Title page

Live cell analysis at sea reveals divergent thermal performance between photosynthetic ocean microbial eukaryote populations

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Running title: Divergent thermal performance of pico-eukaryotes

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Abstract

Experimentation at sea provides insight into which traits of ocean microbes are linked to performance *in situ.* Here we show distinct patterns in thermal tolerance of microbial phototrophs from adjacent water masses sampled in the south-west Pacific Ocean determined using a fluorescent marker for reactive oxygen species (ROS). ROS content of pico-eukaryotes was assessed after 1, 5 and 25 h of incubation along a temperature gradient (15.6 to 32.1 °C). Pico-eukaryotes from the East Australian Current (EAC) had relatively constant ROS and showed greatest mortality after 25 h at 7 °C below ambient, whereas those from the Tasman Sea had elevated ROS in both warm and cool temperature extremes and greatest mortality at temperatures 6 to 10 °C above ambient, interpreted as the outcome of thermal stress. Tracking of water masses within an oceanographic circulation model showed populations had distinct thermal histories, with EAC pico-eukaryotes experiencing higher average temperatures for at least one week prior to sampling. While acclimatization and community assembly could both influence biological responses, this study clearly demonstrates that phenotypic divergence occurs along planktonic drift trajectories.

Text

Studies of marine microbial responses to changing ocean environments have largely focussed on biogeographic shifts in community composition (Dutkiewicz et al. 2013, Fuhrman *et al.* 2015, Barton et al. 2016), or on detailed, short (acclimation) and long (evolutionary) scale responses of single strains in laboratory manipulations (Listmann 2016, Schulte 2014). While insightful, these studies either omit examination of the physiological and ecological mechanisms that influence large-scale *in situ* population persistence, or remove microbes from their ecological context (e.g., investigate them in the absence of competitors or predators) in order to feasibly quantify detailed population-level responses. Observational, modelling, and experimental field studies that capture the legacy of past environmental exposure and the interactions between organisms (Doblin and van Sebille 2016) are therefore critical to understanding the processes regulating microbial population diversity and function in natural environments.

Here we use live cell probing and flow cytometry analyses at sea to examine the response of photosynthetic pico-eukaryotes within intact marine microbial communities (i.e., containing other phototrophs, bacteria, viruses, and grazers), to short-term temperature excursions. We used real-time underway sensing by means of thermosalinograph and acoustic doppler current profiler (ADCP) velocity to target microbial communities in different water masses. A rosette sampler (with attached Seabird SBE19 conductivity temperature and depth profiler) was used to capture surface seawater (6 m) from two sites, one in the EAC, a poleward-flowing western boundary current undergoing relatively rapid long-term warming compared to the global ocean (0.8-1.8 °C per century, 2-3 times the global average; Wu et al. 2012), and another in the adjacent Tasman Sea. Within 2 h of collection, microbial communities were incubated along a thermal gradient of six temperatures ranging from 15.6 to 32.1 °C,

representing a departure from their ambient temperature (~22 °C) of approximately -7 to +10 °C. Using a 'dynamic phenotyping' approach (Cruz et al. 2016; Ruderman 2017), we measured acute rather than acclimated thermal responses to gain insight about the potential for 'preconditioning' to high temperature in the pre-sampling period that would not necessarily be manifest under static conditions (Cruz et al. 2016). Such pre-exposure to high temperature can improve the thermal performance of photosynthetic eukaryotic microbes under heat stress (Middlebrook et al. 2008).

The potential for temperature-induced stress was measured over a 25 h period by quantifying changes in the abundance of reactive oxygen species (ROS) positive cells (Fig. S1), and the size of the photosynthetic pico-eukaryote population. In photosynthetic organisms, the chloroplast, the mitochondrial electron-transport chain, and the peroxisome are the main sources of ROS (Apel and Hirt 2004). Under physiological steady state, the ROS content of cells is controlled through enzymatic (e.g. glutathione peroxidase, catalase) and non-enzymatic scavenging processes (e.g. ascorbic acid; Gill and Tuteja 2010). At low levels, ROS play an important role in pro-survival mechanisms, acting as signalling molecules involved in regulating development and pathogen defence responses, or as secondary messengers that transmit initial stress signals, allowing cells to react and adapt to different environmental cues (Asada 2006, Mittler et al. 2011). However, under stressful conditions (e.g. temperature extremes, UV exposure, pollution), ROS quantities overwhelm the capacity of antioxidants, allowing ROS to accumulate and potentially cause irreversible damage to proteins, DNA, and lipids, triggering programmed cell death processes such as apoptosis (Perez-Perez *et al.*, 2012). Thus, in our experiments, we hypothesised that exposure of populations to temperatures above and below ambient would cause an increase in cellular ROS (i.e., production would exceed scavenging), with potential asymmetry between warming

