1  Mass coral bleaching of *P. versipora* in Sydney Harbour driven by the 2015–

2  2016 heatwave

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**Abstract**

High-latitude coral communities are distinct from their tropical counterparts, and how they respond to recent heatwave events that have decimated tropical reefs remains unknown. In Australia, the 2016 El Niño resulted in the largest global mass coral bleaching event to date, reaching as far south as Sydney Harbour (~34°S). Coral bleaching was observed for the first time (affecting ca., 60% of all corals) as sea surface temperatures (SSTs) in Sydney Harbour remained >2°C above the long-term mean summer maxima, enabling us to examine whether high-latitude corals bleached in a manner described for tropical corals. Responses of the geographically cosmopolitan *Plesiastrea versipora* and southerly-restricted *Coscinaraea mcneilli* were contrasted across two harbour sites, both *in situ* and amongst samples-maintained *ex situ* in aquaria continually supplied with Sydney Harbour seawater. Whilst both coral taxa hosted the same species of microalgal endosymbiont (*Breviolum* spp; formerly clade B), only *P. versipora* bleached both *in situ* and *ex situ* via pronounced losses of endosymbiont cells. Both species displayed very different metabolic responses (growth, photosynthesis, respiration and calcification) and bleaching susceptibilities under elevated temperatures. Bacterial microbiome profiling however revealed a convergence of bacterial community composition across coral species throughout the bleaching. Corals species found in temperate regions, including the generalist *P. versipora*, will therefore likely be highly susceptible to future change as heat waves grow in frequency and severity unless their thermal thresholds increase. Our observations provide further evidence that high-latitude systems are susceptible to community reorganization under climate change.

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**Introduction**

Climate change induced warm-water thermal anomalies are increasing in frequency and

intensity globally (Heron et al. 2016; Hughes et al. 2018). Such "heat wave" events combined

with high solar radiation (Mumby et al. 2001) induce widespread coral bleaching by

disrupting the symbiosis between coral hosts and their micro-algal endosymbionts (e.g.

Warner et al. 2002; Suggett et al. 2011). Subsequent mass coral mortality can result following

bleaching that in turn drives extensive changes in the structure and function of coral reefs

(Pandolfi et al. 2011). The sustained 2015-17 El Niño-Southern Oscillation (ENSO) thermal

anomaly was the most severe to date and resulted in a major global mass coral bleaching

event (Hughes et al. 2018), with the Great Barrier Reef experiencing its worst ever coral

bleaching and mortality (Hughes et al. 2017). In an unprecedented outcome, we observed the

highest latitude coral bleaching event occurring in Sydney Harbour (33ºS), the first ever for

this region, as sea surface temperatures (SST) reached >2ºC above the long-term summer

maxima in February 2016 and remained above long-term average temperatures until July

2016 (U.S. National Oceanic and Atmospheric Administration (NOAA) Coral Reef Watch).

High-latitude corals persist in environments where SSTs, irradiance levels and/or aragonite

saturation states are seasonally lower and much more variable when compared to the tropics

(Kleypas et al. 1999; Sommer et al. 2018; Camp et al. 2018). Corals in these regions survive

under marginal environmental conditions with increased stress tolerance through phenotypic

plasticity and adaptations to colder waters (Beger et al. 2014; Camp et al. 2018, Tuckett et al.

2018). Even so, how such high-latitude coral communities respond to future trajectories of

ocean warming, accompanied with other stressors remains generally unknown (Camp et al.

2018). High-latitude coral populations from the Solitary Islands Marine Park and Lord

Howe Island Marine Park (such as *Isopora cuneata*, *Pocillopora damicornis* and

*Acroporidae*) have been suggested to exhibit lower absolute bleaching thresholds than their

72  tropical counterparts due to differences in thermal histories (Dalton and Carroll 2011).

73  Bleaching in response to thermal anomalies have been reported from high-latitude reefs in the

74  Southern Pacific and Indian Oceans; specifically, the Solitary Islands in 2005-2007 (Dalton

75  and Carroll 2011), Rottnest Island (32°S) in 2011 and 2015-2016 (Thomson et al. 2011; Le

76  Nohaïc et al. 2017), Houtman Abrolhos Islands (28- 29°S) in 2011 (Abdo et al. 2012; Bridge

77  et al. 2014), and Lord Howe Island (31°S) in 2011 (Harrison et al. 2011). Since 2016, coral

78  bleaching has been recorded at 14 high-latitude reefs in New South Wales and SE

79  Queensland (Hughes et al. 2018). However, whilst absolute thresholds for bleaching are

80  likely lower, the extent of anomalous temperature that they can tolerate remains untested.

81  Furthermore, the physiology and bacterial profiles underpinning high-latitude coral bleaching

82  and recovery is still to be described.

83  Sydney Harbour is situated within the Hawkesbury Shelf marine bioregion (33-35°S) where

84  temperate waters are inundated by warm tropical and subtropical waters supplied by the East

85  Australia Current (EAC) ca. 50% of the time (Pollard et al. 1997). Thus, this region alternates

86  between subtropical and temperate extremes (Breen et al. 2007) with large temperature

87  fluctuations ranging from 16°C to 24°C (see Figure 2). Sydney Harbour (33°S) scleractinian

88  coral communities are dominated by two species, the geographically cosmopolitan

89  *Plesiastrea versipora*, with one of the largest latitudinal distribution known for any Indo-

90  Pacific coral species (Veron et al. 2000), and the southerly-restricted temperate species,

91  *Coscinaraea mcneilli*. Both species predominantly grow as veneering coral colonies (Madsen

92  et al. 2014) and are thus non-reef forming on high-latitude reefs (Mizerek et al. 2016). Whilst

93  *Plesiastrea versipora* acclimates to increasing temperatures of up to 21°C with increasing

94  metabolic rates through enhanced autotrophic activity (Howe and Marshall 2001; Howe and

95  Marshall 2002) it remains unknown to what extant their physiology adjusts as colonies

96  experience a full seasonal range of temperatures. Similarly, how metabolic processes respond

once pushed past normal physiological thresholds (i.e. thermal stress) remains largely

unexplored. *Plesiastrea versipora* in Western Australia was recently shown to bleach during

a sustained cold spell where temperatures were >2 °C colder than the 10-year monthly

averages (Tuckett et al. 2017). Thus, a scleractinian coral species with a broad ecological

niche (Sommer et al. 2014) appears susceptible to acute temperature stress, but how

*Plesiastrea versipora* responds to anomalously high temperatures is undocumented.

Whether and how heat wave -induced bleaching processes for temperate corals (both

cosmopolitan and geographically restricted taxa) follows those commonly documented for

tropical corals is unexplored. Therefore, during the Sydney Harbour 2015-2016 El Niño

thermal anomaly, we examined two species, *P. versipora* and *C. mcneilli*, both *in situ* as well

as tank-maintained populations in aquaria supplied with flowing seawater from the Harbour.

We specifically quantified a number of key traits that have been shown to influence coral

bleaching susceptibility and recovery in tropical corals including the photosynthetic

physiology (Scheufen et al. 2017), microbiome composition (Röthig et al. 2017; Ainsworth et

al. 2015; Bourne et al. 2008) and host metabolic rates (Grottoli et al. 2006; Bessell-Browne et

al. 2014; Tremblay et al. 2016) before, during and after (recovery) heat stress from both coral

populations *in situ* and held in aquaria. Intriguingly, of the two species, only the geographical

generalist *P. versipora* exhibited widespread bleaching (but subsequent high recovery

whereas the southerly restricted species was unaffected during the heatwave event). We

discuss how these different susceptibilities reflect differences in their microbiomes and

physiologies.

