

# Protein Potential of *Desmodemus Asymmetricus* Grown in Greenhouse as an Alternative Food Source for Aquaculture

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## Research Article

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# Abstract

During ten months, batch culture of *Desmodesmus asymmetricus* microalgae was carried out under greenhouse conditions. The inoculation ratio was 1:1 inoculum-treated water. The cultures were maintained for 5 days with 12 h:12 h light:dark photoperiod and constant aeration mixed with CO<sub>2</sub>. The biomass was concentrated by centrifugation and dried by lyophilization; subsequently, total proteins and amino acid concentration were determined. A relationship between biomass production and seasonal variation was observed, the lowest dry biomass productivity was recorded in June ( $38.8 \pm 1.0 \text{ mg L}^{-1} \text{ day}^{-1}$ ) and July ( $43.3 \pm 0.1 \text{ mg L}^{-1} \text{ day}^{-1}$ ); while the highest values were greater than  $70 \text{ mg L}^{-1} \text{ day}^{-1}$ . There was a high positive correlation between wet and dry biomass ( $r = 0.97$ ,  $p < 0.001$ ) with a mean conversion of 26%. The mean percentage of protein was  $26.1 \pm 2.6\%$ , the highest percentage was registered in March ( $31.03 \pm 1.48\%$ ) as well as the concentration of amino acids. Regarding amino acids, arginine obtained the highest concentration ( $4.08 \pm 0.43 \text{ g } 100 \text{ g}^{-1}$ ), followed by aspartic acid ( $3.36 \pm 0.23 \text{ g } 100 \text{ g}^{-1}$ ), while the lowest values were for methionine ( $0.55 \pm 0.21 \text{ g } 100 \text{ g}^{-1}$ ), histidine ( $0.77 \pm 0.07 \text{ g } 100 \text{ g}^{-1}$ ) and tyrosine ( $1.01 \pm 0.17 \text{ g } 100 \text{ g}^{-1}$ ). Finally, according to the essential amino acid index (in fish  $\geq 0.90$ , in crustaceans  $> 0.80$ ), the biomass of *D. asymmetricus* has potential as a food supplement for the production of feed in aquaculture.

## Introduction

Microalgae are photosynthetic microorganisms, whose size are between 2–200  $\mu\text{m}$ , however they can grow autotrophically or heterotrophically. There are many types of organization: unicellular, filamentous or colonial. Cells can have external structures such as membranes, cell walls or silica exoskeletons (Randrianarison and Aqeel Ashraf 2017). These organisms are the simplest of the plant kingdom with a great capacity to adapt to different environments, they use the energy of light and can fix carbon dioxide (CO<sub>2</sub>) more efficiently than land plants, so they produce more quantities of biomass (Christaki et al. 2011; Gaignard et al. 2018). Currently, there are more than 100,000 species identified (Guiry et al. 2014) that live in almost all environments and are distributed around the world (Posten and Feng 2016; Geada et al. 2017).

The first records about the use of microalgae as food were around 2000 years ago, when Chinese people used the *Nostoc* genus to alleviate the famine of their people. Another reference on the application of microalgae in nutrition is associated with the treatment of patients with leprosy, who were included in their diet a soup enriched with *Chlorella sp.* and it was observed that they had an increase in their weight, energy and general health (Milledge 2011; Sigamani et al. 2016). The culture of microalgae began to be developed in Japan as a solution to the food shortage caused by the Second World War and there was a development of products enriched with microalgae. Thenceforth, many studies about biomass production have focused on obtaining biofuels, wastewater treatment and as a high protein food source for use in humans and animals, especially in aquaculture (Spolaore et al. 2006; Christaki et al. 2011; Yaakob et al. 2014; Garcia et al. 2017; Koyande et al. 2019).

Several studies have shown that microalgae can represent an attractive source of compounds with biological activity. For example, polyunsaturated fatty acids (PUFA), carotenoids, phycobilins, peptides and polysaccharides. In addition, they are a good source of A, B1, B2 and B12 vitamins (Skjånes et al. 2013; De Morais et al. 2015). Currently, several products are marketed in the form of tablets, powder, solution or in mixtures with snacks, cookies, noodles, drinks, candies, gums, wines and cereals (Sathasivam et al. 2017; Levasseur et al. 2020). The main countries that lead the world production are China, India, Taiwan, Germany and a few in Latin America (Koyande et al. 2019; Levasseur et al. 2020).

There are two well-known genus: *Arthospira* (commercially known as Spirulina) and *Chlorella*, both with a production of 12000 and 5000 tons per year, respectively. In last decades, many researchers have studied other species and produce biomass such as *Haematococcus*, *Dunaliella*, *Botryococcus*, *Phaeodactylum*, *Porphyridium*, *Chaetoceros*, *Cryptothecodinium*, *Isochrysis*, *Nannochloris*, *Nitzschia*, *Schizochytrium*, *Tetraselmis* and *Skeletonema*. (Plaza et al. 2009; Sathasivam et al. 2017; Matos et al. 2017, Levasseur et al. 2020). However, only a small percentage of all species with potential has been studied. In the same way, research about the development of organoleptically acceptable products, their compounds, benefits, isolation techniques, performance improvement, determination of their bioactivity and toxicity are still scarce (Stengel et al. 2011; Buono et al. 2014; Levasseur et al. 2020).

In aquaculture, the protein supplied in the food of aquatic organisms comes from fishmeal; this ingredient increases its price due to the high demand worldwide and its limited production by fisheries (Amaya et al. 2007; FAO 2018; FAO 2020). As an alternative solution, new commodity markets have been developed such as high-quality processed animal by-products (hydrolyzed feather meal, blood meal, meat and bone meal), vegetables flour, protein concentrates obtained from oilseeds and legume grains. Similarly, it is known that in salmonid farming, fish oil in feed has been replaced by other fats of animal and vegetable origin, although these "second generation" ingredients are not without limitations (Hua et al. 2019).

This sector needs to grow, so several institutions have begun to investigate the use of "third generation ingredients", which is defined as ingredients that come from lower trophic levels (Shah et al 2018; Glencross et al. 2020). In this manner, microalgae biorefineries emerge as an option. There is an advantage in the reuse of industrial emissions, since their waste "outlets" (CO<sub>2</sub>, nutrients, heat) turn out to be the essential "inputs" for the microalgal culture, which would allow achieving rapid cell growth and a greater accumulation of biomass. In terms of input costs, microalgae-based ingredients produced for aquafeeds could have competitive advantages over terrestrial crops, due to it uses lower crop area, potential for wastewater remediation and carbon credits from CO<sub>2</sub> conversion (Roy and Pal 2014, Allen et al. 2019; Perez-Velazquez et al. 2019; Levasseur et al. 2020).

Research in the field of microalgae has increased, which shows its potential. In Peru, studies on massive culture of native microalgae are scarce, therefore, it is important to investigate the biotechnological uses of microalgae in different industries such as in aquaculture, food, pharmaceuticals, renewable energy and others. For this reason, the present study evaluated the culture of the native microalgae *Desmodesmus*

*asymmetricus* in greenhouse conditions, the aim was to determine the values of biomass productivity, protein and amino acids concentrations and its potential as ingredient in aquaculture industry.

