

# Physiological and photosynthetic performance of native microalgae strains isolated from Baja California

Desempeño fisiológico y fotosintético de cepas nativas de microalgas aisladas de Baja California

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## ABSTRACT

To assess the physiological status and photosynthetic activity of sixteen microalgae strains isolated from Baja California, growth estimations and *in vivo* chlorophyll *a* (Chl-*a*) fluorescence measurements were performed. The bacillariophyte *Diploneis* sp. exhibited the highest growth rate, while the highest cell densities were observed in *Tetraselmis suecica* and *Navicula* sp. strain 2. Most strains showed effective maximum quantum yield (Fv/Fm) values above 0.50. The highest values of maximum electron transport rate (ETR<sub>m</sub>) and saturation irradiance (I<sub>k</sub>) were recorded for *Amphora* sp. strain 6 and *Heterococcus* sp. The diatom *Navicula* sp. strain 4 showed the highest content of chlorophyll *a* and carotenoids. The culture conditions used in this study were not stressful for the microalgae strains. Notably, *T. suecica* showed high maximum cell density and Fv/Fm values; *Amphora* sp. strain 6 exhibited the highest electron transport rate (ETR<sub>m</sub>) and elevated saturation irradiance (I<sub>k</sub>). This work highlights the interspecific variability in physiological and photosynthetic traits among native strains, which can be promising candidates for aquaculture and biotechnology applications requiring robust photosynthetic performance.

**Keywords:** Microalgae; growth rate; chlorophyll *a* fluorescence; photosynthesis performance; electron transport rate.

## RESUMEN

Para evaluar el estado fisiológico y la actividad fotosintética de dieciséis cepas de microalgas aisladas de Baja California, se realizaron estimaciones de crecimiento y mediciones de fluorescencia *in vivo* de clorofila *a* (Chl-*a*). La bacilariofita *Diploneis* sp. presentó la mayor tasa de crecimiento, mientras que las mayores densidades celulares se observaron en *Tetraselmis suecica* y *Navicula* sp. cepa 2. La mayoría de las cepas mostró valores efectivos de rendimiento cuántico máximo (Fv/Fm) superiores a 0.50. Los valores más altos de tasa máxima de transporte de electrones (ETR<sub>m</sub>) e irradiancia de saturación (I<sub>k</sub>) se registraron en *Amphora* sp. cepa 6 y en *Heterococcus* sp. La diatomea *Navicula* sp. cepa 4 presentó el mayor contenido de clorofila *a* y carotenoides. Las condiciones de cultivo utilizadas en este estudio no fueron estresantes para las cepas de microalgas. En particular, *T. suecica* mostró alta densidad celular máxima y valores elevados de Fv/Fm; *Amphora* sp. cepa 6 presentó la mayor tasa de trans-

porte de electrones (ETR<sub>m</sub>) e irradiancia de saturación (I<sub>k</sub>) elevada. Este trabajo resalta la variabilidad interespecífica en los rasgos fisiológicos y fotosintéticos entre cepas nativas, las cuales pueden ser candidatas promisorias para aplicaciones en acuicultura y procesos biotecnológicos que requieren un desempeño fotosintético robusto.

**Palabras clave:** microalgas; tasa de crecimiento; fluorescencia de clorofila *a*; desempeño fotosintético.

## INTRODUCTION

Marine phytoplankton are diverse communities of microscopic photosynthetic organisms that play a fundamental role in global biogeochemical cycles. They account for approximately 50 % of the Earth's primary productivity (Crockford *et al.*, 2023) and include cells ranging from 0.2 to 2000 μm in size (Haëntjens *et al.*, 2022). The major taxonomic groups contributing to marine productivity include bacillariophytes, dinoflagellates, and coccolithophores. Additional contributors to marine diversity and productivity are green algae, cyanobacteria, haptophytes, cryptophytes, and euglenophytes (Simon *et al.*, 2009; Calbet, 2024). Despite the high diversity of phytoplankton, only a fraction of 30,000 existing species have been formally described to date (Thoré *et al.*, 2023). Thus, continued efforts in isolating, describing, and characterizing marine microalgae species are essential to better understand ocean productivity and to explore their potential applications in aquaculture, biotechnology, and the food industry.

One approach to characterizing microalgae involves assessing their photosynthetic performance through chlorophyll fluorescence measurements. These measurements provide insights into the physiological status of phytoplankton cells (Juneau and Harrison, 2005) and are commonly obtained using Pulse Amplitude Modulation (PAM) fluorometry (Figueredo *et al.*, 2009). These devices focus on Photosystem II (PSII), and key parameters—such as the maximum quantum yield (Fv/Fm), electron transport rate (ETR), saturation irradiance (I<sub>k</sub>), and photosynthetic efficiency (α)—which are widely used to evaluate cellular responses under varying environmental conditions (White *et al.*, 2011; Sánchez-Saavedra *et al.*, 2018; Vani *et al.*, 2023; Krivina *et al.*, 2023).

A previous study by Jiménez-Valera and Sánchez-Saavedra (2016) characterized the growth and fatty acid profiles of

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21 microalgae strains isolated from the northeastern coastal waters of Baja California, Mexico. That study highlighted the biotechnological potential of these strains, particularly for aquaculture, due to favorable attributes such as small cell size, rapid growth, absence of toxicity, and the presence of polyunsaturated fatty acids (PUFAs), which are essential nutrients for fish, crustaceans, and mollusk larvae. However, no information is yet available on the photosynthetic performance of these strains.

The objective of this work was to evaluate the photosynthetic response of 16 microalgae strains isolated from Baja California, Mexico, to identify suitable light levels for their cultivation and optimization. These results provide a basis for future studies on the potential applications of these strains in aquaculture and biotechnology.

## MATERIAL AND METHODS

### Microalgae strain characteristics

We used 16 microalgae strains previously isolated by Jiménez-Valera and Sánchez-Saavedra (2016) from coastal waters of Ensenada and San Quintín in Baja California, and Mulegé in Baja California Sur, Mexico. The strains included the chlorophyte *Tetraselmis suecica*, the xanthophyte *Heterococcus* sp., and 14 bacillariophytes: *Amphora* sp. (strains 1, 2, 4, 5, 6, and 7), *Navicula* sp. (strains 2, 3, and 4), *Cymbella* sp. (strains 1 and 2), *Nitzschia thermalis*, *Diploneis* sp., and *Rhabdonema* sp.

