



Supplement of

Ocean-related global change alters lipid biomarker production in common marine phytoplankton

Rong Bi et al.

Correspondence to: Meixun Zhao (maxzhao@ouc.edu.cn, maxzhao04@yahoo.com)

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| Species | | Treatment | | DIC (µmol kg ⁻¹) | TA (µmol kg ⁻¹) | $pCO_2(\mu atm)$ | pН |
|-----------------------------|------|----------------------|------------|------------------------------|-----------------------------|------------------|-------------------|
| P. tricornutum ^a | 12°C | Low CO ₂ | N:P = 10:1 | 2476 ± 12 | 2505 ± 7.0 | 1479 ± 97 | 7.788 ± 0.008 |
| | | | N:P = 24:1 | 2425 ± 16 | 2551 ± 0.7 | 728 ± 78 | 8.057 ± 0.017 |
| | | | N:P = 63:1 | 2404 ± 18 | 2533 ± 5.6 | 714 ± 72 | 7.996 ± 0.004 |
| | | High CO ₂ | N:P = 10:1 | 2634 ± 17 | 2502 ± 0.4 | 4032 ± 353 | 7.510 ± 0.016 |
| | | | N:P = 24:1 | 2628 ± 28 | 2534 ± 1.4 | 3300 ± 558 | 7.557 ± 0.044 |
| | | | N:P = 63:1 | 2629 ± 5.2 | 2520 ± 3.3 | 3542 ± 159 | 7.417 ± 0.010 |
| | 18°C | Low CO ₂ | N:P = 10:1 | 2392 ± 11 | 2462 ± 0.5 | 1289 ± 103 | 7.856 ± 0.025 |
| | | | N:P = 24:1 | 2273 ± 9.7 | 2535 ± 7.4 | 435 ± 21 | 8.230 ± 0.029 |
| | | | N:P = 63:1 | 2421 ± 22 | 2512 ± 2.3 | 1167 ± 160 | 7.902 ± 0.019 |
| | | High CO ₂ | N:P = 10:1 | 2564 ± 8.2 | 2466 ± 3.9 | 4045 ± 204 | 7.381 ± 0.019 |
| | | | N:P = 24:1 | 2594 ± 8.1 | 2545 ± 2.1 | 3059 ± 128 | 7.584 ± 0.025 |
| | | | N:P = 63:1 | 2551 ± 23 | 2540 ± 2.4 | 2401 ± 346 | 7.598 ± 0.013 |
| | 24°C | Low CO ₂ | N:P = 10:1 | 2350 ± 15 | 2464 ± 1.8 | 1217 ± 110 | 8.033 ± 0.035 |
| | | | N:P = 24:1 | 2280 ± 32 | 2494 ± 1.1 | 704 ± 103 | 8.233 ± 0.043 |
| | | | N:P = 63:1 | 2330 ± 10 | 2470 ± 0.6 | 1015 ± 59 | 8.067 ± 0.009 |
| | | High CO ₂ | N:P = 10:1 | 2521 ± 2.5 | 2480 ± 0.4 | 3473 ± 86 | 7.449 ± 0.025 |
| | | | N:P = 24:1 | 2514 ± 0.04 | 2503 ± 0.8 | 2865 ± 20 | 7.483 ± 0.054 |
| | | | N:P = 63:1 | 2557 ± 14 | 2497 ± 3.5 | 3928 ± 335 | 7.381 ± 0.018 |
| Rhodomonas sp. ^a | 12°C | Low CO ₂ | N:P = 10:1 | 2181 ± 6.5 | 2289 ± 0.2 | 725 ± 20 | 8.016 ± 0.009 |
| | | | N:P = 24:1 | 2238 ± 16 | 2345 ± 2.6 | 745 ± 100 | 8.072 ± 0.017 |
| | | | N:P = 63:1 | 2255 ± 14 | 2317 ± 1.7 | 1012 ± 111 | 7.940 ± 0.005 |
| | | High CO ₂ | N:P = 10:1 | 2368 ± 7.3 | 2298 ± 2.0 | 2726 ± 138 | 7.456 ± 0.003 |
| | | | N:P = 24:1 | 2418 ± 2.7 | 2349 ± 5.0 | 2724 ± 58 | 7.506 ± 0.017 |

Table S1. Measured dissolved inorganic carbon (DIC) and total alkalinity (TA), calculated pCO_2 , and pH (mean \pm SE) in the cultures of *Phaeodactylum tricornutum*, *Rhodomonas* sp. and *Emiliania huxleyi* on the sampling days at steady state. Triplicates were set for each treatment.

