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Effects of blue light on the biochemical composition and photosynthetic activity of *Isochrysis* sp. (T-iso)

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Abstract:

In aquaculture, particularly in bivalve hatcheries, the biochemical composition of algal diets has a strong influence on larval and post-larval development. Biochemical composition is known to be related to culture conditions, among which light represents a major source of variation. The effects of blue light on biochemical composition and photosynthetic rate of *Isochrysis* sp. (T-iso) CCAP 927/14 were assessed in chemostat at a single irradiance (300 µmol photons m^{-2} s^{-1}) and compared with white light. Two different dilution (renewal) rates were also tested: 0.7 and 0.2 d^{-1}. Relative carbohydrate content was lower under blue light than under white light at both dilution rates, whereas chlorophyll *a* and photosynthesis activity were higher. In contrast, carbon quota was lower and protein content higher under blue light than under white light, but only at 0.7 d^{-1}. Despite these metabolic differences, cell productivity was not significantly affected by the spectrum. However, the nitrogen to carbon ratio and photosynthetic activity were higher at 0.7 d^{-1} than at 0.2 d^{-1}, while carbon quota and carbohydrate content were lower. Our results show that blue light may influence microalgal metabolism without reducing productivity for a given growth rate, a result that should be of great interest for microalgal production in aquaculture.

Keywords: *Isochrysis* ; Blue light ; Photosynthesis ; Proximate composition ; Chemostat

1. Introduction

The applications of microalgae have expanded and diversified in recent years. As these organisms have found uses in a broad range of fields, such as healthcare, cosmetics, environmental management, energy production and aquaculture, microalgal metabolic orientation has become a major concern for the scientific community and industry. Indeed, by the manipulation of metabolic pathways through the modulation of environmental factors or genetic engineering, cellular functions can be redirected toward the synthesis of desired end-products and expand the processing capabilities of microalgae (Rosenberg et al., 2008). In aquaculture, a supply of live microalgae is essential for growth and survival of aquatic species of commercial interest. Cultured shellfish larvae require microalgae in the form of multi-species diets (O' Connor and Heasman, 1997; Rico-Villa et al., 2006), but qualitative specifications of their needs are still lacking. This complicates the task of choosing the appropriate proportions of different microalgal species, and a further level of complexity is introduced by variation in nutritional values between cultures of the same species. Many environmental factors affect the metabolism of cultured microalgae and, thus, their nutritional qualities: temperature (Berges et al., 2002; Chen et al., 2008), nutriment source (Fabregas et al., 1986) and concentration in the medium (Fabregas et al., 1985; Durmaz, 2007), and irradiance (Falkowski et al., 1985; Sukenik and Wahnon, 1991; Anning et al., 2000).
Most studies on the effects of light have focused exclusively on the influence of irradiance, although it has been shown that algal metabolism and growth can be also affected by spectrum (Gostan et al., 1986; Wynne and Rhee, 1986; Rivkin, 1989). Furthermore, spectrum quality is known to influence biochemical composition, pigment content and photosynthesis rate of various species (Voskresenskaya, 1972; Humphrey, 1983; Sanchez-Saavedra and Voltolina, 1996). More specifically, when compared with red or white light, blue light radiation induces a higher production of amino acids and lower carbohydrate content (Wynne and Rhee, 1986; Sanchez-Saavedra and Voltolina, 2006).
In our approach for light effects, the experimental design must avoid any confounding effects between light spectrum distribution and any other factor, particularly irradiance itself. Adequately controlled conditions would be nearly impossible to achieve in batch cultures, where irradiance, ambient nutrient concentration and growth rate continuously vary with time. While most of the previous studies were conducted in batch cultures, here we propose to assess the effect of the light spectrum distribution under continuous-flow cultures where the growth conditions can be more readily and independently controlled. Specifically, chemostat cultures of *Isochrysis* sp. (T-iso) were used to compare the influence of blue and white light on the biochemical composition, photosynthetic activity and growth performances of this microalga at two different dilution (renewal) rates. T-iso is a small Prymnesiophyceae (4-6 μm) commonly used in shellfish hatcheries (Borowitzka, 1997; Brown, 2002), where it is often used as part of a mixed diet with other microalgae such as *Chaetoceros* sp.

2. Materials and methods

2.1. Culture of microalgae

The effects of blue light on biochemical composition and photosynthesis of *Isochrysis* sp. (T-iso) CCAP 927/14 in continuous culture was determined using two 3.5-L photobioreactors (PBR). These PBR were made of two transparent Polymethylmetacrylate (PMMA) columns (60 mm diameter) connected by two flanges (for design reference, see the single module in Loubiere et al. (2009)).
Inocula were pre-acclimated to the experimental spectra for 5 days at 25°C in 2-L glass-flasks containing 1.5 L natural 0.22-μm filtered seawater enriched with 1 mL L^{-1} Conway medium (Walne, 1966). Inocula were continuously aerated and maintained under constant

irradiance from white (OSRAM FQ54W/965HO cool daylight) or blue (OSRAM FQ54W/67HO blue) fluorescent tubes at 100 μmol photons m^{-2} s^{-1}.
PBRs were sterilized with 5‰ peroxyacetic solution for 20 min before use and then filled with pre-acclimated cultures at 10^6 cell mL^{-1} in Conway-enriched sea water (3 mL L^{-1}, in order to prevent nutrient limitation). One culture was conducted for each dilution and light condition. Cultures were thermoregulated at 26±1°C by air conditioning and pH was maintained by automated CO_2 injections at 7.2±0.1 with a pH measurement loop (electrode Inpro 4800/225/PT1000, Mettler Toledo and HPT 63, LTH electronics LTD). Light was continuously delivered by six dimmable fluorescent tubes (blue or white ; for reference, see above), which incident irradiance was set at 300 μmol photons m^{-2} s^{-1} using a dimming device. This amount of irradiance was selected on the basis of the results of Marchetti et al. (2012) who reported that, under 300 μmol photons m^{-2} s^{-1}, growth rate was more than 90% of maximal growth rate and that no photoinhibition occurred. Irradiance and s pectral distribution (Figure 1) were measured outside the PBR, between columns and at middle height of the PBR, using a spherical quantum sensor (LI-250 light meter, LI-COR, 3 m m diameter) and a spectroradiometer (USB 200+, Ocean Optic Inc, equipped with a L2 collection lens ; detector range : 200-1000 nm), respectively. Additionally, attenuation was checked for PMMA for both light sources and did not notably modify the emission spectrum. PBR illuminated with white daylight was considered as a control, while PBR illuminated with blue light was the experimental PBR. In order to evaluate the effect of dilution rate on metabolism under the two light conditions, two dilution rates (0.7 d^{-1} or 0.2 d^{-1}) were applied with a dosing pump (KNF stepdos), for both light conditions and checked daily by weighing the harvested volume.
Cellular concentration was assessed daily by two methods (cell counts and light absorbance measurements) to provide accurate growth monitoring and establish steady state reliably. Cell counting was performed by image analysis (IPS 32, Unilog and microscope Diaplan, 307-148001, Leitz; 3CCD color vision camera module, Donpisha) with Malassez slides and Lugol staining solution. To evaluate chlorophyll *a* (chl *a*) content and cellular concentration, absorbances were measured at 680 and 800 nm, respectively (μQuant spectrophotometer, Bio-tek instruments. Inc). Cultures were assumed to be at steady state when the cellular concentration and absorbances were stable for at least three consecutive days with less than 10% variation. Once at steady state, cultures were sampled daily for biochemical analyses for 3 consecutive days, at least.

