Electronic Supplementary Information (ESI)

Differences in Phytoplankton Population Vulnerability to Chemical Activity of Mixtures

Talles Bruno Oliveira dos Anjos[1], Quyen Nham[2], Sebastian Abel[1], Elin Lindehoff [2], Clare Bradshaw[3], Anna Sobek[1]

[1]Department of Environmental Science, Stockholm University, [2]Department of Biology and Environmental Science, Centre of Ecology and Evolution and Microbial Model Systems (EEMiS), Linnaeus University; [3]Department of Ecology, Environment and Plant Sciences, Stockholm University

List of contents

Table ESI 1: Results from LC-MS analysis of PAHs in the exposure medium/water

Species	PAHs freely dissolved concentrations and total chemical activity (mean ± s.d.)				
	Acenaphtene (ng/L)	Fluorene (ng/L)	Phenanthrene (ng/L)	Fluoranthene (ng/L)	Total chemical activity
Prymnesium parvum	44 (± 0.2)	36 (± 0.2)	20 (± 0.2)	4 (± 0.1)	0.02 (± 0.0002)
	103 (± 0.2)	87 (± 0.4)	46 (± 0.2)	10 (± 0.2)	0.06 (± 0.0003)
	174 (± 3.5)	148 (± 3.0)	77 (± 2.2)	16 (± 0.9)	0.09 (± 0.003)
	356 (± 4.6)	309 (± 22.5)	144 (± 10.5)	38 (± 5.5)	0.19 (± 0.015)
Phaeodactylum tricornutum	43 (± 1.0)	34 (± 0.9)	20 (± 0.3)	4 (± 0.1)	0.02 (± 0.0004)
	103 (± 0.7)	86 (± 0.5)	45 (± 0.5)	10 (± 0.3)	0.05 (± 0.0008)
	169 (± 0.7)	144 (± 0.5)	76 (± 0.2)	17 (± 0.5)	0.09 (± 0.001)
	339 (± 5.7)	280 (± 3.5)	130 (± 1.7)	32 (± 0.7)	0.17 (± 0.002)
Nannochloris sp.	48 (± 0.4)	38 (± 0.2)	22 (± 0.4)	4 (± 0.1)	0.02 (± 0.0003)
	110 (± 0.7)	92 (± 0.6)	48 (± 0.4)	10 (± 0.1)	0.06 (± 0.0004)
	178 (± 1.0)	151 (± 0.5)	79 (± 0.3)	17 (± 0.2)	0.09 (± 0.001)
	362 (± 2.7)	299 (± 3.1)	138 (± 1.4)	32 (± 0.8)	0.18 (± 0.002)
Monoraphidium minutum	29 (± 3.2)	25 (± 1.3)	14 (± 0.7)	3 (± 0.2)	0.02 (± 0.002)
	42 (± 0.7)	39 (± 15)	21 (± 7.7)	5 (± 1.8)	0.02 (± 0.009)
	106 (± 28)	96 (± 21)	50 (± 12)	11 (± 2.8)	0.06 (± 0.014)
	297 (± 65)	275 (± 25)	136 (± 1.2)	32 (± 0.4)	0.17 (± 0.011)
Rhodomonas salina	17 (± 1.1)	16 (± 1.0)	9 (± 0.7)	1 (± 0.0)	0.01 (±0.00)
	133 (± 9.7)	114 (± 7.0)	64 (± 1.6)	14 (± 0.3)	0.07 (±0.00)
	145 (± 22)	126 (± 14)	74 (± 4.8)	17 (± 0.6)	0.09 (±0.01)
	258 (± 5.2)	218 (± 2.6)	124 (± 0.5)	28 (± 0.7)	0.15 (±0.00)

Quality control and assurance for LC-MS analysis of PAHs in the exposure medium/water

Analyte	Blank	QC (mean ± st. dev.)	% RSD
Acenaphthene	NF	5.8 ± 0.61 ng/L	10.623
Fluorene	NF	4.8 ± 0.29 ng/L	6.137
Phenanthrene	NF	4.9 ± 0.07 ng/L	1.335
Fluoranthene	NF	4.9 ± 0.04 ng/L	0.742

[a]Mean ± standard deviation, n=3. NF = not found.

