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Research Article

The effect of major nutrients in five levels of an f medium on growth and proximal composition of *Thalassiosira weissflogii*

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ABSTRACT. This work aims to evaluate its effect on cell density, biomass, and proximal composition in semicontinuous cultures. *Thalassiosira weissflogii* was cultivated in five culture media, for which the f medium was taken as reference, and the concentrations of nitrates, phosphates, and silicates were modified up to a factor of four in five treatments (f/4, f/2, f, 2f, and 4f). Cell density and biomass increased as the initial nutrients rose in the treatments. Protein and lipids (mg L^{-1}) in the biomass of *T. weissflogii* were higher in the 2f and 4f treatments for the three partial harvests. The lipid biomass in the cultures was higher than with proteins and carbohydrates for all treatments, with 2f and 4f being the most concentrated. The concentration of nitrates, phosphates, and silicates of the culture media, declined as the cell density increased in all cultures, decreasing more than 90% in phosphates and 94-98% for the silicates for the initial concentrations. At the cell level, the percentage of carbohydrates related to dry weight in *T. weissflogii* increased as the level of the f treatment decreased. Meanwhile, the protein fraction declined when the level of the f medium was either increased or decreased. The data reported in this study can be useful for producing *T. weissflogii* in a 30% semicontinuous system, obtaining more controlled biomass production per day with an estimated value of its proximal composition, based on the level of the f medium.

Keywords: *Thalassiosira weissflogii*; diatoms; proteins; carbohydrates; lipids; nutrient limitation

INTRODUCTION

Microalgae are the primary producers ecologically important and distributed in water bodies worldwide (George et al. 2014). Microalgae have been used for decades as food in aquaculture and as a promising source of active ingredients for drugs, cosmetics, and other industrial applications, including carotenoids and polyunsaturated fatty acids recovery, or most recently, for biofuels production (George et al. 2014).

Microalgae can be grown in open or closed controlled systems, although they are exposed to environmental variability in the former. Open systems are usually built on a large scale and are perhaps the most common systems for producing microalgae commercially (Borowitzka & Moheimani 2013). However, closed systems allow for better control over the culture than open systems, and therefore, their efficiency in biomass production is higher (Tredici 2010). On the other hand, closed systems require a high initial investment cost, but their scaling up is comparatively more complex (Chini-Zittelli et al. 2013).

An appropriate media for microalgae culture shall provide enough nutrients for adequate cell growth (Grobbelaar 2013). These media contain major nutrients called "macronutrients," such as nitrates, phosphates, and silicates. In addition, culture media also contain selected minerals and vitamins in minimal amounts (Silva-Benavides 2016). The most commonly used nutrient medium for microalgae production systems is "f" (Guillard & Ryther 1962).

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Generally, the proximal composition of microalgae varies between 30-50% protein, 0-20% carbohydrates, and between 20-40% lipids (Zhao et al. 2013a). Nevertheless, several techniques for microalgae cultures can alter such biochemical composition (Cheirsilp & Torpee 2012, García et al. 2012). For instance, lack of nutrients such as nitrogen and phosphorus in microalgae cultures increases lipids but diminishes protein, although a lower biomass production may occur (Chen et al. 2011, Jiang et al. 2012). Nutrient limitation diminishes cell density, and therefore, the maximum cumulative division rate ($\sum\mu$) is reduced (López-Elías et al. 2008). On the other hand, the amount of polyunsaturated fatty acids increases when the culture temperature decreases (Aussant et al. 2018); likewise, water salinity modifies the fatty acid composition for some microalgae species (Gu et al. 2012).

The use of artificial lighting is another important issue about microalgae cultures. For example, in photobioreactors, the amount of light can raise microalgal biomass production or modify its proximal (biochemical) composition depending on the light wavelength (Carvalho et al. 2011, Zhao et al. 2013b). The pH is also an important parameter for microalgae cultures since variations in pH can affect cell growth and biochemical composition; high alkaline levels could kill microalgae cells (Khatoon et al. 2014).

Microalga *Thalassiosira weissflogii* is considered by several hatcheries the single best alga for larval shrimp, and it has been widely used in shellfish larviculture and for feeding copepods and *Artemia* (Hemaiswarya et al. 2011). It has also been proposed as a promising candidate for the production of lipids and fucoxanthin (D'Ippolito et al. 2015, Marella & Tiwari 2020). Selected studies have evaluated the effect of major nutrient limitations on the biochemical composition of these microalgae. For example, Suroy et al. (2015) estimated the effect of stress caused by nitrates and silicates on the carbohydrates of *T. weissflogii*, finding that ribose, glucose, and galactose exhibited the highest degradation rate constants. Lin et al. (2018) investigated the effect of nitrates, phosphates, and silicates separately on the accumulation of lipids in *T. weissflogii*, showing a greater accumulation of lipids when phosphate deficiency is present. Similarly, Botte et al. (2017) verified that silicate deficiency and the use of CO_2 enhanced the production of biomass and *T. weissflogii* bioproducts. Although nutrient limitation affects microalgae's growth and biochemical composition, no studies are comparing the effect of nitrates, phosphates, and silicates (major nutrients) together at different f-medium levels using the microalgae *T. weissflogii*.

The objective of this work was to evaluate the effect of five treatments carried out, based on the medium f (in which the concentrations of nitrates, phosphates, and silicates were modified) on the cell growth, biomass, and proximal composition of *T. weissflogii*, cultivated in a closed system with partial 30% harvests.

MATERIALS AND METHODS

Culture system

The experimental units consisted of 15 L carboys installed in a room with a controlled temperature of 25°C. The LED lamps supplied the light intensity, providing the culture 10,000-12,000 lux full time. Permanent aeration was supplied to the experimental cultures by using a 2.5 Hp blower. The salinity of the water in the experiment was monitored by using a salinometer (ATAGO^{MR}), with values of 35.0 ± 0.5 throughout the experiment.

Strain

The *Thalassiosira weissflogii* diatom was selected because laboratories widely use it for shrimp larvae production in Sinaloa and Sonora, Mexico. The strain was provided by the Laboratory of Ecophysiology of Aquatic Organisms and Support Crops of the Autonomous University of Sinaloa (UAS acronym in Spanish), Faculty of Marine Sciences.

f medium

The f medium (Guillard & Ryther 1962) was used as a base for the five treatments (f/4, f/2, f, 2f, and 4f), modifying only its major nutrients, except for treatment f. In the f/2 and f/4 treatments, nitrates, phosphates, and silicates decreased in a proportion of two and four concerning the initial concentration of the medium f. In contrast, treatments 2f and 4f increased by a ratio of two to four.

Sodium nitrate ($NaNO_3$) as a nitrogen source, sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$) as a phosphorus source, and sodium metasilicate nonahydrate ($Na_2O_3Si \cdot 9H_2O$) as a silica source were used as nutrients supply.

For the cultures, seawater was filtered purified through pores of 10, 5, and 1 µm, then purified using an activated carbon filter. The water was disinfected with chlorine for at least 24 h before using it, and the residual chlorine was removed with sodium thiosulfate (Hemerick 1973).

Experimental design

Each experiment consisted of a five-day culture in which the treatments (f/4, f/2, f, 2f, and 4f) were tested. Each treatment comprises four biological replicates,

beginning with an initial inoculum of 10,000 cell mL^{-1} of *T. weissflogii*. Samples from each replicate were taken daily to measure pH, temperature, salinity, cell density, nitrates, phosphates, silicates, nitrites, and ammonium ions. The culture system was semicontinuous from the third to the final day of the experiment with a 30% harvesting rate; biomass, proteins, lipids, carbohydrates, and ash were analyzed in the *T. weissflogii*. The volume added in each harvest was replaced with filtered and disinfected seawater and the corresponding nutrients' amount for the selected treatment. The major nutrients (nitrates, phosphates, and silicates), nitrites, and ammonium ions were also measured after each injection of fresh nutrimental medium.

Population parameters

The cell density (N) of the *T. weissflogii* was determined by a Neubauer chamber. In addition, the growth rate (μ) and cumulative growth rate ($\sum\mu$) were calculated according to Arredondo-Vega & Voltolina (2007).

Proximal analysis

The samples of each *T. weissflogii* culture were filtered by Whatman GF/C 25 mm filters and were stored at -79°C until further processing. Proteins were determined according to Lowry et al. (1951) at 750 nm; carbohydrates according to Dubois et al. (1956) at 485 nm; while lipids were extracted as described by Blight & Dyer (1959) and determined according to Pande et al. (1963) at 590 nm. The dry weight (DW) was determined using Whatman GF/C 47 mm filters to take the samples, and then, such filters were weighed until reaching a constant weight in an oven at 60°C. For the inorganic weight (IW), DW filters were incinerated in an oven at 450°C and then taken to a constant weight. According to Arredondo-Vega & Voltolina (2007), the difference between DW and IW is equal to the organic weight (OW).

