The role of vertical symbiont transmission in altering cooperation and fitness of coral-*Symbiodinium* symbioses

Carly D Kenkel[a,1] and Line K Bay[a,2]

[a]Australian Institute of Marine Science, PMB No 3, Townsville MC, Queensland 4810, Australia

[1]Corresponding author, email: carly.kenkel@gmail.com or c.kenkel@aims.gov.au; phone: +61 07 4753 4268; fax: +61 07 4772 5852

[2]Email: L.Bay@aims.gov.au

RUNNING TITLE: Vertical symbiont transmission in coral

KEYWORDS: Symbiosis, transmission mode, reef corals, calcification, bleaching, metabolite transfer

ABSTRACT

Cooperation between species is regularly observed in nature, but understanding of what promotes, maintains and strengthens these relationships is limited. We used a phylogenetically controlled design to investigate one potential driver in reef-building corals: variation in symbiont transmission mode. Two of three species pairs conformed to theoretical predictions stating that vertical transmission (VT) should maximize whole-organism fitness. *Montipora aequituburculata* (VT) exhibited greater net photosynthetic function, calcification rate and bleaching tolerance than its horizontally transmitting (HT) sister taxa *Acropora millepora*. Similar performance was observed in *Porites lobata* (VT) and *Goniopora columna* (HT), with the VT species showing higher fitness. However, *Galaxea archelia* (VT) was consistently out-performed by its HT congener *G. astreata*. Cooperation, quantified with radioisotope tracing of carbon sharing, was more variable across pairs, perhaps reflecting different optimization strategies necessary to maximize holobiont fitness. Variation in fitness patterns between VT and HT corals was not explained by differences in *Symbiodinium* communities among species; however, reproductive mode may play a role as both *M. aequituburculata* and *P. lobata* transmit symbionts transovarially whereas *G. archelia* broods larvae. Taken together, transmission mode may be an important driver of symbiosis evolution in some corals, but additional evolutionary mechanisms should be investigated.

1. INTRODUCTION

Inter-species cooperation has played a fundamental role in the evolution and diversification of life [1-4]. A continuum of relationships can be found in nature, ranging from fleeting facultative interactions, such as cleaner wrasse and their reef-fish clientele [5] to life-long partnerships, as in *Rhizobium*-legume symbioses [6], to fully integrated organelles, like mitochondria and chloroplasts within eukaryotic cells [7]. Yet despite pervasive examples across all taxonomic levels, surprisingly little is known about the factors that promote increased integration of inter-species partnerships and natural experimental systems for studying evolutionary transitions among cooperative states are lacking [8, 9].

Despite the positive connotation of "cooperative", these relationships are better defined as a spectrum that ranges from negative parasitic interactions to mutually beneficial symbioses, both within the context of a focal inter-species interaction and when comparing relationships across taxa [10-13]. One factor predicted to influence the level of cooperation between partners is the mode of symbiont transmission [14, 15]. Two transmission modes predominate: symbionts can be acquired horizontally from the local environment, usually during a defined larval stage, or vertically from parents, typically through the maternal germ line (reviewed in [16]). Virulence theory suggests that horizontal transmission allows symbionts to adopt selfish strategies, potentially harmful to the host [17]. A transition from horizontal to vertical transmission is predicted to align the reproductive interests of partners (via partner-fidelity feedback *sensu* [18]) and optimize resource sharing to maximize holobiont (the combination of host and symbiont) fitness [19-21]. Vertical transmission also results in the loss of an aposymbiotic life-stage for both hosts and symbionts; consequently, its evolution has also been proposed as a mechanism underpinning major transitions in individuality [9].

Experimental manipulations of transmission mode have provided empirical support for a reduction in pathogen virulence under enforced vertical transmission scenarios [22-25]. Bacteriophages forced into vertical transmission evolved lower virulence and lost the capacity to transmit horizontally [22]. Similarly, *Symbiodinium microadriaticum* under an experimentally enforced horizontal transmission regime proliferated faster within their *Cassiopea* jellyfish hosts while reducing host reproduction and growth [23]. However, studies quantifying the fitness consequences of shifts in transmission mode in natural systems remain rare, with Herre's demonstration of a negative relationship between vertical transmission and virulence in nematodes that parasitize fig wasps a notable exception [26].

Reef-building corals are a potential system in which to study naturally occurring transitions in transmission mode in a mutualistic symbiosis. Corals (Cnidaria: Anthozoa: Scleractinia) are colonial animals that harbour intracellular populations of dinoflagellate algae in the genus *Symbiodinium*. This symbiosis is considered obligate as the breakdown of the relationship between host animals and their intracellular *Symbiodinium* algae, commonly known as coral bleaching, has major fitness consequences for both partners, and can be lethal (reviewed in [27]). This inter-species partnership is ancient (evolved ~ 250 MYA [28]), prolific (600+ species worldwide [29]), and constitutes the foundation of one of the most bio-diverse and productive ecosystems on the planet. The majority of coral species (~85%) acquire their *Symbiodinium* horizontally from the local environment in each generation [30]. However, vertical transmission has evolved repeatedly across lineages, such that both transmission strategies can be exhibited by different coral species within the same genus [31-33].

