

Effect of light quality on the growth and proximal composition of *Amphora* sp.

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Received: 6 August 2016 / Revised and accepted: 30 November 2016 / Published online: 17 December 2016
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Abstract We determined the effects of various light spectra (white, blue, green, yellow, and Grolux) on the growth rate, proximate composition, pigment content, and cell size of the marine benthic diatom *Amphora* sp. during two growth phases of batch cultures. The growth rate was higher under green light and lowest with Grolux and yellow light. Cell size differed significantly between growth phases but remained unaffected by light spectra; the smallest cells were observed on the initial day of culture, whereas larger cells developed in the stationary phase under Grolux treatment. The proximate composition was modified by the light spectra and growth phase. In the exponential growth phase, the protein content was higher with yellow and white light, and lipid content peaked with Grolux. The pigment content (chlorophyll *a*, carotenoids) was also higher under yellow light. In the stationary growth phase, we noted a higher carbohydrate content under Grolux and yellow light. Our results show that light spectra and growth phase influence the metabolism of *Amphora* sp., changing its proximate composition, pigment content, growth rate, and cell size.

Keywords *Amphora* sp. · Diatom · Light spectra · Proximate composition · Growth rate

Introduction

One of the major applications of microalgae cultures is in aquaculture, in which they are used as feed for zooplankton,

crustaceans (larval stages), fish (larval stages), and mollusks (all life stages) (Yoshioka et al. 2012; Welladsen et al. 2014). Benthic diatoms are usually supplied as feed for sea urchin larvae (Lawrence 2006; Carboni et al. 2012), abalone postlarvae (*Haliotis* spp.) (Correa-Reyes et al. 2001; Simental-Trinidad et al. 2001; Mercado et al. 2004), and other organisms. The growth and survival of abalone postlarvae depend on the nutritional value of the diatoms (Daume et al. 2003; Correa-Reyes et al. 2009; Sánchez-Saavedra 2013).

The benthic diatom *Amphora* sp. is commonly ingested by abalone under natural conditions (Siqueiros-Beltrones 2002; Siqueiros-Beltrones and Argumedo-Hernández 2015). *Amphora* sp. cells can live as solitary organisms or in biofilms (Roberts et al. 2000), have low mobility (Hudon and Bourgetm 1983), and produce extracellular polymeric substances (EPSs)—primarily containing fucose and galactose (Zhang et al. 2008)—that act as inductors of abalone larval settlement (Kawamura et al. 1998; Kawamura and Takami 1995). The production of microalgae for the aquaculture industry has been expanding continuously and now represents 40 to 50% of total operation costs (Heasman et al. 2000; Ponis et al. 2008). The mass production of live microalgae cells is the main bottleneck for the culture of several species (Coutteau and Sorgeloos 1992; Borowitzka 1997), and there is a need to optimize the culture technology to increase their production on biomass and their nutritional value.

Biomass production by microalgae cultures and their composition are influenced by several environmental variables (Muller-Feuga et al. 2007). The most significant factors of the growth of microalgae is light, the spectral characteristics and quantity of which can affect their photosynthesis, growth rate, and composition of cellular components, thus affecting their nutritional value for use in aquaculture (Sánchez-Saavedra and Voltolina 1996; Mercado et al. 2004; Muller-Feuga et al. 2007; Dubinsky and Stambler 2009). The effects

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of blue light have been studied extensively; for various microalgae species, blue light usually accelerates their growth, alters their carbon metabolism, and increases protein synthesis (Marchetti et al. 2013; Sánchez-Saavedra et al. 2016). The growth phase of microalgae cultures can also alter their proximate composition as the high quantity of nutrients and light availability in exponential growth phase increases protein content, while in stationary growth the main components are carbohydrate and lipid, which are storage reserves produced by nitrogen or light limitation (Brown 2002; Muller-Feuga et al. 2007; Sánchez-Saavedra et al. 2016). In the aquaculture industry, the use of microalgae cells that are harvested in different growth phases implies modifications of the proximate composition, considering the different times required for the maintenance of the cultures and the impact on economic cost of the production of microalgae.

We hypothesized that the manipulation of spectral properties of light can be used to alter and enhance the metabolism and biochemical composition of *Amphora* sp. cultures for further application in aquaculture.

Materials and methods

Growth conditions

The diatom *Amphora* sp. was isolated from the coastal area of Mulegé Baja California Sur, México (26°38'22.13"N and 111°50'13.92"W) (Jiménez-Valera and Sánchez-Saavedra 2016), and obtained from the algae collection of the Laboratory of Microalgae, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE) Baja California, México. Nonaxenic and monospecies batch cultures were maintained in triplicate under various light spectra in 1000-mL Erlenmeyer flasks with 700 mL of "f" medium in seawater (Guillard and Ryther 1962). The medium was prepared with seawater that had been filtered and sterilized at 121 °C and 1.05 kg cm⁻².