Determining major nutrients

In all water quality tests (nitrate, nitrite, ammonium ion, phosphate, and silicate), Whatman GF/C 47 mm filters removed suspended materials. In addition, a 1 cm quartz cell was used to read the samples in the spectrophotometer (Hach DR5000). Spectrophotometry in the ultraviolet range was performed to determine nitrates, reading at 220 nm and using a correction factor at 275 nm and a detection limit (MDL) of 0.2 mg L^{-1} $N\text{-}NO_3^-$ (Greenberg et al. 1992). Analyses of nitrite silicate, ammonium ion, and phosphate were performed using colorimetric methods. Nitrites were measured at a wavelength of 543 nm with an MDL of 0.05 mg L^{-1} $N\text{-}NO_2^-$; the ammonium ion was determined by using the indophenol blue method at 640 nm, with an MDL 0.01 mg L^{-1} $N\text{-}NH_4^+$; the phosphate quantification was read at 880 nm with an MDL of 0.03 mg L^{-1} $P\text{-}PO_4^{3-}$ (Greenberg et al. 1992). Finally, the silicates were analyzed at 810 nm with an MDL of 0.01 mg L^{-1} $Si\text{-}SiO_4^{4-}$ (Grasshoff et al. 1999). All analyses were performed with minor modifications to adjust the sample volumes.

Statistical analysis

Based on data from the biomass and proximal composition of *T. weissflogii* and water quality from the cultures, the Kolmogorov-Smirnov normality test and Levene homoscedasticity test (Zar 2010) were used to define the application of parametric or nonparametric statistical analytical methods. Data were analyzed using a unidirectional analysis of variance (ANOVA) when parametric or Kruskal-Wallis when nonparametric. When the analysis revealed significant differences, the Student-Newman-Keuls (SNK) multiple comparison tests were used to determine these differences (Zar 2010). These data were processed with SigmaStat 3.5 statistical software.

RESULTS

The effect of the major nutrients on cell density and cumulative growth rate of *Thalassiosira weissflogii*

The cell density number (cell mL^{-1}) of *T. weissflogii* was significantly different between treatments ($P < 0.05$) over time (Fig. 1). On the first day, the cell density in f/4 was significantly lower than that from the other treatments ($P < 0.05$), with a value of $0.118 \pm 0.004\times10^6$ cell mL^{-1} (Fig. 1). On the second day, the f/4 and f/2 treatments ($0.172 \pm 0.005\times10^6$ and $0.212 \pm 0.004\times10^6$ cell mL^{-1}, respectively) had lower cell densities compared to those from the other treatments ($P < 0.05$). Figure 1 shows the partial harvests from day three to five. In the first partial harvest, significant differences ($P < 0.05$) were found in cell density according to the following tendency 4f > 2f > f > f/2 > f/4, with values between $0.180 \pm 0.005\times10^6$ and $0.531 \pm 0.021\times10^6$ cell mL^{-1}. In the last partial harvest, the cell density in 2f and 4f was also significantly higher ($P < 0.05$) than that from the other treatments, with values of $0.476 \pm 0.019\times10^6$ and $0.464 \pm 0.008\times10^6$ cell mL^{-1} respectively (Fig. 1), without any difference between them.

The analysis of the cumulative growth rate ($\sum\mu$) also permitted the evaluation of the growth performance of *T. weissflogii* in the treatments and estimated the exponential growth phase (Table 1).

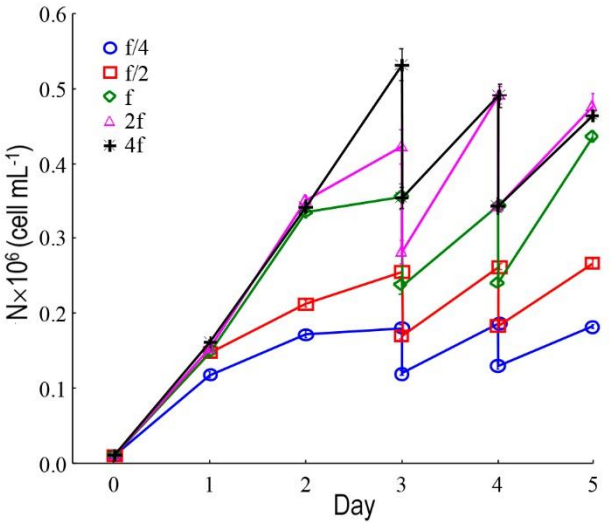

Figure 1. Average values and standard error of the cell density of *Thalassiosira weissflogii* through the five days of culture in the five treatments. N: number of cells.

From the first day to the fifth day, f/4 always had a ∑μ significantly lower ($P < 0.05$) than the other treatments, reaching a final value of 4.19 ± 0.02. On the first day, no significant differences were found in ∑μ between treatments ($P > 0.05$), except for f/4 with 4.10 ± 0.04 (Table 1). On the second day, with a ∑μ of 4.41 ± 0.03, f/2 was significantly lower ($P < 0.05$) than those from the f, 2f, and 4f treatments. On the other hand, in the first harvest, 4f presented a ∑μ of 5.73 ± 0.06, which was significantly higher ($P < 0.05$) than those from the other treatments. During the fourth and fifth days, all treatments were similar in terms of ∑μ to those observed on the day 3, indicating that the cultures reached a "quasi-stationary" state after partial harvests of 30% (Table 1). At the end of the culture, ∑μ in f (5.45 ± 0.02), 2f (5.57 ± 0.06) and 4f (5.54 ± 0.06) were significantly higher ($P < 0.05$) than those found in f/4 and f/2.

The effect of the major nutrients on the biomass and proximal composition of *Thalassiosira weissflogii*

An increase in biomass production and inorganic fraction was found as the initial concentration of the major nutrients increased in the treatments (Table 2, Fig. 2). During the first partial harvest (day 3), 2f and 4f had a biomass production per culture volume significantly greater ($P < 0.05$) than those from the rest of the treatments (Table 2). 4f reached the maximum biomass production (334.8 ± 33.8 mg L^{-1}) on the fourth day, concerning the other treatments. In contrast, f/4 and f/2 had shown the lowest biomass production values ($P < 0.05$), reaching 126.8 ± 4.7 and 140.5 ± 7.8 mg L^{-1}, respectively, at the end of the culture. Aside from the f treatment, the rest of the cultures reached their highest biomass production on day 4 (Table 2).

The percentage of OW in the biomass of *T. weissflogii* showed a significant difference ($P < 0.05$) between treatments (Fig. 2). A tendency where the OW percentage raised as the initial nutrients were lower was observed in the treatments. The exception was from f/4 to f/2 on day 3, possibly due to the lower assimilation of inorganic nutrients caused by the shortage of other nutrients such as nitrates and phosphates. The maximum OW percentage was obtained in f/2 on the day 3 with 71 ± 4%, significantly higher than those in 2f (62 ± 2%) and 4f (52 ± 1) treatments ($P < 0.05$). The percentage of IW recorded during the three partial harvests in the *T. weissflogii* cultures increased as the level of the treatments did (Fig. 2). For example, the IW achieved by 4f was about 48% in the three partial harvests, while f/4 was approximately 35%.

Significant differences were found in the concentrations of proteins, carbohydrates, and lipids between the treatments in the *T. weissflogii* cultures (Table 3). Protein concentration in *T. weissflogii* cultures (mg L^{-1}) increased when the initial nutrients did in the treatments, with approximate values of 27 mg

Table 1. Average values and standard error of the cumulative growth rates of *Thalassiosira weissflogii* in the five treatments per day and during the partial harvests. Equal or common letters indicate there are no significant differences ($P > 0.05$), a<b<c. H1: after harvest 1, H2: after harvest 2.

Day	Treatment				
	f/4	f/2	f	2f	4f
1	$3.56^{a} \pm 0.06$	$3.88^{b} \pm 0.03$	$3.88^{b} \pm 0.07$	$3.94^{b} \pm 0.06$	$4.02^{b} \pm 0.02$
2	$4.10^{a} \pm 0.04$	$4.41^{b} \pm 0.03$	$5.06^{c} \pm 0.03$	$5.13^{c} \pm 0.03$	$5.10^{c} \pm 0.04$
3	$4.17^{a} \pm 0.04$	$4.68^{b} \pm 0.04$	$5.15^{c} \pm 0.07$	$5.40^{d} \pm 0.08$	$5.73^{e} \pm 0.06$
H1	$3.59^{a} \pm 0.04$	$4.09^{b} \pm 0.04$	$4.56^{c} \pm 0.07$	$4.81^{d} \pm 0.08$	$5.14^{e} \pm 0.06$
4	$4.22^{a} \pm 0.05$	$4.71^{b} \pm 0.02$	$5.10^{c} \pm 0.03$	$5.62^{d} \pm 0.03$	$5.61^{d} \pm 0.04$
H2	$3.70^{a} \pm 0.05$	$4.20^{b} \pm 0.02$	$4.59^{c} \pm 0.03$	$5.10^{d} \pm 0.03$	$5.10^{d} \pm 0.03$
5	$4.19^{a} \pm 0.02$	$4.74^{b} \pm 0.03$	$5.45^{c} \pm 0.02$	$5.57^{c} \pm 0.06$	$5.54^{c} \pm 0.03$

Table 2. Average values and standard error of dry weight biomass production (mg L^{-1}) by *Thalassiosira weissflogii* in the five treatments for the three partial harvests. Equal or common letters indicate that there are no significant differences ($P > 0.05$) between treatments per day, a<b<c. *Indicates nonparametric test.

