Journal of Applied Phycology

Physiological responses to light regime of a Mediterranean lagoon strain of *Chaetoceros tenuissimus* and a collection strain of *Chaetoceros calcitrans*

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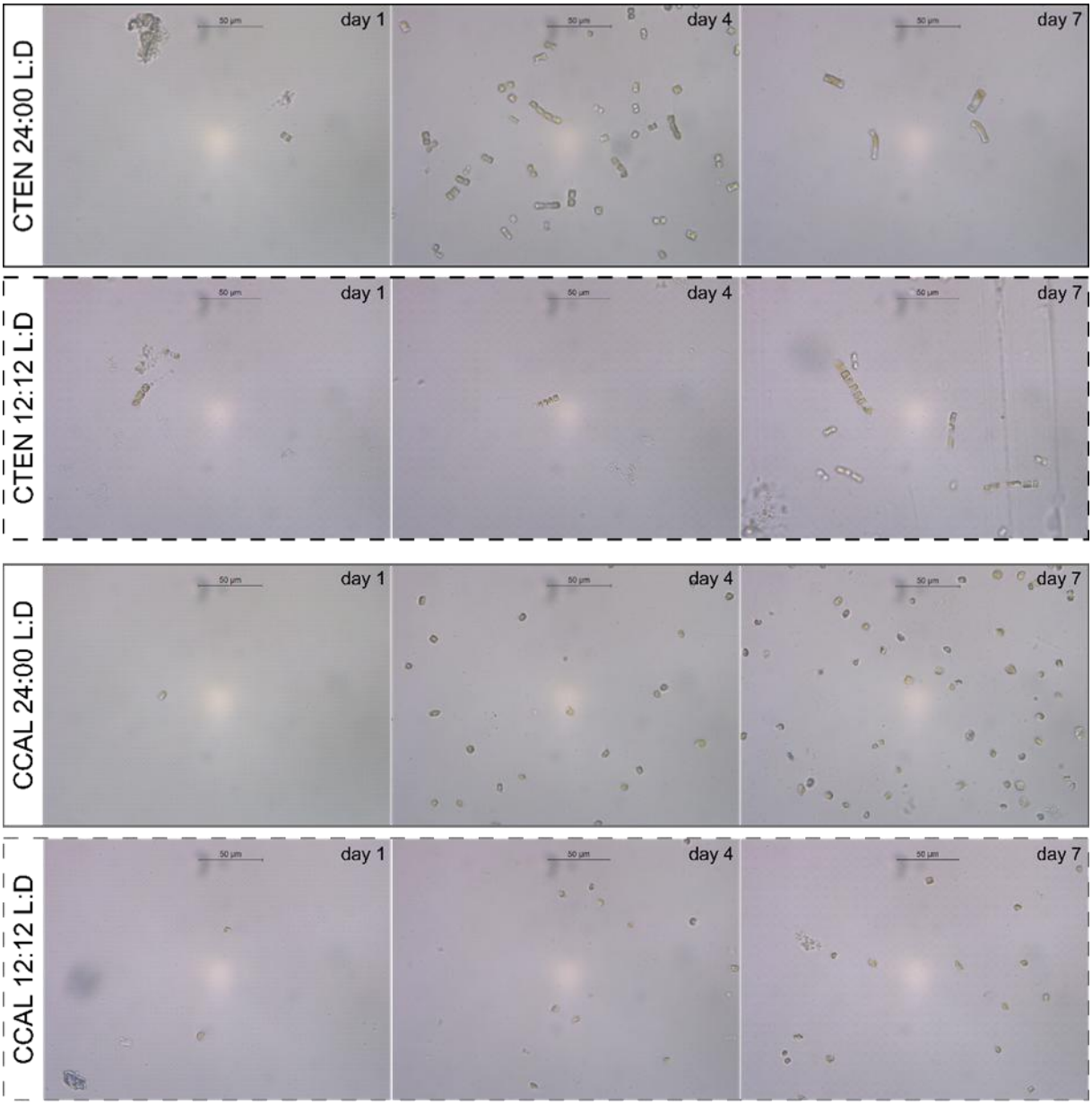
Supplementary Material

Figure S1. Samples of micrographs taken at day 1, day 4 and day 7, in the CTEN and CCAL cultures under the two photoperiod treatments, showing changes in cell morphology, including cell chain elongation in CTEN during exponential growth phase. Micrographs were used for measurements of cell section area.