While corals offer a potential opportunity to further our understanding of the evolution of symbiont transmission mode, there are also pressing applied reasons to study this relationship. Climate change and other anthropogenic processes threaten corals because of the

sensitivity of the coral-dinoflagellate symbiosis to environmental stress [34, 35]. However, there is substantial variation in both intra- and inter-specific bleaching thresholds [36], suggesting that levels of cooperation between host and symbiont may also vary. Significant work has gone into investigating coral bleaching over the past three decades, yet fundamental questions remain unresolved [37]. For example, five different hypotheses exist concerning the cellular mechanisms causing symbionts to be lost or ejected from host tissues and there is no consensus with respect to their relative importance during natural bleaching events [38]. A greater understanding of coral bleaching mechanisms is necessary for predicting and managing coral responses to climate change. Rather than asking what factors promote the breakdown of the coral-*Symbiodinium* relationship, here we investigate a factor predicted to reinforce it: the evolution of vertical symbiont transmission.

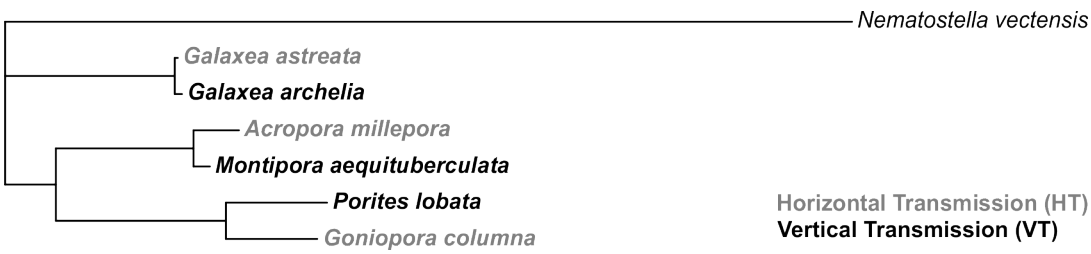


Figure 1. Cladogram of coral species used in this study (redrawn from 39,40) coloured by transmission mode.

We compare physiological components of fitness between horizontal and vertical transmitters in a phylogenetically controlled design of three pairs of related coral species exhibiting different strategies: (1) *Galaxea archelia* (vertical transmitter, VT) and *G. astreata* (horizontal transmitter, HT); (2) *Porites lobata* (VT) *and Goniopora columna* (HT); (3) *Montipora aequituburculata* (VT) *and Acropora millepora* (HT, Fig. 1). Species comparisons were drawn from the same or sister genera and replicate comparisons from more distantly related clades [39, 40]. Species also represented a diversity of reproductive modes (e.g. broadcast spawner, brooder), sexual systems (e.g. hermaphroditic, gonochoric), and morphologies (e.g. massive, branching), and host different subclades of *Symbiodinium* [41],

such that mode of symbiont transmission was the only consistent difference between pairs [31, 33, 42]. We quantified photosynthetic function and coral calcification (i.e. growth) under ambient conditions and changes in symbiont densities during thermal stress, to test the hypothesis that vertical transmission is associated with increased holobiont fitness. To examine patterns of cooperation we also quantified metabolic exchange between host and symbiont using radioisotopes. Finally, we conducted amplicon sequencing of the *Symbiodinium* ITS-2 locus to investigate whether symbiont community could explain patterns of fitness and cooperation among horizontal and vertical transmitters at a species level.

2. MATERIAL and METHODS

(a) *Coral Collection and Acclimation*

Fragments from sixty unique coral colonies (~genotypes), ~ 20 cm in diameter, were collected from reefs on the Central GBR from the 8-22 April 2015 under the Great Barrier Reef Marine Park Authority permit G12/35236.1 and G14/37318.1 (see Supplementary Material for additional details). Ten corals of each species, *Galaxea archelia* and *G. astreata* were collected from Davies Reef (18°49.816', 147°37.888'), ten corals each of *Acropora millepora* and *Montipora aequituburculata* were collected from Pelorus Island (18°33.358', 146°30.276') and ten corals each of *Goniopora columna* and *Porites lobata* were collected from Pandora Reef (18°48.778', 146°25.593') from depths of <10m. Corals were transported to AIMS Sea Simulator facility and placed in shaded holding tanks with 0.2 µM filtered flow-through seawater (FSW, 27°C, 150 µM PAR). Each individual coral was further cut into 6 genotype replicates using a diamond blade band saw and these fragments were mounted on aragonite plugs using either super-glue or marine epoxy. On 1 May 2015, fragments were moved into 12 50-L treatment tanks fitted with 3.5-watt Turbelle nanostream 6015 pumps (Tunze, Germany) with flow-through filtered seawater (FSW, ~25 liters/hour) at 27°C with a

120 12:12 light/dark cycle, peaking at 130-160 μM PAR at midday. Tanks were cleaned daily.
121 Beginning on 7 May, at 20 mins before dark every day, corals were fed *Artemia* naupli at a
122 density of 1-1.5 naupli/5ml and rotifers at a density of 1-3/ml.

123 (b) *Experimental Conditions and Physiological Trait Measurements*

124 Beginning on 28 May 2015, effective quantum yield of *Symbiodinium* photosystem II
125 (EQY) was measured daily for all experimental fragments using a pulse amplitude modulated
126 fluorometer (diving-PAM, Waltz) fitted with a plastic fibre optic cable (Fig. S1).
127 Measurements were made using factory settings with a measuring intensity of 12 and a gain
128 of 5 and taken at peak light intensity. EQY values were used to set the final sampling time
129 point, where a decline reflects an impact on the photosynthetic condition of the *Symbiodinium*
130 [43], indicating onset of the coral bleaching response.

