Supplementary data

Seasonal biochemical fingerprints of outdoor cultivated Spirulina (*Limnospira platensis*) and *Microchloropsis gaditana*: insights into nutritional and functional shifts

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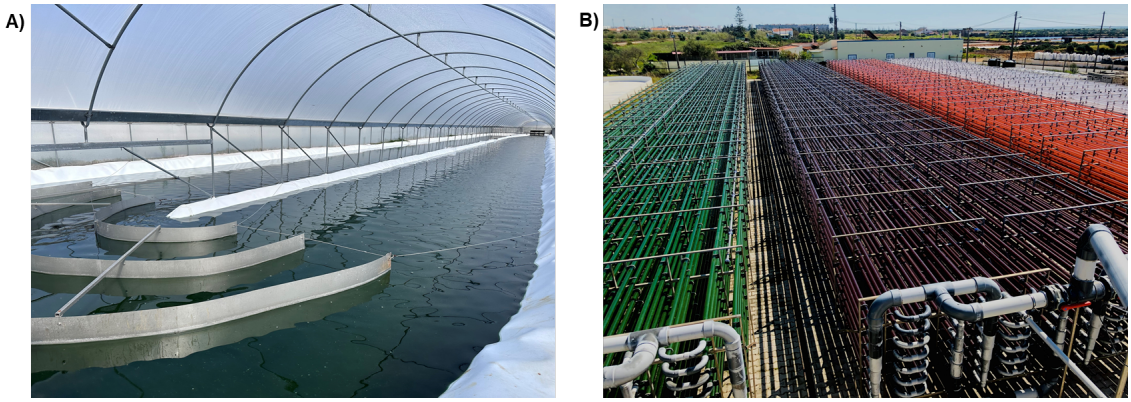
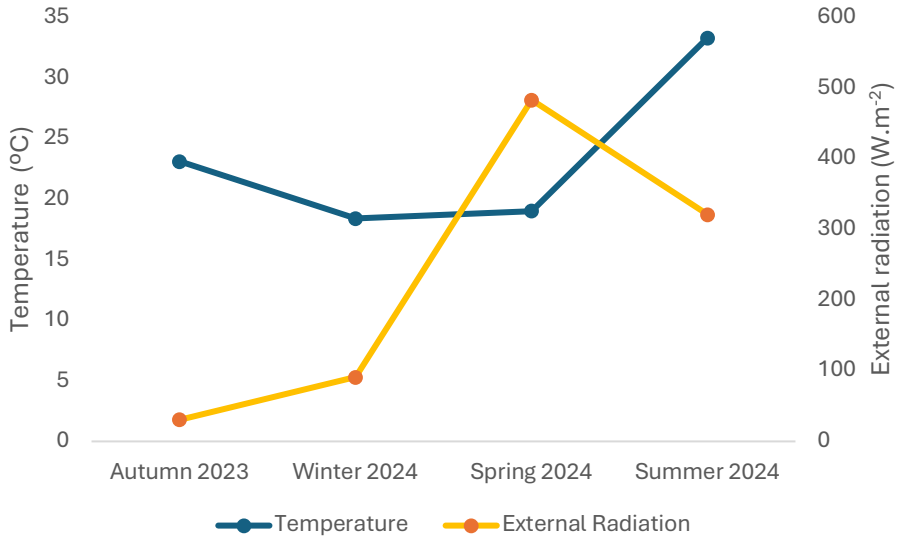
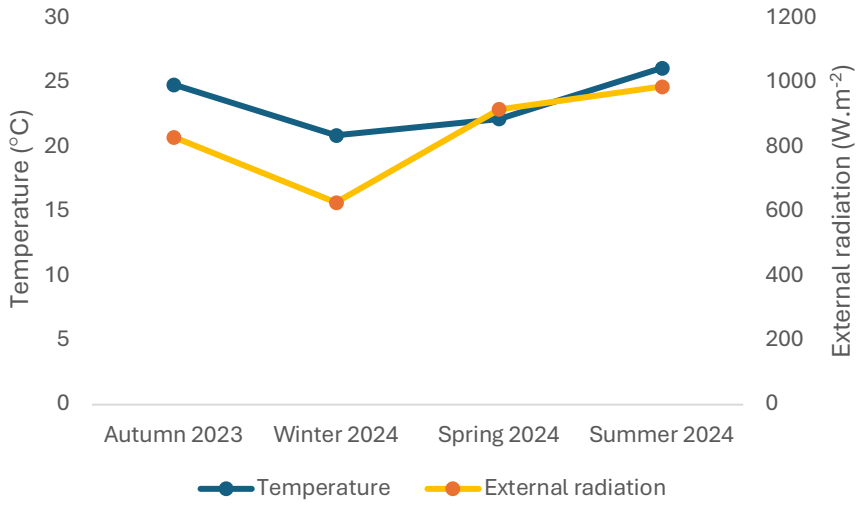
Supplementary Figures



