Effects of the Light Irradiance on the Growth and Lipid Content of *Amphidinium carterae* (Dinophyceae) for Biofuel Production

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Abstract
The irradiance level used in the microalgae cultures can modify the growth and proximate composition; however, this response is specie specific. The dinoflagellate group has the potential to be used as a source of biofuel production. This study evaluated the effect of five irradiance levels (50, 100, 150, 200, and 250 µmol photon m⁻² s⁻¹) on the growth rate, proximal composition, pigment content, and photosynthesis of *Amphidinium carterae*. The highest cell concentration was for the cultures at 150 µmol photon m⁻² s⁻¹ (130 × 10³ cells mL⁻¹) and the lowest value for 50 µmol photon m⁻² s⁻¹ (49 × 10³ cells mL⁻¹). The cultures maintained under the lowest irradiance had the highest values of organic dry weight (ODW) and inorganic dry weight (IDW). The protein and carbohydrate content changes significantly concerning the irradiance level, with the higher values (1599.46 pg cell⁻¹ and 557.24 pg cell⁻¹, respectively) at an irradiance of 200 µmol photon m⁻² s⁻¹. Lipid content was modified by the effect of irradiance, with the highest values (6920.89 pg cell⁻¹) at the lowest irradiance used. As a general trend, the high irradiances increased the photosynthesis rates. These findings demonstrate that the strain of *A. carterae* used in this work can grow in high irradiances (100 to 250 µmol photon m⁻² s⁻¹) and significantly increase the lipid content at low irradiance used.

Keywords *Amphidinium carterae* · Growth rate · Biomass production · Lipids · Photosynthesis · Proximal composition

Introduction
Rapidly exhausting fossil fuels and combined with the increasing energy demand led to the search for alternative energy sources [1]. Microalgae are a promising source of proteins, lipids, and carbohydrates for the food/feed and biofuel industry. For alternative biofuel production, microalgae represent ventsages due to low environmental impact, carbon sequestration, high lipid content, and rapid growth. In comparison with soya and palm oil, microalgae can be grown more efficiently and sustainably [2]. The biodiesel production (L Ha⁻¹ year⁻¹) of microalgae (12,000 with 10 g m⁻² day⁻¹ and 30% triacyl-glycerides) was higher than the obtained from crops such as oil palm (5950), jatropha (1892), sunflower (952), and soybean (446) [3]. However, to obtain a good economic-production benefit, microalgal cultivation requires a good light source, effective mass transfer, minimal or no contamination, low infrastructure, and land cost [1].

Microalgae are photosynthetic organism sources of many high-value-added metabolites. Lipids and fatty acids from microalgae are used for biofuels productions. Proteins, carbohydrates, carotenoids, and pigments can be used as feedstock for applications in cosmetics, pharmaceutical, animal, and human nutrition [4]. Current research is focused on developing cultivation strategies for the production of lipids and other value-added products in laboratory-scale biorefineries [5].

Dinoflagellates are associated with the production of many marine toxins, which are of interest for their commercial impact, potential medical, pharmacological research, and other applications [6]. Also, marine dinoflagellates are a source of numerous bioactive compounds, besides toxins, with...
commercial interest [7]. Peridinin is a carotenoid present in the dinoflagellates and has potential use in medicine as a therapeutic agent against different diseases [8]. The dinoflagellate group can accumulate large amounts of lipids, and they can be used as an alternative source to chemical products used as biofuels [9], pharmaceutical, and nutritional applications. The EPA (eicosapentaenoic acid), ARA (arachidonic acid), and DHA (docosahexaenoic acid) are some of the most important fatty acids in nutrition [10].

The *Amphidinium* genus is the most abundant and diverse benthic dinoflagellate species worldwide [11], and they produce toxins and bioactive compounds with harmful effects [12]. The main bioactive compounds produced by *Amphidinium* cells are the amphidinols (polyketides). The amphidinols are compounds with antifungal and hemolytic activities [12, 13], and in less frequent cases, cytotoxic activity was used for anticancer activity [14]. However, *Amphidinium* also has other bioactive compounds with commercial interest, like polyunsaturated fatty acids and carotenoids [15]. The species *Amphidinium carterae* is the most used of the genus in studies for biomass production with photobioreactors for the production of carotenoids, fatty acids, and amphidinols [15–17].

