Supplementary Information

Supplementary Methods

Site selection and sampling

Samples were collected from two sites during an oceanographic voyage (IV2015_V03) in the East Australia Current (EAC) region in austral winter (June 2015) aboard the *R/V Investigator*, Australia's Marine National Facility managed by CSIRO. Vertical profiles of temperature (SBE3T S/N, Sea-Bird Scientific, USA), salinity (measured as conductivity SBE4C S/N, Sea-Bird Scientific, USA), dissolved oxygen (SBE43 S/N, Sea-Bird Scientific, USA) and chlorophyll-*a* fluorescence (Aquatrack III, Chelsea Technologies Group, UK), were measured using a CTD (conductivity-temperature-depth)-profiler. Sensors were calibrated by on-board analyses using a Guildline Autosal Laboratory Salinometer 8400(B) – SN 71611, and an automated Photometric Oxygen system (Scripps Institute of Oceanography). Mixed layer depth (MLD) was calculated as the depth where potential density is +0.125 kg m^{-3} relative to the surface using the get_mld Matlab function. Absolute temperature was converted to potential temperature using the CSIRO SeaWater library function 'sw_ptmp' and this was then used to calculate potential density 'sw_dens.'

Dissolved nutrients (phosphate, silicate, nitrite, nitrate and ammonium) were analysed from Niskin bottle samples. A segmented flow auto-analyser Seal AA3HR was used, following the standard operational procedures (SOP 001-004) modified from published methods by the CSIRO Oceans and Atmosphere Hydrochemistry Team to optimise nutrient analysis at sea. Briefly, phosphate was determined using the molybdenum blue method, based on Murphy and Riley (1962) with modifications from the NIOZ-SGNOS Practical Workshop (2012). Silicate was also measured using the molybdenum blue method, and nitrite and nitrate using the Copper-cadmium reduction

– Naphthylenediamine photometric method, both based on Armstrong et al (1967). Ammonium was analysed using the ortho-phtaldiadehyde method based on Kérouel and Aminot 1997. The accuracy of nutrient analysis was determined by analysing a certified reference material produced by KANSO, Japan. The RMNS Lot CA (produced 22/02/2013) was measured four times in every analytical run. The RMNS Lot CD (produced 08/04/2015) was analysed twice alongside the CA Lot. RMNS results were converted from μmol/kg to μ mol L^{-1} at 21°C.

Seawater containing microbial communities was collected in 12 L Niskin bottles using a 24 bottle CTD-rosette sampler. From there, samples were gently dispensed via silicon tubing into plastic containers before being aliquoted into replicate borosilicate flat-bottomed glass vials (30 mL capacity). Tubing and all vessels were acid-washed to minimize metal contamination. Vials containing seawater aliquots were then randomly allocated to temperature treatments within a thermal gradient block.

Experimental set up

Microbial communities were incubated within 2 h of collection under ~75 μmol photons m^2 s^{-1} (below the photosynthesis saturation irradiance (Bouman et al. 2017) so as not to induce additional ROS production from high light stress, but likely not representative of the dynamic light conditions in the mixed layer), maintained using LED light panels (Cidley, China). Illumination was set to a 12:12 light dark cycle to reflect the average natural diurnal cycle. The experimental design entailed exposing microbial communities to a range of temperatures spanning 7 °C below and 10 °C above ambient temperature (~22 °C for both sites) using a thermal gradient block. The thermal block was made of solid aluminium machined to form replicate wells to house flat-bottom vials, with the

48 temperature gradient created by pumping cold water into one end and hot water into the other
49 (resulting in a temperature range from 15.6 to 32.1 °C). This design was intended to test the acute,
50 not acclimated, response to temperature as a way of gaining insight into the thermal performance
51 of populations that may diverge due to previous thermal exposure. Microbes were placed into
52 experimental treatments where temperature would have equilibrated within 0.5 h. For comparison,
53 thermal trajectories extracted from a global circulation model using Lagrangian tracking software
54 (Doblin and van Sebille, 2016), show the maximum change in microbial temperature exposure is
55 approximately 5 °C over a 5-d period (i.e., 1 °C per day).
56
57 *Physiological response to short-term temperature excursions*
58 To understand the physiological responses of microbes to temperature changes, we quantified their
59 intracellular reactive oxygen species (ROS) content at 4 time points: at the beginning of the
60 experiment (local time ~10:00, ~4 h after sunrise) and 1, 5, and 25 h later (i.e., T0, T1, T5, T25 h,
61 respectively). This allowed ROS to be measured during the natural light period. Commercially
62 available fluorescent markers for superoxide (488 nm blue excitation; 580 nm orange emission)
63 and other ROS (488 nm blue excitation; 530 nm green emission) (Total ROS/Superoxide detection
64 kit ENZ-51010, Enzo Life Sciences, Inc.,New York, USA) were used within their 6-month shelf
65 life. Prior to the voyage, the protocol was optimised for use with phytoplankton, whereby a matrix
66 of fluorescent dye incubation time and concentration for both dyes was tested. Optimal staining
67 conditions were achieved at 1:1000 for superoxide stain (orange) and 1:2000 for other ROS stain
68 (green) both incubated in the dark at the experimental temperature for 1 h before flow cytometric
69 analysis. Initial samples for positive (induced using kit) and negative (no stain) controls were
70 aliquoted and run on board (confirming stain optimisation for the different samples) using an

Influx flow cytometer (BD Biosciences). T0 samples were also analyzed to measure ambient background ROS within each population (Fig. S2).