Day	Treatment				
	f/4	f/2	f	2f	4f
3	$152.3^{a} \pm 13.0$	$138.8^{a} \pm 9.4$	$162.0^{a} \pm 2.1$	$210.8^{b} \pm 20.9$	$241.1^{b} \pm 1.6$
4*	$167.2^{a} \pm 1.4$	$203.0^{ab} \pm 13.9$	$203.9^{ab} \pm 7.0$	$229.1^{b} \pm 10.3$	$334.8^{c} \pm 33.8$
5	$126.8^{a} \pm 4.7$	$140.5^{a} \pm 7.8$	$217.7^{b} \pm 5.9$	$214.6^{b} \pm 6.3$	$307.0^{c} \pm 9.8$

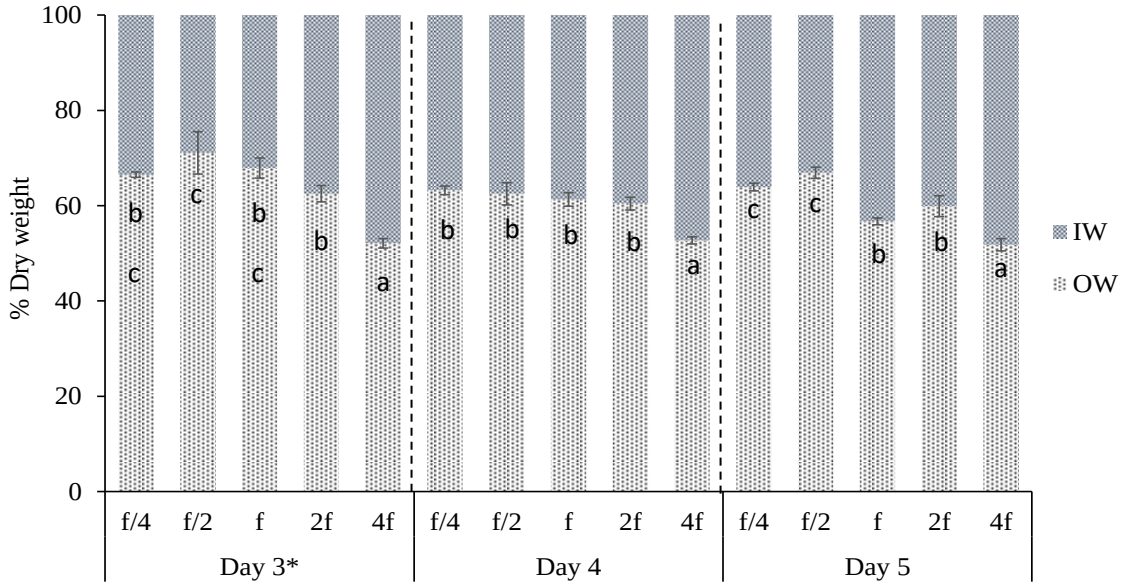

Figure 2. Average values and standard error of the percentage of organic (OW) and inorganic weight (IW) in the biomass of *Thalassiosira weissflogii* cultivated in the five treatments for the three partial harvests. Equal or common letters indicate there are no significant differences between OW percentages of the treatments per day ($P > 0.05$), a<b<c. *Indicates nonparametric test.

L^{-1} for f/4 medium to 52 mg L^{-1} for 4f, showing significant differences ($P < 0.05$) during the partial harvests between the treatments (Table 3). Maximum protein concentration was found at 2f on the day 3 with 54.7 ± 6.2 mg L^{-1}, while f/4 had the lowest concentration ($P < 0.05$) with 27.9 ± 1.1 mg L^{-1} for the same day. On the fourth day, f/4 showed the lowest protein concentration ($P < 0.05$) with 28.6 ± 0.4 mg L^{-1}, while 4f had the highest concentration of 54.1 ± 2.5 mg L^{-1} ($P < 0.05$). By the end of the experiment, the lowest protein concentration was found along time in f/4, 26.5 ± 1.2 mg L^{-1}, being statistically different from those in the other treatments ($P < 0.05$).

As for carbohydrate concentration, *T. weissflogii* cultures showed the least variation between treatments during partial harvests (Table 3). Significant differences ($P < 0.05$) in carbohydrate concentrations between treatments occurred only on the day 3. 2f and 4f showed the highest carbohydrate concentrations with 39.0 ± 4.7 and 37.0 ± 1.4 mg L^{-1} respectively, while the minimum carbohydrate concentration was found in f on the third day with 26.5 ± 1.0 mg L^{-1}. The maximum carbohydrate concentration of 39.0 ± 4.7 mg L^{-1} was found at 2f on the same day (Table 3).

The lipid concentration by volume in *T. weissflogii* cultures on the third and fourth day was significantly higher ($P < 0.05$) in 2f and 4f, reaching on day three the maximum value in the 2f treatment with 68.6 ± 3.4 mg L^{-1} (Table 3). At the end of the experiment, a lipid concentration of 42.0 ± 2.3 mg L^{-1} was observed in f/4 and 39.7 ± 1.6 mg L^{-1} in f/2, concentrations significantly lower than in the other treatments ($P < 0.05$).

Water quality in *Thalassiosira weissflogii* cultures

At the beginning of the culture, significant differences ($P < 0.05$) were found as to pH values among the treatments (Fig. 3), these differences were observed in f/4 and f/2 treatments with significantly lower pH values

Table 3. Average values and standard error (mg L^{-1}) of the amount of proteins, carbohydrates, and lipids of *Thalassiosira weissflogii* in the five treatments during partial harvests. Equal or common letters indicate that there are no significant differences ($P > 0.05$) between treatments per day, a<b<c. *Indicates nonparametric test.

Day	Proximal variable	Treatment				
		f/4	f/2	f	2f	4f
3	Protein*	$27.9^{a} \pm 1.1$	$38.3^{b} \pm 1.8$	$48.4^{b} \pm 4.6$	$54.7^{b} \pm 6.2$	$52.4^{b} \pm 4.1$
	Carbohydrates*	$30.6^{a} \pm 0.8$	$28.7^{a} \pm 2.1$	$26.5^{a} \pm 1.0$	$39.0^{b} \pm 4.7$	$37.0^{b} \pm 1.4$
	Lipids	$41.6^{a} \pm 3.6$	$40.5^{a} \pm 2.5$	$47.4^{a} \pm 0.6$	$68.6^{b} \pm 3.4$	$64.4^{b} \pm 2.5$
4	Protein	$28.6^{a} \pm 0.4$	$39.1^{b} \pm 2.5$	$45.2^{b} \pm 2.9$	$47.7^{b} \pm 2.6$	$54.1^{c} \pm 2.5$
	Carbohydrates	$33.3^{a} \pm 0.5$	$32.6^{a} \pm 2.3$	$30.0^{a} \pm 2.4$	$34.7^{a} \pm 2.4$	$38.6^{a} \pm 2.2$
	Lipids	$42.4^{a} \pm 0.6$	$42.6^{a} \pm 1.6$	$48.9^{a} \pm 0.3$	$65.7^{b} \pm 3.0$	$65.6^{b} \pm 3.7$
5	Protein*	$26.5^{a} \pm 1.2$	$37.0^{b} \pm 0.8$	$37.8^{b} \pm 2.0$	$43.6^{b} \pm 3.9$	$47.0^{b} \pm 3.0$
	Carbohydrates	$36.1^{a} \pm 0.3$	$34.2^{a} \pm 1.7$	$28.9^{a} \pm 2.5$	$33.6^{a} \pm 3.0$	$34.2^{a} \pm 1.7$
	Lipids	$42.0^{a} \pm 2.3$	$39.7^{a} \pm 1.6$	$64.7^{b} \pm 2.3$	$65.3^{b} \pm 2.0$	$63.1^{b} \pm 1.2$

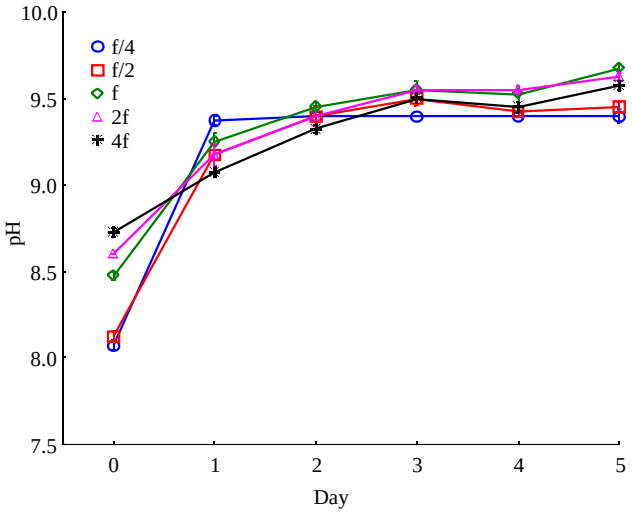

Figure 3. Average values and standard error of pH in the five treatments with *Thalassiosira weissflogii* throughout the five days of the culture.

of about 8.1 ($P < 0.05$), while 4f showed the highest value of 8.7. On the first day, only f/4 was significantly different ($P < 0.05$), having the highest pH value of 9.4 ± 0.0 among the treatments. From days 2 to 4, pH values ranged from 9.3 to 9.6 for all treatments with no significant differences between them (Fig. 3). For the fifth day, the pH value in 4f, 2f, and f was significantly higher ($P < 0.05$) than those in f/4 and f/2 media (Fig. 3).