The knowledge of the photosynthesis efficiency of the microalgae strain is a critical parameter in the mass microalgae cultures [9]. In closed growing systems, the light conditions can be changed, which can be used to increase the productivity of microalgae cultivation [18]. An increase in irradiance stimulates growth; however, if present in excess, the growth is not stimulated, having an inhibitory effect causing a reduction in duplication rate, and may lead to the formation of harmful reactive oxygen species (ROS) and oxidative stress [19].

The studies of microalgae in small cultures are useful for predicting the physiological response to different environmental variables. The information obtained in small cultures will be used to extrapolate the cultures in photobioreactors, and to produce any metabolite with satisfactory productivity, which is necessary to define the culture conditions.

We hypothesized that *A. carterae* at high irradiances (150 to 250 μmol photon m⁻² s⁻¹) decrease the growth rate and photosynthesis, altering the metabolism and increasing the lipids content. This work evaluated the effect of five irradiances levels (50, 100, 150, 200, and 250 μmol photon m⁻² s⁻¹) on the growth rate, proximal composition, pigment content, and photosynthesis of *Amphidinium carterae*.

**Materials and Methods**

**Culture Conditions**

*Amphidinium carterae* was isolated from Bahía Todos Santos, Ensenada, Mexico, in 2015, from a bloom. Batch cultures were maintained in 125-mL flasks with 110 mL of “f” media [20] in seawater at 20 ± 1 °C, with 12:12-h light:dark photoperiod. The pH was measured daily at initial values of 7.8, which increased throughout the culture to reach values of 8.2.

Triplicate batch cultures were grown with the conditions described above in five different irradiances: 50, 100, 150, 200, and 250 μmol photons m⁻² s⁻¹; the light was provided by 40-W white fluorescent lamps (Philips F40 T12/DX). The five irradiances were obtained, adjusting the distance of the culture to the lamp, and measured at the center of the surface of the flask with a 4π QSL-100 quantum radiometer (Biospherical Instruments, USA).

**Growth Rate**

Every day 1 mL of the cultures was fixed with Lugol iodine, and cell density was measured by direct count using a hemocytometer with a microscope Olympus CX31. The exponential growth rates were calculated according to Guillard [21]:

$$\mu = \log_2 \left( \frac{N_2 - N_1}{t_2 - t_1} \right)$$

where $N_1$ and $N_2$ are the cell concentration at times $t_1$ and $t_2$.

**Pigments and Photosynthesis**

For pigment analyses, 5 mL of each culture at day 8 of growth was filtered through GF/F glass microfiber filters and frozen immediately at −20 °C. Chlorophylls and carotenoids were extracted with 3 mL of acetone 90%, and the concentrations were determined according to Sánchez-Saavedra et al. [22].

Samples for photosynthesis (15 mL) were collected on day 8 of the culture. Photosynthesis was analyzed using rapid light curves on a pulse amplitude modulation (PAM) fluorometer (Walz, junior PAM). The samples were acclimated for 20 min in darkness before photosynthesis curves were analyzed. Photosynthetic efficiency ($\alpha$), irradiance of saturation ($I_\alpha$), the relative electron transport rate (rETR), and the maximum quantum yield of photosystem II ($F_v/F_m$) values were obtained for each treatment [23]. The maximum quantum yield of photosystem II ($F_v/F_m$) was determined by the following equation:

$$\frac{F_{v\alpha}}{F_m} = \frac{F_m - F_0}{F_m}$$

where $F_m$ is the maximal chlorophyll fluorescence yield when the reaction centers of PS II are closed due to a strong light pulse, and $F_0$ is the basic fluorescence yield that was recorded with low light. $F_v/F_m$ values were obtained by calculating the absorbance with the Solver program [23].
Dry Weight and Proximate Composition

To measure total dry weight (TDW), triplicate 13-mL samples on day 8 of growth were passed through washed and preweighted 47-mm Whatman GF/C glass fiber filters (1-µm pore), rinsed with 25-mL ammonium formate (3%) to remove salt residues, and dried at 60 °C to a constant weight. To measure the ash content or inorganic dry weight (IDW), the filters with cell biomass were incinerated at 450 °C for 4 h. The organic dry weight (ODW) was calculated as the difference between the total dry weight and ash content [22].

