Effects of thermal stress on amount, composition, and antibacterial properties of coral mucus

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The surface mucus layer of reef-building corals supports feeding, sediment clearing, and protection from pathogenic invaders. As much as half of the fixed carbon supplied by the corals' photosynthetic symbionts is incorporated into expelled mucus. It is therefore reasonable to expect that coral bleaching (disruption of the coral–algal symbiosis) would affect mucus production. Since coral mucus serves as an important nutrient source for the entire reef community, this could have substantial ecosystem-wide consequences. In this study, we examined the effects of heat stress-induced coral bleaching on the composition and antibacterial properties of coral mucus. In a controlled laboratory thermal challenge, stressed corals produced mucus with higher protein (β = 2.1, $p < 0.001$) and lipid content (β = 15.7, $p = 0.02$) and increased antibacterial activity (likelihood ratio = 100, $p < 0.001$) relative to clonal controls. These results are likely explained by the expelled symbionts in the mucus of bleached individuals. Our study suggests that coral bleaching could immediately impact the nutrient flux in the coral reef ecosystem via its effect on coral mucus.

1 Effects of thermal stress on amount, composition, and antibacterial properties of coral mucus

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13 **ABSTRACT**

14 The surface mucus layer of reef-building corals supports feeding, sediment clearing, and

15 protection from pathogenic invaders. As much as half of the fixed carbon supplied by the corals'

16 photosynthetic symbionts is incorporated into expelled mucus. It is therefore reasonable to

17 expect that coral bleaching (disruption of the coral–algal symbiosis) would

18 affect mucus production. Since coral mucus serves as an important nutrient source for the entire

19 reef community, this could have substantial ecosystem-wide consequences. In this study, we

20 examined the effects of heat stress-induced coral bleaching on the composition and antibacterial

21 properties of coral mucus. In a controlled laboratory thermal challenge, stressed corals

22 produced mucus with higher protein ($\beta = 2.1$, $p < 0.001$) and lipid content ($\beta = 15.7$, $p = 0.02$)

23 and increased antibacterial activity (likelihood ratio $= 100$, $p < 0.001$) relative to clonal controls.

24 These results are likely explained by the expelled symbionts in the mucus of bleached

25 individuals. Our study suggests that coral bleaching could immediately impact the nutrient flux

26 in the coral reef ecosystem via its effect on coral mucus.

28 **INTRODUCTION**

29   Rising sea surface temperature has increased the global risk of coral bleaching, the

30 breakdown of the symbiosis between a coral host and its algal symbiont, to alarming levels

31 (Hughes et al., 2018). The direct impacts of bleaching on the animal host and algal symbiont are

32 well studied. For example, coral bleaching has been shown to downregulate the expression of

33 genes related to host immunity (Pinzón et al., 2015) and alter host metabolism (Kenkel, Meyer,

34 & Matz, 2013; Rodrigues & Grottoli, 2007). Symbionts (family Symbiodiniaceae) expelled

35 during bleaching produce elevated amounts of reactive oxygen species but are otherwise

36 physiologically similar to healthy endosymbionts (Nielsen, Petrou, & Gates, 2018). Sustained

37 coral bleaching often leads to host mortality and subsequent shifts in the reef community

38 structure (Harriott, 1985). For example, coral bleaching and mortality causes shifts in reef-fish

39 assemblage structure and alters recruitment success (Booth & Beretta, 2006; Richardson,

40 Graham, Pratchett, Eurich, & Hoey, 2018).

41   Coral mucus is a complex mixture of proteins, lipids, and carbohydrates that is produced

42 by mucocytes in the coral epidermal layer and secreted by coral surface tissues (reviewed in

43 Brown & Bythell, 2005). Arabinose and glucose primarily compose the monosaccharide portion

44 of coral mucus (Wild, Woyt, & Huettel, 2005). Arabinose is likely a symbiont-specific

45 contribution to coral mucus, as aposymbiotic, heterotrophic cold-water corals produce mucus

46 nearly identical in composition to mucus from symbiotic warm-water corals, except for the

47 presence of arabinose in the latter (Wild, Naumann, Niggl, & Haas, 2010). Up to about half of

48 the photosynthetically fixed carbon supplied by a coral's algal symbiont is expelled as mucus

49 (Crossland, 1987; Crossland, Barnes, & Borowitzka, 1980; Davies, 1984). This coral surface

50 mucus layer acts as a defense against desiccation and pathogens (Shnit-Orland & Kushmaro,

51 2009), and is also released into the water column where it traps suspended particles and acts as

52 an energy source for benthic communities (Wild, Huettel, et al., 2004). Given the integral role of

53 photosynthetically fixed carbon in mucus production of most reef-building corals, it is predicted

54 that coral bleaching events will reduce mucus production and subsequently impact the flow of

55 energy throughout the reef ecosystem (Bythell & Wild, 2011). However, one study has shown

56 that acroporid corals exposed to an experimental heat stress released almost twice as much

57 dissolved and particulate organic carbon relative to unchallenged controls (Niggl, Glas, Laforsch,

58 Mayr, & Wild, 2009). One explanation for this finding is that increased mucus release in heat-

59 stressed corals augments defense against pathogens when other immune defense mechanisms

60 may be compromised by thermal challenge (Palmer, Bythell, & Willis, 2010).

