2.3	23.0 ± 1.7	22.7 ± 1.6
17:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0*
18:0	0.8 ± 0.1	2.8 ± 0.3	0.5 ± 0.0	0.5 ± 0.0	1.8 ± 0.7	14.0 ± 1.9	1.5 ± 0.2	6.8 ± 0.2*
20:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0*
22:0	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.0*	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
24:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2	0.3 ± 0.2	0.6 ± 0.3
14:1n-5	0.0 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0*	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0*
16:1n-13 t	0.6 ± 0.0	1.0 ± 0.0	0.7 ± 0.1	2.0 ± 0.3*	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1*
16:1n-5	0.2 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.4 ± 0.1	0.3 ± 0.3
16:1n-7	16.9 ± 1.1	13.5 ± 0.4	18.6 ± 0.5	17.2 ± 1.9	33.4 ± 0.7	19.6 ± 1.7	40.4 ± 0.5	28.2 ± 1.0*
16:1n-9	0.3 ± 0.1	0.6 ± 0.1	0.1 ± 0.0	0.1 ± 0.0*	0.4 ± 0.3	0.9 ± 0.1	0.1 ± 0.0	2.0 ± 0.5*
18:1n-7	4.4 ± 0.4	13.5 ± 1.5	1.1 ± 0.1	1.4 ± 0.2	0.6 ± 0.2	0.7 ± 0.0	0.3 ± 0.0	0.7 ± 0.2
18:1n-9	0.8 ± 0.1	2.2 ± 0.1	0.4 ± 0.0	0.2 ± 0.0*	0.3 ± 0.1	0.7 ± 0.0	0.3 ± 0.1	0.4 ± 0.1
22:1n-9	0.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
16:2n-4	2.9 ± 0.1	2.5 ± 0.2	5.7 ± 0.7	10.4 ± 0.6*	2.4 ± 0.3	1.5 ± 0.1	2.0 ± 0.5	3.3 ± 0.2*
16:2n-7	3.6 ± 0.5	1.9 ± 0.2	2.8 ± 0.7	3.0 ± 0.6	1.9 ± 0.1	1.4 ± 0.1	1.6 ± 0.4	1.6 ± 0.2
16:3n-3	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0*	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0*
16:3n-4	12.7 ± 0.1	10.1 ± 0.6	11.9 ± 0.5	15.4 ± 0.9*	5.0 ± 0.8	4.7 ± 0.2	1.4 ± 0.2	2.5 ± 0.3*
16:4n-1	1.2 ± 0.2	1.3 ± 0.0	0.4 ± 0.1	1.0 ± 0.4*	0.7 ± 0.0	1.0 ± 0.2	0.1 ± 0.0	0.1 ± 0.2
16:4n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	2.2 ± 0.3*
18:2n-6	1.0 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.3 ± 0.0*	0.4 ± 0.1	0.8 ± 0.2	0.2 ± 0.0	0.3 ± 0.1
18:3n-4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:3n-6	0.3 ± 0.0	0.0 ± 0.0	2.6 ± 0.8	0.5 ± 0.2*	0.1 ± 0.0	0.0 ± 0.0	0.6 ± 0.2	0.1 ± 0.1*
18:4n-3	0.9 ± 0.1	0.8 ± 0.3	1.2 ± 0.3	1.9 ± 0.2*	0.4 ± 0.1	1.8 ± 0.07	0.5 ± 0.2	0.7 ± 0.6
18:5n-3	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:3n-6	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0*
20:4n-3	0.4 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0*
20:4n-6	1.1 ± 0.1	0.0 ± 0.0	4.7 ± 0.9	0.5 ± 0.1*	0.6 ± 0.1	0.0 ± 0.0	1.7 ± 0.3	0.1 ± 0.2*
20:5n-3	15.9 ± 4.0	18.3 ± 0.5	22.1 ± 2.2	22.2 ± 3.2	13.4 ± 4.5	5.9 ± 0.88	6.1 ± 0.4	4.5 ± 1.2
22:4n-6	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:5n-3	0.4 ± 0.2	0.1 ± 0.2	0.1 ± 0.0	0.2 ± 0.1*	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
22:5n-6	0.6 ± 0.0	1.1 ± 0.2	0.1 ± 0.0	0.0 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:6n-3	5.8 ± 1.6	3.1 ± 0.5	2.5 ± 0.2	1.9 ± 0.2*	0.9 ± 0.1	1.1 ± 1.30	0.3 ± 0.1	0.9 ± 0.3

Results are shown for each lipid fraction (Mean ± SD; n = 3). Statistical comparisons (Kruskal–Wallis test) were made between photoperiod treatments (*; p-value < 0.05) within strains

pg cell⁻¹) and mostly in the form of PL (Fig. 5, Tables 2 and 3).