To measure the proximate composition, 10-mL samples for proteins, carbohydrates, and lipids were passed through washed 25-mm Whatman GF/C glass microfiber filters and stored at −20 °C for further analysis. Water-soluble proteins were extracted with 0.1 N NaOH at pH 12 and 80 °C for 20 min, and the content was quantified by Folin [22]. A calibration curve was generated using bovine serum albumin (98%) as the standard. Carbohydrates were extracted with sulfuric acid and quantified by the phenol–sulfuric method [22]. Lipids were extracted with chloroform–methanol and quantified by sodium dichromate [22]. A calibration curve for lipids was generated using tripalmitin (99%) as standard.

The productivity was obtained by the multiplication of the specific growth rate by the TDW values for each irradiance.

Statistical Analysis

All data were tested for homoscedasticity and normality. Differences in growth rate, TDW, IDW, ODW, proximate composition (proteins, carbohydrates, and lipids), chlorophyll a, carotenoid, and photosynthetic parameters (α, ETRr, Ic, and Fv/Fm) were analyzed for each variable in a triplicate set by one-way analysis of variance (ANOVA). Differences in cell concentration were analyzed by covariance analysis (ANCOVA). When significant differences were obtained, Tukey a posteriori test was performed. The data analysis was performed using the software Statistica 7.0, and the significance level was set to $p < 0.05$ in all cases.

Results

Growth Rate

The cell densities of *Amphidinium carterae* maintained under the five irradiances differed significantly ($p < 0.05$, Fig. 1), increasing rapidly depending on the irradiance used, and the culture day. The highest cell concentration ($130 \times 10^3$ cell mL$^{-1}$) was measured for the cultures at 150 µmol photon m$^{-2}$ s$^{-1}$ and the lowest values for 50 µmol photon m$^{-2}$ s$^{-1}$ ($49 \times 10^3$ cells mL$^{-1}$) (Fig. 1).

The highest growth rate values (0.63 to 0.67 div day$^{-1}$) were obtained at the irradiances of 150 to 250 µmol photon m$^{-2}$ s$^{-1}$ without significant differences between them ($p > 0.05$, Fig. 2), and the lowest (0.43 div day$^{-1}$) at 50 µmol photon m$^{-2}$ s$^{-1}$ with a significant difference respect to the other irradiances ($p < 0.05$, Fig. 2).

Dry Weight and Proximal Composition

The TDW ($p < 0.05$), ODW ($p < 0.05$), and IDW ($p < 0.05$) values differed significantly by the effect of irradiance (Fig. 3). The cultures at low irradiance (50 µmol photon m$^{-2}$ s$^{-1}$) had the highest values of TDW (13,418 pg cell$^{-1}$), ODW (8159.88 pg cell$^{-1}$), and IDW (5258.68 pg cell$^{-1}$). The TDW, ODW, and IDW values obtained for the irradiances from 100 to 250 µmol photon m$^{-2}$ s$^{-1}$ were unchanged (Fig. 3).

The proximate composition differed significantly ($p < 0.05$) depending on the irradiance level (Fig. 3). The protein content was significantly different by the effect of the irradiance ($p < 0.05$), with the highest value (1599.46 pg cell$^{-1}$) at the irradiance of 200 µmol photon m$^{-2}$ s$^{-1}$ and the lowest value (815.97 pg cell$^{-1}$) at 50 µmol photon m$^{-2}$ s$^{-1}$.