61   In the Florida Keys, annual mass bleaching events are predicted to begin by the mid-

62 century (Manzello, 2015). Currently, multiple anthropogenic factors including thermal stress,

63 increased storms, and disease outbreaks have led to a near 80% decline in reef cover in the

64 Florida Keys since the 1980s (Porter et al., 2001; Williams & Miller, 2011; Williams, Miller, &

65 Kramer, 2008). In particular, corals in the genus *Acropora* have faced some of the most dramatic

66 declines in this region (Greenstein, Curran, & Pandolfi, 1998). The staghorn coral, *Acropora*

67 *cervicornis*, has been selected as a focal species for multiple active restoration programs, such as

68 the Coral Restoration Foundation, due to its relatively fast asexual growth through fragmentation

69 (Lirman et al., 2010). As restoration efforts aim to replenish stands of *A. cervicornis*, it is critical

70 to assess this species' greater role in coral reef ecosystem. This study aims to characterize coral

71 mucus release, composition, and antibacterial activity during acute thermal stress in *A.*

72 *cervicornis* as a reference to guide further research on the potential consequences of bleaching-

73 mediated mucus changes on the larger coral reef ecosystem.

74

MATERIALS & METHODS

Corals

77 Fifteen *Acropora cervicornis* genets (n = 3 fragments per genet) were shipped from the Coral

78 Restoration Foundation (Key Largo, Florida USA, Project ID CRF-2016-021) on 7 September

79 2016 to the University of Texas at Austin. Upon arrival, corals were immediately tagged with

80 colored zip ties to uniquely identify each genet and allowed to recover for 12 days in artificial

81 seawater (ASW; 30–31 ppt) at 25°C under 12000K LED lights on a 12 h/12 h day/night cycle.

82 Corals were fed weekly with Ultimate Coral Food (Coral Frenzy, LLC).

83

Experimental conditions

85 Coral fragments were partitioned into experimental and control tanks. One genet (U10)

86 experienced mortality during the recovery period, so only one U10 fragment remained when the

87 experiment began. For all other genets, one fragment was placed in a control tank (26°C) and one

88 fragment was placed in an experimental tank. Any remaining fragments from the shipment of n =

89 3 per genet were retained in a holding tank, though many genets developed tissue loss or

90 experienced damage on a single fragment during shipping. The single remaining U10 fragment

91 was placed in the experimental tank. The temperature in the experimental tank was ramped from

92 26°C to 31°C over 33 h. High summer temperature in the Florida Keys often reaches 31°C

93 (Manzello, 2015). Therefore, a 31°C heat treatment was chosen to represent an ecologically

94 relevant stressor.

95   After corals had been exposed to experimental conditions for four days, corals appeared visibly

96   pale relative to initial photographs and paired control fragments (Figure 1). At this time, the

97   temperature in the experimental tank was reduced to 26°C over 6 h.

98

99   **Image analysis**

100   Prior to the experiment, photographs of each fragment were taken using a Nikon D5100 camera.

101   Images of the front and back of each fragment were taken using the same camera, settings, and

102   lighting each day of the experiment. Brightness values in images were measured for the front and

103   back sides of each fragment using image analysis software (ImageJ, Schneider, Rasband, &

104   Eliceiri, 2012). Corals become brighter (paler) as their symbioses with pigmented algae break

105   down. Therefore, changes in coral brightness reflect changes in algal densities (Winters,

106   Holzman, Blekhman, Beer, & Loya, 2009). A standard curve of brightness values was

107   constructed using standard Coral Health Charts that were included in each image. Briefly,

108   brightness (white value intensity measured in unitless values ranging 0–255) was measured for a

109   series of standard color cards (arbitrary values 1–6) in each photograph to construct a linear

110   curve of brightness to compare fragments. Brightness values were standardized to color cards to

111   normalize for any minor differences in lighting across days.

112

113   **Mucus collection**

114   After the experiment, each coral fragment was placed within a pre-weighed 50 mL conical tube

115   containing 5 mL ASW from the respective tank. Tubes were placed on their sides and secured to

116   a gently rocking incubator plate (135 RPM, 28°C) for 20 minutes, rotating the tubes every 5

117   minutes to ensure that all sides of the coral fragment were submerged in water. After rocking,

118   fragments were inverted dry above the liquid in the conical for 20 minutes and lightly

119   centrifuged (200 RPM) for 2 minutes to pull down mucus adhering to the surface of the coral,

120   modified from (Wild, Huettel, et al., 2004).The volume and mass of mucus from each fragment

121   was measured and stored at -80°C. Coral fragments were returned to their tanks. The mucus

122   collection procedure was repeated six days later, exactly as described above. During mucus

123   collection, algal cells were clearly visible in some samples. All mucus aliquots were briefly

124   centrifuged at 3500 g for 2 minutes to sediment the algal pellet and decant algal cell-free mucus

125   before the experiments described below.