During the experiment, subsamples were removed from glass vials and placed into tubes, stain added, and tubes incubated in the dark for 1 h under incubation conditions. Following incubation, a 10 µl aliquot of standard 1.0 µm yellow-green fluorescent beads was added (Fluoresbrite® YG Microspheres 1.00µm (Cat#17154-10); Polysciences Inc., Taipai, Taiwan) to tubes and stained samples interrogated using a flow cytometer (BD Influx, Becton Dickson, Brussels, Belgium) equipped with a 50 mW blue laser emitting at a fixed wavelength of 488 nm. Picoplankton populations were discriminated as low phycoerythrin (PE-580/30 nm) high chlorophyll-*a* (Chl-692/20 nm) cells and gated according to Fig. S1A.

Gated picoeukaryotes were then investigated for their ROS content using "daughter" biplots of green (530 ± 20 nm; 530/40 nm) vs orange (530 ± 15 nm; 580/30 nm) fluorescence (Fig. S1; FlowJo, LLC, Ashland Oregon). To estimate ROS accumulation, unstained T0 populations were used to define 'healthy' cells so that ROS expression would be quantified as an increase from background (Fig. S2). The stained samples were then used to determine ROS content of cells under incubation conditions; a gate depicting 'stressed' cells was made using boolean logic (Fig. S1).

The median forward scatter and fluorescence (580, 530 nm) were extracted for standard beads and 'healthy' and 'stressed' cells in all samples. To quantify changes in the relative fluorescence of 'stressed' cells over time, scatter and fluorescence values were normalized to forward scatter (FSC) and fluorescence of the standard bead using Equation 1:

Equation 1: ROS fluorescence (RFU) = ((pico-eukaryote median pop fluor 580* nm/median bead fluor 580* nm)/(pico-eukaryote median pop FSC/median bead FSC))

* same equation for 530 nm

To assess temperature-induced stress within water masses, normalised fluorescence values for each sample were summed (yielding 530 + 580 fluorescence; Fig. 1A and C) and analysed using ANOVA. T0 values were subtracted from all subsequent time points in order to determine change from the initial condition. We note that PE-containing eukaryotes may change their orange fluorescence with temperature via phycoerythrin pigment content (Chaloub et al. 2015) or through potential changes in the association of phycobilisomes with the thylakoid membrane (Li et al. 2001). In this study, we define the pico-eukaryote population as relatively low PE and relatively high Chl-*a* (Fig. S1). As such the relative changes in PE quantified during our ship-board assays should be due to relative changes in ROS content, however care should be taken when applying this method to other studies.

Microbial diversity determination

To characterise the diversity of initial microbial communities used in experiments, sampled seawater (4 L) was filtered immediately (within 1 h of arriving on deck) through 0.22 µm Durapore filters (Merck Millipore, Bayswater, VIC, Australia). Filters were folded, placed in cryovials, snap frozen in liquid nitrogen, and stored at -80 ^{0}C (<3 months). DNA was extracted using the MoBio PowerWater DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) with the following modifications to the manufacturer's instructions. After the addition of PW1, filters were incubated for 10 min at 60 °C. Following Step 10, 650 µL phenol:chloroform:isoamyl alcohol (25:24:1, pH

8, Sigma-Aldrich, Castle Hill, NSW, Australia) was added to the sample, vortexed to mix, and centrifuged for 5 min at room temperature. The aqueous phase containing the sample was transferred to a sterile microcentrifuge tube, and the previous step was repeated using 650 µL chloroform:isoamyl alcohol (24:1, Sigma-Aldrich). The aqueous phase was again transferred to a fresh sterile microcentrifuge tube and the manufacturer's protocol was resumed from Step 15. DNA concentration and purity was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and DNA was stored at -20 ˚C. 16S rRNA amplicon sequencing was performed on the variable regions V1-V3 using the primer pair 27F (Lane 1991) and 519R (Turner et al. 1999) on an Illumina MiSeq (Illumina, San Diego, CA, USA; Molecular Research LP, Shallowater, TX, USA). 16S rRNA gene sequencing reads were analysed using the QIIME pipeline (Caporaso et al. 2010; Kuczynski et al. 2012). Briefly, paired-end DNA sequences were joined, *de novo* OTUs were defined at 97% sequence identity using UCLUST (Edgar 2010) and taxonomy was assigned against the SILVA database (version 128) using the BLAST algorithm. To estimate the diversity of microbial phototrophs enumerated in experiments, chloroplast OTUs were then filtered out to a separate file and taxonomy was assigned against PhytoREF (Decelle et al. 2015) in QIIME (Caporaso et al. 2010). Chimeric sequences were detected using usearch61 (Edgar 2010) and filtered from the dataset. Sequences were aligned, filtered and alpha diversity parameters were calculated in Primer v6.1 (Clarke & Gorley 2006). OTUs were subsequently grouped at the genus level, and the contribution of specific taxa to each water mass was calculated using the SIMPER routine (Primer v6.1; Clarke & Gorley 2006).