The culture conditions were as follows: temperature 20 ± 1 °C, salinity 33 ± 1‰, daily manual stirring, and pH between 7.8 and 8.9. Light with various spectral characteristics was supplied continuously to the cultures (24 h) at several energy levels by 40-watt white (Philips F40T12/DX), blue (General Electric F40B), green (General Electric F40G), yellow (Sylvania Gold F40/GO), and GroLux (Sylvania F40/GRO/WS) fluorescent lamps (Fig. 1).

Light spectra were measured with an International Light Technologies ILT 900W spectroradiometer between 350 and 750 nm at 1-nm intervals. Irradiance was continuous at 50 μmol photons m⁻² s⁻¹ and was measured at the center of the surface of the Erlenmeyer flasks with a 4π QSL-100 quantum radiometer (Biospherical Instruments, USA). The irradiance value was based on the photosynthetically usable

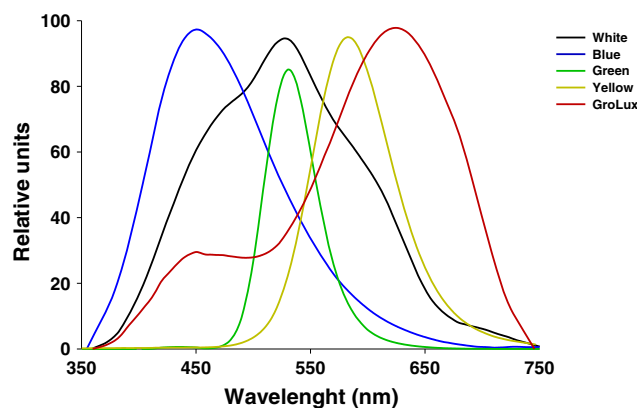


Fig. 1 Light spectra (in relative units) of white, blue, green, yellow, and GroLux fluorescent lamps

radiation (PUR) and optical properties of *Amphora* sp. cells (Romero-Romero 2015).

Growth rate and cell size

For each culture condition, we measured the cell concentration daily by direct count using a hemocytometer. Clumps of cells were dislodged by ultrasonication for 5 min at 100 kHz with an L&R Solid State/Ultrasonic model T-9B sonicator (L&R Manufacturing Company, USA). The cell concentrations were log₂-transformed to determine the growth rates (μ); for this calculation, we considered the cell concentration in the exponential growth phase using the equation described by Fogg and Thake (1987).

Cell size was measured for each culture condition on various days in culture (0, 3, and 8). We randomly selected 30 cells and measured the length and width under a compound microscope using a digital camera with the program Image-Pro Discovery, version 5.1.

Dry weight, proximate composition, and pigment content

The dry weight, proximate composition, and pigment content of the cells in each culture were measured in the exponential (day 3) and stationary growth phases (day 8).

To measure the total dry weight, 25 mL of culture was passed through washed and preweighed 47-mm Whatman GF/C glass microfiber filters (≈1 μm pore), rinsed with 15 mL of ammonium formate (3%) to remove salt residues, and dried at 60 °C. To measure the ash content, filters with cell biomass were incinerated at 450 °C for 4 h. The organic dry weight was calculated as the difference between the total organic dry weight and ash content per Sorokin (1973).

To determine the proximate composition of *Amphora* sp. for each culture condition, triplicate 10 mL samples were filtered through washed and preweighed 25-mm Whatman GF/C glass microfiber filters and stored at -20 °C for further analysis. Proteins were extracted with 0.1 N NaOH at 100 °C

for 15 min, and the content was quantified as per Lowry et al. (1951). A calibration curve was generated using bovine serum albumin (98%) as a standard. Carbohydrates were extracted according to White (1987) and quantified by the phenol-sulfuric method (Dubois et al. 1956). A calibration curve for carbohydrates was drawn using glucose (99%) as the standard. Lipids were extracted per Blight and Dyer (1959) and Chiaverini (1972) and quantified per Pande et al. (1963). The calibration curve for lipids was generated using tripalmitin (99%) as the standard.

Chlorophyll *a* and carotenoid contents were measured as described by Parsons et al. (1984).

Statistical analysis

Before the statistical analysis, all data were tested for homoscedasticity and normality (Sokal and Rohlf 1995). Differences in cell concentration were examined by analysis of covariance (ANCOVA), using the time in culture as a covariable. Differences in proximate composition (proteins, carbohydrates, lipids, organic dry weigh, ash content, chlorophyll *a*, and carotenoids), growth rate, and cell size were compared by two-way analysis of variance (ANOVA) for each variable (light quality and growth phase). When significant differences were obtained, Tukey *a posteriori* test was used. The significance level was set to $P < 0.05$ in all cases.