Non-axenic, monospecific cultures were maintained in 250 mL Erlenmeyer flasks containing 100 mL of "f" medium (Guillard and Ryther, 1962) at 20 °C, salinity of 33 ± 1 ‰, and under continuous light (24 h) at an irradiance of 50 μmol photons m<sup>-2</sup> s<sup>-1</sup>, provided by cool white fluorescent lamps. After 10 d of preculture, the microalgae were inoculated into fresh 100 mL "f" medium in new 250 mL flasks and maintained under identical culture conditions with daily manual stirring. All microalgae strain cultures were carried out in triplicate. Initial cell densities were 0.05 × 10<sup>6</sup> cells mL<sup>-1</sup> for *Rhabdonema* sp. and *Diploneis* sp., 0.1 × 10<sup>6</sup> cells mL<sup>-1</sup> for *Navicula* sp. strain 3 and *Amphora* species, 0.25 × 10<sup>6</sup> cells mL<sup>-1</sup> for *N. thermalis* and *Cymbella* sp. strains 1 and 2, 0.4 × 10<sup>6</sup> cells mL<sup>-1</sup> for *Heterococcus* sp., and 1.0 × 10<sup>6</sup> cells mL<sup>-1</sup> for *T. suecica*.

Cell density and maximum cell density (MCD) were measured every 48 h over 12 d using direct cell counts with a hemocytometer and compound microscope (Olympus CX-31, Japan). Growth rate (μ) and generation time (GT) were calculated using the equations described by Fogg and Thake (1987). For the growth rate:

$$\mu = \frac{(\log_2 N_2) - (\log_2 N_1)}{t_2 - t_1} \quad \text{Eq. (1)}$$

Where, μ is the specific growth rate; N<sub>2</sub> is the cell concentration at the end of the exponential growth phase; N<sub>1</sub> is the cell concentration at the beginning of the exponential growth phase; Log<sub>2</sub> is the logarithm base 2 of the cell concentration; t<sub>2</sub> the final time of the exponential growth phase; and t<sub>1</sub> initial time of the exponential growth phase.

Generation time was calculated according to the following equation:

$$\text{TG} = 1/\mu \quad \text{Eq. (2)}$$

Where TG is the generation time and μ is the growth rate.

### Pigment determination

Chlorophyll *a* (Chl-*a*), chlorophyll *b* (Chl-*b*), chlorophyll *c* (Chl-*c*), and total carotenoids were extracted following Parsons *et al.* (1984). Samples of 5 mL of each microalgae culture were filtered through 25 mm glass fiber filters (GF/C, 1 μm pore size). The filtered samples were extracted with 3 mL of 90 % acetone solution, incubated overnight at 4 °C in darkness, and spectra (400 to 750 nm) were recorded using a spectrophotometer (HACH DR-6000, HACH, USA). Data obtained were used to calculate pigment concentrations according to Jeffrey and Humphrey (1975):

$$\text{Chl- } a \text{ (}\mu\text{g mL}^{-1}\text{)} = -0.08 A_{630} - 1.54 A_{647} + 11.85 A_{664} \quad \text{Eq. (3)}$$

$$\text{Chl- } b \text{ (}\mu\text{g mL}^{-1}\text{)} = -2.66 A_{630} + 21.03 A_{647} - 5.43 A_{664} \quad \text{Eq. (4)}$$

$$\text{Chl- } c \text{ (}\mu\text{g mL}^{-1}\text{)} = 24.52 A_{630} - 7.60 A_{647} - 1.67 A_{664} \quad \text{Eq. (5)}$$

$$\text{Carotenes (}\mu\text{g mL}^{-1}\text{)} = 7.6 A_{480} - 1.49 A_{510} \quad \text{Eq. (6)}$$

$$\text{Final concentration} = \text{mg pigment m}^{-3} = \frac{C \times v}{V \times 10} \quad \text{Eq. (7)}$$

where: A is the corrected absorbance at the wavelength indicated; C is the concentration of each pigment calculated according to equations 3 to 6; v is the 90 % acetone volume used for the extraction (expressed in mL), and V is the sample volume filtered (expressed in liters). Wavelength corrections were applied by subtracting 1x the absorbance of 750 nm from the absorbances of 630, 647, and 664 nm; 2x from the absorbance at 510 nm, and 3x from the absorbance at 480 nm. Pigment concentrations were expressed in μg mL<sup>-1</sup> to represent the content for each microalgae strain. For absorption measurements used in the estimation of photosynthetic parameters, pigment concentrations were expressed as mg m<sup>-3</sup>.

### In vivo chlorophyll *a* fluorescence measurements

Photosynthetic activity was assessed on day 3 by measuring *in vivo* chlorophyll *a* fluorescence. Triplicates of 10 mL samples, a sample from each flask, were dark-adapted for 20 min to oxidize the PSII reaction centers. Rapid light curves (RLC) were obtained with a pulse-amplitude modulation fluorometer (Junior-PAM, Heinz Walz, GmbH, Germany) operated with WinControl software. To ensure optimal signal quality across replicates and species, settings of intensity, frequency, and gain of actinic light were adjusted to achieve a fluorescence yield (Ft) between 200 and 400 mV. The RLC measurements followed the Universal Light curve protocol (WinControl), and electron transport rate (ETR) was calculated according to Schreiber *et al.* (1995):



$$ETR = \frac{\Delta F}{F_m} * a^*(\lambda) * E * FII \text{ (}\mu\text{mol } e^- \text{ (mg Chl-}a\text{)}^{-1} \text{ m}^{-2} \text{ s}^{-1}\text{)} \quad (\text{Eq. 8})$$

For this, the effective quantum yield ( $\Delta F/F'_m$ ) is calculated according to Schreiber *et al.* (1995) as:

$$\frac{\Delta F}{F'_m} = (F'_m - F_t) / F'_m \quad (\text{Eq. 9})$$

Where,  $F'_m$  is the maximum fluorescence induced by a saturating light pulse;  $F_t$  is the steady-state fluorescence of light-adapted algae;  $a^*(\lambda)$  is the chlorophyll *a* (Chl *a*) specific absorption of phytoplankton based on the chlorophyll *a* content (expressed in  $\text{mg m}^{-3}$ );  $E$  is the photosynthetically active radiation (PAR) (expressed in  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ); FII is the fraction of light absorbed by photosystem II. The FII values were obtained from Johansen and Sakshaug (2007) and were 0.8 for bacillariophytes and 0.5 for chlorophyte and xanthophyte. To calculate the absorption coefficient  $a(\lambda)$  was obtained as follows:

$$a(\lambda) = (2.303 OD_\lambda I^{-1}) / \text{Chl } a \text{ content (mg m}^{-3}\text{)} \quad (\text{Eq. 10})$$

where  $OD_\lambda$  is the spectral optical density in the visible range (400 to 750 nm), and 2.303 is the conversion factor from base-10 logarithm to natural logarithm ( $\log_{10}/\log_e$ ). The maximum quantum yield of PSII ( $F_v/F_m$ ) was calculated using the following equation:

$$F_v/F_m = (F_m - F_0) / F_m \quad (\text{Eq. 11})$$

where  $F_m$  is the maximum fluorescence and  $F_0$  is the minimum fluorescence.