| | | | N:P = 63:1 | 2420 ± 14 | 2313 ± 2.0 | 3420 ± 315 | 7.333 ± 0.005 |
|-------------------------|-------|-----------------------|------------|--------------|----------------|----------------|-------------------|
| | 18°C | Low CO ₂ | N:P = 10:1 | 2211 ± 15 | 2287 ± 0.3 | 1138 ± 136 | 7.958 ± 0.019 |
| | | | N:P = 24:1 | 2255 ± 8.0 | 2342 ± 2.1 | 1073 ± 70 | 7.979 ± 0.017 |
| | | | N:P = 63:1 | 2209 ± 7.7 | 2311 ± 1.5 | 945 ± 61 | 7.970 ± 0.021 |
| | | High CO ₂ | N:P = 10:1 | 2387 | 2285 ± 2.3 | 4028 | 7.399 ± 0.013 |
| | | | N:P = 24:1 | 2423 ± 17 | 2347 ± 1.7 | 3489 ± 414 | 7.472 ± 0.014 |
| | | | N:P = 63:1 | 2388 ± 2.5 | 2310 ± 1.1 | 3486 ± 74 | 7.448 ± 0.012 |
| | 24°C | Low CO ₂ | N:P = 10:1 | 2188 ± 7.4 | 2302 ± 0.5 | 1083 ± 56 | 7.939 ± 0.010 |
| | | | N:P = 24:1 | 2201 ± 17 | 2306 ± 3.2 | 1160 ± 109 | 7.927 ± 0.016 |
| | | | N:P = 63:1 | 2189 ± 12 | 2288 ± 4.6 | 1210 ± 126 | 7.920 ± 0.009 |
| | | High CO ₂ | N:P = 10:1 | 2383 ± 15 | 2297 ± 1.3 | 4395 ± 347 | 7.372 ± 0.011 |
| | | | N:P = 24:1 | 2398 ± 8.4 | 2318 ± 5.1 | 4259 ± 95 | 7.372 ± 0.010 |
| | | | N:P = 63:1 | 2408 ± 3.4 | 2306 ± 2.2 | 4814 ± 138 | 7.789 ± 0.183 |
| E. huxleyi ^b | 12 °C | Low pCO_2 | N:P = 10:1 | 1302 ± 54 | 1269 ± 57 | 1509 ± 35 | 7.803 ± 0.007 |
| | | | N:P = 24:1 | 1328 ± 18 | 1292 ± 27 | 1564 ± 149 | 7.710 ± 0.039 |
| | | | N:P = 63:1 | 1374 ± 25 | 1349 ± 24 | 1412 ± 21 | 7.832 ± 0.004 |
| | | High pCO ₂ | N:P = 10:1 | 1956 ± 46 | 1962 ± 50 | 1357 ± 14 | 7.504 ± 0.034 |
| | | | N:P = 24:1 | 2042 ± 17 | 2053 ± 17 | 1357 ± 76 | 7.466 ± 0.001 |
| | | | N:P = 63:1 | 1829 ± 22 | 1801 ± 49 | 1041 ± 191 | 7.494 ± 0.014 |
| | 18 °C | Low pCO_2 | N:P = 10:1 | 763 ± 15 | 793 ± 4 | 552 ± 118 | 8.032 ± 0.044 |
| | | | N:P = 24:1 | 885 ± 6 | 922 ± 12 | 567 ± 84 | 7.906 ± 0.015 |
| | | | N:P = 63:1 | 1065 ± 3 | 1108 ± 8 | 633 ± 44 | 8.072 ± 0.001 |
| | | High pCO ₂ | N:P = 10:1 | 1415 ± 154 | 1454 ± 121 | 1113 ± 489 | 7.704 ± 0.077 |
| | | | N:P = 24:1 | 1278 ± 13 | 1196 ± 18 | 2944 ± 330 | 7.581 ± 0.079 |
| | | | N:P = 63:1 | 1613 ± 35 | 1620 ± 32 | 1507 ± 332 | 7.705 ± 0.010 |
| | 24 °C | Low pCO_2 | N:P = 10:1 | 785 ± 13 | 808 ± 10 | 845 ± 256 | 8.105 ± 0.007 |

| | N:P = 24:1 | 809 ± 10 | 682 ± 11 | - | 8.042 ± 0.017 |
|-------------------------------|------------|-------------|-------------|--------------|-------------------|
| | N:P = 63:1 | 1243 ± 16 | 1231 ± 10 | 1734 ± 163 | 7.980 ± 0.039 |
| High <i>p</i> CO ₂ | N:P = 10:1 | 1266 ± 22 | 1240 ± 20 | 2079 ± 406 | 7.625 ± 0.004 |
| | N:P = 24:1 | 1596 ± 63 | 1691 ± 36 | 1163 ± 190 | 7.608 ± 0.007 |
| | N:P = 63:1 | 1616 ± 27 | 1550 ± 34 | 3295 ± 171 | 7.553 ± 0.029 |

^a Data for *Phaeodactylum tricornutum* and *Rhodomonas* sp. are modified after Bi et al. (2017). ^b Data for *Emiliania huxleyi* are from Bi et al. (2018).

