The effect of high temperatures on the growth and biochemical composition of cultivated *Palmaria palmata* (Rhodophyta) – **Supplementary Information**

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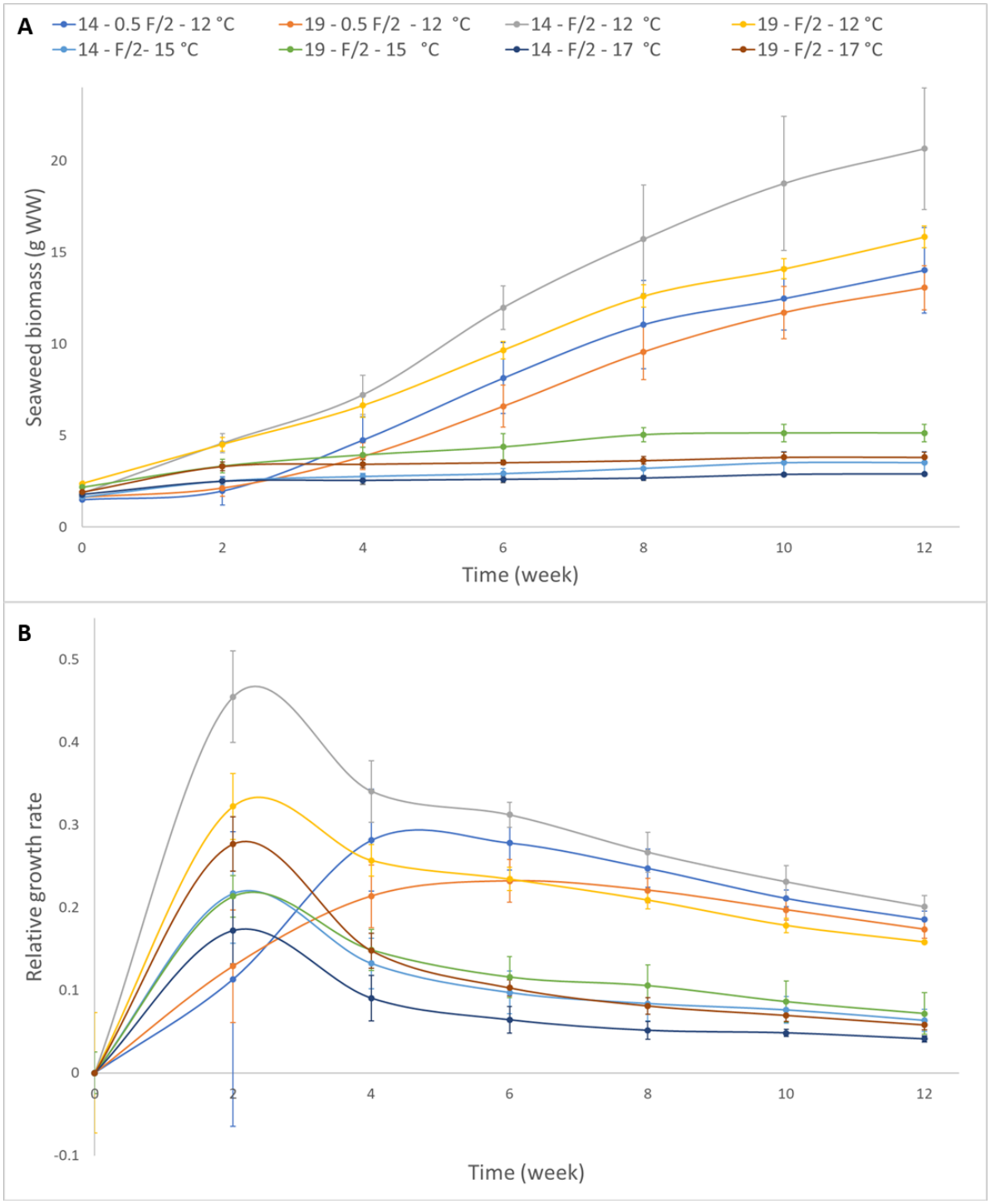
Supplementary table S1. Composition and concentrations of F/2 medium stock solutions and their respective final molar concentration when added to filtered natural seawater. F/2 medium does not contain silicate by default because most algae do not require it to grow. Hence, stock D was omitted from the medium in the current study.

