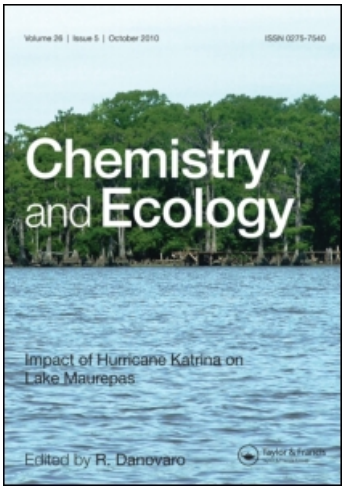

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The microalgae *Tetraselmis suecica* in mesocosms under different light regimes

Francesca Borghini[a]*, Andrea Colacevich[a], Nadia Bergamino[b], Primo Micarelli[c], Arduino Massimo Dattilo[b], Silvia Focardi[b], Silvano Focardi[a] and Steven Arthur Loiselle[b]

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Pigment profiles and pheopigment accumulation of the microalga *Tetraselmis suecica* were studied under different spectral irradiances. Irradiance conditions typical of coastal waters with ('clear') and without ('coloured') chromophoric dissolved organic matter (C-DOM) were simulated in mesocosm cultures. The lag, growth and stationary phases were found to follow a logistic model, as light limitation controlled growth where maximum carrying capacity and incremental growth were sensitive to irradiance conditions, both being higher in mesocosms with full irradiance conditions (clear sea conditions without attenuation due to C-DOM). Through daily measurements of chlorophylls, carotenoids and pheopigments, it was found that the highest concentrations of pigments occurred in the growth phase: in particular in the clear sea conditions. Lower concentrations of lutein were measured in the coloured mesocosms showing their protective function against photo-oxidation. Pheopigment concentrations increased linearly throughout the experiment, being highest in the mesocosm with clear sea conditions. In general, clear sea conditions, including ultraviolet irradiance, showed the higher production of primary and secondary pigments, demonstrating the high tolerability of *T. suecica* to a range of solar irradiance conditions.

Keywords: *Tetraselmis suecica*; mesocosm; light limitation; spectral irradiance; logistic growth

1. Introduction

T. suecica (Chlorodendrales, Prasinophyta) grows in a wide range of nutrient and salinity conditions [1–4] and is common in coastal waters. Some *Tetraselmis* species are euryhaline whereas others (e.g. *T. contracta*) are able to grow in salinities ranging from 1–4%. *Tetraselmis* is an important marine quadriflagellate both ecologically as well as in commercial aquaculture, where it is utilised as live feed for bivalve molluscs, zooplankton and larval/early juvenile stages of crustaceans and fish [5]. The nutritional quality of the microalgae is linked to its biochemical composition, which is directly influenced by a series of environmental factors: in particular nutrient and irradiance conditions [6–8]. Because of its high content of tocopherol (vitamin E), *T. suecica* has also been proposed as source of vitamins for human and animal consumption [9].

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Extracts from *T. suecica* have been reported to inhibit pathogenic bacteria in fish [10] and exhibited significant cytotoxic activity on tumour cell cultures [11]. It has also been used in assays to evaluate the effects of sediment toxicity [12]. *T. suecica* has been shown to have good accumulation properties for cadmium, suggesting the possibility of using this microalgae in bioremediation processes [13,14].

By quantifying the photosynthetic pigments, it is possible to gain information on species productivity and community composition, making it possible to use this information as taxonomic markers [15]. Changes in the relative ratios of specific pigments and their degradation products provide information on growth dynamics and physiological state in different environmental conditions (e.g. nutrient and the spectral irradiance) [16]. Modifications in the spectral composition and intensity of incident radiation can lead to changes in pigment composition within the same species in relation to photoacclimation and photoinhibition mechanisms [17]. In particular, carotenoids may dissipate excess energy via the xanthophyll cycle, protecting photosynthetic reaction centres and the antenna complexes against photochemical damage. Carotenoids may also have a structural role by stabilising and photoprotecting chloroplast membranes as well as acting as accessory pigments [18]. Several species of microalgae can synthesise large quantities of secondary carotenoids under unfavourable culture conditions [19]. In particular, elevated UVB irradiance has been shown to induce a rapid increase in intracellular carotenoids, a marked decline in chlorophyll *a* (chl *a*) and a suppression of polyunsaturated fatty acid synthesis in *T. suecica*. These changes can occur over very short periods. Following a reduction in UVB, *T. suecica* has been shown to rapidly reorganise cellular processes [20]. However, *T. suecica* has not shown mobility changes in relation to exposure of cells to UVR in the water column, where cells tend to accumulate in the surface layer [21].

As light penetrates the water column, its intensity is reduced and its spectral quality changes in relation to the optically active components of the water column. In clear sea waters, the solar spectrum is shifted towards shorter visible wavelengths due to the higher absorption by liquid water in the infrared and higher visible wavelengths. In coastal environments near rivers or wetlands, dissolved organic matter may increase attenuation in the shorter wavelengths (blue and ultraviolet) [22,23].