Photosynthetic parameters—maximum electron transport rate ( $ETR_m$ ), photosynthetic efficiency ( $\alpha$ ), and saturation irradiance ( $I_k$ )—were estimated from  $F_0$  and  $F_m$  values obtained from rapid light curves, from absorbance  $a^*(\lambda)$  as calculated as previously described, and from the fraction of light absorbed by photosystem II (FII), depending on the microalgae group analyzed. This information was integrated, and photosynthetic parameters were calculated using the hyperbolic tangential function of Eilers and Peeters (1988).

### Statistical analysis

Normality and homoscedasticity of data were verified. Differences in growth, pigment concentrations, and photosynthetic parameters were analyzed using the Kruskal-Wallis test, followed by a Tukey *a posteriori* test when significant differences were found. Statistical significance was set at  $p < 0.05$ . Data were analyzed using Statistica 7.0, and graphs were generated with Origin Pro 8.0.

## RESULTS AND DISCUSSION

Microalgae strains exhibited significant differences in growth parameters ( $p < 0.05$ ) (Table 1, Figure 1). The highest growth rates ( $\mu$ ) were observed in the diatoms *Diploneis* sp. ( $0.52 \pm 0.02$  divisions  $\text{d}^{-1}$ ), *Navicula* sp. strain 3 ( $0.41 \pm 0.04$

divisions  $\text{d}^{-1}$ ), and *Amphora* sp. strain 5 ( $0.35 \pm 0.04$  divisions  $\text{d}^{-1}$ ). In contrast, the xanthophyte *Heterococcus* sp. showed the lowest growth rate ( $0.16 \pm 0.01$  divisions  $\text{d}^{-1}$ ). An inverse trend was observed for generation time (GT): *Heterococcus* sp. had the longest GT ( $6.19 \pm 0.25$  ds), while *Diploneis* sp. showed the shortest ( $1.69 \pm 0.38$  ds). On average, most strains remained in exponential growth for 8 ds; however, *Amphora* sp. strain 26, *Nizschia thermalis*, and *Cymbella* sp. strain 2 showed extended exponential phases of 13, 11, and 11 d, respectively. The shortest exponential growth phases were observed in *Navicula* sp. strain 3, *Cymbella* sp. strain 1, and *Amphora* sp. strain 2, with durations of 7, 7, and 5 d, respectively. The growth rates of all microalgae strains in this study were lower than those reported by Jiménez-Valera and Sánchez-Saavedra (2016), who conducted a preliminary characterization of the same strains. This reduction may be attributed to longer exponential growth phases observed in our cultures, which typically lower the calculated growth rate. For *Navicula* sp. ( $0.35$  divisions  $\text{day}^{-1}$ ) and *Cymbella* sp. ( $0.30$  divisions  $\text{day}^{-1}$ ), growth rates were similar to those reported by other authors (Correa-Reyes *et al.*, 2001; Khatoon *et al.*, 2010). The chlorophyte *Tetraselmis suecica* and the diatoms *Navicula* sp. strain 2 and *Nizschia thermalis* reached the highest cell densities ( $46.79 \pm 0.38$ ,  $46.37 \pm 2.18$ , and  $44.49 \pm 1.59 \times 10^5$  cells  $\text{mL}^{-1}$ , respectively). Conversely, *Cymbella* sp. strain 1 and *Diploneis* sp. exhibited the lowest densities ( $3.65 \pm 0.25 \times 10^5$  cells  $\text{mL}^{-1}$  for both). In this study, *Tetraselmis suecica*, *Navicula* sp. strain 2, and *Nizschia thermalis* reached values close to  $4 \times 10^6$  cells  $\text{mL}^{-1}$ , which were higher than those observed by Jiménez-Valera and Sánchez-Saavedra (2016). Temperature, medium, and agitation conditions used in this study were the same as those used by Jiménez-Valera and Sánchez-Saavedra (2016), except for the irradiance level. Thus, differences in growth rate may be attributed to variations in irradiance conditions: we used  $50 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , whereas the previous study used  $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ .

*Tetraselmis suecica* is a widely studied species due to its importance in aquaculture and biotechnology (Grabowsky, 2017; Rentería-Mexía *et al.*, 2022). In aquaculture, *T. suecica* is one of the most commonly used microalgae species as feed for fish, crustaceans, and mollusk larvae due to its cell size, biochemical composition, ease of culture, and digestibility (Yigitkurt *et al.*, 2025). This microalgae species is widely used in wastewater bioremediation (Andreotti *et al.*, 2020), as a strategy to control the density of pathogenic *Vibrio* species (Smahajcsik *et al.*, 2025), and for the production of compounds with anticancer, antibacterial, and anti-inflammatory activities (Rentería-Mexía *et al.*, 2022). The higher cell densities observed here, compared with previous studies, are likely related to differences in nutrient availability, salinity, photoperiod, and possibly strain variation.

Another relevant factor influencing growth is the initial inoculum density. In our study, higher inoculum concentrations may have contributed to lower growth rates, consistent with findings by Jiménez-Valera and Sánchez-Saavedra (2016) and Michels *et al.* (2012), who reported that high initial

**Table 1.** Mean values and standard deviation of growth rate ( $\mu$ : divisions day<sup>-1</sup>), generation time (GT: d), maximum cell density (MCD: cells mL<sup>-1</sup> × 10<sup>5</sup>) and days in exponential growth phase (EGP: days) for 16 microalgae strains isolated from Baja California, Mexico. Letters indicate significant differences by non-parametric ANOVA Kruskal Wallis,  $n = 3$ ,  $\alpha = 0.05$ ,  $a > b > c > d > e > f > g > h > i$ .