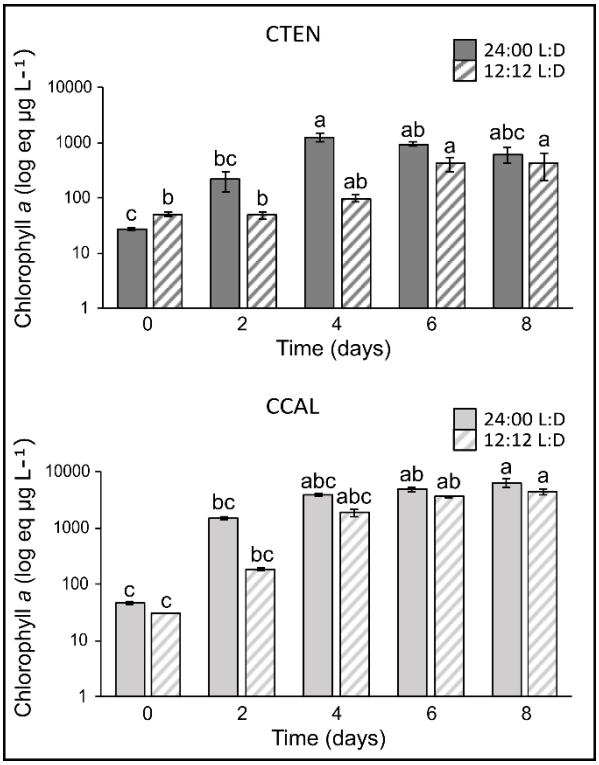


Figure S2. Variation in chl *a* biomass (eq µg L^{-1}; mean ± SD) estimated from in vivo PAM fluorescence, in triplicate cultures during photoperiod experiments for CTEN (dark grey; top figure) and CCAL (light grey; bottom figure), under 24:00 L:D (full bar) or 12:12 L:D (hatched bar) photoperiod. Error bars represent the standard deviation (n = 3). Statistical comparisons (Kruskal-Wallis test) were made between days for each photoperiod treatment and within strains (different letters represent significant differences (p-value < 0.05) between days).

Table S1. Evolution of cell concentrations (cell mL^{-1}) in the experiments carried out for setting the concentration of the inoculum, in the CTEN (10×10^3; 50×10^3; 100×10^3; 300×10^3 cell mL^{-1}) and CCAL (10×10^3; 50×10^3; 100×10^3 cell mL^{-1}) cultures.

Time	**CTEN** *(cell mL^{-1})*				**CCAL** *(cell mL^{-1})*		
(day)	**CTEN 10**	**CTEN 50**	**CTEN 100**	**CTEN 300**	**CCAL 10**	**CCAL 50**	**CCAL 100**
0	1.04E+04	5.00E+04	1.00E+05	2.91E+05	1.00E+04	6.27E+04	1.00E+05
1	3.40E+04	8.00E+04	2.09E+05	9.36E+05	2.67E+04	8.00E+04	1.97E+05
2	8.00E+04	2.73E+05	6.44E+05	2.83E+06	5.93E+04	1.62E+05	4.36E+05
3	1.97E+05	7.29E+05	1.25E+06	4.48E+06	1.19E+05	3.65E+05	9.18E+05
4	4.02E+05	1.87E+06	2.80E+06	4.34E+06	1.33E+05	6.53E+05	2.23E+06
5	9.24E+05	3.67E+06	4.33E+06	4.33E+06	2.81E+05	1.42E+06	5.04E+06
6	1.67E+06	3.95E+06	3.68E+06	4.59E+06	5.76E+05	2.38E+06	8.91E+06
7	3.17E+06	4.37E+06	4.57E+06	3.66E+06	1.35E+06	4.28E+06	1.23E+07
8	3.99E+06	3.81E+06	3.85E+06	3.61E+06	2.77E+06	6.76E+06	1.59E+07
9	4.21E+06	2.55E+06	3.71E+06	2.95E+06	5.86E+06	9.02E+06	1.71E+07
10	3.87E+06	2.00E+06	3.35E+06	2.49E+06	9.29E+06	1.44E+07	2.01E+07
11	3.47E+06		2.36E+06		1.21E+07		2.13E+07
12	2.61E+06		2.02E+06		1.72E+07		2.64E+07
13	2.42E+06		1.75E+06		2.00E+07	2.80E+07	3.14E+07
14	1.92E+06		1.52E+06		2.56E+07		3.04E+07
Exponential k	1.15	1.38	1.37	1.64	1.01	0.98	1.17
Period *(days)*	*(0 – 7)*	*(1 – 5)*	*(0 – 4)*	*(0 – 2)*	*(0 – 8)*	*(1 – 6)*	*(1 – 5)*