126

127   **Mucus composition**

128   Total protein was measured following the Coomassie (Bradford) Protein Assay Kit (Thermo

129   Scientific, Waltham, MA, USA). Total carbohydrate was measured using the Total Carbohydrate

130   Quantification Assay Kit (Abcam, Cambridge, UK). Total lipids were extracted and the dry mass

131   of each mucus sample were measured. A standard curve was prepared using reagent grade

132   cholesterol in a 2:1 chloroform:methanol mixture and an aliquot of 2:1 chloroform:methanol was

133   added to each sample tube. After mixing, the solvent was evaporated from all standard and

134   sample tubes on a heat block at 90°C. Concentrated sulfuric acid was added to each tube, then

135   incubated at 90°C for 20 minutes. Samples were cooled, then plated in triplicate into wells of a

136   96-well plate. Background absorbance was measured at 540 nm. After incubating each sample

137   with 50 µL of vanillin-phosphoric acid for 10 minutes, absorbance was measured again at 540

138   nm. The concentrations of protein, carbohydrate, and lipid in the mucus were estimated using

139   standard curves constructed by measuring absorbance for known concentrations of protein,

140   carbohydrate and lipids, per manufacturer's recommendations. All measurements were

141   normalized to the volume of mucus expelled and the surface area of the fragment.

142

143   **Antibacterial activity**

144   Cultures of laboratory *E. coli* (K-12) were grown overnight in Luria broth (LB), then washed

145   twice in sterile ASW to remove remaining culture media. Coral mucus (140 μL) and washed *E.*

146   *coli* culture (60 μL) was added to triplicate wells in 96-well plates. The covered plates were

147   incubated at 37°C for 12 h. Every 30 minutes the plate was shaken and the absorbance at 600 nm

148   was measured. The plates were completely dry by the end of the incubation, so only the first 5 h,

149   the latest time at which we observed liquid in the wells, are included in these analyses. Control

150   wells included bacteria in LB or coral mucus without added bacteria to demonstrate bacterial

151   growth or quantify endogenous mucus bacteria, respectively.

152

153   **Coral surface area**

154   Fragment surface area was estimated using a 3D scanner and accompanying ScanStudioPro

155   software (NextEngine, Santa Monica, CA, USA). Each scan was completed using a 360 degree

156   scan with 16 divisions and 10,000 points/inch^2. Scans were then trimmed, polished to fill holes,

157   fused and then surface area was estimated based on a size standard.

158

159   **Real-time quantitative PCR**

160   The forward primer 5'-TCTGTACGCCAACACTGTGCTT-3' and reverse primer 5'-

161   AGTGATGCCAAGATGGAGCCT-3' was used to amplify the *Acropora cervicornis* actin

162   sequence as developed in (Winter, 2017). The forward primer 5'-

163   GTGAATTGCAGAACTCCGTG-3' and reverse primer 5'-CCTCCGCTTACTTATATGCTT-3'

164   was used to amplify the Symbiodiniaceae ITS2 sequence. Primer pair specificity was verified by

165   gel electrophoresis and melt curve analysis of the amplification product obtained with *A.*

166   *cervicornis* holobiont DNA. Primer efficiencies were determined by amplifying a series of four-

167   fold dilutions of *A. cervicornis* holobiont DNA and analyzing the results using *PrimEff* function

168   in the *MCMC.qpcr* package (Matz, Wright, & Scott, 2013) in R. Briefly, C_T (threshold cycle)

169   results were plotted as CT vs. $\log_2$[DNA], and amplification efficiencies (amplification factor per

170   cycle) of each primer pair were derived from the slope of the regression using formula:

171   efficiency $= 2^{-(1/slope)}$ (Pfaffl, 2001).

172   Mucus aliquots were centrifuged to remove any cell debris. A 14 µL aliquot of coral mucus was

173   combined with SYBR Green PCR Master Mix (Applied Biosystems), 1.5 µM forward and

174   reverse primers, and water. The Roche LightCycler 480 system was used to carry out the PCR

175   protocol (95°C for 40 seconds, then 40 cycles of 60°C for 1 minute and 72°C for 1 minute) and

176   detect the fluorescence signal.

177

178   **Statistics**

179   All statistical analyses were performed in R (3.4.0, R Core Team, 2016)). Bayesian models

180   implemented using the MCMCglmm package (Hadfield, 2010) was used to explain variation in

181   coral color and mucus composition, with treatment as a fixed effect and genotype as a random

182   effect under a Gaussian distribution for coral color and metabolic assays and a Poisson

183   distribution for marker gene counts. A nonlinear mixed-effects model followed by ANOVA was

184   implemented using the nlme package (Pinheiro, Bates, DebRoy, & Sarkar, 2017) was used for

185   time-series analysis of antibacterial activity, with time, treatment, and their interaction as fixed

186   effects and plate well as a random effect. Experimental data and R scripts are included as

187   Supplemental Data S1 and S2, respectively. Data and scripts can also be accessed on GitHub:

188   https://github.com/rachelwright8/bleached_coral_mucus.

189

190   **RESULTS**

191   **Coral bleaching and mucus collection**

192   Corals from both treatments produced similar amounts of mucus by volume ($\beta = 0.01$, $p = 0.25$,

193   Figure 2A) and mass ($\beta = 0.14$, $p = 0.27$, Figure 2B). After a six-day recovery period, corals in

194   both treatments produced significantly less mucus by volume ($\beta = -0.14$, $p < 0.001$, Figure 2A)

195   and mass ($\beta = -1.1$, $p < 0.001$, Figure 2B) compared to the first time point. After four days in the

196   experimental treatment at 31°C, corals paled significantly compared to corals in the control

197   condition ($\beta = -1.16$, $p < 0.001$, Figure 3A).