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*Fig. 1* Mean values ($n = 3$) and standard deviations of cell concentration during the growth of *Amphidinium carterae* cultured under five irradiances (50, 100, 150, 200, and 250 µmol photons m$^{-2}$ s$^{-1}$). Different letters indicate significant differences by irradiance (covariance analysis ANCOVA and a Tukey a posteriori test, $\alpha=0.05$: $a>b>c$)
Carbohydrate content was significantly different by the effect of irradiance ($p < 0.05$), with the highest values (557.24 pg cell$^{-1}$) at the irradiance of 200 µmol photon m$^{-2}$ s$^{-1}$. The lipid content was modified significantly by the effect of irradiance ($p < 0.05$), with the highest values (6920.89 pg cell$^{-1}$) at the low irradiance (50 µmol photon m$^{-2}$ s$^{-1}$).

The productivity differed significantly ($p < 0.05$) by the effect of the irradiance, with the lowest value at 50 µmol photon m$^{-2}$ s$^{-1}$, and increase the productivity gradually until rising the highest value at the irradiance of 150 µmol photon m$^{-2}$ s$^{-1}$ (Fig. 3).

**Pigments and Photosynthesis**

The chlorophyll a content was significantly affected by the irradiance level ($p < 0.05$), and the content of chlorophyll a decreased from the irradiance of 50 to 100 µmol photon m$^{-2}$ s$^{-1}$, and decrease again from the irradiance of 150 to 200 µmol photon m$^{-2}$ s$^{-1}$ (Fig. 4). The chlorophyll c content ($p > 0.05$) and carotenoid content ($p > 0.05$) were not significantly modified by the effect of irradiance (Fig. 4).

The $F_{v}/F_{m}$ values were significantly different by the effect of irradiance ($p < 0.05$, Fig. 5), where the lowest irradiance (50 µmol photon m$^{-2}$ s$^{-1}$) had the lower value (0.21). The $F_{v}/F_{m}$ were maintained with similar values for the other irradiance levels used (100 to 250 µmol photon m$^{-2}$ s$^{-1}$).

The photosynthesis curves were significantly affected by the irradiance level of the cultures ($p < 0.05$, Fig. 6). The photosynthetic curves measured as relative electron transport (ETR) had the lowest values at the irradiance of 50 µmol photon m$^{-2}$ s$^{-1}$ and the highest at the irradiance of 200 µmol photon m$^{-2}$ s$^{-1}$.

The photosynthetic parameters also differed among irradiance levels ($p < 0.05$). The photosynthetic efficiency ($\alpha$) and maximum relative electron transport (ETRm) had significant difference between irradiances ($p < 0.05$), with the lowest value at the irradiance of 50 µmol photon m$^{-2}$ s$^{-1}$ and the highest value at 200 µmol photon m$^{-2}$ s$^{-1}$. The irradiance of saturation ($I_{s}$) presented the highest values at the irradiances of 50 and 200 µmol photon m$^{-2}$ s$^{-1}$, and then decreased without significant differences ($p > 0.05$).
at irradiances of 100, 150, and 250 µmol photon m\(^{-2}\) s\(^{-1}\) (Table 1).

![Fig. 4 Mean values and standard deviations (n=3) of pigment content of chlorophyll a (chl a), chlorophyll c (chl c), and carotenoid content (pg cell\(^{-1}\)) of Amphidinium carterae cultures maintained under five irradiances. Different letters indicate significant differences by irradiance (one-way ANOVA and a Tukey a posteriori test, \(\alpha=0.05\): a>b)](image)

**Fig. 4** Mean values and standard deviations \((n=3)\) of pigment content of chlorophyll a (chl a), chlorophyll c (chl c), and carotenoid content \((\text{pg cell}^{-1})\) of Amphidinium carterae cultures maintained under five irradiances. Different letters indicate significant differences by irradiance (one-way ANOVA and a Tukey a posteriori test, \(\alpha=0.05\): a>b)

**Discussion**

This study describes the effects of five different irradiances on the growth and lipid content of *Amphidinium carterae* maintained in culture conditions. This work obtained changes in the growth rate of *A. carterae* by the effect of irradiance, with the highest values from 150 to 200 µmol photon m\(^{-2}\) s\(^{-1}\) and the lowest values at 50 µmol photon m\(^{-2}\) s\(^{-1}\).