and cooling because of the temperature dependence of enzymatic processes. Furthermore, the increase in ROS would potentially trigger a cascade leading to an increase in cell mortality – i.e., resulting in a decrease in the population relative to its initial abundance. The acute response to temperature excursions was captured by subsampling populations over a time-course (0, 1, 5, 25 h), staining them with a commercially available kit that uses two stains to specifically target ROS produced in eukaryotic cells (superoxide, and all other ROS except superoxide; Enzo Life Sciences, Inc., New York, USA), and quantifying their fluorescence intensity (relative to a standard microsphere; see Supplementary Information, Fig. S1) by immediately analysing them with a flow cytometer (Influx Mariner, BD Biosciences).

Initially, there was a relatively low proportion of cells in the Tasman Sea versus EAC pico-eukaryote population that contained ROS above background levels (10.9 ± 2.7 % compared to 44.0 ± 3.2 %, respectively; mean ± SD, n=3; Fig. S4), with both populations showing effective upregulation of ROS production (Fig. S2) in the induced positive control (92.1 ± 0.8 % and 84.4 ± 4.5 % in the Tasman Sea and EAC, respectively). During the experiment, ROS expression in the EAC population remained relatively constant under upwards or downwards temperature excursions at all time points (Fig. 1A), suggesting scavenging processes were effectively maintained. In the Tasman Sea population however, ROS fluorescence was immediately (1 h) higher at +10 °C above ambient (ANOVA, $F = 4.67$, $p = 0.013$), with a relatively high proportion of stressed cells during the first 5 h of the assay (Fig. S4B). Furthermore, in contrast to the EAC, the time course of ROS expression over 25 h showed a significantly different pattern amongst temperatures, with ROS fluorescence declining with time at high temperature (Fig 1A and C; ANOVA temperature x time interaction, $F = 4.16$, $p = 0.001$).

After 25 h, the size of pico-eukaryote populations had changed significantly across temperatures (Fig. 1B). The Tasman Sea population declined by 80-90 % at 32.1 °C but at other temperatures, the population was similar in size to the T0 samples (ANOVA, $F = 31.19$, $p < 0.001$). The EAC population however, experienced 40-50 % mortality across temperatures between 17.4 and 32.1 °C, with ~90 % loss of cells at the lowest temperature of 15.6 °C (Fig. 1B; ANOVA $F = 15.43$, $p < 0.001$). Overall, the Tasman Sea population appeared most sensitive to temperatures from +6 to +10 °C above ambient, with the EAC population most sensitive to the coldest temperature (-7 °C below ambient). There was a significant positive relationship between the amount of ROS fluorescence and the percentage of cells remaining in both the EAC and the Tasman Sea population (Fig. 1C; Generalised Additive Mixed Model, $p < 0.001$ and $p = 0.030$, respectively; Table S6).

It was apparent that temperature was not the only factor influencing cell survival during incubations—population dynamics within assays were likely mediated by the presence of other phototrophs, viruses, bacteria and grazers within experimental vessels. However, the dynamics of ROS within pico-eukaryotes was clearly influenced by temperature and revealed divergent physiology between water masses, suggesting that Tasman Sea cells experienced thermally-induced stress at the highest temperature which led to greatest mortality at 32.1 °C (Fig. 1C). In contrast, the EAC population appeared to maintain ROS scavenging processes across all temperatures (Fig. 1A).