The present study examines the impact of two different spectral irradiance conditions on the pigment profile of *T. suecica* cultures. This was achieved using controlled incident irradiance with cut-off filters to simulate the spectral irradiance of coastal waters with and without chromophoric dissolved organic matter (C-DOM). These experiments were conducted in vertical mesocosms under constant mixing.

2. Materials and methods

2.1. *Culture conditions*

T. suecica was precultivated for several generations under laboratory conditions. Light (12 hour photoperiod 100 mmole photon $m^{-1}\,s^{-1}$) and nutrient conditions were controlled throughout the cultivation. A single light tube (30 W, 4900 K) was located 15 cm from the 2-litre flasks used for the preculture. Filtered (Ø 0.22 μm) artificial seawater (Instant Ocean, Aquarium System Inc.) was used and the temperature of the culture was maintained at $20 \pm 2\,^{\circ}C$. Glass-wool filtered air was provided continuously at a flow rate of $1.5\,l\,min^{-1}$ for gas exchange and to maintain microalgae in suspension. Prior to inoculation and at regular intervals throughout the study, microscopic analysis was performed to verify monoculture conditions.

Plexiglas cylinder mesocosms (0.19 m diameter × 1 m) were filled with filtered (0.22 μm) artificial seawater and enriched with $NaNO_3$ ($75\,mg\,L^{-1}$), $K_2HPO_4\ 2\ H_2O$ ($5\,mg\,L^{-1}$) and $FeCl_3$

($3\,mg\,L^{-1}$). Nutrient concentrations were maintained throughout the experiment, determined daily by UV-Vis spectrometer following standard methods [24]. Salinity was maintained at $36\,g\,L^{-1}$. Dissolved oxygen (range from 5.5–8.4 $mg\,L^{-1}$), pH (8.5), and temperature ($20 \pm 2\,^{\circ}C$) were measured twice daily by portable metres in order to ensure constant conditions during the culture. Inoculation of the mesocosms was performed using $50\,ml\,L^{-1}$ (50000 cells mL^{-1}) of the precultured *T. suecica* obtained during the logarithmic growth phase.

A light source was positioned 60 cm directly over each mesocosm. A metal halide 150 W lamp (OSRAM HQI-TS) was used with a photoperiod of 12 hours. Light intensity was measured at the beginning and conclusion of the experiment using a PUV541 spectroradiometer (Biospherical Instruments, San Diego, CA), as well as continuously using a 4 band radiometer (SKR 1850) positioned externally to the columns.

Two different spectral irradiances were used, one to simulate coastal waters containing C-DOM (coloured) and one to simulate clear coastal waters without C-DOM (clear). Appropriate spectral attenuation was achieved using spectral filters (Figure 1) which in one case removed UVC/UVB/UVA/blue wavelengths to create optical conditions similar to coloured coastal waters while the other attenuated irradiance in the UVC/UVB (clear coastal waters). The elevated absorption in the former is typical of inland and coastal waters where the concentration of terrigenous based C-DOM is elevated. Duplicate mesocosms were used to examine 'coloured' conditions while a single mesocosm was used to simulate 'clear' waters. Glass-wool filtered air was provided continuously to each mesocosm at a flow rate of $3\,L\,min^{-1}$ for gas exchange and to maintain the microalgae in suspension. Complete mixing within each column was checked prior to the experiment using neutral density plastic chips. The experiment was performed for 18 days until the logarithmic growth period was concluded.

Incident spectral irradiance was measured at two depths in the water column to examine modifications in spectral attenuation during the growth phase. Measurements were made in the centre of each column just below the water surface (to reduce reflection and refraction artefacts) and at half column depth (0.50 m) using a spectroradiometer EPP2000C -100 (StellarNet Inc) operating in

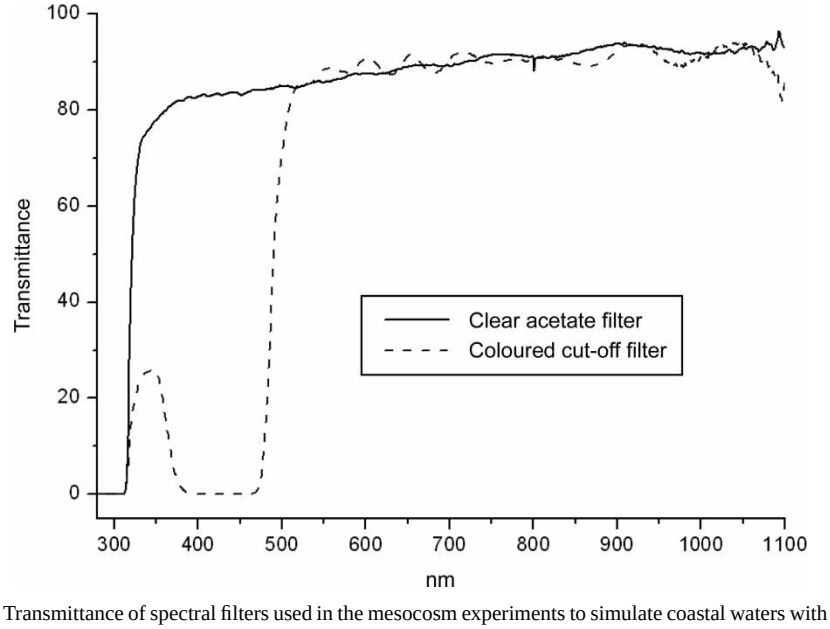

Figure 1. Transmittance of spectral filters used in the mesocosm experiments to simulate coastal waters with (coloured) and without (clear) chromophoric dissolved organic matter.