**Tabla 1.** Valores promedio y desviación estándar de la tasa de crecimiento ( $\mu$ : divisiones día<sup>-1</sup>), tiempo de generación (GT: días), densidad celular máxima (MCD: células mL<sup>-1</sup> × 10<sup>5</sup>) y días en fase de crecimiento exponencial (TEP: días) de 16 cepas de microalgas aisladas de Baja California, México. Letras indican diferencias significativas por la prueba no paramétrica ANOVA Kruskal Wallis,  $n = 3$ ,  $\alpha = 0.05$ ,  $a > b > c > d > e > f > g > h > i$ .

Group	Species	$\mu$		GT		MCD		EGP	
Chlorophytes	<i>Tetraselmis suecica</i>	0.20	± 0.01 gh	4.97	± 0.35 b	46.79	± 1.68 a	9.00	
Xanthophytes	<i>Heterococcus</i> sp.	0.16	± 0.01 h	6.19	± 0.25 a	10.16	± 0.19 cd	8.00	
Bacillariophytes	<i>Amphora</i> sp. strain 1	0.28	± 0.02 def	3.53	± 0.25 def	6.62	± 0.21 efgh	8.00	
	<i>Amphora</i> sp. strain 2	0.34	± 0.01 bcd	2.88	± 0.15 efg	6.59	± 0.15 efgh	5.00	
	<i>Amphora</i> sp. strain 4	0.31	± 0.01 cde	3.20	± 0.05 def	5.94	± 0.02 fghi	8.00	
	<i>Amphora</i> sp. strain 5	0.35	± 0.04 bc	2.82	± 0.40 fg	8.15	± 0.27 def	9.00	
	<i>Amphora</i> sp. strain 6	0.21	± 0.01 gh	4.71	± 0.04 b	8.97	± 0.06 cde	8.00	
	<i>Amphora</i> sp. strain 7	0.30	± 0.01 cde	3.26	± 0.12 def	4.87	± 0.58 hi	8.00	
	<i>Navicula</i> sp. strain 2	0.29	± 0.01 cdef	3.42	± 0.14 def	46.37	± 2.18 a	8.00	
	<i>Navicula</i> sp. strain 3	0.41	± 0.04 b	2.45	± 0.27 gh	8.02	± 1.41 defg	7.00	
	<i>Navicula</i> sp. strain 4	0.22	± 0.02 fg	4.38	± 0.45 bc	11.45	± 0.52 bc	10.00	
	<i>Cymbella</i> sp. strain 1	0.31	± 0.01 cde	3.20	± 0.11 def	14.07	± 0.39 b	7.00	
	<i>Cymbella</i> sp. strain 2	0.27	± 0.01 efg	3.76	± 0.16 cd	3.65	± 0.25 i	11.00	
	<i>Nitzschia thermalis</i>	0.29	± 0.01 cdef	3.39	± 0.03 def	44.49	± 1.59 a	11.00	
	<i>Diploneis</i> sp.	0.52	± 0.02 a	1.69	± 0.38 h	3.65	± 0.25 i	5.00	
	<i>Rhabdonema</i> sp.	0.28	± 0.02 def	3.54	± 0.33 de	5.27	± 0.29 ghi	13.00	

cell densities reduce light availability within bioreactors, limiting cell division. For *Navicula* sp., the growth rate obtained in this work was 0.35 divisions day<sup>-1</sup>, which is similar to the 0.29 divisions day<sup>-1</sup> reported by Correa-Reyes *et al.* (2001) and 0.37 divisions day<sup>-1</sup> obtained by Khatoon *et al.* (2010). For *Cymbella* sp. strain 1, the growth rate was 0.31 divisions day<sup>-1</sup>, whereas for *Cymbella* sp. strain 2 was 0.27 divisions day<sup>-1</sup>. These results are consistent with the 0.35 divisions day<sup>-1</sup> reported by Khatoon *et al.* (2010). It is important to note that the irradiance used by Correa-Reyes *et al.* (2001) was 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , whereas the culture medium and temperature conditions were the same as those used in this study. In the study of Khatoon *et al.* (2010), cultures were maintained at 28 °C, using Conway medium, and an irradiance of 32  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , which is similar to that used in this study.

#### Pigment content and photosynthetic activity

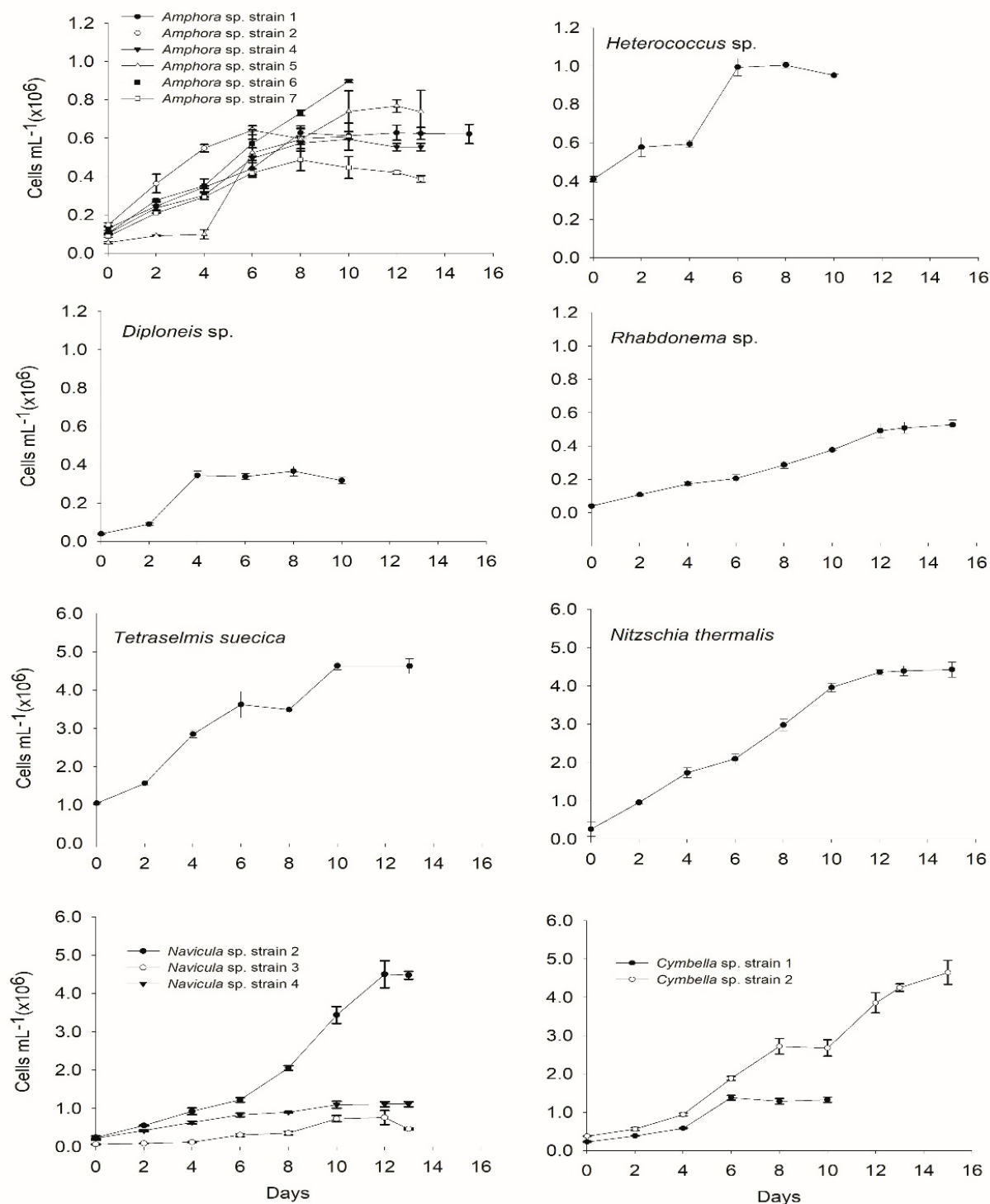
Chlorophyll *a* (Chl-*a*) was the most abundant pigment across all strains, with concentrations ranging from  $2.42 \pm 0.35 \mu\text{g mL}^{-1}$  in *Navicula* sp. strain 4 to  $0.18 \pm 0.03 \mu\text{g mL}^{-1}$  in *Navicula* sp. strain 3. Chlorophyll *b* (Chl-*b*) was consistently lower, with the highest concentration found in *Heterococcus* sp. ( $0.52 \pm 0.03 \mu\text{g mL}^{-1}$ ). The highest carotenoid content was recorded in *Navicula* sp. strain 4 ( $0.71 \pm 0.00 \mu\text{g mL}^{-1}$ ), while the lowest values were observed in *Tetraselmis suecica* ( $0.04 \pm 0.00$ ), *Amphora* sp. strain 4 ( $0.05 \pm 0.00$ ), *Cymbella* sp. strain 1 ( $0.05 \pm 0.04$ ), and *Nitzschia thermalis* ( $0.05 \pm 0.01 \mu\text{g mL}^{-1}$ ) (Table 2). Pigments found in diatom species stu-