Results

Growth rate and cell size

Cell concentrations of the *Amphora* sp. cultures under the five light spectra differed significantly over time ($P < 0.05$; Fig. 2,

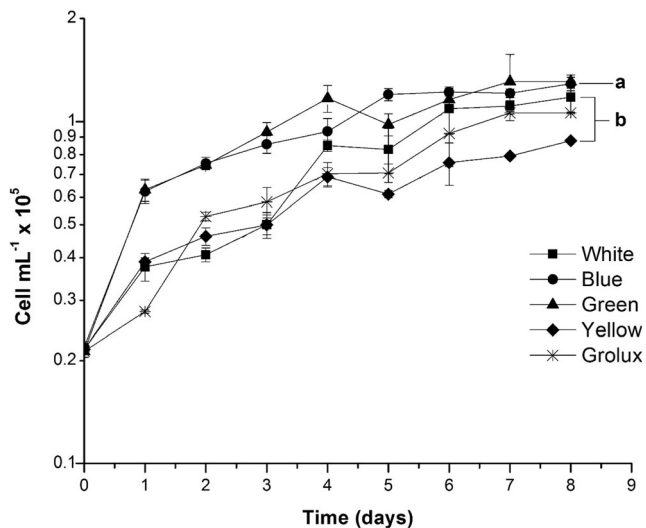


Fig. 2 Mean values ($n = 3$) and standard deviation of cell concentrations during the growth of *Amphora* sp. under various light spectra

Table 1), increasing rapidly during the first 2 days of culture, depending on the light. The exponential growth phase lasted for 4 days for all light spectra, except for blue light, under which it had a duration of 5 days. The highest cell density was obtained under blue light ($1.29 \pm 0.06 \times 10^6$ cells mL^{-1}) on the last day of culture (day 8). The growth rate was significantly influenced by light spectra ($P < 0.05$; Table 1) and was higher with green light spectra (0.61 ± 0.02 divisions day^{-1}).

Cell size, measured as length and width, differed significantly between growth phases ($P < 0.05$; Table 2) but was unaffected by light spectra. *Amphora* sp. cells were longer with respect to the initial size in both growth phases (Table 2). Cell width also rose over culture time for all light treatments (Table 2). Cell size was greater with GroLux treatment, rising to 24.76 ± 2.61 μm length by 15.30 ± 1.48 μm width (Table 2).

Dry weight, proximate composition, and pigment content

The organic dry weight (ODW) content was significantly influenced by light spectra and growth phase ($P < 0.05$; Fig. 3). ODW was higher in the exponential growth phase under yellow light (9.99 ± 1.47 pg cell^{-1}). Ash content (AC) was also significantly altered by light spectra and growth phase ($P < 0.05$; Fig. 3). In general, the AC was higher in the exponential growth phase for all treatments, peaking under yellow light treatment in the exponential growth phase (24.02 ± 3.26 pg cell^{-1}) (Fig. 3).

The proximate composition of *Amphora* sp. underwent changes between light spectra and growth phases ($P < 0.05$; Fig. 4). The highest protein content was obtained under yellow light in both growth phases (52.02 ± 7.19 pg cell^{-1} in exponential and 54.81 ± 4.76 pg cell^{-1} in stationary) and with white light (51.09 ± 6.33 pg cell^{-1} in exponential) (Fig. 4). In the stationary phase, the carbohydrate content was higher with GroLux (12.592 ± 2.34 pg cell^{-1}) and yellow light (12.40 ± 1.50 pg cell^{-1}) compared with the exponential growth phase for all light spectra.

Table 1 Growth rate (μ : divisions day^{-1}), time in exponential growth phase (ET as days), and higher cell concentration (HCD: cells $\text{mL}^{-1} \times 10^6$) in two growth phases: exponential (E) and stationary (S) phase of *Amphora* sp. cultured under various light spectra

Light spectra	μ	ET	HCD	
			E	S
White	$0.49 \pm 0.01\text{b}$	4	$0.85 \pm 0.03\text{b}$	$1.18 \pm 0.12\text{ab}$
Blue	$0.49 \pm 0.01\text{b}$	5	$1.19 \pm 0.05\text{a}$	$1.29 \pm 0.06\text{a}$
Green	$0.61 \pm 0.02\text{a}$	4	$1.16 \pm 0.08\text{a}$	$1.26 \pm 0.98\text{ab}$
Yellow	$0.42 \pm 0.02\text{c}$	4	$0.69 \pm 0.05\text{c}$	$0.88 \pm 0.03\text{c}$
GroLux	$0.43 \pm 0.03\text{c}$	4	$0.70 \pm 0.05\text{c}$	$1.06 \pm 0.01\text{bc}$