Nitrates

During the experiment, nitrate concentration in the treatments decreased according to the following arrangement 4f > 2f > f > f/2 > f/4 (Table 4). In addition, significant differences ($P < 0.05$) were found, as indicated in Table 4. The highest nitrate consumption was found at the 4f level with a decrease of 45 mg L^{-1} from day zero to the day 3 (first partial harvest). Instead, f/4 and 2f showed the lowest nitrate consumption, with 6 and 7 mg L^{-1}, respectively.

Phosphates

Phosphate consumption in the experimental cultures was higher than nitrate consumption regarding their initial concentration (Table 5). Phosphate concentration was lower in f/4 during the five days of the culture compared to that in the others treatments. The highest phosphate concentration reduction was found in the first partial harvest in 4f, thus, decreasing 1.89 mg L^{-1} concerning the initial concentration (Table 5). Furthermore, f/4 and f/2 showed a decrease in their phosphate concentration close to 0 mg L^{-1} in their first partial harvest. Partial nutrient rechanges after microalgae biomass harvesting affected the phosphate concentration, increasing the different treatments (Table 5). The last partial harvest showed significant differences ($P < 0.05$) between f/4 and 2f.

Silicates

Silicate concentration in *T. weissflogii* cultures was almost entirely consumed on the second and day 3 of the culture (Table 6). On the day 3, all treatments had silicate concentrations below 1 mg L^{-1}; f/4, f/2 had the least ($P < 0.05$). On the day 3, no silicates in f/4 and f/2 were detected; by the end of the experiment were found in 4f. At the beginning of the experiment, silicate concentration did not show significant differences between the different treatments, except for f/2 (Table 6). At the same time, silicate concentration did not show a tendency to rise as the level of the treatments increased (Table 6).

Nitrites and ammonium ion

Throughout the experiment, nitrite concentration increased as initial concentrations of major nutrients

Table 4. Average values and standard error of nitrate concentration (mg L^{-1} $N\text{-}NO_3^-$) in the treatments throughout the research period. Equal or common letters indicate that there are no significant differences ($P > 0.05$) between treatments per day, a<b<c. *Indicates nonparametric test. H1: after harvest 1, H2: after harvest 2.

Treatment	Day							
	0	1*	2*	3*	H1*	4*	H2*	5*
f/4	$7.94^{a} \pm 0.49$	$5.85^{a} \pm 0.06$	$2.48^{a} \pm 0.52$	$1.65^{a} \pm 0.73$	$5.23^{a} \pm 1.60$	$0.37^{a} \pm 0.19$	$5.85^{a} \pm 1.08$	$2.19^{a} \pm 0.97$
f/2	$19.45^{b} \pm 1.13$	$12.99^{b} \pm 1.05$	$7.41^{b} \pm 0.29$	$6.46^{b} \pm 1.32$	$10.82^{a} \pm 1.15$	$4.49^{b} \pm 0.99$	$10.56^{a} \pm 1.35$	$7.71^{b} \pm 0.60$
f	$32.33^{c} \pm 2.19$	$24.98^{c} \pm 0.80$	$19.78^{c} \pm 0.84$	$21.35^{c} \pm 1.08$	$26.02^{b} \pm 0.18$	$28.58^{c} \pm 2.48$	$32.05^{b} \pm 2.31$	$21.56^{c} \pm 0.60$
2f	$54.92^{d} \pm 0.84$	$51.39^{d} \pm 1.27$	$43.37^{d} \pm 2.45$	$48.22^{d} \pm 5.11$	$47.55^{c} \pm 3.52$	$62.20^{d} \pm 3.98$	$54.16^{c} \pm 3.88$	$48.93^{d} \pm 2.19$
4f	$104.91^{e} \pm 1.62$	$95.64^{e} \pm 1.95$	$85.15^{e} \pm 2.95$	$60.44^{d} \pm 2.61$	$85.70^{d} \pm 4.08$	$91.69^{e} \pm 5.16$	$89.86^{d} \pm 5.02$	$82.66^{e} \pm 2.67$

Table 5. Average values and standard error of the phosphate concentration (mg L^{-1} $P\text{-}PO_4^{3-}$) in the treatments throughout the research period. Equal or common letters indicate that there are no significant differences ($P > 0.05$) between treatments per day, a<b<c. *Indicates nonparametric test. H1: after harvest 1, H2: after harvest 2.

Treatment	Day							
	0	1*	2*	3*	H1*	4*	H2*	5
f/4	$0.09^{a} \pm 0.02$	$0.09^{a} \pm 0.01$	$0.03^{a} \pm 0.00$	$0.03^{a} \pm 0.00$	$0.03^{a} \pm 0.0$	$0.15^{a} \pm 0.00$	$0.18^{a} \pm 0.01$	$0.44^{a} \pm 0.03$
f/2	$0.49^{b} \pm 0.06$	$0.32^{bn} \pm 0.00$	$0.11^{b} \pm 0.00$	$0.03^{a} \pm 0.00$	$0.09^{b} \pm 0.00$	$0.15^{a} \pm 0.00$	$0.26^{b} \pm 0.02$	$0.81^{ab} \pm 0.09$
f	$0.92^{c} \pm 0.04$	$0.57^{c} \pm 0.06$	$0.25^{c} \pm 0.26$	$0.12^{b} \pm 0.00$	$0.31^{c} \pm 0.01$	$0.27^{b} \pm 0.02$	$0.66^{cd} \pm 0.08$	$0.73^{ab} \pm 0.14$
2f	$1.77^{d} \pm 0.02$	$0.81^{c} \pm 0.11$	$0.32^{c} \pm 0.05$	$0.18^{c} \pm 0.00$	$0.38^{c} \pm 0.04$	$0.35^{c} \pm 0.02$	$0.53^{c} \pm 0.01$	$0.89^{b} \pm 0.10$
4f	$2.13^{e} \pm 0.09$	$0.86^{c} \pm 0.05$	$0.43^{d} \pm 0.11$	$0.24^{c} \pm 0.00$	$0.56^{d} \pm 0.04$	$0.47^{c} \pm 0.08$	$0.70^{d} \pm 0.02$	$0.78^{ab} \pm 0.08$

Table 6. Average values and standard error of silicate concentration (mg L^{-1} $Si\text{-}SiO_4^{4-}$) in the treatments throughout the research period. Equal or common letters indicate that there are no significant differences ($P > 0.05$) between treatments per day, a<b<c. *Indicates nonparametric test. H1: after harvest 1, H2: after harvest 2. Nd: Not detected.

Treatment	Day							
	0*	1	2	3	H1	4*	H2	5
f/4	$7.97^{b} \pm 3.26$	$2.09^{a} \pm 0.59$	$0.63^{a} \pm 0.16$	Nd	$1.07^{a} \pm 0.31$	$0.39^{a} \pm 0.10$	$1.75^{a} \pm 0.56$	Nd
f/2	$3.80^{a} \pm 0.30$	$2.01^{a} \pm 0.08$	$1.11^{a} \pm 0.10$	Nd	$0.81^{a} \pm 0.14$	$0.14^{a} \pm 0.11$	$0.82^{a} \pm 0.25$	Nd
f	$9.12^{b} \pm 1.94$	$3.95^{b} \pm 0.26$	$2.28^{b} \pm 0.18$	$0.15^{a} \pm 0.06$	$2.25^{b} \pm 0.42$	$0.75^{a} \pm 0.41$	$4.43^{b} \pm 0.84$	Nd
2f	$14.98^{b} \pm 4.64$	$5.22^{b} \pm 0.63$	$2.45^{b} \pm 0.30$	$0.33^{b} \pm 0.05$	$3.78^{c} \pm 0.24$	$3.73^{b} \pm 0.95$	$6.51^{c} \pm 0.27$	Nd
4f	$15.44^{b} \pm 1.88$	$8.37^{c} \pm 0.42$	$3.43^{c} \pm 0.22$	$0.93^{c} \pm 0.02$	$6.80^{d} \pm 0.51$	$3.6^{b} \pm 0.23$	$9.35^{d} \pm 0.17$	2.80 ± 1.0

did (Table 7). At the beginning of the culture, there were no significant differences in the nitrite concentration ($P > 0.05$) between treatments (Table 7). From the first to the last day, 4f showed significant increases in nitrite concentration compared to the other treatments ($P < 0.05$), with values above 1.60 mg L^{-1} $N\text{-}NO_2^-$ from the day 3. In the last day of the culture, nitrite concentration varied significantly according to the following tendency: 4f > 2f > f > f/2 > f/4.