Assessment of relationship between temperature and population variables

Relationships between % cells remaining and temperature after 1, 5, and 25 h of exposure were analysed using Generalized Additive Models (GAMs). Specifically, independently at each time point, we allowed for a smoothed effect of temperature on % survival, varying around a parametric mean. Our initial k for determining the dimension of the smoothed effect was 4. Models were fit separately for the EAC and Tasman Sea, using the gam() function from the mgcv package in R (Wood 2006, 2011). An identical approach was used to analyse the relationship between ROS production and temperature over all three time points. Finally, to consider the relationship between % cells remaining and ROS production, we used a GAM with an additional random effect to capture variation among temperature treatments, employing the gamm() function. Prior to analysis, we averaged replicate survival and fluorescence values within time and temperature levels. We elected to treat temperature as a random effect rather than a fixed effect because: (i) we wished to avoid overfitting a relatively limited data set (n = 36), and (ii) we considered the explicit effects of temperature in the preceding analyses.

Trajectory analysis

A real-time ocean circulation model was used to determine the source of water sampled at both sites and estimate the thermal exposure of entrained microbes in the weeks before sampling. A total of 100 virtual particles were released at the surface at each of the two sites, and then tracked backwards in time with the Parcels tool (Lange and Van Sebille, 2017) by integrating the surface velocity fields of the HYCOM + NCODA Global 1/12° Analysis (Bleck, 2002). This HYCOM dataset assimilates observational data from satellites, Argo floats and other instruments, and is designed to be as similar to the real ocean flow as possible.

In order to establish the thermal history of the samples, the virtual particles were tracked backward in time for 85 days, storing positions and *in-situ* temperatures every day. Subgrid scale diffusion is represented by a Brownian random walk process, with a diffusivity constant of $K = 100$ m^2s^{-1}. In order to test the sensitivity of the tracking results to the date of sampling, a sensitivity analysis was performed where similar virtual particle experiments were done where the starting dates were moved up to four weeks earlier and later. These data are shown in Fig. S5. All code used in the particle tracking and creation of the plots can be downloaded from https://github.com/OceanParcels/Microbes_EAC.

Supplementary Tables

Table S1: Surface seawater properties at the time of sampling and descriptive thermal history parameters for water and resident organisms arriving to the sampling sites.

	EAC	Tasman Sea
Oceanographic conditions at sampling sites		
Latitude (˚S)	30.621	32.788
Longitude (˚E)	153.371	153.785
Bottom depth (m)	504	4798
Temperature ^{0}C	22.29	21.48
Salinity	35.65	35.68
Dissolved Oxygen (mmol L^{-1})	218.92	220.29
Mixed Layer Depth (m)	50	100
NO_3 (µmol L^{-1})	0.19	0.34
PO_4 (µmol L^{-1})	0.08	0.09
Eukaryote community attributes		
Picoeukaryote (cells ml^{-1})	4372 ± 200	5985 ± 1221
# OTUs (97%)	151	242
Shannon's Diversity	4.011	4.037
Pielou's Evenness	0.800	0.735
Margalef's Richness	24.192	40.197
Thermal History within real-time ocean (previous 85 days)		
Mean (± SD) temperature ^{0}C of trajectories (n = 100)	24.35 ± 1.26	22.68 ± 0.97

Table S2: Generalised Additive Mixed Model summary for analysis of % cells remaining in EAC. Overall, the model fitted 81% of the variance in cell survival across temperatures and time points. Intercept (1 h) is the average % cells remaining across temperatures at T1, one hour after the incubation started. Change by 5 h/25 h is how much the average % cells remaining has declined (relative to the value at 1 h) at T5/T25 (5 or 25 h after the incubation started). Smoothed effect of temperature is the deviation in % cells remaining across temperatures (relative to the mean % remaining at 1 hr) using a smooth function. A non-significant p value indicates that the trend with temperature is not significantly different from a flat line (with no slope). Therefore, at 1 h, temperature does not explain additional variation in % cells remaining. The estimated df values describe the shape of the relationship between %cells remaining and temperature– a value of 1 suggests that the relationship is linear; a value of 2 suggests that the relationship is quadratic. Significant p values indicate that the variation in % cells remaining (after accounting for the mean value) relates to temperature.

Adjusted R2	**Deviance explained**	**GCV**	**Scale estimate**	**n**
0.779	81%	168.23	142.39	53
Parametric terms	**Estimate**	**Std. Error**	**t-value**	**p-value**
Intercept (1 h)	86.47	2.82	30.7	< 0.001
Change by 5 h	-10.20	3.98	-2.56	0.0138
Change by 25 h	-48.92	4.04	-12.1	< 0.001
Smoothed effect of temperature	**Estimated df**	**Reference df**	**F-value**	**p-value**
At 1 h	2.28	2.63	0.834	0.332
At 5 h	1.00	1.00	9.524	0.003
At 25 h	1.86	2.23	5.970	0.004

Table S3: Generalised Additive Mixed Model summary for analysis of % cells remaining in Tasman Sea. Legend as for Table S2. The non-significant p value for change by 5 h indicates that the average % cells remaining in the Tasman Sea after 5 h is not different from the value at 1 h. However, at 5 h, there is now a significant relationship between % cells remaining and temperature.

Adjusted R2	**Deviance explained**	**GCV**	**Scale estimate**	**n**
0.71	75%	228.22	192.84	53
Parametric terms	**Estimate**	**Std. Error**	**t-value**	**p-value**
Intercept (1 h)	98.61	3.27	30.1	< 0.001
Change by 5 h	-3.38	4.63	-0.73	0.469
Change by 25 h	-36.00	4.71	-7.65	< 0.001
Smoothed effect of temperature	**Estimated df**	**Reference df**	**F-value**	**p-value**
At 1 h	1.05	1.11	0.372	0.601
At 5 h	1.76	2.11	11.55	< 0.001
At 25 h	2.40	2.74	15.43	< 0.001

Table S4: Generalised Additive Mixed Model summary for analysis of changes in ROS production: 530+580 fluorescence (RFU) in the EAC. Legend as for Table S2.