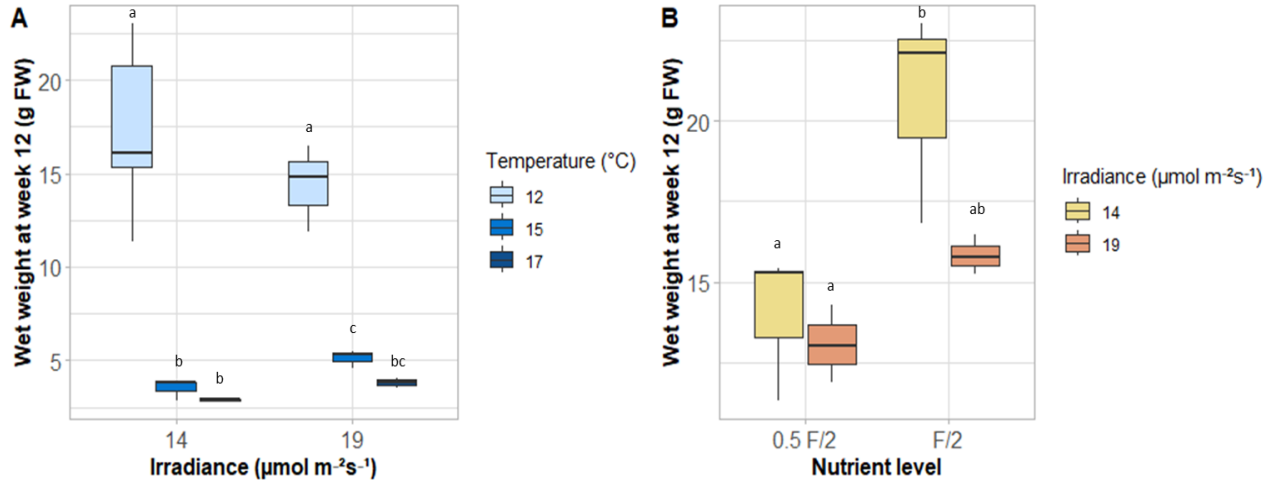
Label	Components	Concentration (g L^{-1} dH_2O)	Molar concentration in final medium (M)
Stock A	$NaNO_3$	75	$8.82 \cdot 10^{-4}$
Stock B	$NaH_2PO_4 \cdot 2H_2O$	5.69	$3.62 \cdot 10^{-5}$
Stock C	Na_2CO_3	30.00	$2.83 \cdot 10^{-4}$
Stock D	$Na_2SiO_3 \cdot 9H_2O$	30.00	$1.06 \cdot 10^{-4}$
Stock E	*Trace metals*		
	$FeCl_3 \cdot 6H_2O$	3.15	$1.17 \cdot 10^{-5}$
	$Na_2EDTA \cdot 2H_2O$	4.36	$1.17 \cdot 10^{-5}$
	$CuSO_4 \cdot 5H_2O$	$9.8 \cdot 10^{-3}$	$3.93 \cdot 10^{-8}$
	$Na_2MoO_4 \cdot H_2O$	$6.3 \cdot 10^{-3}$	$2.60 \cdot 10^{-8}$
	$ZnSO_4 \cdot 7H_2O$	$2.2 \cdot 10^{-2}$	$7.65 \cdot 10^{-8}$
	$CoCl_2 \cdot 6H_2O$	0.01	$4.20 \cdot 10^{-8}$
	$MnCl_2 \cdot 4H_2O$	0.18	$9.10 \cdot 10^{-7}$
Stock F	*Vitamins*		
	Thiamine HCl (Vitamin B1)	0.2	$2.96 \cdot 10^{-7}$
	Biotin (Vitamin H)	$1.0 \cdot 10^{-4}$	$2.05 \cdot 10^{-9}$
	Cyanocobalamin (Vitamin B12)	$1.0 \cdot 10^{-3}$	$3.69 \cdot 10^{-10}$

Supplementary table S2. Average relative growth rate (± SD) of *Palmaria palmata* over 12 weeks compared to start of the experiment in the different treatments.