On the other hand, ammonium ion concentrations were found after the day 3 of the culture (Table 8). This compound was found in the highest concentration at the end of the experiment in f with 0.04 ± 0.02 mg L^{-1} $N\text{-}NH_4^+$. However, ammonium ion concentration in f/4 on the fourth day showed the highest value with 0.19 mg L^{-1} (Table 8).

DISCUSSION

Cell density results in *Thalassiosira weissflogii* for this study (Fig. 1) are comparable to those found by Vella et al. (2019) with the same microalgae in axenic cultures within a 40 L closed tubular photobioreactor. After a year of cultivation, the authors achieved average cell densities of nearly 0.400×10^6 cell mL^{-1} in their batch system. In this study, the cellular density of *T. weissflogii* increased as it did the macronutrients in the treatments, with similar results as those obtained by López-Elías et al. (2008) when analyzing the growth of *Chaetoceros muelleri* in the f/2 and f media in an open system.

The small increase in $\sum\mu$ of *T. weissflogii* in all treatments between the first and second day showed that this microalga completed the exponential growth

Table 7. Average values and standard error of nitrite concentration (mg L^{-1} $N\text{-}NO_2^-$) in the treatments throughout the research period. Equal or common letters indicate that there are no significant differences ($P > 0.05$) between treatments per day, a<b<c. *Indicates nonparametric test. H1: after harvest 1, H2: after harvest 2.

Treatment	Day							
	0	1	2	3	H1	4	H2	5*
f/4	$0.08^a \pm 0.00$	$0.68^a \pm 0.04$	$0.74^a \pm 0.04$	$0.76^a \pm 0.07$	$0.67^a \pm 0.05$	$0.53^a \pm 0.06$	$0.49^a \pm 0.03$	$0.46^a \pm 0.00$
f/2	$0.08^a \pm 0.00$	$0.71^a \pm 0.05$	$0.97^b \pm 0.08$	$1.20^b \pm 0.10$	$0.66^a \pm 0.02$	$0.93^b \pm 0.13$	$0.80^b \pm 0.11$	$0.82^b \pm 0.08$
f	$0.08^a \pm 0.00$	$0.73^a \pm 0.02$	$1.02^b \pm 0.10$	$1.28^b \pm 0.04$	$1.03^b \pm 0.04$	$1.37^{bc} \pm 0.13$	$0.97^b \pm 0.10$	$1.00^c \pm 0.04$
2f	$0.08^a \pm 0.00$	$0.72^a \pm 0.03$	$1.07^b \pm 0.04$	$1.42^b \pm 0.09$	$1.03^b \pm 0.03$	$1.38^{bc} \pm 0.14$	$1.11^b \pm 0.09$	$1.28^d \pm 0.09$
4f	$0.09^a \pm 0.01$	$0.86^b \pm 0.02$	$1.37^c \pm 0.05$	$1.78^c \pm 0.06$	$1.45^c \pm 0.05$	$1.71^c \pm 0.15$	$1.51^c \pm 0.08$	$1.60^e \pm 0.08$

Table 8. The treatments' average values and standard error of ammonium ion concentration (mg L^{-1} $N\text{-}NH_4^+$). Equal or common letters indicate that there are no significant differences ($P > 0.05$) between treatments per day, a<b<c. *Indicates nonparametric test. H1: after harvest 1, H2: after harvest 2. Nd: Not detected.

Treatment	Day							
	0	1	2	3	H1	4*	H2	5*
f/4	Nd	Nd	Nd	Nd	Nd	$0.19^a \pm 0.11$	$0.10^c \pm 0.01$	$0.02^{bc} \pm 0.00$
f/2	Nd	Nd	Nd	Nd	Nd	$0.07^a \pm 0.01$	$0.03^b \pm 0.01$	$0.01^{bc} \pm 0.00$
f	Nd	Nd	Nd	Nd	Nd	$0.07^a \pm 0.01$	$0.06^b \pm 0.01$	$0.04^c \pm 0.02$
2f	Nd	Nd	Nd	Nd	Nd	$0.08^a \pm 0.01$	$0.05^b \pm 0.00$	$0.01^a \pm 0.00$
4f	Nd	Nd	Nd	Nd	Nd	$0.12^a \pm 0.01$	$0.01^a \pm 0.00$	$0.01^{bc} \pm 0.00$

stage between the experiment beginning and the first day (Table 1). This exponential phase growth occurred rapidly in all cultures concerning other microalgae species, such as *Nannochloropsis* sp. (Navarro-Peraza et al. 2017). In that work, *Nannochloropsis* sp. reached the maximum growth on the fifth day of culture, with $\sum\mu$ of 4.80 ± 0.14 in an f medium at 25°C. The $\sum\mu$ value is similar to those reached during the five treatments on the second day of cultivation for this study (Table 1). Moreover, Supramaetakorn et al. (2019) found maximum specific growth rates of 0.59 and 1.18 below different irradiances for *Chaetoceros* sp., lower than those reported in this study. López-Elías et al. (2008) also found differences in the cumulative division rate for *C. muelleri* in open cultures, where the $\sum\mu$ was greater in f medium than in f/2. Likewise, Piña et al. (2007) reported that batch cultures of *T. weissflogii* reached a $\sum\mu$ of 3.71 ± 0.23 and 5.27 ± 0.68 at the third and fourth day respectively. These differences may be due to the type of light and culture volume used (Piña et al. 2007). In addition, size may be another influencing factor since *Chaetoceros* sp. and *Nannochloropsis* sp. is smaller than *T. weissflogii*, and each species has its growth curve (Lee & Kim 2002).

Regarding microalgae biomass production, studies such as Lin et al. (2018) found a biomass production of *T. weissflogii* of 496 mg L^{-1} on the day 6 of culture by using 1.6 L bottles, f/2 medium and with cycles of light/darkness of 14:10 h, reaching higher biomass values than those obtained in this study, and suggesting that the volume of culture has a direct influence on biomass production of *T. weissflogii*. Likewise, the biomass results of *T. weissflogii* cultivated at 25°C (Table 2) were like those found by Vella et al. (2019) for the same microalgae, reporting biomass values around 200 and 400 mg L^{-1} at similar temperatures. However, they also registered biomass concentrations that exceeded those obtained in this study with approximate values of 1,000 mg L^{-1} when the temperature reached a minimum of 4°C in the coldest months. Nevertheless, in other species such as *Chlorella vulgaris*, *Nannochloropsis granulata*, and *Skeletonema marinoi*, their biomass remained constant or exhibited a decrease as the temperature of the cultures did (Xu et al. 2019, Cheregi et al. 2021).

The organic weight values (OW) of this study are comparable to those found by Ortega-Salas & Flores-Nava (2017), with 59% of OW for *T. weissflogii* in an f medium. In this work, the percentage of the inorganic weight values (IW) in *T. weissflogii* biomass increased as it did the level of the treatments, attributed to the higher contribution of inorganic components, mostly silicates that increased according to treatment. Previous studies with cultures of *C. muelleri* in the f/2 and f media found differences up to 22% in IW, which increased as the f medium raised (López-Elías et al. 2008).

For this study, protein concentration values in *T. weissflogii* cultures are consistent with those in other works, like Piña et al. (2007), who found protein concentrations of 49.3 mg L^{-1} in an f medium by using the same species with different nitrogen sources. These results were comparable to those recorded in this study, with values between 48 and 55 mg L^{-1} in f, 2f and 4f (Table 3). Similarly, the amount of protein (38 mg L^{-1}) in f/2 in this study was comparable to that obtained by García et al. (2012). They found an approximate value of 35 mg L^{-1} in f/2, with the same microalgae in the stationary phase. Meanwhile, Botte et al. (2017) reported protein levels similar and higher than those described in this study. For example, in a seven-day f/2 culture, protein content was approximately 28 mg L^{-1}, similar to that achieved in f/4 with about 27 mg L^{-1} (Table 3). However, in another treatment, nutrients were injected daily for 14 days with a working volume of 2 L, reaching a protein concentration of approximately 370 mg L^{-1}, a value above any reported in this and other studies with *T. weissflogii* (Piña et al. 2007, García et al. 2012). These differences were probably due to the daily nutrient supplementation and higher light availability because of the smaller volume, making biomass production more efficient.

On the other hand, carbohydrate results (26.5-39.0 mg L^{-1}) in this study (Table 3) were similar to those found by Piña et al. (2007) and García et al. (2012), who recorded values of 33.9 and 25.3 mg L^{-1}, respectively. However, Botte et al. (2017) reported carbohydrate concentrations of approximately 50 mg L^{-1} in cultures of *T. weissflogii* for seven days in an f/2 medium without nutrient rechange, and values of 60 mg L^{-1} for the same species with 14 days culture and a daily injection of nutrients, which were higher to those obtained in this work. These differences were probably due to the lack of major nutrients during the seven days the culture lasted, and such limitation stimulated carbohydrates production, an effect observed in the f/4 and f/2 treatments of this work (Table 3). The culture with a continuous supply of nutrients exhibited a higher biomass production, regardless of its low carbohydrate percentage.