Table ESI 2: Quality control and assurance for GC-MS analysis of PAHs in biota

Species	Analyte	Recovery range (%)	Blank Level (ng)	LOD (ng)	LOQ (ng)
Prymnesium parvum	Acenaphtene	133 ± 46	0.08	0.47[a]	1.56[a]
	Fluorene	109 ± 22	NF	0.49[a]	1.64[a]
	Phenanthrene	120 ± 17	NF	0.37[b]	0.69[b]
	Fluoranthene	139 ± 23	NF	0.62[b]	1.19[b]
Monorahidium minutum	Acenaphtene	166 ± 73	NF	0.47[a]	1.56[a]
	Fluorene	107 ± 18	NF	0.49[a]	1.64[a]
	Phenanthrene	114 ± 14	NF	0.24[b]	0.37[b]
	Fluoranthene	134 ± 22	NF	0.49[b]	0.98[b]
Phaeodactylum tricornutum	Acenaphtene	117 ± 46	0.05	0.47[a]	1.56[a]
	Fluorene	84 ± 14	NF	0.49[a]	1.64[a]
	Phenanthrene	91 ± 9.7	NF	0.36[b]	0.80[b]
	Fluoranthene	111 ± 11	NF	0.31[b]	0.47[b]
Nannochloris sp.	Acenaphtene	147 ± 65	NF	0.47[a]	1.56[a]
	Fluorene	107 ± 18	NF	0.49[a]	1.64[a]
	Phenanthrene	129 ± 8.6	NF	0.23[b]	0.26[b]
	Fluoranthene	152 ± 11	NF	0.44[b]	0.59[b]

Not found (NF). Calibration curve (min – max): Acenaphthene: 0.152 – 192.7 ng; Fluorene: 0.152-192.7 ng; Phenanthrene: 0.154 – 185.7 ng; Fluoranthene: 0.137-188.3 ng; blank consists of solvent only.

[a]LOD and LOQ based on the method blank. Acenaphthene were found in 2 of 8 blanks, therefore the blank method was used. LOD = mean (blanks) + 3 x Std. Deviation (blanks); LOQ = mean (blanks) + 10 x Std. Deviation (blanks)

[b]LOD and LOQ based on the three lowest point of the calibration curve,

$$\text{LOD} = \frac{[\,mean\,(diff.\,between\,specified\,and\,calculated\,amounts)\,]}{slope} \times 3;$$

$$\text{LOQ} = \frac{[\,mean\,(diff.\,between\,specified\,and\,calculated\,amounts)\,]}{slope} \times 10$$

Treatment	*M. minutun*	*P. parvum*	*P. tricornutum*	*Pichlorum* sp./*Nannochloris* sp.
Control	10.17 (± 0.015)[a]	10.07 (± 0.049)[a]	10.43 (± 0.043)[a]	8.96 (± 0.053)[a]
Solvent Control	10.39 (± 0.045)[b]	9.91 (± 0.070)[a]	10.46 (± 0.038)[a]	9.09 (± 0.110)[ab]
0.02	10.35 (± 0.094)[b]	9.86 (± 0.024)[a]	10.52 (± 0.060)[a]	9.03 (± 0.019)[ab]
0.06	10.30 (± 0.052)[ab]	9.55 (± 0.0162)[b]	10.49 (± 0.056)[a]	8.93 (± 0.30)[b]
0.09	10.18 (± 0.023)[a]	9.19 (± 0.049)[c]	10.48 (± 0.041)[a]	8.90 (± 0.032)[b]
0.17	8.87 (± 0.056)[c]	8.68 (± 0.195)[d]	9.66 (± 0.013)[b]	8.49 (± 0.003)[c]

Table ESI 3: pH measured in the medium

Note: Differences between the treatments were assessed by one-way ANOVA. Different letters indicate significant differences ($p<0.05$) between treatments according to the Tukey´s post-hoc test.

Table ESI 4: Concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) in Various Algal Species at Different Chemical Activities