Supplementary Figure S1. Representative photographs of A) open raceway ponds (ORP) at the facilities of Allmicroalgae - Natural Products S.A. (Pataias, Portugal) and B) tubular photobioreactors (TPBR) the facilities of Necton S.A (Olhão, Portugal).



Supplementary Figure S2. Temperature and external radiation recorded on the day for *L. platensis* produced at the facilities of Allmicroalgae - Natural Products S.A. (Pataias, Portugal) across different seasons.



Supplementary Figure S3. Temperature and external radiation recorded on the day for *M. gaditana* produced at Necton S.A. (Olhão, Portugal) across different seasons.

Supplementary Tables

Supplementary Table S1. Elemental composition (% of DW biomass) of Spirulina (*L. platensis*) and *M. gaditana* along the seasons. The values represent the mean ± standard deviation (SD) of three replicates (n=3). Significant differences between the groups were revealed using one-way ANOVA with Tukey's post hoc test. Values in the same row followed by the same letter are significantly different at *p-value* < 0.05.

	Autumn 2023	**Winter 2024**	**Spring 2024**	**Summer 2024**
L. platensis				
N	9.07 ± 0.03 ^{a,b}	5.65 ± 0.05 ^{a,c,d}	10.00 ± 0.16 ^{b,c}	9.51 ± 0.26 ^{d}
C	45.91 ± 0.24 ^{a,b}	43.98 ± 0.06 ^{c}	43.95 ± 1.01 ^{a,d}	48.82 ± 0.62 ^{b,c,d}
H	7.14 ± 0.12 ^{a}	6.93 ± 0.05 ^{b}	6.88 ± 0.22 ^{c}	7.75 ± 0.11 ^{a,b,c}
M. gaditana				
N	7.48 ± 0.08 ^{a}	6.42 ± 0.63 ^{a,b}	7.82 ± 0.16 ^{b}	7.01 ± 0.02
C	51.13 ± 0.20 ^{a}	43.95 ± 4.29 ^{a}	50.85 ± 0.67	44.75 ± 0.49
H	7.96 ± 0.04 ^{a}	6.67 ± 0.61 ^{a,b}	8 ± 0.18 ^{b}	7.21 ± 0.08

Supplementary Table S2. Proximal composition (% of DW biomass) of Spirulina (*L. platensis*) and *M. gaditana* along the seasons. The values represent the mean ± standard deviation (SD) of three replicates (n=3). Significant differences between the groups were revealed using one-way ANOVA with Tukey's post hoc test. Values in the same row followed by the same letter are significantly different at *p-value* < 0.05.

	Autumn 2023	**Winter 2024**	**Spring 2024**	**Summer 2024**
L. platensis				
Carbohydrates	25.72 ± 0.68 ^{a,b}	50.85±1.47 ^{a,c,d}	10.32±0.05 ^{b,c,e}	29.62±2.78 ^{d,e}
Lipids	8.63±0.52 ^{a}	4.56±0.49 ^{a,b}	9.51±0.35 ^{b,c}	6.30±1.54 ^{c}
Protein	43.34±0.13 ^{a,b}	27.01±0.22 ^{a,c,d}	47.82±0.74 ^{b,c}	45.48±1.24 ^{d}
Ash	13.78±0.42 ^{a,b,c}	6.34±0.11 ^{a,d}	14.92±0.13 ^{b,d,e}	5.63±0.08 ^{c,e}
Others	8.53±0.89 ^{a,b}	11.24±1.41 ^{c,d}	17.43±0.33 ^{a,c}	16.32±0.61 ^{b,d}
M. gaditana				
Carbohydrates	14.56±0.69 ^{a,b}	11.23±0.12 ^{a,c}	13.35±0.56 ^{c}	12.08±0.25 ^{b}
Lipids	21.67±1.35 ^{a,b}	18.92±0.42 ^{c,d}	15.8±0.96 ^{a,c}	15.93±0.6 ^{b,d}
Protein	35.77±0.39 ^{a}	30.71±2.99 ^{a,b}	37.36±0.75 ^{b}	33.49±0.12
Ash	11.41±0.12 ^{a,b}	13.3±0.60 ^{c}	13.92±1.15 ^{a,d}	18.23±0.74 ^{b,c,d}
Others	16.59±1.28 ^{a}	25.84±3.79 ^{a}	19.57±0.77	20.27±0.41