died here are in consistent with those reported for diatoms (Sharma *et al.*, 2023), whereas the pigments detected in *T. suecica* and *Heterococcus* sp. are similar to those reported in the literature for these microalgae species (Serive *et al.*, 2017; Casian-González, 2020).

ETR curves showed high variability among species, with values ranging from 3 to 25  $\mu\text{mol e}^{-} \text{mg Chl-}a^{-1} \text{s}^{-1}$ . The light intensities used did not lead to photoinhibition (Figure 2). Variations in ETR curves are related to differences in the photosynthetic apparatus, pigment content, and light adaptation of the analyzed microalgae strain. For example, the light-harvesting antenna of diatoms differs from green algae due to the presence of fucoxanthin-chlorophyll *a/c* protein complexes, and there is evidence that the organization and structure of the photosynthetic apparatus can vary among different diatom species. It is plausible that this heterogeneity in pigment composition and architecture of the photosynthetic machinery may lead to wide photosynthetic responses among different microalgae groups or even among species within the same group (Arshad *et al.*, 2021).

Significant differences were observed among strains in Fv/Fm, photosynthetic efficiency ( $\alpha$ ), maximum electron transport rate (ETR<sub>m</sub>), and saturation irradiance ( $I_k$ ) ( $p < 0.05$ ) (Table 3). Fv/Fm ranged from  $0.74 \pm 0.01$  in *Tetraselmis suecica* to  $0.47 \pm 0.03$  in *Navicula* sp. strain 3. Most strains exhibited photosynthetic efficiency values ( $\alpha$ ) between 1.0 and  $2.0 \times 10^{-2}$ , although *Tetraselmis suecica*, *Rhabdonema* sp., and *Nitzschia thermalis* recorded higher values (7.0, 3.0, and





**Figure 1.** Mean values and standard deviation of cell density (cells mL<sup>-1</sup> × 10<sup>6</sup>) for 16 microalgae strains isolated from Baja California, Mexico.

**Figura 1.** Valores promedio y desviación estándar de la densidad celular (células mL<sup>-1</sup> × 10<sup>6</sup>) de 16 cepas de microalgas aisladas de Baja California, México.

**Table 2.** Mean values and standard deviations of chlorophyll *a*, *b*, *c*, and carotenoids (Chl-*a*, Chl-*b*, Chl-*c*, and carotenoids, respectively;  $\mu\text{g mL}^{-1}$ ) in 16 microalgae strains isolated from Baja California, Mexico. Letters indicate significant differences based on non-parametric ANOVA (Kruskal-Wallis test),  $n = 3$ ,  $\alpha = 0.05$ ;  $a > b > c > d > e > f > g > h > i > j$ .

**Tabla 2.** Valores promedio y desviación estándar del contenido de clorofila *a*, *b*, *c* y carotenoides (Chl-*a*, Chl-*b*, Chl-*c* y carotenos, respectivamente, en  $\mu\text{g mL}^{-1}$ ) de 16 cepas de microalgas aisladas de Baja California, México. Letras indican diferencias significativas por ANOVA no paramétrico Kruskal Wallis,  $n = 3$ ,  $\alpha = 0.05$ ,  $a > b > c > d > e > f > g > h > i > j$ .