131 On 31 May 2015, temperatures in the heat treatment tanks were increased at a rate of
132 1°C per day until temperatures reached 31°C (day 4, Fig S2). Sample time points occurred on
133 day 2 (29°C, 1 Jun), day 4 (31°C, 3 Jun) and day 17 (31°C, 16 Jun, Fig S2). On each
134 sampling day, one replicate of each genotype (n=10) and species (n=6) from each
135 temperature treatment (n=2; n=120 total per sampling day) were used to measure net
136 photosynthesis and light-enhanced calcification following the protocol described in Strahl et
137 al. [44].

138 To quantify net photosynthesis and instant calcification, corals were incubated in
139 enclosed acrylic chambers at their respective treatment temperatures and light levels for 1.5
140 h. Four chambers without corals were used as blanks to account for potential changes in
141 oxygen content and alkalinity due to the metabolic activity of other microorganisms in the
142 seawater. For net photosynthesis measures, the O_2 concentration of the seawater in each
143 chamber was measured at the end of the run using a hand-held dissolved oxygen meter

144 (HQ30d, equipped with LDO101 IntelliCAL oxygen probe, Hach, USA). Values from blank
145 chambers were subtracted from measures made in coral chambers and the subsequent rate of
146 net photosynthesis was related to coral surface area, calculated in μg $O_2/cm^2/min$. To
147 determine total alkalinity, a 120 ml subsample of seawater from each incubation chamber was
148 fixed with 0.5 mg mercuric chloride. Light calcification rates were determined from changes
149 in alkalinity quantified with the alkalinity anomaly technique [45] using a Titrando 855
150 Robotic Titrosampler (Metrohm AG, Switzerland). Values from blank chambers were
151 subtracted from measures made in coral chambers and calcification rate was related to coral
152 surface area and calculated in μM $CaCO_3/cm^2/min$.

153 Tissue was removed from snap frozen coral skeletons using an air gun and
154 homogenized for 60 s using a Pro250 homogenizer (Perth Scientific Equipment, AUS). A
155 300-μl aliquot of the tissue homogenate was fixed with 5% formalin in FSW and used to
156 quantify *Symbiodinium* cell density. The average cell number was obtained from four
157 replicate haemocytometer counts of a 1-mm^3 area and cell density was related to coral surface
158 area and expressed as cells/cm^2. The remaining homogenate was centrifuged for 2 min at
159 3500 rcf to separate host and symbiont fractions. For ^{14}C bicarbonate treated corals, 2 ml of
160 the host tissue slurry was frozen at -20°C. For ^{14}C-artemia/rotifer treated corals, the host
161 slurry was discarded and the *Symbiodinium* cell pellet was washed with FSW, resuspended in
162 2 ml FSW and frozen at -20°C. Coral skeletons were rinsed with 5% bleach then dried at
163 room temperature. Skeletal surface area was quantified using the single wax dipping method
164 [46] and skeletal volume was determined by calculating water displacement in a graduated
165 cylinder.

166 To measure resource sharing from algal symbionts to host animals, five genotypes of
167 each species from each treatment (n=30 control, n=30 heat) were placed into 18-L of FSW
168 with a 5-W aquarium pump for circulation and ^{14}C-bicarbonate (specific activity: 56

169 mCi/mmol) was added to a final concentration of 0.28 µCi/ml. Corals were incubated for 5
170 hours in light conditions identical to experimental tanks, rinsed with flow-through FSW for
171 one hour to remove remaining unfixed ^{14}C then snap frozen in liquid nitrogen. To measure
172 resource sharing from host animal to algal symbionts, freshly hatched *Artemia* (~600
173 naupli/ml) and rotifers (~600/ml) were incubated for 30 hours pre-feeding in FSW with 0.63
174 µCi/ml ^{14}C-glycine at 21°C in closed falcon tubes with gentle rotation (10 rpm), following
175 the protocols of [47, 48]. This radioactive feed mix was washed for 5 min in FSW and added
176 to treatment tanks containing the remaining five genotypes per species (n=30 control, n=30
177 heat) in place of the normal afternoon feed (1-1.5 naupli/5ml and 1-1.5 rotifers/ml).
178 Aquarium pumps were turned off for one hour and water flow was turned off for two hours to
179 allow for food capture by corals. Corals were snap frozen in liquid nitrogen the following
180 morning, 18 hrs post-feeding, following the protocol of [47].

181 Sample radioactivity was determined using a liquid scintillation counter (Tri-Carb
182 2810TR v2.12, Perkin Elmer, USA). For host samples, triplicate 300 µl aliquots were mixed
183 with 3.5 ml Ultima Gold XR liquid scintillation cocktail (Perkin Elmer, USA). Samples were
184 temperature and light adapted for 1 hour and then counted for 1.5 min using the default
185 parameters. Counts per minute were converted to disintegrations per minute (DPM) using a
186 standard curve derived from a ^{14}C Ultima Gold Quench Standards Assay (Perkin Elmer,
187 USA). For *Symbiodinium* samples, 500 µl aliquots were mixed with 500 µl 45% Sodium
188 hypochlorite. Triplicate technical replicates were run for day 17 samples, duplicates for day 4
189 samples and only a single replicate for day 2 samples. Samples were incubated for 2 hrs at
190 60°C to bleach chlorophyll. Samples were then mixed with 3 ml Ultima Gold XR liquid
191 scintillation cocktail; temperature and light adapted for 1 hour and counted using the same
192 conditions as host samples. Technical replicates were averaged and counts were related to

193 total *Symbiodinium* cell number and coral surface area, calculated in ^{14}C DPM/cell/cm^2 to
194 control for variation in host size and symbiont cell density.