2.2. Productivity

For each dilution rate (D), 0.2 and 0.7 d^{-1}, cellular productivity (P_c) was calculated at steady state according to equation 1.

$$P_c = X.D \qquad \text{equation 1}$$

Where X is the steady-state cell concentration (cell mL^{-1}) measured by image analysis.

2.3. Total carbon and nitrogen

At steady state, approximately 100 10^6 cells were filtered through pre-combusted Whatman GF/C glass filters and dried at 70°C for 48 h. Particulate nitrogen (Q_N) and carbon (Q_C) were determined by elemental analysis (EAGER 300, Thermo Scientific, CHN analyzer) and Q_N and Q_C were computed on the basis of the mean cell concentration at steady state (X).

2.4. Total chlorophyll *a*

Chlorophyll *a* (Chl *a*) was determined using 150 10^6 cells previously filtered on a GF/C pre-combusted (450°C) glass-filter. Filter was introduced in a 15mL–tube with 10 mL 90% acetone. Then, filter was grinded and sample was further kept 24 hours in dark at 4°C for complete pigment extraction. Quantification was performed following the Lorenzen (1967) spectrophotometric method.

2.5. Photosynthetic activity

Photosynthetic activity was measured at steady state using a DW3 Oxylab unit (Hansatech, UK) fitted with a Clark electrode disc and a 36 red LED array LH36/2R (λ=660 nm). After electrode calibration, nitrogen was flushed for 1 min into the sample to lower oxygen concentration to 50% of saturation. Oxygen production was measured in 1.3 mL of sample (5 10^6 cells mL^{-1}) mixed with 0.055 mL of a 144 mmol L^{-1} $NaHCO_3$ solution. Then, 20 min-light/10 min-dark cycles were applied, with different levels of irradiance (*I*) ranging from 0 to 361 μmol photons m^{-2} s^{-1} within the sample.
To evaluate photosynthetic activity (*P*) and to take photoinhibition into account, experimental data were fitted with a Haldane model, as modified by Papacek et al. (2010) (equation 2).

$$P = P_m \times \frac{I}{K_s + I + I^2/K_i} - R_d \qquad \text{equation 2}$$

where P_m is the maximal photosynthetic capacity (μmol O_2 chl a^{-1} h^{-1}), K_i the inhibition constant (μmol photons m^{-2} s^{-1}), K_s the half-saturation constant (μmol photons m^{-2} s^{-1}) and R_d the dark respiration, expressed as an oxygen consumption (μmol O_2 chl a^{-1} h^{-1}).
Light-saturated photosynthetic rate P_{max} (μmol O_2 chl a^{-1} h^{-1}), initial slope of the *P-I* curve at limiting irradiance α (μmol O_2 chl a^{-1} h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$), light saturation index I_k (μmol photons m^{-2} s^{-1}), light saturation irradiance I_s, and compensation irradiance I_c were then calculated according to equations 3 to 7 (Papacek et al., 2010).

$$P_{max} = \frac{P_m}{2\sqrt{K_s K_i} + 1} - R_d \qquad \text{equation 3}$$

$$I_S = \sqrt{K_s K_i} \qquad \text{equation 4}$$

$$I_C = \frac{P_m - R_d + \sqrt{(R_d - P_m)^2 - (2R_d\sqrt{K_s K_i})^2}}{2R_d/K_i} \qquad \text{equation 5}$$

$$\alpha = R_d / I_C \qquad \text{equation 6}$$

$$I_k = \frac{P_m - R_d}{\alpha} \qquad \text{equation 7}$$

2.6. Total carbohydrates

At steady state, 60 10^6 cells were sampled and carbohydrate content was analyzed according to the sulfuric acid colorimetric method (Dubois et al., 1956), based on phenolphthalein absorbance at 490 nm.

2.7. Total proteins

Protein extraction was more complex than expected due to T-iso pigment content. A new method for protein evaluation was therefore used, based on the protocol of Barbarino and Lourenço (2005), whereby samples (60 10^6 cells) were first centrifuged at 2,500 *g* for 20 min (5°C) to remove the culture medium and then stored at -20°C.
To collect total cellular protein content, cells had to be broken: 0.5 mL ultra-pure water and up to 2% SDS were added to each defrosted sample, which was then sonicated for 10 min in an iced bath. After a 10-min centrifugation step at 15,000 *g* (5°C), the proteins in the supernatant were collected and stored at 4°C for 12 h.
Proteins were then precipitated by the addition of 100% acetone (2.5:1, v:v), sonicated for 30 min in an iced bath, and collected following re-centrifugation and elimination of the supernatant. The proteins were then rinsed with 70% acetone (2.5:1, v:v) and centrifuged for 2 min at 15,000 *g* (5°C) followed by supernatant elimination. The proteins were finally solubilized in 0.5 mL of ultra-pure water.
Solubilized proteins were quantified with a BCA protein assay kit (Pierce) based on alkaline copper colorimetric quantification at 562 nm (Lowry et al., 1951).

2.8. Total lipids

Samples of 300 10^6 cells were filtered through 450°C pre-combusted GF/C glass-filters (Whatman, diameter 47 mm). Lipids were extracted with 6 mL chloroform-methanol mix (2:1 v/v), following Folch et al. (1957), and stored at -20°C under nitrogen. The Bligh and Dyer protocol (1959) was used, in which, dichloromethane and ethanol were replaced by chloroform and methanol, respectively, in the same solvent-mixing proportions. Hence, 1 mL $CHCl_3$ and 0.9 mL water were added to 1 mL of Folch extract. After centrifugation, the organic phase was recovered. The water/methanol phase was rinsed twice with 1 mL $CHCl_3$ in order to collect the residual organic phases, which were then added to the previous one. The whole collected organic phase was then totally dried under nitrogen flow and stored in a desiccator for 24-48 h. Lipids were weighed to 0.01 mg precision.