When *A. carterae* (Amphi 1) was grown in “f/2” media at 19 °C, and from 10 to 50 µmol photon m\(^{-2}\) s\(^{-1}\), the growth rate was linearly proportional to irradiance, and the maximum growth was at 80 µmol photon m\(^{-2}\) s\(^{-1}\) in batch cultures [24].

The growth rate obtained in this study for *A. carterae* at high irradiances (100 to 250 µmol photon m\(^{-2}\) s\(^{-1}\)) was highest (0.59 to 0.67 divisions day\(^{-1}\)), with regard to the growth rate values (0.15 to 0.55 divisions day\(^{-1}\)) reported to *A. carterae* isolated from macroalgae and grow in low irradiances (35 to 70 µmol photon m\(^{-2}\) s\(^{-1}\)) [25]. Also, the growth rates obtained in this study were higher than the values measured for the cultures of *A. carterae* (CCMP1314) maintained at 20 °C with 100 µmol photon m\(^{-2}\) s\(^{-1}\) supply 14:10 h light:dark under P-repleted (0.52 divisions day\(^{-1}\)) and P-deprived (0.27 divisions day\(^{-1}\)) [26]. For *A. carterae* cultures maintained with irradiances of 50 to 750 µmol photon m\(^{-2}\) s\(^{-1}\), the highest growth rate was at 300 µmol photon m\(^{-2}\) s\(^{-1}\) during the first 5 days of culture [27]. The growth rate values obtained for *A. carterae* in this work differed from those mentioned by other authors, possibly for
the differences in the origin of the strain and the different culture conditions used.

In this work, the specific growth rate and cell concentration increased proportionally at irradiance from 50 to 150 µmol photon m⁻² s⁻¹, but the rise of light intensity from 150 to 250 µmol photon m⁻² s⁻¹ did not increase the growth rates. This fact suggests that saturation photosynthesis was achieved; although the irradiance curves and \( I_k \) values indicate that \( A. \) carterae can tolerate high irradiances, this will not be reflected in high growth rates. Photoacclimation probably supports the tolerance to high irradiances and tolerates a wide range of light intensities for different phytoplankton species. In the benthic dinoflagellate \( Gambierdiscus \) spp., photoacclimation permits that grow at high irradiances [28]. Photoacclimation of \( A. \) carterae in batch cultures allowed at high irradiances (300 µmol photon m⁻² s⁻¹) the maximum growth rate of 0.65 divisions day⁻¹, however, increase the irradiances until 250 µmol photon m⁻² s⁻¹ does not increase the growth rates [16].

If the light intensity is too low in phytoplankton cultures, logarithmic growth will not prevail [29]. The results obtained with \( A. \) carterae showed that at an irradiance of 50 µmol photon m⁻² s⁻¹ grows lower than the different irradiances used; thus, the low irradiance level used was insufficient to promote a high growth rate. The \( F_v/F_m \) value and photosynthetic efficiency (\( \alpha \)) at the irradiance of 50 µmol photon m⁻² s⁻¹ indicate that the light intensity is low to promote the growth rate and increase of cell density, and consequently, the cells accumulate metabolites instead to be used to cell reproduction and increased the values of dry weight. \( A. \) carterae cultured in 12:12 LED-lighted raceway photobioreactor had high growth at irradiances between 100 and 289 µmol photon m⁻² s⁻¹, reaching the stationary growth phase at 9 days of culture [16]. The results obtained with \( A. \) carterae showed that the logarithmic growth phase was achieved at least for 8 days of culture and that irradiances below 100 µmol photon m⁻² s⁻¹ are limiting for cell growth.

In cultures of \( Scenedesmus \) obliquus, when the cells are stressed, they decrease or stop reproduction and increase cellular weight [19]. This pattern was observed in the cultures of \( A. \) carterae maintained at an irradiance of 50 µmol photon m⁻² s⁻¹; in this irradiance, the cell decreases their growth, and the cell components used to reproduce are storage and consequently increase cell weight; under unfavorable environmental condition, many algae increase and accumulate neutral lipids which serve primarily as a storage form of carbon and energy [30]. In this work, the TDW, ODW, and IDW were not modified by irradiance at values between 100 and 250 µmol photon m⁻² s⁻¹; this trend was due to the cells maintaining a similar growth under these irradiance values.