198

199   **Mucus biochemistry**

200   The mucus produced by heat-stressed fragments contained significantly more total protein ($\beta =$

201   2.1, $p < 0.001$, Figure 3B) and total lipid ($\beta = 15.7$, $p = 0.02$, Figure 3C). There was also a slight

202   increase in carbohydrate content in mucus from heat-stressed corals, though this difference was

203   not significant ($\beta = 0.64$, $p = 0.10$, Figure 3D).

204

205   **Mucus antibacterial activity**

206   Antibacterial activity increased in thermally stressed corals from the experimental treatment

207   relative to healthy corals (likelihood ratio $= 100$, $p < 0.001$, Figure 4). Optical density at 600 nm

208   (OD_{600}), an absorbance measure that reflects bacterial density, decreased throughout the

209   incubation period in all mucus samples. However, bacterial density declined significantly faster

210   in mucus samples from heat-stressed corals. Bacterial density remained low throughout the

211   sampling time period in negative control samples, which contained mucus from control corals

212   with no added bacteria, suggesting that decreases in observed bacterial density were due to

213   elimination of the experimentally added *E. coli* (Supplementary Figure 1). Positive control wells

214   that contained *E. coli* in growth media with no coral mucus increased in OD, showing that the

215   bacteria were alive and capable of growth under the experimental conditions (Supplementary

216   Figure 1).

217

218   **Presence of host and symbiont DNA in mucus**

219   Real-time quantitative PCR (qPCR) was performed to determine the relative abundances of

220   coral- and symbiont-derived marker gene copies present in the mucus released by healthy and

221   heat-stressed corals. Primers were designed to target a coral-specific actin gene and the

222   Symbiodiniaceae ITS2 region. Presumably, copies of the coral-specific actin gene would

223   represent lysed coral cells, while copies of the ITS2 region would represent material released

224   from Symbiodiniaceae cells in the mucus. We did not detect any ITS2 sequences in the mucus of

225   unchallenged corals (Figure 5). Mucus released by heat-stressed coral fragments contained

226   significantly more marker gene copies of both coral-derived (95% credible interval (CI) = 1.6–

227   4.6, p = 0.001) and symbiont-derived sequences (95% CI = 4.3–25.7, p < 0.001, Figure 5).

228

229   **DISCUSSION**

230   **Coral mucus stores take a long time to replenish.**

231   Mucus volumes released from both control and heat-stressed fragments during the initial

232   collection (0.18 ± 0.04 mL/cm², or about 1.8 L/m²) are consistent with daily mucus release

233   values previously reported for submerged acroporid corals (1.7 L/m² in Wild, Huettel, et al.,

234   2004) . However, following a six-day recovery period, less than half of the original mucus

235   volume was collected from both control and heat-stressed fragments (0.18 ± 0.04 mL/cm² initial

236   collection *vs*. 0.08 ± 0.02 mL/cm² post-recovery, $p_{\mathrm{MCMCglmm}} < 0.001$, Figure 2A), suggesting that

237   mucus stores were not completely replenished to the original volume in this amount of time. The

238   methods used in this study to extract mucus left the coral nubbins completely dry. Therefore, the

239   results presented here represent the total volume of stored mucus at the time of collection, which

240   may not correspond directly to daily mucus release in natural settings. These results emphasize

241   the importance of measuring mucus release over time to confidently estimate daily release rates

242   and predict daily energetic flow throughout the reef ecosystem.

243

244   **Stressed corals produce mucus high in protein and lipid.**

245   We found no difference in the quantity of mucus produced by stressed corals compared to

246   healthy corals after four days of heat stress or after a six-day recovery period (Figure 2),

247   suggesting that the quantity of mucus produced by a coral is relatively unaffected by thermal

248   stress and that mucus stores cannot be replenished within a week. Given the energetic cost of

249   producing mucus (Riegl & Branch, 1995), it is reasonable to predict that corals with low

250   densities of autotrophic symbionts would produce less, or lower nutritional quality, mucus than

251   healthy corals. These findings of enhanced protein and lipid content in mucus from heat-stressed

252   corals relative to mucus produced by healthy corals (Figure 3) support a previous study that

253   found increased organic carbon release by thermally stressed corals (Niggl et al., 2009).

254   Symbiodiniaceae store reserve energy as lipid droplets and starch granules that are translocated

255   from the algal membrane to coral cells in a healthy coral–algae symbiotic relationship (Patton &

256   Burris, 1983). We observed a pellet of algal cells in the mucus of thermally stressed corals, but

257   not in mucus produced by healthy corals. Though algal cells were pelleted and removed from all

258   mucus collections, extracellular lipid droplets would remain in the mucus and could represent a

259   potential explanation for the increased abundance of lipids in mucus from stressed corals.

260   Likewise, proteins and lipids released from damaged Symbiodiniaceae and host cells would also

261   be present in the mucus of stressed corals. This particular possibility is supported by finding both

262   coral and Symbiodiniaceae DNA in the mucus of stressed corals (Figure 5). Future studies

263   should measure long-term effects of bleaching to determine the duration of this observed

264   enrichment in coral mucus quality following thermal stress.