Table S2. Results of Akaike information criterion corrected (AICc) in the generalized linear mixed models (GLMMs) testing for the effects of temperature, N:P supply ratios and pCO_2 on carbon-normalized and per-cell contents of brassicasterol/epi-brassicasterol (brassi./epi-brassi./POC and brassi./epi-brassi./cell; μ g mg C⁻¹ and pg cell⁻¹) in *Phaeodactylum tricornutum*, *Rhodomonas* sp. and *Emiliania huxleyi*, and carbon-normalized and per-cell contents of C₃₇ - C₃₉ total alkenones (alkenones/POC and alkenones/cell), per-cell contents of C₃₇ alkenones (C₃₈/Cell), C₃₈ ethyl ketones (C₃₈ Et/Cell) and C₃₈ methyl ketones (C₃₈ Me/Cell), C₃₇/C₃₈ alkenone ratios, and C₃₈ Et/C₃₈ Me (C₃₈ Et/Me) in *E. huxleyi*.

| Species | Response variable | Effect builder | AICc |
|----------------|--------------------------|-----------------------------|------|
| P. tricornutum | Brassi./epi-brassi./POC | Main, two way and three way | 271 |
| | | Main, two way | 248 |
| | | Main | 207 |
| | Brassi./epi-brassi./cell | Main, two way and three way | 63 |
| | | Main, two way | 39 |
| | | Main | -21 |
| Rhodomonas sp. | Brassi./epi-brassi./POC | Main, two way and three way | 197 |
| | | Main, two way | 194 |
| | | Main | 191 |
| | Brassi./epi-brassi./cell | Main, two way and three way | 94 |
| | | Main, two way | 66 |
| | | Main | 5 |
| E. huxleyi | Brassi./epi-brassi./POC | Main, two way and three way | 272 |
| | | Main, two way | 248 |
| | | Main | 200 |
| | Brassi./epi-brassi./cell | Main, two way and three way | 116 |
| | | Main, two way | 89 |
| | | Main | 31 |
| | Alkenones/POC | Main, two way and three way | 575 |

| | Main, two way | 558 |
|--|-----------------------------|-----|
| | Main | 534 |
| Alkenones/Cell | Main, two way and three way | 109 |
| | Main, two way | 82 |
| | Main | 26 |
| C ₃₇ /Cell | Main, two way and three way | 105 |
| | Main, two way | 77 |
| | Main | 22 |
| C ₃₈ /Cell | Main, two way and three way | 115 |
| | Main, two way | 88 |
| | Main | 32 |
| C ₃₈ Et/Cell | Main, two way and three way | 125 |
| | Main, two way | 98 |
| | Main | 42 |
| C ₃₈ Me/Cell | Main, two way and three way | 100 |
| | Main, two way | 73 |
| | Main | 15 |
| C ₃₇ /C ₃₈ alkenone ratios | Main, two way and three way | 79 |
| | Main, two way | 50 |
| | Main | -13 |
| C ₃₈ Et/Me | Main, two way and three way | 83 |
| | Main, two way | 55 |
| | Main | -5 |

The selected models are shown in bold.