The highest values of productivity in \( A. \) carterae obtained in this work were in the irradiances of 150 and 200 µmol photon m⁻² s⁻¹ in small-scale cultures. These values were four orders of magnitude higher than those obtained in large-scale photobioreactor cultures of \( A. \) carterae, used for metabolites production [31]. These differences are due to the scale of the cultures, when increased the culture volume usually decreases the cell densities, that produces low productivity.

The lipid content of \( A. \) carterae had significant modifications by the effect of light irradiances and showed the highest values (6920.90 pg cell⁻¹) with the low irradiance. Previous reports mentioned that \( A. \) carterae could produce lipid content from 7.2 to 9.2% of dry weight [32]; in this study, lipids represent between 35.4 and 84.9% of dry weight (1080 to 6920 pg cell⁻¹), and the lipid content of some marine diatoms can change from 22.7% when grows under normal conditions to 44.6% when was maintained under stress conditions [30]. The lipid percentage in \( A. \) carterae is high compared with other microalgae like diatoms; however, the cultivation of \( A. \) carterae in photobioreactors has been tested, with good results in large-scale culture [15], besides, not only the lipids can be used, other metabolites like carotenoids and amphidinols which are produced by \( A. \) carterae can be exploited.

Microalgae like \( Scenedesmus \) obliquus and \( Dunaliella \) salina present the highest lipid content in high irradiances [19, 33]. However, \( Nanochlorella \) sp. products high lipid content at low irradiances [29]. The effect of the light irradiance on the proximate composition is species-specific, and their modifications are related to each strain characteristic.

| Irradiance (µmol photons m⁻² s⁻¹) | \( a \) (O₂ photon⁻¹) | rETRm (µmol electrons m⁻² s⁻¹) | \( I_0 \) (µmol photons m⁻² s⁻¹) |
|-----------------------------------|----------------------|--------------------------------|-------------------------------|
| 50                                | 0.0035 ± 0.0006c      | 6.15 ± 3.88c                  | 2032.68 ± 778.07ab            |
| 100                               | 0.0072 ± 0.0014b      | 11.10 ± 5.58b                 | 1906.70 ± 108.48b             |
| 150                               | 0.0076 ± 0.0017b      | 9.20 ± 0.23b                  | 1918.63 ± 633.85b             |
| 200                               | 0.0140 ± 0.0051a      | 21.06 ± 7.04a                 | 2249.70 ± 460.50a             |
| 250                               | 0.0086 ± 0.0015b      | 12.70 ± 3.12b                 | 1858.86 ± 400.80b             |

Values with different letters indicate significant differences by irradiance (one-way ANOVA and a Tukey \( a \) posteriori test, \( a=0.05 \); \( a>b>c \)).
In this work, *A. carterae* decreases the growth rate at the lowest irradiance, consequently increasing the storage products as lipids.

The neutral lipids in the main form of triacylglycerols can be converted to fatty acid methyl esters and used as biofuel [34]. Lipids serve in two ways; as energy reserves and structural components of the membranes of the cell [35], triacylglycerols typically provide a storage form of carbon to structural components of the membranes of the cell [35], triacylglycerols were found in irradiances of 60 µmol photon m⁻² s⁻¹ [37]. However, in the same species diatom *P. tricornutum* when it grows with nitrogen starvation, the highest triacylglycerols were found in irradiances of 60 µmol photon m⁻² s⁻¹ [38].

In this study, *A. carterae* had the highest lipid content in the lowest irradiance (50 µmol photon m⁻² s⁻¹). Maltsve et al. [18] describe that the optimal light intensity for the highest lipid content and productivity is not the same for different taxa of microalgae.