Values with lowercase letters indicate significant differences between light spectra (one-way ANOVA and Tukey *a posteriori* test, $\alpha = 0.05$; $\text{a} > \text{b} > \text{c}$. Mean values \pm SD, $n = 3$)

Table 2 Cell size (μm) of *Amphora* sp. cultured under various light spectra

Light spectra	Day 0		Day 3		Day 8	
	Length	Width	Length	Width	Length	Width
White	22.96 \pm 3.13b	12.88 \pm 1.72c	24.12 \pm 2.96a	12.64 \pm 1.41c	24.44 \pm 2.66a	13.67 \pm 1.97bc
Blue	22.96 \pm 3.13b	12.88 \pm 1.72c	24.12 \pm 3.02a	13.55 \pm 1.44bc	24.13 \pm 2.33a	14.56 \pm 2.13ab
Green	22.96 \pm 3.13b	12.88 \pm 1.72c	23.18 \pm 3.10a	12.52 \pm 1.37c	23.65 \pm 2.70a	14.33 \pm 1.81ab
Yellow	22.96 \pm 3.13b	12.88 \pm 1.72c	23.78 \pm 3.61a	12.36 \pm 1.20c	23.56 \pm 2.67a	14.71 \pm 1.56ab
Grolux	22.96 \pm 3.13b	12.88 \pm 1.72c	24.09 \pm 3.32a	12.55 \pm 1.76c	24.76 \pm 2.51a	15.30 \pm 1.48a

Values with lowercases indicates significant differences between light spectra and culture day (two-way ANOVA and Tukey *a posteriori* test, $\alpha = 0.05$; $a > b > c$. Mean values \pm SD, $n = 3$)

The lipid content decreased over time in culture, peaking in the exponential growth phase under Grolux ($27.06 \pm 6.31 \text{ pg cell}^{-1}$).

The chlorophyll *a* content of *Amphora* sp. was significantly affected by light spectra and growth phase ($P < 0.05$; Fig. 5), with the highest value found during the exponential growth phase under yellow light ($0.26 \pm 0.03 \text{ pg cell}^{-1}$). Similarly, the carotenoid content was significantly modified by light spectra ($P < 0.05$; Fig. 5), with higher values under yellow light ($0.07 \pm 0.01 \text{ pg cell}^{-1}$) in both growth phases.

Discussion

Growth rate and cell size. Light regimens have been altered to effect changes in the growth, pigment content, and proximate composition of microalgae (Mercado et al. 2004; Seyfabady et al. 2011). In this study, we noted differences in the growth of *Amphora* sp. with green, blue, white, Grolux, and yellow light. Our results are consistent with other reports of blue and green light spectra enhancing the cell concentration and growth rate of other diatoms such as *Chaetoceros* sp., *Skeletonema costatum*, *Haslea ostrearia*, *Chaetoceros gracilis*, and *Coscinodiscus granii* (Sánchez-Saavedra and

Voltolina 2002; Mouget et al. 2005; Gorai et al. 2014; Su et al. 2015).

Generally, blue light and similar wavelengths alter microalgae cultures, increasing DNA and RNA synthesis (Wallen and Geen 1971), modifying their photosynthetic properties (Mercado et al. 2004), enhancing respiration and degradation of reserve carbohydrates (Kowallik 1982), and altering the morphology and thickness of the cell wall (Talarico 1996).

In our study, green and blue light spectra increased the growth rate and cell concentration of *Amphora* sp. due to changes in their metabolism, and we assume that in *Amphora* sp. cells, increasing the DNA and RNA synthesis then stimulated the population growth or cell production as was mentioned for other microalgae strains (Wallen and Geen 1971; Voskresenkaya 1972). The effects of blue and green light on growth rate are related to pigment composition, photoacclimation, physiological responses, and biofilm formation in *Amphora* sp.—the combination of these factors defines the capacity for cell light absorption during metabolism (Becker 1994; Silva-Aciares and Riquelme 2008; Doiron et al. 2012).