the wavelength range of 300–800 nm. The receiving sensor (CR1 UV-VIS) with a cosine response and fibre optic cable with silica core (diameter 400 μm) was calibrated prior to the experiment using a LI-COR spectral irradiance lamp (model #1800-02L) and a StellarNet deuterium lamp (model SL3, serial #091403) with a 10%TND filter.

2.2. *Pigment and nutrient analysis*

Pigment samples were obtained 3 hours after the conclusion of the light period. This was done to avoid sampling in different periods of the diel cycle as well as potential impacts of recent light history related to photoinhibition at the surface layer. Diel variability in the concentrations of photosynthetic pigments of *T. gracilis* has been documented [25]. Water samples were obtained daily at half column depth (0.50 m) and filtered immediately using GF/F filters. Filters were extracted by sonication with 5 mL of 90% acetone and stored overnight at 5 °C. Throughout the extraction procedure, samples were protected from light. The extracts were filtered and analysed by atmospheric pressure chemical ionisation liquid chromatography-mass spectrometry (APCI LC-MS). A reversed phase column (Spherisorb ODS2 Hypersil, 150 × 2.1 mm ID, 5 μm particle size equipped with ODS2 pre-column) was used [26] with slight modifications in the mobile phase, flow and sample volume (10 mM ammonium acetate, a 200 μL min^{-1} flow rate and 10 μL of sample). LC-MS was performed using a Thermo system (Finnigan surveyor autosampler, a MS pump and a Finnigan LTQ). APCI LC-MS analysis was performed in positive ion mode: capillary temperature 250 °C, APCI vaporiser temperature 450 °C, discharge current 10 μA, sheat gas flow 40 (arbitrary units), auxiliary gas flow 14 (a.u.).

Pigment concentrations were determined by comparing HPLC peak areas with those of standard solutions prepared with commercially available purified pigments (canthaxanthin, fucoxanthin, echinenone, pheophytin *a*, pheophorbide *a*, lutein, zeaxanthin) from the International Agency for 14C Determination VKI in Hoersholm, Denmark and (chl *a* and *b*, α- and β-carotene) from Sigma Aldrich. Samples were analysed in triplicate and the relative standard deviation was below 20%. Solvent blanks were analysed every six samples and resulted below the minimum detection limit for each studied pigment.

Nutrient concentrations were analysed at the beginning and at the conclusion of the experiment following APHA standard methods [24]. Nutrient concentrations were maintained in excess throughout the experiment through weekly additions. Filtered (0.22 μm) artificial sea water was added daily to maintain a constant water level in the water columns and therefore distance from the illumination source.

2.3. *Statistical analysis*

A Mann–Whitney *U* test was used to investigate the differences in pigment concentrations between the two light regimes. Statistical analyses were performed using the Statistica 6.0 program.

3. Results

3.1. *Light conditions*

After inoculation, spectral irradiance at 0.50 m shows the effects of the filter on the incident light field (Figure 1). Irradiance in the UVA (340–400 nm) and in a limited amount in UVB (280–340 nm) was present in the mesocosm with a clear acetate filter. The two mesocosms with

coloured acetate filters had initial light conditions (at 0.50 m) which showed a strong reduction in the UV and short visible wavelengths (Figure 2). After 18 days of growth, spectral attenuation in all three columns increased significantly, reaching $3\,m^{-1}$ at 490 nm. No significant differences in spectral attenuation between columns were present at the conclusion of