Species	PAH Mixture	Concentrations of PAHs in the organic carbon (mean ± s.d.)			
	Chemical activity	Acenaphtene (mg/ Kg C)	Fluorene (mg/ Kg C)	Phenanthrene (mg/ Kg C)	Fluoranthene (mg/ Kg C)
Prymnesium parvum	0.02	272 (± 93)	364 (± 138)	472 (± 288)	224 (± 167)
	0.06	285 (± 94)	372 (± 126)	349 (± 84)	122 (± 22)
	0.1	2051 (± 710)	2362 (± 534)	1801 (± 125)	1036 (± 67)
	0.18	4974 (± 1936)	5385 (± 1680)	3616 (± 727)	2958 (± 160)
Phaeodactylum tricornutum	0.02	N/A	N/A	N/A	N/A
	0.06	548 (± 153)	784 (± 271)	999 (± 355)	1146 (± 292)
	0.1	1660 (± 407)	2314 (± 304)	2736 (± 166)	2801 (± 170)
	0.18	4818 (± 1204)	6708 (± 1371)	7429 (± 364)	7505 (± 26)
Nannochloris sp.	0.02	1209 (± 173)	1307 (± 268)	1298 (± 230)	945 (± 159)
	0.06	1340 (± 343)	1667 (± 332)	1740 (± 322)	1611 (± 241)
	0.1	2620 (± 1764)	3164 (± 1692)	2761 (± 748)	2368 (± 484)
	0.18	7151 (± 2086)	7504 (± 2102)	5080 (± 1344)	4367 (± 1711)
Monoraphidium minutum	0.02	1010 (± 334)	1393 (± 548)	1741 (± 799)	1612 (± 880)
	0.06	1460 (± 385)	2241 (± 383)	2755 (± 171)	2776 (± 80)
	0.1	2454 (± 171)	3629 (± 171)	4354 (± 150)	4449 (± 236)
	0.18	3778 (± 653)	5505 (± 724)	6149 (± 128)	7323 (± 589)
Rhodomonas salina	0.01	345 (± 37)	444 (± 52)	446 (± 28)	318 (± 9)
	0.08	4144 (± 235)	4740 (± 343)	3584 (± 288)	1166 (± 106)
	0.1	3567 (± 247)	4452 (± 494)	3579 (± 303)	1179 (± 134)
	0.13	5993 (± 640)	7523 (± 1293)	5676 (± 755)	2781 (± 289)

Not applicable (N/A)

Table ESI 5: Summary of the effects of the nominal[a] chemical activity exerted by a PAH mixture on five phytoplankton species.

Treatment	*Prymnesium parvum*		*Monoraphidium minutum*	
	Growth rate. d^{-1}	% inhibition	Growth rate. d^{-1}	% inhibition
Control	0.529 (± 0.146)	-	0.499 (± 0.061)	-
Solvent Control	0.451 (± 0.137)	-	0.691 (± 0.133)	-
0.02	0.452 (± 0.023)	-0.2 (± 5.12)	0.833 (± 0.086)	-20.5 (± 12.5)
0.05	0.320 (± 0.054)	29.5 (±11.89)	0.779 (± 0.027)	-12.7 (± 3.9)
0.09	- 0.134 (± 0.042)	129.8 (± 9.28)	0.738 (± 0.084)	-6.84 (± 12.2)
0.17	- 0.727 (± 0.122)	261 (± 27.11)	0.073 (± 0.034)	89.4 (± 4.90)

	Nannochloris sp.		*Phaeodactylum tricornutum*	
	Growth rate. d^{-1}	% inhibition	Growth rate. d^{-1}	% inhibition
Control	0.428 (± 0.006)	-	0.565 (± 0.188)	-
Solvent Control	0.493 (± 0.009)	-	0.470 (± 0.052)	-
0.02	0.453 (± 0.016)	4.74 (± 3.40)	0.527 (± 0.025)	- 8.81 (± 5.38)
0.05	0.409 (± 0.012)	14.1 (± 2.53)	0.527 (± 0.023)	- 8.72 (± 1.44)
0.09	0.344 (± 0.004)	27.8 (± 0.86)	0.416 (± 0.034)	11.5 (± 7.17)
0.17	0.089 (± 0.039)	81.2 (± 8.16)	0.281 (± 0.027)	40.2 (± 5.76)

	Rhodomonas salina	
	Growth rate. d^{-1}	% inhibition
Control	0.420 (± 0.017)	-
Solvent Control	0.453 (± 0.024)	-
0.01	0.422 (± 0.023)	6.86 (± 0.023)
0.08	0.271 (± 0.007)	40.1 (± 1.57)
0.1	0.167 (± 0.017)	63.1 (± 3.72)
0.15	- 0.139 (± 0.088)	130 (± 19.4)

Note: Growth rate and % of inhibition were generated from the cell density determined by flow cytometer (n=3). except for *Pichlorum* sp./*Nannochloris* sp. (n=3) and *R. salinas* (n=4), for which optical density was used. [a]True values are displayed in Table ESI 2.

Table ESI 6: Reduction in the Particulate Organic Carbon (POC) in five phytoplankton species exposed to a PAH mixture at different chemical activities[a].