## Materials And Methods

### Culture of microalgae

The batch culture mode was performed for *D. asymmetricus* (family Scenedesmaceae) microalgae, whose code is IMP-BG-249. The inoculum of *D. asymmetricus* were obtained from Laboratorio de Alimento Vivo of the Instituto del Mar del Perú (IMARPE), the volumes were in exponential phase ( $\sim 1.0 \pm 0.5 \times 10^6$  cells/mL). Then, in the Laboratorio de Cultivo de Microalgas-IMARPE, the microalgae were cultivated in vertical tubular bioreactors of 30 L, it was considered to have a number of bioreactors necessary to reach 900 L of harvest. The inoculation ratio was 1:1 (microalgal inoculum-treated freshwater); freshwater was filtered with 10, 5 and 1  $\mu\text{m}$  cartridges and irradiated with ultraviolet light. The culture medium used was liquid foliar fertilizer Bayfolan®, dose of  $0.20 \text{ mL L}^{-1}$ . Constant aeration mixed with  $0.5 \text{ mL L}^{-1} \text{ CO}_2$  flow were supplied. The conditions of cultures were 12 h:12 h light:dark photoperiod,  $7.02 \pm 0.53$  pH,  $8.62 \pm 0.42 \text{ mg L}^{-1}$  dissolved oxygen and  $0.36 \pm 0.15$  ppm salinity.

### Monitoring of abiotic parameters

Greenhouse growth was conducted from January to November 2018 in the IMARPE, whose seasons are distributed as follows: summer (January, February, March), autumn (April, May), winter (June, July, August, September) and spring (October, November, December). The cultures were maintained for 5 days. A weekly harvest was carried out.

Abiotic parameters were recorded daily at 8:00, 12:00 and 16:00 h. Culture temperature (CT), pH, salinity and dissolved oxygen (DO) were measured with multiparameter WTW (Multi 350i, Weilheim, Germany). Greenhouse temperature (GT) was register with max/min wall thermometer (Ootdty, Shangay, China), Illuminance (IL) was measured with a lux meter Control Company (CC-3252, Texas, EEUU) and photosynthetically active radiation (PAR) were register with a radiation meter LI-COR (LI-1400, Nebraska, EEUU).

### Biomass Productivity

The cultures were harvested in 500 L tanks and concentrated by means of centrifugation method. For this, a Westfalia semi-industrial separator (OTC3, Oelde, Germany) was used at 10000 rpm and output flow between  $120\text{--}200 \text{ L h}^{-1}$ . The samples were placed in steel trays, they were spread in layers of 1 cm maximum thickness and were frozen ( $-20^\circ\text{C}$ ) and stored for later drying. The harvest cell density (HCD) and the wet biomass productivity (WBP) were determined in this stage.

Subsequently, the biomass obtained was dried in a Labconco lyophilizer (18L, Kansas, USA) at a vacuum pressure between  $0.022\text{--}0.070$  mbar. The collector temperature was  $-56^\circ\text{C}$ . The biomass was dried

using the following temperature ramp: -15°C for 8 h; then 0.5°C min<sup>-1</sup> to 5°C for 15 h; finally, 0.5°C min<sup>-1</sup> until 25°C for 7 h. The samples were withdrawn and homogenized in a mortar until the pulverized dry biomass was obtained. The dry biomass productivity (DBP) was determined according to Eq. 1 where: DB = dry biomass, HV = harvest volume and DC = days of culture. The percentage of conversion from wet to dry biomass (%WB/DB) was determined. The chemical analyzes of the samples were carried out in the Laboratorio de Analisis instrumental.

$$DBP \text{ (mg L}^{-1} \text{ day}^{-1}\text{)} = \left[ \left( \frac{DB \text{ (g L}^{-1}\text{)}}{HV \text{ (L)}} \times DC \right) \times 1000 \right] \quad (1)$$

## Protein content and amino acid profile

The percentage of protein was estimated as the sum of the values of the determined amino acids. Concerning to determination of the amino acid profile, 30 mg of dry biomass of the microalgae were mixed with 2 mL of 6 M hydrochloric acid and incubated in a dry bath at 112°C during 22 h for the acid hydrolysis reaction (Hirs et al. 1954). Then, 50 µL of the hydrolyzed sample was transferred to a test tube to which 100 µL of 2.5 mM L-aminobutyric acid (internal standard) and 4850 µL of ultrapure water were added. The solution was filtered on a 0.45 µm PTFE syringe filter and 10 µL of the filtrate was derivatized, using the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate reagent (Cohen and Michaud 1993) following the instructions of the AccQ-Fluor reagent kit (Waters Corporation, Milford, MA, USA). Next, 5 µL of the derivatized amino acid solution was injected into the Hitachi Elite LaChrom HPLC (Hitachi High Technologies, Tokyo, Japan), equipped with an L-2200 autosampler, L-2130 HTA pump, L-2350 column oven and fluorescence detector L-2485. A Thermo Scientific™ Hypersil GOLD™ C18 column of 150 mm × 4.6 mm × 5 µm (Thermo Fisher Scientific, Waltham, MA, USA) was used. The HPLC equipment was programmed to operate with the following conditions: column temperature 37°C, excitation wavelength 250 nm, emission wavelength 395 nm, flow rate 1 mL min<sup>-1</sup>. The mobile phases were as follows: (A) 140 mM sodium acetate pH 5.1 and (B) Acetonitrile with the following gradient: 0–2 min, 100% A; 2–24 min, 100 – 83.5% A; 24–30 min, 83.5–75% A; 30.1–38 min 100% A. Finally, the data acquisition and analysis were performed with the EZChrom Elite v3.2.1 software (Agilent Technologies, Santa Clara, CA, USA). The tryptophan (Trp) and cysteine (Cys) amino acids were not identified by the applied analytical technique.

Once the amino acid values were determined, the chemical score and the essential amino acid index (EAAI) were found, the chemical score is determined as the ratio of the value of each essential amino acid (EAA) of the microalgal sample with the value of the corresponding amino acid of a standard protein as described by Peñaflores (1989), in the event that the chemical score is greater than 1, this value was approximated as 1. As standard protein, the EAA requirements recommended in Table 1 were used, suggested by Kaushik (1998): for Gilthead seabream (*Sparus aurata*), which is a species belonging to the same order as Peruvian grunt (*Anisotremus scapularis*); Turbot (*Psetta maxima*), which is a type of flatfish similar to Fine flounder (*Paralichthys adspersus*); according to Hossain et al. (2011) for Silver pomfret (*Pampus argentus*); Bicudo and Cyrino (2009) mentioned the values for freshwater fish such as Streaked prochilod (*Prochilodus lineatus*); and Xie et al. (2012), in shrimp (*Penaeus vannamei*). The EAAI

evaluates the degree of EAA of the whole sample in relation to the composition or requirements of EAA of the organism (Ju et al. 2008; Zhang et al. 2009). The formula proposed by Peñaflores (1989) was used to calculate the EAAI (Eq. 2), where:  $aa_i$  = percentage of EAA<sub>i</sub> in the total of EAA of the sample,  $AA_i$  = percentage of EAA<sub>i</sub> in the total EAA in the standard and  $n$  = quantity of essential amino acids determined. The Table 1 shows the EAA required by different aquaculture species.