2.9. Statistical analysis

Following an examination of homogeneity of variance and normal distribution, a multi-factor analysis of variance (5% significance level) was used to assess effects of light quality (white and blue) and dilution rate (D = 0.2 and 0.7 d^{-1}) treatments, as well as interactions. Then, a Fisher's least significant difference (LSD) procedure was run to determine which of the experimental conditions were significantly different. To compare chlorophyll *a* content and biochemical composition on per-carbon and per-cell bases, linear least square regressions were performed with Statgraphics centurion XV software. The same procedure was used for comparison of photosynthesis normalized to chlorophyll *a*, carbon or cells. The correlation coefficient, *p*-value and number of observations were calculated for each regression analysis.

3. Results

As shown in Figure 2, cell concentration mean was significantly affected (Table 1) by dilution rate *D* and was 1.5 fold lower at 0.7 d^{-1} than at 0.2 d^{-1} (32 *vs* 48 10^6 cell mL^{-1}). In contrast, light quality did not significantly influence cell concentration (Table 1).
This difference in cell concentration resulted in a 2.5-fold increase in productivity at 0.7 d^{-1} compared with 0.2 d^{-1} (data not shown: 22 *vs* 10 10^9 cell L^{-1} d^{-1}). Indeed, despite the lower cell concentration, productivity was higher at 0.7 d^{-1} because of the increased volume collected. In contrast, productivity remained unaffected by light spectrum.

Cellular carbon quota Q_C was significantly enhanced at high *D* (0.7 d^{-1}), as shown in Figure 3a. Thus, Q_C was 508 fmol C $cell^{-1}$ and 673 fmol C $cell^{-1}$ for 0.2 and 0.7 d^{-1}, respectively, under white light, while it was 507 fmol C $cell^{-1}$ and 563 fmol C $cell^{-1}$ under blue light. Additionally a significant interaction was found between spectrum and dilution resulting in Q_C significantly lower under blue light than under white light at 0.7 d^{-1} (Table 1).
Nitrogen cell quota Q_N (Figure 3b) was significantly enhanced at 0.7 d^{-1} (32 fmol N $cell^{-1}$ at 0.2 d^{-1} and 80 fmol N $cell^{-1}$ at 0.7 d^{-1}) but remained unaffected by light quality (Table 1: $p>0.05$). Conversely, when nitrogen quota was expressed per carbon unit (q_N) (Figure 3c), the same significant effect was recorded for *D* (0.063 mol N mol C^{-1} at 0.2 d^{-1} and twice as much at 0.7 d^{-1}) as well as a significant effect for light quality (Table 1) under the high dilution rate, as a consequence of carbon quota enhancement under the same conditions. A significant interaction demonstrated that spectrum induced differences for q_N were significantly increased under the high dilution rate .

Chlorophyll a per carbon unit (chl a:C) was significantly lower under blue light than under white light (Figure 4a). Again, a significant interaction (Table 1) was found for Chl a:C : indeed, Chl a:C was only 60% of the value obtained under white light at 0.2 d^{-1} and this difference was reduced at 0.7 d^{-1}. In contrast, when normalized to a per-cell basis (Figure 4b), chlorophyll a was significantly affected by light quality only.

Regardless of the calculation basis (carbon, cell or chlorophyll *a*), the same overall trends were recorded for photosynthesis. Therefore, only the chlorophyll *a*-normalized results are discussed hereafter.
In general, photosynthetic activity increased with *D*, though the difference was more marked for cultures grown under blue light (Figure 5).
Datasets of photosynthesis on irradiance were fitted with the Haldane model (equation 2) and the resulting parameters are given in Table 2. R_d, P_{max} and α increased with *D* while I_c decreased by a factor of 3. Increase in dilution rate resulted in striking enhancements in P_{max} and α, which were more than 10 fold and 3.8 fold higher, respectively, at 0.7 d^{-1} compared with 0.2 d^{-1}.
Only R_d and P_{max} were significantly affected by light quality, while other parameters were not modified. We recorded a significant interaction for P_{max} , reflecting that P_{max} was more sensitive to *D* under blue light. Besides, photoinhibition was more pronounced under blue light (Figure 5) and this was confirmed by the low K_i recorded under blue light at 0.7 d^{-1} (Table 2).

Biochemical composition is presented both on a per-cell basis (pg $cell^{-1}$) and on a per-carbon basis (pg pg C^{-1}) in Table 3. A significant linear correlation was found between the two calculation basis used for the biochemical composition (lipids: $p<0.05$, $r=0.65$, $n=12$;

carbohydrates: $p<0.01$, $r=0.92$, $n=12$; proteins: $p<0.01$, $r=0.89$ and $n=12$); therefore, all subsequent analyses are presented on a per-carbon basis only.
Carbohydrate content was significantly affected by *D* (Table 3), being reduced by more than 40 % at high *D* (0.7 d^{-1}) under both blue (from 0.85 to 0.47 g gC^{-1}) and white light (from 1.08 to 0.64 g gC^{-1}). Light quality also significantly influenced carbohydrate content, since it was depressed under blue light for both dilution rates (from 1.08 to 0.85 g g C^{-1} at D=0.2 d^{-1} and from 0.64 to 0.47 g g C^{-1} at D=0.7 d^{-1}). No significant interaction was found for carbohydrate. This reflected that light spectrum and dilution rate acted independently on carbohydrate content.
When expressed on a per carbon basis, protein content was not significantly affected by *D,* while a s ignificant difference was recorded for protein content per cell (Table 3). This discrepancy may result from higher SD for protein on a per carbon basis. Protein content on a per carbon basis was significantly affected by light spectrum, being higher under blue light. A significant interaction was also found for protein on a per carbon basis, resulting in higher protein content under high *D* and blue light.
Finally, neither *D* nor light quality had a significant effect on lipid content (Table 3).