We considered two possible explanations for the contrasting thermal responses of populations in the EAC and Tasman Sea: differences in the taxonomic composition of the phototrophic pico-eukaryote populations at the start of the incubation, and differences in their thermal acclimation status. Amplicon sequencing of the chloroplast 16S rRNA gene from replicate

160 samples at each site and subsequent bioinformatic analysis (PhytoREF, Decelle et al 2015)
161 revealed that the two water masses shared 55% of photosynthetic microbial genera (Fig. S3;
162 SIMPER, Primer v6; PRIMER-E, Plymouth, UK). At higher taxonomic resolution (OTUs
163 with 97% nucleotide identity) the two water masses shared 17% of taxa, with greater
164 diversity of OTUs in the Tasman Sea (Table S1). The taxonomic dissimilarity between the
165 two water masses was primarily due to differences in the abundances of two chlorophyte
166 OTUs: *Bathycoccus* (Mamiellales) and *Prasinococcales*, which comprised 15% and 1%
167 (respectively) of EAC photosynthetic eukaryotes, and 5% and 8% of those in the Tasman
168 Sea. Based on the known biogeography of *Bathycoccus* ecotypes, it is possible that the
169 divergent pico-eukaryote thermal responses in the EAC and Tasman Sea were influenced by
170 differences in the relative abundance of strains TOSAG39-1 and RCC1105, the latter of
171 which prefers warmer water (Vannier et al. 2016).
172
173 To assess the potential for differences in acclimatization between water masses, a high-
174 resolution ocean circulation model with particle tracking software was used to estimate the
175 thermal exposure of virtual microbes arriving to the sampling sites (Supplementary
176 Information). Although surface seawater temperature at the time of sampling was similar
177 (Table S1), microbial communities had distinct thermal histories, as shown by their different
178 transport trajectories (Fig. 2A). Organisms sampled in the EAC likely originated from
179 northerly locations and experienced higher average temperatures for several weeks (i.e., up to
180 approximately 20 generations) prior to sampling, as compared to the Tasman Sea population
181 (Fig. 2B). A sensitivity analysis was undertaken to assess how different the thermal history
182 would be if the populations were sampled up to 4 weeks prior/post the voyage (Fig. S5). The
183 analysis shows that for 6 out of 9 scenarios, temperature exposure one week prior to sampling
184 (equivalent to numerous pico-eukaryote generations) is consistently different between the

sampling locations, with the exception of 2-4 weeks after the voyage. This indicates that thermal exposure immediately prior to sampling is most likely to influence the physiology of picoeukaryotes observed in our study.

Although it is difficult to rank the importance of community assembly versus physiological acclimatization processes along drift trajectories, our data clearly demonstrate that advected populations diverge in their thermal performance, with pico-eukaryotes in a relatively warm western boundary current being less sensitive to high temperature induced stress compared to those in adjacent waters. Furthermore, we determined that differences in ROS expression after 1 h of warming are indicative of mortality 24 h later, with largest increases in cellular ROS fluorescence corresponding to greatest population decline (Fig. S6). Differences in ROS production amongst picoeukaryotes may have been due to a direct effect of heat on the photosystems (a source of ROS; Pospíšil 2009), or an indirect effect via intermolecular interactions (Feder and Hoffman 1999). For example, prior exposure to relatively high but non-lethal temperatures could induce greater non photochemical quenching (resulting in more effective heat dissipation), as has been demonstrated in symbiont zooxanthellae (Middlebrook et al. 2008), or it could cause upregulation of heat shock proteins and molecular chaperones (Henkel and Hoffman 2008) that moderate impacts on enzymes and other proteins involved in ROS scavenging.

This study highlights the physiological differences in adjacent microbial populations, and suggests that the ability to tolerate high temperature may be an important trait influencing fitness and the capacity for range expansion amongst natural populations. While the exact mechanism remains to be elucidated, our results have clear implications for predicting the impacts of marine heat waves and long-term warming on ocean microbes. Further field

experimentation will help bridge the gap between models and culture studies, revealing the limits of plasticity for microbes to dynamically respond to changing ocean conditions.

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Conflict of interest

The authors declare no conflict of interest.

References

Apel K, Hirt H (2004). Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction. *Ann Rev Plant Biol* **55**: 373-399.

Asada K (2006). Production and scavenging of Reactive Oxygen Species in chloroplasts and their functions. *Plant Physiol* **141**: 391-396.

Barton AD, Irwin AJ, Finkel ZV, Stock CA (2016). Anthropogenic climate change drives shift and shuffle in North Atlantic phytoplankton communities. *Proc Nat Acad Sci* **113**: 2964–2969.