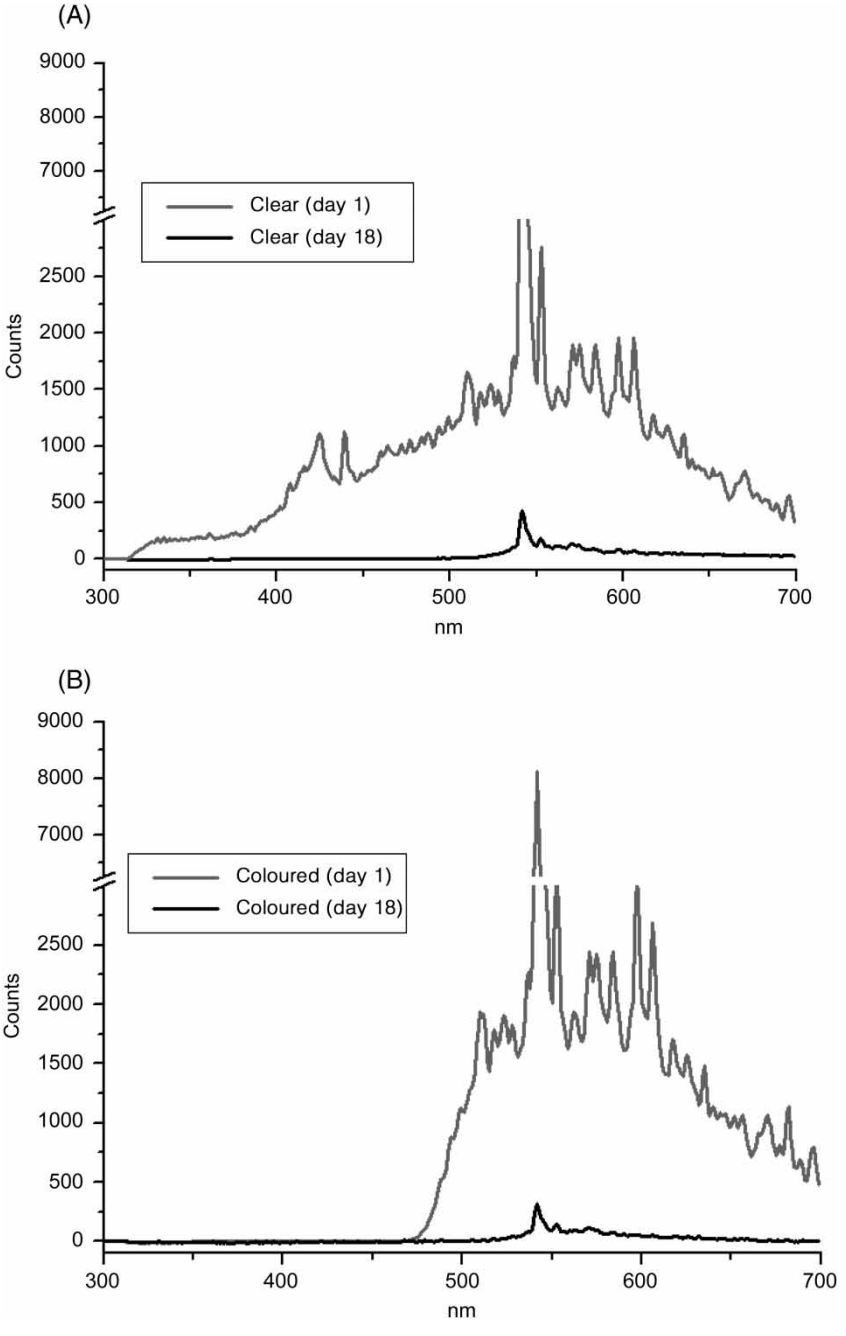

Figure 2. Spectral irradiance measured at 0.50 m in mesocosm experiments before and after the growth phase of *T. suecica* mesocosms with irradiance conditions similar to clear (A) and coloured (B) conditions.

the experiment. Measurements at 0.50 m on day 18 showed irradiance values below the detection limit of the spectroradiometer ($0.001\,Wm^{-2}\,nm^{-1}$) for wavelengths below 520 nm and above 660 nm.

3.2. *Pigment concentrations*

The concentrations of both chlorophylls and carotenoids, determined daily, were found to be similar in the two coloured filter mesocosms, with no statistically significant differences (Mann–Whitney U test, $p < 0.01$). The mean values of both mesocosms are used in the overall analysis.

Culture conditions were analysed at the conclusion of the experiment using fluorescence microscopy and pigment analysis. Qualitative microscopic observations showed that the cultures presented a limited (<1%) diatom contamination. This was confirmed by pigment analysis with the presence of fucoxanthin and diadinoxanthin, marker pigments for diatoms. Daily cell counts were not performed during this experiment [20].

Chl *a* concentrations showed an exponential increase after 3 days and a maximum increase between the fifth and sixth days in each mesocosm. Chl *a* concentrations were constantly lower in the coloured mesocosms. The stationary phase extended to the final day of measurements. The increase of chl *a* concentrations during the experiment followed a logistic growth curve, indicating monoculture conditions, where each species grows to its carrying capacity, limited only by intraspecies competition.

By fitting the biomass growth with a logistic curve (Equation (1)), several aspects of the growth dynamics can be quantified.

$$P = A_2 + \frac{A_1 - A_2}{(1 + (d/d_m)^{\mu})}. \tag{1}$$

P is phytoplankton biomass, A_1 is the background concentration, A_2 is the biomass concentration upper limit (ecosystem carrying capacity), d is the measurement day, d_m is the day of maximum incidental growth and μ is the slope of the incremental growth. It should be noted that the use of chl *a* as an estimate for biomass growth has several limitations, as chl *a* concentrations per cell may vary in relation to light acclimatation, nutrient availability and other physiological conditions [27]. However, it is often used in ecological studies as a common proxy for biomass dynamics [28].