	Prymnesium parvum		*Monoraphidium minutum*	
Treatment	POC (µg/ml)	% reduction	POC (µg/ml)	% reduction
Control	13.4 (± 0.9)	-	18.1 (± 2.5)	-
Solvent Control	12.3 (± 2.9)	-	20.0 (± 1.8)	-
0.02	12.7 (± 0.4)	-3.5 (± 3.0)	18.6 (± 1.8)	6.91 (± 8.8)
0.05	10.2 (± 1.1)	16.8 (± 8.6)	19.0 (± 1.0)	4.91 (±5.0)
0.09	6.2 (± 0.3)	49.2 (± 2.2)	17.1 (± 1.4)	14.5 (± 7.0)
0.17	3.8 (± 0.1)	68.7 (± 0.7)	6.6 (± 0.5)	66.7 (± 2.3)

	Nannochloris sp.		*Phaeodactylum tricornutum*	
	POC (µg/ml)	% reduction	POC (µg/ml)	% reduction
Control	4.8 (± 0.6)	-	-	-
Solvent Control	5.1 (± 0.4)	-	28.0 (± 1.2)	-
0.02	3.6 (± 0.7)	29.2 (± 14.4)	-	-
0.05	3.8 (± 0.2)	25.2 (± 4.1)	33.6 (± 6.9)	-20 (± 24.7)
0.09	3.5 (± 0.7)	32.0 (± 14.0)	24.3 (± 0.7)	13.1 (± 2.7)
0.17	2.4 (± 1.3)	52.2 (± 25.5)	14.3 (± 1.0)	48.8 (± 3.5)

	Rhodomonas salina	
	POC (µg/ml)	% reduction
Control	-	-
Solvent Control	17.5 (± 0.7)	-
0.01	17.5 (± 0.8)	0 (± 4.4)
0.08	13.9 (± 0.4)	20 (± 2.3)
0.1	13.7 (± 0.9)	21 (± 5.1)
0.15	6.2 (± 6.2)	64 (± 3.0)

Note: The reduction in the POC content relative to the solvent control on day 3 of exposure.
[a]True values are displayed in Table ESI 2.

Table ESI 7: Principal Component Analysis summary of 441 lipid metabolites

Table Analyzed	**441 Metabolites**			
PC summary	PC1	PC2	PC3	PC4
Eigenvalue	203.3	121.5	56.31	31.98
Proportion of variance	46.10%	27.55%	12.77%	7.25%
Cumulative proportion of variance	46.10%	73.65%	86.42%	93.67%
Component selection	Selected	Selected	Selected	
Data summary				
Total number of variables	441			
Total number of components	14			
Component selection method	Parallel analysis			
Random seed	775065250			
Number of simulations	1000			
Number of selected components	3			
Rows in table	15			
Rows skipped (missing data)	0			
Rows analyzed (# cases)	15			

Table ESI 8: Principal Component Analysis summary of 16 lipid classes

Table Analyzed	**Lipid classes**			
PC summary	PC1	PC2	PC3	PC4
Eigenvalue	8.964	3.576	2.508	0.5542
Proportion of variance	56.02%	22.35%	15.68%	3.46%
Cumulative proportion of variance	56.02%	78.37%	94.05%	97.51%
Component selection	Selected	Selected		
Data summary				
Total number of variables	16			
Total number of components	14			
Component selection method	Parallel analysis			
Random seed	775033203			
Number of simulations	1000			
Number of selected components	2			
Rows in table	15			
Rows skipped (missing data)	0			
Rows analyzed (# cases)	15			

Table ESI 9: Principal Component Analysis summary of 23 methylated free fatty acids

Table Analyzed	**Met FA**			
PC summary	PC1	PC2	PC3	PC4
Eigenvalue	13.52	4.360	3.734	0.6921
Proportion of variance	58.76%	18.96%	16.24%	3.01%
Cumulative proportion of variance	58.76%	77.72%	93.95%	96.96%
Component selection	Selected	Selected		
Data summary				
Total number of variables	23			
Total number of components	14			
Component selection method	PCs that together explain this percent of the total variance: 75.00			
Number of selected components	2			
Rows in table	15			
Rows skipped (missing data)	0			
Rows analyzed (# cases)	15			

Table ESI 10: Principal Component Analysis summary of 23 trimethylated fatty acids