Overall, CCAL contained more EPA than CTEN and the other way around for DHA (Fig. 5, Table 2). However,

under continuous light CTEN showed twice the proportion of DHA in polar and neutral lipids compared to CCAL (Tables 2 and 3).

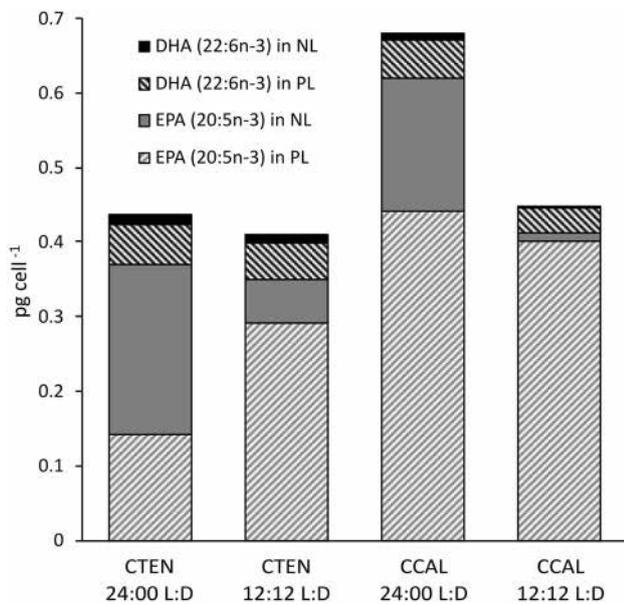


Fig. 5 Cellular EPA (grey) and DHA (black) content ratio (by weight) in CTEN and CCAL cultures, depending on the photoperiod treatment, on the fourth day of cultivation. Lipid content (which is detailed in Tables 2 and 3) is expressed in pg cell^{-1} and distinct between the different lipid fractions: the polar (striped fill) and neutral (solid fill) fractions

Discussion

Effects of photoperiod

Cell growth and morphology

Between continuous light and the 12:12 L:D cycle, photoperiod treatment had a strong effect on the growth of both strains. Under the 12:12 L:D photoperiod, growth occurred after an additional day of lag-phase, and growth rate was significantly reduced compared to continuous light: these two parameters induced a longer growth period but maximum cell concentrations achieved or to be expected (by extrapolating growth curves) at stationary phase were similar between the two light regimes. These observations are consistent with other studies on the influence of photoperiod on the growth of microalgae (Krzemińska et al. 2014; Fakhri et al. 2015; Beigbeder and Lavoie 2022). The growth rates obtained in our study were in the same range as those recorded in other studies with similar experimental conditions on the same two species and on other small species of *Chaetoceros*, i.e., often between 1 and 3 div day^{-1} assessed at the start of exponential growth for few days, before a rapid decrease until the stationary phase (McGinnis et al. 1997; Doblin et al. 1999; Orlova and Aizdaicher 2000; Anning et al. 2001; Robert et al. 2004; Liang et al. 2006; Shirai et al. 2008; Ragg et al. 2010;