The logistic equation for phytoplankton growth in the mesocosms showed different growth dynamics for each light environment (Figures 3 and 4). The clear irradiance conditions showed a higher upper limit (A_2) as well as a higher slope in the exponential growth phase (μ), the latter indicating higher productivity. The day of maximum incremental change (d_m) was found to be similar in all mesocosms, as was the lag (3 days) prior to the growth phase. Additionally, the increased availability of irradiance in the UVA and short visible wavelengths allowed for higher incremental growth (μ) with respect to the reduced irradiance environment present in the coloured mesocosms. Light limitation was confirmed by spectral irradiance measurements, with a near total attenuation at 0.50 m on the final day of measurements in all mesocosms (Figure 2). Spectral irradiance appears, therefore, to influence overall phytoplankton carrying capacity and productivity, with coloured conditions showing lower productivity and carrying capacity.

The temporal dynamics of the concentrations of chl *b* coincided with that of chl *a*, including a 3 day lag period followed by a growth period in all columns. Concentrations reached the stationary phase quickly and remained stable over the remaining 2 weeks. No significant differences were found between the concentrations of chl *b* in the two light regimes.

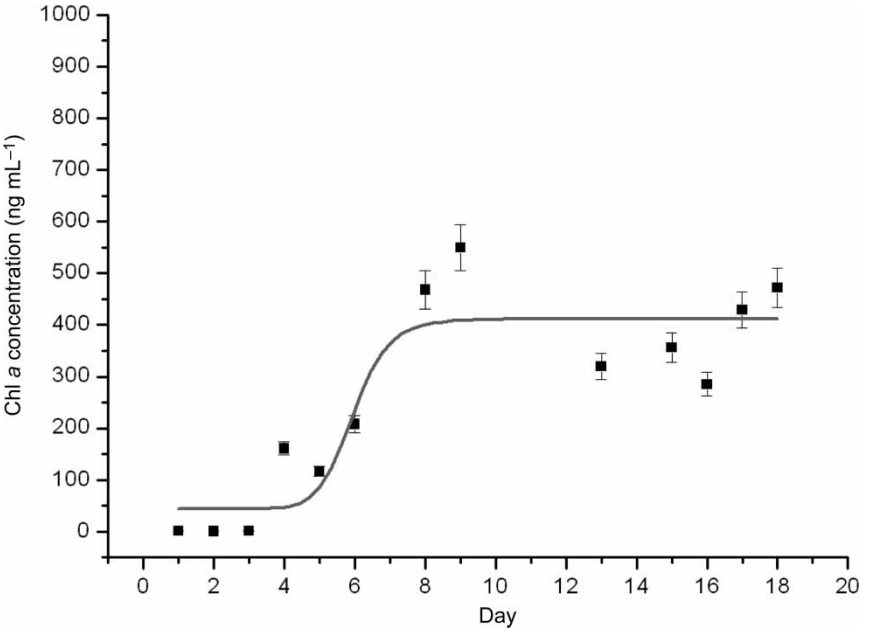

Figure 3. Logistic growth of phytoplankton biomass (measured as chl *a* concentrations) in mesocosms with irradiance conditions representing coastal waters with dissolved organic matter ($\chi^2 = 7963$, $R^2 = 0.83$, $A_2 = 410 \pm 38$ ng mL^{-1}, $d_m = 5.4 \pm 0.5$ days, $\mu = 6.8 \pm 4.4$).

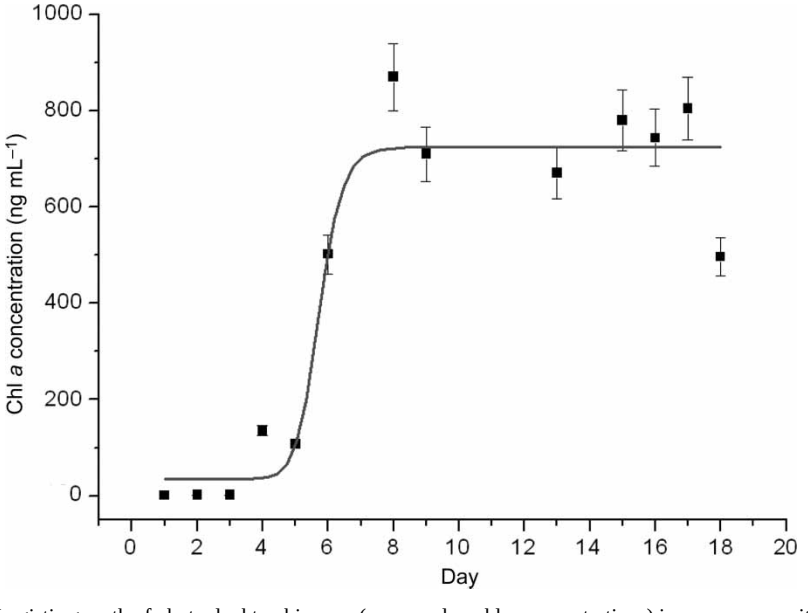

Figure 4. Logistic growth of phytoplankton biomass (measured as chl *a* concentrations) in mesocosms with irradiance conditions representing clear coastal waters ($\chi^2 = 10474$, $R^2 = 0.93$, $A_2 = 725 \pm 39$ ng mL^{-1}, $d_m = 5.6 \pm 0.2$ days, $\mu = 14.0 \pm 6.9$).