| | Treatm | ent | 1 | P. tricornutum | Rh | Rhodomonas sp. | | E. huxleyi | |
|-------|------------------------------|------------|----------------------------|-----------------------|--|-----------------------|----------------------------|-----------------------|--|
| | | | μ g mg C ⁻¹ | pg cell ⁻¹ | $\mu \mathrm{g} \mathrm{mg} \mathrm{C}^{-1}$ | pg cell ⁻¹ | $\mu g mg C^{-1}$ | pg cell ⁻¹ | |
| 12°C | Low <i>p</i> CO ₂ | N:P = 10:1 | 8.00 ± 1.59 | 0.06 ± 0.01 | 4.02 ± 0.18 | 0.37 ± 0.02 | 6.08 ± 0.69 | 0.11 ± 0.01 | |
| | | N:P = 24:1 | 8.34 ± 0.10 | 0.05 ± 0.002 | 4.49 ± 0.42 | 0.48 ± 0.11 | 6.24 ± 0.44 | 0.11 ± 0.01 | |
| | | N:P = 63:1 | 6.33 ± 0.30 | 0.06 ± 0.01 | 4.31 ± 0.34 | 0.50 ± 0.10 | 3.99 ± 1.66 | 0.08 ± 0.04 | |
| | High pCO ₂ | N:P = 10:1 | 6.51 ± 0.29 | 0.05 ± 0.002 | 3.96 ± 0.28 | 0.50 ± 0.12 | 5.46 ± 0.55 | 0.08 ± 0.01 | |
| | | N:P = 24:1 | $\boldsymbol{6.40\pm0.18}$ | 0.06 ± 0.003 | 4.30 ± 0.04 | 0.29 ± 0.06 | 5.28 ± 0.51 | 0.11 ± 0.01 | |
| | | N:P = 63:1 | 6.36 ± 0.78 | 0.08 ± 0.02 | 3.64 ± 0.52 | 0.51 ± 0.18 | 4.77 ± 0.38 | 0.11 ± 0.01 | |
| 18°C | Low <i>p</i> CO ₂ | N:P = 10:1 | 8.53 ± 0.11 | 0.08 ± 0.002 | 4.47 ± 0.80 | 0.41 ± 0.06 | 5.73 ± 0.16 | 0.04 ± 0.001 | |
| | | N:P = 24:1 | 7.43 ± 0.94 | 0.05 ± 0.01 | 4.91 ± 0.13 | 0.32 ± 0.03 | 7.19 ± 0.21 | 0.08 ± 0.01 | |
| | | N:P = 63:1 | 7.42 ± 0.82 | 0.11 ± 0.02 | 4.01 ± 0.23 | 0.48 ± 0.05 | 4.85 ± 0.44 | 0.15 ± 0.03 | |
| | High pCO_2 | N:P = 10:1 | 9.29 ± 0.44 | 0.08 ± 0.003 | 4.95 ± 0.12 | 0.51 ± 0.10 | $\boldsymbol{6.33\pm0.70}$ | 0.04 ± 0.01 | |
| | | N:P = 24:1 | 8.26 ± 0.52 | 0.08 ± 0.01 | 1.91 ± 0.34 | 0.32 ± 0.16 | 7.70 ± 0.92 | 0.14 ± 0.01 | |
| | | N:P = 63:1 | 7.35 ± 0.64 | 0.10 ± 0.01 | 4.61 ± 0.69 | 0.59 ± 0.17 | 4.17 ± 0.35 | 0.13 ± 0.05 | |
| 24 °C | Low pCO_2 | N:P = 10:1 | 6.31 ± 0.75 | 0.05 ± 0.01 | 3.36 ± 0.16 | 0.25 ± 0.04 | 7.54 ± 0.24 | 0.06 ± 0.003 | |
| | | N:P = 24:1 | $\boldsymbol{6.36\pm0.91}$ | 0.07 ± 0.02 | 9.56 ± 0.55 | 0.29 ± 0.03 | 7.15 ± 0.56 | 0.07 ± 0.002 | |
| | | N:P = 63:1 | 7.47 ± 0.44 | 0.13 ± 0.01 | 4.79 ± 0.40 | 0.54 ± 0.16 | 4.25 ± 0.43 | 0.09 ± 0.01 | |
| | High pCO_2 | N:P = 10:1 | 7.66 ± 0.17 | 0.07 ± 0.01 | 4.44 ± 0.44 | 0.38 ± 0.03 | 6.83 ± 0.46 | 0.05 ± 0.003 | |
| | | N:P = 24:1 | 8.88 ± 2.10 | 0.11 ± 0.004 | 4.10 ± 0.39 | 0.32 ± 0.06 | 7.85 ± 0.31 | 0.19 ± 0.02 | |
| | | N:P = 63:1 | 7.90 ± 0.40 | 0.09 ± 0.01 | 2.94 ± 0.08 | 0.42 ± 0.03 | 4.03 ± 0.30 | 0.12 ± 0.02 | |

Table S3. Brassicasterol/epi-brassicasterol contents (each μ g mg C⁻¹ and pg cell⁻¹; mean ± SE) in *Phaeodactylum tricornutum*, *Rhodomonas* sp. and *Emiliania huxleyi* as a function of temperature, N:P supply ratios and *p*CO₂. Triplicates were set for each treatment.