Cruz JA, Savage LJ, Zegarac R, Hall CC, Satoh-Cruz M, Davis GA et al. (2016). Dynamic environmental photosynthetic imaging reveals emergent phenotypes. *Cell Systems* **2**: 365-377.

Decelle J, Romac S, Stern RF, Bendif EM, Zingone A, Audic S, et al. (2015). PhytoREF: a reference database of the plastidial 16S rRNA gene of photosynthetic eukaryotes with curated taxonomy. *Mol Ecol Res* **15**: 1435-1445.

Doblin MA, van Sebille E (2016). Drift in ocean currents impacts intergenerational microbial exposure to temperature. *Proc Nat Acad Sci* **113**: 5700-5705.

Dutkiewicz S, Scott J, Follows MJ (2013). Winners and losers: Ecological and biogeochemical changes in a warming ocean. *Glob Biogeochem Cycles* **27**: 463-477.

Feder ME, Hoffman GE (1999). Heat shock proteins, molecular chaperones, and the stress response. *Ann Rev Physiol* **61**: 243-282.

Fuhrman JA, Cram JA, Needham DM (2015). Marine microbial cymmunity dynamics and their ecological interpretation. *Nature Microbiol* **13**: 133-146.

Gill SS, Tuyeja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* **48**: 909-930.

Henkel SK, Hoffman GE (2008). Differing patterns of hsp70 gene expression in invasive and native kelp species: evidence for acclimation-induced variation. *J Appl Phycol* **20**: 915-924.

Listmann L, LeRoch M, Schluter L. Thomas M, Reusch TBH (2016). Swift thermal reaction norm evoluation in a key marine phytoplankton species. *Evol Appl* **9**: 1156-1164.

Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoele K et al. (2011). ROS signaling: the new wave? *Trends Plant Sci* **16**: 300-309.

Perez-Perez ME, Lemaire SD, Crespo JL (2012). Reactive Oxygen Species and Autophagy in Plants and Algae. *Plant Physiol* **160**: 156-164.

Pospíšil P (2009) Production of reactive oxygen species by photosystem II. *Biochimica et Biophysica Acta* **1787**: 1151-1160.

Ruderman D (2017). The emergence of dynamic phenotyping. *Cell Biol Toxicol* **33**: 507-509.

Schulte PM, Healy TM, Fangue NA (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr Comp Biol* **51**: 691-702.