$$EAAI = \sqrt[n]{\frac{aa_1}{AA_1} \times \frac{aa_2}{AA_2} \dots \times \frac{aa_n}{AA_n}} \quad (2)$$

Table 1  
Amino acid requirement in different aquaculture species (g 100 g<sup>-1</sup> dry weight)

Amino acids	<i>S. aurata</i>	<i>P. maxima</i>	<i>P. argentus</i>	<i>P. lineatus</i>	<i>P. vannamei</i>
Arginine (Arg)	5.4	4.8	3.5	4.0	2.0
Histidine (His)	1.7	1.5	1.4	1.5	0.8
Isoleucine (Ile)	2.6	2.6	2.0	2.9	1.7
Leucine (Leu)	4.5	4.6	4.0	4.9	2.4
Lysine (Lys)	5.0	5.0	4.5	5.8	1.6
Methionine (Met) + Cysteine (Cys)	2.4	2.7	1.9	1.6	0.7
Phenylalanine (Phe) + Tyrosine (Tyr)	2.9	5.3	3.8	4.6	1.4
Threonine (Thr)	2.8	2.9	2.9	2.8	1.2
Valine (Val)	3.0	2.9	2.8	3.5	1.4

## Statistic analysis

The Pearson correlation index ( $r$ ) was calculated to determine the correlation of the variables. The data from the mean comparisons were analyzed with the analysis of variance test (ANOVA) with a significance level of 0.05 and the Tukey post-hoc test was used. The Kruskal Wallis test was applied for the data that did not meet the ANOVA assumptions. Finally, in order to know the interrelationships between the factors analyzed (abiotic parameters and amino acid profile), the Principal Component Analysis - PCA was applied. The statistical programs Minitab 17 (Minitab® Statistical Software, Pennsylvania, USA) and RStudio 1.2.5001 (RStudio, Boston, USA) were used.

## Results

### Abiotic parameters

A moderate positive correlation was observed between IL-GT and PAR-GT, while a high correlation between PAR-IL and PAR-CT (Fig. 1). The highest temperature inside greenhouse (GT) was above 26°C during January and February. While, the lowest temperature was 19°C during June and July (Fig. 1A), the GT for these last months were significantly lower compared to the other months evaluated ( $p = 1.000$ ). Regarding the CT values were between 26–28°C in warm seasons and ~20°C in cold seasons (Fig. 1D). The highest IL average was obtained in March ( $221 \pm 137$  Klux) with significant differences compared to June ( $p < 0.001$ ) (Fig. 1A); in the same way with PAR, the highest average was obtained in March ( $336 \pm 162 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with significant differences compared to June ( $p < 0.001$ ) (Fig. 1B). When graphing PAR with IL, the same trend is also observed.

## Biomass productivity

The total volume of 31370 L was harvested in ten months, which represented a final production of 45 kg of wet biomass and 10 kg of dry biomass. The lowest dry biomass productivity (DBP) was recorded during the months with the lowest temperature: June ( $38.8 \pm 1.0 \text{ mg L}^{-1} \text{ day}^{-1}$ ) and July ( $43.3 \pm 0.1 \text{ mg L}^{-1} \text{ day}^{-1}$ ); in addition, no significant differences were obtained between them ( $p = 0.999$ ). While the highest values of DBP were obtained in months of warm temperatures, whose values were higher than 70  $\text{mg L}^{-1} \text{ day}^{-1}$  (Fig. 2). A moderate positive correlation was observed between DBP – IL ( $r = 0.845$ ,  $p = 0.002$ ); while, there is a high correlation between DBP with PAR and CT ( $r = 0.920$ ,  $p < 0.005$ ;  $r = 0.937$ ,  $p < 0.005$  respectively). There is a high correlation between the dry and wet biomass weight ( $r = 0.971$ ,  $p < 0.010$ ), with an average conversion of 26% (Fig. 3).

## Proteins

According to our results, the mean protein percentage of the 10 months of culture was  $26.1 \pm 2.6\%$  (Fig. 4), its highest value was obtained in March ( $31.03 \pm 1.48\%$ ) with significant differences compared to April, May and July ( $p = 0.001$ ).

Apparently, according to Fig. 5, there would be a direct relationship between WBP and total protein concentration, however, when performing a correlation between the variables, a low relationship was found. In the same way, it happened with CT ( $r = 0.433$ ,  $p = 0.211$ ) and PAR ( $r = 0.521$ ,  $p = 0.122$ ), only a significant direct correlation was observed with IL ( $r = 0.796$ ,  $p = 0.026$ ). However, when the outlier corresponding to June was eliminated a significant positive correlation was obtained between the mean percentage of proteins and DBP ( $r = 0.832$ ,  $p < 0.001$ ).

## Amino acids profile

The highest concentration of amino acids was recorded in March ( $Z = 2.84$ ) (Fig. 6). Arginine (Arg) was the amino acid with the highest concentration ( $4.08 \pm 0.43 \text{ g } 100 \text{ g}^{-1}$ ), followed by aspartic acid (Asp) ( $3.36 \pm 0.23 \text{ g } 100 \text{ g}^{-1}$ ), in contrast to methionine (Met) ( $0.55 \pm 0.21 \text{ g } 100 \text{ g}^{-1}$ ), histidine (His) ( $0.77 \pm 0.07 \text{ g } 100 \text{ g}^{-1}$ ) and tyrosine (Tyr) ( $1.01 \pm 0.17 \text{ g } 100 \text{ g}^{-1}$ ) ( $p < 0.001$ ), which were the lowest concentration. The final average of all amino acids is shown in Table 3. In our study, the chemical score was determined

for different species (Table 2), it was observed that for fish the lowest value was > 0.70, while the limiting amino acid for *P. vannamei* was isoleucine (chemical score = 0.57). Concerning the EAAI, the highest value was for *P. maxima* (0.92) and the lowest value was for *P. vannamei* (0.85). Likewise, Fig. 7 shows the principal component analysis (PCA), it was observed that first component obtained direct correlation with the variables CT, GT, IL and with two amino acids: methionine (Met) and tyrosine (Tyr); while, the other amino acids are in the second component with direct correlation.

Table 2  
Chemical score and essential amino acids index (EAAI) of different species evaluated with the essential amino acids (EAA) of the biomass of *D. asymmetricus*.

<b>EAA</b>	<b><i>S. aurata</i></b>	<b><i>P. maxima</i></b>	<b><i>P. lineatus</i></b>	<b><i>P. argentus</i></b>	<b><i>P. vannamei</i></b>
Arg	1.00	1.00	1.00	1.00	1.00
His	0.75	0.90	0.90	0.82	0.71
Ile	0.85	0.91	0.81	0.98	0.57
Leu	0.85	0.88	0.83	0.85	0.72
Lys	0.76	0.81	0.70	0.75	1.00
Phe + Tyr	1.00	0.92	1.00	1.00	1.00
Thr	0.96	0.99	1.00	0.84	1.00
Val	1.00	1.00	0.94	0.96	0.96
EAAI	0.90	0.92	0.90	0.90	0.85

Table 3

Comparison of the protein and amino acid concentration of microalgae *D. asymmetricus* with other food sources (g 100 g<sup>-1</sup> dry weight).