| Treatment | | C ₃₇ | :4 Me | C ₃₇ | _{7:3} Me | C ₃₇ | ₂₂ Me | С | _{38:3} Et | C38 | 3:3 Me | |
|-----------|-----------------------|-----------------|----------------------------|-----------------------|----------------------------------|-----------------------|--|-----------------------|----------------------------------|-----------------------|--|-----------------------|
| | | | μ g mg C ⁻¹ | pg cell ⁻¹ | $\mu { m g}~{ m mg}~{ m C}^{-1}$ | pg cell ⁻¹ | $\mu \mathrm{g} \ \mathrm{mg} \ \mathrm{C}^{-1}$ | pg cell ⁻¹ | $\mu { m g}~{ m mg}~{ m C}^{-1}$ | pg cell ⁻¹ | $\mu \mathrm{g} \ \mathrm{mg} \ \mathrm{C}^{-1}$ | pg cell ⁻¹ |
| 12°C | Low pCO_2 | N:P = 10:1 | 5.10 ± 0.61 | 0.09 ± 0.01 | 52.6 ± 9.17 | 0.91 ± 0.12 | 13.3 ± 2.63 | 0.23 ± 0.03 | 22.5 ± 3.95 | 0.39 ± 0.05 | 16.2 ± 3.21 | 0.28 ± 0.04 |
| | | N:P = 24:1 | 4.90 ± 0.38 | 0.09 ± 0.01 | 43.3 ± 3.67 | 0.79 ± 0.09 | 10.5 ± 0.83 | 0.19 ± 0.02 | 21.1 ± 1.95 | 0.39 ± 0.05 | 13.6 ± 1.47 | 0.25 ± 0.03 |
| | | N:P = 63:1 | 7.17 ± 1.22 | 0.15 ± 0.05 | 56.9 ± 3.28 | 0.80 ± 0.42 | 18.7 ± 0.31 | 0.26 ± 0.13 | 24.3 ± 2.20 | 0.51 ± 0.15 | 19.3 ± 1.28 | 0.40 ± 0.11 |
| | High pCO ₂ | N:P = 10:1 | 5.38 ± 0.25 | 0.08 ± 0.01 | 40.9 ± 7.59 | 0.61 ± 0.11 | 11.0 ± 3.32 | 0.16 ± 0.05 | 18.0 ± 3.28 | 0.27 ± 0.05 | 16.5 ± 3.34 | 0.25 ± 0.05 |
| | | N:P = 24:1 | 4.05 ± 0.19 | 0.09 ± 0.01 | 39.5 ± 1.67 | 0.86 ± 0.07 | 11.3 ± 0.46 | 0.24 ± 0.02 | 18.8 ± 1.04 | 0.41 ± 0.03 | 14.3 ± 0.68 | 0.31 ± 0.03 |
| | | N:P = 63:1 | 6.15 ± 0.63 | 0.14 ± 0.02 | 49.7 ± 6.17 | 1.13 ± 0.16 | 19.8 ± 4.47 | 0.45 ± 0.11 | 18.4 ± 1.79 | 0.42 ± 0.05 | 18.1 ± 2.35 | 0.41 ± 0.05 |
| 18°C | Low pCO_2 | N:P = 10:1 | 1.01 ± 0.06 | 0.01 ± 0.001 | 41.1 ± 8.74 | 0.29 ± 0.08 | 25.3 ± 6.89 | 0.18 ± 0.06 | 10.7 ± 2.25 | 0.08 ± 0.02 | 13.2 ± 3.02 | 0.09 ± 0.03 |