The concentration of individual carotenoids varied over the course of the experiment. The highest concentrations were present during the growth phase of the phytoplankton biomass and lowest concentrations in the stationary phase. Lutein concentrations showed an increase beginning on the third day of measurements (Figure 5(A)). The concentrations in the mesocosm representing clear

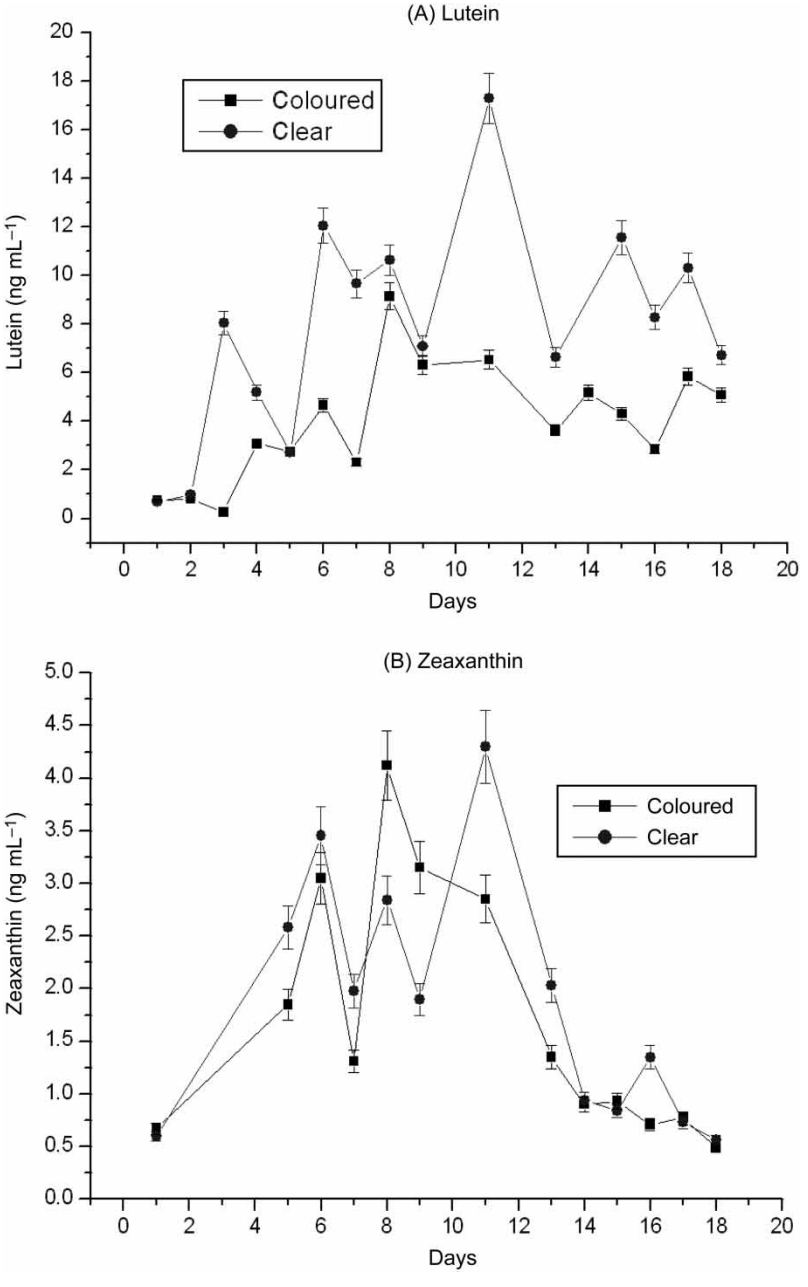

Figure 5. Modifications in the concentration of carotenoids in mesocosms with light environments similar to coastal waters with (coloured) and without (clear) chromophoric dissolved organic matter.

sea conditions were significantly higher (Mann–Whitney U test, $p < 0.01$) than those measured in the mesocosms with coloured filters. No significant differences (Mann–Whitney U test) were found between the concentrations of zeaxanthin in the two irradiance regimes (Figure 5(B)). In general, the average concentrations of all pigments (with the exception of zeaxanthin) were higher in the clear mesocosm throughout the experiment (Table 1).

Table 1. Arithmetic average (± standard deviation) of the concentrations of pigments and pheopigments (expressed in ng mL^{-1}) obtained during 18 days of measurements in mesocosms simulating coastal waters containing chromophoric dissolved organic matter ('coloured') and without organic matter ('clear').

Pigment	Coloured mesocosms	Clear mesocosms
Chlorophyll *a*	281 ± 227	533 ± 407
Chlorophyll *b*	4.2 ± 3.1	8.0 ± 7.4
Lutein	4.0 ± 2.3	7.5 ± 4.5
Zeaxanthin	1.7 ± 1.2	1.9 ± 1.2
Pheophytin *a*	2.4 ± 2.6	5.4 ± 7.9
Pheophorbide *a*	0.5 ± 0.2	0.7 ± 0.3

In the present experiment, the ratios of carotenoids and chl *b* to chl *a* decreased throughout the lag and growth phase but were not significantly affected during the stationary phase. The chl *b*/chl *a* ratio was found to decrease exponentially (Figure 6).

