

Supplementary material for the manuscript, ‘Prevalence and Preferred Niche of Small Eukaryotes with Mixotrophic Potentials in the Global Ocean’, accepted for publication in the *Microorganisms* journal by Dong et al.

Supplementary Table S1. Species and cellular 18S rRNA gene copy numbers used in this study to retrieve gene-cell abundance correction factors for small eukaryotes.

| Taxa | Group | Reference | Method | Biovolume (μm^3) | Gene Copies per Cell |
|----------------------------------|------------------|-------------------|----------------|-------------------------------|----------------------|
| <i>Ostreococcus tauri</i> | | Gong et al. 2019 | bioinformatics | 0.5 | 3.4 |
| <i>Trebouxia</i> sp. | | Gong et al. 2019 | bioinformatics | 1767.1 | 78.1 |
| <i>Bathycoccus prasinos</i> | | Zhu et al. 2005 | qPCR | 3.8 | 5.2 |
| <i>Emiliania huxleyi</i> | | Zhu et al. 2005 | qPCR | 64.3 | 2.5 |
| <i>Mesopedinella arctica</i> | | Zhu et al. 2005 | qPCR | 252.8 | 7.4 |
| <i>Micromonas pusilla</i> | | Zhu et al. 2005 | qPCR | 4.0 | 3.0 |
| <i>Nannochloropsis salina</i> | | Zhu et al. 2005 | qPCR | 4.0 | 1.0 |
| <i>Ostreococcus tauri</i> | | Zhu et al. 2005 | qPCR | 0.3 | 2.5 |
| <i>Pelagomonas calceolata</i> | | Zhu et al. 2005 | qPCR | 7.7 | 1.3 |
| <i>Rhodomonas salina</i> | other eukaryotes | Zhu et al. 2005 | qPCR | 217.0 | 28.8 |
| <i>Tetraselmis</i> sp. | eukaryotes | Zhu et al. 2005 | qPCR | 208.7 | 7.6 |
| <i>Chrysochromulina</i> sp. | | Li et al., 2022 | qPCR | 20.6 | 2.0 |
| <i>Chrysochromulina</i> sp. | | Li et al., 2022 | qPCR | 41.6 | 2.0 |
| ChrysoH | | Li et al., 2022 | qPCR | 5.6 | 1.0 |
| DictyX | | Li et al., 2022 | qPCR | 78.0 | 4.5 |
| <i>Florenciella Parvula</i> | | Li et al., 2022 | qPCR | 26.5 | 1.2 |
| Hap2 | | Li et al., 2022 | qPCR | 47.7 | 3.0 |
| <i>Rhizochromulina</i> | | Li et al., 2022 | qPCR | 14.1 | 1.2 |
| <i>Triparma</i> | | Li et al., 2022 | qPCR | 8.2 | 0.8 |
| <i>Florenciella</i> sp. | | Li et al., 2022 | qPCR | 31.1 | 2.0 |
| <i>Symbiodinium kawagutii</i> | | Gong et al. 2019 | bioinformatics | 288.7 | 160.5 |
| <i>Symbiodinium minutum</i> | | Gong et al. 2019 | bioinformatics | 220.9 | 116.0 |
| <i>Amphidinium carterae</i> | dinoflagellate | Zhu et al. 2005 | qPCR | 432.0 | 83.7 |
| <i>Prorocentrum nux</i> | | Zhu et al. 2005 | qPCR | 79.9 | 22.1 |
| <i>Extubocellulus spinifer</i> | | Godhe et al. 2008 | qPCR | 150.0 | 19.0 |
| <i>Phaeodactylum tricornutum</i> | | Gong et al. 2019 | bioinformatics | 101.6 | 4.2 |
| <i>Thalassiosira oceanica</i> | diatom | Gong et al. 2019 | bioinformatics | 179.5 | 17.2 |
| <i>Nitzschia closterium</i> | | Zhu et al. 2005 | qPCR | 640.0 | 28.0 |
| <i>Thalassiosira weissflogii</i> | | Zhu et al. 2005 | qPCR | 471.0 | 14.8 |

Supplementary Table S2. Stepwise regression analysis revealed significant variables contributing to the prevalence of mixotrophs in both surface and chlorophyll maximal layer (SUR and CML) samples, and surface (SUR) samples alone. Significance of P values and coefficient for 8 selected predictors were given in each row.