Adjusted R2	**Deviance explained**	**GCV**	**Scale estimate**	**n**
0.85	87.4%	8.21e-5	6.83e-5	53
Parametric terms	**Estimate**	**Std. Error**	**t-value**	**p-value**
Intercept (1 h)	0.076	0.0020	38.88	< 0.001
Change by 5 h	-0.031	0.0028	-11.26	< 0.001
Change by 25 h	-0.045	0.0028	-16.17	< 0.001
Smoothed effect of temperature	**Estimated df**	**Reference df**	**F-value**	**p-value**
At 1 h	1.70	2.05	1.17	0.304
At 5 h	2.13	2.49	5.01	0.006
At 25 h	2.00	2.37	2.95	0.046

Table S5: Generalised Additive Mixed Model summary for analysis of changes in ROS production: 530+580 fluorescence (RFU) in the Tasman Sea. Legend as for Table S2.

Adjusted R2	**Deviance explained**	**GCV**	**Scale estimate**	**n**
0.893	90.7%	2.10e-4	1.79e-4	53
Parametric terms	**Estimate**	**Std. Error**	**t-value**	**p-value**
Intercept (1 h)	0.111	0.0032	35.14	< 0.001
Change by 5 h	-0.066	0.0045	-14.78	< 0.001
Change by 25 h	-0.082	0.0045	-18.18	< 0.001
Smoothed effect of temperature	**Estimated df**	**Reference df**	**F-value**	**p-value**
At 1 h	2.34	2.68	19.855	< 0.001
At 5 h	1.46	1.76	0.292	0.681
At 25 h	1.00	1.00	0.083	0.775

Table S6: Generalised Additive Mixed Model summary for analysis of the relationship between % survival and ROS production in the EAC and Tasman Sea. Intercept represents the average % survival of pico-eukaryotes from the EAC population across time points and temperatures. The difference between the average % survival in the Tasman Sea and EAC is represented by (Tasman Sea – EAC). The smoothed effect of 530+580 fluorescence shows that % cells remaining declines with ROS fluorescence; in the EAC it declines approximately linearly, but in the Tasman Sea the relationship is more curvilinear. The random effect represents the portion of variation in % survival that is attributed to a random effect of temperature.

Adjusted R^2	**Scale estimate**	**n**		
0.358	328.08	36		
Parametric terms	**Estimate**	**Std. Error**	**t-value**	**p-value**
Intercept (EAC)	71.55	5.68	12.61	< 0.001
(Tasman Sea – EAC)	12.04	6.39	1.88	0.069
Smoothed effect of 530+580 fluorescence	**Estimated df**	**Reference df**	**F-value**	**p-value**
EAC	1.00	1.00	15.56	< 0.001
Tasman Sea	1.21	1.21	5.56	0.030
Random effect	**Std. deviation**			
Temperature	8.004			