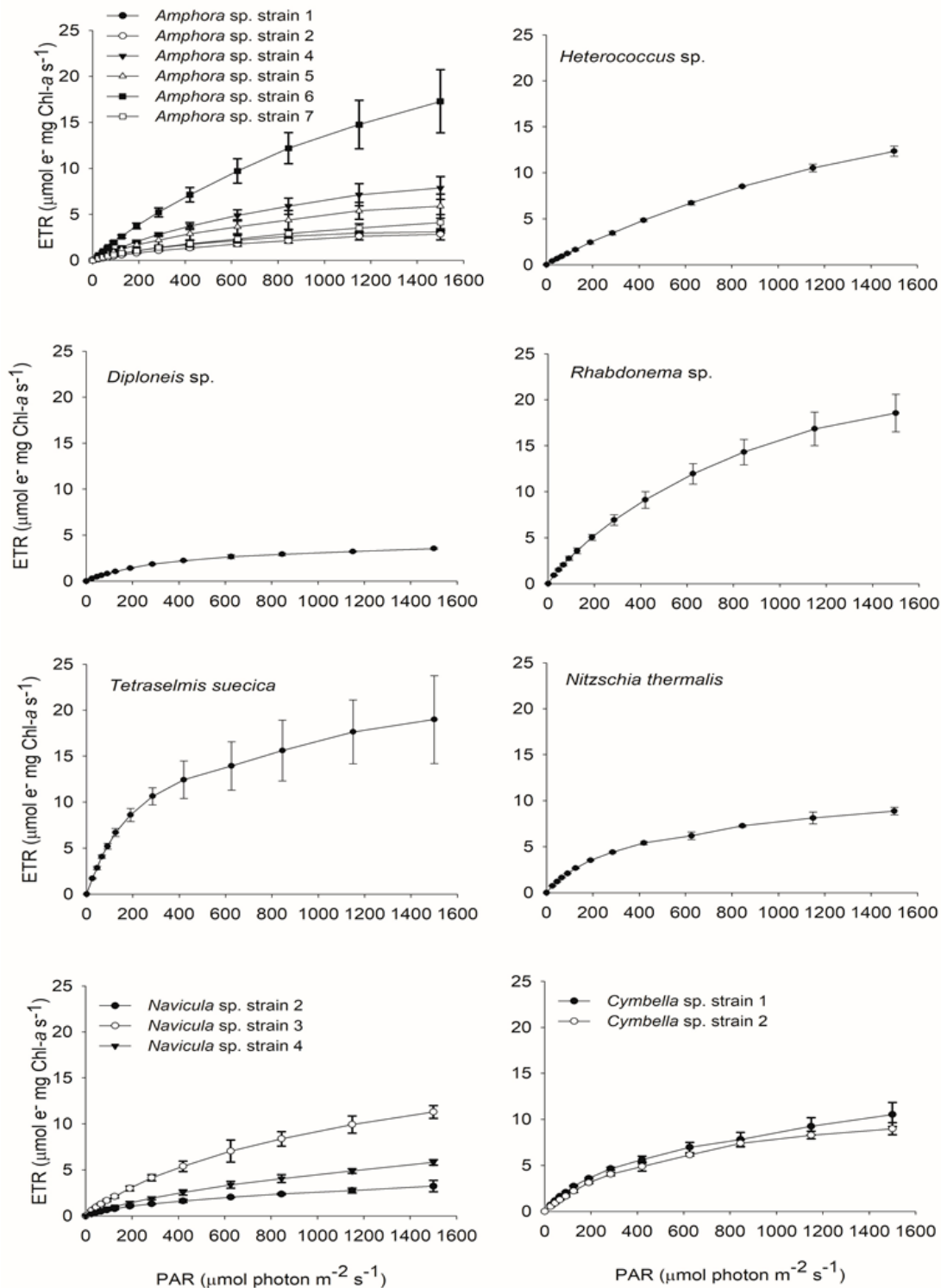
Group	Species	Chl- <i>a</i>		Chl- <i>b</i>		Chl- <i>c</i>		Carotenos	
Chlorophytes	<i>Tetraselmis suecica</i>	0.36	± 0.01 fg	0.15	± 0.01 b	0.02	± 0.00 j	0.04	± 0.00 e
Xanthophytes	<i>Heterococcus</i> sp.	0.73	± 0.11 cd	0.52	± 0.08 a	0.05	± 0.02 hij	0.09	± 0.02 cd
Bacillariophytes	<i>Amphora</i> sp. strain 1	1.14	± 0.13 b	0.02	± 0.01 c	0.12	± 0.01 cdefg	0.21	± 0.01 b
	<i>Amphora</i> sp. strain 2	0.94	± 0.09 bc	0.07	± 0.05 c	0.11	± 0.04 defgh	0.16	± 0.02 bc
	<i>Amphora</i> sp. strain 4	0.70	± 0.11 cd	0.06	± 0.01 c	0.16	± 0.03 cde	0.05	± 0.00 e
	<i>Amphora</i> sp. strain 5	0.22	± 0.10 g	0.06	± 0.01 c	0.06	± 0.03 ghij		ND
	<i>Amphora</i> sp. strain 6	0.43	± 0.06 ef	0.03	± 0.01 c	0.08	± 0.01 fghij	0.05	± 0.00 e
	<i>Amphora</i> sp. strain 7	0.92	± 0.06 bc	0.07	± 0.01 c	0.26	± 0.02 b	0.21	± 0.07 b
	<i>Navicula</i> sp. strain 2	0.48	± 0.06 def		ND	0.09	± 0.01 efgh	0.16	± 0.02 bc
	<i>Navicula</i> sp. strain 3	0.18	± 0.03 g	0.03	± 0.01 c	0.03	± 0.00 ij		ND
	<i>Navicula</i> sp. strain 4	2.42	± 0.35 a		ND	0.48	± 0.05 a	0.71	± 0.00 a
	<i>Cymbella</i> sp. strain 1	0.35	± 0.02 fg	0.02	± 0.01 c	0.06	± 0.01 ghij	0.05	± 0.04 e
	<i>Cymbella</i> sp. strain 2	0.64	± 0.08 cde	0.02	± 0.01 c	0.14	± 0.01 cdef	0.16	± 0.03 bc
	<i>Nitzschia thermalis</i>	0.46	± 0.09 def	0.02	± 0.01 c	0.17	± 0.02 cd	0.05	± 0.01 e
	<i>Diploneis</i> sp.	0.61	± 0.05 de	0.04	± 0.01 c	0.18	± 0.02 c	0.11	± 0.05 cd
	<i>Rhabdonema</i> sp.	0.36	± 0.06 fg	0.03	± 0.02 c	0.09	± 0.02 fghi	0.08	± 0.03 cd

$3.0 \times 10^{-2}$ , respectively). The highest  $\text{ETR}_m$  was obtained in *Amphora* sp. strain 6 ( $44.34 \pm 1.51 \mu\text{mol e}^- \text{mg Chl-}a^{-1} \text{s}^{-1}$ ), followed by *Heterococcus* sp. and *Rhabdonema* sp. ( $34.37 \pm 1.24$  and  $30.72 \pm 4.27 \mu\text{mol e}^- \text{mg Chl-}a^{-1} \text{s}^{-1}$ , respectively). The lowest  $\text{ETR}_m$  values ( $3\text{--}4 \mu\text{mol e}^- \text{mg Chl-}a^{-1} \text{s}^{-1}$ ) were observed in *Amphora* sp. strains 1, 2, and 7, and *Navicula* sp. strain 2. Regarding saturation irradiance ( $I_k$ ), most strains had values between 400 and 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Exceptions included *Heterococcus* sp. ( $2582.12 \pm 5.77 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), *Amphora* sp. strain 6 ( $2059.59 \pm 249.21$ ), and *Navicula* sp. strain 4 ( $1653.13 \pm 321.15$ ), which exhibited the highest  $I_k$  values. The lowest  $I_k$  values were recorded in *Tetraselmis suecica* ( $235.81 \pm 24.59$ ) and *Navicula* sp. strain 2 ( $310.17 \pm 162.08 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) (Table 3).