Table Analyzed	Trans FA			
PC summary	PC1	PC2	PC3	PC4
Eigenvalue	8.947	3.611	2.479	0.7543
Proportion of variance	55.92%	22.57%	15.49%	4.71%
Cumulative proportion of variance	55.92%	78.49%	93.98%	98.70%
Component selection	Selected	Selected		
Data summary				
Total number of variables	16			
Total number of components	14			
Component selection method	Parallel analysis			
Random seed	775053375			
Number of simulations	1000			
Number of selected components	2			
Rows in table	15			
Rows skipped (missing data)	0			
Rows analyzed (# cases)	15			

Table ESI 11: Lipid composition responses of various phytoplankton species to different stressors found in literature

	Organism	Effect	stressor	Reference
glycerol-3-phosphate	*R. subcapitata*	High contribution SIMPER	ɤ-radiation	[1]
Polyunsaturated fatty acids (PUFAS)	microalgae	eicosapentaenoic acid (EPA) ↑ Linoleic acid ↑	pesticides	[2]
	R. subcapitata	High contribution SIMPER 18:2 (n-6) Linoleic acid 18:0 Stearic acid	ɤ-radiation	[1]
	Chlorella sp.	18:0 Stearic acid (High abundance)	waste water	[3]
	Nannochloropsis oculata	18:2 Linoleic acid↑ C18:1 Oleic acid ↑	nitrogen limitation	[4]
	Chlorella sp.	C 18:1 Oleic acid C 18:2 Linoleic acid C 18:3 (high abundance)	waste water	[3]
	Nannochloropsis oculata	C 20:5↓	nitrogen limitation	[4]
	microalgae	PUFA Decrease ↓	heavy metals	[5]
Monounsaturated fatty acids (MUFA)	microalgae	16:1 Palmitoleic acid ↑ Oleic acid ↑	pesticides	[2]
	Thalassiosira pseudonata	C16:1 Palmitoleic acid ↑	PCBs	[6]
	Nannochloropsis sp.	C16:1 Palmitoleic acid ↑	nitrogen limitation	[7,8]
	Chlorella sp.	C16:1 Palmitoleic acid (high abundance)	waste water	[3]

Table ESI 11: Lipid composition responses of various phytoplankton species to different stressors found in literature (continuation)

	Organism	Effect	stressor	Reference
Saturated fatty acids (SFAs)	microalgae	SFA Increase ↑	heavy metals	[5]
	Thalassiosira pseudonata	Palmitic acid C16:0 ↑	PCBs	[6]
	N. oculata	Palmitic acid C16:0 ↑	nitrogen limitation	[4]
	Chlorella sp.	Palmitic acid C16:0 (high abundance)	waste water	[3]
	Nannochloropsis sp	Palmitic acid C16:0 ↑	nitrogen limitation	[7,8]

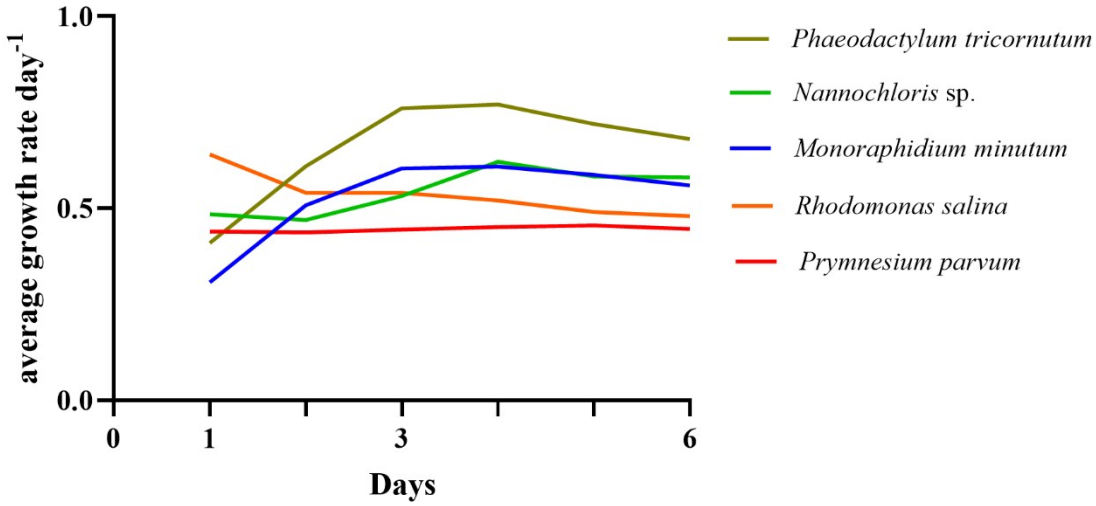

Figure ESI 1: Growth of the algal cultures during the cultivation time (non-exposed)