Kaspar et al. 2014; Miller et al. 2014; Tomaru et al. 2014; Remize et al. 2021). Considering cell size, this range of growth rates is consistent with the allometric growth-size relationship described in algae, including diatoms (Tang 1995; Sarthou et al. 2005). As a corollary, in batch culture, following the steep increase in cell concentration shortly after inoculation, stationary phase is often reached after few days (less than a week) under continuous light (Robert et al. 2004; Ragg et al. 2010; Miller et al. 2014; Remize et al. 2021) and twice as long under a 12:12 h L:D photoperiod (Zhang et al. 2017; Kong et al. 2021). Maximum cell concentration reached by CTEN at stationary phase (around $4\text{--}5 \times 10^6 \text{ cells mL}^{-1}$) was in the same range as other *C. tenuissimus* strains (Doblin et al. 1999; Tomaru et al. 2014; Hano and Tomaru 2019), *C. calcitrans* strains (Phatarpekar et al. 2000; Banerjee et al. 2011) and other small *Chaetoceros* species (McGinnis et al. 1997; Orlova and Aizdaicher 2000). Maximum cell concentrations at stationary phase were higher by one order of magnitude in our CCAL strain (up to $\sim 3.2 \times 10^7 \text{ cells mL}^{-1}$), in a similar range ($> 10^7 \text{ cell mL}^{-1}$) with various *C. calcitrans* strains (Robert et al. 2004; Ragg et al. 2010; Kaspar et al. 2014; Miller et al. 2014; Zhang et al. 2017; Kong et al. 2021) and other small *Chaetoceros* species (Remize et al. 2021).

During growth and depending on the photoperiod treatment, variations in cell size (assessed by the section area) were observed in both strains but with different patterns and magnitude. Morphological plasticity was very low in CCAL, with little variation in cell size during growth and under both light regimes. In contrast, in CTEN, strong variations in cell size were related to growth stage and light regime. Under both light regimes and more markedly under continuous light, CTEN cell size was minimal just after the peak of growth rate and then increased until the stationary phase as a result of cell elongation. Observations of elongated cells have been documented in CTEN strain by scanning electron microscopy (SEM), also showing that elongated cells can subsequently divide into smaller daughter cells (Grzebyk et al. 2022). The increase of cell size during late growth to stationary phase was indicative of the sufficiency of N- and P-nutrients and of no CO_2 limitation (supplied by bubbling resulting in no significant pH variation in the culture medium), nor even Si. Therefore, this raises the question of what prevented the elongated cells from dividing before reaching the stationary phase, capping the maximum cell concentration at around $4\text{--}5 \times 10^6 \text{ cells mL}^{-1}$, regardless of the growth conditions implemented in our experiments or with other *C. tenuissimus* strains (Shirai et al. 2008; Tomaru et al. 2014). These observations suggest that growth inhibition occurred due to cell-to-cell contact starting from a certain cell concentration level, probably taking place at the long setae of this organism (up to 25–30 μm long; Grzebyk

et al. 2022) and being increased by small-scale turbulence inside aerated cultures. Towards stationary phase, when cell volume went up to double due to cell elongation, the increase in equivalent spherical volume occupied at the tips of setae was much less than it would do after an additional cell division. This could correspond to a kind of quorum sensing phenomenon, a cell-to-cell communication well documented in bacteria (Waters and Bassler 2005). In unicellular eukaryotes, the phenomenon of self-control of the density of organisms by contact inhibition, resulting from cell proliferation, has been reported in yeasts (Avbelj et al. 2016). In microalgae, a first report of growth inhibition due to cell-to-cell contact came from the benthic dinoflagellate *Prorocentrum lima* (Costas et al. 1993).

Chlorophylls a and c, carotenoids and photosynthetic activity

Chlorophyllide *a* (chl *a*), resulting from chl *a* degradation, was only detected in CTEN samples, and cell contents were similar under continuous light at the end of growth phase and under the 12:12 L:D cycle in the mid-exponential growth phase. In diatoms, chl *a* is usually associated with senescence and mortality of cells (Bidigare et al. 1986). However, this is in contradiction with the state of the CTEN cells in our cultures, in particular those under the 12:12 L:D regime being in exponential growth and with a high F_v/F_m value. Different rates of degradation of chl *a* to chl *a* have been reported in various *Chaetoceros* species following a standard procedure for cell harvesting and HPLC pigment analysis (Jeffrey and Hallegraeff 1987). Therefore, it is suggested that these measurements of chl *a* might not be related to the physiological state of CTEN cells, and rather be due to specific structures of fucoxanthin-chlorophyll-binding protein complexes (FCP) or photosystems (PS), making a fraction of chl *a* molecules prone to degradation during the analysis process, unlike CCAL.

In this study, photoacclimation occurred in both strains in response to the photoperiod treatment, through the adjustment of the cellular content of chl *a* with that of chl *c* and carotenoid pigments (mostly xanthophylls), and consequently varying the pigment molar ratios.

In both strains, the cellular chl *a* content (measured on day 4) was higher under 12:12 L:D photoperiod than continuous light, particularly for CTEN cells despite their smaller size.