Yellow light yielded the lowest cell concentration in *Amphora* sp. cultures, similar to other studies on

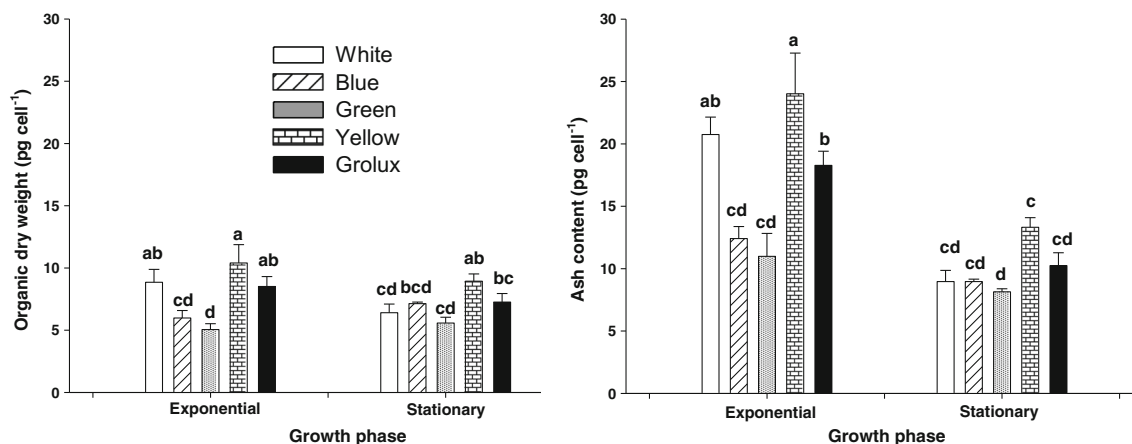


Fig. 3 Organic dry weight and ash content of *Amphora* sp. cultured under various light spectra and two growth phases. Values with lowercase letters indicate significant differences between light spectra and growth phase (two-way ANOVA and Tukey *a posteriori* test, $\alpha = 0.05$; $a > b > c$, $n = 3$)

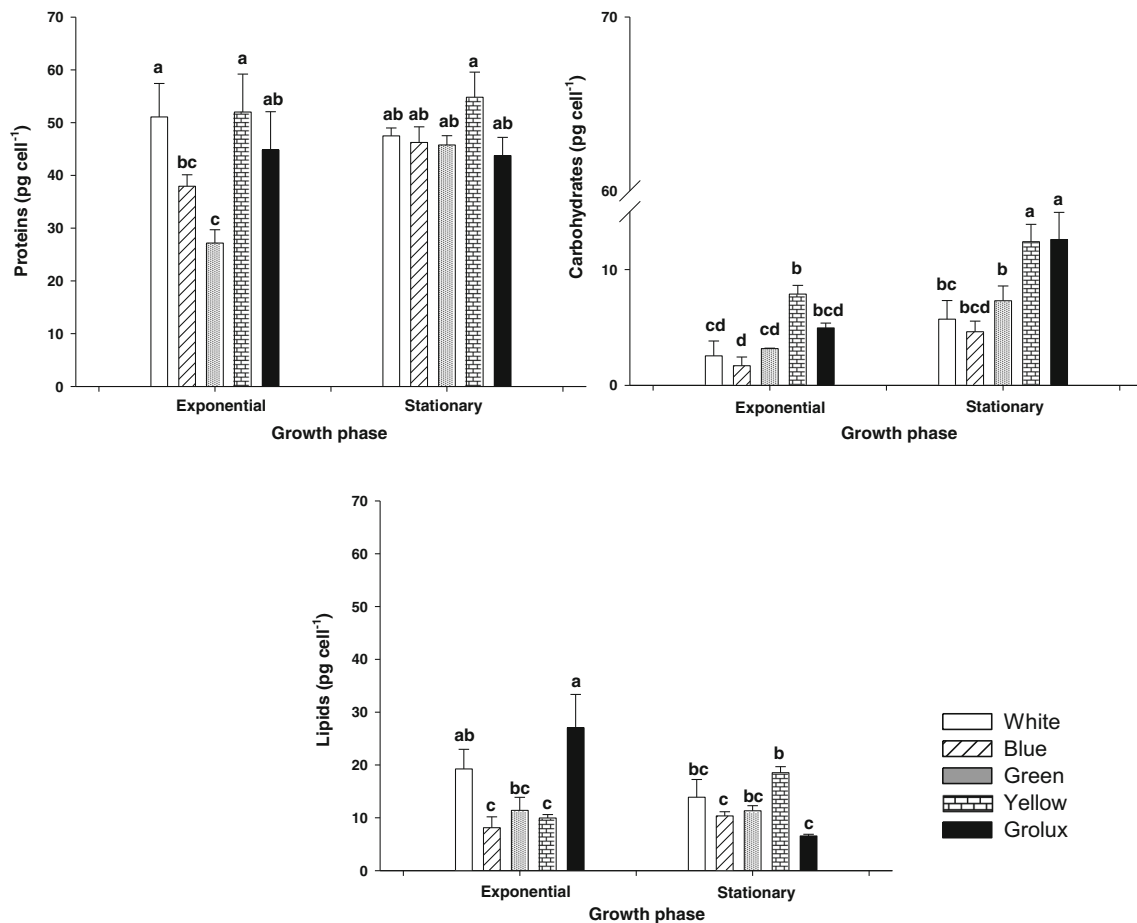


Fig. 4 Proximate composition (proteins, carbohydrates, and lipids) of *Amphora* sp. cultured under various light spectra and two growth phases. Values with lowercase letters indicate significant differences

between light spectra and growth phase (two-way ANOVA and Tukey *a posteriori* test, $\alpha = 0.05$; $a > b > c$, $n = 3$)