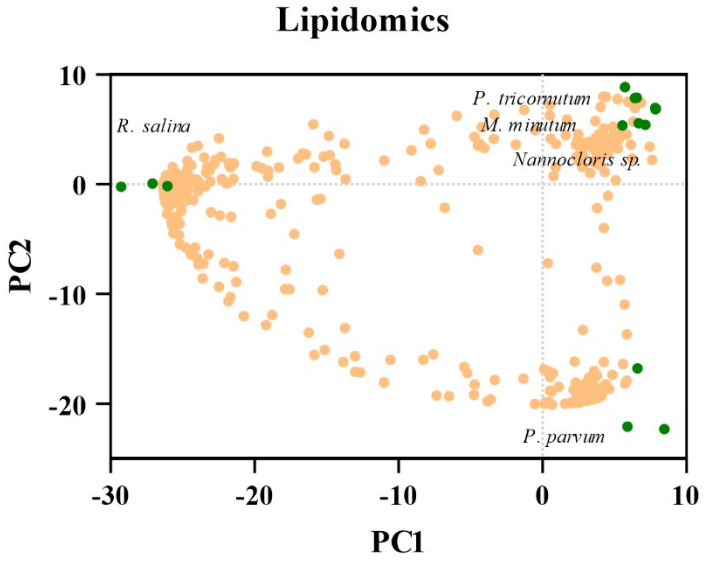

Figure ESI 2: Analysis of 441 lipids based on signal intensity (non-target method, mass and retention time) PC1 + PC2 = 65.3 % of variance. PC scores are represented by orange dots associated to the lipids. Loadings are represented by green dots and are associated to the species (n=3).

Text ESI 1: Methodology and instrumentation overview for the lipidomic analysis

The lipid extraction process involved a chloroform: methanol (2:1) phase extraction, with the chloroform phase (extract) stored at -80°C until LC-MS analysis, following the Folch extraction method. The extraction mix included four internal standards (IS): 13C3-TG (16:0/16:0/16:0) (Larodan, Solna, Sweden); D30-Cer (d18:1/16:0), D70-PC (18:0/18:0), D5-DG (18:0/0:0/18:0) (Avanti polar Lipids, Birmingham; AL, USA) added in identical concentrations to all samples before extraction. Additionally, a quality control (QC) sample, a mix of extracts from all samples, and a dilution series of QC were prepared. For UHPLC-QTOF/MS analysis, a 1290 Infinity UHPLC (Agilent) with a plasma lipid-optimized gradient and an Acquity UPLC CSH 2.1x50 1.7um C18 column (Waters) were utilized. The QTOF 6546 instrument ("ODIN") (Agilent) employed electrospray ionization in both positive and negative modes. The batch underwent an initial run in positive mode, followed by switching to negative mode. Major peaks in the QC sample underwent additional sample injections with two different collision energies (25V and 40V). Software ProFinder 10.0 (Agilent MassHunter) facilitated batch targeted feature extractions, referencing internal databases for various lipid classes. MSMS analysis with Agilent Mass Hunter Qualitative Analysis 10.0 aimed to verify major lipid

classes in the samples, with preliminary identity suggestions based on searches against SMC databases. Lipids were annotated with lipid class and total carbon and double bond counts. For the fatty acid analysis, samples underwent methylation for free fatty acids and transmethylation for bound fatty acids.

Text ESI 2: Algae toxicity test quality control

The pH increased during the exposure, from the starting pH of 8, to a maximum of 10.52 (*P. tricornutum*; 0.02) and a minimum of 8.49 (*Pichlorum* sp./*Nannochloris* sp.; 0.17) (Table ESI 1). All algae species displayed exponential growth in the control across all tests, with specific growth ranging from 0.42 d^{-1} for *R. salinas* to 0.56 d^{-1} *P. tricornutum* (Table 1). We investigated the effect of the silicone (PDMS) loaded only with methanol on the growth rate and found that *Pichlorum* sp./*Nannochloris* sp. and *M. minutum* show increase in growth in the presence of the PDMS (Table 1). Previous studies have reported a growth-promoting effect of silicone, although this effect was considered negligible. [9,10]