**Materials and methods**

*Site Characterisation*

122    As part of a long-term assessment program, coral populations were periodically examined

123    from February to August 2016 at two sites within Sydney Harbour, Fairlight Beach

124    (33°48'3"S, 151°16'30"E) and Middle Head (33°49'29"S, 151°15'47"E) for physiological and

125    microbiological metrics. Benthic video transects at both Middle head (n = 4, length 50 m at

126    depths 2-4 and 4-6 m) and Fairlight (n = 4, 30 m at depths 3-5 and 5-7 m) were initially

127    conducted in February, prior to the peak in SSTs and on-set of observed bleaching. Benthic

128    transects followed the site substrate contours and video footage (GoPro Hero3) was taken

129    ~30 cm above the substrate. *P. versipora* forms high density patch communities generally

130    clustered within each site. Therefore, to ensure adequate habitat representation, transects

131    began at a *P. versipora* colony and continued to include surrounding habitat and capture

132    habitat variance. Video transects were visually analysed following a continuous line intercept

133    method (Gardner et al. 2019) to calculate percentage cover of coral. For each transect, the

134    total number of colonies were counted (bleached and healthy colonies). The total number of

135    colonies intercepted is expressed as the amount of coral intercepted relative to the total

136    distance. Repeat transects were performed in April during the bleaching event and again in

137    August during recovery. Coral bleaching is defined in this manuscript as atypical colouration

138    where 'bleached corals' include corals undergoing bleaching which are paler than those seen

139    during seasonal norms (see S1).

140    *In situ sample collections*

141    Coral fragments (n = 5) were sub-sampled from each site (~3-5m depth at Middle Head, 4-

142    6m at Fairlight) by SCUBA using a hammer and chisel (2 cm$^2$) (Figure 1). Healthy *P.*

143    *versipora* and *C. mcneilli* fragments were sampled in February 2016 (pre-bleaching) and June

144    (recovery), while bleached (*P. versipora* only) and unbleached colonies were sampled in

145    April (during bleaching). Fragments were removed from the edges of the colonies and were

146    held in falcon tubes filled with seawater. On the surface, water was removed from the tubes

147 and samples were snap frozen immediately in liquid nitrogen and stored at -80 degrees in the

148 laboratory until DNA extraction.  2 x 2 L of seawater was collected from around the coral for

149 16S rDNA analysis and kept on ice until processing.

150 *16S rDNA amplicon sequencing and analysis*

151 *DNA Extraction:* Tissue was removed from coral fragments (n = 4, bleached and unbleached

152 corals) using an air gun and 1x phosphate buffer saline (PBS) and further homogenised with a

153 sterile syringe. Samples were centrifuged for eight mins at 3000 RPM to pellet the coral

154 tissue and mucus. Pellets were resuspended in 2 mL of PBS and centrifuged as above and the

155 PBS supernatant was removed. A PowerPlant® Pro DNA isolation kit (cat # 13400) (Qiagen,

156 USA) was used following manufacturer's protocol. The optional phenolic separation solution

157 was also used to combat the high polyphenolic compound content of corals, as per the

158 manufacturer's protocol. Mechanical tissue lysis was performed using a Qiagen TissueLyser

159 LT (Hilden, Germany) at 50 Hz for 3 mins. Equal volume of phenol: chloroform: isoamyl

160 alcohol (pH 8) was added to the supernatant and then centrifuged for 13000 x g for five

161 minutes. The supernatant was then extracted with an equal volume of chloroform/isoamyl

162 alcohol and centrifuged again as above. These additional DNA extraction steps were found to

163 improve DNA yield and quality and were used on all samples. Genomic DNA concentrations

164 were assessed using the Qubit® High-sensitivity dsDNA assay kit (Life Technologies, NSW,

165 Australia). DNA template was screened for PCR efficiency using the barcoded primer pair

166 27F and 519R (Tout et al. 2015; Prazeres et al. 2017).

167 *Bioinformatics & data analysis:* Bacterial 16S extracted from the coral holobiont was

168 sequenced using the Illumina MiSeq v3 platform (Ramaciotti Centre for Genomics). The

169 universal Eubacterial primers 27 F (5′ -AGAGTTTGATCMTGGCTCAG) and 519R

170 (5′GWATTACCG CGGCKGCTG)) were used for PCR amplification, targeting the highly

171 variable V1-V3 regions of the 16S rRNA. Reads were processed as outlined in (Kahlke et al.

172   2018) (https://github.com/timkahlke/ampli-tool). Briefly, sequences were joined using

173   FLASh (Magoč et al. 2011) and subsequently filtered and trimmed using mothur

174   (PARAMETERS: maxhomop = 6, maxambig = 0, minlength = 469, maxlength = 503).

175   Fragments were clustered into operational taxonomic units (OTUs) at a 97% identity

176   threshold and chimeric sequences were removed using vsearch (Rognes et al. 2016) and the

177   Silva v128 database. Taxonomies were assigned to the OTUs using QIIME (Caporaso et al

178   2010) and the BLAST algorithm against the Silva v128 database. Negative kit controls and

179   seawater controls were used to identify any laboratory or seawater contamination and these

180   OTUs were removed from the analysis. Coral host mitochondrial sequences and 16S

181   sequences identified as mitochondria or chloroplasts were also removed from the analysis.

182   Data were rarefied to 3000 reads per sample, representing the lowest number of reads among

183   all samples.

184   PRIMER+PERMANOVA (version 6.1; UK) was used to statistically analyse the data.

185   Relative abundances were square root transformed and clustered with a Bray Curtis

186   resemblance matrix. Permutational multivariate analysis of variance (PERMANOVA) with

187   pairwise comparisons identified the effect time and species have on microbiome composition.

188   Similarity percentages analysis (SIMPER) uncovered dissimilarities based on OTUs over

189   time and between species.

190   The core microbiome was defined as OTUs that were found in a minimum of 3 out of 4

191   replicates with a minimum relative abundance of 0.001%, identified using QIIME. Venn

192   diagrams depicting the core microbiome were constructed with the online software from

193   Bioinformatics and Evolutionary Genomics

194   (http://bioinformatics.psb.ugent.be/software/details/Venn-Diagrams). To determine the

195   differential abundance of OTUs between the two coral species the Statistical Analysis of

196   Metagenomic Profiles (STAMP) software package was used for univariate analysis (Parks

197    and Beiko et al. 2010). P-values were calculated using the Kruskal-Wallis test with Storey's

198    False Discovery Rate (FDR) multiple test correction method and a $p$-value $< 0.05$. The P-

199    value and FDR values are output of these tests was visualised as extended error bar plots (CI

200    = DP: Bootstrap 95%) which display the relative abundance of each bacterial taxa, specified

201    for each sample grouping as bars, with the difference in proportions with 95% confidence

202    interval error bars displayed for each bacterial taxon.

203    *Genetic identifications of the resident endosymbiont*

204    Colonies of *P. versipora* from Fairlight (n = 15) and Middle Head (n = 15and colonies of *C.*

205    *mcneilli* from Fairlight (n = 5) were sampled in June 2015 and samples processed for DNA

206    analyses to determine the identity of the micro-algal endosymbiont common to each species.