H. ostrearia and *C. granii* diatom cultures, for which the lowest concentrations were also obtained under yellow light (Mouget et al. 2005; Su et al. 2015). The effect of yellow light on *Amphora* sp. cell concentration might be attributed to the accumulation of amino acids that are used for synthesis of pigment proteins, and not for an increase in proteins and cell biomass (Figs. 4 and 5). The lowest cell concentration of *Amphora* sp. is possible due to the lower absorption of yellow wavelengths by the algae due to their pigment profile. Additionally, *Amphora* sp. cells under yellow light had a low growth rate but a high organic dry weight and ash content without modifications in cell size. Also, with the use of yellow light, *Amphora* sp. produced heavier cells.

The maximum nitrogen uptake rate between wavelengths for several benthic diatom strains (*Achnanthes* sp., *Amphora* sp., *Navicula* sp., and *Nitzschia* sp.) was high under blue light, followed by (in descending order) mixed, red, and yellow wavelengths (Kwon et al. 2011). Apparently, yellow light is insufficient to promote nitrogen uptake and protein synthesis (Kim et al. 2013). Another important consideration is that

Amphora sp. lives as a benthic diatom that needs to assimilate nutrients efficiently at low irradiances, which is more common with yellow light (Stevenson et al. 1996; Seckbach 2007).

The cell concentrations in this study under various light spectra used were similar to those for the same genus of *Amphora* sp. when cultured with f/2 medium at $62 \pm 8 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Courtois de Viçose et al. 2012) and with f medium at $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Jiménez-Valera and Sánchez-Saavedra 2016).

The growth rates of *Amphora* sp. under the light spectra were on the same order of magnitude as those reported in other benthic diatoms, independent of the irradiance and light quality (Gilstad and Sakshaug 1990; Mouget et al. 1999; Correa-Reyes and Sánchez-Saavedra 2001; Mercado et al. 2004).

Dry weight, proximate composition, and pigment content. The organic dry weight (ODW) and ash content (AC) of *Amphora* sp. were affected by light spectra and growth phase, and these values were similar to those in other studies (Sánchez-Saavedra and Voltolina 1996; Fernández-Robledo 2013; Sánchez-Saavedra et al. 2016). The ODW represents the total carbon content that is stored in the cells; we measured

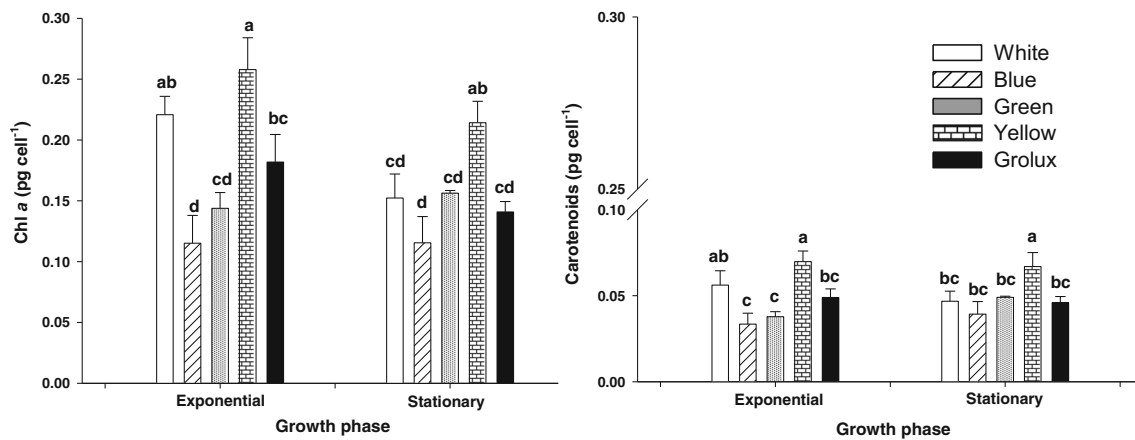


Fig. 5 Cell content of chlorophyll *a* and carotenoids in *Amphora* sp. cultured under various light spectra and two growth phases. Values with lowercase letters indicate significant differences between light spectra and growth phase (two-way ANOVA and Tukey *a posteriori* test, $\alpha = 0.05$; $a > b > c$, $n = 3$)

higher ODW values in the exponential growth phase under yellow light, which was associated with a low growth rate and high protein content. The high AC in many benthic diatom strains is attributed to the silica requirements for frustule formation, and AC rises with the age of the culture (Simental-Trinidad et al. 2001; Sánchez-Saavedra and Voltolina 2006; Correa-Reyes et al. 2009). However, in our study, *Amphora* sp. had a higher AC under the various light spectra in the exponential growth phase, possibly due to the higher reproduction of cells, causing an increase in cell wall production.