The lipid values observed in this study (39.7-68.6 mg L^{-1}) were higher than those lipid concentrations published by García et al. (2012) with the same microalgae. However, Vella et al. (2019) presented a concentration range between 25-100 mg L^{-1} of lipids in *T. weissflogii* cultures, values that in some cases exceed those reported in this study. These differences regarding this work (Table 3) were attributed to the different culture conditions such as volume, irradiance, temperature, growth phase, nutrient availability, and cultivation time.

In this study, pH variations at the beginning of the experiment were attributed to the concentration of nutrients contained in the different treatments, especially silicates, which alkalinized the water (Fig. 3). As days of cultivation went by, the highest pH in all *T. weissflogii* cultures was attributed to the biomass concentration because a higher cell density means more CO_2 consumption by microalgae for photosynthesis, thus increasing the alkalinity of the water (Bartley et al. 2013, Franchino et al. 2013).

Microalgae are some of the most used organisms as live food in aquaculture (Martínez-Córdova et al. 2014) because they contain compounds such as essential fatty acids and carotenoids, required by the species in culture. One of the most used species in aquaculture is *T. weissflogii*, preferred by laboratories working with shrimp larvae and other shellfish due to its larger size than those of other species. Besides, they can be used in more advanced larval stages (Asha-Shalini et al. 2019). The reason why it is so important to know the effects of limiting certain nutrients to obtain compounds of interest in *T. weissflogii*. One of the main limiting nutrients is nitrogen because it modifies the biochemical composition of microalgae. Nitrate concentration in *T. weissflogii* cultures with the 2f and 4f treatments rose after each partial harvest because of the added nutrients (Table 4).

Nevertheless, in the other treatments, nitrate concentration stabilized close to 0 in f/4, so it was not considered a limiting nutrient in some treatments of this work. Similarly, Botte et al. (2017) found that nitrates were not limiting factors in their cultures of *T. weissflogii*; on the contrary, they accumulated daily added nutrients. Although other studies have researched nitrate consumption in microalgae cultures (Lin et al. 2018), information on the simultaneous effect of the three major nutrients (nitrates, phosphates, and silicates) is quite scarce. Therefore, this study evaluated the consumption of these three nutrients in the five treatments.

That nutrient concentration in the culture medium affects microalgal metabolism, and bioactive compounds production has been reported (Kang et al. 2011, Jiang et al. 2015). Previous studies have documented that nitrogen limitation in microalgae cultures affects both cell density and biomass production. For example, Lin et al. (2018) reported lower biomass production in *T. weissflogii* and *C. muelleri* cultures when the control (f/2) was compared to similar cultures but limited by nitrates and nitrites. In another study, Marella & Tiwari (2020) found in *T. weissflogii* cultures that the lower the concentration of nitrates, the lower the cell density. In this study, the *T. weissflogii* cultures that were least limited by nitrates

were those exhibiting the highest cell density (Fig. 1, Table 4).

Likewise, nitrate deficiency in microalgae cultures causes a decrease in protein concentration and an increase in the lipid concentration inside the cell (Jian et al. 2012, Botte et al. 2017). On the third and fourth days, this study showed that the percentage of proteins at the cellular level was lower in f/2 and f/4 treatments (Table 9) than in f because the availability of nitrates was lower (Table 4). However, this decrease was not found for 4f, 2f, and f, probably because in these treatments, the *T. weissflogii* cells consumed the silicates contained in the nutrient solutions (Table 6). Thus, a higher percentage of inorganic weight in the biomass of these microalgae was found (Fig. 2). Although f/2 and f/4 are the most limited in nitrates (Table 4), the percentage of lipids in *T. weissflogii* cells did not increase (Table 9), which can be attributed to nitrate limitation in microalgae cultures that can take up to 12 to 14 days to derive an increase in lipid concentration (Lai et al. 2011, Botte et al. 2017) and the replenishments with nutrients from day 3 onwards conducted in this work. Moreover, nutrient limitation (e.g. nitrogen, phosphorus, and silicates) in some microalgae cultures influences lipid or carbohydrate accumulation (Thajuddin et al. 2015, Zhu et al. 2016, Li et al. 2018). This limitation can even lead to a higher concentration of polyunsaturated fatty acids such as docosahexaenoic acid in some species of microalgae (Wang et al. 2018), very important in the diet of marine species, especially in the larval stage of fish and crustaceans, where they play an important role in many biological functions such as development, survival, and stress tolerance (Hamre et al. 2013).

In this work, carbohydrate percentage within the *T. weissflogii* cell decreased when the level of macronutrients in the treatments rose (Table 9). Our findings demonstrated that throughout the three partial harvests, differences became more pronounced as days went by (Table 9). These observations could be attributed to the lack of nutrients in f/4 and f/2. Thereby, Botte et al. (2017) observed an increase in carbohydrate percentage of about 30% of its DW in *T. weissflogii* cultures grown in an f/2 medium with and without daily nutrient injections. Biomass concentration (DW) at the cell level of *T. weissflogii* in this study increased as the level of the treatments decreased (Table 9). In the first harvest, the dry weight concentration of *T. weissflogii* cells of f/4 was significantly higher ($P < 0.05$) concerning that of the other treatments, doubling the amount of biomass from f and 4f (Table 9). At the end of the experiment, the *T. weissflogii* cell in f/4 had the highest DW concentration of 695.7 ± 33.9 pg cell^{-1} (Table 9), probably due to the low availability of nitrates in f/4, since other studies have found that the lack of nitrogen can originate larger diatoms (Litchman et al. 2009), and consequently, higher biomass.

This study showed that phosphate concentration (Table 5) decreased as cell density increased, and it was more limiting than that of nitrates, as observed by other authors (Lin et al. 2018). In microalgae polyculture, Ramachandra et al. (2011) observed phosphate concentration increase twice as much of that in the initial concentration, similar to f/4 and f/2 in this work with *T. weissflogii*. However, studies, such as the one of Lai et al. (2011), have found that phosphate deficiency does not influence the protein content of the *Prorocentrum donghaiense* dinoflagellate. However, this study shows a reduction in protein content in f/4 and f/2 compared to f on the third and fourth days (Table 9). These results could be attributed to the synergistic effect caused by low concentrations of phosphates and nitrates in the treatments (Tables 4-5). In diatom culture media, silicates are present as silicic acid to contribute to the formation of frustules (Petrou et al. 2019). Therefore, a detriment in cell growth rate can occur when silicate concentrations are limited. For instance, Lin et al. (2018) observed a decline in biomass production of *T. weissflogii* when silicates limited cultures. In this work, the significant decrease ($P < 0.05$) in cell density (Fig. 1) and biomass (Table 2) for f/4 and f/2 could be explained by the limitation of the three major nutrients. In addition, Lin et al. (2018) observed the accumulation of lipids in the cell of *T. weissflogii*. The authors explained these findings as a result of silicate, nitrate, or phosphate limitation. At the cell level of *T. weissflogii,* no significant ($P > 0.05$) accumulation of lipids was found between the treatments (Table 9) in this work. The microalgae were harvested on the day 3 for this study, so the addition of nutrients did not allow enough time for the microalgae to stress and reflect it by the accumulation of lipids; while Lin et al. (2018) carried out their harvesting procedure in the day 6 without nutrient supply after the beginning of the experiment.

Alternatively, nitrite consumption was never observed in this study (Table 7) since when nitrate concentrations were high, the enzymes that process this nutrient in microalgae were activated (Sanz-Luque et al. 2015). Li et al. (2020) observed in *Chlorella* sp. an accumulation of nitrites when these were also present in the culture medium. On the other hand, a decrease in nitrites in the culture medium when there were no nitrates was found. A low concentration of ammonium ions occurred in this study because *T. weissflogii* achieved the stationary growth phase only; therefore, not much decomposing organic matter was there as a source of this compound.

Table 9. Average values and standard error of dry weight (pg cell^{-1}) per *Thalassiosira weissflogii* cell and proximal composition (%) in the five treatments for the partial harvests. DW: dry weight, P: protein, C: carbohydrates, L: lipids. Equal or common letters indicate that there are no significant differences ($P > 0.05$) between treatments per day, a<b<c. *Indicates nonparametric test.