Supplementary Table S3. Amino acids profile (g *per* 100 g DW biomass^{-1}) of *L. platensis* and *M. gaditana* along the seasons determined by liquid chromatography. Values are the mean ± standard deviation (SD) of three replicates (n=3). Significant differences between the groups were revealed using one-way ANOVA with Tukey's post hoc test. Values in the same row followed by the same letter are significantly different at *p-value* < 0.05.

Aminoacids	Autumn 2023	Winter 2024	Spring 2024	Summer 2024
L. platensis				
Aspartic acid	2.75±0.04 ^{a,b,c}	2.55±0.01 ^{a,d,e}	3.88±0.03 ^{b,d,f}	3.71±0.04 ^{c,e,f}
Glutamic acid	3.73±0.02 ^{a,b}	2.38±0.03 ^{a,c,d}	3.63±0.07 ^{c}	3.47±0.1 ^{b,d}
Cysteine	2.78±0.05 ^{a,b,c}	2.01±0.01 ^{a,d,e}	3.06±0.08 ^{b,d,f}	3.93±0.04 ^{c,e,f}
Serine	0.27±0.01 ^{a,b}	0.27±0.04 ^{c,d}	0.49±0.03 ^{a,c}	0.47±0.04 ^{b,d}
Glycine	1.26±0.01 ^{a,b,c}	2.05±0.09 ^{a,d,e}	3.12±0.03 ^{b,d,f}	2.98±0.04 ^{c,e,f}
Threonine	4.15±0.05 ^{a,b,c}	3.13±0.08 ^{a,d,e}	4.76±0.02 ^{b,d,f}	4.55±0.03 ^{c,e,f}
Arginine	3.61±0.02 ^{a,b,c}	2.61±0.02 ^{a,d,e}	3.98±0.04 ^{b,d,f}	3.8±0.07 ^{c,e,f}
Alanine	2.12±0.03 ^{a,b,c}	1.53±0.06 ^{a,d,e}	2.33±0.02 ^{b,d,f}	2.23±0.03 ^{c,e,f}
Tyrosine	4.19±0.10 ^{a,b,c}	3.03±0.06 ^{a,d,e}	4.61±0.03 ^{b,d,f}	4.41±0.04 ^{c,e,f}
Valine	3.58±0.01 ^{a,b,c}	2.59±0.01 ^{a,d,e}	3.94±0.03 ^{b,d,f}	3.77±0.05 ^{c,e,f}
Methionine	4.63±0.05 ^{a,b,c}	3.36±0.02 ^{a,d,e}	5.11±0.01 ^{b,d,f}	4.88±0.01 ^{c,e,f}
Phenylalanine	2.09±0.09 ^{a,b,c}	1.20±0.01 ^{a,d,e}	1.82±0.03 ^{b,d}	1.74±0.04 ^{c,e}
Isoleucine	3.21±0.09 ^{a,b,c}	1.23±0.03 ^{a,d,e}	1.88±0.06 ^{b,d}	1.80±0.10 ^{c,e}
Leucine	3.11±0.02 ^{a,b,c}	2.25±0.02 ^{a,d,e}	3.43±0.04 ^{b,d,f}	3.28±0.07 ^{c,e,f}
Lysine	1.02±0.04 ^{a}	0.74±0.03 ^{a,b,c}	1.12±0.09 ^{b}	1.07±0.07 ^{c}
Total	42.50 ± 0.30 ^{a,b,c}	30.94 ± 0.17 ^{a,d,e}	47.15 ± 0.11 ^{b,d,f}	46.09 ± 0.32 ^{c,e,f}
M. gaditana				
Aspartic acid	2.71±0.03 ^{a,b}	2.63±0.07 ^{c,d}	2.93±0.07 ^{a,c,e}	2.13±0.02 ^{b,d,e}
Glutamic acid	2.53±0.06 ^{a,b}	2.46±0.02 ^{c,d}	2.74±0.01 ^{a,c,e}	2.88±0.04 ^{b,d,e}
Cysteine	2.14±0.03 ^{a}	2.23±0.06	2.31±0.05 ^{a,b}	2.15±0.04 ^{b}
Serine	0.34±0.02 ^{a}	0.33±0.08 ^{b}	0.37±0.05 ^{c}	1.01±0.02 ^{a,b,c}
Glycine	2.18±0.01 ^{a,b}	2.11±0.1 ^{c,d}	2.36±0.06 ^{a,c,e}	0.98±0.02 ^{b,d,e}
Threonine	3.32±0.02 ^{a,b}	3.23±0.06 ^{c}	3.59±0.04 ^{a,c,d}	3.21±0.01 ^{b,d}
Arginine	2.53±0.03 ^{a,b}	2.7±0.12 ^{c}	3.00±0.09 ^{a,c,d}	2.79±0.04 ^{b,d}
Alanine	2.78±0.04 ^{a,b,c}	1.58±0.07 ^{a,d}	1.76±0.03 ^{b,d}	1.64±0.07 ^{c}
Tyrosine	3.22±0.02 ^{a}	3.13±0.06 ^{b,c}	3.48±0.04 ^{a,b,d}	3.24±0.02 ^{c,d}
Valine	2.75±0.03 ^{a}	2.67±0.08 ^{b}	2.98±0.06 ^{a,b,c}	2.77±0.03 ^{c}
Methionine	3.57±0.08 ^{a}	3.46±0.04 ^{b}	3.86±0.02 ^{a,b,c}	3.59±0.09 ^{c}
Phenylalanine	1.27±0.03 ^{a}	1.23±0.09 ^{b}	1.37±0.05 ^{c}	1.62±0.02 ^{a,b,c}
Isoleucine	1.31±0.06 ^{a,b}	1.27±0.01 ^{c,d}	1.42±0.01 ^{a,c,e}	2.48±0.02 ^{b,d,e}
Leucine	2.40±0.04 ^{a}	2.33±0.01 ^{b}	2.59±0.07 ^{a,b,c}	2.41±0.04 ^{c}
Lysine	0.78±0.03	0.76±0.02	0.85±0.03	0.79±0.09
Total	33.86 ± 0.25 ^{a,b}	32.13 ± 0.14 ^{a,c,d}	35.58 ± 0.11 ^{b,c,e}	33.67 ± 0.09 ^{d,e}