Supplementary Figures

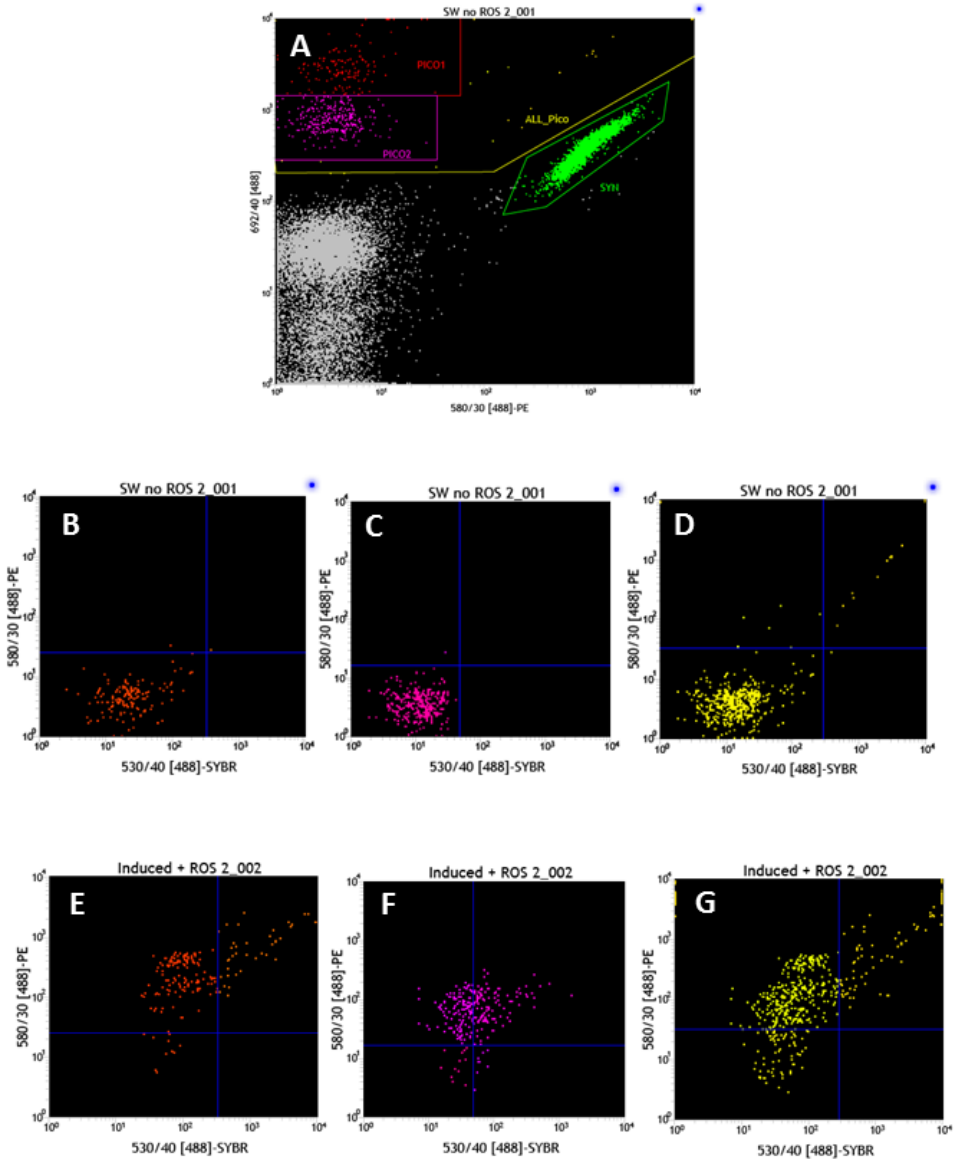


Fig S1. Gating logic for flow cytometric analysis of pico-eukaryote populations and ROS production. Pico-eukaryotes were discriminated from other phototrophs by their relatively low phycoerythrin and relatively high chlorophyll-*a* content (A). These target cells were divided into two populations (Pico1 and Pico2) based on chlorophyll-*a* fluorescence, but analyses presented in the text use the combined Pico1 and 2 population. Cells without any ROS stain (B-D) are shown, separated into Pico1 (B) Pico2 (C) and all Pico (D). Gates were set on these populations to account for any autofluorescence in these channels. Positive controls, where cells were induced to produce ROS are shown in the lower panel, including Pico1 (E), Pico2 (F), all Pico (G), with a positive shift in orange (580/30 nm) fluorescence indicative of superoxide, and a positive shift in green (530/30 nm) indicative of all ROS except superoxide.

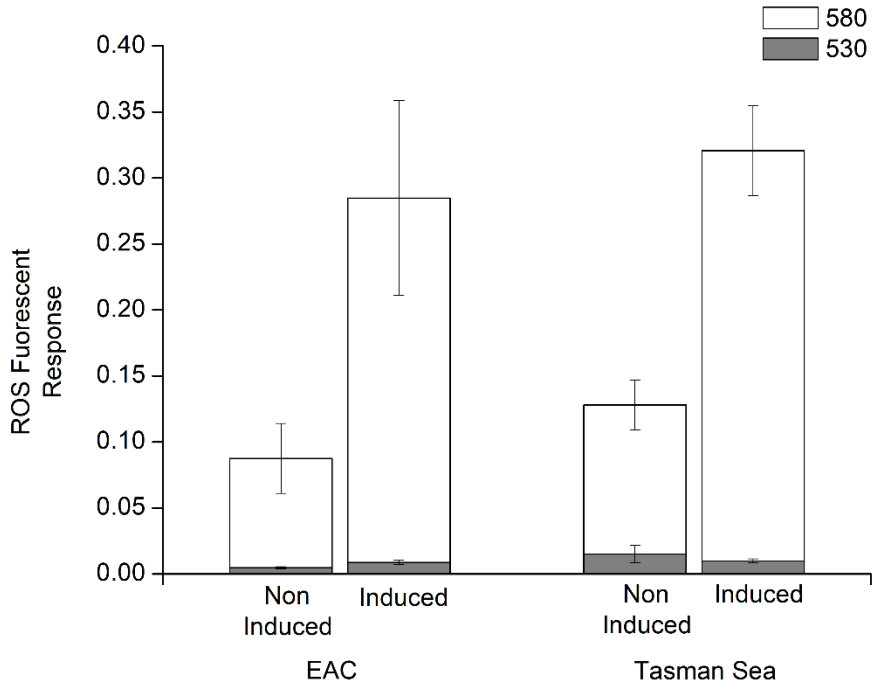


Fig S2. Experimental controls showing initial background ROS in sampled picoeukaryote populations versus induced ROS staining. The commercial kit contains an "induction solution" that causes cells to produce large amounts of ROS (positive control). The non-induced sample (negative control) indicates that there is some ROS already present in the population before they were used in experiments. This base level ROS was both expected and accounted for in our time-course analyses. Plot shows the median fluorescence (normalised to standard fluorescent microspheres) of EAC and Tasman Sea picoeukaryote populations; 580 nm (white) and 530 nm (grey).