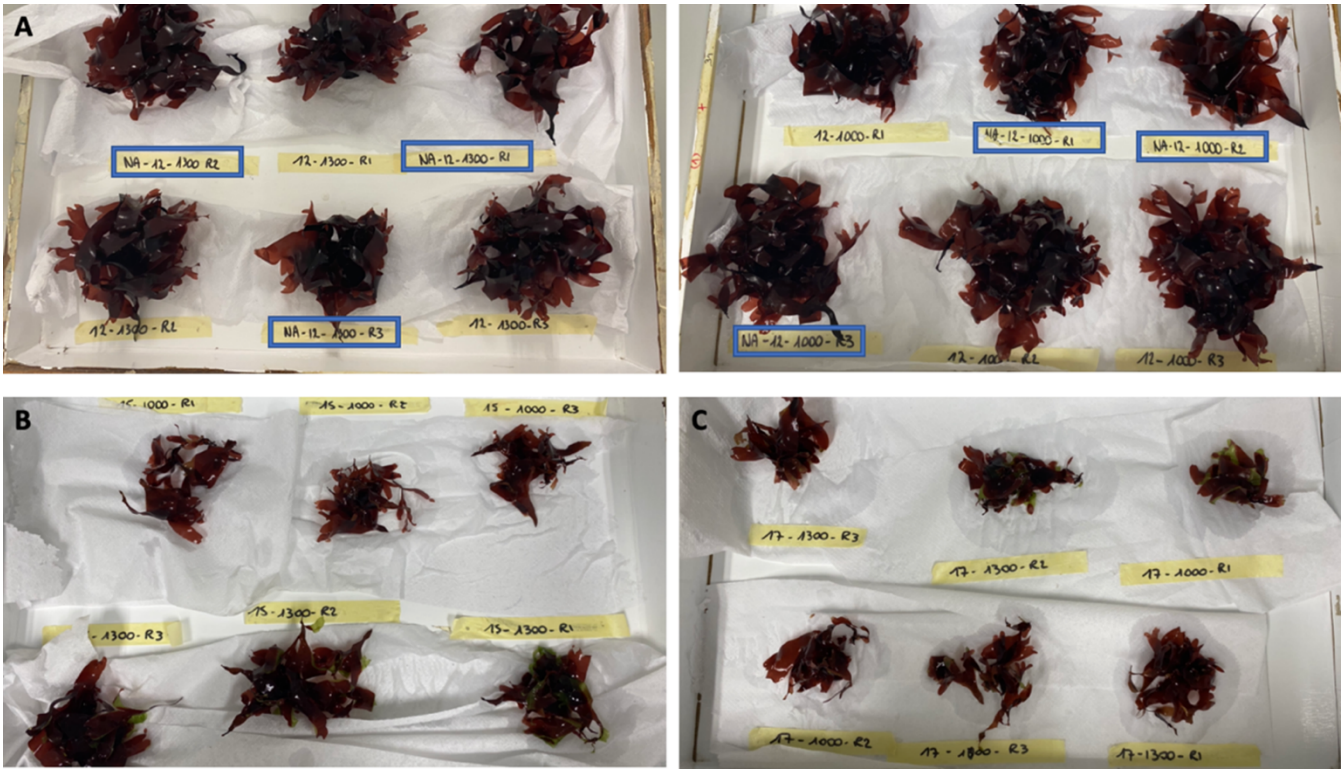
Growth conditions			Relative growth rate (day^{-1})					
Temperature (°C)	Irradiance (µmol m^{-2} s^{-1})	Medium	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
12	14	0.5 F/2	0.11 ± 0.10	0.28 ± 0.06	0.28 ± 0.03	0.25 ± 0.02	0.21 ± 0.01	0.19 ± 0.01
	14	F/2	0.45 ± 0.06	0.34 ± 0.04	0.31 ± 0.01	0.27 ± 0.02	0.23 ± 0.02	0.20 ± 0.01
	19	0.5 F/2	0.13 ± 0.07	0.21 ± 0.04	0.23 ± 0.03	0.22 ± 0.01	0.20 ± 0.01	0.17 ± 0.01
	19	F/2	0.32 ± 0.07	0.26 ± 0.04	0.23 ± 0.02	0.21 ± 0.01	0.18 ± 0.01	0.16 ± 0.01
15	14	F/2	0.22 ± 0.06	0.13 ± 0.03	0.10 ± 0.03	0.08 ± 0.02	0.08 ± 0.02	0.06 ± 0.01
	19	F/2	0.21 ± 0.06	0.15 ± 0.03	0.12 ± 0.03	0.11 ± 0.01	0.09 ± 0.01	0.07 ± 0.01
17	14	F/2	0.17 ± 0.04	0.09 ± 0.03	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.00
	19	F/2	0.28 ± 0.03	0.15 ± 0.02	0.10 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01



Supplementary figure S1. A) Average biomass of exposed *Palmaria palmata* over twelve weeks. B) Average relative growth rate (day^{-1}) compared to start of the experiment. Error bars indicate associated standard deviations (analyses in triplicate). Abbreviations used: 14 = 14 µmol m^{-2} s^{-1}, 19 = 19 µmol m^{-2} s^{-1}; 0.5 F/2 = low nutrient supply, only half the nutrients present in F/2 medium were administered; F/2 = normal nutrient supply, administration of F/2 medium.