207    Whole coral tissue DNA extractions were performed as described by (LaJeunesse et al.

208    2003), consisting of a 2 min bead-beating step (0.4-0.6 mm glass beads) and a modified and

209    abreviated DNA wizard extraction protocol (Promega). Nuclear large-subunit ribosomal

210    DNA (LSU) and chloroplast large-subunit rDNA (cp23S) were amplified and sequenced

211    (LaJeunesse et al. 2012). Conditions for amplifying the LSU are provided in (Zardoya et al.

212    1995); and conditions for amplifying cp23S are provided by (Zhang et al. 2005).

213    To amplify DNA, reactions were performed in 25 µL volumes containing 2.5 µL of 2.5 mM

214    dNTPs, 2.5 µL of 25 mM $MgCl_2$, 2.5 µL standard *Taq* Buffer (New England Biolabs,

215    Ipswich, MA, USA), 0.13 µL of 5 U • µL$^{-1}$ *Taq* DNA Polymerase (New England Biolabs), 1

216    µL of each forward and reverse primer at 10 µM, and 1 µL of 5–100 ng DNA template.

217    Products were cleaned with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and directly

218    sequenced on an Applied Biosciences sequencer (Applied Biosciences, Foster City, CA,

219    USA) at the Pennsylvania State University Genomics Core Facility. Sequence

220    electropherograms were each examined manually and nucleotide sequences aligned by eye.

221    Raw sequences and alignments for each gene are available from the Dryad Digital

222  Repository. Phylogenetic analyses using maximum parsimony were then performed on

223  aligned data sets in PAUP* v.4.0d151 (Swofford 2014) with indels in rDNA included as a 5th

224  character state. Bootstrap support was calculated based upon 1000 replicates.


225

226  *Coral metabolism*

227  Colonies of *P. versipora* and *C. mcneilli* were collected from Fairlight Beach (4-6 m depth)

228  in September 2015 and allowed to acclimate for six weeks prior to long-term experimentation

229  in aquaria at Manly SeaLife Sanctuary. Three replicate colonies of each species were

230  fragmented to yield a total of n = 20 fragments per species, which were then distributed

231  randomly across aquaria. The experimental setup consisted of four tanks on a continuous

232  flow through system supplied by fresh Sydney Harbour water (passing through two rapid

233  sand filters and a sedimentation tank). Flow rate was maintained at ~2.5 L min$^{-1}$ and light

234  intensity ($\mu$mol photons m$^{-2}$ s$^{-1}$), measured with a Li-250A with $4\pi$ sensor ($\mu$mol Li-Cor,

235  Lincoln, Nebraska, USA), was changed seasonally as determined by modelling the

236  underwater light field adjusted for 7 m depth based on the diffuse attenuation coefficient (as

237  per Hennige et al. 2010).

238  Rates of net photosynthesis ($P_G$), dark respiration (R) and light calcification (G) were

239  obtained by respirometry for five fragments of *P. versipora* and *C. mcneilli* at each timepoint

240  as per (Camp et al. 2015). Timepoints $t_0$, $t_1$ and $t_2$ represent the pre-bleaching months

241  December, January and February respectively, $t_3$ is during bleaching in April and $t_4$ is

242  recovery in August. Importantly, both tank and *in situ* corals had bleached by April, however

243  metabolism measurements of bleached corals in aquaria were not made until June. Thus, the

244  rates measured and reported here reflect metabolic responses during a long term (> 2 months)

245  bleaching event. Each colony was individually incubated for three hours in a 500ml gas tight

246  chamber submerged in a flowthrough experimental tank acting as a water bath and stirred

247   manually every five minutes (as well as two control chambers containing only seawater to

248   correct for any biological activity of the seawater). Rates of $P_N$ and R ($\mu mol\ O_2\ cm^{-2}\ h^{-1}$)

249   were quantified for each fragment simultaneously at the start and end of each incubation

250   using a FireSting optical oxygen meter with a needle-type microsensor (Pyroscience,

251   Germany). Dark incubations were conducted 30 minutes after the light incubations by

252   covering the experimental tanks with blackout material. $P_G$ was calculated as $P_N$ + R (Camp

253   et al. 2017) . Calcification rates ($\mu mol\ CaCO_3\ cm^{-2}\ h^{-1}$), determined as changes in TA, and

254   $O_2$ for each chamber during each 3 h incubation was corrected for any changes in TA or $O_2$

255   from the seawater controls ($n = 3$) (as per Camp et al. 2017 ). Surface area was determined

256   for each fragment at the end of the experiment using the advanced geometric technique

257   (Naumann et al. 2013) and were used to normalise metabolic rates for each fragment.

258   Following a normality check using the Shapiro test, One-way ANOVAs and Tukey's post

259   hoc tests were performed for *C. mcneilli*, on each metabolic variable ($P_G$, R and G), to test for

260   differences between time points for each species. Data for *P. versipora* was not normally

261   distributed therefore non-parametric Kruskal-Wallis tests with pairwise comparisons were

262   used.

263   Abiotic measurements were made in triplicate for each experimental tank bi-monthly (WTW

264   Multiprobe 3630 and sensors, calibrated for each sensor before each use). Total alkalinity

265   was measured using an auto-titrator (Metrohm 916 Ti-Touch Autotitrator) with accuracy and

266   precision of less than or equal to 2 $\mu mol\ kg^{-1}$ as verified with Dickson standards and pH

267   (total) was measured with a Metrohm iUnitrode electrode and calibrated with tris buffers

268   (precision *ca*. $\pm$ 0.0005 pH units). Aragonite saturation state and dissolved inorganic carbon

269   were calculated using $CO_2SYS$ software (Lewis et al. 1998). Temperature and light of each

270   experimental tank was also continuously measured using HOBO pendent loggers (Microdaq,

271  USA) set at a 10-minute interval, calibrated before each use and values given in LUX were

272  converted to PAR using the daylight coefficient (Camp et al. 2015).

273

274  *Common measurements in situ and aquaria*

275  A number of common metrics were used to ensure data sets between *in situ* and aquaria

276  experiments were comparable.

277  *Environmental variables*: Water temperature data was recorded semi-continuously at both

278  sites from July 2015- August 2016 using temperature/light loggers (HOBO Pendant,

279  Microdaq, USA) set to measure at 30-minute intervals and are reported here as monthly

280  averages. Monthly composite SST data were obtained from the MODIS (MODerate

281  Resolution Imaging Spectroradiometer) platform using the Giovanni online data system and

282  used to calculate temperature anomalies from 2010-2017 and average SST from 2015-2017

283  (Figure 2). Degree Heating Weeks (DHW) were obtained from the National Environmental

284  Satellite Data and Information Service (NESDIS) of the U.S. National Oceanic and

285  Atmospheric Administration (NOAA) (50km). Water temperatures were strongly correlated

286  between the experimental system and *in situ* temperatures as determined with a Pearson

287  correlation coefficient analysis ($r^2 = 0.924$, not shown*).* All other environmental parameters

288  were measured bi-monthly at both sites as explained above. Triplicate water samples were

289  taken at each site using a nutrient analyser (Quikchem QC8500 Automated Ion Analyser)

290  following manufactures protocol and quality control procedures (LACHAT Instruments,

291  USA). Data were interpreted using Omnion version 3 software (LACHAT Instruments,

292  USA).

293  *Symbiodinium Cell density:* Tissue (~1 cm$^2$) was removed from fragments (additional

294  fragments to those used for physiology measurements) using a waterpik and GF/F- filtered

295    seawater. The resulting tissue slurry was homogenised, and an aliquot taken for cell

296    quantification using a haemocytometer (Neubauer Haemocytometer, Fisher Scientific,

297    Loughborough, UK) with eight replicate quadrats counted for each sample.

298    *Photophysiology:* A diving-PAM underwater fluorometer (Waltz, Germany) was used to

299    obtain maximal quantum yields ($F_v/F_m$) of chlorophyll *a* fluorescence of colonies *in situ*

300    (measurements taken early morning prior to direct sunlight exposure) and *ex situ* following

301    20 minutes of low light acclimation (settings: measuring light intensity = 8, saturation pulse

302    intensity = 11, saturating width = 0.8, damp = 2, gain = 4) (e.g. Suggett et al. 2012).