| | | N:P = 24:1 | 0.90 ± 0.07 | 0.01 ± 0.002 | 39.0 ± 3.39 | 0.45 ± 0.09 | 25.5 ± 2.14 | 0.30 ± 0.06 | 14.3 ± 1.07 | 0.17 ± 0.03 | 10.8 ± 0.88 | 0.13 ± 0.02 |
| | | N:P = 63:1 | 1.10 ± 0.15 | 0.03 ± 0.01 | 36.9 ± 8.36 | 1.15 ± 0.38 | 22.8 ± 9.16 | 0.72 ± 0.30 | 10.4 ± 2.95 | 0.33 ± 0.12 | 9.72 ± 2.41 | 0.31 ± 0.10 |
| | High pCO ₂ | N:P = 10:1 | 1.58 ± 0.34 | 0.01 ± 0.00 | 51.8 ± 2.14 | 0.35 ± 0.07 | 27.3 ± 2.51 | 0.19 ± 0.06 | 14.1 ± 0.43 | 0.10 ± 0.02 | 16.4 ± 0.58 | 0.11 ± 0.03 |
| | | N:P = 24:1 | 0.72 ± 0.16 | 0.01 ± 0.002 | 28.7 ± 2.65 | 0.51 ± 0.04 | 14.7 ± 1.55 | 0.26 ± 0.01 | 14.4 ± 0.90 | 0.25 ± 0.01 | 8.06 ± 0.70 | 0.14 ± 0.01 |
| | | N:P = 63:1 | 0.97 ± 0.09 | 0.03 ± 0.01 | 38.0 ± 11.2 | 0.93 ± 0.10 | 30.8 ± 13.3 | 0.69 ± 0.21 | 11.4 ± 3.37 | 0.28 ± 0.03 | 10.8 ± 3.34 | 0.26 ± 0.03 |
| 24 °C | Low pCO_2 | N:P = 10:1 | 0 ± 0 | 0 ± 0 | 18.7 ± 1.22 | 0.15 ± 0.01 | 78.3 ± 9.84 | 0.62 ± 0.09 | 3.90 ± 0.24 | 0.03 ± 0.003 | 3.91 ± 0.21 | 0.03 ± 0.003 |
| | | N:P = 24:1 | 0 ± 0 | 0 ± 0 | 17.4 ± 2.55 | 0.16 ± 0.01 | 60.6 ± 19.4 | 0.56 ± 0.15 | 5.50 ± 0.78 | 0.05 ± 0.004 | 2.51 ± 0.39 | 0.02 ± 0.002 |
| | | N:P = 63:1 | 0 ± 0 | 0 ± 0 | 12.8 ± 3.66 | 0.26 ± 0.05 | 41.7 ± 13.8 | 0.85 ± 0.17 | 3.05 ± 1.02 | 0.06 ± 0.02 | 1.69 ± 0.48 | 0.03 ± 0.01 |
| | High pCO ₂ | N:P = 10:1 | 0 ± 0 | 0 ± 0 | 20.2 ± 5.41 | 0.15 ± 0.03 | 47.8 ± 13.0 | 0.69 ± 0.31 | 3.26 ± 0.50 | 0.04 ± 0.01 | 3.01 ± 0.39 | 0.03 ± 0.01 |
| | | N:P = 24:1 | 0 ± 0 | 0 ± 0 | 15.7 ± 1.41 | 0.37 ± 0.05 | 76.1 ± 9.40 | 1.82 ± 0.31 | 5.36 ± 0.38 | 0.13 ± 0.01 | 2.65 ± 0.31 | 0.06 ± 0.01 |
| | | N:P = 63:1 | 0 ± 0 | 0 ± 0 | 20.2 ± 3.18 | 0.56 ± 0.04 | 53.0 ± 1.03 | 1.96 ± 0.21 | 5.32 ± 0.90 | 0.15 ± 0.01 | 2.91 ± 0.46 | 0.08 ± 0.01 |
| | | | | | | | | | | | | |