195 To identify major *Symbiodinium* clades hosted by focal species, DNA was extracted
196 from symbiont fractions of the tissue homogenate for each genotype of each species (n=60)
197 using Wayne's method [49]. A restriction digest of the LSU region of *Symbiodinium* rRNA
198 [50, 51] across individuals consistently revealed single bands indicating the dominance of a
199 single *Symbiodinium* clade for four of the six species (*A. millepora*, *M. aequituburculata*, *G.*
200 *columna* and *P. lobata*) whereas communities in *G. astreata* and *G. archelia* appeared more
201 variable (Fig. S3). Therefore, DNA was pooled in equal proportions by species (n=6 samples)
202 to identify general species-specific communities and additional DNA samples for each of the
203 *G. astreata* and *G. archelia* individuals (n=20 samples) were submitted for amplicon
204 sequencing of the ITS2 region of *Symbiodinium* rRNA at the Genome Sequencing and
205 Analysis Facility at the University of Texas at Austin.

206 (c) *Statistical Analyses*

207 All statistical analyses were performed in R, version 2.15.3 [52]. A series of linear
208 mixed models were used to determine the effect of transmission mode on physiological
209 components of fitness and cooperation. First, we determined the effect of transmission mode
210 under ambient conditions. For corals from control tanks, we modelled the fixed effects of
211 transmission mode (levels: vertical, horizontal), sampling day (levels: day 2, day 4, day 17)
212 and their interaction on *Symbiodinium* cell density, net photosynthesis, light calcification and
213 host-symbiont resource sharing using the *lme* command of the *nlme* package [53]. Symbiont
214 densities and DPM values were log-transformed prior to statistical analyses to satisfy
215 assumptions of linearity and homoscedasticity of model residuals. Patterns were evaluated
216 within each focal species pair (*G. archelia* and *G. astreata; A. millepora* and *M.*

217 *aequituburculata*; *G. columna* and *P. lobata*), including a random effect of coral genotype
218 within species.

219 Next, we explored changes in each of these traits for each of the focal species pairs at
220 the end of a 17-day time-course of exposure to temperature stress. We modelled changes in
221 *Symbiodinium* cell density, net photosynthesis, light calcification and host-symbiont resource
222 sharing as a function of the fixed effects of transmission mode, temperature treatment, and
223 their interaction, again including a random effect of genotype within species. Symbiont
224 densities, and DPM values were log-transformed prior to statistical analyses.

225 All models were assessed for normality of residuals and homoscedasticity. To assess
226 goodness-of-fit, we used the function *rsquared.glmm* to calculate the conditional R^2 value for
227 each of our mixed models [54]. Significance of fixed factors within models was evaluated
228 using Wald tests and Tukey's post-hoc tests were used to evaluate significance among levels
229 within factors and interactions when warranted.

230 Amplicon sequencing data were analysed following methods described in [55]. A
231 total of 858,110 paired-end reads were generated and the program *cd-hit* [56] was used to
232 trim, quality filter and assemble raw reads into 100% identical groups, which resulted in 38
233 operational taxonomic units (OTUs). This stringent cut-off was used in order to distinguish
234 closely-related sub-groups within Clade C since C1 and C15, for example, are not resolved
235 with lesser thresholds. For pooled species samples, 59% of reads were mapped to these 38
236 OTUs, with total numbers ranging from 6213 to 20403 reads per sample pool (mean =
237 14479). For individual *Galaxea* spp. samples, 62% of reads were mapped, with 277 to 57728
238 reads per individual (mean = 23231). The *MCMC.OTU* package (ref) was used to analyse
239 pooled samples and individual *Galaxea* spp. sample sets independently, discarding both
240 outlier samples (those with total counts below z-score cutoff of -2.5) and OTUs (those

241 comprising less than 0.1% of the total sum of counts as per [57]). We then compared
242 normalized proportions of remaining OTUs represented across remaining samples and used a
243 blastn search against NCBI's *nr* database [58] to identify *Symbiodinium* types represented by
244 dominant OTUs. To determine the impact of *Symbiodinium* communities on fitness responses
245 within and among species pairs, OTU data were fit to a model incorporating a fixed effect of
246 performance (categorized as 'high' or 'low' fitness within each pair, e.g. Fig. 2).

247 3. RESULTS

248 (a) *The effect of transmission mode on physiological components of fitness*

249 (i) *Acropora millepora* and *Montipora aequituberculata*

250 Net photosynthesis was elevated in the vertical transmitting (VT) coral *M.*
251 *aequituberculata* compared to *A. millepora*, its horizontally transmitting (HT) relative
252 (R^2_{GLMM}=0.51, P<0.001, Fig 2A). *M. aequituberculata* also exhibited significantly elevated
253 rates of calcification (R^2_{GLMM}=0.47, P<0.0001, Fig 2B) and showed greater resilience to 17
254 days of elevated temperature treatment, retaining higher *Symbiodinium* densities than *A.*
255 *millepora* (Tukey's HSD<0.05, Fig. 2C).