303    Triplicate measurements were made for each colony with a total of eight colonies measured

304    at each timepoint. All measurements were made at a constant distance from the coral (3mm),

305    standardised using a fibre optic adaptor and taken from the middle of the colony.

306

307    **Results**

308    *Environmental conditions*

309    Satellite-observed SST from February-August 2016 consistently exceeded long-term (10

310    year) monthly averages, peaking at 3.7°C above the average in February, resulting in DHWs

311    of 5.4°C and bleaching alert level 1 (bleaching likely) (NOAA Coral Reef Watch. 2000,

312    updated twice-weekly). In March, DHWs reached 12.8°C and bleaching Alert level 2. SSTs

313    continued to exceed monthly averages until August 2016, resulting in a Bleaching Watch

314    status over this time period. Sydney is subjected to warm and cold thermal anomalies yearly.

315    However, warm SST anomalies were greater in 2015 and 2016 than the previous six years

316    coinciding with the study period of interest (Figure 2a). As expected, most physico-chemical

317    conditions within the aquaria closely matched the *in-situ* conditions (Table S1) since the

318    aquaria were supplied by continuously flowing seawater from Sydney Harbour. Notably

319    temperature ranged from 17.2 – 25.9°C *in situ* and 17.8 – 25.9°C in aquaria and pH ranged

320    from 7.96 - 8.11 *in situ* and 7.76 - 7.97 in aquaria.  However, phosphate levels were

321    consistently higher in the tank experiment (by a factor of ca. 2.4 – 14.8).

322

323    *Bleaching and endosymbiont dynamics*

324    *Plesiastrea versipora* account for $40.0 \pm 17.0\%$ (range 11.0-88.0%) and $33.0 \pm 15.0\%$ (range

325    0-62.0%) of total benthic cover at sites targeted within Fairlight and Middle Head

326    respectively. In April, up to $60 \pm 8.1\%$ of colonies displayed signs of bleaching undergoing

327    bleaching orcompletely white) across the two harbour sites (Figure 3). By July, corals had

328    begun to regain normal colour (Figure 3) with bleached corals making up only $25 \pm 1.2\%$ of

329    colonies. Healthy coral cover increased from $40.4 \pm 8.1$ % of colonies during April to $77.4 \pm$

330    1.4% in August.

331    Changes in maximum quantum yield of PSII $(F_v/F_m)$ values are commonly used to describe

332    coral responses to heat stress (e.g. Suggett et al. 2011), and values for both the aquarium

333    housed and *in situ* colonies were equal at the beginning of the experiment ($0.65 \pm 0.02$, mean

334    $\pm$ SE, and $0.63 \pm 0.02$ respectively, Figure 3). Healthy colonies of *P. versipora in situ*

335    maintained $F_v/F_m$ values greater than 0.6 for the duration of the study. In April, mean values

336    for $F_v/F_m$ for *in situ* and tank housed fragments were reduced to $0.24 \pm 0.03$ and $0.35 \pm 0.03$,

337    respectively. These values remained significantly lower than the $F_v/F_m$ of healthy colonies

338    until the end of the experiment. $F_v/F_m$ values returned to within pre-bleaching values for *in*

339    *situ* corals, whereas tank corals only increased to $0.45 \pm 0.04$, with a slower overall recovery.

340    Changes of *in situ Symbiodinium* cell densities followed the loss and recovery trends in $F_v/F_m$

341    during heat stress. *Symbiodinium* cell densities were significantly reduced for bleached

342    colonies of *P. versipora* in April (during bleaching) (Figure 3) ($3.55 \times 10^6 \pm 7.83 \times 10^4$ to

343    $3.77 \times 10^5 \pm 1.49 \times 10^4$ cells/cm$^{-2}$) for bleached colonies before returning to within pre-

344    bleaching values by June.

345    In contrast to *P. versipora*, *C. mcneilli*, accounting for <10 % of total benthic cover at sites

346    targeted within Fairlight did not show any visual or physiological signs of bleaching in either

347    tank experiment or *in situ*, where values of $F_v/F_m$ (ca. 0.65-0.76)) and symbiont cell densities

348    ($3.6 \times 10^6$ - $3.85 \times 10^6$) remained generally constant throughout (Figure 3).

349    *Identity of endosymbiotic micro-algae*

350    Sampling from a variety of colonies across all sites revealed that *P. versipora* and *C. mcneilli*

351    contained a *Symbiodinium* sp. whose LSU rDNA were identical, indicating the presence of a

352    single undescribed species of *Breviolum* (= formerly *Symbiodinium* clade B), provisionally

353    referred to as type B18a (Goyen 2018, unpublished). However, phylogenetic analyses of the

354    cp23S marker exhibited a fixed difference that distinguished symbionts in *P. versipora* from

355    *C. mcneilli*. In addition, the identity of *Symbiodinium* was determined for *P. versipora*

356    colonies pre-bleaching and during bleaching (Fujise 2018, unpublished), showing no

357    difference in the composition of *Symbiodinium* clades using DNA metabarcoding based on

358    the cp23S primer set between the two health states.

359    *Holobiont bacterial communities*

360    Potential shifts in the holobiont microbiomes of *P. versipora* and *C. mcneilli* were determined

361    using 16S rRNA gene sequencing. After quality control, a total of 5,414,105 reads were

362    matched to reference sequences within the Silva 128 database with an average of 30 000

363    reads for each sample (non-rarefied). Seawater controls samples contained significantly

364    distinct microbial communities compared to coral samples across the study period

365    (PERMANOVA; $p <0.05$, t = 1.65) indicating low-levels of sample contamination with non-

366    holobiont DNA. At the class taxonomic level, *Gammaproteobacteria* and *Actinobacteria*

367    dominate the microbiomes of both *P. versipora* and *C. mcneilli* (Figure S4, S5).