The cells of *Amphora* sp. maintained under yellow light had the highest proximate composition; this is explained by the higher organic dry weight of the cells produced as a consequence of the low growth rate. The *Amphora* sp. cells were heavier under yellow light. Also, the cell size did not change by effect of light spectra.

Blue light effects significant changes in the cell size of certain species of benthic diatoms, eliciting an increase (*Nitzschia thermalis*) or decrease (*Nitzschia laevis*) in cell surface area (Mercado et al. 2004). The cell size in *Amphora* sp. was not affected by the spectral composition of light, but the cell size was influenced by the growth phase—smaller cells were obtained at the beginning of the experiment (day 0). The differences in cell size might be due to their rapid growth and, as a consequence, an increase in protein content and decrease in cell size. The greater size of the cells is related to the higher ODW and AC values of the cells for all treatments.

Two of the main factors that modify the metabolism of microalgae are the spectral composition of light and the growth phase of the culture—both elements produce variations in proximate composition and pigment content (Voskresenskaya 1972; Sánchez-Saavedra et al. 2016; Vadiveloo et al. 2015). In our study, the spectral composition of light modified the proximate composition of *Amphora* sp. Slight differences in protein content were obtained between growth phases; these results are comparable with the findings of other groups (Mercado et al. 2004; Sánchez-Saavedra and

Voltolina 2006; Sánchez-Saavedra et al. 2016; Jiménez-Valera and Sánchez-Saavedra 2016). The low carbohydrate and lipid content in *Amphora* sp. under the various light spectra are consistent with other findings on diatoms that have been maintained under blue light (Sánchez-Saavedra and Voltolina 1996). In our study, the carbohydrate content was higher in the stationary growth phase, similar to what has been reported for certain diatom species under white and blue light (Simental-Trinidad et al. 2001; Sánchez-Saavedra and Voltolina 2006; Courtois de Viçose et al. 2012).

The lipid content in our study peaked in the exponential growth phase, in contrast to the common pattern in benthic diatoms, wherein the lipid content increases with the age of the culture as a consequence of storage products in the cells, due to the limited availability of light and nutrients (Lourenço 2002).

The proximate composition of *Amphora* sp. maintained under white light resulted with a similar trend as those obtained in the *f/2* medium produced highest protein and lipid content in the exponential growth phase (Courtois de Viçose et al. 2012; de la Peña 2007). As far as we know, no previous studies related to the effect of light spectra on cultures of *Amphora* sp. and the biochemical composition have been reported. The only work about *Amphora* sp. that describes the effect of light spectra mentioned that the growth rate and nutrient removal were increased under blue light, and lower values of growth and nutrient removal were obtained under yellow light (Kwon et al. 2013). The trend in growth rate of *Amphora* sp. previously mentioned by Kwon et al. (2013) was similar to that obtained in the present study. Apparently, the metabolism of *Amphora* sp. is different from those mentioned in various other microalgae. The blue and blue-green light spectra enhanced the protein content. An effect that has been studied widely with this light spectrum is the carboxylase enzyme and oxaloacetate production, which increases and induces the continuous regeneration of the Krebs cycle (Voskresenskaya 1972; Sánchez-Saavedra and Voltolina

1996; Marchetti et al. 2013). In *Amphora* sp., we found that blue and green light did not increase protein levels, but increased the growth rate. The protein content in *Amphora* sp. was higher with most of the light spectra, possibly because benthic diatoms live as a conglomeration of cells and because they are adapted to various light conditions. Studies on benthic communities indicate that there is a correlation between maximum photosynthesis rates and photosynthetic efficiency—a strategy for photoacclimation is based on the optimization of photosynthesis and photosynthetic efficiency, permitting diatoms to often become predominant in subtidal communities (Blanchard and Montagna 1992).

In our study, the highest carbohydrate content was seen in the stationary growth phase under GroLux and yellow light spectra, which is comparable with results by Sánchez-Saavedra and Voltolina (2006), who indicated that GroLux light increases the carbohydrate content in the stationary growth phase in *Chaetoceros* sp. Further, blue light treatment decreased the carbohydrate content, confirming its ability to increase the levels of respiration enzymes, promoting the use of stored carbohydrates (Kowallik 1982).