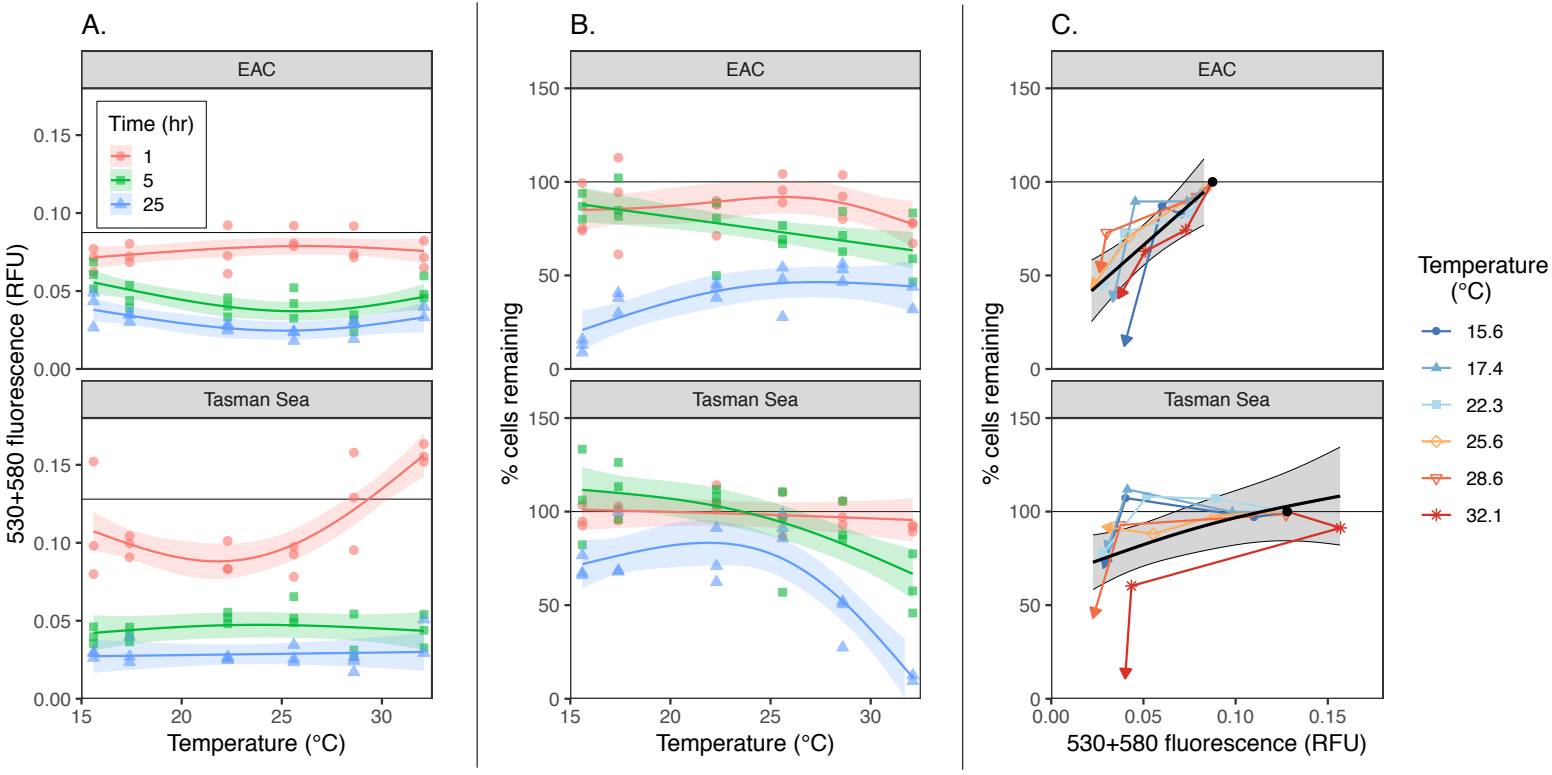
Vannier T, Leconte J, Seeleuthner Y, Mondy S, Pelletier E, Aury JM et al. (2016). Survey of the green picoalga *Bathycoccus* genomes in the global ocean. *Nature Sci Rep* **6**: 37900.