Thus, relative carbohydrate content (percentage of the total proximate composition) decreased strongly with *D*. This resulted in a 6% increase in relative lipid content for both light qualities. A 10% increase in relative protein content was recorded under blue light, while the increase was only 2% under white light (Figure 6).
In summary, protein was the least abundant biochemical component in T-iso (roughly 20%) under all conditions. For the high dilution rate under blue light (Table 3, and Figure 6), protein content increased to 34% of the total proximate composition, whereas carbohydrate content decreased to 22%.

4. Discussion

In the present study, potential changes of biochemical composition and photosynthetic activity of T-iso were investigated at steady state under different qualities of light (blue or white) and at two dilution rates (0.2 and 0.7 d^{-1}). The effects of the two factors and their interactions are discussed hereafter. Indeed interaction between spectrum and dilution rate were significant for the maximum photosynthetic rate as well as for most carbon based quotas. It is stressed, however, that the interactions resulted in different response intensities but not in reversal trends.

4.1. Growth performances

Under both light spectra, productivity increased with *D*. Since productivity depends on algal species and light attenuation, *i.e.,* geometry of PBR (Fernandes et al. 2010), and particularly surface to volume ratio (Yongmanitchai and Ward, 1991), it was difficult to compare our results with most earlier studies because these had been done on other species. However, Loubière *et al*. (2009), who carried out their experiments with T-iso in a similar PBR, reported a hyperbolic function for volumetric productivity according to *D* under daylight, with an optimal value close to 0.84 d^{-1} (column diameter D=0.06 m). Our results are therefore consistent with those of Loubière et al. (2009) as we found productivity to be an increasing function of *D*, the higher volume collected compensating for the lower cell concentration in the range we investigated.
In contrast, cellular concentration and productivity did not vary significantly with spectrum quality at steady state. Biomass production was therefore unaffected by the use of blue light, as already reported for a number of different diatoms (Wallen and Geen, 1971; Gostan et al., 1986; Aidar et al., 1994; Mercado et al., 2004), *Tetraselmis gracilis* (Aidar et al., 1994) and

Dunaliella tertiolecta (Wallen and Geen, 1971). Although these studies were carried out in batch culture where light changed continuously during growth, preventing rigorous light measurement, similar biomasses were produced under blue and white light.

4.2. Proximate composition

In the following, carbon and nitrogen quota are discussed through q_N, the nitrogen quota on a per-carbon basis. The range of variation we found for q_N was consistent with previous results recorded for *Isochrysis galbana*, where q_N ranged from 0.10 to 0.08 mole N mole C^{-1} in batch cultures (Fidalgo et al., 1998).

In the present study, q_N increased with D, slightly more under blue light than under white light (0.06 at 0.2 d^{-1} for both spectra and 0.12 and 0.15 at 0.7 d^{-1} under white and blue light, respectively). These trends were difficult to compare with other studies, since most that dealt with q_N concerned nutrient-limited (Chalup and Laws, 1990; Elrifi and Turpin, 1985; Sakshaug et al., 1989; Terry, 1980; Terry et al., 1985). Increase for q_N resulted from the differential increase in Q_N and Q_C. Indeed, we recorded that Q_C also increased with D, mainly under white light. This result has already been reported by Laws and Bannister (1980) for *Thalassiosira fluviatilis* under light limitation, where a linear relationship was found. We can assume that, since cultures were light-limited in the present study, increase in D (*i.e.* increase in light availability) resulted in higher C fixation rate, and higher Q_C. This assumption was further confirmed by increased P_{max} under high D. It is unclear why increase in Q_C was lower under blue light, as highlighted by the significant interaction (Table 1), although P_{max} was higher. The apparent discrepancy could be solved assuming that stocked Carbon was utilized to support the higher metabolic rate under blue light, as suggested by the lower carbohydrate content.

Changes in q_N were consistent with the biochemical alteration we recorded. Under white light, q_N increased with D, while the relative amount of carbohydrate, the main energy storage material in T-iso (Sukenik and Wahnon, 1991), decreased with D. Similarly, q_N increased with D under blue light and was closely related to protein enhancement and a concomitant reduction in carbohydrate, whereas lipids were unchanged. These results are in agreement with observations reported elsewhere for higher plants (Voskresenskaya, 1972), macroalgae (Korbee et al., 2005), diatoms (Gostan et al., 1986; Sanchez-Saavedra and Voltolina, 1994) and other marine phytoplanktonic species (Wynne and Rhee, 1986; Rivkin, 1989). Decrease in carbohydrate content under blue light was already reported by Sanchez-Saavedra and Voltolina (1994) for *Chaetoceros sp.* Besides, Rivkin (1989) observed in *Dunaliella tertiolecta* and *Thalassiosira rotula*, that blue light allows higher photosynthetic carbon incorporation into protein than white light. According to Zhou et al. (2009), the protein increase under blue light could be related to the enhancement of the light collection system, *i.e.,* the structural protein of PSII (Miyachi et al., 1978). However, this phenomenon has not been conclusively demonstrated and must be treated with caution.

The changes in proximate composition we recorded here agree with earlier studies performed in semi-continuous cultures at different D (Chrismadha and Borowitzka, 1994 ; Fabregas et al., 1996 ; Otero et al., 1997), although light-limited cultures have rarely been used to measure the effect of D on biochemical composition. Other studies using light-limited cultures reported conflicting results on the influence of irradiance on biochemical composition of different microalgae: carbohydrate content strongly increased with irradiance in *Chaetoceros protuberans* (Gostan et al., 1986; Rivkin, 1989), but decreased in *Thalassiosira rotula* and *Dunaliella tertiolecta* (Rivkin 1989); in T-iso, protein content could be negatively affected by irradiance (Rivkin, 1989) or not at all (Sukenik et al., 1990). However, these differing modifications in biochemical composition in relation to irradiance could be interpreted as being due to (1) species-dependent photo-acclimation, as already demonstrated by (Falkowski et al., 1985; Dubinsky and Stambler, 2009)), and/or (2) the

different bases used for computation of the biochemical composition (per *C*, per cell or as a percentage of dry weight).