The lipid content in *Amphora* sp. was higher under GroLux light during exponential growth, in contrast to another diatom species, *Chaetoceros* sp., which when cultured with GroLux light had a decreased lipid content (Sánchez-Saavedra and Voltolina 2006). Our finding can be explained by the lower energy that GroLux lamps produce compared with blue light.

The pigment content of *Amphora* sp. also was altered by light spectra and growth phase. The chlorophyll *a* content of diatoms rises under blue or green light (Sánchez-Saavedra and Voltolina 1996; Mercado et al. 2004; Godínez-Ortega et al. 2008), but in *Amphora* sp., we noted an opposite trend, with blue light decreasing such levels. Chlorophyll *a* levels were higher under yellow light in the exponential growth phase, similar to other groups of algae (Sánchez-Saavedra and Voltolina 2002; Lafarga-de la Cruz et al. 2006; Fernández-Robledo 2013; Sánchez-Saavedra et al. 2016).

The carotenoid content in *Amphora* sp. was higher under yellow light during exponential growth, which has been attributed to a photoprotective mechanism in response to high availability of light and low cell concentration in the exponential growth phase as was described by other authors (Sánchez-Saavedra and Voltolina 2002; Fernández-Robledo 2013).

The chlorophyll *a* and carotenoid content in *Amphora* sp. was higher under yellow light, due to the low light absorption by the cells, effecting low growth rates; in contrast, pigment levels were lower under blue and green light, based on the high efficiency of light absorption, reflected in higher growth concentration of the population. Other studies have found that the carotenoid content of the benthic diatoms *Nitzscha thermalis*, *Navicula incerta*, and *N. laevis* (strains 1 and 2) is unaffected by blue and white light (Mercado et al. 2004).

The irradiance used to maintain the cultures of *Amphora* sp. was an intermediate irradiance (between 50 and 70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) as a higher irradiance may saturate its photosystems (Gilstad and Sakshaug 1990; Mouget et al. 1999). We considered that the cultures of *Amphora* sp. maintained under a different light condition on this work were not modified by the effect of saturation of irradiance as all culture conditions promoted the growth of the cells.

Other factors include the optical properties of the cell and biofilm, the self-shading effect due to the increase in cell concentration, the package effect, which results in a combination of photosynthetic pigments, and cell size, which directly affects the light absorption capacity (Dawes 1991; Geider and Osborne 1992; Kirk 1994; Sakshaug et al. 1997). The photosynthetic apparatus can adapt to light spectra, the photoperiod (including light-shade adaptation), the photosynthetic electron transfer chain, and the light-harvesting complex, and this adaptation differs between taxa and species (Falkowski and Owens 1980; Raven and Geider 2003).

Aquaculture uses

The size of *Amphora* sp. in this report demonstrates that it is adequate for use as abalone postlarvae nutrition, based on studies of the size of diatoms that are used for abalone feed (Kawamura and Takami 1995; Kawamura et al. 1998; Xing et al. 2008; Siqueiros-Valencia et al. 2015).

The use of monochromatic light for the maintenance of benthic diatoms allows their proximate composition to be modified by the light source and increases their growth rate and biomass production significantly. All *Amphora* sp. cultures produced under the various light spectra on this study had the required cell size and proximate composition for several organisms (e.g., abalone, sea urchin, shrimp larvae) under culture conditions (Sánchez-Saavedra and Voltolina 1995; Brown 2002; Muller-Feuga 2007).

Our study has shown the effect of light spectra and growth phase (exponential and stationary) on population growth and the proximate composition of *Amphora* sp., demonstrating that both factors alter its metabolism. White and yellow light spectra induce proteogenesis, GroLux and yellow light increase carbohydrate and lipid synthesis in *Amphora* sp., and yellow light elicits high synthesis of pigments.

We concluded that the manipulation of spectral properties of light can be used to alter the metabolism and proximate composition of *Amphora* sp. cultures. The use of different light spectra on the *Amphora* sp. cultures can provide differences on the nutritional value for further application in aquaculture. Thus, if *Amphora* sp. cells are used to enhance the nutrition of abalone postlarvae, it is more suitable to maintain the cultures with GroLux, as this produces high protein and carbohydrate contents during the exponential growth phase.

Acknowledgements This work has been funded by Consejo Nacional de Ciencia y Tecnología (CONACyT, project: SEP-CONACyT 2009-01-130074) and Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE, project: 623108). C.C. Romero-Romero acknowledges her Master's in Science from CONACyT and CICESE.

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