Source	<i>D. asymmetricus</i>	Soy <sup>1</sup>	Egg <sup>2</sup>	<i>Scenedesmus</i> <sup>3</sup>	<i>Arthrospira</i> <sup>4</sup>	<i>Chorella</i> <sup>5</sup>
<b>Protein (%)</b>	<b>20–31</b>	36	47	8–56	42–73	14–67
<b>Essential amino acids</b>						
<b>Arginine (Arg)</b>	<b>4.1</b>	<b>7.4</b>	<b>6.2</b>	<b>6–7</b>	<b>4–8</b>	<b>3–14</b>
Histidine (His)	0.8	2.6	2.4	2–3	1–5	1–6
Isoleucine (Ile)	1.4	5.3	6.6	4–5	<1–7	<1–4
Leucine (Leu)	2.4	7.7	8.8	7–9	5–14	3–9
Lysine (Lys)	2.4	6.4	5.3	5–6	3–8	2–10
Methionine (Met)	0.5	1.3	3.2	1–2	1–5	<1–2
Phenylalanine (Phe)	1.9	5.0	5.8	5–7	3–7	2–8
Threonine (Thr)	1.7	4.0	5.0	5–6	3–7	<1–6
Triptofano (Trp)	ND	1.4	1.7	<1–2	<1–3	1–10
Valine (Val)	1.9	5.3	7.2	6	3–7	2–7
<b>Non-essential amino acids</b>						
Alanine (Ala)	3.1	5.0	-	9.0	6.8	7.9
Asparagine (Asn)	3.4	1.3	11.0	8.4	8.6	9.0
Cysteine (Cys)	ND	1.9	2.3	0.6	0.4	1.4
Glutamine (Gln)	3.1	19.0	12.6	10.7	12.6	11.6
Glycine (Gly)	2.1	4.5	4.2	7.1	4.8	5.8
Proline (Pro)	1.7	5.3	4.2	3.9	3.9	4.8
Tyrosine (Tyr)	1.0	3.7	4.2	3.2	3.9	3.4
Serine (Ser)	2.2	5.8	6.9	3.8	4.2	4.1

Extracted from the following references:

<sup>1</sup> Barka A, Blecker C (2016) Microalgae as a potential source of single-cell proteins. A review. *Biotechnol Agron Soc Environ* 20(3): 427–436.

Source	<i>D. asymmetricus</i>	Soy <sup>1</sup>	Egg <sup>2</sup>	<i>Scenedesmus</i> <sup>3</sup>	<i>Arthrospira</i> <sup>4</sup>	<i>Chorella</i> <sup>5</sup>
<sup>2</sup> Christaki E, Florou-Paneri P, Bonos E (2011). Microalgae: a novel ingredient in nutrition. <i>Int J Food Sci Nutr</i> 62(8): 794–799.						
<sup>3</sup> Tibbetts SM (2018). The Potential for “Next-Generation”, Microalgae-Based Feed Ingredients for Salmonid Aquaculture in Context of the Blue Revolution. In: Lopes E, Queiroz L, Queiroz M. <i>Microalgal Biotechnology</i> . IntechOpen, London, pp 152–175.						
<sup>4</sup> Kent M, Welladsen HM, Mangott A et al (2015) Nutritional Evaluation of Australian Microalgae as Potential Human Health Supplements. <i>Plos one</i> 10(2): e0118985.						
<sup>5</sup> Koyande A, Chew K, Rambabu K et al (2019) Microalgae: A potential alternative to health supplementation for humans. <i>Food Sci Hum Well</i> 8: 16–24.						

## Discussion

### Abiotic parameters

For the production of microalgae biomass, mainly for proteins, we should consider that the concentration of nutrients, composition of the culture medium, temperature and light are factors that influence the biochemical composition of biomass, as well as their interrelationships with other environmental parameters; therefore, these are parameters that should be optimized to improve biomass and protein production (Barbosa et al. 2003, Rasdi and Qin 2015, Khan et al. 2018, Sarker and Salam 2020). Light (Illuminance (IL) and PAR) is the source of energy to carry out photosynthesis, it mainly affects the growth and metabolism of microalgae. Temperature affects enzyme activity, nutritional requirements and gas solubility in cultures (Ras et al. 2013). For this reason, it is very important to know the optimal conditions and the tolerance limits of different parameters for each species (Ma et al. 2016, Jazzar et al. 2016).

Illuminance values between 20–500 klx should be used for the light to be effective, while a range of 27–500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  should be considered for PAR (Singh and Singh 2015). In our study, the greenhouse cultures of *D. asymmetricus* remained within the average of these values. In addition, the temperature, illuminance and PAR showed the highest values in warm months and the lowest values in cold months. On the other hand, some species of microalgae resist wide pH ranges, such as the genus *Chlorella* and *Desmodesmus*. The optimal growth of microalgae under normal conditions occurs between pH of 7 to 9 (Park et al. 2011; Ma et al. 2014; Beltrán-Rocha et al. 2017; Morales et al. 2018). In our study, an adequate pH of  $7.02 \pm 0.53$  was maintained. Most microalgal species survive under mesophilic temperature conditions (16–27°C), a value > 35 ° C would cause a collapse in the microalgal growth (Ras et al. 2013; Hernández-Pérez and Labbé 2014; IMARPE 2018; Barten 2020). According to the latter, in the case of genus *Desmodesmus*, the culture temperature between 25–28°C was adequate.

### Biomass productivity

Seasonal variation throughout the year influences biomass productivity (Eustance et al. 2015; Janssen 2016). In winter, productivity decreases due to the low intensity of light and temperature, while in summer it increases (Pérez - López et al. 2017). Light intensity is necessary for photosynthesis to take place, at low intensity the photosynthesis rate decreases because photochemical reactions are limited by the availability of light and the ability of cells to absorb them, which affects microalgal growth (Masojídek et al. 2013).

According to our results, DBP changes as a function of seasonal variations and it would be affected by CT and PAR, since there is a direct correlation with them. Authors such as Gomez et al. (2016) mentioned that the relationship between light intensity and photosynthesis rate are independent of temperature at low intensities ( $< 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). It depends on the dark reactions of photosynthesis and the level of nutrients. The microalgae will grow slowly in low luminosity, but it will be more efficient at capturing light, despite of this energy will not be used properly. If the light intensity is high ( $> 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), the development of microalgae will be limited by carbon fixation and the cells will use less of their own resources as in the synthesis of chlorophyll. Furthermore, Ras et al. (2013) indicate that the effect of temperature in the cultures responds constantly to the environmental temperature; therefore, the cell temperature will be equal to the temperature of the culture medium in comparison with other physicochemical parameters. In addition, the optimal temperature range has low influence on biomass productivity, in contrast to light (light intensity, photoperiod, wavelength, light source and photosynthetically active radiation) (Ma et al. 2016; Vecchi et al. 2020).

On the other hand, the dry biomass productivity of *D. asymmetricus* ( $38\text{--}78 \text{ mg L}^{-1} \text{ day}^{-1}$ ) coincides with reported by Mata et al. (2010), who obtained values between  $30\text{--}260 \text{ mg L}^{-1} \text{ day}^{-1}$  of *Desmodesmus sp.* cultured in different systems.