Table S2. Variation in cell concentration, day per day growth rate, cell section area, maximum quantum yield and chl*a* content for CTEN and CCAL cultures under the two photoperiod treatments (24:00 L:D; 12:12 L:D). Data are the mean ± SD, n = 3.

Photoperiod		Time *(days)*	Cell concentration *(10^3cell mL^{-1} ± SD)*	Growth rate *(div d^{-1} ± SD)*	Cell section area *(μm^2 ± SD)*	Fv/Fm *(± SD)*	Chlorophyll *a* *($\mu g\ L^{-1}$ ± SD)*
CTEN	24:00 L:D	0	100 ± 13		59.1 ± 21.2	0.39 ± 0.02	27.47 ± 1.14
		1	136 ± 30	0.43 ± 0.40	42.4 ± 21.9		
		2	1043 ± 445	2.86 ± 0.36	33.6 ± 10.0	0.62 ± 0.02	215.97 ± 88.50
		3	2652 ± 663	1.41 ± 0.30	34.1 ± 9.7		
		4	3903 ± 452	0.58 ± 0.35	46.3 ± 16.1	0.55 ± 0.03	1264.82 ± 198.29
		5	3910 ± 92	0.01 ± 0.17	66.1 ± 34.8		
		6	3528 ± 128	-0.15 ± 0.09	78.2 ± 28.7	0.35 ± 0.03	941.86 ± 76.44
		7	3230 ± 128	-0.13 ± 0.11	77.9 ± 42.5		
		8	2370 ± 814	-0.50 ± 0.42	91.0 ± 41.8	0.21 ± 0.04	621.67 ± 189.39
	12:12 L:D	0	100 ± 1		66.1 ± 19.7	0.41 ± 0.02	50.76 ± 4.14
		1	130 ± 14	0.36 ± 0.15	53.3 ± 22.6		
		2	146 ± 14	0.18 ± 0.23	47.2 ± 35.6	0.47 ± 0.02	48.75 ± 6.67
		3	214 ± 26	0.55 ± 0.04	40.9 ± 20.7		
		4	403 ± 56	0.91 ± 0.12	27.9 ± 6.6	0.60 ± 0.01	97.69 ± 13.66
		5	863 ± 43	1.11 ± 0.20	34.7 ± 11.6		
		6	1868 ± 431	1.09 ± 0.39	38.2 ± 8.8	0.61 ± 0.04	422.06 ± 119.84
		7	2203 ± 504	0.24 ± 0.05	48.9 ± 16.0		
		8	2565 ± 854	0.19 ± 0.15		0.50 ± 0.01	426.23 ±216.64
CCAL	24:00 L:D	0	117 ± 9		40.3 ± 7.9	0.61 ± 0.06	47.81 ± 2.86
		1	402 ± 14	1.78 ± 0.16	45.0 ± 10.4		
		2	2010 ± 339	2.31 ± 0.25	43.2 ± 11.3	0.60 ± 0.03	1504.64 ± 105.59
		3	7097 ± 565	1.83 ± 0.14	37.3 ± 7.7		
		4	12555 ± 1110	0.82 ± 0.01	37.9 ± 7.9	0.66 ± 0.03	3862.78 ± 160.59
		5	19313 ± 2506	0.62 ± 0.06	38.3 ± 7.1		
		6	24220 ± 1682	0.33 ± 0.10	39.9 ± 8.1	0.68 ± 0.02	4823.87 ± 436.15
		7	28600 ± 3776	0.23 ± 0.18	39.0 ± 9.0		
		8	32567 ± 4004	0.19 ± 0.02	34.7 ± 8.7	0.67 ± 0.02	6236.47 ± 1046.70
	12:12 L:D	0	96 ± 1		34.5 ± 8.1	0.56 ± 0.04	30.44 ± 0.06
		1	151 ± 14	0.65 ± 0.16	33.3 ± 9.7		
		2	444 ± 25	1.56 ± 0.22	34.6 ± 8.7	0.65 ± 0.01	184.95 ± 15.16
		3	1424 ± 173	1.68 ± 0.20	36.1 ± 9.0		
		4	4563 ± 1690	1.63 ± 0.34	30.0 ± 5.1	0.67 ± 0.00	1872.28 ± 301.69
		5	8420 ± 1206	0.93 ± 0.36	28.8 ± 5.7		
		6	13230 ± 2485	0.64 ± 0.11	37.6 ± 6.0	0.68 ± 0.01	3606.04 ± 134.45
		7	14950 ± 1653	0.19 ± 0.15	32.3 ± 8.3		
		8	18660 ± 2421	0.32 ± 0.24		0.71 ± 0.04	4448.91 ± 568.62