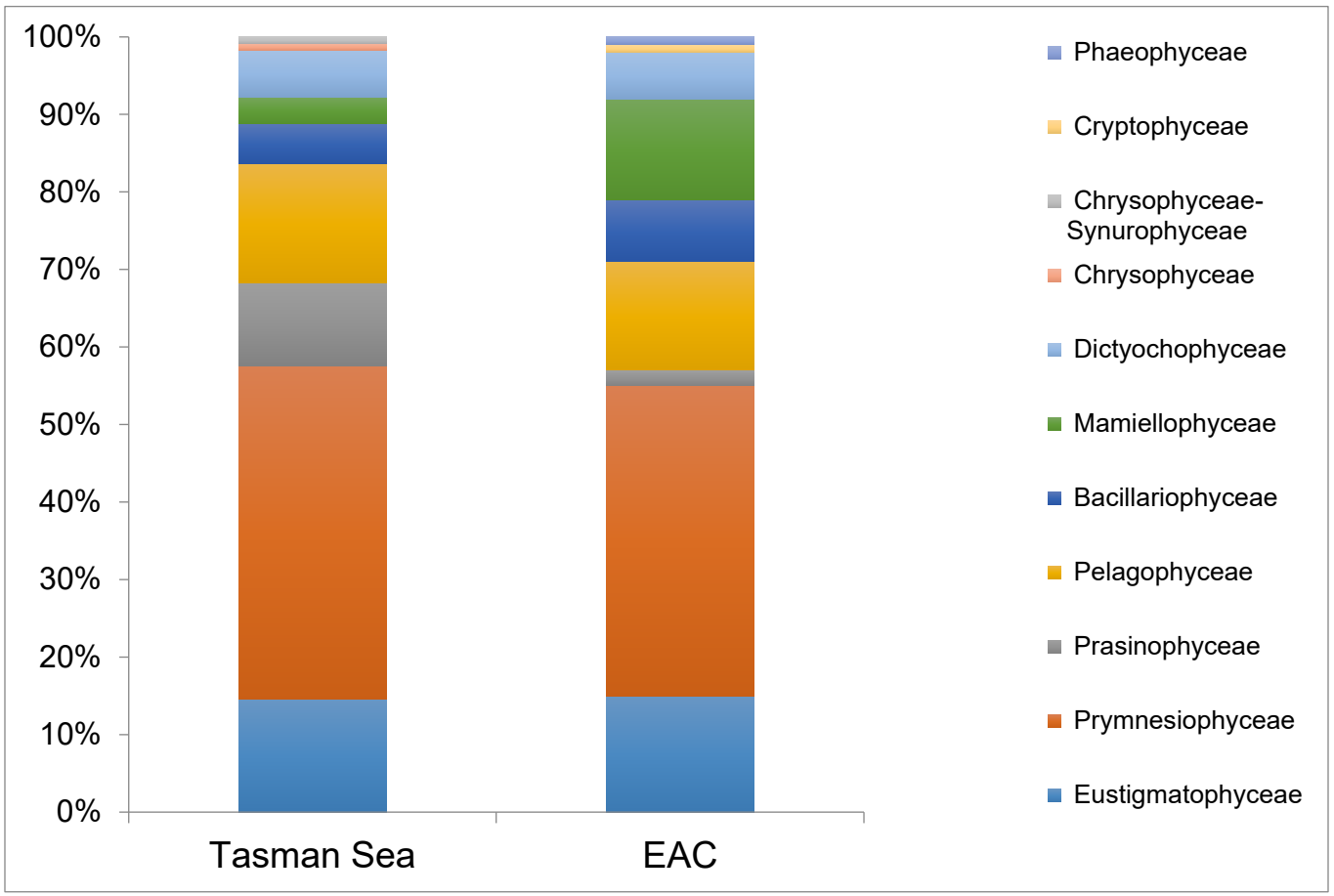
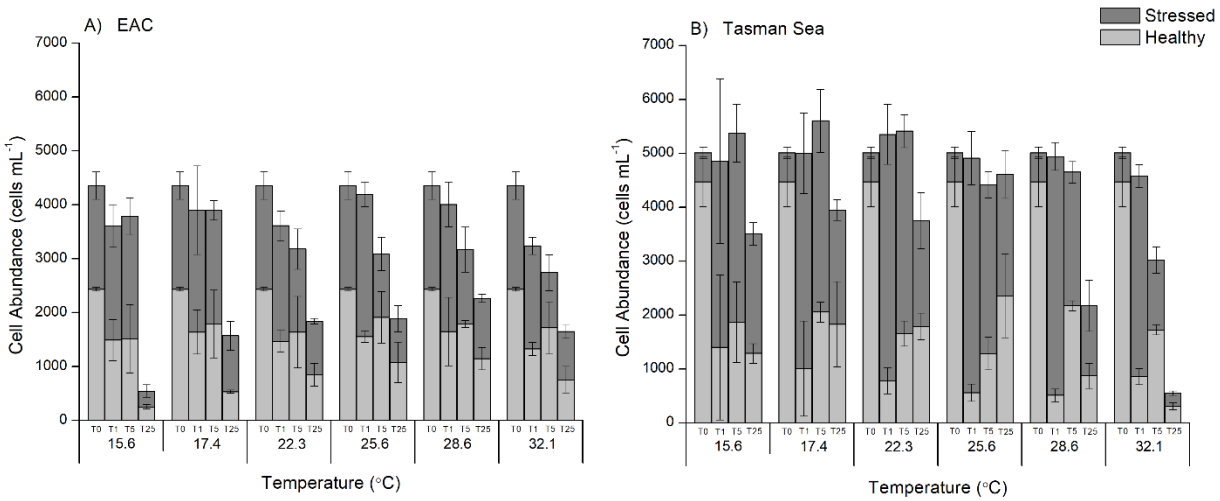


Fig S3. Diversity of phototrophic microbes in the EAC and Tasman Sea. Relative abundance of pico-eukaryote OTUs (97% nucleotide identity) based on the chloroplast 16S rRNA gene (Decelle et al. 2015). Dominant taxa are labelled at the Family level.