368    In February ($t_2$), prior to bleaching, *P. versipora* and *C. mcneilli* had significantly distinct

369    microbiomes (Table 1) driven primarily (as shown with SIMPER and supported by STAMP

370    analysis, Figure S6, S7) Flavobacteriaceae (2.67%, STAMP 13.33%, p = 0.004)

371    *Oceanospirralles* (3.59%, STAMP 5.8% p = 0.003) and *Photobacterium* (2.68%, STAMP

372    4.68% p = 0.002) associated with *C. mcneilli*, and Xanthomonadales (2.41%, STAMP 5.73%

373    p < 0.001) and *Geothermobacter* (2%, STAMP 4.69%, p = 0.0003) associated with *P.*

374    *versipora*. Core taxa that distinguished the host microbiomes were unclassified (*UC*)

375    *Flavobacteriaceae* and *UC Oceanospirillales* for *C. mcneilli* and UC Xanthomonadales, and

376    *Geothermobacter* for *P. versipora* (Figure 4). The core microbiome of *C. mcneilli* was richer

377    than that for *P. versipora* (5 core OTUs in contrast to 2 core OTUs respectively), yet the

378    Shannon index indicates similar overall diversity (3.8 for *P. versipora* and 4.0 for *C.*

379    *mcneilli). Escherichia/Shigella* were core taxa common to both hosts [10% ± 4% SE (here on

380    in denoted as % ± SE) and 5% ± 4% for *P. versipora* and *C. mcneilli* respectively, Figure 4].

381    *Pseudomonas* was also a core taxon for both coral species but consistently represented in

382    abundances <1% for the duration of the experiment. Chlorobiaceae was a dominant taxon

383    present across both species at this time (Table 1).

384    In April ($t_3$) during the bleaching event, despite certain colonies of *P. versipora* showing

385    visual and photophysiological signs of stress, overall microbial composition did not differ

386    significantly between bleached and healthy *P. versipora* colonies or across species (Table 1).

387    *Escherichia/Shigella* increased in abundance from 10% ± 4% to 16% ± 7% for *P. versipora*

388    and 5% ± 4% to 26% ± 3% for *C. mcneilli* and remained as shared core bacterial taxa from

389    February. This taxon was also in extremely high abundance in bleached colonies of *P.*

390    *versipora* (14% ± 6%) together explaining much of the similarity in microbial community

391    composition across species and between bleached and healthy colonies of *P. versipora.*

392    *Geothermobacter*, no longer a core taxon for *P. versipora,* and Chlorobiaceae were

393    overrepresented in bleached *P. versipora* colonies (6% ± 4% and 9 ± 4% respectively), these

394    taxa thought to be key drivers between healthy and bleached coral in our study (Randall et al.

395    2016; Cai et al. 2015). Overall, Shannon diversity indices are the same between unbleached

396    and bleached colonies (2.8).

397    Convergence of bacterial communities in April was also attributed largely to

398    Anaerolineaceae, which increased in unbleached *P. versipora* and decreased in *C. mcneilli*.

399    Flavobacteriaceae decreased in *C. mcneilli* without parallel increases in *P. versipora* (Table

400    1). Such changes reflect shifts towards an increased microbiome similarity between the two-

401    host species suggesting that the thermal anomaly exerted general selection pressure on the

402    structure of these bacterial communities regardless of host identity. *C. mcneilli* however

403    contained a higher overall diversity (Shannon: 3.78 vs 2.82 for *P. versipora,* Table 1).

404    In August ($t_4$) as Harbour waters cooled, the two-host species were again characterised by

405    distinct bacterial communities (Table 1) with shifts in the microbiome potentially indicative

406    of seasonal changes. This was driven by *Escherichia/Shigella* which explained 5% of the

407    dissimilarity according to SIMPER, and which no longer represented a shared core taxon,

408    only a core for *P. versipora. Anaerolineaceae* showed decreasing average relative abundance

409    for both species and *Geothermobacter* and Flavobacteriaceae decreased for *P. versipora,*

410    while Flavobacteriaceae and Chlorobiaceae disappeared entirely from *C. mcneilli* (Table 1).

411    *Marinicella* became part of the core microbiome for *C. mcneilli,* but was absent in *P.*

412    *versipora*.

413    A number of taxa appeared (<1% relative abundances) for the first time associating only with

414    *C. mcneilli* further driving the dissimilarity between the two-host species. Of these,

415    Porphyromonadaceae (2.13% of the dissimilarity in SIMPER and 8.86% p = 0.002 in

416    STAMP) accounted for a large proportion (~19% ± 5%) of relative abundance and

417    *Blastopirellula* (3.16% of the dissimilarity in SIMPER and 2.2% p < 0.01 in STAMP)

418    accounting for 4% ± 1.8% relative abundance. Increases in Flavobacteriaceae for *C. mcneilli*

419    explained 2.42% (0.4%, p = 0.007 in STAMP) of the dissimilarity respectively. During

420    recovery, *P. versipora* had a greater diversity of taxa than *C. mcneilli* (Shannon = 5.25 and

421    3.99 respectively) and a lower diversity of unique core OTUs (1 OTUs compared to 3 OTUs

422    for *C. mcneilli*).

423    In summary, *Escherichia/Shigella* was a dominant taxon for both coral species in February

424    and increased during the thermal stress event in April, suggesting the importance of this

425    taxon in bleaching resistance (abundances were higher in unbleached *P. versipora* and *C.*

426    *mcneilli* than bleached *P. versipora*). *Escherichia/Shigella* remained dominant in *P. versipora*

427    with the recovery of this coral in June while disappearing entirely from the comparatively

428    bleaching- resistant *C. mcneilli.* Overall, changes in the microbiome of *P. versipora* are

429    characterised by shifting abundances of a few key taxa, indicating a highly conserved

430    microbiome throughout the thermal anomaly. In contrast, these key taxa also found in *C.*

431    *mcneilli* were lost by June and a number of new taxa, such as *Marinicella,* appeared

432    indicating a less conserved microbiome for this coral species, and potentially seasonal shifts

433    in the bacterial community as waters cooled.

434

435    *Holobiont physiology*

436    December, January and February (pre-bleaching) net rates of Respiration (R) and light driven

437    calcification ($G_L$) and gross photosynthesis ($P_G$) were higher for *P. versipora* than *C. mcneilli*

438      (Table S2). The photosynthesis to respiration ratio (P: R) values for *P. versipora* and *C.*

439      *mcneilli* remained above 1 during this period ranging from 1.5 – 2.2 and 2.1 – 2.3

440      respectively (Figure. 5). In June (during bleaching), *P. versipora* exhibited reduced

441      photosynthesis (53 - 56% reduction in $P_G$) that corresponded to a similar decrease in

442      symbiont cell density (85% decrease) relative to pre-bleaching levels (Figure S2).

443      *Symbiodinium* cell normalised gross photosynthesis rates ($P_G$ cell$^{-1}$) were enhanced for the

444      remaining *Symbiodinium* cells ($P_G$ cell$^{-1}$ was 4.85 x$10^{-7}$ pre-bleaching and 1.71 x$10^{-6}$ $P_G$/cell

445      during bleaching, Figure S3). These trends were accompanied by a decrease in $G_L$ by 51 –

446      58% ($T_0$ and $T_2$) from pre-bleaching levels, and active calcification appeared to largely cease.

447      Values of P: R decreased during this time (25 - 48% decrease to 1.16 ± 0.08) via a

448      reduction in $P_G$ (53 - 56% reduction) while respiration remained relatively unchanged

449      (Figure 5).