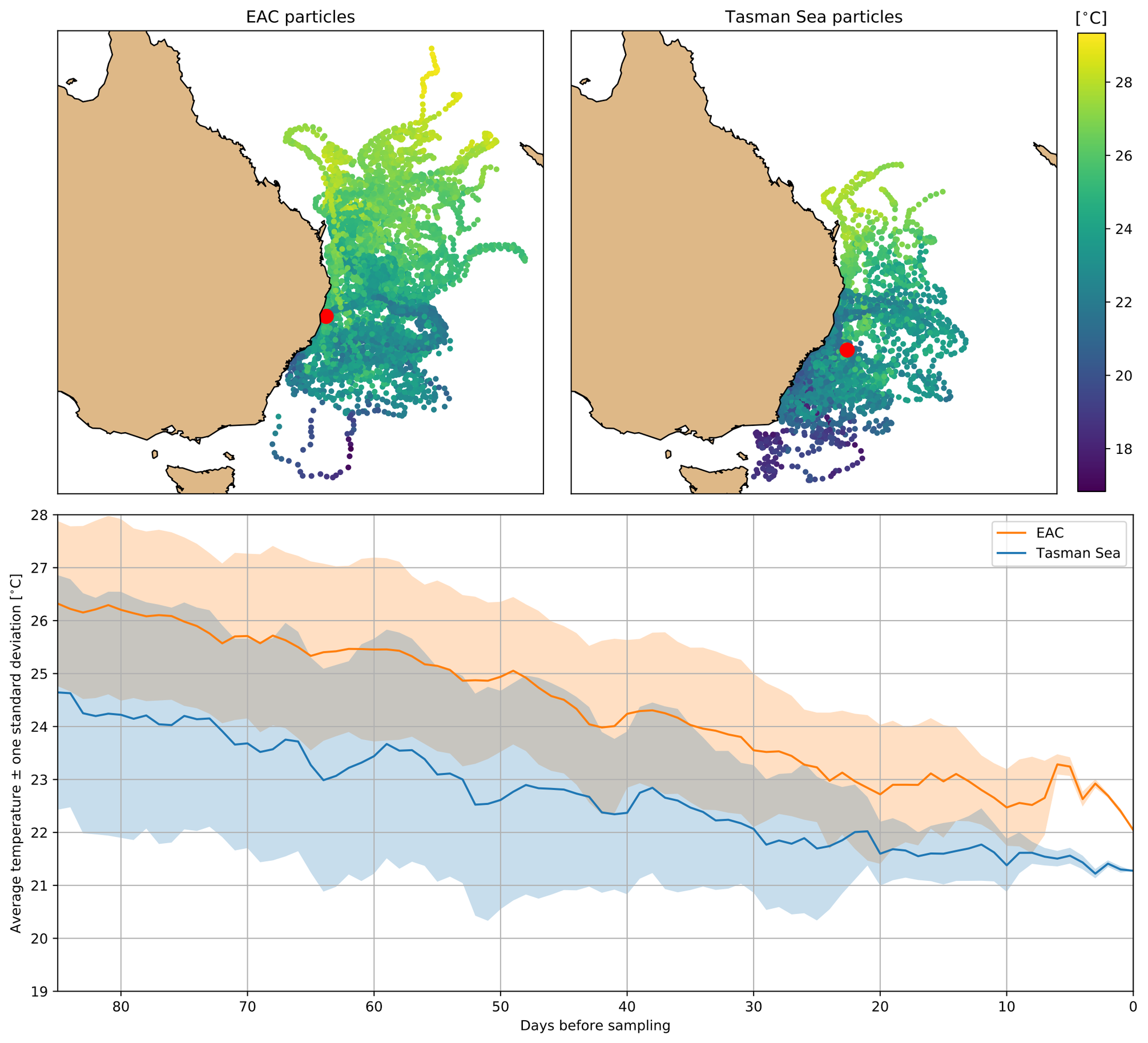
Wu L, Cai W, Zhang W, Nakamura H, Timmermann A, Joyce T et al. (2012). Enhanced warming over the global subtropical western boundary currents. *Nature Clim Change* **2**: 161-166.

Figure legends

Figure 1. Time-course (1, 5, 25 h) of the (A) bead-normalised Reactive Oxygen Species (ROS) fluorescence (530 + 580 nm relative fluorescence units, RFU) of cells in the EAC and Tasman Sea pico-eukaryote population at temperatures above and below ambient (~22 °C) (Tables S1 and S2; Fig. S1); (B) % cells remaining in the EAC and Tasman Sea pico-eukaryote population after 1, 5, and 25 h of incubation at different temperatures (Tables S3 and S4). (C) The relationship between % pico-eukaryotes remaining and bead-normalised ROS fluorescence at 0, 1, 5 and 25 h (arrows connect observations over time taken within the same water mass and temperature treatment, indicated by color/symbols with global average across all temperatures shown in black). Over time, in both water masses and across temperatures, the population size declined with decreasing bead-normalised fluorescence (Table S4 and S5); in the EAC it declines approximately linearly, but in the Tasman Sea the relationship is more curvilinear (Table S6).

Figure 2. Thermal exposure of virtual microbes arriving to the EAC (A) and Tasman Sea (B). A total of 100 virtual particles released at each oceanographic sampling site (red symbols) were backtracked for 85 days before the date of sampling (June 2015 austral winter), recording the temperature of their locations. Panel C shows mean temperature (solid line) ± one standard deviation (shaded area) experienced by particles from the EAC (orange) and Tasman Sea (blue).


A.
B.
C.
EAC
Tasman Sea
Time (hr)
1
5
25
530+580 fluorescence (RFU)
% cells remaining
Temperature (°C)
Temperature (°C)
15.6
17.4
22.3
25.6
28.6
32.1


EAC particles
Tasman Sea particles
[˚C]
28
26
24
22
20
18
EAC
Tasman Sea
Average temperature ± one standard deviation [˚C]
28
27
26
25
24
23
22
21
20
19
80
70
60
50
40
30
20
10
0
Days before sampling