256 (ii) *Goniopora columna* and *Porites lobata*

257 Trait patterns in *G. columna* and *P. lobata* were highly similar to the *M.*
258 *aequituberculata* - *A. millepora* pair. *P. lobata* (VT) exhibited greater net photosynthesis than
259 its HT counterpart, *G. columna* (R^2_{GLMM}=0.68, P_{Trans}<0.0001, Fig 2D), though an interaction
260 with sampling day was also observed, as the effect size was slightly less pronounced on the
261 final day of sampling (0.11 μg/cm^2/min, Tukey's HSD<0.01) when compared to the earlier
262 days (day 1: 0.15 μg/cm^2/min, Tukey's HSD<0.001; day 3: 0.21 μg/cm^2/min, Tukey's
263 HSD<0.001, Fig S4). Coral host calcification also tended to be elevated in *P. lobata*, but the

difference was not significant and the effect varied among sampling days (R^2_{GLMM}=0.42, P=0.06, Fig 2E, Fig. S5). Thermal tolerance, however, was significantly greater in *P. lobata* when compared to *G. columna* (Tukey's HSD<0.05, Fig. 2F).

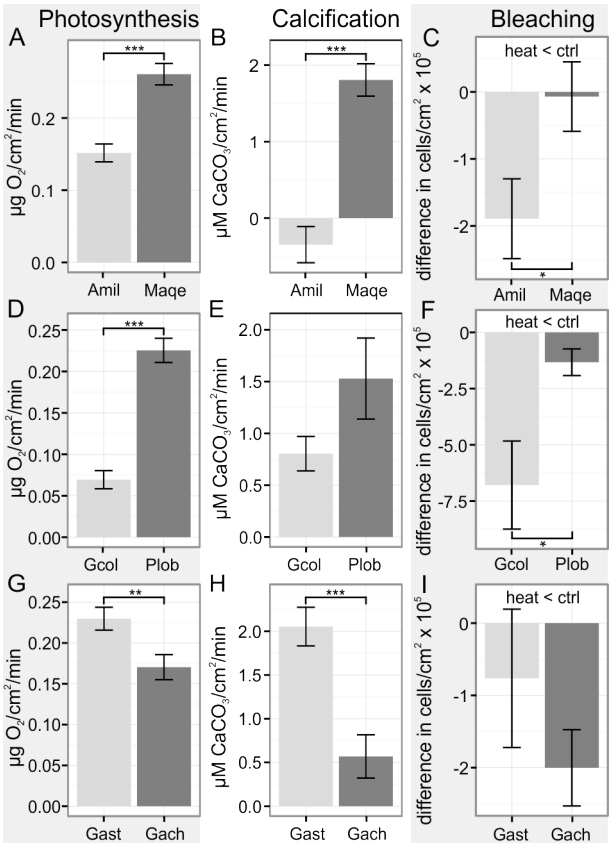


Figure 2. Effect of transmission mode on fitness-related traits across species pairs. (A,D,G) Mean net photosynthesis (±SEM, n=30 per species), (B,E,H) mean calcification (±SEM, n=15 per species) under ambient conditions (27°C) and (C,F,I) mean bleaching (±SEM, n=30 per species), quantified as the loss of *Symbiodinium* in heat-treated corals (31°C) relative to their paired controls in (A-C) *Acropora millepora* and *Montipora aequituberculata* , (D-F) *Goniopora columna* and *Porites lobata*, and (G-H) *Galaxea astreata* and *G. archelia*. In bleaching panels, 'heat<ctrl' indicates a significant effect of temperature treatment (P<0.05), independent of transmission mode. Stars indicate significant difference between species pairs (*<0.05, **<0.01, ***<0.001).

(iii) *Galaxea astreata* and *Galaxea archelia*

Contrary to predictions, the HT congener, *G. astreata*, exhibited greater net photosynthesis (R^2_{GLMM}=0.21, P<0.01, Fig 2G) and calcification (R^2_{GLMM}=0.30, P<0.001, Fig 2H) than *G. archelia* (VT). Thermal tolerance was not significantly different between these species (Fig. 2I).

(b) *The effect of transmission mode on host-symbiont cooperation*

(i) *Acropora millepora* and *Montipora aequituberculata*

Symbiodinium densities did not differ between these species (R^2_{GLMM}=0.51, Fig 3A). *A. millepora* (HT) received more photosynthetically fixed carbon from its symbionts than *M. aequituberculata* (VT, R^2_{GLMM}=0.51, P<0.01, Fig 3B) and hosts exhibited a non-significant trend of sharing more heterotrophically acquired carbon with each of their symbionts (R^2_{GLMM}=0.29, P=0.17, Fig 3C).