265   This short-term enrichment in nutrition could invoke shifts in trophic interactions and

266   density dependent foraging behavior in coralivorous fish and invertebrates. In the Florida Keys,

267   previous studies have observed increased predation intensity by corallivorous fishes and

268   invertebrates as coral cover declines (Baums, Miller, & Szmant, 2003; Burkepile, 2012). In

269   addition, there is established prey preference asymmetries in the Caribbean, where *A. cervicornis*

270   is already the preferred prey of a corallivorous gastropod (Johnston & Miller, 2014). Our results

271   implicate that as heat-stressed corals release more nutrients into their mucus, this may drive

272   stronger predation pressure, at least in bursts associated with the bleaching event itself. This may

273   only maintain a benefit to higher trophic levels at very short time scales, as predation on coral

274   reef dwelling fish is higher when they reside in bleached corals (Coker, Pratchett, & Munday,

275   2009) and corallivorous fish are well known to suffer population declines in response to coral

276   bleaching (Pratchett, Thompson, Hoey, Cowman, & Wilson, 2018). Therefore, future studies

277 should investigate how bleaching impacts predator–prey dynamics between corals and

278 corallivorous fish and invertebrates.

279

280 **Stressed corals produce mucus with high antibacterial activity.**

281 This study found that mucus collected from stressed coral fragments eliminated bacteria faster

282 than mucus from healthy fragments from matched genets (Figure 4). Antibacterial activity of

283 coral mucus is attributed to antimicrobial substances produced by commensal microbes living on

284 the coral surface (Nissimov, Rosenberg, & Munn, 2009; Shnit-Orland & Kushmaro, 2009). A

285 previous study of mucus collected from *A. palmata* during a summer bleaching event in the

286 Florida Keys found that mucus from bleached corals lacked antibiotic properties normally found

287 in healthy coral mucus (Ritchie, 2006). The difference in findings between this study and the

288 investigation of antibiotic activity in naturally bleached corals could be attributed to the timing of

289 collections. Mucus in this study was collected as soon as corals became pale, whereas the

290 previous study collected mucus after corals in the Florida Keys had been experiencing high

291 levels of thermal stress and bleaching for about a month according to NOAA reports of the

292 thermal stress event (Eakin et al., 2010). Long-term thermal stress is known to promote coral

293 disease by altering bacterial pathogenicity and host susceptibility (Bruno et al., 2007; Maynard et

294 al., 2015). In our short-term bleaching conditions, the increased protein and lipid content in the

295 mucus (Figure 3B–C) may have temporarily improved the antibacterial activity of commensal

296 microbes that exist in the coral mucus (Krediet, Ritchie, Paul, & Teplitski, 2013). Though the

297 mechanism is unclear, Symbiodiniaceae do appear to play a role in a coral's ability to manage

298 immune stress and regulate microbial communities (Littman, Bourne, & Willis, 2010; Wright et

299 al., 2017).

300   Other studies that have investigated bacterial growth in coral mucus have found that

301   mucus supports the growth of bacterial species found on corals (Wild, Rasheed, et al., 2004),

302   such as *Pseudoalteromonas* and *Vibrio* spp. (Allers, Niesner, Wild, & Pernthaler, 2008). Both

303   *Pseudoalteromonas* and *Vibrio* are commonly observed in corals, though they are usually

304   associated with coral stress and disease suggesting that these bacteria employ mechanisms to

305   resist antimicrobial properties of coral mucus (Ritchie, 2006) that our lab *E. coli* strain, which

306   was naïve to coral mucus, presumably lacks. This difference in bacterial species could explain

307   the discrepancy in findings. Additionally, the previous experiments measured bacterial growth

308   over 40–50 h. It is possible that we would have found similar results had we measured bacterial

309   growth beyond 5 h.

310

311   **CONCLUSIONS**

312   Our results show that thermal stress does not significantly affect the volume of mucus produced

313   by *A. cervicornis* immediately following a bleaching event. Stressed corals produced mucus with

314   higher protein content, higher lipid content, and increased antibacterial activity relative to

315   unstressed controls. Additional lipids and proteins likely come from Symbiodiniaceae and host

316   cells damaged during bleaching rather than from additional investment by the coral host. Short-

317   term nutritional enrichments of mucus released from bleaching corals could promote growth of

318   heterotrophic microbes at the lowest trophic levels of marine ecosystems and thus cause large-

319   scale shifts in a reef's nutrient cycle. Changes in the nutritional composition and antibacterial

320   properties of the mucus should influence coral-associated microbes that contribute to coral

321   disease susceptibility. Future experiments should investigate longer-term effects of thermal stress

322   on mucus production and content to further investigate reef-wide consequences of coral

323   bleaching.

324

325

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329

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478

Figure 1

Experimental design and representative coral image.