Supplementary Table S4. Fatty acids profile (mg.g DW biomass^{-1}) of Spirulina (*L. platensis*) along the seasons determined by gas chromatography–mass spectrometry (GC–MS). The values represent the mean ± standard deviation (SD) of three replicates (n=3). Significant differences between the groups were revealed using the Kruskal-Wallis test, followed by Dunn's post-hoc comparisons. Values in the same row followed by the same letter are significantly different at *p-value* < 0.05.

Fatty acids	Autumn 2023	Winter 2024	Spring 2024	Summer 2024
FA 16:0	13.92 ± 0.22 [a]	4.63 ± 0.48 [a]	12.01 ± 2.47	9.37 ± 1.98
FA 16:1 *n*-5	0.66 ± 0.03	0.55 ± 0.07	0.58 ± 0.15	0.57 ± 0.14
FA 16:1 *n*-7	1.93 ± 0.07	2.07 ± 0.24	1.67 ± 0.42	1.40 ± 0.34
FA 16:2 *n*-4	0.09 ± 0.01	0.17 ± 0.02	0.10 ± 0.02	0.09 ± 0.02
FA 18:0	1.38 ± 0.31 [a]	0.70 ± 0.25	0.70 ± 0.15	0.42 ± 0.08 [a]
FA 18:1 *n*-9	2.12 ± 0.02 [a]	0.25 ± 0.03 [a]	0.62 ± 0.13	0.59 ± 0.15
FA 18:1 *n*-7	0.41 ± 0.01 [a]	0.15 ± 0.03	0.30 ± 0.05	0.12 ± 0.03 [a]
FA 18:2 *n*-6	6.95 ± 0.03	2.53 ± 0.43	6.90 ± 1.41	5.78 ± 1.40
FA 18:3 *n*-6	8.82 ± 0.30 [a]	5.15 ± 0.57 [a]	7.37 ± 1.46	6.51 ± 1.58
FA 20:2 *n*-6	0.09 ± 0.05	0.05 ± 0.01	0.07 ± 0.02	0.06 ± 0.02
FA 20:3 *n*-6	0.13 ± 0.01	0.07 ± 0.01	0.10 ± 0.02	0.13 ± 0.03
SFA	15.31 ± 0.41 [a]	5.32 ± 0.46 [a]	12.72 ± 2.60	9.79 ± 2.05
MUFA	5.13 ± 0.10 [a]	3.02 ± 0.37 [a]	3.16 ± 0.74	2.68 ± 0.66
PUFA	16.06 ± 0.28	7.97 ± 1.03	14.55 ± 2.94	12.55 ± 3.08
Total FA	36.50 ± 0.49	16.32 ± 1.77	30.42 ± 6.27	25.02 ± 5.79