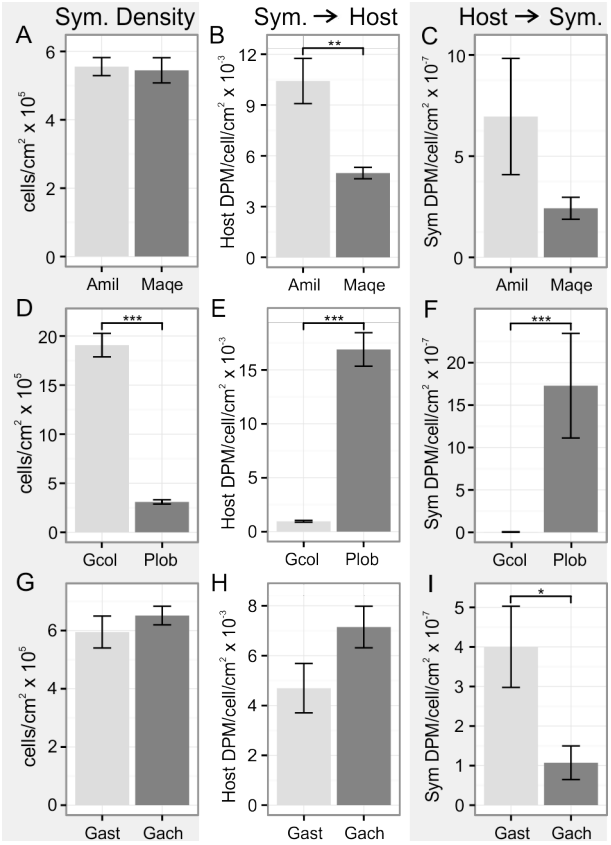


Figure 3. Effect of transmission mode on cooperation across species pairs in ambient conditions (27°C). (A,D,G) Mean *Symbiodinium* cell density (±SEM, n=30 per species), (B,E,H) mean carbon transfer from symbiont to host (±SEM, n=15 per species) and (C,F,I) mean carbon transfer from host to symbiont (±SEM, n=15 per species) in (A-C) *Acropora millepora* and *Montipora aequituberculata* , (D-F) *Goniopora columna* and *Porites lobata*, and (G-H) *Galaxea astreata* and *G. archelia*. Stars indicate significant difference between species pairs (*<0.05, **<0.01, ***<0.001).

297 (ii) *Goniopora columna* and *Porites lobata*

298 *P. lobata* (VT) exhibited reduced *Symbiodinium* densities in comparison to *G.*
299 *columna* (HT, R^2_{GLMM}=0.89, P<0.0001, Fig 3D). However, each *P. lobata* symbiont
300 transferred more photosynthetically fixed carbon to its host (R^2_{GLMM}=0.94, P<0.0001, Fig 3E)
301 and hosts shared more heterotrophically acquired carbon with each of their symbionts
302 (R^2_{GLMM}=0.97, P<0.0001, Fig. 3F) than *G. columna*, although the absolute amount shared
303 varied among sampling days (P=0.02, Fig. S6).

304 (iii) *Galaxea astreata* and *Galaxea archelia*

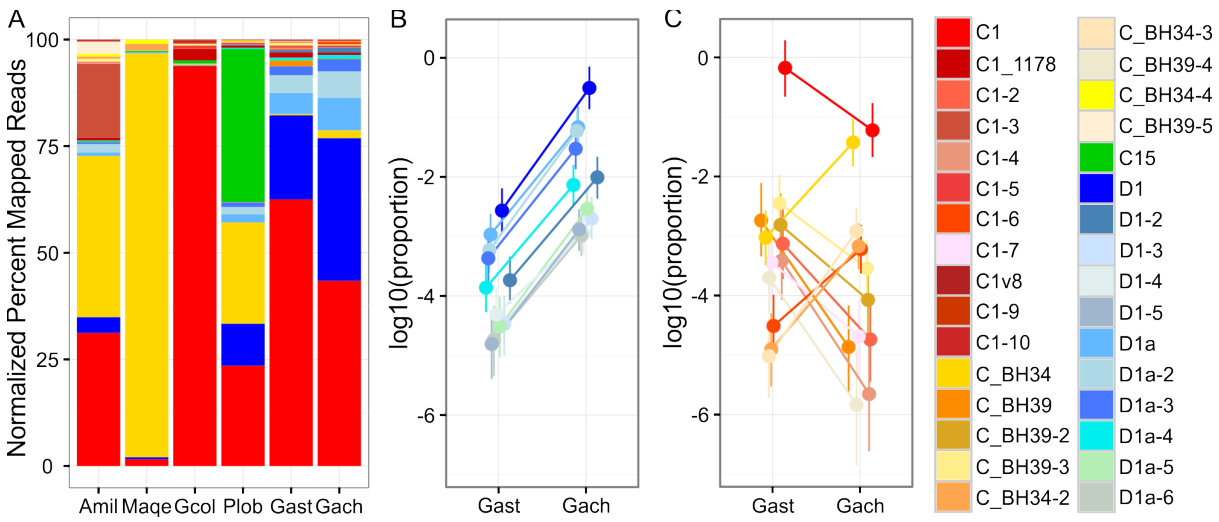
305 *Symbiodinium* densities did not differ between *G. archelia* (VT) and *G. astreata* (HT,
306 R^2_{GLMM}=0.46, Fig 3G). *G. archelia* symbionts tended to share more carbon with their hosts,
307 but this trend was not significant (R^2_{GLMM}=0.49, P=0.1, Fig 3H) whereas *G. astreata*
308 exhibited greater host to symbiont carbon transfer, although this pattern varied among
309 sampling days (R^2_{GLMM}=0.66, P<0.05, Fig 3I, Fig. S6).

310 (c) *Does Symbiodinium community composition explain fitness differences?*

311 For species pools, 24 operational taxonomic units (OTUs) of sufficient representation
312 (>0.1% abundance, *sensu* [57]) were identified and consisted of clade C1 and D1-type
313 *Symbiodinium* (Table S1). *Symbiodinium* communities varied among species (Fig. 4A) and a
314 model comparing high performing (*M. aequituberculata, P. lobata, G. astreata*) and low
315 performing species (*A. millepora, G. columna, G. archelia*, Fig. 2) found no relationship
316 between observed fitness differences and changes in abundance of these OTUs.