*In vivo* chlorophyll *a* fluorescence is a rapid, non-invasive method to assess photosynthetic performance and physiological status in algae. Light intensity, temperature, and nutrient levels affect the photosynthetic apparatus and, consequently, fluorescence (Malapascua *et al.*, 2014; Gebara *et al.*, 2023). To our knowledge, only one study (Mercado *et al.*, 2004) has examined the photosynthetic activity of microalgae strains from the Baja California Peninsula. That study measured  $I_k$  values ranging from 12 to 43  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in benthic diatoms, consistent with values reported for other subtidal communities. Species such as *T. suecica* and *N. thermalis* showed high values of photosynthetic efficiency ( $\alpha$ ) and lower values of  $I_k$ . This response is expected for low-light acclimation cells, which adjust their physiology

to optimize light-harvesting efficiency (Perkins *et al.*, 2006). High values of  $\alpha$ ,  $\text{ETR}_m$ , and  $I_k$  can be associated with efficient light utilization and the capacity of photoadaptation to high irradiances (Pérez-Varillas and Sánchez-Saavedra, 2025). This suggests that the diversity of photosynthetic strategies in the microalgae studied here is species-specific and can be linked to the environmental sites from which they were isolated. In the benthic diatom *Navicula phyllepta*,  $I_k$  values around 800  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  were obtained under light intensities of 25 and 400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , and no photoinhibition was detected in the rapid light curves (Perkins *et al.*, 2006). Similar  $I_k$  values were obtained in this study for *Amphora* sp. strains 2, 4, 5, 6, and 7, *Navicula* sp. strains 3 and 4, *Cymbella* sp. strains 1 and 2, and for *Rhabdonema* sp.  $\text{ETR}_m$  values observed in *Amphora* sp. strain 6 and *Rhabdonema* sp. in this study were comparable to the  $31.6 \pm 1.1 \mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$  reported for *Amphora coffeaeformis* (Torres *et al.*, 2013).

The Fv/Fm ratio, widely used to assess cellular health, typically ranges from 0.5 to 0.8 under non-stressful conditions; lower values suggest stress or cell death (Bobco, 2014). Tan *et al.* (2019) reported average Fv/Fm values by algal group: Chlorophyta (0.71) > Cryptophyta (0.62) > Bacillariophyta  $\approx$  Chrysophyta (0.60) > Xanthophyceae (0.54) > Pyrrophyta (0.51). Our Fv/Fm results for the chlorophyte *T. suecica* ( $0.74 \pm 0.01$ ) align with these values. Most strains studied here had Fv/Fm values between 0.50 and 0.72, suggesting that our culture conditions were generally non-stressful. Only *Navicula* sp. strains 2 and 3 showed slightly lower values (0.49 and



**Figure 2.** Mean values and standard deviation of electron transport rate (ETR,  $\mu\text{mol e}^- \text{mg Chl-a s}^{-1}$ ) versus Photosynthetic Active Radiation (PAR,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 16 microalgae strains isolated from Baja California, Mexico.

**Figura 2.** Valores promedio y desviación estándar de la tasa de transporte de electrones (ETR,  $\mu\text{mol e}^- \text{mg Chl-a s}^{-1}$ ) contra la radiación fotosintéticamente activa (PAR,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) de 16 cepas de microalgas aisladas de Baja California, México.

**Table 3.** Mean values and standard deviation of maximum quantum yield of photosystem II (Fv/Fm), photosynthetic efficiency ( $\alpha$  ( $\times 10^{-2}$ )  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ), maximum electron transport rate (ETR<sub>m</sub>:  $\mu\text{mol e}^{-} \text{mg Chl-}a \text{s}^{-1}$ ) and irradiance of saturation ( $I_k$ :  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) for 16 microalgae strains isolated from Baja California, Mexico. Letters indicate significant differences by non-parametric ANOVA Kruskal Wallis,  $n = 3$ ,  $\alpha = 0.05$ ,  $a > b > c > d > e > f > g > h > i$ .

**Tabla 3.** Valores promedio y desviación estándar del rendimiento cuántico máximo del fotosistema II (Fv/Fm), eficiencia fotosintética ( $\alpha$  ( $\times 10^{-2}$ )  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ), tasa de transporte de electrones máxima (ETR<sub>m</sub>:  $\mu\text{mol e}^{-} \text{mg Chl-}a \text{s}^{-1}$ ) e irradiancia de saturación ( $I_k$ :  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) de 16 cepas de microalgas aisladas de Baja California, México. Letras indican diferencias significativas por ANOVA no paramétrico Kruskal-Wallis,  $n = 3$ ,  $\alpha = 0.05$ ,  $a > b > c > d > e > f > g > h > i$ .