The ratio of lutein and zeaxanthin to chl *a* was higher in the clear sea mesocosm with respect to the coloured mesocosms, but this increase had limited significance ($p < 0.2$). However, the ratio of lutein to zeaxanthin was significantly higher ($p < 0.01$) in the clear mesocosm (higher UV irradiance), in particular in the stationary phase (Figure 7).

Generally pheopigments reached the highest values during the stationary phase and displayed a rapid increase when algae entered the senescence phase. In the present experiment, both pheophytin *a* and pheophorbide *a* increased in a linear manner throughout the experiment (Figure 8). Concentrations of both phaeopigments were slightly higher in the clear mesocosm with respect to the mesocosms with coloured filters ($p < 0.1$), probably in relation to the higher incident irradiance. There is no evidence that the senescence phase was reached during the experiment.

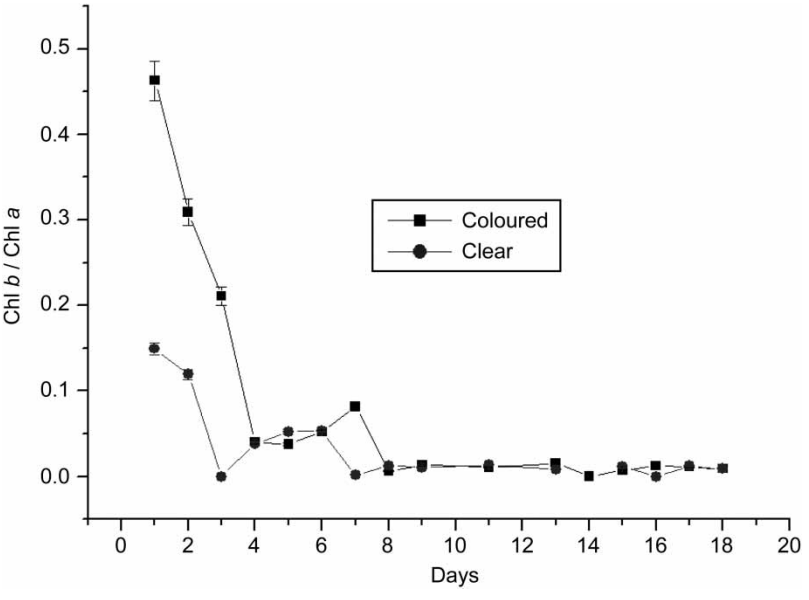

Figure 6. Modifications in the relative concentrations of chl *b* to chl *a* in mesocosms with light environments simulating coastal waters with (coloured) and without (clear) chromophoric dissolved organic matter.

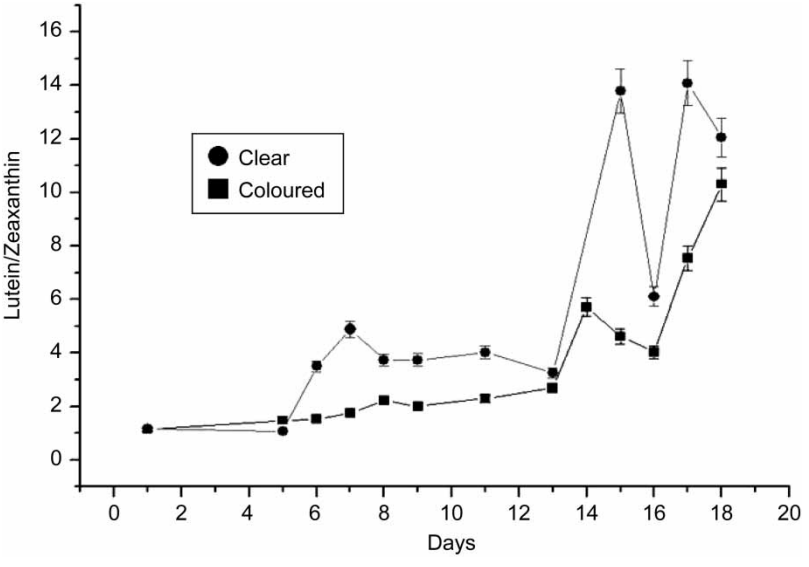

Figure 7. Modifications in the ratio of carotenoid concentrations of lutein to zeaxanthin in mesocosms with light environments similar to coastal waters with (coloured) and without (clear) chromophoric dissolved organic matter.