Table S3. Cellular pigment content (pg cell^{-1}; mean ± SD; n = 3), in CTEN and CCAL cultures under the two photoperiod treatments (24:00 L:D; 12:12 L:D) on day 4. Statistical comparisons (Kruskal-Wallis test) of the content of each pigment were carried out between the two photoperiod treatments for each strain (*; p-value < 0.05).

	Pigments	**CTEN**		**CCAL**	
	(pg cell^{-1})	**24:00 L:D**	**12:12 L:D**	**24:00 L:D**	**12:12 L:D**
Pigment composition	Chlorophyll *a*	0.333 ± 0.134	0.512 ± 0.031*	0.514 ± 0.055	0.623 ± 0.049*
	Chlorophyll *c*	0.119 ± 0.041	0.113 ± 0.009	0.077 ± 0.008	0.102 ± 0.010*
	Xanthophylls[(1)]	0.093 ± 0.036	0.201 ± 0.012*	0.324 ± 0.036	0.335 ± 0.027
	Fucoxanthin (F)	0.083 ± 0.034	0.167 ± 0.011*	0.288 ± 0.034	0.295 ± 0.024
	Diadinoxanthin (Dd)	0.008 ± 0.003	0.019 ± 0.001*	0.035 ± 0.003	0.040 ± 0.003
	Diatoxanthin (Dt)	< LD[(2)]	0.008 ± 0.001	0.001 ± 0.000	< LD[(2)]
	Zeaxanthin (Z)	0.001 ± 0.000	0.007 ± 0.000*	< LD[(2)]	< LD[(2)]
	β-carotene	0.009 ± 0.000	0.011 ± 0.001*	0.014 ± 0.003	0.019 ± 0.001*

[(1)] Sum of pigments fucoxanthin, diadinoxanthin and diatoxanthin, and zeaxanthin.
[(2)] Below the detection limit

Table S4. Pigment and lipid concentrations (mg L^{-1}; mean ± SD) in CTEN and CCAL cultures under the two photoperiod treatments (24:00 L:D; 12:12 L:D) on day 4. The sum of the pigments, n-3 PUFA and n-6 PUFA are described below the table.