Supplementary figure S2. (A) Effect of temperature and irradiance on harvested biomass (fresh weight) at week 12, cultivated in regular F/2 medium. (B) Effect of irradiance and nutrient supply on *Palmaria palmata* biomass at week 12, at a water temperature of 12 (± 1) °C. Abbreviations used: F/2 = normal nutrient supply, i.e. in regular F/2 medium; 0.5 F/2 = lower nutrient supply, only half of nutrients were administered compared to the standard levels. The horizontal bold line in each box represents the median, the box itself represents upper and lower (25% and 75%) quartiles, the bars encompass the data range, while dots placed past the bar edges indicate outliers. Different letters indicate statistically significant differences (adjusted $p < 0.05$).



Supplementary figure S3. The habit of the *Palmaria palmata* thalli at harvest reared at three different temperatures 12 °C (A), 15 °C (B) and 17 °C (C) for twelve weeks at two different light irradiances, i.e. 14 µmol $m^{-2}s^{-1}$ (approximating an illuminance of 1000 lux) and19 µmol $m^{-2}s^{-1}$ (illuminance of 1300 lux). Blue squares indicate that only half of the usually supplied nutrients of the F/2 medium were administered.

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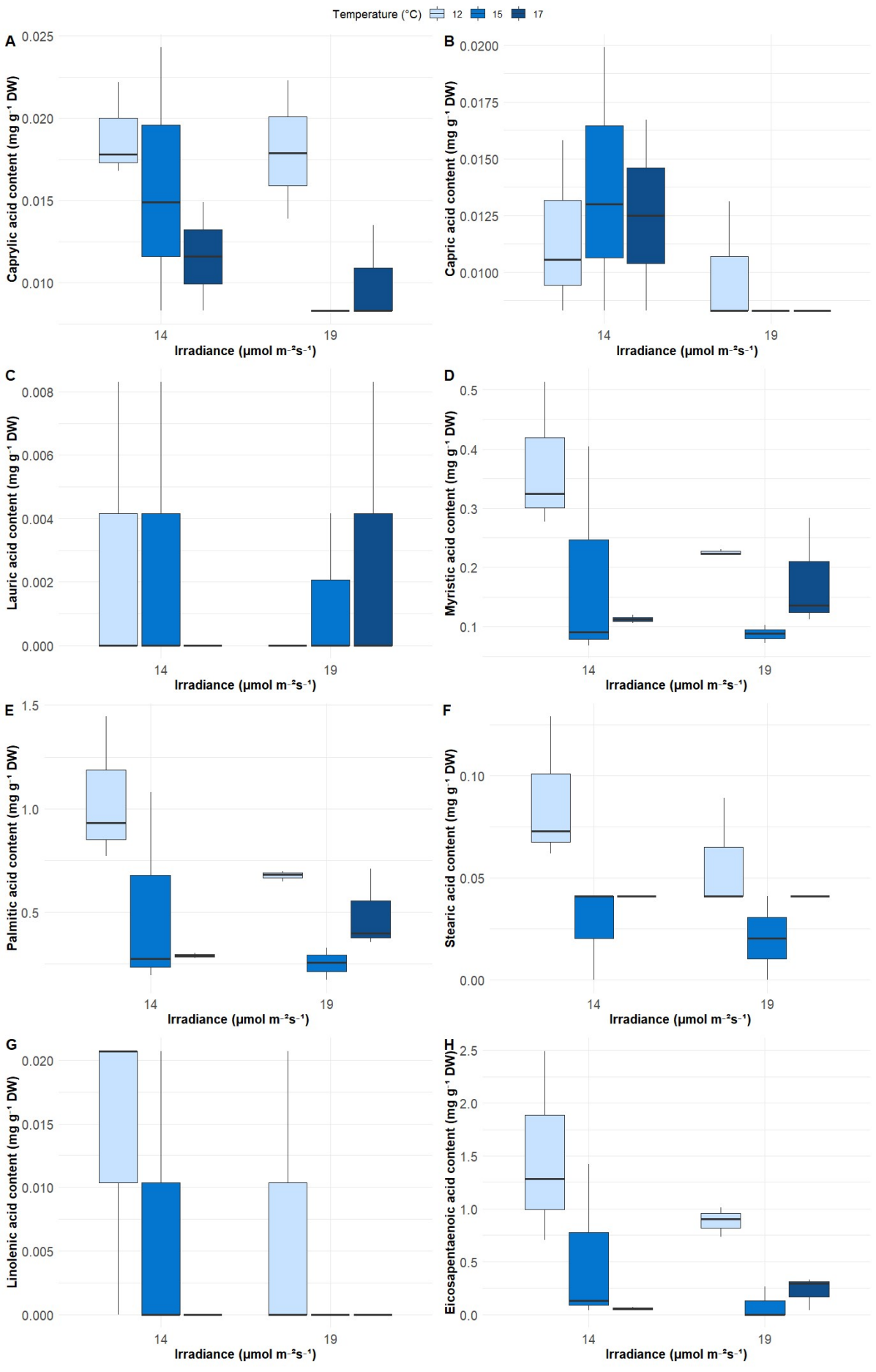
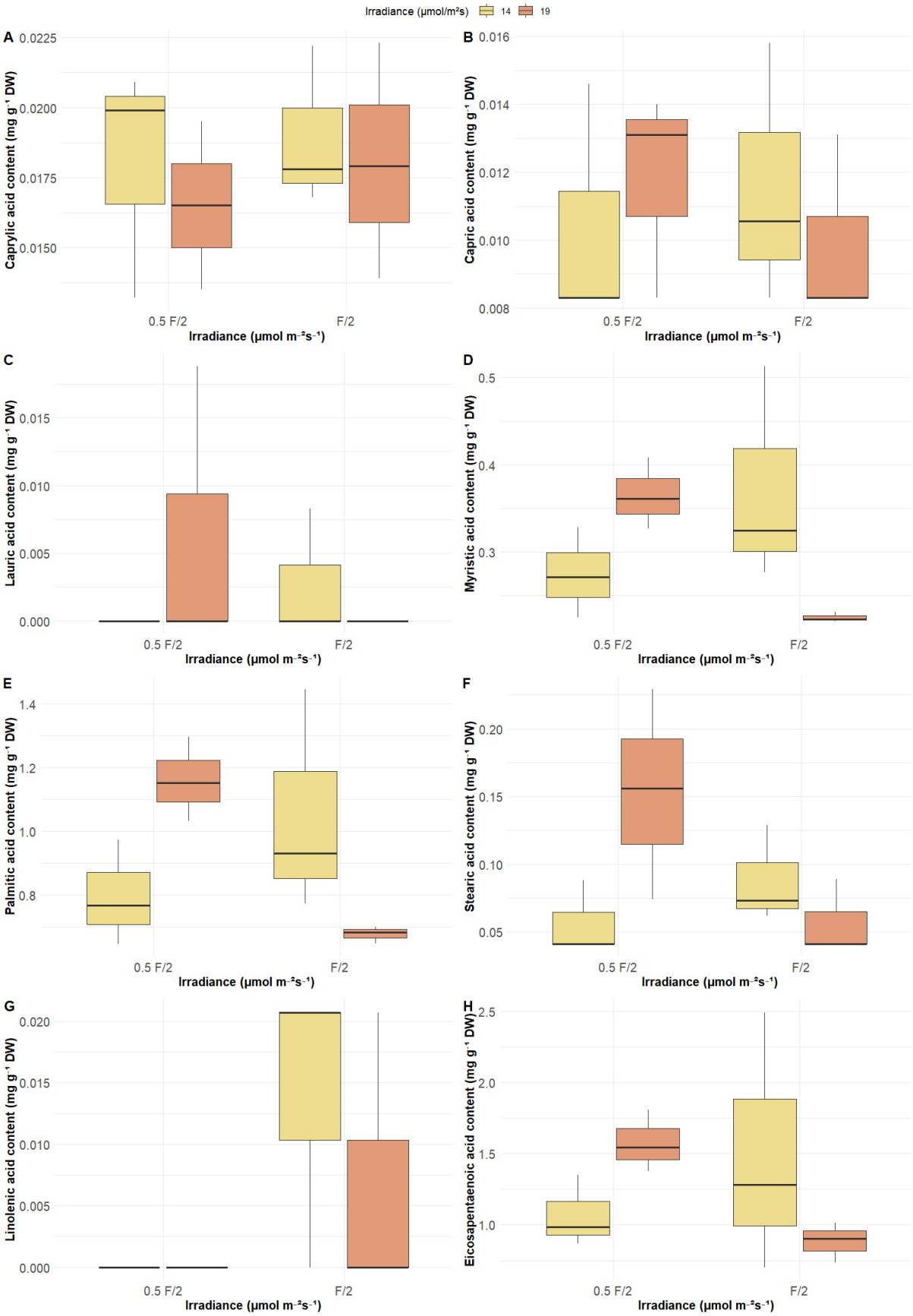
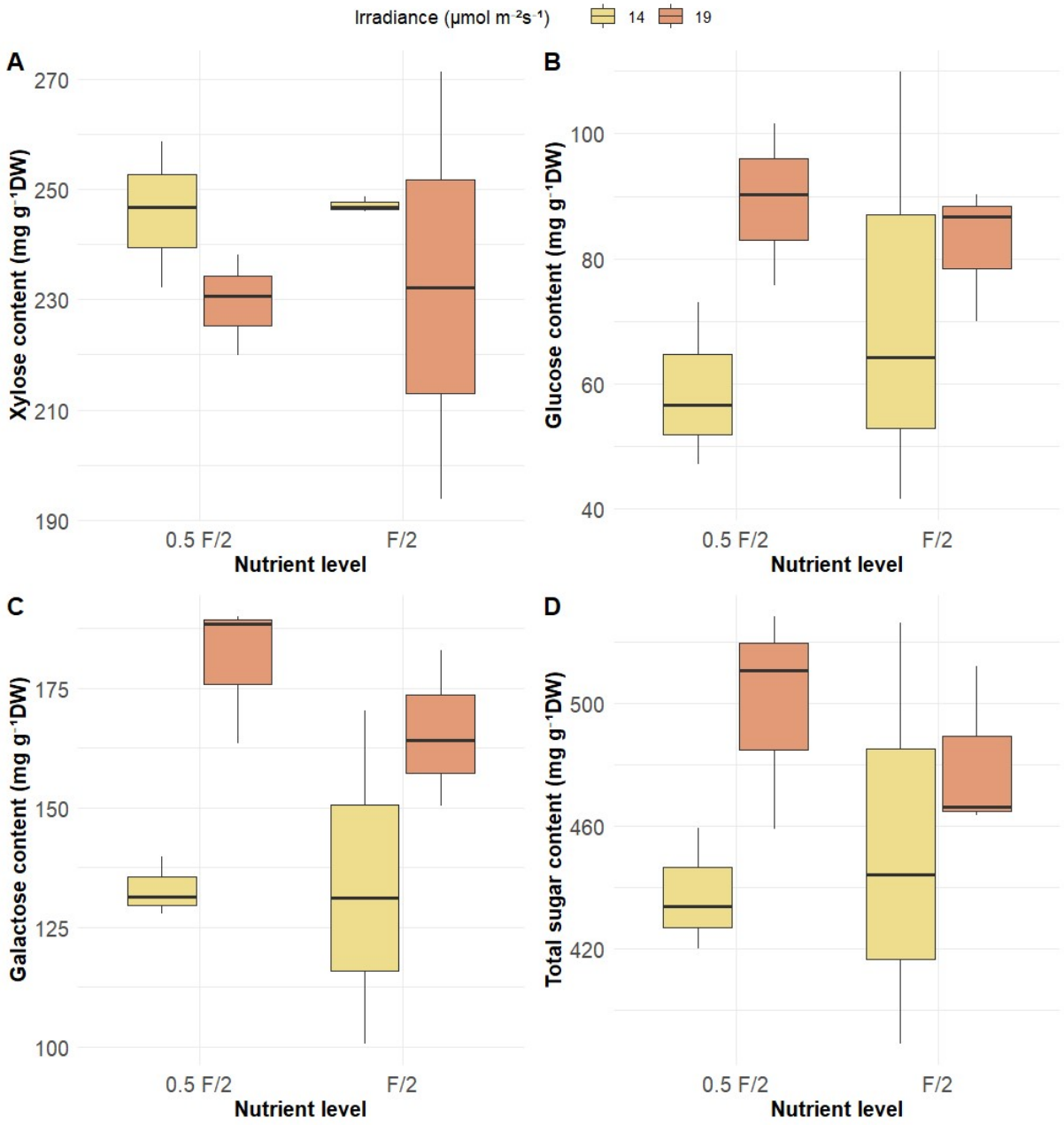