| SUR and CML | | | SUR | | |
|---------------------------------------------------------------|----------------|-------------|------------------------------------------------------------|----------------|-------------|
| Predictor [†] | P [‡] | Coefficient | Predictor [†] | P [‡] | Coefficient |
| PAR | ** | 0.1 | CML | *** | 0.15 |
| NO ₃ ⁻ and NO ₂ ⁻ | *** | -0.08 | <i>Prochlorococcus</i> | ** | -0.82 |
| Density | *** | 0.80 | NO ₃ ⁻ &NO ₂ ⁻ | *** | -0.15 |
| PIC | ** | 207.5 | PIC | * | 218.3 |
| CML | *** | 0.10 | PO ₄ ³⁻ | * | 0.28 |
| Hbac | ** | 1.25 | Density | * | 0.78 |
| <i>Prochlorococcus</i> | * | -0.53 | PAR | ** | 0.12 |
| / | / | / | NPP | . | 0.08 |

[†]Abbreviations: PAR, photosynthetically available radiation; PIC, particulate inorganic carbon; Hbac, heterotrophic bacteria; NPP, net primary production

[‡]Significant codes: ‘***’ p<0.001, ‘**’ p<0.01, ‘*’ p<0.05, ‘.’ p<0.1

Supplementary figures

Figure S1. Redundancy analysis for environmental impact on the distribution of trophic groups in surface oceans, derived from 18S rRNA gene corrected cell abundances. Only 17 *Tara Oceans* lineages passed Escoufier selectin (0.9 threshold) were shown, and adjusted R^2 of the analysis is of 0.54. Environmental variables selected were denoted in cyan-blue arrows and text. Abbreviations: PAR, photosynthetically active radiations; CML, chlorophyll maximum layer; MLD, mixed layer depth; POC, particulate organic carbon; PIC, particulate inorganic carbon; Pro, *Prochlorococcus*.

Figure S2. Linear regression between $TI_{A/H}$ (all in cell abundances) and resource variables retrieved from the SUR (104 samples) and SUR and CML dataset (165 samples). Different significance levels were marked with different number of red stars (one star if $0.001 < p < 0.05$ and two stars if $p < 0.001$), and shaded bands are pointwise 95% confidence interval on the fitted values (the line). Abbreviations: PAR, photosynthetically active radiations; N: inorganic nutrients of nitrate (NO_3^-) and nitrite (NO_2^-); Hbac: heterotrophic bacteria; CML, chlorophyll maximal layer.

Figure S3. Linear regression between TI_g (all in cell abundances) and single environmental variables that have shown significant correlations, retrieved from all 165 SUR and CML samples. Different significance levels were marked with different number of red stars (one star if $0.001 < p < 0.05$ and two stars if $p < 0.001$), and shaded bands are pointwise 95% confidence interval on the fitted values (the line). Abbreviations: CML, chlorophyll maximal layer; Chl a , total chlorophyll a ; Pro, *Prochlorococcus*.