317 In *A. millepora*, 86% of reads were accounted for by three major OTUs all matching
318 to clade C-type *Symbiodinium*, identified as variants of C1 (Fig. 4A). *M. aequituberculata*
319 reads nearly all mapped to one of these same variants (C_BH34, 95%, Fig. 4A). Reads were

320 similarly uniform in *G. columna*, with 94% mapping to the most common C1-type OTU
321 across samples (Fig. 4A). In *P. lobata*, read diversity was more variable. 36% of reads
322 mapped to a C15-type OTU (Fig. 4A), a common associate of *Porites* spp. in the Indo-Pacific
323 [59]. An additional 47% of *P. lobata* reads also mapped to two other C1-type symbiont
324 OTUs, while 10% were identified as the most abundant clade D-type OTU, D1 (Fig. 4A).
325 Reads from both *Galaxea* spp. predominantly mapped to the major C1-type OTU identified
326 across species (63% of *G. astreata* reads and 43% of *G. archelia* reads, Fig. 4A) but these
327 species also contained major fractions of an OTU identified as a D1-type *Symbiodinium* (*G.*
328 *astreata*: 20%, *G. archelia*: 33%, Fig. 4).

329 

330 Figure 4. *Symbiodinium* community composition. (A) Normalized proportion of reads mapping to each
331 *Symbiodinium* OTU (>0.1% abundance) from pooled samples of all individuals within each focal species. Log_{10}
332 proportion of (B) D-type and (C) C-type OTUs showing significant differences in abundance between individual
333 samples of *G. astreata* and *G. achrelia* by species (FDR<0.05).

334

335 Significant differences in OTU abundance were detected between *G. astreata* and *G.*
336 *archelia* when individual coral samples were analysed (Fig. 4B,C). For this sample set, 27
337 OTUs passed the abundance threshold, consisting of the same major OTUs identified in the
338 species pools and a few additional low abundance variants (<1%, Table S1). Of these, 23
339 showed significant differences in abundance between the two species (FDR<0.05). All of the

340 significant D-type OTUs showed increased abundance in *G. archelia* relative to *G. astreata*
341 (11/11, Fig. 4B) whereas the majority of C-type OTUs showed decreases (8/12, Fig. 4C). The
342 most notable exception to the increased D/decreased C pattern was the second most abundant
343 C-type OTU across samples, C_BH34, which was elevated in *G. archelia* (Fig. 4C, Table
344 S1).

345 4. DISCUSSION

346 Understanding variation in the degree of cooperation between corals and their
347 *Symbiodinium* will be critical for assessing survival potential among species and populations
348 in the face of increasing environmental change [12]. As in other mutualisms [19-21], vertical
349 transmission has been proposed as an evolutionary mechanism for enhancing holobiont
350 fitness in the Cnidarian-algal symbiosis [60]. This study is the first to empirically test these
351 predictions by quantifying photosynthesis, calcification, thermal tolerance and carbon sharing
352 using a phylogenetically controlled design that pits vertically transmitting corals against their
353 closest horizontally transmitting relatives (Fig. 1).

354 On average, trait patterns support theoretical predictions for the evolution of vertical
355 transmission (Fig. S7), but individual species pairs differ in their degree of conformity. Both
356 *P. lobata* and *M. aequituburculata* (VT) exhibited elevated net photosynthesis, calcification
357 and thermal tolerance, or greater holobiont fitness, than their HT counterparts (Fig. 2A-F),
358 consistent with predictions. However, the VT *G. archelia* provides no support as it was
359 consistently out-performed by its HT relative, *G. astreata* (Fig. 2G-I). Possible explanations
360 for these results include variation in *Symbiodinium* community or in life-history traits among
361 species.

362 *Symbiodinium community composition does not explain differential fitness and cooperation*
363 *among taxa*

364 Previous work has shown that variation in *Symbiodinium* community composition can
365 impact holobiont fitness and cooperation. For example, conspecific corals hosting clade D
366 *Symbiodinium* exhibit greater thermal tolerance than those hosting C1 or C2-types [61, 62],
367 however they generally grow more slowly under non-stressful conditions [63, 64] and receive
368 less photosynthetically fixed carbon from their symbionts [65]. We investigated
369 *Symbiodinium* community composition in our six focal species (Fig. 4) but did not detect any
370 consistent differences that could explain differential performance in high vs. low fitness
371 species (Fig. 2). The low-performing *A. millepora* and *G. archelia* did host more D-type
372 *Symbiodinium* than their paired counterparts, but so did *P. lobata*, the only species to exhibit
373 both elevated fitness and cooperation (Fig. 2,3,4). The overall diversity of symbionts hosted
374 was also unrelated to fitness, as both the high performing *M. aequituburculata* and low
375 performing *G. columna* hosted highly homogenous communities, with a single OTU
376 comprising 94-95% of reads in each (Fig. 4A). Taken together, these results support prior
377 observations that *Symbiodinium* community composition alone is not sufficient to explain
378 variation in coral fitness and that interactions with the host must also be considered [66-68].