4.3. Photosynthesis activity

In the opposite way to q_N, a decreasing *chla:C* ratio was observed under white light when *D* was higher, *i.e.,* with increasing light availability, as it has already been reported by other authors (Laws and Bannister, 1980; Rivkin, 1989 ; Anning et al., 2000; Dubinsky and Stambler, 2009). Indeed, under low irradiance, the light collection apparatus (mainly *chla:C*) is enhanced, as previously reported in *Phaeodactylum tricornutum* and *Skeletonema costatum* (Beardall and Morris, 1976; Anning et al., 2000). However, no significant change could be recorded for *chla:C* under blue light. This difference between the effects of the two light spectra was already reported for *Thalassiosira rotula* and *Dunaliella tertiolecta* by Rivkin (1989), who recorded a smaller difference in *chla:C* ratio in relation to light availability under blue light than under white light. In the present work, we found that chl *a* content (per cell and per C) was higher under white light than under blue light, which contrasts with some previous studies (Wynne and Rhee, 1986; Rivkin, 1989 ; Sanchez-Saavedra and Voltolina, 1994 ; Mercado et al., 2004). However, Aidar et al. (1994) reported that blue light resulted in chlorophyll *a* decrease in *Tetraselmis gracilis*, while it remained constant in *Thalassiosira gravida* and *Phaeodactylum tricornutum* (Holdsworth (1985). As far as we know, there is no consensus on chl *a* content behaviour among microalgal species with respect to their potential for chromatic adaptation.
Results associated to PI curves demonstrated that under high *D*, the irradiance amount used in our experiment was photoinhibiting. It is unclear why photoinhibition occurred here for irradiance higher than 150 μmol photons m^{-2} s^{-1}, since previous study demonstrated that photoinhibition did not occur for irradiance as high as 300 μmol photons m^{-2} s^{-1} (Marchetti et al., 2012). However, direct comparison between these two experiments suffers from methodological differences (such as different light spectra, long-term turbidostat versus short-term photosynthesis experiments) that may give rise to inconsistencies. Nevertheless, from the results reported here, we may assume that reducing irradiance to 150 μmol photons m^{-2} s^{-1} would increase productivity as well as protein content. Indeed, Terry et al (1983) reported that photoinhibiting conditions decreased synthesis rate for protein in

4.4. Phaeodactylum tricornutum

Photosynthetic activity increased with *D*, revealing a higher carbon fixation rate at higher dilution. Photosynthetic parameters showed different trends with *D*. First, P_{max} was enhanced at high *D,* as previously reported for *Skeletonema costatum* (Anning et al., 2000). Since light availability and *D* are closely related in light-limited chemostat, this result is consistent with the report by (Dubinsky and Stambler, 2009) that P_{max} increases under bright light in correlation with the decreasing quantity of photosynthetic units.
On the other hand, we recorded a negative correlation between chl *a* specific photosynthesis efficiency (α) and *chla:C*. Increase in α was due to variation in both I_c and *Rd*. Indeed, I_c decrease with increasing *D* was concomitant with *Rd* enhancement. The sensitivity of *Rd* to *D* has already been shown in several studies, with a positive correlation between R_d and *D* at steady state for light-limited cultures (Laws and Bannister, 1980; Falkowski et al., 1985; Anning et al., 2000). The increase of *Rd* and the parallel decrease in carbohydrate content is thus consistent with the use of carbohydrate as energy to support high growth rates.
A general enhancement in photosynthetic activity was recorded when microalgae were exposed to the blue spectrum. The highest P_{max} was recorded under blue light, as already reported for some other marine microalgae (Wallen and Geen, 1971; Humphrey, 1983; Vogel and Sager, 1985), indicating that cultures grown under blue light were more

photosynthetically active at saturating irradiance. According to Voskresenskaya (1972), this might result from activation of photosynthetic electron transfer chain reactions and a high activity of ribulose-1,5-diphosphate carboxylase (Rubisco). Indeed, the increase in Rubisco synthesis under blue light has already been shown in plants and microorganisms (Senger, 1982), but also in green algae (Roscher and Zetsche, 1986) where the light promoter effect was confirmed for wavelengths ranging from 430 to 510 nm and the maximal effect was at 460 nm. Blue light also increased the amount of mRNA for the large and small subunits of this enzyme (Roscher and Zetsche, 1986) and nitrate-reductase activity (Figueroa et al., 1995), explaining the higher protein and nitrogen quota reported here.
In the literature, modeling of the *P* on *I* curve often relies on the Michaelis-Menten model, where the initial slope α is computed from the ratio of P_{max} to I_k (Aidar et al., 1994; Anning et al., 2000; Dubinsky and Stambler, 2009). Using this modeling approach, computation of α from our data set resulted in higher α under blue relative to white light, as already reported in *Scenedesmus obliqus* (Brinkmann and Senger, 1978), *Prorocentrum mariae lebouriae* (Vogel and Sager, 1985), *Dunaliella tertiolecta* and *Cyclotella nana* (Wallen and Geen, 1971). Conversely, use of the Haldane model (equation 2) did not reveal any significant effect of spectrum on α. According to equation 6, this result is in contradiction with the constancy of I_c and the increase in R_d under blue light. However, it should be stressed that α increased significantly in blue light at 0.7 d^{-1} (Table 2). Hence, the lack of an overall significant effect of spectrum on α might reflect the strongly reduced photosynthetic activity at 0.2 d^{-1} and the resulting lack of precision in α assessment. An increased number of observations in the subsaturating region would have circumvented this issue.
Thus, in the present study, P_{max} and α were enhanced by blue light at 0.7 d^{-1} while I_k was not affected, showing the "I_k-independent" variability of the *P vs I* curve for T-iso (Behrenfeld et al., 2004). Physiological bases of this phenomenon are poorly known but appear to result from growth-rate-dependent variability in the metabolic processing of photosynthetically generated reducing agents (Behrenfeld et al., 2004). Blue light also enhanced R_d under blue light, as previously reported for *Scenedesmus obliquus* (Brinkmann and Senger, 1978), *Rhodomonas salina* (Hammer et al., 2002) or *Dunaliella tertiolecta* and *Thalassiosira rotula* (Rivkin, 1989), confirming a higher rate of carbohydrate degradation in blue light.
In summary, blue light resulted in an enhancement of photosynthetic activity as well as an alteration in carbon metabolism in close relation with an increase in protein synthesis. Newly-synthesized proteins that allow high growth rate and favour photosynthetic structures could be involved in enzyme production (Senger, 1982; Mercado et al., 2004).

4.5. Implications for mollusk feeding

In spite of numerous studies on mollusk requirements, optimal biochemical composition of microalgae required for improving mollusks rearing is still unclear (Brown et al., 1989; Knauer et al., 1999). However, requirement of mollusk larvae for proteins seems to be higher relative to that required by adults. In addition, protein-enriched microalgae (30-60% of cell weight) seem to meet requirement for larvae (Brown et al., 1989; Utting, 1986). Therefore, we assume that protein increase in T-iso, resulting from a high *D* under blue light, will be appropriate for feeding mollusks, especially larvae. In order to increase rearing performance, aquaculturist faces two possibilities : first, a high cell concentration with a low protein content and, second, a lower cell concentration but protein-enriched. It was previously shown that larval ingestion rate was an hyperbolic function of the phytoplankton density, with a maximum ingestion rate of 50 10^3 cells $larvae^{-1}$ d^{-1} for a density of 25 phytopklankton cells mL^{-1} (Rico-Villa et al., 2009). Hence, we may assume that the maximum density of protein-enriched cells, should favour larval rearing. Further experiments on larvae remain to be carried out in order to test this assumption.