Corals were maintained in either (A) control (26°C) or (B) experimental (31°C) conditions for four days. Paling was observed for fragments in the experimental treatment, but not under control conditions.
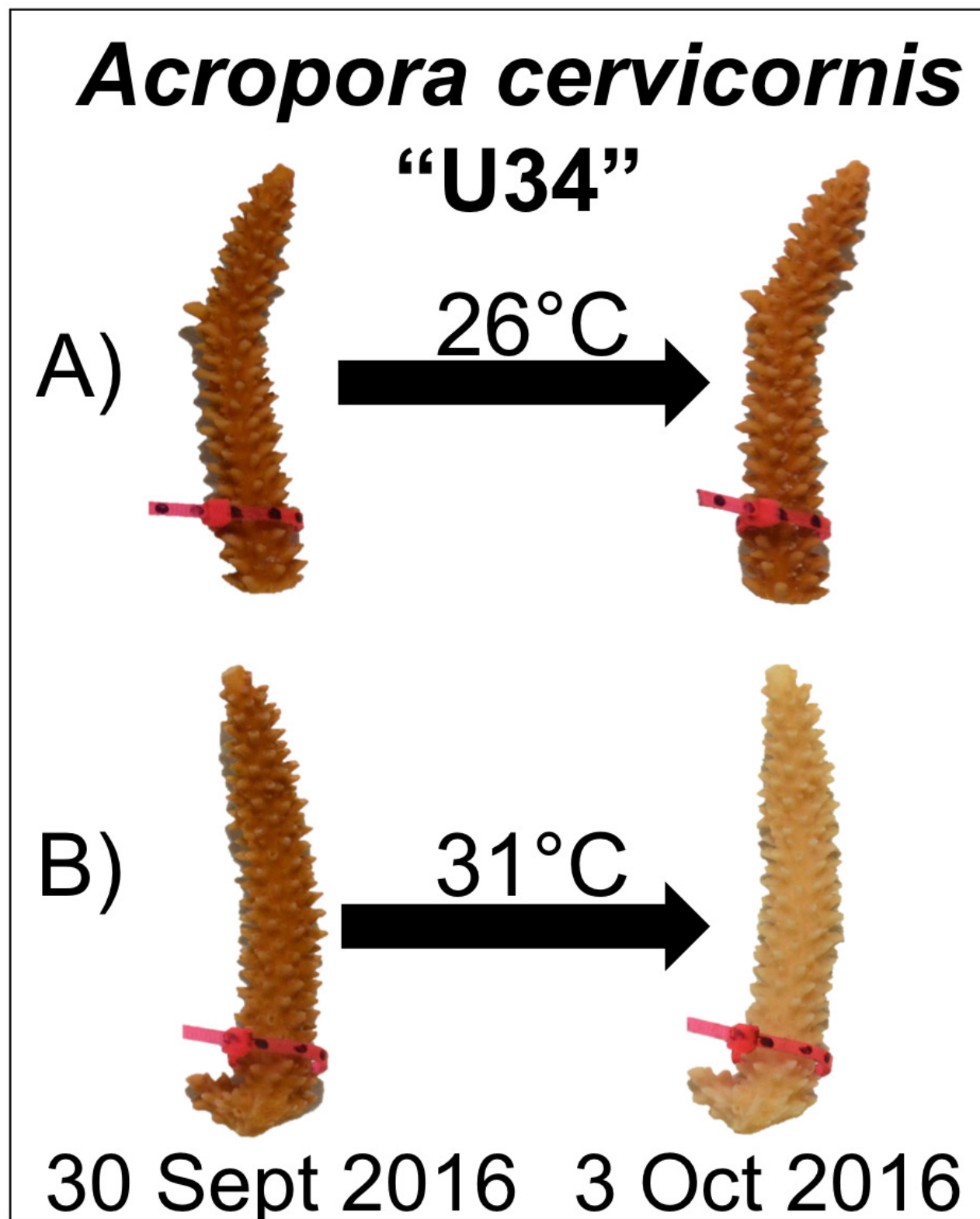
Figure 2

Mucus production.

Mucus was collected immediately after paling was observed ("Initial") and six days after the challenged corals were returned to control conditions ("Recovery"). The volume (**A**) and mass (**B**) of the recovered mucus was normalized to the surface area of the coral fragment. Red boxes represent fragments in control conditions; beige boxes represent heat-stressed fragments. Error bars represent standard error.
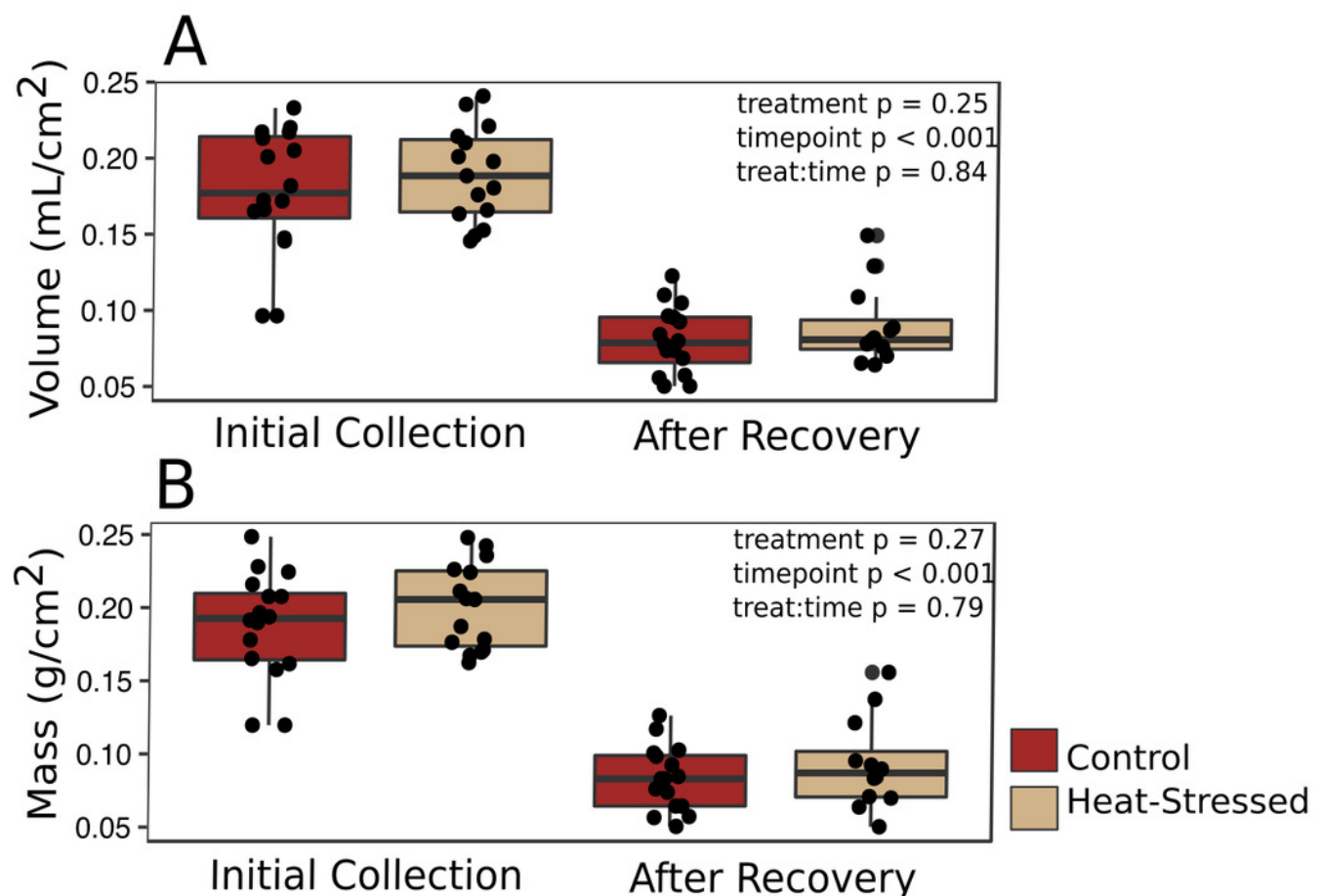
Figure 3