Table S4. Alkenone contents (each μ g mg C⁻¹ and pg cell⁻¹; mean ± SE) in *Emiliania huxleyi* as a function of temperature, N:P supply ratios and <u>*p*CO</u>₂. Triplicates were set for each treatment.

| | | Mean per-cell content | | | | |
|---------------|----------------------|-----------------------|----------------------|----------------------|----------------------|--|
| | C _{37:4} Me | C _{37:3} Me | C _{37:2} Me | C _{38:3} Et | C _{38:3} Me | |
| All treatment | 0 - 0.15 | 0.15 - 1.15 | 0.16 - 1.96 | 0.03 - 0.51 | 0.02 - 0.41 | |

| Table | e S4 . | Continued. | |
|-------|---------------|------------|--|
| | | | |

| Treatment | | (| C _{38:2} Et | C3 | _{8:2} Me | C _{39:3} Et* | | C _{39:2} Et* | | |
|-----------|-----------------------|------------|----------------------|-----------------------|----------------------------------|-----------------------|----------------------------------|-----------------------|-------------------|-----------------------|
| _ | | | $\mu g mg C^{-1}$ | pg cell ⁻¹ | $\mu { m g}~{ m mg}~{ m C}^{-1}$ | pg cell ⁻¹ | $\mu { m g}~{ m mg}~{ m C}^{-1}$ | pg cell ⁻¹ | $\mu g mg C^{-1}$ | pg cell ⁻¹ |
| 12 °C | Low pCO_2 | N:P = 10:1 | 13.4 ± 2.85 | 0.23 ± 0.04 | 4.08 ± 1.01 | 0.07 ± 0.01 | 3.81 ± 0.82 | 0.07 ± 0.01 | 2.29 ± 0.53 | 0.04 ± 0.01 |
| | | N:P = 24:1 | 10.9 ± 0.63 | 0.20 ± 0.02 | 3.27 ± 0.50 | 0.06 ± 0.01 | 3.29 ± 0.33 | 0.06 ± 0.01 | 1.88 ± 0.19 | 0.03 ± 0.005 |
| | | N:P = 63:1 | 16.3 ± 0.43 | 0.33 ± 0.06 | 5.71 ± 0.01 | 0.12 ± 0.02 | 4.50 ± 0.21 | 0.09 ± 0.02 | 3.28 ± 0.05 | 0.07 ± 0.01 |
| | High pCO ₂ | N:P = 10:1 | 10.6 ± 3.72 | 0.16 ± 0.05 | 4.10 ± 1.28 | 0.06 ± 0.02 | 3.84 ± 0.96 | 0.06 ± 0.01 | 2.37 ± 0.78 | 0.04 ± 0.01 |
| | | N:P = 24:1 | 12.1 ± 0.30 | 0.27 ± 0.03 | 3.81 ± 0.12 | 0.08 ± 0.01 | 3.60 ± 0.19 | 0.08 ± 0.01 | 2.33 ± 0.07 | 0.05 ± 0.01 |
| | | N:P = 63:1 | 16.4 ± 4.37 | 0.37 ± 0.10 | 6.42 ± 1.34 | 0.15 ± 0.03 | 3.77 ± 0.64 | 0.09 ± 0.01 | 3.37 ± 0.86 | 0.08 ± 0.02 |
| 18 °C | Low pCO_2 | N:P = 10:1 | 16.5 ± 4.73 | 0.12 ± 0.04 | 7.89 ± 2.21 | 0.06 ± 0.02 | 1.95 ± 0.49 | 0.01 ± 0.004 | 3.06 ± 0.93 | 0.02 ± 0.01 |
| | | N:P = 24:1 | 20.3 ± 1.70 | 0.23 ± 0.05 | 6.66 ± 0.47 | 0.08 ± 0.01 | 2.03 ± 0.19 | 0.02 ± 0.005 | 3.08 ± 0.27 | 0.04 ± 0.01 |
| | | N:P = 63:1 | 15.0 ± 6.30 | 0.48 ± 0.21 | 5.85 ± 2.29 | 0.19 ± 0.08 | 1.50 ± 0.42 | 0.05 ± 0.02 | 2.48 ± 1.01 | 0.08 ± 0.03 |
| | High pCO ₂ | N:P = 10:1 | 18.1 ± 2.53 | 0.13 ± 0.05 | 8.85 ± 1.18 | 0.06 ± 0.02 | 2.54 ± 0.27 | 0.02 ± 0.01 | 3.49 ± 0.63 | 0.03 ± 0.01 |
| | | N:P = 24:1 | 13.5 ± 1.64 | 0.24 ± 0.02 | 3.94 ± 0.37 | 0.07 ± 0.003 | 1.99 ± 0.09 | 0.04 ± 0.001 | 2.07 ± 0.23 | 0.04 ± 0.003 |
| | | N:P = 63:1 | 21.5 ± 9.85 | 0.47 ± 0.16 | 8.24 ± 3.62 | 0.18 ± 0.06 | 1.75 ± 0.59 | 0.04 ± 0.01 | 3.68 ± 1.66 | 0.08 ± 0.03 |
| 24 °C | Low pCO ₂ | N:P = 10:1 | 40.2 ± 5.94 | 0.32 ± 0.05 | 15.0 ± 1.88 | 0.12 ± 0.02 | 0.25 ± 0.13 | 0.002 ± 0.001 | 4.49 ± 0.63 | 0.04 ± 0.01 |
| | | N:P = 24:1 | 41.3 ± 14.1 | 0.38 ± 0.11 | 7.14 ± 2.07 | 0.07 ± 0.02 | 0.23 ± 0.11 | 0.002 ± 0.001 | 2.87 ± 0.83 | 0.03 ± 0.01 |
| | | N:P = 63:1 | 23.3 ± 8.87 | 0.46 ± 0.12 | 4.87 ± 1.71 | 0.10 ± 0.02 | 0 ± 0 | 0 ± 0 | 1.68 ± 0.68 | 0.03 ± 0.01 |
| | High pCO ₂ | N:P = 10:1 | 25.3 ± 8.55 | 0.39 ± 0.19 | 9.00 ± 2.73 | 0.12 ± 0.05 | 0.17 ± 0.17 | 0.002 ± 0.001 | 2.75 ± 0.88 | 0.04 ± 0.02 |
| | | N:P = 24:1 | 51.0 ± 5.79 | 1.22 ± 0.19 | 9.59 ± 1.15 | 0.23 ± 0.04 | 0 ± 0 | 0 ± 0 | 4.21 ± 0.48 | 0.10 ± 0.02 |
| | | N:P = 63:1 | 41.2 ± 11.7 | 1.10 ± 0.12 | 8.84 ± 2.21 | 0.24 ± 0.03 | 0.11 ± 0.11 | 0.002 ± 0.002 | 3.24 ± 0.91 | 0.09 ± 0.01 |