Under the 12:12 L:D photoperiod with respect to continuous light, the chl *c*:chl *a* ratio decreased in CTEN due to the fact that the cellular chl *c* content did not increase along with that of chl *a*. In contrast, in CCAL the chl *c* content increase almost proportionally followed that of chl *a*. The differences in the variation of chl *c*:chl *a* ratio between CTEN and CCAL likely reflected different ways of adjustment of their

photosynthesis apparatus (photosystems I and II and associated light-harvesting complexes) in the photoacclimation process. In *Chaetoceros gracilis*, different FCP complexes associated with PS I and II have been characterized with different chl *c*:chl *a* molar ratios (Ikeda et al. 2013; Nagao et al. 2013). This suggests that the variation of chl *c* content and chl *c*:chl *a* ratio in response to light conditions could result from the change in the distribution (i.e., numbers and proportions) of such different FCP complexes in the thylakoid membrane around the PS reaction centers: apparently, under the L:D photoperiod, providing a lower cumulative light energy supply than continuous illumination, CTEN implemented particular PS units with a lower chl *c*:chl *a* ratio. In contrast, CCAL under the L:D photoperiod increased the number of PS units identical to those implemented under continuous illumination.

Conspicuous differences were observed between the content of carotenoid pigments of CTEN and CCAL strains, and in their variations for adaptation to the light regime. The biosynthesis pathway of diatom xanthophylls is not yet well known. In the last decades, several hypothetical pathways have been proposed from the β -carotene precursor to the last transformation steps leading to fucoxanthin (F), diadinoxanthin (Dd) and diatoxanthin (Dt) (Lohr and Wilhelm 1999, 2001; Bertrand 2010; Dambek et al. 2012; Bai et al. 2022). Xanthophyll compounds in green plastids, (i.e., zeaxanthin (Z), antheraxanthin (A), violaxanthin (V) and neoxanthin (N)) have been inferred as usually undetectable intermediates, accumulating and undergoing dynamic interconversions in the photoprotection process under experimental high light stress (Lohr and Wilhelm 1999, 2001). Nevertheless, V was more recently reported as a trace component in FCP complexes in *C. gracilis* (Ikeda et al. 2013), and the presence of Z (the de-epoxidized form of V) in the CTEN strain (which we also observed in the Japanese *C. tenuissimus* strain NIES-3715; unpublished data) seems consistent with this observation. Functionally, F is the main light-harvesting pigment capturing photons for transmission to photosynthetic reaction centers. Dd and Dt function as a dynamic pool, where Dd (epoxidised form) converts to Dt (de-epoxidised form) under light, and reverses in darkness or low light (Lohr and Wilhelm 2001; Goss and Jakob 2010). This diadinoxanthin photoprotective cycle typically activates under light stress, but can also be effective at low irradiance, with activity varying depending on the taxa or strains (Lavaud and Lepetit 2013), and Dt being widely found in diatoms grown under low irradiance (Stauber and Jeffrey 1988). Additionally, Dd exists in two pools, with some molecules serving as F precursors (Goericke and Welschmeyer 1992; Olaizola and Yamamoto 1994), and its amounts in PS remain more stable than in FCPs under different light regimes (Lavaud et al. 2003).

Under the 12:12 L:D photoperiod compared to continuous light, photoacclimation occurred through enhanced

carotenoid synthesis to increase the cellular content of light-harvesting pigments, but this occurred differently, with respect to the intrinsic level of xanthophylls content of each strain. CCAL showed a relatively high xanthophyll content and few variations between the two photoperiods, contrary to CTEN. Carotenoid cell content increased (not significantly) by only ~5% (including ~3.6% for xanthophylls) in CCAL under the 12:12 L:D photoperiod, while it doubled (of which more than a doubling of xanthophylls) in CTEN. Accordingly, in both strains under the 12:12 L:D cycle, the increase of β -carotene content fueled the higher xanthophyll content, in agreement with observations below growth-saturating irradiances (Goericke and Welschmeyer 1992). Subsequently in the process, the Dd pool (i.e., Dd + Dt) also increased, congruently with the increase of the final product F, but proportionally less than the latter so that the F:Dd pool mole ratio decreased; this occurred more strongly in CTEN than in CCAL. Under the 12:12 L:D cycle compared to continuous light, despite the increase in chl *a* content in both strains, the higher cellular content of F in CTEN increased the F:chl *a* and (Dd + Dt):chl *a* ratios to significantly strengthen light-harvesting antennas, while for CCAL the stable F content led to a lower F:chl *a* and (Dd + Dt):chl *a* ratios, but adjusted to improve the conversion of the captured light flux.