Group	Species	Fv/Fm		$\alpha$ ( $\times 10^{-2}$ )		ETR <sub>m</sub>		$I_k$	
Chlorophytes	<i>Tetraselmis suecica</i>	0.74	± 0.01 a	7.1	± 0.30 a	17.31	± 0.98 c	235.81	± 24.59 h
Xanthophytes	<i>Heterococcus</i> sp.	0.67	± 0.03 ab	1.00	± 0.01 d	34.37	± 1.24 b	2582.12	± 5.77 a
Bacillariophytes	<i>Amphora</i> sp. strain 1	0.63	± 0.01 bc	1.00	± 0.01 d	3.83	± 0.89 e	500.85	± 5.25 efg h
	<i>Amphora</i> sp. strain 2	0.61	± 0.02 bcde	1.00	± 0.01 d	3.34	± 0.65 e	690.07	± 157.17 def
	<i>Amphora</i> sp. strain 4	0.60	± 0.02 bcdef	1.00	± 0.01 d	10.15	± 2.71 d	910.21	± 132.72 d
	<i>Amphora</i> sp. strain 5	0.57	± 0.03 cdefg	1.00	± 0.01 d	9.63	± 3.14 d	859.15	± 131.30 d
	<i>Amphora</i> sp. strain 6	0.60	± 0.02 bcdef	2.00	± 0.01 c	44.34	± 1.51 a	2059.59	± 249.21 b
	<i>Amphora</i> sp. strain 7	0.55	± 0.02 defgh	1.00	± 0.01 d	4.13	± 1.34 e	918.99	± 196.46 d
	<i>Navicula</i> sp. strain 2	0.49	± 0.05 hi	1.00	± 0.01 d	3.02	± 0.66 e	310.17	± 162.08 gh
	<i>Navicula</i> sp. strain 3	0.47	± 0.03 i	2.00	± 0.01 c	13.86	± 3.18 cd	788.83	± 56.19 de
	<i>Navicula</i> sp. strain 4	0.52	± 0.02 ghi	1.00	± 0.01 d	12.04	± 0.52 d	1653.13	± 321.15 c
	<i>Cymbella</i> sp. strain 1	0.54	± 0.02 fghi	2.00	± 0.01 c	11.32	± 2.00 d	648.30	± 148.86 def
	<i>Cymbella</i> sp. strain 2	0.54	± 0.03 efghi	2.00	± 0.01 c	13.19	± 0.04 cd	640.79	± 38.48 defg
	<i>Nitzschia thermalis</i>	0.58	± 0.01 cdefg	3.00	± 0.01 b	11.02	± 0.11 d	421.62	± 19.47 fgh
	<i>Diploneis</i> sp.	0.60	± 0.01 bcdef	1.00	± 0.01 d	3.93	± 0.94 e	450.24	± 95.06 fgh
	<i>Rhabdonema</i> sp.	0.62	± 0.03 bcd	3.00	± 0.01 b	30.72	± 4.27 b	967.70	± 92.06 d

0.47, respectively). Based on Fv/Fm and  $I_k$  values, the light intensity used in this study ( $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) was not a limiting or stressful factor for most strains. However, some strains, such as *T. suecica* and *Navicula* sp. strain 2, did not reach high  $I_k$  values, indicating potential for adaptation to lower irradiance. In contrast, *Heterococcus* sp., *Amphora* sp. strain 6, and *Navicula* sp. strain 4 exhibited higher  $I_k$  values, consistent with adaptation to high-light environments, such as the shallow coastal waters of Ensenada, San Quintín, and Mulegé, where they were originally isolated (Bermúdez-Contreras *et al.*, 2008; Perea-Moreno and Hernández-Escobedo, 2016).

ETR<sub>m</sub> values varied among strains and were likely influenced by pigment composition, cell size, and environmental origin. For example, *Amphora* sp. strain 6, a small benthic diatom ( $5.70 \mu\text{m}$  length,  $3.68 \mu\text{m}$  width), showed the highest ETR<sub>m</sub> ( $44.34 \pm 1.51 \mu\text{mol e}^{-} \text{mg Chl-}a^{-1} \text{s}^{-1}$ ) and a moderate Chl-*a* content ( $0.43 \pm 0.06 \mu\text{g mL}^{-1}$ ). In contrast, *Amphora* sp. strain 7, a larger-celled strain ( $13.83 \mu\text{m}$  length,  $3.99 \mu\text{m}$  width), had the lowest ETR<sub>m</sub> ( $4.13 \pm 1.34$ ), indicating that larger cells may be less efficient in light capture due to self-shading and greater pigment packaging (Yun *et al.*, 2010). An interesting exception was the xanthophyte *Heterococcus* sp., which exhibited high photosynthetic activity and moderate pigment content despite being the largest strain ( $1903.13 \mu\text{m}$  in length,  $13.44 \mu\text{m}$  in width). *Heterococcus* species are known for their morphological plasticity, capable of shifting between spherical, elongated, and irregular forms depending on their life cycle stage, even under controlled

conditions (Darling *et al.*, 1987). The results of growth and photosynthetic parameters suggest that the culture conditions applied in this study did not impose stress on the microalgae strains analyzed. Therefore, these conditions can serve as a reference for laboratory cultivation of microalgae in aquaculture or biotechnology facilities.

## CONCLUSION

This work provides a comprehensive physiological and photosynthetic characterization of 16 native microalgae strains isolated from coastal environments of the Baja California Peninsula, México. The results reveal marked interspecific variability in growth, pigment composition, and photosynthetic performance, supporting their potential for diverse biotechnological applications.

Among the analyzed strains, *Tetraselmis suecica* stood out for its high Fv/Fm value ( $0.74 \pm 0.01$ ), indicating excellent physiological status under the tested conditions, and its elevated maximum cell density ( $46.79 \times 10^5 \text{ cells mL}^{-1}$ ), reinforcing its suitability for aquaculture and biomass production. Similarly, the benthic diatom *Amphora* sp. strain 6 exhibited the highest maximum electron transport rate (ETR<sub>m</sub>) at  $44.34 \pm 1.51 \mu\text{mol e}^{-} \text{mg Chl-}a^{-1} \text{s}^{-1}$  and one of the highest  $I_k$  values ( $2059.59 \pm 249.21 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), suggesting strong adaptation to high-irradiance environments. In contrast, strains such as *Navicula* sp. strain 3 exhibited both low Chl-*a* content ( $0.18 \pm 0.03 \mu\text{g mL}^{-1}$ ) and low Fv/Fm ( $0.47 \pm 0.03$ ), pointing to a reduced photosynthetic efficiency under the tested conditions.



The strain *Heterococcus* sp., despite its unusually large cell size, displayed notable photosynthetic capacity ( $ETR_m$ :  $34.37 \pm 1.24 \mu\text{mol e}^- \text{mg Chl-}a^{-1} \text{s}^{-1}$ ) and high light saturation ( $I_k$ :  $2582.12 \pm 5.77 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), highlighting its morphological plasticity and potential adaptability to fluctuating environmental conditions.

Taken together, these findings not only broaden our understanding of the physiological diversity among native microalgae from this region, but also provide a valuable baseline for selecting strains with optimal traits for targeted uses in aquaculture, sustainable bioresource development, and industries such as pharmacology and cosmeceuticals. Future studies should evaluate these strains under stress conditions or in large-scale culture systems to confirm their robustness and commercial applicability.

## CONFLICTS OF INTEREST

The authors declare that they have not conflicts of interest.

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