Conclusions

The effects of light quality and dilution rate on the biochemical composition of T-iso were assessed at steady state. Spectrum quality did not alter T-iso productivity, but resulted in metabolic changes. Indeed, under blue light, chlorophyll *a*-specific photosynthetic activity and respiration were enhanced, resulting in higher carbon fixation rates, with photosynthates preferentially incorporated into proteins. Protein synthesis was consequently enhanced at the expense of carbon storage compounds.
Growing T-iso at high *D* resulted in enhanced productivity, with biomass containing more protein and less carbohydrate than at low *D*.

Different combinations of light quality and *D* resulted could lead to a 3-fold increase in the carbohydrate to protein ratio. This large change in biochemical composition could be a starting point for future studies aiming to examine the nutritional needs of mollusks, since the nutritional value of microalgae is a key point for larval development and survival (Brown et al., 1993) .

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Figures

Figure 1

Figure 1 White and blue light spectra of the fluorescent sources used during the experiment. Blue light (solid line) showed higher photon counts in the range 420-500 nm, while white light source (dashed line) mainly emitted radiation below 580 nm.

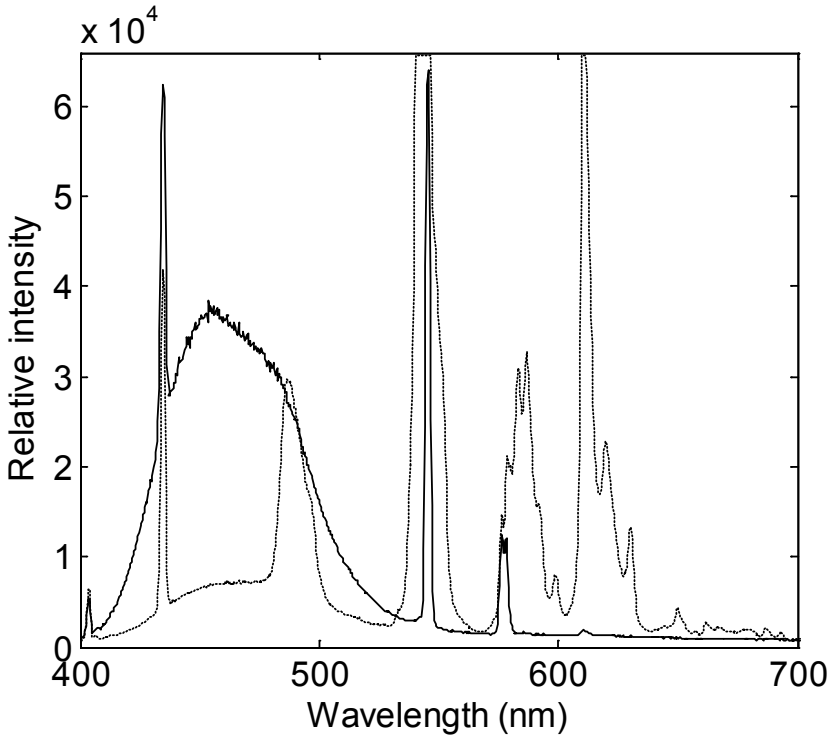


Figure 2

Figure 2 Cell concentration as a function of dilution rate D (0.2 or 0.7 d^{-1}) and spectrum quality. White bars : white light ; grey bars : blue light.

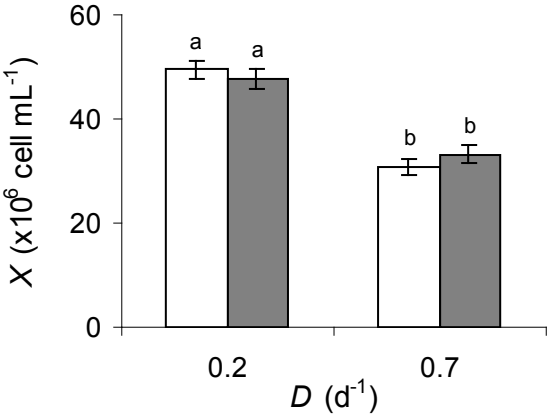


Figure 3

Figure 3 Cellular carbon quota Q_C (a), cellular nitrogen quota Q_N (b) and nitrogen quota per carbon q_N (c) as a function of D (0.7 or 0.2 d^{-1}) and spectrum quality. White bars : white light ; grey bars : blue light. Values with the same letter are not significantly different (p>0.05).