Text ESI 3: Analysis of PAH freely dissolved concentrations in the medium

PAH determination in equilibrated Milli-Q water samples was carried out on a high-performance liquid chromatograph coupled to a photodiode array detector (HPLC-PDA, Shimadzu i-Series LC 2040C 3D, Shimadzu. Sweden). The samples were analyzed directly after vortexing, with an injection volume of 50 µL. Chromatographic separation was achieved on a HALO 90 Å PAH column (particle size of 2.7 µm, 2.1 x 50 mm, HALO. USA). The mobile phases consisted of Milli-Q water (A) and acetonitrile (B). The gradient started with 50% B. Starting from 0.31 min the gradient was linearly ramped first to 70% B until 5.0 min then to 99% B until 5.2 min and maintained for 1.3 min, followed by a linear decrease back to 50% B within 0.1 min and maintained for another 1.2 min. The total run time was 8 min. Throughout the whole separation the flow rate was 0.5 mL/min, the sample compartment temperature was 15 ℃, and the column temperature was 30 ℃. PDA detection wavelength was set at 225 nm, 234 nm, 249 nm, and 261 mm for measuring acenaphthene, fluoranthene, phenanthrene, and fluorine, respectively, with bandwidth of +/- 4 nm. Quantification was carried out using an external calibration curve prepared with the 4 PAH analytes at 8 concentration points in a range of 1-1000 µg/L. PAHs solubilities in water were also adjusted to 17℃, by extrapolating the solubility-temperature correlation based on Wauchope et al., (1972) [11] and the adjusted solubility was applied in the equation (1) to obtain the adjusted subcooled liquid solubility.

Text ESI 4: Characterization of Lipid Profiles in Non-Exposed Algae Species

The lipidomic analysis identified 441 lipids from samples of non-exposed algae (Figure 4), demonstrating good reproducibility of internal standards across samples. The lipid profile in *P. parvum* was predominantly influenced by the polar lipids such as DGCC (di-galactocyl-diacylglycerol) and PG (phosphatidylglycerol) (Figure 4-B). According to Lowenstein et al., (2021) [12], the high abundance of DGCC in *P. parvum* is a shared characteristic with other haptophytes in coastal areas, reflecting the ecological niche and regional macronutrient availability. Compared to the other species, *P. parvum* exhibits notable richness in free fatty acids, comprising a diverse profile of more than ten distinct fatty acids (Figure 4-C, Table ESI 7). Free fatty acids are often indicative of the immediate availability of substrate for energy production or other metabolic processes, for example production of toxins. Renowned for its importance in harmful algal blooms, *P. parvum* has been associated with the production of toxins identified as fatty acid amides. [13] Although our analysis did not achieve a comparable level of identification for these amide fatty acids, several fatty acids identified in *P. parvum*, such as palmitic acid (C16:0), stearic acid (C 18:0), oleic acid (C18:1 ω-9), linoleic acid (C 18:2 ω-6), can exist with an amine group ($-NH_2$).

R. salina exhibited high relative levels of DGTS (diacyl-glycerol-trimethyl-hermoserine), which is a type of glycerolipid belonging to the phospholipid group along with PM (phosphatidylmethanol), PS (phosphatidylserine), and PE (phosphatidylethanol) (Figure 1-B). These lipids contribute to the structural integrity of cell membranes. Additionally, *R. salina* showed elevated levels of TG (triacylglyceride), one of the major forms of neutral lipids for energy storage in organisms. *R. salina* displays high diversity in terms of bound fatty acids compared to the other species (Figure 4-D, Table ESI 8), with few free fatty acids (C 17:1 ω-7, C 17:0, C:17:0, C:18:0) appearing in high abundance (Figure 4-C). Bound fatty acids are typically associated with structural lipids like phospholipids and glycolipids and their abundance might reflect the cells long-term adaptive strategy for lipid storage and membrane composition. [14] Upon closer analysis of the structures of both free and bound fatty acids in *P. parvum* and *R. salina*, the chemical formula indicates chloroplast diol fatty acids such as palmitic acid (C16:0), stearic acid (C 18:0), oleic acid (C18:1 ω-9), linoleic acid (C 18:2 ω-6) and alpha-linoleic acid (C18:3 ω-3). These plastid fatty acids are essential components of the thylakoid membranes where the photosynthetic process takes place. Additionally, they can serve as precursors for signaling molecules and contribute to the organism response to environmental stress. [15]