Effects of heat stress.

(**A**) Effect of treatment on coral color (decrease in color score indicates bleaching). (**B–D**) Effects on mucus composition: protein (**B**), in mg/cm^2 fragment surface area, and lipid (**C**) and carbohydrate (**D**), in µg/cm^2 fragment surface area. Red boxes represent fragments in control conditions; beige boxes represent heat-stressed fragments. Error bars represent standard error.
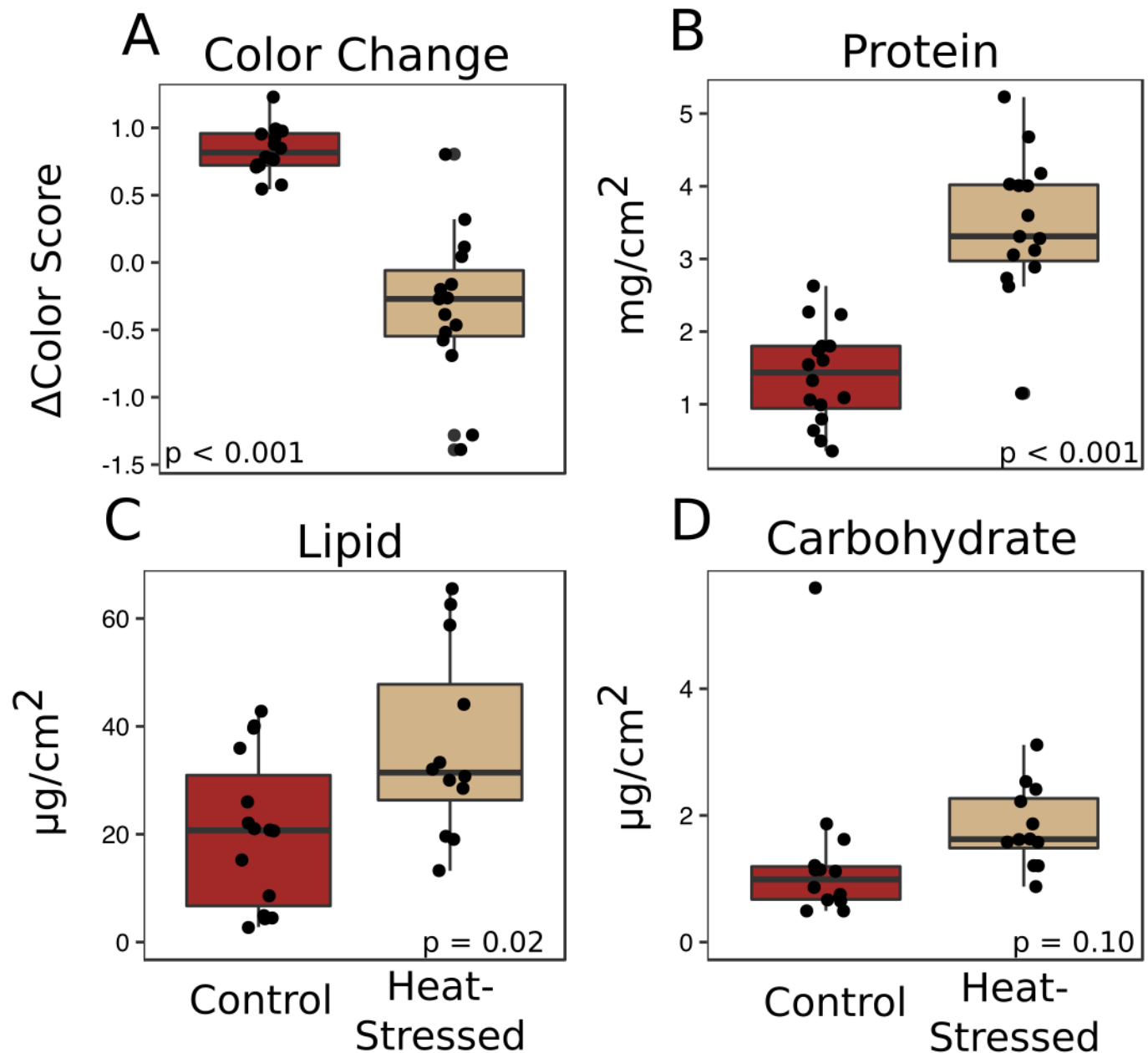
Figure 4