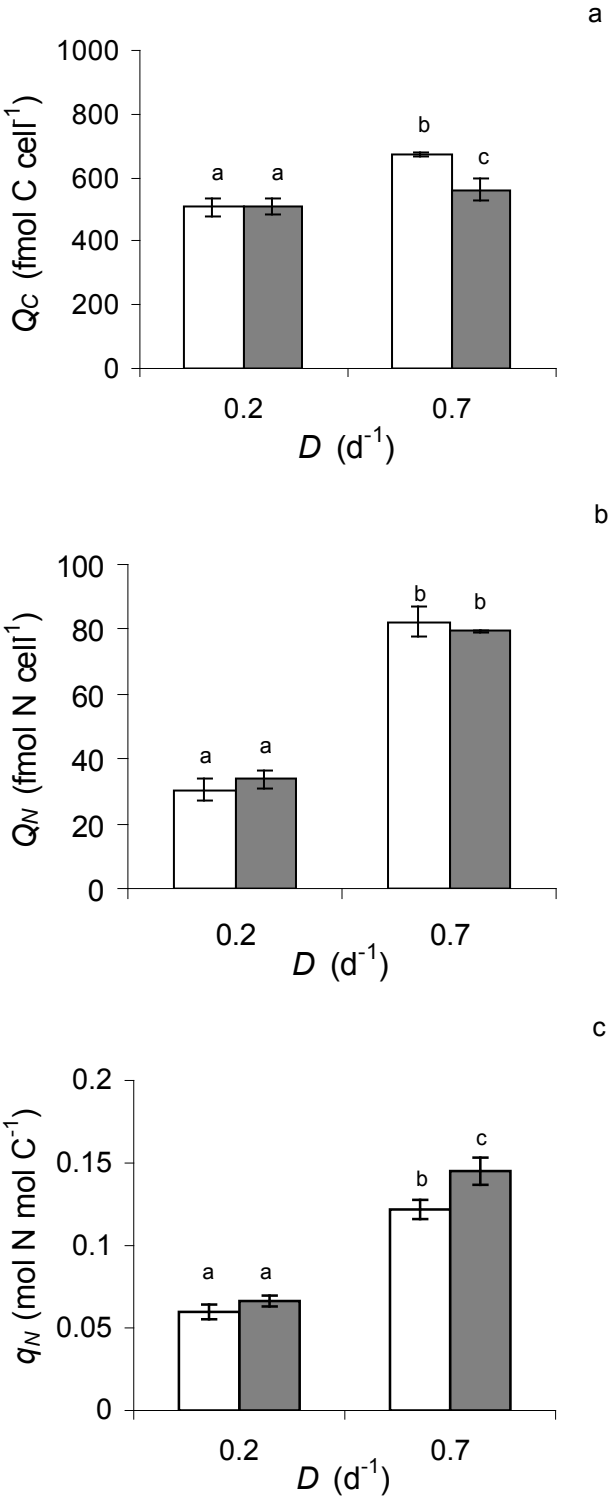


Figure 4

Figure 4 Chlorophyll *a* content expressed on a per-carbon basis (a) or on a per-cell basis (b) as a function of *D* (0.7 or 0.2 d^{-1}) and spectrum quality (white or blue). White bars : white light ; grey bars : blue light. Values with the same letter are not significantly different ($p>0.05$).

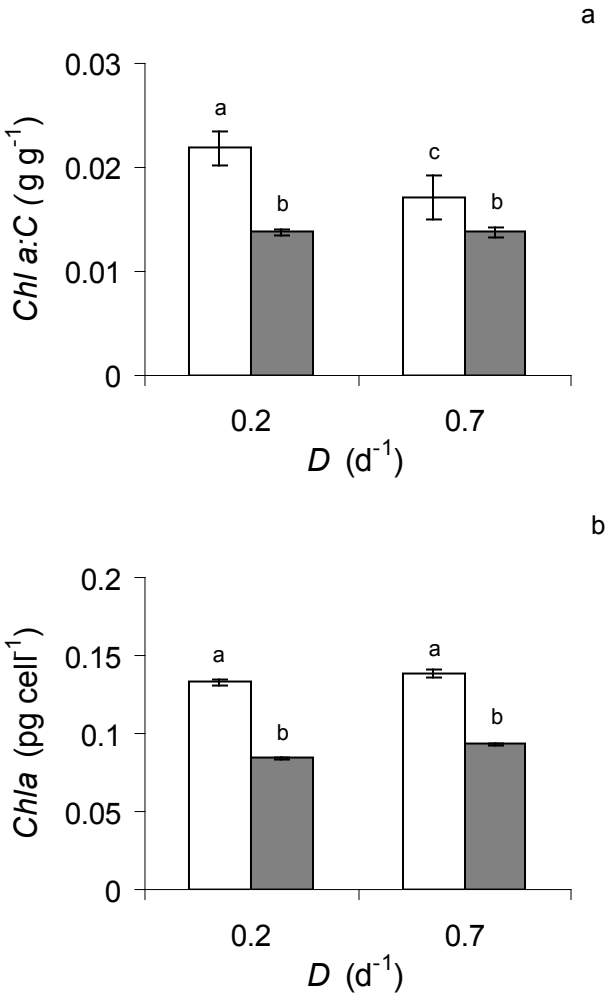


Figure 5

Figure 5 Photosynthetic activity (µmol O_2 mg chl a^{-1} h^{-1}) for the four conditions as a function of irradiance. Data points and error bars represent mean values and standard errors of three independent replicates. Solid and dotted lines represent the fitting of the Haldane model (equation 3) to experimental data for blue and white light, respectively.

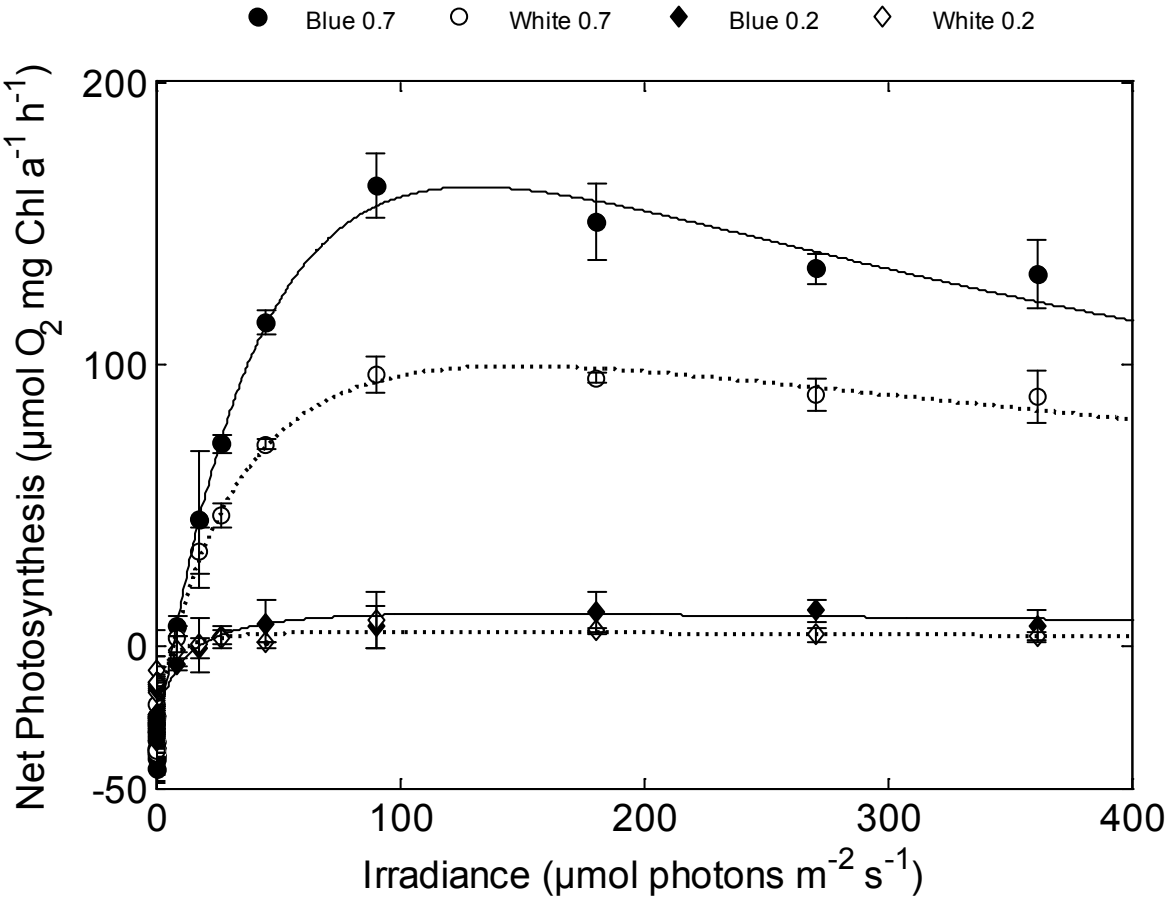
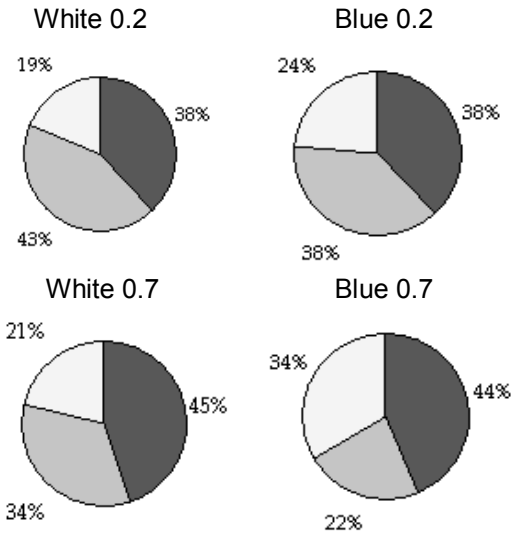