Temperature (°C) 12 15 17
A
Caprylic acid content (mg g⁻¹ DW)
0.025
0.020
0.015
0.010
B
Capric acid content (mg g⁻¹ DW)
0.0200
0.0175
0.0150
0.0125
0.0100
C
Lauric acid content (mg g⁻¹ DW)
0.008
0.006
0.004
0.002
0.000
D
Myristic acid content (mg g⁻¹ DW)
0.5
0.4
0.3
0.2
0.1
E
Palmitic acid content (mg g⁻¹ DW)
1.5
1.0
0.5
F
Stearic acid content (mg g⁻¹ DW)
0.10
0.05
0.00
G
Linolenic acid content (mg g⁻¹ DW)
0.020
0.015
0.010
0.005
0.000
H
Eicosapentaenoic acid content (mg g⁻¹ DW)
2.5
2.0
1.5
1.0
0.5
0.0
14
19
Irradiance (µmol m⁻²s⁻¹)

Supplementary figure S4. Fatty acid content of a selection of fatty acids in *Palmaria palmata* grown in F/2 medium (mg g^{-1} DW). (A) The effect of temperature and irradiance on caprylic acid (A), capric acid (B), lauric acid (C), myristic acid (D), palmitic acid (E), stearic acid (F), Linolenic acid (G), and eicosapentaenoic acid (H) content. The horizontal line represents the median, the box represents upper and lower (25% and 75%) quartiles, bars encompass the data range, while dots placed past the bar edges indicate outliers.



Supplementary figure S5. Fatty acid content (mg g^{-1} DW) of a selection of fatty acids in *Palmaria palmata* grown in F/2 of 0.5 F/2 medium. (A) The effect of nutrient supply and irradiance on caprylic acid (A), capric acid (B), lauric acid (C), myristic acid (D), palmitic acid (E), stearic acid (F), Linolenic

acid (G), and eicosapentaenoic acid (H) content. The horizontal line represents the median, the box represents upper and lower (25% and 75%) quartiles, bars encompass the data range, while dots placed past the bar edges indicate outliers.



Supplementary figure S6. Box plots illustrating monosaccharide and carbohydrate content of *Palmaria palmata* grown under different nutrient and irradiance conditions (mg g^{-1} DW). (A) xylose content. (B) glucose content. (C) galactose content. (D) total carbohydrate (sum of monosaccharides) content. The horizontal line represents the median, the box represents upper and lower (25% and 75%) quartiles, bars encompass the data range, while dots placed past the bar edges indicate outliers. No significant differences between the treatments were observed.

Supplementary table S3. Fatty acid composition of *Palmaria palmata* (grown in F/2 medium), expressed as means (mg g^{-1} DW) and standard deviations under different temperature and irradiance conditions. Two-way ANOVA on the effects of temperature (A, df = 2), irradiance level (B, df = 1) and their interaction (AxB, df = 2) are reported, or nonparametric alternatives (underlined) Kruskal-Wallis, Wilcoxon and Dunn's test. Asterisks indicate significant p-values. Abbreviations used: LOD = limit of detection. Abbreviations: n.d. = not detectable; LNA = linoleic acid; ALA = linolenic acid; EPA = eicosapentaenoic acid; n = omega classification.

Fatty acid (GC-MS)	14 µmol m^{-2} s^{-1}			19 µmol m^{-2} s^{-1}			Source of variation	p-value
	12 °C	15 °C	17 °C	12 °C	15 °C	17 °C		
C8:0 Caprylic acid	0.019 ± 0.0029	0.016 ± 0.0080	0.012 ± 0.0047	0.018 ± 0.0042	0.0083	0.010 ± 0.0030	A B AxB	0.0048* 0.10 0.30
C10:0 Capric acid	0.011 ± 0.0034	0.014 ± 0.0058	0.013 ± 0.0060	0.011 ± 0.0028	0.0083	0.0083	A B	0.71 0.124
C12:0 Lauric acid	0.0014 ± 0.0034	0.0028 ± 0.0048	< LOD	0.0031 ± 0.0077	0.0014 ± 0.0024	0.0028 ± 0.0048	A B	0.84 0.73
C14:0 Myristic acid	0.32 ± 0.10	0.19 ± 0.19	0.11 ± 0.0099	0.30 ± 0.081	0.087 ± 0.15	0.178 ± 0.092	A B	0.0065* 0.65
C15:0 Pentadecylic acid	0.010 ± 0.0024	0.0083 ± 0.0072	0.012	0.012	0.010 ± 0.0036	0.012	A B	0.34 0.15
C15:1 Pentadecenoic acid	0.0014 ± 0.0035	< LOD	< LOD	< LOD	< LOD	< LOD	A B	0.63 0.34
C16:0 Palmitic acid	0.92 ± 0.28	0.52 ± 0.49	0.29 ± 0.017	0.92 ± 0.28	0.25 ± 0.077	0.49 ± 0.19	A B AxB	0.0014* 0.80 0.42
C16:1 Palmitoleic acid	0.15 ± 0.095	< LOD	< LOD	0.12 ± 0.073	< LOD	< LOD	A B	9.55×10^{-5}* 0.67
C18:0 Stearic acid	0.072 ± 0.033	0.027 ± 0.024	0.041	0.11 ± 0.074	0.21 ± 0.21	0.041	A B	0.0025* 1
C18:1 Cis-9 oleate	0.082 ± 0.050	0.049 ± 0.013	< LOD	0.072 ± 0.053	0.014 ± 0.024	0.027 ± 0.024	A B	0.014* 0.46
C18:2n-6 LNA	0.085 ± 0.051	0.041	< LOD	0.060 ± 0.58	0.014 ± 0.024	0.014 ± 0.024	A B	0.0095* 0.27
C18:3n-6 gamma-Linolenate	0.21 ± 0.023	< LOD	< LOD	0.21 ± 0.023	< LOD	0.014 ± 0.024	A B	0.090 0.79
C18:3n-3 ALA	0.0069 ± 0.011	0.0069 ± 0.012	< LOD	0.0035 ± 0.0085	< LOD	< LOD	A B	0.48 0.26
C20:4n-6 Arachidonic acid	0.047 ± 0.014	0.014 ± 0.024	< LOD	0.041	< LOD	< LOD	A B	0.00012* 0.42
C20:5n-3 EPA	1.28 ± 0.64	0.53 ± 0.77	0.055 ± 0.020	1.23 ± 0.41	0.087 ± 0.15	0.22 ± 0.16	A B	0.0021* 0.73