Supplementary Table S5. Fatty acids profile (% relative abundance) of Spirulina (*L. platensis*) along the seasons determined by gas chromatography–mass spectrometry (GC–MS). The values represent the mean ± standard deviation (SD) of three replicates (n=3). Significant differences between the groups were revealed using the Kruskal-Wallis test, followed by Dunn's post-hoc comparisons. Values in the same row followed by the same letter are significantly different at *p-value* < 0.05.

	Autumn 2023	Winter 2024	Spring 2024	Summer 2024
FA 16:0	38.15±0.12	28.38±0.12 [a]	39.49±0.37 [a]	37.56±0.60
FA 16:1 *n*-5	1.81±0.08 [a]	3.38±0.06 [a]	1.89±0.08	2.28±0.04
FA 16:1 *n*-7	5.30±0.19	12.68±0.21	5.45±0.22	5.59±0.06
FA 16:2 *n*-4	0.25±0.02 [a]	1.07±0.04 [a]	0.32±0.01	0.36±0.01
FA 18:0	3.79±0.70	4.33±1.37	2.31±0.22	1.68±0.11
FA 18:1 *n*-9	5.82±0.06 [a]	1.53±0.05 [a]	2.03±0.04	2.36±0.05
FA 18:1 *n*-7	1.13±0.03 [a]	0.92±0.07	0.99±0.04	0.48±0.01 [a]
FA 18:2 *n*-6	19.06±0.19	15.45±0.9 [a]	22.68±0.05	23.07±0.26 [a]
FA 18:3 *n*-6	24.17±0.45	31.56±0.17	24.26±0.32	25.97±0.24
FA 20:2 *n*-6	0.26±0.01	0.29±0.03	0.24±0.02	0.21±0.03
FA 20:3 *n*-6	0.35±0.02	0.41±0.01	0.34±0.02 [a]	0.51±0.01 [a]
SFA	41.94±0.64 [a]	32.71±1.49 [a]	41.80±0.17	39.24±0.68
MUFA	14.05±0.33	18.51±0.37 [a]	10.36±0.23 [a]	10.70±0.13
PUFA	44.00±0.39 [a]	48.79±1.12	47.84±0.3	50.06±0.56 [a]

Supplementary Table S6. Fatty acids profile (mg.g DW biomass^{-1}) of *M. gaditana* along the seasons determined by gas chromatography–mass spectrometry (GC–MS). The values represent the mean ± standard deviation (SD) of three replicates (n=3). Significant differences between the groups were revealed using the Kruskal-Wallis test, followed by Dunn's post-hoc comparisons. Values in the same row followed by the same letter are significantly different at *p-value* < 0.05.