Antibacterial activity of coral mucus.

Optical density (OD) at 600 nm indicates the density of inoculated bacteria in coral mucus samples. Red lines represent fragments in control conditions; beige lines represent heat-stressed fragments. Error bars represent standard error.
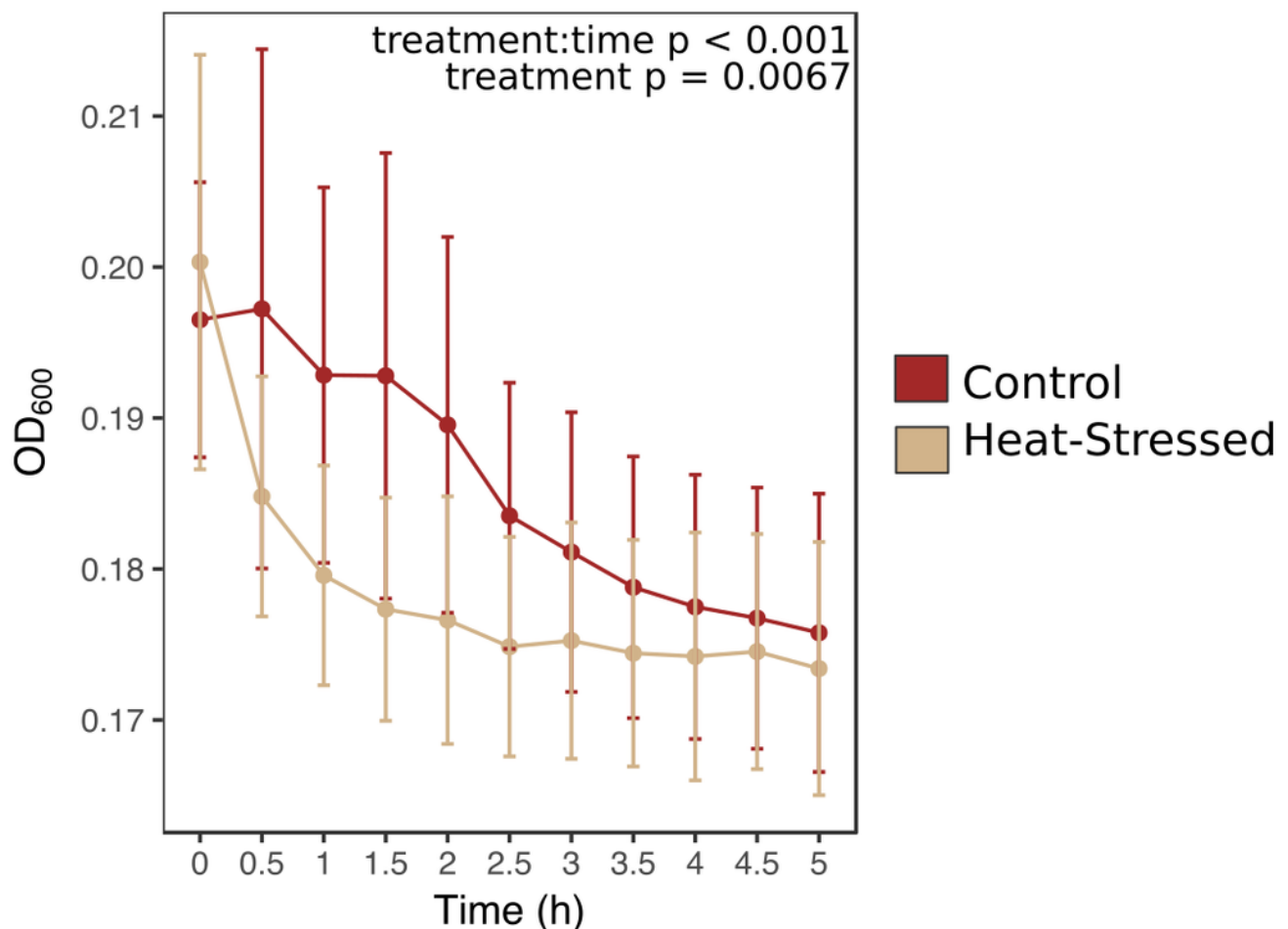
Figure 5

Symbiodiniaceae and coral DNA in mucus.

Real-time quantitative PCR detected *A. cervicornis* actin (**A**) or Symbiodiniaceae ITS2 (**B**) marker gene copies in the coral mucus of healthy and pale corals. Red boxes represent fragments in control conditions; beige boxes represent heat-stressed fragments. Error bars represent standard error.