Light intensity in this work was likely below saturation and photoinhibition did not occur in either photoperiod treatment, nevertheless, conversion of Dd to Dt occurred in both strains but not under the same light regime. In CCAL, the formation of Dt (accounting for only 2.9% of the Dd + Dt pool) occurred solely under continuous light, and might have resulted from a background level of de-epoxidase activity whereas the absence of a dark period prevented a complete conversion back from Dt to Dd. In contrast in CTEN, Dt formed only under the 12:12 L:D photoperiod, resulting in a higher Dt:(Dt + Dd) ratio. This seems to be a consequence of the doubling of xanthophyll pool, including the tripling of the Dd + Dt which took place in the photoacclimation process, likely making Dd (or a part of the pool) more prone to de-epoxidation after entering the light period. The presence of zeaxanthin in CTEN was unexpected under the moderate light exposure used in our experiments, because in diatoms, zeaxanthin accumulation has been shown to occur under high-light stress (Lohr and Wilhelm 1999). Under continuous light, only trace amounts of Z were found in CTEN cells, which can be paralleled with those of Dt in CCAL. In contrast under the 12:12 L:D cycle, CTEN cells exhibited a Z content comparable to that of Dt, suggesting that the formation of both pigments occurred by the same process. De-epoxidized pigments located in light-harvesting antennas (mostly Dt, and Z and β -carotene) would contribute to protection of photosystems from overexcitation through

nonphotochemical quenching and heat dissipation (Olaizola and Yamamoto 1994; Falkowski and Raven 2007).

The maximal photosynthetic efficiency of photosystem II (F_v/F_m) is widely used as a proxy for the estimation of photosynthetic stress in plants and values ranging between 0.6–0.65 are universally considered indicators of good health in algal organisms (Schreiber 1983; Maxwell and Johnson 2000; Parkhill et al. 2001; White et al. 2011). During our experiments in both photoperiod treatments, CCAL maintained high and stable F_v/F_m values from exponential to late growth (before stationary phase was reached). This has previously been reported for other strains of *C. calcitrans* where F_v/F_m started to decrease (below 0.6) at the beginning of the last doubling of cell concentration before stationary phase and below 0.5 during culture decay (Zhang et al. 2017; Kong et al. 2021). In contrast in CTEN, under both photoperiods, the F_v/F_m value varied during growth in a similar pattern but lagged over time (later under the 12:12 L:D cycle than under continuous illumination), briefly peaking at 0.6 during the exponential phase, before dropping as the stationary phase approached (to ~0.5) and further more dramatically (below 0.4) in the decay phase under continuous illumination.

Differences in photoacclimation behavior linked to the underwater light climate of the native marine habitat of phytoplankton species have previously been reported in diatoms and other lineages (Lavaud et al. 2007; Dimier et al. 2009). In the same way, it is suggested that the differences in pigment contents and ratios and their variations in response to the two light regimes between the CTEN and CCAL strains, indicate different light niches for these two organisms. In the CCAL strain, the high F content and the high F:chl *a* ratio suggest large size antennas (light-harvesting chlorophyll-protein complexes) better adapted to low light irradiance. In contrast in the CTEN strain, considering the low F content and the low F:chl *a* ratio, the photosynthesis apparatus appears to consist of small-sized antennas, better adapted to high light irradiance with small fluctuations: these conditions are generally found in the Mediterranean lagoon where it was isolated, which is characterized by high irradiance and low turbidity. Lower capability of CTEN to further increase the xanthophyll pool and the size of light-harvesting antennas in order to acclimate to limited light, would explain the lower growth rate obtained under the 12:12 L:D cycle due to light-limitation.

Lipid content

The cellular concentrations of total lipids and their structure and reserve proportions can vary widely, depending on the growth phase and culture conditions (Muller-Feuga et al. 2003; Miller et al. 2014). Muller-Feuga et al. (2003)

estimated that *Chaetoceros* genus shows total lipid concentrations of around 2 pg cell^{-1} on average.