Supplementary table S4. Fatty acid composition of *Palmaria palmata* (grown at 12 °C), expressed as mean (mg g^{-1} DW) and standard deviation. Two-way ANOVA on the effects of nutrient supply (A), irradiance level (B) and their interaction (AxB, df = 1) are reported. Nonparametric alternatives (underlined) are Kruskal-Wallis and Wilcoxon test. Asterisks indicate significant p-values. Abbreviations used: LOD = limit of detection; LNA = linoleic acid; ALA = linolenic acid; EPA = eicosapentaenoic acid; n = omega classification.

Fatty acid (GC-MS)	0.5 F/2		F/2		Source of variation	p-value
	14 µmol m^{-2} s^{-1}	19 µmol m^{-2} s^{-1}	14 µmol m^{-2} s^{-1}	19 µmol m^{-2} s^{-1}		
C8:0 Caprylic acid	0.018 ± 0.0042	0.017 ± 0.0030	0.019 ± 0.0029	0.018 ± 0.0042	A B AxB	0.57 0.58 0.89
C10:0 Capric acid	0.010 ± 0.0036	0.012 ± 0.0031	0.012 ± 0.0038	0.0099 ± 0.0028	A B AxB	0.85 0.95 0.45
C12:0 Lauric acid	< LOD	0.0063 ± 0.11	0.0028 ± 0.0048	< LOD	A B	1 1
C14:0 Myristic acid	0.27 ± 0.052	0.37 ± 0.041	0.37 ± 0.12	0.23 ± 0.0053	A B AxB	0.61 0.51 0.020*
C15:0 Pentadecylic acid	0.0080 ± 0.00031	0.012	0.012	0.012	A B	0.074 0.074
C15:1 Pentadecenoic acid	< LOD	< LOD	0.0028 ± 0.0049	< LOD	A B	0.40 0.40
C16:0 Palmitic acid	0.80 ± 0.17	1.16 ± 0.13	1.05 ± 0.35	0.68 ± 0.026	A B AxB	0.36 0.97 0.015*
C16:1 Palmitoleic acid	0.084 ± 0.051	0.18 ±0.042	0.22 ± 0.083	0.060 ± 0.035	A B AxB	0.82 0.35 0.0045*
C18:0 Stearic acid	0.057 ± 0.027	0.153 ± 0.078	0.088 ± 0.036	0.057 ± 0.028	A B AxB	0.27 0.26 0.047*
C18:1n-9 Cis-9 oleate	0.041	0.10 ± 0.065	0.12 ± 0.034	0.041	A B	0.79 0.79
C18:2n-6 LNA	0.041	0.092 ± 0.069	0.13 ± 0.026	0.028 ± 0.024	A B AxB	0.63 0.29 0.0094*
C18:3n-6 gamma-Linolenate	< LOD	0.028 ± 0.024	0.041	0.014 ± 0.024	A B AxB	0.20 1 0.022*
C18:3n-3 ALA	< LOD	< LOD	0.014 ± 0.012	0.0069 ± 0.012	A B AxB	0.067 0.50 0.50
C20:4n-6 Arachidonic acid	0.041	0.041	0.053 ± 0.020	0.041	A B	0.40 0.40
C20:5n-3 EPA	1.07 ± 0.25	1.58 ± 0.22	1.49 ± 0.91	0.88 ± 0.14	A B AxB	0.65 0.87 0.085

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