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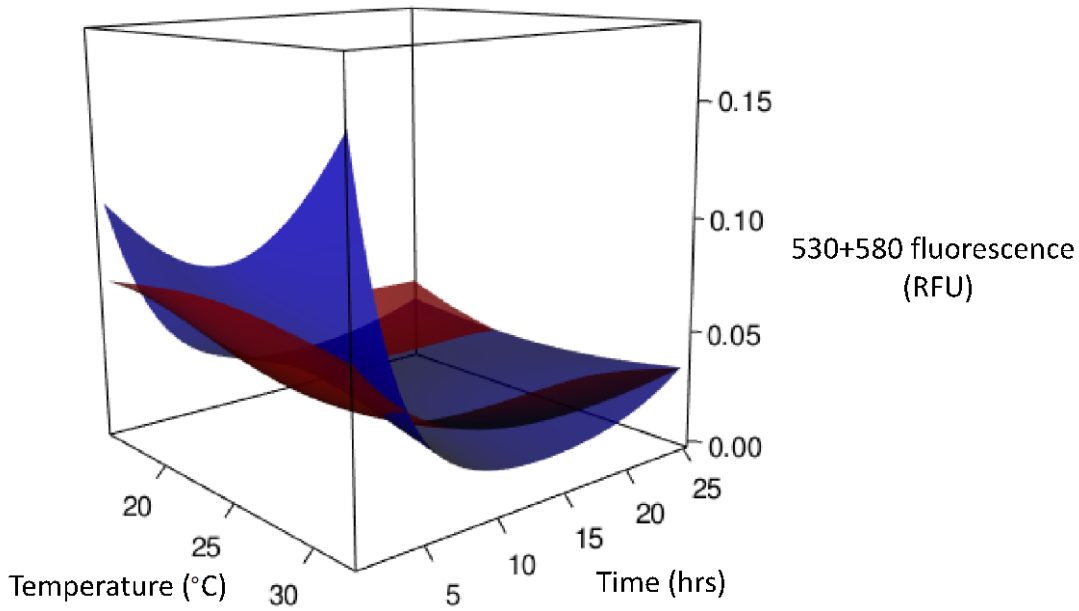

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262 **Fig. S4. Pico-eukaryote response to temperature excursion. A:** Change in the number of ROS
263 negative (healthy) and ROS positive cells (stressed) in the EAC (A) and Tasman Sea (B)
264 picoeukaryote populations over the 25 h assay at different temperatures. (C) Contour plot showing
265 the relationship between ROS expression (530 + 580 nm fluorescence) across temperature and
266 time in the EAC (red) and Tasman Sea (blue).

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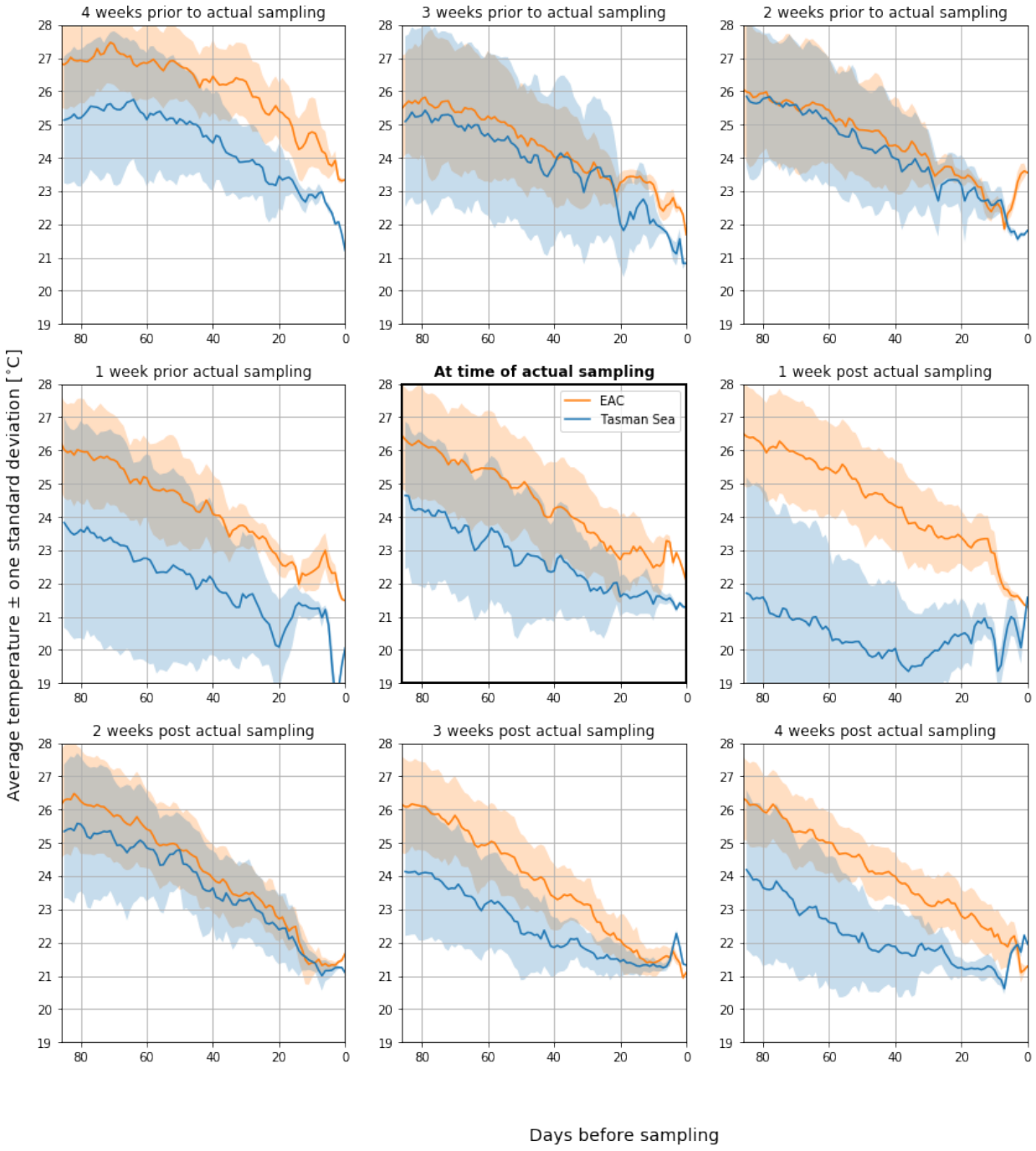