Figure 6

Figure 6 Relative gross biochemical composition of T-iso as a function of *D* (0.2 or 0.7 d^{-1}) and spectrum quality (blue or white). Dark grey : lipids ; light grey : carbohydrates ; white : proteins.



Tables

Table 1 Multifactor ANOVA for cell concentration, cellular quota and chlorophyll *a* content. Probabilities (p) are given for α=0.05; numbers in bold type denote a significant effect of the corresponding factor.

		X	Q_C	Q_N	N :C	Chl *a*:C
D (d^{-1})	p (α=0.05)	**0.0000**	**0.0001**	**0.0000**	**0.0000**	**0.0143**
Spectrum	p (α=0.05)	0.7043	**0.0051**	0.9271	**0.0041**	**0.0001**
Interaction	p (α=0.05)	0.0830	**0.0057**	0.1855	**0.00432**	**0.0157**

Table 2 Estimated parameters (K_s, K_i, R_d and P_m) from Haldane modeling for the four experimental conditions. P_{max}, α, E_k, I_s and I_c were computed according to equations 14 to 18. Standard deviations are presented in brackets. A multifactor ANOVA analysis provided the probabilities (p) for $\alpha = 0.05$. Numbers in bold type denote a significant effect for the corresponding factor.

D (d^{-1})	Spectrum	K_s	K_i	R_d	P_m	P_{max}	α	I_k	I_s	I_c
0.2	B	16 (15)	6213[2] (5069)	27 (2)	49 (17)	12 (6)	1.4 (0.6)	58 (21)	211 (88)	21 (8)
	W	11 (5)	1317[2] (692)	13 (3)	23 (1)	6 (4)	1.1 (0.9)	49 (32)	120 (56)	19 (14)
0.7	B	74 (37)	279 (97)	36 (4)	422 (136)	163 (14)	5.4 (0.7)	88 (40)	135 (5)	7 (1)
	W	45 (17)	819[2] (781)	29 (6)	204 (31)	100 (2)	4.2 (0.9)	59 (17)	164 (39)	7(1)
Multifactor ANOVA										
D (d^{-1})	p($\alpha = 0.05$)	**0.0061**	0.0634	**0.0009**	**0.0001**	**0.0000**	**0.0001**	0.2716	0.6474	**0.0239**
Spectrum	p($\alpha = 0.05$)	0.2036	0.1827	**0.0022**	**0.0171**	**0.0001**	0.1313	0.2914	0.3601	0.8709
Interaction	p($\alpha = 0.05$)	0.3518	0.1063	0.2311	**0.0454**	**0.0002**	0.3339	0.5486	0.1017	0.8500

[1] μmol photons m^{-2} s^{-1}; [2] μmol O_2. mg chl a^{-1} h^{-1} ; [3] μmol O_2 mg chl a^{-1} h^{-1} (μmol photons m^{-2} s^{-1})

[2] high values for K_i indicate that no photoinhibition occured. In these cases a Monod model would advantageously replace the Haldane formulation

Table 3 Gross composition of T-iso as a function of dilution rate (0.2 and 0.7 d^{-1}) and light spectral quality (blue, B, and white, W). Results are expressed on a per-cell and a per-carbon basis and standard deviations given in brackets. Numbers in bold type denote a significant effect for the corresponding factor.

		pg $cell^{-1}$			pg pg C^{-1}		
D (d^{-1})	Spectrum	Lipids	Carbohyd.	Proteins	Lipids	Carbohyd.	Proteins
0.2	B	5.19 (0.30)	5.16 (0.57)	3.25 (0.14)	0.85 (0.05)	0.85 (0.11)	0.53 (0.04)
	W	5.81 (0.50)	6.58 (0.17)	2.86 (0.13)	0.95 (0.07)	1.08 (0.06)	0.47 (0.03)
0.7	B	6.26 (1.30)	3.19 (0.33)	4.81 (0.40)	0.92 (0.14)	0.47 (0.03)	0.72 (0.10)
	W	6.74 (1.28)	5.15 (0.40)	3.23 (0.47)	0.83 (0.16)	0.64 (0.06)	0.40 (0.06)
Multifactor ANOVA							
D (d^{-1})	p (α=0.05)	0.1066	**0.0001**	**0.0020**	0.7243	**0.0000**	0.2132
Spectrum	p (α=0.05)	0.3509	**0.0001**	**0.0017**	0.9323	**0.0017**	**0.0014**
Interaction	p (α=0.05)	0.9073	0.3041	**0.0240**	0.2023	0.4772	**0.0134**

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