450      In contrast to *P. versipora*, *C. mcneilli* upregulated $P_G$ and R during the bleaching period (24-

451      31% and 6-11% respectively, Table S2) and did not display signs of coral bleaching (no

452      paling, no change in *Symbiodinium* density and no decrease in $F_v/F_m$; Figure. 2). As a result,

453      P: R was increased by 21% during the bleaching period independent of *Symbiodinium* cell

454      density. $P_G$ per cell increased during the bleaching period (4.01 x $10^{-7}$ pre-bleaching to 5.55 x

455      $10^{-7}$ during the bleaching period) although this was not significant and was less than the rates

456      of $P_G$/cell for bleached *P. versipora* colonies (5.55 x $10^{-7}$ and 1.71 x$10^{-6}$ $P_G$/cell respectively,

457      Table S2). $G_L$ for *C. mcneilli* was highly variable across the experiment with measurements

458      for June ($T_3$) 93 - 98% lower (resulting in net dissolution) than the other time points.

459      In August during recovery ($T_4$), $P_G$ increased back to pre-bleaching levels for *P. versipora*

460      (with a 74% increase in *Symbiodinium* density) (Table S2). P: R increased during recovery

461      (74% increase to 4.4 ± 0.2) largely from a decrease in respiration (Figure 6), which was

462    significantly different to all other time points. For *C. mcneilli,* $G_L$ increased, while R

463    significantly decreased, leading to only a 26% increase in P: R (with *Symbiodinium* cell

464    density remaining stable) (Figure 5).

465

466

467    ***Discussion***

468    Tropical coral communities have been increasingly subjected to heat wave events that induce

469    mass bleaching (Hughes et al. 2018). Consequently, there has been renewed focus on

470    understanding why and how tropical corals are sensitive to thermal stress. In 2016, the

471    greatest heat wave to date in Australia extended to temperate systems where we witnessed the

472    first documented temperate coral bleaching event. In tropical systems, heat stress sensitivity

473    is influenced by the host (Grottoli et al. 2006; Bessell-Browne et al. 2014; Tremblay et al.

474    2016), symbiont (Scheufen et al. 2017, Sampayo et al. 2008, Silverstein et al. 2017) and/or

475    bacterial (Röthig et al. 2017, Ainsworth et al. 2015, Bourne et al. 2008) responses. Our data

476    examining how metabolism versus microbial dynamics suggests that heat sensitivity of

477    temperate taxa may similarly reflect a complex interplay of factors.

478

479    *Coral bleaching dynamics of corals in temperate regions.*

480    Tropical corals typically show a reduction in $F_v/F_m$, symbiont densities and net

481    photosynthesis of *Symbiodinium* in response to thermal stress (e.g.  Scheufen et al. 2017;

482    Gardner et al. 2017). Such suppression of holobiont photosynthesis via coral bleaching is

483    similarly characteristic for the coral *P. versipora* in temperate regions, which bleached in this

484    study. Rates of gross photosynthesis per cell have been shown to decrease during thermal

485    stress for some tropical corals (i.e. *M. cavernosa;* Scheufen et al. 2017) and increase for

486    others (i.e. *O. faveolata,* Scheufen et al. 2017; *P. damicornis*, Wangpraseurt et al. 2017). In

487    our study, rates of $P_G$ cell$^{-1}$ for *P. versipora* increased with coral bleaching (Figure S2),

488    indicating that the remaining symbionts retained cell functionality and are likely critical in

489    maintaining metabolic capacity (Sampayo et al. 2008). Whether, that with a loss of symbiont

490    cells, carbon limitation is relieved (or there is more carbon available per *Symbiodinium* cell)

491    for the fraction of cells that remain (that have not been impacted by heat stress) is unclear

492    (Levas et al. 2016, Suggett et al. 2013). However, P: R ratios decreased for this coral species

493    at this time suggesting a transition to heterotrophic carbon acquisition.

494    Bleaching susceptibility and recovery, while not well resolved into a single model, is strongly

495    dictated by the photosynthetic physiology (Scheufen et al. 2017) and species of microalgal

496    endosymbiont harboured within the coral. It is known from extensive research on tropical

497    corals that associations with thermally tolerant endosymbionts (Sampayo et al. 2008;

498    Silverstein et al. 2017) influence bleaching susceptibility and post bleaching mortality. In

499    Western Australia, *P. versipora* is known to associate with clade B18 (Silverstein et al.

500    2011). In Sydney, *P. versipora* and *C. mcneilli* maintain specific associations with a

501    particular undescribed species of *Breviolum* spp., provisionally referred to as type B18a. This

502    symbiont remained dominant throughout the thermal stress event (Fujise 2018, unpublished).

503    However, DNA sequence analyses revealed fixed differences in the cp23S gene that

504    differentiated symbiont populations associated with each species. *Breviolum* spp. appears

505    relatively rare in the south east Pacific (https://sites.google.com/site/geosymbio/home) and

506    thus clearly warrants further study given the ability to rapidly recover from stress by

507    increasing cell productivity ($P_G$/Cell) that we observed here.

508    An intriguing outcome was that the widely geographically distributed species *P. versipora*

509    exhibited greater signs of stress, whereas the southerly-restricted species *C. mcneilli* appeared

510     unaffected. These differential responses were observed both in the field and in the aquaria

511     exposed to the same temperatures and light levels. We initially considered that due to the

512     cryptic nature of *C. mcneilli*, which typically grows under boulders and overhangs or in

513     deeper water, this taxon was spared the synergistic impact of high temperature and irradiance,

514     well reported as a driver of coral bleaching (Mumby et al. 2001; Wooldridge et al. 2009).

515     *Plesiastrea versipora* colonies were most abundant on the upwards facing section of

516     boulders, exposed to increased solar radiation, which reaches summer levels of ~300 µmol

517     photons $m^{-2}$ $s^{-1}$. However, given that *C. mcneilli* did not bleach *ex situ*, under the same

518     irradiance levels as *P. versipora*, this taxon therefore appears to have attributes that provide

519     greater thermal tolerance.

520     *Coral bacterial communities*

521     We identified a number of dominant bacterial taxa present during the thermal anomaly and

522     temporal dynamics, consistent with the notion that highly stable microbiomes may confer

523     bleaching resilience (Ziegler et al. 2017; Grottoli et al. 2018), and that bleaching sensitivity is

524     also paralleled by the emergence of opportunistic bacteria species. Bacterial taxa

525     *Anaerolineaceae* and *Flavobacteriaceae* were dominant in the coral *C. mcneilli* in February

526     (summer), but rare in *P. versipora*. Surprisingly, these taxa exhibit increased abundance at

527     anthropogenically impacted sites (Ziegler et al. 2016), and are found commonly in diseased

528     tissues (Ainsworth et al. 2007; Roder et al. 2014). This pattern is commonly observed for

529     corals under thermal stress (Bourne et al. 2008; Thurber et al. 2009), but clearly in our study

530     the physiological functioning of *C. mcneilli* did not seem to be impacted by these potential

531     pathogen-associated bacteria. The convergence of *P. versipora* and *C. mcneilli*

532     microbiomes in April (during the bleaching period), was driven by increases in

533     *Anaerolineaceae* and *Flavobacteriaceae* in *P. versipora* and their corresponding decrease

534    in *C. mcneilli* (in August). Therefore, the true nature of these bacterial groups remains to

535    be determined. Overall, colonies of *C. mcneilli* did not exhibit significant shifts in their

536    microbiome between February (summer) and April (autumn- bleaching period), whereas *P.*

537    *versipora* had significant shifts in their microbiome over this period (driven by changes in the

538    relative abundances of dominant taxa with decreasing diversity). Thus, the more stable

539    microbiomes in *C. mcneilli* provide further and independent evidence that this taxon

540    experienced less stress than *P. versipora*.