Likewise, *Desmodesmus obliquus* has registered values between  $4\text{--}740 \text{ mg L}^{-1} \text{ day}^{-1}$  and  $10\text{--}190 \text{ mg L}^{-1} \text{ day}^{-1}$  for *Desmodesmus quadricauda* (Rodolfi et al. 2009; Zhou et al. 2015). Similarly, the dry biomass of other freshwater species such as *Chlorella vulgaris* ( $20\text{--}200 \text{ mg L}^{-1} \text{ day}^{-1}$ ) and *Haematococcus pluvialis* ( $50\text{--}60 \text{ mg L}^{-1} \text{ day}^{-1}$ ) were similar with the values of our study (Dos Santos et al. 2021); although, the value was lower compared to *Chlorella pyrenoidosa* ( $2900\text{--}3640 \text{ mg L}^{-1} \text{ day}^{-1}$ ) (Mata et al. 2010).

## Proteins

Proteins were the first substances recognized as vital for all organisms, hence their name comes from the Greek word "protos" which means "of first importance" and they constitute one of the most abundant components of most living beings. Proteins are macromolecules constituted by hydrogen, carbon, oxygen and nitrogen; sometimes, it can include elements such as iron, cobalt or phosphorus. They are formed by a specific sequence of their own constituent elements: amino acids. There are 20 different amino acids that are linked through peptide bonds, all of them have an amino group ( $-\text{NH}_2$ ) and a carboxyl group (-

COOH) attached to the same carbon atom (alpha carbon) and differ in their side chains or R groups (Allison 2011).

In aquaculture, the evaluation of protein and amino acid requirements are important for the formulation of diets with greater efficiency and thus achieve sustainable aquaculture (Hua et al. 2019). An adequate intake of amino acids (from proteins) stimulates growth in fish. In addition, the percentage of protein determines the production costs, mainly in the diet of marine fish, since they require high levels; for this reason, this compound is considered the most important for fish culture (Sanz 2000). The nutritional value of a protein source is a function of its digestibility and its amino acid profile (Trushenki et al 2006). However, like other organisms, fish do not have a true requirement for protein, but have a specific requirement for a well-balanced profile of essential and nonessential amino acids in the diet (Gaye-Siessegger et al. 2007; De Almeida and Possebon 2014; Massamitu et al. 2015).

In fish farming, the protein requirement is influenced by the energy-protein balance, amino acid composition, digestibility of the diet, quantity of non-protein energy in the diet, muscle composition, blood histochemistry, intestinal health and gene expression (Glencross 2009; Conde-Sieira and Soengas 2017). The excess of energy in the diets can be a limit of consumption, since the fish ingest until they cover their energy requirements. Many authors affirm that there is a decrease in the protein requirement of fish as they increase their weight and age. Protein requirement can be altered due to a change in water temperature (Singh et al. 2009; Mizanur et al. 2014). Many studies report that salmonids can only tolerate low inclusion levels (< 10% of the diet) of microalgae meal such as *Arthrospira*, *Chlorella*, *Entomoneis*, *Isochrysis*, *Nannochloropsis*, *Phaeodactylum* and *Tetraselmis*. Also, there are some studies that indicate that the diet can be replaced up to 20% with a mixture of *Scenedesmus/Chlamydomonas* and *Desmodesmus/Nanofrustulum* (generally considered a benthic genus, however, they can be found in water columns) (FAO 2009; Walker and Berlinsky 2011; Tibbetts 2018).

In Table 3, a compilation of several investigations about the nutritional composition of different species of microalgae is shown, the Scenedesmaceae family (genus *Scenedesmus* and *Desmodesmus*) has reported between 8–56% of dry weight protein, which coincides with our study ( $26.1 \pm 2.6\%$  of protein). On the other hand, genus such as *Chlorella* and *Arthrospira* obtained values between 67–73%, respectively (Tibbetts 2018; Batista et al. 2020; Colombo et al. 2020; Glencross et al. 2020; Raji et al. 2020). In the microalgae, the percentage of protein is dependent on the supplied nitrogen source; the protein decreases if the nitrogen concentration decreases in the medium and its assimilation is strongly dependent on light, it incorporates or reduces nutrients such as nitrate ( $\text{NO}_3^-$ ) through the regulation of the synthesis and activity of the enzyme nitrate reductase. Another form of assimilation and incorporation occurs when light supplies ATP through photophosphorylation. Another indirect pathway occurs through the photosynthetic production of carbon compounds to accept the reduced nitrogen. This last methodology is carried out because light and carbon dioxide are needed to obtain the maximum rates of incorporation of nitrate, nitrite and ammonium, (Lachman et al. 2019; Kumar and Bera 2020).

According to our results, a significant direct correlation was observed between the percentage of total proteins and the illuminance (IL). In addition to the above, the intensity of light influences the biochemical composition of biomass due to the effect on photosynthesis, a high intensity of light produces stress in microalgae and increases storage compounds such as lipids, carbohydrates and carotenoids as a replacement for proteins (He et al. 2015; Nzayisenga et al. 2020; Sui et al. 2021). In June (atypical data respect to the percentage of proteins), probably the lower IL compared to the other months ( $94.5 \pm 64.4$  klux) has doubled the chlorophyll content, since the species of the genus *Scenedesmus* adapt their photosynthetic apparatus between 6–8 hours of culture (Sense and Senger 1990; Singh and Singh 2015), as a consequence, they would fix more nitrogen by increasing the protein content (Evans 1989, Evans and Clarke 2019).

## Amino acids profile

Amino acids are structural units of proteins, they are generally classified as: essential, non-essential and conditional, the latter has been proposed by some researchers in recent years (Li et al. 2009; Wu et al. 2014). A deficiency of amino acids results in inadequate use of the nutrients in the diet; therefore, there is low growth and low feeding efficiency in organisms (Kaushik and Seiliez 2010). Essential amino acids have carbon skeletons that are not synthesized by organisms or are synthesized in insufficient amounts; therefore, they must be provided from the diet. In contrast, non-essential amino acids are specific for each organism and they are synthesized *de novo* to satisfy their requirements (Wu 2010).

For many years, without much evidence, it was assumed that organisms (including humans) could synthesize sufficient amounts of all non-essential amino acids and that they were unnecessary in diets to achieve adequate growth or maintain good health. However, the increase in the number of investigations in various organisms has shown that some amino acids, traditionally classified as non-essential (glutamine, glutamic acid and arginine), play important roles in multiple cell signaling pathways, thus regulating gene expression, intracellular protein turnover, nutrient metabolism and oxidative immune defense. In addition, they can generate a benefit in the species of interest when it is supplied in adequate amounts in the diet (Li et al. 2009; Pianesso et al. 2015; Alagawany et al. 2020; Hoseini et al. 2020).

In fish farming, fish must be fed balanced levels of nutrients in their diets for successful aquaculture (Hixson 2014). The balance of proteins and amino acids is important to achieve optimal performance as in fish growth as in physiological and immune function (Hoseini et al. 2019). Amino acids are important molecules for living organisms with functions in protein and hormone synthesis, immune and antioxidant responses, and other physiological functions (Li et al. 2007; Wu 2009). A balanced amino acid profile in the diet is more important than protein levels in teleost diets, as each amino acid has some vital functions in the fish body (Wilson 2003; Andersen et al. 2016).