In *C. calcitrans* forma *pumilum* under continuous light, Ponis et al. (2003) estimated a mean cellular concentration of total lipids of $3.0 \pm 0.2 \text{ pg cell}^{-1}$ during the stationary phase, while Miller et al. (2014) reported concentrations between 3.5 and 7.4 pg cell^{-1} following the growth phase. In a previous study (Robert et al. 2004), cellular concentrations of total lipids in the same CCAL strain were estimated to be 2.2 pg cell^{-1} and 5.1 pg cell^{-1} during exponential and stationary phases, respectively. Our results are hence in the range of total lipids' concentrations described in literature for *C. calcitrans*.

For lipid content in CTEN, total lipid cellular concentrations did not show a marked distinction between the two photoperiods. In their work on a species defined as *Chaetoceros* "tenuissimus-like", Robert et al. (2004) estimated total lipids contents of 1.1 pg cell^{-1} and 1.3 pg cell^{-1} during the exponential and stationary phases, respectively. Our results are consistent with these previous observations, even though CTEN showed slightly higher values than those documented in literature for other strains of *Chaetoceros* genus.

In both studied *Chaetoceros* species, FA in the form of storage lipids were higher under 24:00 L:D photoperiod, especially in CCAL and at the expense of polar lipids in CTEN. Variation in lipid composition and content during culture growth (i.e., in successive growth phases) is well documented. Generally, as it was observed in our study, lipid content, along with accumulation of SAFA and MUFA in neutral lipids increases during stationary phase (Liang et al. 2006; Li et al. 2014; Miller et al. 2014; Remize et al. 2021). These neutral lipids are assumed to be used as energy resources for the aerobic metabolism of the cells (Miller et al. 2014). Fatty acid compositions in both strains were fairly similar to that of various *Chaetoceros* strains considered for shellfish feed in aquaculture, in view of the variety of culture conditions used between different studies (Renaud et al. 1999; Kaspar et al. 2014; da Costa et al. 2023). Like other species of the *Chaetoceros* genus, *C. calcitrans* is known for its high content in essential lipids, as notably EPA (Volkman et al. 1989; Brown 2002).

High proportions of PUFA, including n-3 PUFA and EPA, were found in polar lipids of both studied *Chaetoceros* species. Multiple studies have described high PUFA proportions under photoperiods including long dark phases (Krzemińska et al. 2014). Lim and Zaleha (2013) hypothesized that under light-limiting conditions (12:12 L:D), the organisms increase thylakoid membrane surface, the expansion of which is hence associated to a strong need of polar PUFA as major component of membranes. In CCAL, the highest proportions of 16 PUFA in PL under 12:12 L:D cycle may reflect a higher cellular content of thylakoid

membrane as compared to cell constantly exposed to light (24:00 L:D regime). Diatoms are known to be enriched in 16:2 and 16:3 PUFA (Guschina and Harwood 2006; Liang et al. 2014). As shown by Abida et al. (2015) on the diatom *Phaedactylum tricorutum*, 16:2, 16:3 and 16:4 fatty acids are specifically associated to monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), which glycolipids are predominating in the thylakoids membranes of chloroplast. Furthermore, 16 PUFA are almost exclusively found on the sn-2 of MGDG and DGDG while the sn-1 position is dominated by EPA (20:5n-3) (Abida et al. 2015). In a recent study, González-Fernández et al. (2020) reported that 16 PUFA (16:2n-7, 16:2n-4 and 16:3n-4) in polar lipids of *C. neogracile* were found in higher proportions during the exponential phase (9.2%) along with a higher chl *a* content, than during stationary phase (4.3%). Similarly, Remize et al. (2021) observed a drastic decrease of 16 PUFA in polar lipids of *C. neogracile* during a 14 days culture; concomitantly with an increase of neutral lipids from 1.7 pg cell^{-1} at day 0 to $11.8 \text{ pg cell}^{-1}$ at day 14.

Altogether, this reveals that CCAL cells under 12:12 L:D cycle responded to a lower illumination condition by increasing their thylakoid membranes. Based on these observations regarding the relative richness of essential FA in CCAL cultures, the continuous light regime is a better condition in term of PUFA production, of EPA in particular, compared to 12:12 L:D photoperiod.