541    Gammaproteobacteria *Escherichia/Shigella* represent a shared core taxon between the two

542    species over time until August (recovery) where it disappears from *C. mcneilli* entirely.

543    From February to April this bacterium increased in average relative abundance from 10% to

544    16% in colonies of *P. versipora* and 14% to 26% for *C. mcneilli.* With a shift to greater

545    heterotrophy in colonies of *P. versipora* with diminished symbionts cell densities, the

546    complementary increase in abundance of *Escherichia/Shigella sp.* may enhance nutrient

547    acquisition by supplementing host feeding (Röthig et al. 2017), although this would need

548    to be proven with isotopic analysis. *Escherichia spp.* have been previously shown to

549    associate with healthy corals at high temperatures (Kimes et al. 2013; Littman et al. 2011)

550    however increased abundance can generally be attributed to human impact, specifically fecal

551    contamination and are considered as potentially pathogenic (Kegler et al. 2017). Sydney

552    Harbour is an urban centre therefore it is perhaps not surprising that *Escherichia/Shigella sp.*

553    are found associating with these corals. Interestingly, *Escherichia/Shigella sp.* was not

554    detected in seawater samples and thus the functional role of this bacteria to coral health

555    warrants further investigation.


556    A shift in metabolic pathways from autotrophy to heterotrophy under thermal stress was

557    also seen in a metagenomic study by Yang et al. (2016) showing increases in bacterial

558    genes responsible for the metabolism of proteins, simple carbohydrates, phosphorous and

559    sulphur. On comparing bleached vs unbleached colonies of *P. versipora*, there is no

560    difference in the relative abundances of *Escherichia/Shigella sp.* (14% and 16% respectively)

561    and the complete absence of *Escherichia/Shigella sp*. in *C. mcneilli* in August (no thermal

562    stress) together could indicate that this taxon is only abundant in times of stress aiding

563    thermal tolerance, and further consistent with the restoration of normal physiological

564    functioning by August for *C. mcneilli*.

565    In addition, *Chlorobiaceae* and *Geothermobacter* were consistently abundant in *P. versipora*,

566    and became more abundant in April associated with bleached colonies. Chlorobiaceae, a

567    phylum of bacteria *Chlorobi*, are well known green sulphur bacteria with the ability to

568    supply nutrients through nitrogen fixation and are capable of anoxygenic photosynthesis

569    (Randall et al. 2016). *Geothermobacter* is likely a thermophilic of the family

570    Geobacteraceae, and has been previously found associating with both healthy and diseased

571    tropical corals (Hernández-Zulueta et al. 2016; Kashefi et al. 2003) although little is known

572    about the function of this bacteria. It has been proposed that this bacterium is a

573    predominant Fe (III)-reducing microorganism in many environments and plays are role in

574    nitrogen cycling (Vega Thurber et al. 2014). With the increased abundance of these taxa

575    associating with bleached colonies, increases in nitrogen fixation may increase the N:P ratio

576    in corals, altering coral physiology and resulting in increased susceptibility to bleaching and

577    disease (McDevitt-Irwin et al. 2017).

578    *Marinicella (Gammaproteobacteria)* was a dominant taxon associated only with *P.*

579    versipora in February (4% average abundance) that was lost entirely in bleached colonies

580    and did not return in August during recovery. *Gammaproteobacteria* has been associated

581    with enriched functional profiles of corals subjected to heat stress (Ziegler et al. 2017) and

582     the disappearance of *Marinicella* from bleached *P. versipora* may increase the bleaching

583     susceptibility of this coral. It has been shown that this class of bacteria are strongly

584     associated with healthy corals and dominate corals recovering from bleaching (Bourne et

585     al. 2008), which may explain the abundance of *Marinicella* in *C. mcneilli* in August (7%

586     average abundance). It is likely that the services provided by the microbiome, in particular

587     *Escherichia/Shigella sp*. enabled the rapid and widespread recovery of *P. versipora*. In

588     addition, this species had a highly diverse microbiome (Shannon 5.3 ± 1, Table 1) during

589     the recovery period consistent with previous evidence of higher microbial diversity

590     associated with healthy corals compared to bleached corals (Bourne et al. 2008; Castillo et

591     al. 2005), a seemingly important metric dictating coral health state.


592


593     *Holobiont metabolic rate-responses to heat stress*

594     Photosynthesis to respiration ratios (P: R) of *P. versipora* and *C. mcneilli* ranged from 1.5 –

595     2.2 and 2.1 – 2.3 respectively, over 20 – 26°C (December – February), indicating persistent

596     net autotrophy for both species throughout. P: R values closer to 2 suggest that these species

597     are largely autotrophic at these temperatures, likely explaining the success of *P. versipora*

598     over such a wide geographical range (Howe and Marshall et al. 2001). Intriguingly, rates of

599     photosynthesis, respiration and calcification (normalised to surface area) in *P. versipora* and

600     *C. mcneilli* did not show significant variation with increasing temperature, which may

601     indicate efficient thermal compensation as a way to sustain the symbiotic relationship over a

602     wide temperature range (Anthony et al. 2009; Gibbin et al. 2018). Rates of $P_G$ for *P.*

603     *versipora* and *C. mcneilli* in our study were comparable to those previously reported by

604     (Howe and Marshall 2001) at ~ 19°C and 21°C and are within the range of most reef corals

605     (Camp et al. 2017) despite the lower temperature and light intensities. Respiration rates of

606     both *P. versipora* and *C. mcneilli* obtained in this study were similar to or higher than many

607    reef corals (Camp et al. 2017; Hoogenboom et al. 2010) potentially due to the slow

608    calcification rate of these corals (as also shown by (Howe and Marshall 2002), and likely

609    driving the enhanced autotrophic capabilities seen.

610    In our study during coral bleaching, the decrease in P: R to 1.2 for *P. versipora* was largely

611    driven by a significant decrease in $P_G$ (53 - 56%) and R remained relatively unchanged. The

612    reduction in $P_G$ could be attributed to a decrease in cell density. Here, *P. versipora* went from

613    a highly autotrophic carbon acquisition mode to increased reliance on heterotrophy between

614    February and June 2016 when this taxon bleached. This metabolic shift is widely reported in

615    for tropical corals under heat stress (Anthony and Fabricius 2000; Grottoli et al. 2006;

616    Tremblay et al. 2016) as well as corals in extreme environments characterised by high-

617    sediment/reduced light conditions (Camp et al. 2017). Enhanced heterotrophic feeding yields

618    additional nutrients (i.e. nitrogen and phosphorus), especially in highly turbid and sediment-

619    enriched environments (e.g. Sydney Harbour). In doing so, these  corals from temperate

620    regions may be able to increase in symbiont cell numbers to maximise light capture and

621    restore a stable symbiosis (Rädecker et al. 2015).

622    For both *C. mcneilli* and *P. versipora*, metabolic re-adjustments appear to occur at the

623    expense of calcification under thermal stress. For *P. versipora*, this could be a direct

624    consequence of the inability to maintain photosynthesis, while respiration remained

625    unchanged (Camp et al. 2017) or it may be an indication that by June 2016 (winter), stored

626    energy reserves had been depleted, and a loss of symbionts resulted (Schoepf et al. 2015). In

627    contrast, *C. mcneilli* up-regulated $P_G$ and $R$ during bleaching and as a result P: R was

628    increased by ~20% and was > 2, maintaining high autotrophic rates during thermal stress.