Table 3 shows the analysis of amino acid profile of *D. asymmetricus* and the values were compared with other food sources such as: soy (Barka and Blecker 2016), egg (Christaki et al. 2011), *Scenedesmus* (Tibbetts 2018), *Arthrospira* (Kent et al. 2015; Tibbetts 2018) and *Chlorella* (Kent et al. 2015; Koyande et al. 2019), which shows that microalgae can produce the amino acids commonly found in proteins. In our

results, the essential amino acids values were lower compared to other organisms. Highlights the value of arginine, which is in the average value of  $4.1 \pm 0.43 \text{ g } 100 \text{ g}^{-1}$  dry weight. Arginine is an essential amino acid in fish with different physiological roles in health and growth performance (Wilson 2003).

Concerning the EAAI, authors such as Oser 1959, Zhang et al. (2009) and Ju et al. (2008) proposed that values of EAAI  $> 0.95$  in different organisms and a value close or equal to 1.0 for shrimp, indicate that the diet contains an amino acid profile similar to that found in the entire body of the organism; ingredients with EAAI  $> 0.90$  were considered good quality protein foods; EAAI equal 0.80 was considered as useful and values  $< 0.70$  was considered inadequate. However, the characteristics of an ingredient to satisfy the amino acid requirements of an animal depends on the rate of consumption, bioavailability (digestibility) and composition (Li et al. 2010). Likewise, the values obtained from the chemical score indicate that foods with low EAA require the addition of these, either by other sources of protein or synthetic amino acid supplements. In this way, you seek to obtain a performance similar to a high-quality diet (Nunes et al. 2014). The EAAI found for the different organisms was greater than 0.80, therefore classified as useful protein material according to Ju et al. (2008). The EAAI value was equal to the biofloc of bacteria reported by Ju et al. (2008) and it was close to the value of soybean meal (0.87) for *Penaeus monodon*, informed by Peñaflores (1989). In this study, the result was in the range reported for different microalgae (marine and freshwater), between 0.76–1.00 (Kent et al. 2015; Tibbetts et al 2015; Cobos et al. 2020). Therefore, *D. asymmetricus* proteins can be classified as good quality and useful in the growth of different aquatic organisms.

Many investigations confirmed that the concentration of amino acids differs between species. In addition, the temperature used in the culture system can influence its concentration (Morançais et al. 2018). The PCA results showed a direct correlation between temperature (CT and IL) and the amino acids methionine and tyrosine. These results agree with those reported by James et al. (1989), who observed a similar behavior for *Chlorella* at temperatures between 25 and 30°C. Similarly, Uslu et al. (2006) indicated similar results for these amino acids; they conducted studies on a *Spirulina* species cultured at different temperatures, in warm ( $33.9 \pm 0.4 \text{ }^\circ\text{C}$ ) and cold ( $18.6 \pm 0.5 \text{ }^\circ\text{C}$ ) months.

## Conclusions

*Desmodesmus asymmetricus* is a eukaryotic microalga, which is among the most common freshwater genus. It has many advantages such as high biomass productivity and tolerance to high temperatures in greenhouse conditions. The best conditions for increasing biomass occurred in warm months, the biomass harvested presented an acceptable level of total protein. It has potential as a food supplement in the production of animal feed due to its high EAAI (in fish  $\geq 0.90$ , in crustaceans  $> 0.80$ ); the amino acid profile determined was similar to the values of other species currently used in food and aquaculture. It is recommended to carry out studies on the formulation of diets in aquaculture according to the cultivated species. In addition, should evaluate the profitability of the microalgae production, in order to obtain a new food input for aquaculture.

# Declarations

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## AUTHORS ROLES

AOH, MCG, LFR and ARS: performed experimental designs, performed the manuscript and data processing; AOH and MCG: collected and analyzed the samples; AOH, MCG, LFR and ARS: reviewed and approved the manuscript.

## CONFLICT OF INTEREST

The authors do not incur conflicts of interest.

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## ETHICS / LEGALS

The authors declare not to have incurred in ethical or legal faults during the development of the investigation and writing of this work.

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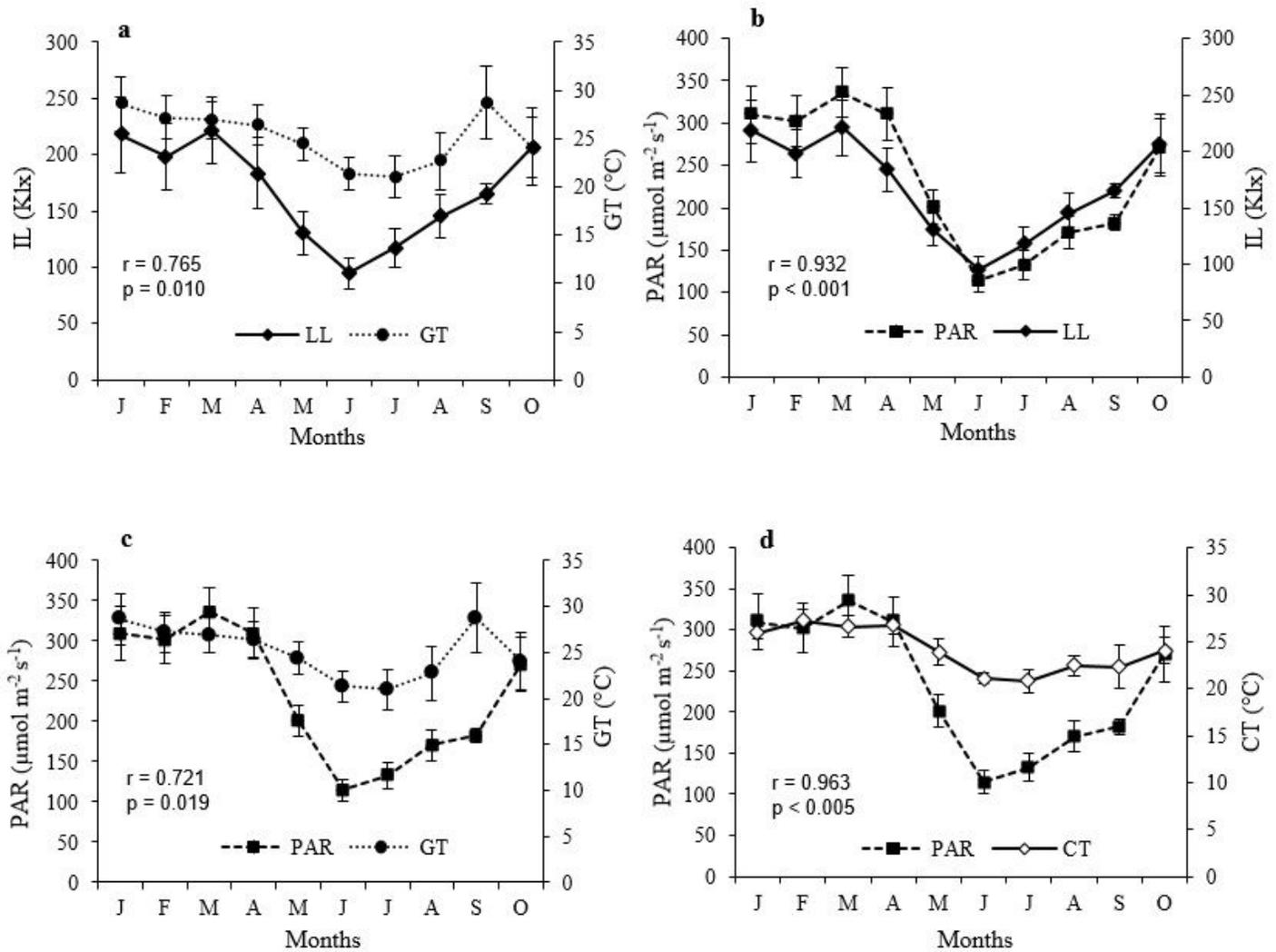
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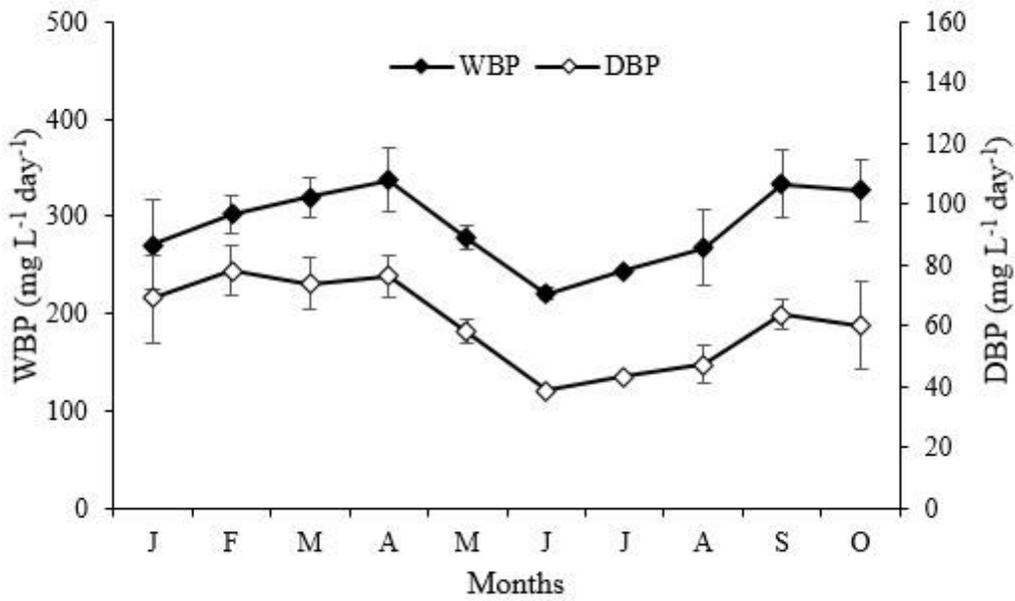
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## Figures