On the potential use of *Chaetoceros tenuissimus* CT16ED strain for aquaculture purposes

Evaluating the potential for use for aquaculture of a microalgal strain requires taking into account a set of intrinsic characteristics of the strain and their suitability with the conditions for implementing the culture production and the subsequent use of microalgae.

Regarding growth parameters, compared to similarly sized *Chaetoceros* strains used for aquaculture, the CTEN strain had similar growth rates under both photoperiods, although under a 12:12 L:D cycle, the high-light ecotype of the strain might require higher illumination than we used in this study. Scaling up of this microalgae production might seem challenging owing to cell concentrations observed in our study, but other criteria do prevail in the choice of a prey strain that can offset this reserve.

The size of microalgal prey is also an important parameter with respect to the organisms fed, considering the diversity of mollusks and crustaceans in hatcheries, which often have several larval stages present simultaneously in the rearing tanks (Galgani and AQUACOP 1988; Phatarpekar et al. 2000; Muller-Feuga et al. 2003; González-Araya et al. 2011, 2012; Gomes et al. 2021). Thus, certain

morphological changes of CTEN observed during its growth in culture, could be beneficial to facilitate the capture of algal prey by fed organisms of different sizes, by offering a variety of size of prey particles, with small solitary cells and larger particles made of small chains during the exponential phase, or with elongated (larger) cells at the end of the growth phase. Moreover, these size-increased cells can be nutrient-replenished in terms of nitrogen, phosphorus, and carbon content (Riemann et al. 1989), including high lipid and probably protein content, which are particularly interesting for the hatchery-rearing of mollusks and shrimp larvae (Brown 2002; Muller-Feuga et al. 2003; Tocher 2003; Ragg et al. 2010).

Regarding lipid quality, CTEN's advantage over other *Chaetoceros* strains lies in its relatively stable lipid content from the growth phase to the stationary phase, and little altered by the photoperiodic cycle. In addition, this good lipid content was obtained in cells grown in a standard rich medium without the need for nutrient limitation, making it easier to control this quality factor in aquaculture nutrition. Another advantage of CTEN is the high percentages of EPA and DHA, and high DHA:EPA ratio, compared to other *Chaetoceros* strains that are generally low in DHA. In practice for feeding aquaculture organisms, the supply of *Chaetoceros* food must often be compensated by mixing with another algal species, often haptophytes, which are richer in DHA (for example, the genera *Isochrysis* or *Tisochrysis*) (Volkman et al. 1989). Thus, the CTEN strain could reduce the need for mixed cultures, particularly with haptophytes which are also smaller in size and possibly less easy to capture by fed organisms.

Another interesting aspect of CTEN for its use as live-stock feed, is that a simple light microscope observation can be used to assess the state of culture development (i.e., by the presence of cell chains or elongated cells), providing insights into the biochemical quality (including lipids) of the cells, and helping to decide whether to use this culture for feeding.

Conclusion

Despite the relatively low cellular densities obtained with the *C. tenuissimus* CTEN strain, which may be challenging for its use for aquaculture production, our study revealed a promising nutritional potential for CTEN and interesting physiological characteristics. The study indicated a marked influence of photoperiod on the growth and metabolism of the CTEN strain and the *C. calcitrans* CCAL strain. The biochemical response to the two contrasting light regimes revealed the specific light niche of the CTEN strain, probably linked to its original environment, showing an adaptation

of this strain to high irradiance environments consistent with its Mediterranean lagoon origin. Moreover, CTEN showed an interesting lipid content, rich in essential PUFA, including a higher DHA proportion. This makes CTEN a quality food for use in aquaculture.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10811-025-03505-0>.

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Authors' contributions C.P.†: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – Original draft, Visualization. V.L.†: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – Original draft, Writing – Review & Editing, Visualization. D.G.: Conceptualization, Validation, Formal analysis, Data curation, Writing – Original draft, Writing – Review & Editing. P.S.: Conceptualization, Validation, Formal analysis, Data curation, Writing – Original draft, Writing – Review & Editing. A.A.: Methodology, Resources, Project administration, L.A.: Methodology, Resources, Project administration. P.C.: Conceptualization, Methodology, Validation, Formal analysis, Writing – Review & Editing, Supervision. V.P.: Conceptualization, Methodology, Validation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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