Fig. S5. Sensitivity analysis of estimated thermal exposure of microbes sampled in this study. Central plot shows the estimated thermal trajectories of microbes before they were sampled at EAC site (orange) on 2015-06-14 (YY-MM-DD) and Tasman Sea site (blue) on 2015-06-13. Previous and subsequent plots show estimated thermal trajectories from the same sites if they were sampled 1, 2, 3, or 4 weeks prior or post the actual sampling date.

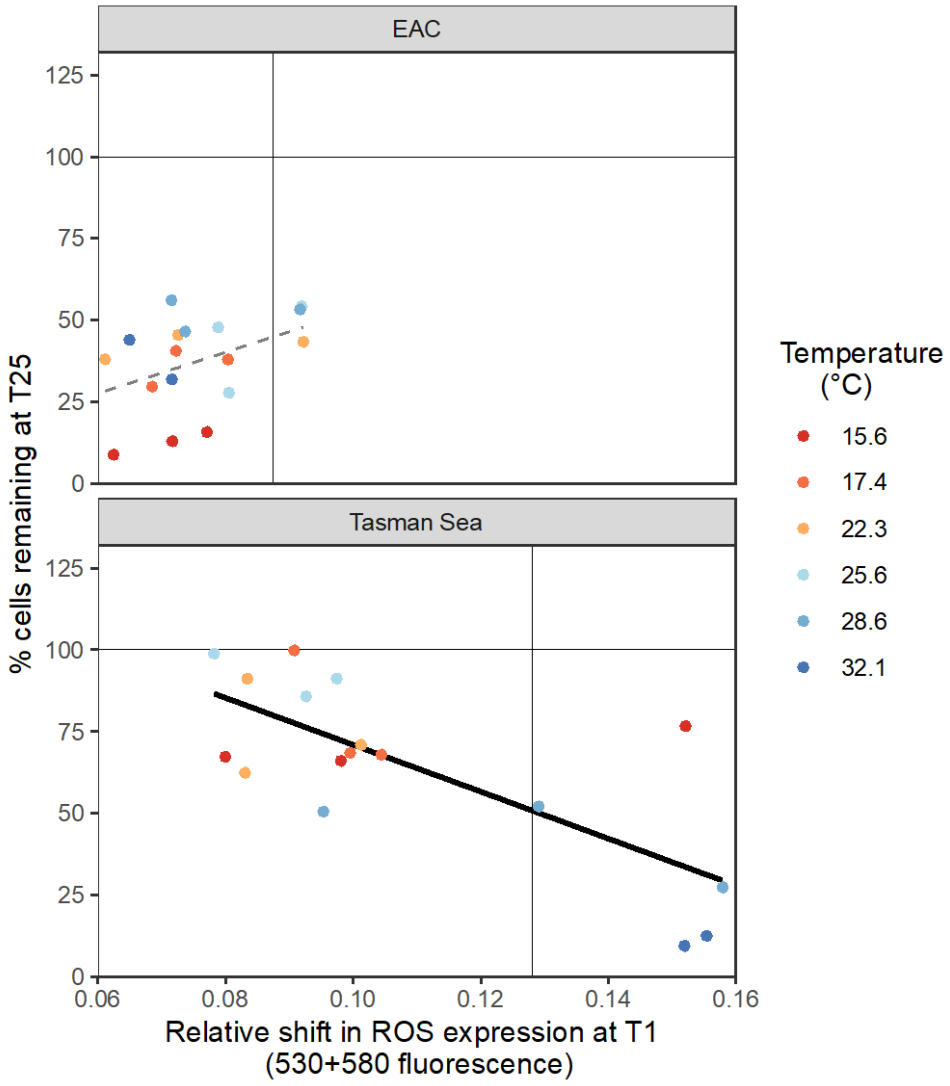

Fig. S6. ROS expression is an early indicator of pico-eukaryote mortality. A large increase in 530 + 580 nm fluorescence of pico-eukaryote cells at T1 relative to T0 is correlated with the lowest % cells remaining at 25 h. ROS production 1 h after exposure to new temperatures (T1) predicts longer-term population dynamics (% cells remaining at T25) in pico-eukaryote populations from the Tasman Sea (solid line, $p < 0.001$, Adjusted $R^2 = 0.528$) but not the EAC (dashed line, $p = 0.094$).