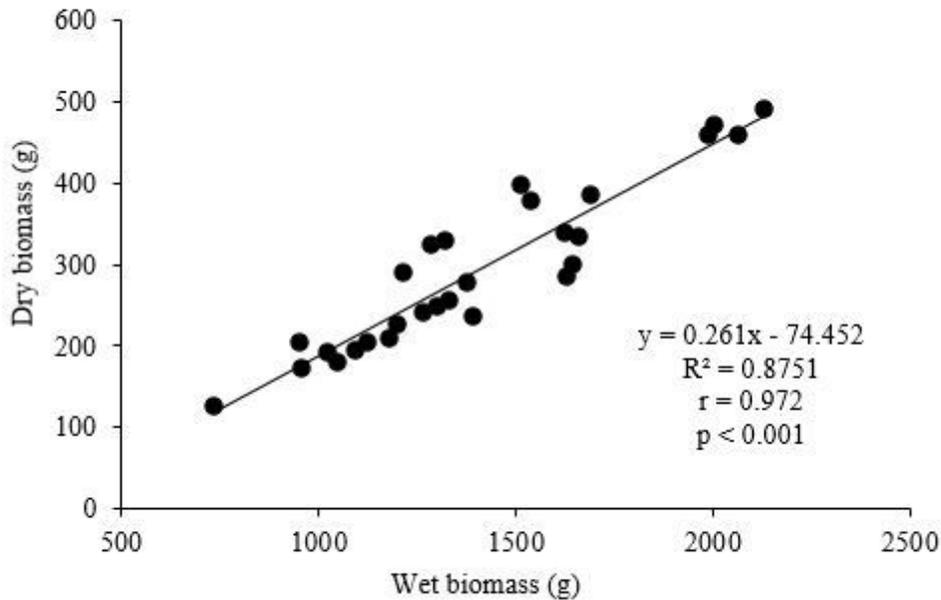
**Figure 1**

Monthly comparison of abiotic parameters in massive culture of *D. asymmetricus*. **a** Illuminance (IL) and greenhouse temperature (GT), **b** photosynthetically active radiation (PAR) and IL, **c** PAR and GT, and **d** PAR and culture temperature (CT). Vertical bars represent the standard deviation.



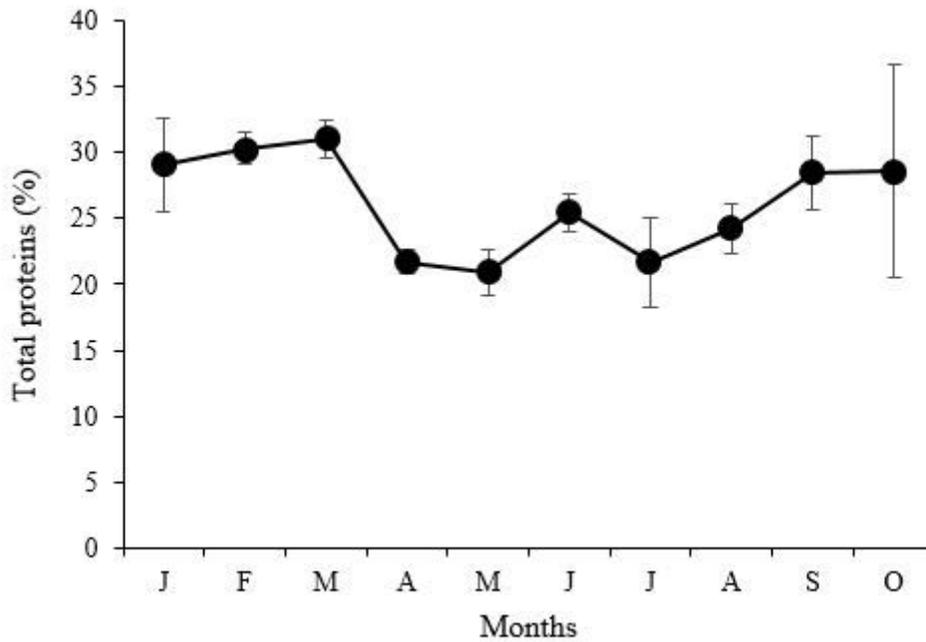
**Figure 2**

Monthly comparison of the final biomass concentrations in massive cultures of *D. asymmetricus*, wet biomass productivity (WBP) and dry biomass productivity (DBP). Vertical bars represent the standard deviation.