Other environmental parameters can influence the growth and proximate composition of different microalgae strains, e.g., *Porphyridium cruentum* maintained with low irradiance (50 µmol photon m⁻² s⁻¹) and ammonium as nitrogen source, increasing lipid content [22]. However, Metsoviti et al. [39] mentioned that it is difficult to generalize the specific influence of the environmental factors on growth and biochemical composition in microalgae species due to differences in their metabolism. The results obtained in this work show that the lowest irradiances used on the cultures of *A. carterae* induce a low growth rate, increasing TDW, ODW, and lipid content by cell. In this work, protein content for *A. carterae* was high at the irradiance of 200 µmol photon m⁻² s⁻¹. This trend previously described was also observed in *Scenedesmus obliquus* [19]. Protein synthesis can be stimulated by factors like the nitrogen source, temperature, and light [39]. Carbohydrate content obtained in this work had slight variation by the effect of the five irradiances used.

Chlorophyll a content decreases concerning the irradiance level used for *A. carterae* cultures in this work. One general response of microalgae to increase the photon flux densities is reducing cellular pigment content, regulating the light-harvesting antennas size, and modifying the cell size [40]. The chlorophyll a content obtained in this study (3.1 to 4.02 pg cell⁻¹) was higher than the previously reported for *A. carterae* [15, 24, 41]. We considered these differences due to the characteristics of the strain used and distinct culture conditions.

Under high light irradiance, the excess energy absorbed can cause a destructive effect in the photosynthetic apparatus; carotenoids and their antioxidant capacity protect the photosystem from damage [42]. The carotenoid content was not modified by the effect of the irradiance in this work; thus, the light irradiance used is not too high to cause photodamage in the photosynthetic apparatus of *A. carterae*. The carotenoid content measured in this work (1.5 to 1.84 pg cell⁻¹) was similar to the values mentioned in *Amphidinium carterae* by other authors (2 to 5 pg cell⁻¹) [15, 41].

For several microalgae groups, *Fv/Fm* values ranged from 0.50 to 0.80 with mean values of 0.65 and indicating that the cells are without stress, when decreasing the values of *Fv/Fm* indicates stress [43]. For the dinoflagellate *Karlodinium veneficum* was evaluated the effect of the light spectrum (white, red, and blue) and light intensity (50, 100, and 200 µmol photon m⁻² s⁻¹) was found that high light intensities inhibited the photosynthetic photochemical process by decreasing the *Fv/Fm* values from 0.50 to 0.32 when increasing the irradiance level [44]. The yield varies significantly, depending on the irradiance regime, physiological treatment, and species [45]. The values of *Fv/Fm* for *A. carterae* in this work show that at irradiances from 100 to 250 µmol photon m⁻² s⁻¹ were obtained similar values of *Fv/Fm* (0.33 to 0.38) and decreased at the low irradiance (0.21); this trend indicates that at irradiances from 100 to 250 µmol photon m⁻² s⁻¹, the cells are under similar maximum photochemical quantum efficiency of photosystem II. The growth rate had a similar trend to those obtained for *Fv/Fm* values for *A. carterae*. Those results indicated that irradiances from 100 to 250 µmol photon m⁻² s⁻¹ promote a similar growth rate by the effect of the light. The *Fv/Fm* ratio obtained in this work (0.21 to 0.38) was similar to the values previously mentioned for *A. carterae* (0.30 to 0.40) [25]; however, the *Fv/Fm* values were lower than those obtained by Li et al. [26] (0.30 to 0.70) and by Molina-Miras et al. [15] (0.62). The lowest *Fv/Fm* values obtained in the irradiance of 50 µmol photon m⁻² s⁻¹ indicate that *A. carterae* is under the unfavorable light condition with low photosynthetic efficiency.

This study provides information about the effect of the irradiance in small-scale culture, which can be used as a base to extrapolate results to big-scale cultures. Garcia-Camacho [46] mentions that small-scale cultures are potentially relevant in the result extrapolation to photobioreactor cultures in the dinoflagellate *Protoceratium reticulatum*.

**Conclusion**

In conclusion, the results obtained in this study indicate that the strain of *A. carterae* used in this work can grow in high irradiances (100 to 250 µmol photon m⁻² s⁻¹). When the cultures of *A. carterae* were maintained with low irradiance (50 µmol photon m⁻² s⁻¹), they had low growth rates, productivity, and photosynthetic activity, however, the content
of lipids increases, and those can be used in the production of biodiesel or ethanol.

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

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Declarations

Competing Interests The authors declare no competing interests.

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