References

1. Golz AL, Bradshaw C. Gamma radiation induced changes in the biochemical composition of aquatic primary producers and their effect on grazers. *Front Environ Sci*. 2019;7(JUL):451867. doi:10.3389/FENVS.2019.00100/BIBTEX

2. Filimonova V, Gonçalves F, Marques JC, De Troch M, Gonçalves AMM. Fatty acid profiling as bioindicator of chemical stress in marine organisms: A review. *Ecol Indic*. 2016;67:657-672. doi:10.1016/J.ECOLIND.2016.03.044

3. Nzayisenga JC, Farge X, Groll SL, Sellstedt A. Effects of light intensity on growth and lipid production in microalgae grown in wastewater. *Biotechnol Biofuels*. 2020;13(1):1-8. doi:10.1186/S13068-019-1646-X/FIGURES/5

4. Olofsson M, Lamela T, Nilsson E, et al. Combined Effects of Nitrogen Concentration and Seasonal Changes on the Production of Lipids in Nannochloropsis oculata. *Marine Drugs 2014, Vol 12, Pages 1891-1910*. 2014;12(4):1891-1910. doi:10.3390/MD12041891

5. Rocchetta I, Mazzuca M, Conforti V, Ruiz L, Balzaretti V, De Molina MDCR. Effect of chromium on the fatty acid composition of two strains of Euglena gracilis. *Environmental Pollution*. 2006;141(2):353-358. doi:10.1016/J.ENVPOL.2005.08.035

6. Fisher NS, Schwarzenbach RP. FATTY ACID DYNAMICS IN THALASSIOSIRA PSEUDONANA (BACILLARIOPHYCEAE): IMPLICATIONS FOR P HYSIOLOGICAL ECOLOGY. *J Phycol*. 1978;14(2):143-150. doi:10.1111/J.1529-8817.1978.TB02439.X

7. Sukenik A, Carmeli Y. LIPID SYNTHESIS AND FATTY ACID COMPOSITION IN NANNOCHLOROPSIS SP. (EUSTIGMATOPHYCEAE) GROWN IN A LIGHT-DARK CYCLE1. *J Phycol*. 1990;26(3):463-469. doi:10.1111/J.0022-3646.1990.00463.X

8. Sukenik A, Carmeli Y, Berner T. REGULATION OF FATTY ACID COMPOSITION BY IRRADIANCE LEVEL IN THE EUSTIGMATOPHYTE NANNOCHLOROPSIS SP.1. *J Phycol*. 1989;25(4):686-692. doi:10.1111/J.0022-3646.1989.00686.X

9. Kreutzer A, Faetsch S, Heise S, Hollert H, Witt G. Passive dosing: Assessing the toxicity of individual PAHs and recreated mixtures to the microalgae Raphidocelis subcapitata. *Aquatic Toxicology*. 2022;249:106220. doi:10.1016/j.aquatox.2022.106220

10. Niehus NC, Floeter C, Hollert H, Witt G. Miniaturised Marine Algae Test with Polycyclic Aromatic Hydrocarbons − Comparing Equilibrium Passive Dosing and Nominal Spiking. *Aquatic Toxicology*. 2018;198:190-197. doi:10.1016/j.aquatox.2018.03.002

11. Wauchope RD, Getzen FW. Temperature Dependence of Solubilities in Water and Heats of Fusion of Solid Aromatic Hydrocarbons. *J Chem Eng Data*. 1972;17(1):38-41. doi:10.1021/JE60052A020

12. Lowenstein DP, Mayers K, Fredricks HF, Van Mooy BAS. Targeted and untargeted lipidomic analysis of haptophyte cultures reveals novel and divergent nutrient-stress adaptations. *Org Geochem*. 2021;161:104315. doi:10.1016/J.ORGGEOCHEM.2021.104315

13. Bertin MJ, Zimba P V., Beauchesne KR, Huncik KM, Moeller PDR. Identification of toxic fatty acid amides isolated from the harmful alga Prymnesium parvum carter. *Harmful Algae*. 2012;20:111-116. doi:10.1016/J.HAL.2012.08.005

14. De Carvalho CCCR, Caramujo MJ. The Various Roles of Fatty Acids. *Molecules 2018, Vol 23, Page 2583*. 2018;23(10):2583. doi:10.3390/MOLECULES23102583

15. Li J, Liu LN, Meng Q, Fan H, Sui N. The roles of chloroplast membrane lipids in abiotic stress responses. *Plant Signal Behav*. 2020;15(11). doi:10.1080/15592324.2020.1807152