| | Mean per-cell content | | | | |
|---------------|-----------------------|----------------------|-----------------------|-----------------------|--|
| | C _{38:2} Et | C _{38:2} Me | C _{39:3} Et* | C _{39:2} Et* | |
| All treatment | 0.12 - 1.22 | 0.06 - 0.24 | 0 - 0.09 | 0.02 - 0.10 | |

* Tentatively identified due to low molecular ion abundance.

| Tabla | C/ | Continued |
|-------|-------------|-----------|
| Laure | DH . | Commucu. |

| Treatment | | | Total alkenones | | C ₃₇ /C ₃₈ | C ₃₈ Et/Me | $U_{37}^{K'}$ |
|-----------|-----------------------|------------|----------------------------|-----------------------|----------------------------------|-----------------------|-----------------|
| | | | μ g mg C ⁻¹ | pg cell ⁻¹ | | | |
| 12 °C | Low pCO_2 | N:P = 10:1 | 133.2 ± 24.8 | 2.29 ± 0.31 | 1.27 ± 0.02 | 1.79 ± 0.04 | 0.20 ± 0.003 |
| | | N:P = 24:1 | 112.7 ± 9.69 | 2.06 ± 0.24 | 1.20 ± 0.01 | 1.92 ± 0.08 | 0.20 ± 0.004 |
| | | N:P = 63:1 | 156.2 ± 7.51 | 3.24 ± 0.80 | 1.34 ± 0.08 | 1.55 ± 0.07 | 0.24 ± 0.01 |
| | High pCO ₂ | N:P = 10:1 | 112.8 ± 24.0 | 1.68 ± 0.34 | 1.20 ± 0.09 | 1.37 ± 0.05 | 0.20 ± 0.03 |
| | | N:P = 24:1 | 109.9 ± 4.17 | 2.38 ± 0.22 | 1.12 ± 0.01 | 1.71 ± 0.03 | 0.22 ± 0.004 |
| | | N:P = 63:1 | 142.1 ± 21.4 | 3.24 ± 0.53 | 1.29 ± 0.04 | 1.41 ± 0.05 | 0.28 ± 0.03 |
| 18 °C | Low pCO_2 | N:P = 10:1 | 120.7 ± 29.3 | 0.85 ± 0.25 | 1.41 ± 0.04 | 1.28 ± 0.02 | 0.37 ± 0.02 |
| | | N:P = 24:1 | 122.6 ± 10.1 | 1.42 ± 0.28 | 1.26 ± 0.01 | 1.98 ± 0.04 | 0.40 ± 0.005 |
| | | N:P = 63:1 | 105.7 ± 32.1 | 3.33 ± 1.22 | 1.58 ± 0.15 | 1.53 ± 0.17 | 0.34 ± 0.08 |
| | High pCO ₂ | N:P = 10:1 | 144.1 ± 7.07 | 0.99 ± 0.27 | 1.42 ± 0.10 | 1.28 ± 0.03 | 0.35 ± 0.03 |
| | | N:P = 24:1 | 88.1 ± 6.21 | 1.55 ± 0.04 | 1.10 ± 0.05 | 2.34 ± 0.17 | 0.34 ± 0.02 |
| | | N:P = 63:1 | 127.0 ± 47.0 | 2.96 ± 0.60 | 1.41 ± 0.09 | 1.65 ± 0.10 | 0.41 ± 0.06 |
| 24 °C | Low pCO_2 | N:P = 10:1 | 164.7 ± 20.0 | 1.30 ± 0.19 | 1.55 ± 0.03 | 2.31 ± 0.12 | 0.80 ± 0.01 |
| | | N:P = 24:1 | 137.6 ± 39.2 | 1.27 ± 0.30 | 1.40 ± 0.04 | 4.72 ± 0.37 | 0.76 ± 0.04 |
| | | N:P = 63:1 | 89.1 ± 29.9 | 1.80 ± 0.38 | 1.73 ± 0.10 | 3.90 ± 0.21 | 0.76 ± 0.02 |
| | High pCO ₂ | N:P = 10:1 | 138.3 ± 35.9 | 0.85 ± 0.14 | 1.52 ± 0.09 | 2.54 ± 0.22 | 0.79 ± 0.04 |
| | | N:P = 24:1 | 164.6 ± 18.8 | 3.93 ± 0.63 | 1.34 ± 0.01 | 4.62 ± 0.09 | 0.83 ± 0.01 |
| | | N:P = 63:1 | 117.2 ± 3.17 | 4.19 ± 0.39 | 1.60 ± 0.03 | 3.89 ± 0.19 | 0.77 ± 0.02 |

| C_{37} Me C_{38} Et C_{38} Me C_{39} EtAll treatments $48 - 65\%$ $18 - 35\%$ $6.6 - 19\%$ $0 - 5.9\%$ | | % of total alkenones | | | | |
|--|----------------|----------------------|--------------------|--------------------|--------------------|--|
| All treatments 48 - 65% 18 - 35% 6.6 - 19% 0 - 5.9% | | C ₃₇ Me | C ₃₈ Et | C ₃₈ Me | C ₃₉ Et | |
| | All treatments | 48 - 65% | 18 - 35% | 6.6 - 19% | 0 - 5.9% | |

Table S5. Dimension 1 (Dim1) and Dim 2 loadings for each variable in PCA (Fig. 3) testing the responses of carbon-normalized contents of the major sterol component (brassicasterol/epi-brassicasterol), C_{37} - C_{39} total alkenones and total fatty acids (TFAs) (μ g mg C⁻¹) in *Phaeodactylum tricornutum*, *Rhodomonas* sp. and *Emiliania huxleyi* to changes in temperature, N:P molar ratios and pCO_2 .

| Variables | Dim 1 | Dim 2 |
|--|--------|--------|
| % of variance | 26.4 | 17.7 |
| Temperature | -0.320 | 0.493 |
| pCO_2 | 0.185 | 0.151 |
| N:P supply ratio | 0.668 | 0.559 |
| Brassicasterol/epi-brassicasterol (P. tricornutum) | -0.024 | -0.420 |
| Brassicasterol/epi-brassicasterol (Rhodomonas sp.) | -0.402 | 0.393 |
| Brassicasterol/epi-brassicasterol (E. huxleyi) | -0.833 | -0.040 |
| Alkenones (E. huxleyi) | -0.292 | 0.317 |
| TFAs (P. tricornutum) | 0.608 | 0.426 |
| TFAs (Rhodomonas sp.) | 0.819 | -0.104 |
| TFAs (E. huxleyi) | 0.279 | -0.753 |

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Fig. S1
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Fig. S1. Experimental setup. The three phytoplankton species were grown semicontinuously under a full-factorial combination of three temperatures (12, 18 and 24 °C), three N:P supply ratios (molar ratios 10:1, 24:1 and 63:1) and two pCO_2 levels (560 and 2400 µatm). Triplicates were set for each treatment.