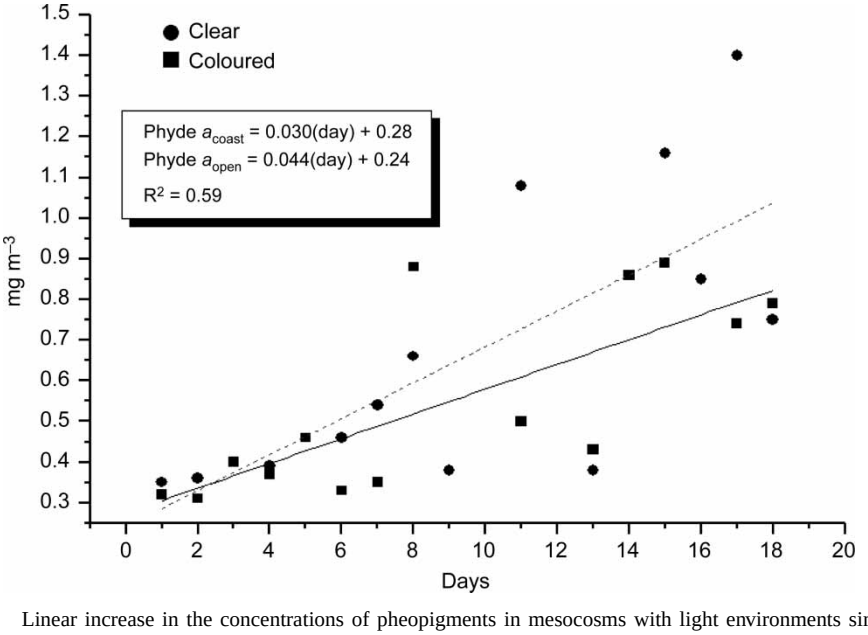

Figure 8. Linear increase in the concentrations of pheopigments in mesocosms with light environments simulating coastal waters with (coloured) and without (clear) chromophoric dissolved organic matter.

4. Discussion and conclusions

Pigment profiles were comparable with those reported for the same genus [29–32]. Chl *a* concentrations were in the same range reported by other authors (0.05–1.14 $\mu g\,mL^{-1}$) [20,33] or for species of the same genus [7] (0.38–3.57 $\mu g\,mL^{-1}$) cultured in different media.

The average chl *b*/chl *a* ratios were found to be slightly lower than reported in similar studies [26,34,35]. The higher relative concentration of lutein in the clear mesocosm may be linked to its protective function from photo-oxidation as previously reported for Prasinophyta [36] and Chlorophyta [37]. The average ratios of carotenoids to chl *a*, lutein (0.11) and zeaxanthin (0.06) were comparable to those for similar Prasinophyta [8,38]. The effects of nutrient and light regimes on biomass specific pigment composition in marine phytoplankton cultures isolated from temperate coastal waters have shown that the ratios of chl *b*, fucoxanthin and neoxanthin to chl *a* decreased with increasing irradiance while the ratios of zeaxanthin, violaxanthin, lutein and alloxanthin increased. These latter pigments have been associated to photoprotection [8]. Phytoplankton populations of the upper euphotic waters undergo variable degrees of photoinhibition in relation to their exposition to solar irradiance. In fact, the distribution of phytoplankton communities in the North Atlantic has shown marked differences in photoadaptation and photoacclimatation in relation to different intensities of irradiance, as indicated by ratios of photosynthetic and photoprotective carotenoids to chl *a* [39].

Growth rates and pigment content have been shown to be affected by both light intensity and spectral quality [39–41], in relation to the species specific capacity for light adaptation [42–44]. A reduction in the cellular contents of chlorophylls and accessory pigments with a concomitant increase in photoprotective carotenoids has often been demonstrated in laboratory studies in relation to increases in incident irradiance [27,36,45,46]. The available data also reveal considerable between-species variability [47] which, together with physiological differences between taxa, contribute to their ecological success.

The use of two markedly different light regimes allowed us to explore the tolerance of *T. suecica* to modified irradiance regimes. Changes in growth dynamics under different irradiances and spectral quality have been shown for several algal species. You and Barnett [48] demonstrated an increase in growth rate as well as the production of extracellular polysaccharides with changing spectral intensity for the red algae *Porphyridium cruentum*. Aidar et al. [49] found that *T. gracilis* had a higher growth rate under red light and synthesised more pigments and proteins when incubated under a full visible spectrum. Chemical composition, the patterns and rates of synthesis of proteins, polysaccharides and lipids have been shown to be sensitive to spectral distribution [50]. Growth efficiency of coastal diatoms can change with increased irradiance in the short visible wavelengths, generally linked to an increase in the DNA and RNA synthesis [51]. However this response is not common to all microalgae, even for those of the same family, as the third species of this study [46] showed the highest relative growth efficiency with white light. Similar to our experiment, the increase of pigment concentrations in clear irradiance conditions could be due to a chromatic adaptation, as blue light not only participates in the rearrangement of chloroplasts but also stimulates the synthesis of chlorophyll. Blue-light-enhanced pigment formation has been reported for macroalgae [52], while chromatic adaptation has been shown to occur for diatoms [41] and Cyanobacteria [53].

In the present experiment, a *T. suecica* culture demonstrated a reduced productivity in spectral conditions where lower wavelengths (UVA and short visible) were strongly attenuated. Increased exposure to UV irradiance may be offset by the production of protective-pigments such as lutein and zeaxanthin, which improves *T. suecica* fitness. In other studies with this species at higher irradiances, an increased production of tocopherol was observed [54]. In the present experiment, *T. suecica* was shown to be tolerant to a range of light environments, giving this species an ecological advantage in changing coastal environments and may help to explain its widespread distribution.

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