Day	Proximal variable	Treatment				
		f/4	f/2	f	2f	4f
3	DW	$931.7^{b} \pm 28.7$	$544.0^{a} \pm 41.6$	$459^{a} \pm 23.3$	$496^{a} \pm 21.8$	$456^{a} \pm 19.3$
	P	$18.6^{a} \pm 1.3$	$27.8^{bc} \pm 1.2$	$29.9^{c} \pm 2.8$	$25.9^{bc} \pm 1.6$	$21.7^{ab} \pm 1.7$
	C	$20.7^{a} \pm 2.5$	$20.7^{a} \pm 1.3$	$16.4^{a} \pm 0.7$	$18.5^{a} \pm 1.2$	$15.3^{a} \pm 0.6$
	*L	$28.1^{a} \pm 3.5$	$29.5^{a} \pm 2.5$	$29.3^{a} \pm 0.7$	$33.4^{a} \pm 3.5$	$26.7^{a} \pm 0.9$
4	DW	$901.4^{c} \pm 32.1$	$773.5^{bc} \pm 41.9$	$594.2^{a} \pm 24.6$	$466.3^{a} \pm 14.3$	$688.9^{b} \pm 83.6$
	P	$17.1^{a} \pm 0.3$	$19.3^{ab} \pm 1.1$	$22.1^{b} \pm 0.9$	$20.8^{b} \pm 0.4$	$16.6^{a} \pm 1.5$
	C	$19.9^{c} \pm 0.3$	$16.2^{b} \pm 1.3$	$14.6^{ab} \pm 0.9$	$15.2^{ab} \pm 0.9$	$11.8^{a} \pm 1.0$
	L	$25.4^{ab} \pm 0.6$	$21.4^{a} \pm 2.0$	$24.1^{ab} \pm 0.8$	$28.8^{b} \pm 1.6$	$20.1^{a} \pm 1.9$
5	DW	$695.7^{b} \pm 33.9$	$529.4^{a} \pm 42.1$	$499.2^{a} \pm 5.4$	$451.7^{a} \pm 6.2$	$661.6^{b} \pm 11.9$
	P	$20.9^{b} \pm 1.2$	$26.5^{c} \pm 1.2$	$17.4^{ab} \pm 0.9$	$20.3^{b} \pm 1.7$	$15.3^{a} \pm 0.6$
	C	$28.6^{d} \pm 0.9$	$24.4^{c} \pm 0.6$	$13.2^{ab} \pm 0.8$	$15.7^{b} \pm 1.3$	$11.2^{a} \pm 0.6$
	L	$33.4^{b} \pm 2.6$	$28.4^{b} \pm 1.2$	$29.7^{b} \pm 1.0$	$30.5^{b} \pm 0.9$	$20.6^{a} \pm 0.6$

CONCLUSIONS

Major nutrients had a direct effect on *T. weissflogii* production. Moreover, the biomass in terms of inorganic weight fraction of *T. weissflogii* rose as the initial nutrients increased in the treatments. Nitrates were the lowest consumed from the major nutrients, while phosphates and silicates were the most demanded compounds. Nitrites and ammonium ions were not detected at concentrations comparable to nitrates, so they were not used as a nitrogen source. Protein and lipid concentrations in *T. weissflogii* biomass rose as the treatments increased. In contrast, the carbohydrate percentage in the cell of *T. weissflogii* increased as the level of treatment decreased.

The 30% partial harvest with nutrient addition selected for this study allowed the *T. weissflogii* cultures to maintain cell density and biomass production throughout the culture for the treatments studied. As a result, a more controlled biomass production per day and an approximate value of its fundamental biochemical composition, based on the treatment used for the culture of *T. weissflogii*. The data reported in this study may be mainly useful for larval shrimp producers or other hatcheries interested in this diatom, allowing them to switch from an open system to a closed system for producing microalgae in a semicontinuous way.

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REFERENCES

Arredondo-Vega, B.O. & Voltolina, D. 2007. Métodos y herramientas analíticas en la evaluación de la biomasa microalgal. Editorial CIBNOR, Baja California Sur.

Asha-Shalini, A., Syed-Ali, M., Anuradha, V., Yogananth, N. & Bhuvana, P. 2019. GCMS analysis and in vitro antibacterial and anti-inflammatory study on methanolic extract of *Thalassiosira weissflogii*. Biocatalysis and Agricultural Biotechnology, 19: 10114. doi: 10.1016/j.bcab.2019.101148

Aussant, J., Guihéneuf, F. & Stengel, D.B. 2018. Impact of temperature on fatty acid composition and nutritional value in eight species of microalgae. Applied Microbiology and Biotechnology, 102: 5279-5297. doi: 10.1007/s00253-018-9001-x

Bartley, M.L., Boeing, W.J., Dungan, B.N., Holguin, F.O. & Schaub, T. 2013. pH effects on growth and lipid accumulation of the biofuel microalgae *Nannochloropsis salina* and invading organisms. Journal of Applied Phycology, 26: 1431-1437. doi: 10.1007/s10811-013-0177-2

Bligh, E.G. & Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37: 911-917. doi: 10.1139/o59-099

Borowitzka, M.A. & Moheimani, N.R. 2013. Open pond culture systems. In: Borowitzka, M.A. & Moheimani, N.R. (Eds.). Algae for biofuels and energy. Springer, Amsterdam, pp. 133-152.

Botte, P., D'Ippolito, G., Gallo, C., Sardo, A. & Fontana, A. 2017. Combined exploitation of CO_2 and nutrient replenishment for increasing biomass and lipid productivity of the marine diatoms *Thalassiosira*

weissflogii and *Cyclotella cryptica*. Journal of Applied Phycology, 30: 243-251. doi: 10.1007/s10811-017-1221-4

Carvalho, A.P., Silva, S.O., Baptista, J.M. & Malcata, F.X. 2011. Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. Applied Microbiology and Biotechnology, 89: 1275-1288. doi: 10.1007/s00253-010-3047-8

Cheirsilp, B. & Torpee, S. 2012. Enhanced growth and lipid production under mixotrophic culture condition: effect of light intensity, glucose concentration and fed-batch cultivation. Bioresource Technology, 110: 510-516. doi: 10.1016/j.biortech.2012.01.125

Chen, M., Tang, H., Ma, H., Holland, T.C., Ng, K.Y.S. & Salley, S.O. 2011. Effects of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. Bioresource Technology, 102: 1649-1655. doi: 10.1016/j.biortech.2010.09.062

Cheregi, O., Engelbrektsson, J., Andersson, M.X., Strömberg, N., Ekendahl, S., Godhe, A. & Spetea, C. 2021. Marine microalgae for outdoor biomass production-A laboratory study simulating seasonal light and temperature for the west coast of Sweden. Physiologia Plantarum, 173: 543-554. doi: 10.1111/ppl.13412

Chini-Zittelli, G., Rodolfi, L., Bassi, N., Biondi, N. & Tredici, M.R. 2013. Photobioreactors for microalgal biofuel production. In: Borowitzka, M.A. & Moheimani, N.R. (Eds.). Algae for biofuels and energy. Springer, Amsterdam, pp. 115-131.

D'Ippolito, G., Sardo, A., Paris, D., Vella, F.M., Adelfi, M.G., Botte, P., et al. 2015. Potential of lipid metabolism in marine diatoms for biofuel production. Biotechnology for Biofuels, 8: 1-10. doi: 10.1186/s13068-015-0212-4

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. 1956. Colorimetric method for the determination of sugars and related substances. Analytical Chemistry, 18: 350-356. doi: 10.1021/ac60111a017

Franchino, M., Comino, E., Bona, F. & Riggio, V.A. 2013. Growth of three microalgae strains and nutrient removal from an agro-zootechnical digestate. Chemosphere, 92: 738-744. doi: 10.1016/j.chemosphere.2013.04.023

García, N., López-Elías, J.A., Miranda, A., Martínez-Porchas, M., Huerta, N. & García, A. 2012. Effect of salinity on growth and chemical composition of the diatom *Thalassiosira weissflogii* at three culture phases. Latin American Journal of Aquatic Research, 40: 435-440. doi: 10.3856/vol40-issue2-fulltext-18

George, B., Pancha, I., Desai, C., Chokshi, K., Paliwa, C., Ghosh, T. & Mishra, S. 2014. Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus* - A potential strain for biofuel production. Bioresource Technology, 171: 367-374. doi: 10.1016/j.biortech.2014.08.086

Grasshoff, K., Kremling, K. & Ehrhardt, M. 1999. Methods of seawater analysis. John Wiley & Sons, New Jersey.

Greenberg, A.E., Clesceri, L.S. & Eaton, A.D. 1992. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

Grobbelaar, J.U. 2013. Inorganic algal nutrition. In: Richmond, A. & Hu, Q. (Eds.). Handbook of microalgal culture. John Wiley & Sons, New Jersey.

Gu, N., Lin, Q., Li, G., Tan, Y., Huang, L. & Lin, J. 2012. Effect of salinity on growth, biochemical composition and lipid productivity of *Nannochloropsis oculata* CS 179. Engineering in Life Sciences, 12: 631-637. doi: 10.1002/elsc.201100204

Guillard, R.R.L. & Ryther, J.H. 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Canadian Journal of Microbiology, 8: 229-239. doi: 10.1139/m62-029

Hamre, K., Yufera, M., Ronnestad, I., Boglione, C., Conceicao, L.E.C. & Izquierdo, M. 2013. Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. Reviews in Aquaculture, 5: 26-58.

Hemaiswarya, S., Raja, R., Ravi-Kumar, R., Ganesan, V. & Anbazhagan, C. 2011. Microalgae: a sustainable feed source for aquaculture. World Journal of Microbiology and Biotechnology, 27: 1737-1746.

Hemerick, G. 1973. Mass culture. In: Stein, J.R. (Ed.). Handbook of phycological methods. Cambridge University Press, Cambridge, pp. 255-273.