Figure S1

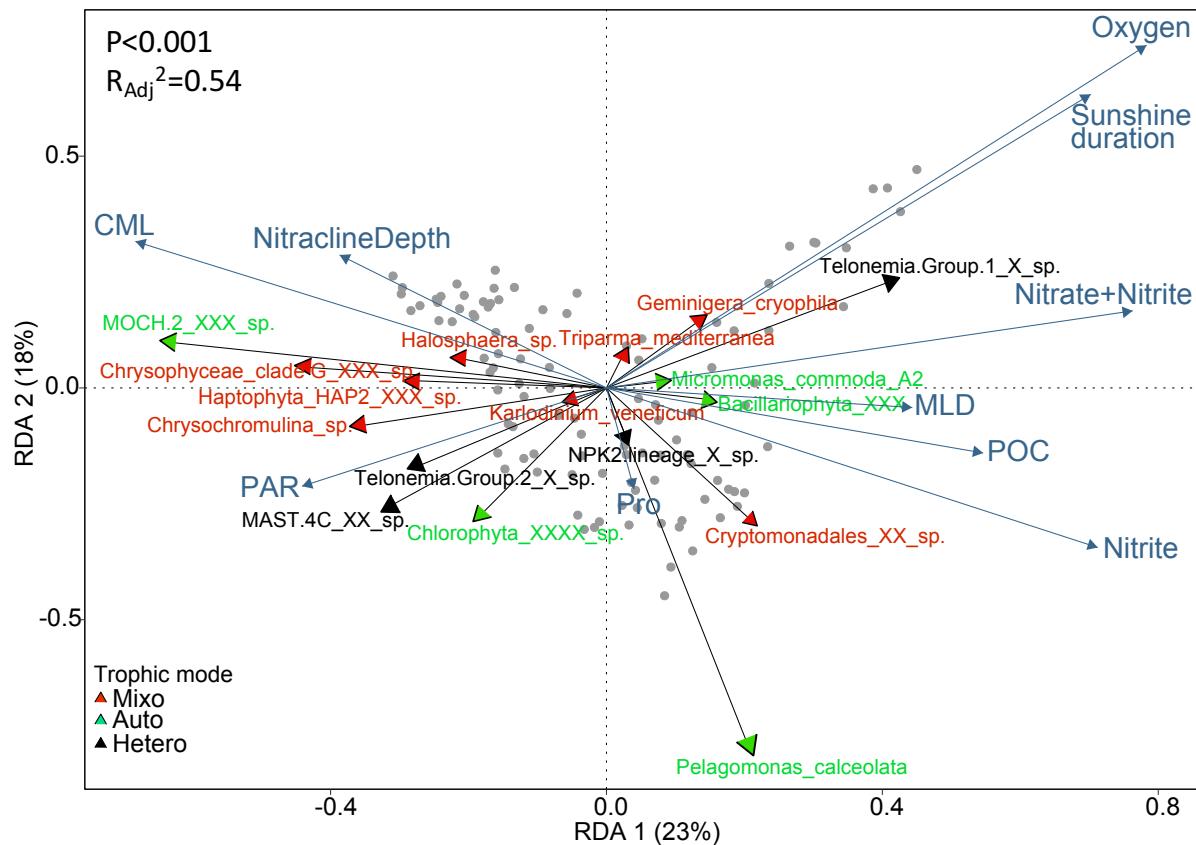


Figure S2

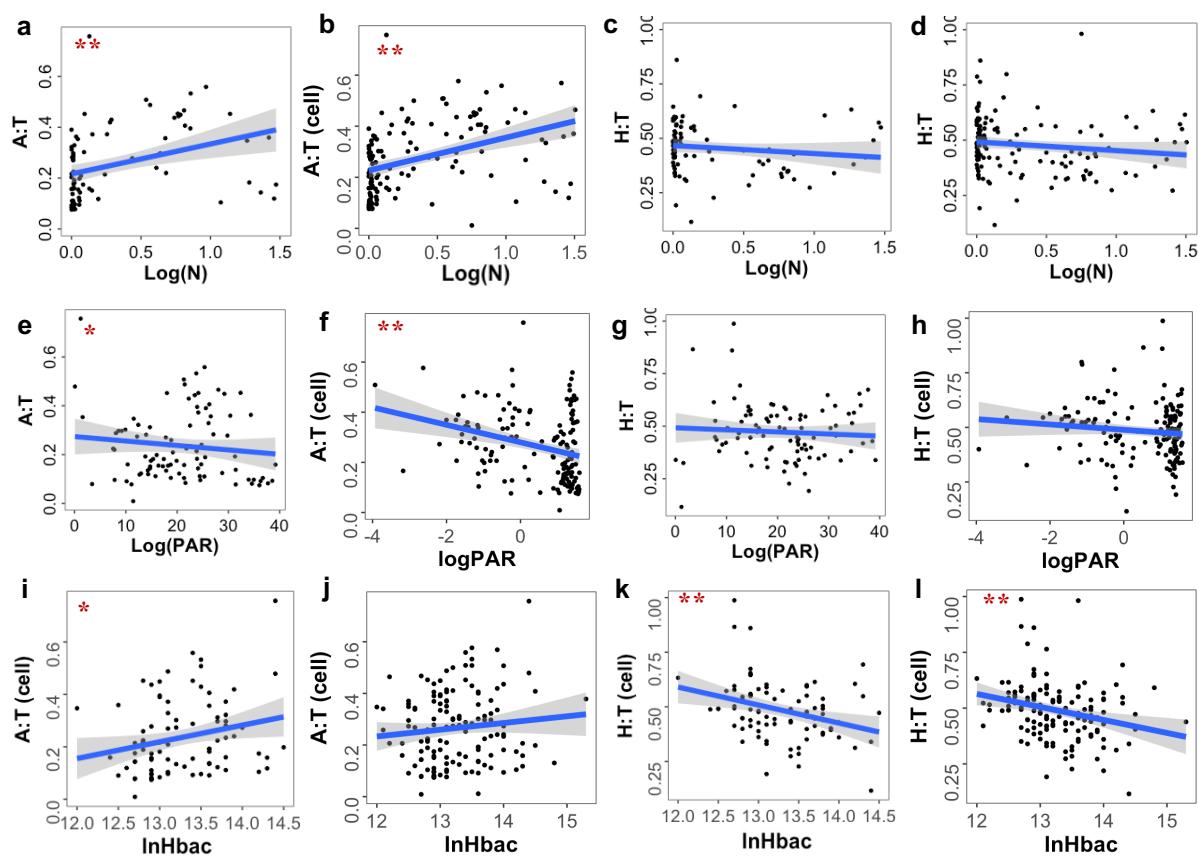