Fatty acids	Autumn 2023	Winter 2024	Spring 2024	Summer 2024
FA 14:0	2.98 ± 0.84	1.85 ± 0.45	2.01 ± 0.30	2.97 ± 0.17
FA 15:0	0.16 ± 0.04	0.14 ± 0.02 [a]	0.37 ± 0.07 [a]	0.20 ± 0.02
FA 16:0	15.70 ± 3.28	12.53 ± 1.62	11.95 ± 1.46	11.77 ± 0.87
FA 16:1*n*-7	18.21 ± 4.07	14.90 ± 2.18	11.74 ± 1.50	10.69 ± 0.78
FA 16:1	0.08 ± 0.04	0.09 ± 0.02	0.10 ± 0.01	0.52 ± 0.03
FA 16:1*n*-9	1.01 ± 0.26	0.92 ± 0.14	0.59 ± 0.09	0.31 ± 0.03
FA 16:2*n*-6	0.38 ± 0.10	0.18 ± 0.03	0.27 ± 0.03	0.10 ± 0.01
FA 16:2*n*-4	0.16 ± 0.04 [a]	0.13 ± 0.02	0.13 ± 0.02	0.09 ± 0.01 [a]
FA 18:0	1.02 ± 0.02	1.32 ± 0.26	1.60 ± 0.45	3.01 ± 0.53
FA 18:1*n*-9	2.87 ± 0.73 [a]	2.19 ± 0.28	2.58 ± 0.33	2.28 ± 0.24 [a]
FA 18:1*n*-7	0.24 ± 0.06	0.22 ± 0.02	0.19 ± 0.03	0.41 ± 0.05
FA 18:2n-6	1.87 ± 0.48	2.24 ± 0.28	1.17 ± 0.17 [a]	1.09 ± 0.13 [a]
FA 18:3*n*-6	0.74 ± 0.19	0.62 ± 0.09	0.45 ± 0.06	0.17 ± 0.03
FA 20:3*n*-6	0.19 ± 0.07	0.39 ± 0.05	0.11 ± 0.10	0.12 ± 0.03 [a]
FA 20:4*n*-6	2.48 ± 0.69	4.57 ± 0.58	1.89 ± 0.30	1.16 ± 0.15
FA 20:5*n*-3	33.49 ± 7.47	31.64 ± 3.69 [a]	20.54 ± 2.52	11.44 ±1.19 [a]
SFA	19.86 ± 4.13	15.84 ± 2.10	15.94 ± 2.10	17.95 ± 0.76
MUFA	22.41 ± 5.16	18.31 ± 2.64	15.20 ± 1.96	14.21 ± 1.11
PUFA	39.30 ± 9.05	39.76 ± 4.73	24.57 ± 3.17	14.16 ± 1.55
Total FA	81.56 ± 18.34	73.91 ± 9.43	55.71 ± 7.19	46.33 ± 3.31

Supplementary Table S7. Fatty acids profile (% relative abundance) of *M. gaditana* along the seasons determined by gas chromatography–mass spectrometry (GC–MS). The values represent the mean ± standard deviation (SD) of three replicates (n=3). Significant differences between the groups were revealed using the Kruskal-Wallis test, followed by Dunn's post-hoc comparisons. Values in the same row followed by the same letter are significantly different at *p-value* < 0.05.

Fatty acids	Autumn 2023	Winter 2024	Spring 2024	Summer 2024
FA 14:0	3.62±0.16	2.47±0.25 [a]	3.60±0.06 [a]	6.43±0.29
FA 15:0	0.19±0.00	0.19±0.01	0.67±0.04	0.42±0.01
FA 16:0	19.30±0.24	16.95±0.08 [a]	21.47±0.17	25.40±0.38 [a]
FA 16:1 *n*-7	22.34±0.03	20.13±0.44 [a]	21.07±0.07	23.06±0.21 [a]
FA 16:1	0.10±0.02 [a]	0.12±0.01	0.19±0.00	1.13±0.04 [a]
FA 16:1 *n*-9	1.23±0.04	1.24±0.03 [a]	1.06±0.03	0.66±0.02 [a]
FA 16:2 *n*-6	0.46±0.02	0.24±0.01 [a]	0.49±0.00 [a]	0.21±0.01
FA 16:2 *n*-4	0.19±0.01	0.17±0.01	0.23±0.00 [a]	0.18±0.01 [a]
FA 18:0	1.29±0.24 [a]	1.81±0.31	2.87±0.54	6.56±1.22 [a]
FA 18:1 *n*-9	3.50±0.08	2.96±0.01 [a]	4.63±0.06	4.92±0.14 [a]
FA 18:1 *n*-7	0.29±0.01 [a]	0.29±0.01	0.33±0.01	0.89±0.03 [a]
FA 18:2 *n*-6	2.28±0.06	3.03±0.01 [a]	2.09±0.06	2.34±0.09 [a]
FA 18:3 *n*-6	0.90±0.02 [a]	0.84±0.01	0.81±0.02	0.37±0.03 [a]
FA 20:3 *n*-6	0.22±0.03	0.53±0.01	0.19±0.13	0.25±0.04
FA 20:4 *n*-6	3.01±0.14	6.18±0.05 [a]	3.39±0.14	2.50±0.12 [a]
FA 20:5 *n*-3	41.07±0.11	42.84±0.45 [a]	36.90±0.20	24.67±0.76 [a]