629    This was not accompanied with an increase in symbiont density, and may suggest that the

630    symbiont has reduced translocation to the host, suppressing potential growth, forcing the

631    host to utilize energy stores or require shifts in the bacterial community to regulate energy

632    transfer within the holobiont (Sorek et al. 2012). Alternatively, *C. mcneilli* may have

633    rapidly decreasing calcification rates and redirected the products of photosynthesis as a

634    strategy to cope with stress. Previous studies have in fact shown that coral species

635    compensate for temperature changes in their natural environment by exchanging one set of

636    rate-determining reactions for another (Howe and Marshall 2001; Houlbrèque et al. 2009).

637    *P. versipora* had regained its colour by August (winter), recovering photosynthetic capacity

638    rapidly even though *Symbiodinium* cell density was still ~50% less than in February (pre-

639    bleaching). It is plausible that heterotrophy helped to restore and maintain the nutritional

640    exchange between host and symbiont by promoting symbiont growth and density, which in

641    turn would allow for increased carbon translocation and lipid storage with an eventual return

642    to autotrophy, ultimately preventing coral mortality as shown previously by (Tremblay et al.

643    2016). For *C. mcneilli*, "recovery" (even through this coral did not bleach) showed increasing

644    P: R. Together, the decrease in respiration during this period for both species suggests that

645    these corals increased their metabolic efficiency through decreasing respiration as a means to

646    conserve energy stores (Rodrigues and Grottoli 2007), which were most likely depleted

647    during bleaching. The marked return to active calcification could be attributed to a return to

648    normal levels of aragonite saturation or heterotrophy, through the supply of organic

649    molecules and energy or as a direct response to increasing photosynthesis for *P. verispora*

650    (Tremblay et al. 2016). High adult survivorship has recently been shown as particularly

651    important for the persistence of *P. versipora* populations in Sydney Harbour (Precoda et al.

652    2018). Thus, whilst the physiological patterns seen in this study are highly consistent with

653    those shown for tropical corals under heat stress, clearly a more targeted investigation is

654    needed to resolve the exact nature of the processes driving these metabolic patterns

655    throughout bleaching and recovery for  corals on temperate rocky reefs which are

656    acclimatized to extremes.

657

In summary, by examining the metabolic rates and microbial community properties of high-latitude corals we observed strong consistencies with bleaching dynamics commonly described for tropical corals, suggesting that there is a common set of general constraints on the physiologies of scleractinian corals under thermal stress irrespective of latitude. Intriguingly, the southerly restricted species (*C. mcneilli)* proved to be the most tolerant to heat stress and was able to up-regulate autotrophic capability during thermal stress. In contrast, the more widely distributed species (*P. versipora*) metabolic patterns were similar to those observed in tropical corals with coral bleaching, but exhibiting restored photosynthetic capacity rapidly when anomalous temperatures subsided. Our results suggest that as thermal anomaly events grow in frequency and intensity, 'tropicalisation' (Vergés et al. 2014, Vergés et al. 2016) will likely occur through the simultaneous arrival of subtropical coral species and the loss of temperate coral fitness perhaps for some species only, highlighting the variability and complexity of responses to thermal anomaly events.

**Conflict of Interest**

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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1043 **Figure 1**. Overview of sampling locations within Sydney Harbour represented by letters a (Fairlight)

1044 and b (Middle Head). All other locations represented on the map are sites with coral populations as

1045 observed by the authors. Inserts show specific sampling sites within locations. The base data for the

1046 map (**a**) were collected from map tiles at www.openstreetmap.org (© OpenStreetMap contributors,

1047 www.openstreetmap.org/copyright) under the Creative Commons Attribution-ShareAlike 2.0 licence

1048 (http://creativecommons.org/licenses/by-sa/2.0/), and customized in Adobe Illustrator (version 16).

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1050 **Figure 2.** Satellite-derived data characterising (A) Sea Surface Temperature (SST) anomalies during

1051 the period 2010-2017 with the study period highlighted in red, (B) 10-year average SST (open

1052 circles), observed SST (Fairlight) (blue squares), and bars of degree heating weeks (DHWs) from

1053 June 2015 (winter)-June 2017. Monthly composite SST data were obtained from the MODIS

1054 (MODerate Resolution Imaging Spectroradiometer) platform and DHWs (50-km) data from the

1055 National Environmental Satellite Data and Information Service (NESDIS) of the U.S. National

1056 Oceanic and Atmospheric Administration (NOAA). Alert level bars correspond to satellite

1057 bleaching alert levels based on current 50-km Hotspot data.

1058 **Figure 3. Top panel -** *Symbiodinium* cell density (cells/cm$^2$ surface area) of *C. mcneilli* (circles) *in*

1059 *situ* and *ex situ*, *P. versipora* (squares) (bleached, red) and *P. versipora* (healthy, blue) (*in situ*)

1060 expressed as means ± SEM (*P. versipora* n = 8, *C. mcneilli* n = 4, *C. mcneilli* was sampled from

1061 Fairlight only). **Bottom panel -** Maximum quantum yield of PSII ($F_v/F_m$) expressed as means ± SEM

1062 of healthy (*in situ*), bleached (*ex situ*),bleached (*in situ*) colonies of *P. versipora* and *C. mcneilli* (*ex*

1063 *situ*) (*P. versipora* n = 8, *C. mcneilli* n = 4, *C. mcneilli* was sampled from Fairlight only).. *In situ*

1064 measurements are of eight colonies in total, four colonies from each harbour site and *ex situ*

1065 measurements are of eight coral fragments maintained in aquaria. ANOVA with Tukey's post hoc

1066 tests were used for statistical analysis with numbers indicating significant differences ($p < 0.05$).

1067 Measurements were taken in February (pre-bleaching), April (during bleaching), June and August

1068 (recovery period) 2016.  Image of bleached (April) and non-bleached (July, recovery) colony of *P.*

1069 *versipora* at Middle Head (depth 4 m).


1070 **Figure 4.** Venn diagram of the core microbiomes of healthy *P. versipora* and *C. mcneilli* colonies

1071 pre-bleaching. The number of OTUs for each species core and the overall shared core are shown.

1072 Only core taxa with >1% abundance are shown. Each OTU is presented to genus level where possible.

1073 UC: Unclassified. *Escherichia/Shigella* is unclassified (UC) at species level.


1074 **Figure 5.** Metabolic comparisons of photosynthesis and respiration (P: R) and calcification (G) rates

1075 ($\mu$mol cm$^{-2}$ h$^{-1}$) (± SEM) over the experimental period (December- August) for A) *P. versipora* and B)

1076 *C. mcneilli.* Dashed lines connect metabolic shifts through time. The bleaching timepoint is indicated

1077 with a red data point and recovery with a blue data point. All other timepoints are pre-bleaching.


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1079 **Table 1**: Relative abundances (%) of dominant bacterial taxa, core OTU number and

1080 Shannon diversity values for *P. versipora* and *C. mcneilli* during February (pre-bleaching),

1081 April (during bleaching) and August (recovery). Statistical significance is given for February

1082 and August (PERMANOVA).

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