	Pigments & Lipids	**CTEN**		**CCAL**	
	(day 4; mg L^{-1} ± SD)	**24:00 L:D**	**12:12 L:D**	**24:00 L:D**	**12:12 L:D**
Pigment composition	Chlorophyll *a*	0.357 ± 0.204	0.116 ± 0.014	6.436 ± 0.701	2.789 ± 0.796
	Chlorophyll *c*	0.467 ± 0.166	0.046 ± 0.007	0.959 ± 0.107	0.462 ± 0.153
	Xanthophylls[(1)]	0.359 ± 0.131	0.081 ± 0.006	4.063 ± 0.494	1.500 ± 0.420
	Fucoxanthin (F)	0.322 ± 0.119	0.067 ± 0.004	3.612 ± 0.46	1.320 ± 0.368
	Diadinoxanthin (Dd)	0.032 ± 0.013	0.008 ± 0.001	0.442 ± 0.037	0.18 ± 0.052
	Diatoxanthin (Dt)	< LD[(4)]	0.003 ± 0.001	0.009 ± 0.004	< LD[(4)]
	Zeaxanthin (Z)	0.005 ± 0.000	0.003 ± 0.000	< LD[(4)]	< LD[(4)]
	β-carotene	0.034 ± 0.006	0.004 ± 0.001	0.178 ± 0.029	0.085 ± 0.028
Total Lipids	Total FA	9.760 ± 3.498	1.005 ± 0.028	61.342 ± 13.944	9.205 ± 2.213
	BRFA	0.161 ± 0.052	0.017 ± 0.001	0.429 ± 0.092	0.157 ± 0.056
	SAFA	3.137 ± 1.024	0.378 ± 0.011	21.053 ± 6.903	2.086 ± 0.439
	MUFA	3.015 ± 1.193	0.285 ± 0.014	20.535 ± 5.816	2.078 ± 0.430
	PUFA	3.448 ± 1.229	0.325 ± 0.009	19.325 ± 2.099	4.883 ± 1.314
	n-3 PUFA[(2)]	1.880 ± 0.743	0.176 ± 0.008	9.345 ± 1.287	2.271 ± 0.704
	20:5n-3 (EPA)	1.463 ± 0.592	0.139 ± 0.005	7.811 ± 1.039	1.859 ± 0.618
	22:6n-3 (DHA)	0.268 ± 0.103	0.024 ± 0.003	0.747 ± 0.120	0.163 ± 0.028
	n-6 PUFA[(3)]	0.184 ± 0.035	0.013 ± 0.002	3.030 ± 0.956	0.119 ± 0.047
Polar Lipids	Total FA	3.707 ± 0.311	0.618 ± 0.013	24.983 ± 3.161	8.042 ± 1.919
	BRFA	0.034 ± 0.009	0.011 ± 0.000	0.051 ± 0.010	0.015 ± 0.009
	SAFA	1.037 ± 0.105	0.160 ± 0.004	5.766 ± 0.449	1.614 ± 0.348
	MUFA	0.887 ± 0.138	0.196 ± 0.012	5.311 ± 0.626	1.718 ± 0.351
	PUFA	1.749 ± 0.059	0.252 ± 0.004	13.855 ± 2.102	4.694 ± 1.244
	n-3 PUFA[(2)]	0.870 ± 0.014	0.141 ± 0.006	6.619 ± 1.391	2.174 ± 0.658
	20:5n-3 (EPA)	0.586 ± 0.101	0.116 ± 0.001	5.575 ± 1.250	1.806 ± 0.590
	22:6n-3 (DHA)	0.218 ± 0.079	0.020 ± 0.003	0.630 ± 0.117	0.153 ± 0.026
	n-6 PUFA[(3)]	0.115 ± 0.005	0.010 ± 0.001	2.045 ± 0.415	0.112 ± 0.040
Neutral Lipids	Total FA	6.053 ± 3.187	0.387 ± 0.029	36.359 ± 15.375	1.163 ± 0.295
	BRFA	0.127 ± 0.043	0.006 ± 0.001	0.378 ± 0.095	0.142 ± 0.047
	SAFA	2.100 ± 0.918	0.219 ± 0.015	15.288 ± 7.035	0.473 ± 0.093
	MUFA	2.128 ± 1.056	0.088 ± 0.013	15.223 ± 6.226	0.359 ± 0.085
	PUFA	1.699 ± 1.169	0.073 ± 0.010	5.470 ± 2.208	0.189 ± 0.070
	n-3 PUFA[(2)]	1.010 ± 0.757	0.035 ± 0.008	2.726 ± 1.241	0.096 ± 0.046
	20:5n-3 (EPA)	0.877 ± 0.693	0.023 ± 0.004	2.237 ± 1.017	0.053 ± 0.028
	22:6n-3 (DHA)	0.050 ± 0.024	0.004 ± 0.005	0.117 ± 0.042	0.010 ± 0.004
	n-6 PUFA[(3)]	0.069 ± 0.029	0.003 ± 0.001	0.985 ± 0.616	0.007 ± 0.007

[(1)] Sum of pigments fucoxanthin, diadinoxanthin, diatoxanthin and zeaxanthin
[(2)] Sum of lipids 16:3n-3; 16:4n-3; 18:4n-3; 18:5n-3; 20:4n-3; 20:5n-3 (EPA); 22:5n-3; 22:6n-3 (DHA)
[(3)] Sum of lipids C18:2n-6; C18:3n-6; C20:3n-6; C20:4n-6; C22:4n-6; C22:5n-6
[(4)] Below detection limit