SFA	24.40±0.31	21.42±0.14 [a]	28.62±0.42	38.82±1.26 [a]
MUFA	27.45±0.11	24.75±0.47 [a]	27.29±0.15	30.66±0.28 [a]
PUFA	48.15±0.20	53.83±0.47 [a]	44.10±0.27	30.52±1.00 [a]

Supplementary Table S8. Pigments profile (mg.g DW biomass^{-1}) of Spirulina (*L. platensis*) along the seasons determined by HPLC-DAD (carotenoids) and spectrophotometry (chlorophylls). The values represent the mean ± standard deviation (SD) of three replicates (n=3). Significant differences between the groups were revealed using one-way ANOVA with Tukey's post hoc test. Values in the same row followed by the same letter are significantly different at *p-value* < 0.05. Canthaxanthin, fucoxanthin and astaxanthin were not detected in all seasons.

Pigments	Autumn 2023	Winter 2024	Spring 2024	Summer 2024
Chlorophyll *a*	6.59 ± 0.15	2.29 ± 0.05 [a]	10.88 ± 0.19	12.35 ± 0.38 [a]
Zeaxanthin	- [a]	- [b]	- [c]	1.64 ± 0.02 [a,b,c]
β-carotene	1.42 ± 0.01 [a]	0.11 ± 0.00	0.10±0.00	- [a]
Neoxanthin	4.55 ± 0.02 [a]	2.35 ± 0.03	2.14 ± 0.05	- [a]
Violaxanthin	0.04 ± 0.00 [a]	0.02 ± 0.00	0.02 ± 0.00	- [a]
Lutein	1.18 ± 0.00 [a]	0.52 ± 0.01	0.52 ± 0.00	- [a]

Supplementary Table S9. Pigments profile (mg g DW biomass^{-1}) of *M. gaditana* along the seasons determined by HPLC-DAD (carotenoids) and spectrophotometry (chlorophylls). The values represent the mean ± standard deviation (SD) of three replicates (n=3). Significant differences between the groups were revealed using one-way ANOVA with Tukey's post hoc test. Values in the same row followed by the same letter are significantly different at *p-value* < 0.05. Canthaxanthin, fucoxanthin and astaxanthin were not detected in all seasons.

Pigments	Autumn 2023	Winter 2024	Spring 2024	Summer 2024
Chlorophyll *a*	12.70 ± 0.01 [a]	1.28 ± 0.00 [a]	4.84 ± 0.04	9.22 ± 0.16
Zeaxanthin	0.19 ± 0.00	0.23 ± 0.00	0.87 ± 0.02 [a]	- [a]
β-carotene	1.26 ± 0.01	1.68 ± 0.02 [a]	0.83 ± 0.07	- [a]
Neoxanthin	18.75 ± 0.15 [a,b]	17.73 ± 0.16	- [a]	- [b]
Violaxanthin	0.19 ± 0.09	0.01 ± 0.00 [a]	20.73 ± 1.36 [a]	6.30 ± 0.37
Lutein	2.34 ± 0.06 [a]	2.02 ± 0.02	0.96 ± 0.09	0.17 ± 0.00 [a]

Supplementary Table S10. Phycocyanin content (mg g DW biomass^{-1}) in *L. platensis* along the seasons. The values represent the mean ± standard deviation (SD) of three replicates (n=3). Significant differences between the groups were revealed using one-way ANOVA with Tukey's post hoc test. Values in the same row followed by the same letter are significantly different at $p < 0.05$.

	Autumn 2023	Winter 2024	Spring 2024	Summer 2024
Phycocyanin	40.23 ± 1.60 [a,b,c]	36.13 ± 1.59 [a,d,e]	67.68 ± 0.80 [b,d,f]	110.06 ± 1.22 [c,e,f]