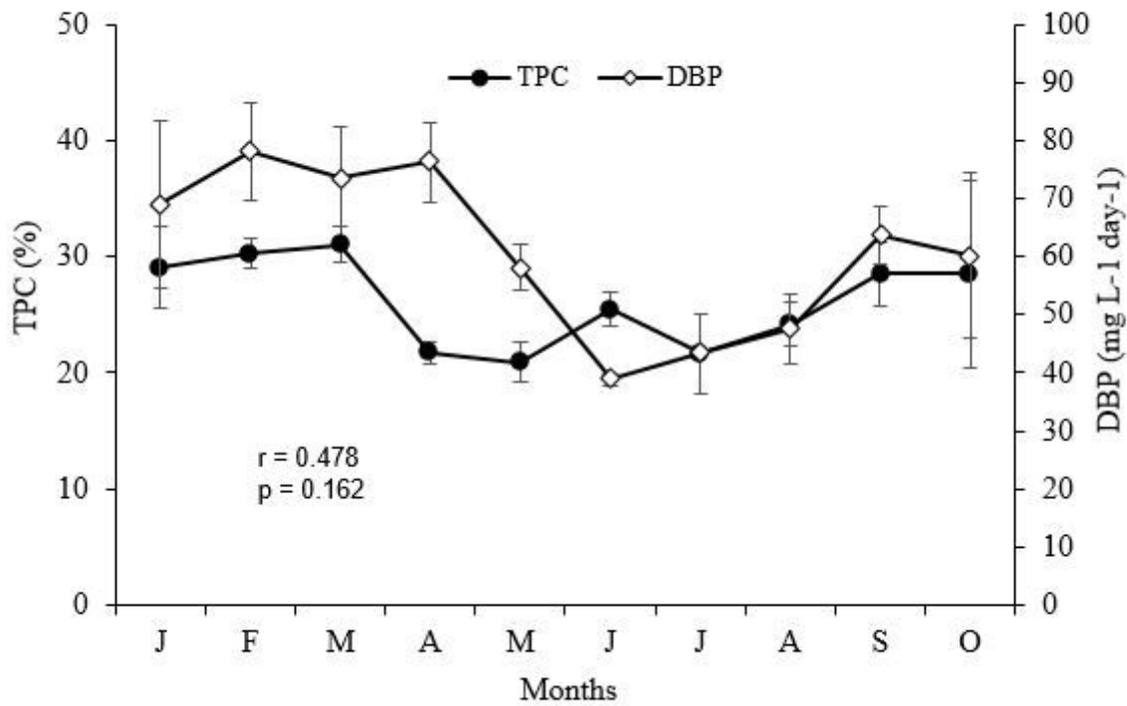
**Figure 3**

Conversion ratio of wet to dry biomass of *D. asymmetricus* grown under greenhouse conditions.



**Figure 4**

Monthly variation of the concentration of total proteins of the biomass of *D. asymmetricus* from the massive cultures. Vertical bars represent the standard deviation.

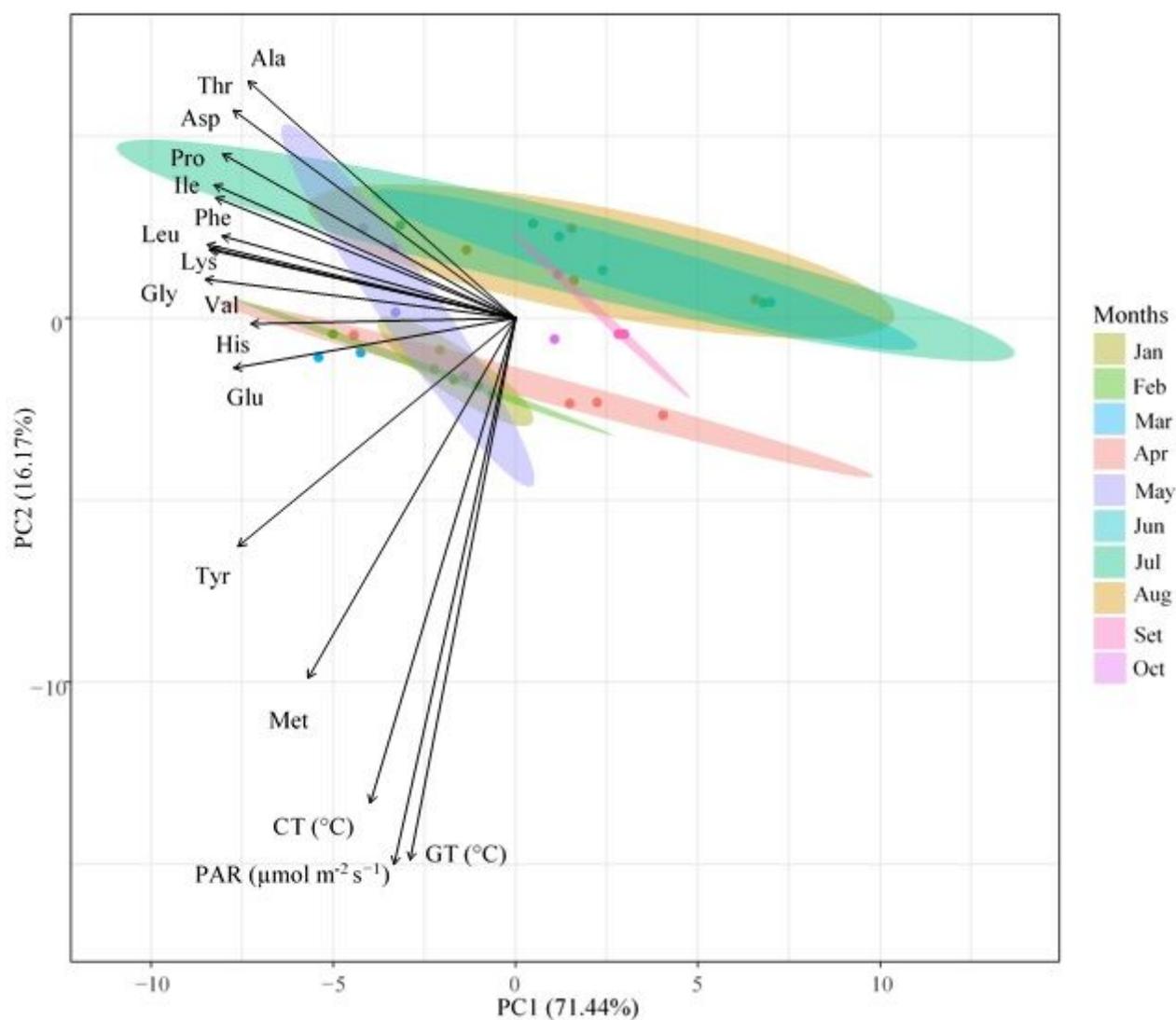


**Figure 5**

Monthly comparison of total protein concentration (TPC) and dry biomass productivity (DBP) in massive cultures of *D. asymmetricus*. Vertical bars represent the standard deviation.

**Figure 6**

Monthly variation of amino acid concentrations (g 100 g<sup>-1</sup>) in massive cultures of *D. asymmetricus*.



**Figure 7**

Principal component analysis (PCA) of abiotic parameters (CT, GT and PAR) and amino acids for *D. asymmetricus*.