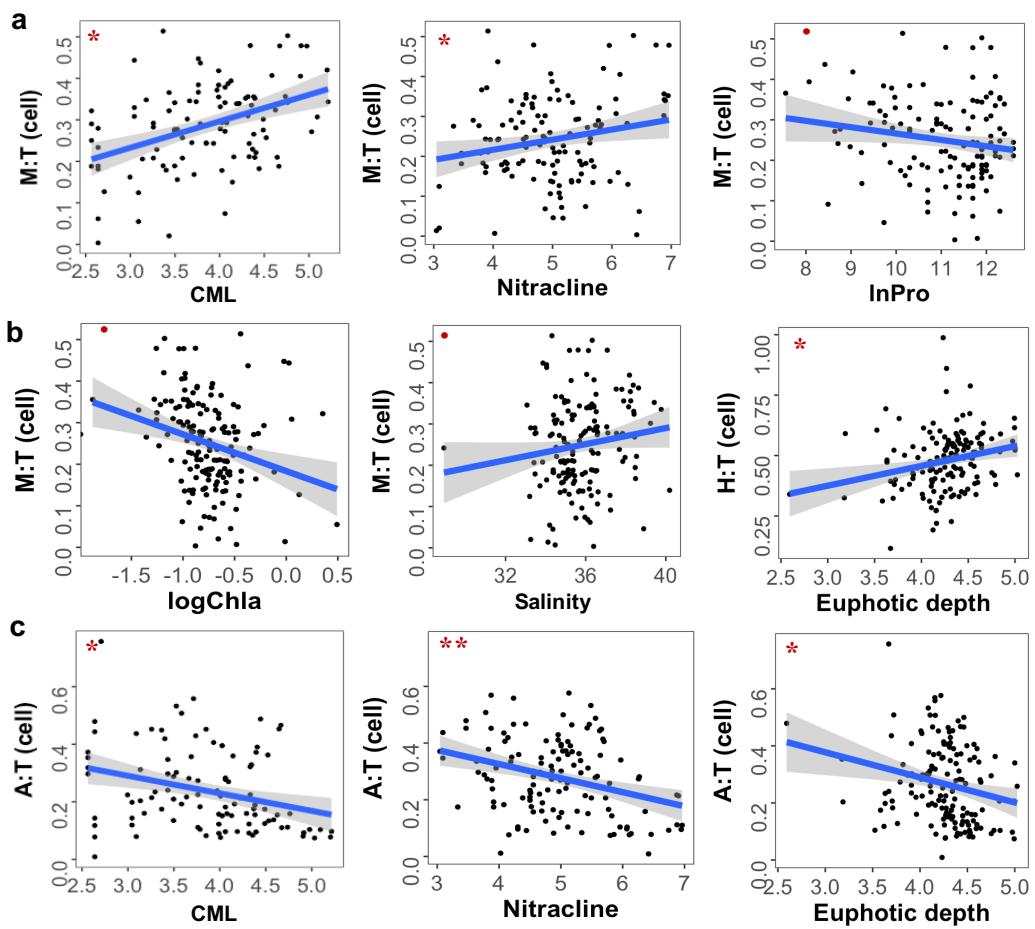


Figure S3



References

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