Jiang, Y., Yoshida, T. & Quigg, A. 2012. Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. Plant Physiology and Biochemistry, 54: 70-77. doi: 10.1016/j.plaphy.2012.02.012

Jiang, Y., Nuñez, M., Starks, L.K. & Quigg, A. 2015. Coupled effect of silicate and nickel on the growth and lipid production in the diatom *Nitzschia perspicua*. Journal of Applied Phycology, 27: 1137-1148. doi: 10.1007/s10811-014-0412-5

Kang, K.H., Qian, Z.J., Ryu, B.M. & Kim, S.K. 2011. Characterization of growth and protein contents from microalgae Navicula incerta with the investigation of antioxidant activity of enzymatic hydrolysates. Food Science and Biotechnology, 20: 183-191. doi: 10.1007/s10068-011-0025-6

Khatoon, H., Norazira, A.R., Sanjoy, B., Nazurah, H., Siti, S.S., Nur, H.Z., et al. 2014. Effects of different salinities and pH on the growth and proximate

composition of *Nannochloropsis* sp. and *Tetraselmis* sp. isolated from South China Sea cultured under control and natural condition. International Biodeterioration & Biodegradation, 95: 11-18. doi: 10.1016/j.ibiod.2014.06.022

Lai, J., Yu, Z., Song, X., Cao, X. & Han, X. 2011. Responses of the growth and biochemical composition of *Prorocentrum donghaiense* to different nitrogen and phosphorus concentrations. Journal of Experimental Marine Biology and Ecology, 405: 6-17. doi: 10.1016/j.jembe.2011.05.010

Lee, J.B. & Kim, B.Y. 2002. Growth characteristics of five microalgal species isolated from Jeju Island and four microalgal stock strains in hatchery. Algae, 17: 117-125. doi: 10.4490/algae.2002.17.2.117

Li, X., Li, W., Zhai, J. & Wei, H. 2018. Effect of nitrogen limitation on biochemical composition and photosynthetic performance for fed-batch mixotrophic cultivation of microalga *Spirulina platensis*. Bioresource Technology, 263: 555-561. doi: 10.1016/j.biortech.2018.05.046

Li, S., Zheng, X., Chen, Y., Song, C., Lei, Z. & Zhang, Z. 2020. Nitrite removal with potential value-added ingredients accumulation via *Chlorella* sp. L38. Bioresource Technology, 313: 123743. doi: 10.1016/j.biortech.2020.123743

Lin, Q., Zhuo, W.-H., Wang, X.-W., Chen, C.-P., Gao, Y.-H., & Liang, J.-R. 2018. Effects of fundamental nutrient stresses on the lipid accumulation profiles in two diatom species *Thalassiosira weissflogii* and *Chaetoceros muelleri*. Bioprocess and Biosystems Engineering, 41: 1213-1224. doi: 10.1007/s00449-018-1950-z

Litchman, E., Klausmeier, C.A. & Yoshiyama, K. 2009. Contrasting size evolution in marine and freshwater diatoms. Proceedings of the National Academy of Sciences, 106: 2665-2670. doi:10.1073/pnas.0810891106

López-Elías, J.A., Enríquez-Ocaña, F., Pablos-Mitre, M.N., Huerta-Aldaz, N., Leal, S., Miranda-Baeza, A., et al. 2008. Growth and biomass production of *Chaetoceros muelleri* in mass outdoor cultures: effect of the hour of the inoculation, size of the inoculum and culture medium. Revista de Investigaciones Marinas, 29: 171-177.

Lowry, O.H., Rosebrough, J., Farr, A.L. & Randall, R.L. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193: 265-275.

Marella, T.K. & Tiwari, A. 2020. Marine diatom *Thalassiosira weissflogii* based biorefinery for co-production of eicosapentaenoic acid and fucoxanthin. Bioresource Technology, 307: 123245. doi: 10.1016/j.biortech.2020.123245

Martínez-Córdova, L.R., Martínez-Porchas, M., López-Elías, J.A. & Enríquez-Ocaña, L.F. 2014. Uso de microorganismos en el cultivo de crustáceos. Revista de Ciencias Biológicas y de la Salud, 16: 50-55.

Navarro-Peraza, R.S., Soto-León, S., Contreras-Andrade, I., Piña-Valdez, P., Viveros-García, T., Cuevas-Rodriguez, E.O. & Nieves-Soto, M. 2017. Effects of temperature and nitrogen limitation on growth kinetics, proximate composition and fatty acid profile of *Nannochloropsis* sp. Revista Mexicana de Ingeniería Química, 16: 359-369.

Ortega-Salas, A.A. & Flores-Nava, P. 2017. Cultivation of the microalgae *Thalassiosira weissflogii* to feed the Rotifer *Brachionus rotundiformis*. Journal of Aquaculture & Marine Biology, 6: 1-2. doi: 10.15406/jamb.2017.06.00169

Pande, S.V., Khan, R.P. & Venkitasubramanian, T.A. 1963. Micro determination of lipids and serum total fatty acid. Analytical Biochemistry, 6: 415-423. doi: 10.1016/0003-2697(63)90094-0

Petrou, K., Baker, K.G., Nielsen, D.A., Hancock, A.M., Schulz, K.G. & Davidson, A.T. 2019. Acidification diminishes diatom silica production in the Southern Ocean. Nature Climate Change, 9: 781-786. doi: 10.1038/s41558-019-0557-y

Piña, P., Medina, M.A., Nieves, M., Leal, S., López-Elías, J.A. & Guerrero, M.A. 2007. Cultivo de cuatro especies de microalgas con diferentes fertilizantes utilizados en acuicultura. Revista de Investigaciones Marinas, 28: 225-236.

Ramachandra, T.V., Sajina, K. & Supriya, G. 2011. Lipid composition in microalgal community under laboratory and outdoor conditions. Indian Journal of Science and Technology, 4: 1488-1494. doi: 10.17485/ijst/2011/v4i11/30276

Sanz-Luque, E., Chamizo-Ampudia, A., Llamas, A., Galvan, A. & Fernandez, E. 2015. Understanding nitrate assimilation and its regulation in microalgae. Frontiers in Plant Science, 6: 1-17. doi: 10.3389/fpls.2015.00899

Silva-Benavides, A.M. 2016. Evaluación de fertilizantes agrícolas en la productividad de la microalga *Chlorella sorokiniana*. Agronomía Mesoamericana, 27: 265-275. doi: 10.15517/am.v27i2.24361

Supramaetakorn, W., Meksumpun, S., Ichimi, K., Thawonsode, N. & Veschasit, O. 2019. Potential fucoxanthin production from a marine diatom. Journal of Fisheries and Environment, 43: 1-10.

Suroy, M., Panagiotopoulos, C., Boutorh, J., Goutx, M. & Moriceau, B. 2015. Degradation of diatom carbohydrates: a case study with N- and Si-stressed *Thalassiosira weissflogii*. Journal of Experimental Marine Biology and Ecology, 470: 1-11. doi: 10.1016/j.jembe.2015.04.018

Thajuddin, N., Ilavarasi, A., Baldev, E., MubarakAli, D., Alharbi, N.S., Chinnathambi, A. & Alharbi, S.A. 2015. Stress induced lipids accumulation in naviculoid marine diatoms for bioenergy application. International Journal of Biotechnology for Wellness Industries, 4: 18-24. doi: 10.6000/1927-3037.2015.04.01.3

Tredici, M.R. 2010. Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. Future Science Biofuels, 1: 143-162. doi: 10.4155/bfs.09.10

Vella, F.M., Sardo, A., Gallo, C., Landi, S., Fontana, A. & D'Ippolito, G. 2019. Annual outdoor cultivation of the diatom *Thalassiosira weissflogii*: productivity, limits and perspectives. Algal Research, 42: 1-7. doi: 10.1016/j.algal.2019.101553

Wang, X., Fosse, H.K., Li, K., Chauton, M., Vadstein, O. & Reitan, K. 2018. Influence of nitrogen limitation on lipid accumulation and EPA and DHA content in four marine microalgae for possible use in aquafeed. Frontiers in Marine Science, 6: 95. doi: 10.3389/fmars.2019.00095

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Xu, K., Zou, X., Wen, H., Xue, Y., Qu, Y. & Li, Y. 2019. Effects of multi-temperature regimes on cultivation of microalgae in municipal wastewater to simultaneously remove nutrients and produce biomass. Applied Microbiology and Biotechnology, 103: 8255-8265. doi: 10.1007/s00253-019-10051-6

Zar, J.H. 2010. Biostatistical analysis. Prentice-Hall, New Jersey.

Zhao, C., Brück, T. & Lercher, J.A. 2013a. Catalytic deoxygenation of microalgae oil to green hydrocarbons. Green Chemistry, 15: 1720-1739. doi: 10.1039/C3GC40558C

Zhao, Y., Wang, J., Zhang, H., Yan, C. & Zhang, Y. 2013b. Effects of various LED light wavelengths and intensities on microalgae-based simultaneous biogas upgrading and digestate nutrient reduction process. Bioresource Technology, 136: 461-468. doi: 10.1016/j.biortech.2013.03.051

Zhu, L.D., Li, Z.H. & Hiltunen, E. 2016. Strategies for lipid production improvement in microalgae as a biodiesel Feedstock. BioMed Research International, 2016: 1-8. doi: 10.1155/2016/8792548