379 *Does reproductive mode influence the mode of symbiont transmission in corals?*

380 Among the selected vertically transmitted species used in this study, *Porites lobata*
381 and *Montipora aequituburculata* exhibit a different reproductive life-history strategy than
382 *Galaxea archelia*, which may provide an alternate explanation for the lack of conformity in
383 the *Galaxea* spp. pairing relative to the other two. Two reproductive modes predominate in
384 reef-building corals: broadcast spawning and internal brooding. Broadcast spawning, the
385 majority strategy, is characterized by an annual release of gametes that undergo fertilization
386 at the ocean surface to produce planktonic larvae [33]. Brooders release only sperm,
387 fertilization is internal and larvae develop within parental tissue [69]. *P. lobata* and *M.*
388 *aequituburculata* are broadcast spawning corals that transmit *Symbiodinium* in oocytes. *G.*

389 *columna*, *A. millepora* and *G. astreata* are also broadcast spawning corals, but do not exhibit
390 vertical transmission of *Symbiodinium*. Whereas *G. archelia* is a brooding coral that releases
391 planulae larvae infected with the maternal symbiont type.

392 Predictions regarding the cooperative and fitness benefits of evolving vertical
393 transmission are based on the assumption that the host-symbiont relationship becomes
394 exclusive: symbiont populations are substantially reduced, resulting in genetic uniformity,
395 more rapid co-evolution of partner traits and reduction in intra-symbiont community
396 competition [4, 20]. Different mechanisms of symbiont infection are likely utilized in
397 brooders and broadcasters that could impact the strictness of vertical transmission. In
398 *Montipora* corals, *Symbiodinium* have been observed entering the oocytes between 8 and 32
399 hours prior to spawning [70] suggesting a specific mechanism with a defined window of
400 infectivity in broadcasting vertical transmitters. In horizontally transmitting corals,
401 phagocytosis is considered the predominant mode of symbiont uptake whereby larvae or
402 juvenile coral polyps consume *Symbiodinium* via active feeding [71] though developmentally
403 earlier acquisitions have also been observed [72]. The time-window for uptake and
404 establishment of symbiosis in these HT broadcast-spawned juveniles spans days to weeks and
405 the initial symbiotic relationships they establish can be highly promiscuous, suggesting that
406 the early establishment process may be passive on the part of the host [73, 74]. It is possible
407 that vertical transmission in brooding corals, such as *G. archelia,* occurs by a similar passive
408 larval uptake mechanism, save that the first infection occurs in the maternal environment.
409 Two recent studies suggest that acquisition of additional *Symbiodinium* types can occur after
410 the initial maternal infection [75, 76], supporting this conjecture. A meta-analysis by Fabina
411 *et al.* [77] also observed more diverse symbiotic interactions in brooding vertical transmitters
412 than in broadcasting vertical transmitters, further suggesting that the strictness of vertical
413 transmission may be dependent on the mode of infection across life-histories.

414 Competition among maternally-derived and environmentally-acquired *Symbiodinium*
415 would negate the fitness benefits associated with strict vertical transmission in brooding
416 corals, providing a possible explanation for the observed difference between *G. archelia* and
417 the two transovarial transmitters, *A. millepora* and *P. lobata*. However, as only one brooding
418 coral was used in the present study, it is difficult to draw any firm conclusions. Additional
419 comparative data on fitness in brooding corals and the mechanism of uptake and
420 establishment of symbiosis in general [78] are needed to test this hypothesis.

421 *The disconnect between cooperation and fitness*

422 The role of cooperation, defined here as resource sharing between hosts and
423 symbionts, in determining fitness outcomes of horizontal and vertical transmitters also
424 requires additional study as patterns of host-symbiont cooperation did not necessarily match
425 fitness patterns across species pairs. Cooperation and fitness were aligned in *P. lobata*:
426 *Symbiodinium* densities were naturally lower (Fig. 3D), suggesting reduced symbiont
427 virulence (*sensu* [23]), while carbon sharing between partners was greater (Fig. 3E,F),
428 suggesting enhanced cooperation. However, resource sharing in the other high-fitness vertical
429 transmitter, *M. aequituburculata*, was characterized by reduced symbiont to host carbon
430 transfer (Fig. 3B) and a tendency towards reduced host to symbiont transfer (Fig. 3C), though
431 *Symbiodinium* densities were not significantly different between species in this focal pair
432 (Fig. 3A). For *Galaxea* spp., *Symbiodinium* densities were also similar between species, but
433 symbiont to host carbon transfer tended to be greater in *G. archelia*, while host to symbiont
434 transfer was greater in *G. astreata* (Fig. 3G-I).

435 Quantifying cooperation between symbiotic partners in terms of biologically realistic
436 costs and benefits remains an outstanding question for many symbioses [20]. The transfer of
437 photosynthetically fixed carbon has long been known as a major cooperative benefit to the

438 coral host as up to 95% of a coral's energy requirements can be met through this mechanism
439 [79]; however, reciprocal products shared by hosts with their symbionts remain largely
440 unknown [80]. We chose to enrich our heterotrophic treatment (*Artemia*+Rotifers) with ^{14}C-
441 glycine because it is one of the few products known to be translocated from invertebrate hosts
442 to *Symbiodinium* [81, 82], but this metabolite may not be the most critical for *Symbiodinium*
443 fitness. The recently sequenced *Symbiodinium kawagutii* genome lacks biosynthesis
444 pathways for lysine and histidine [83], which may be better candidate host-derived amino
445 acids to investigate. Similarly, heterotrophic feeding can offset the need for symbiont-derived
446 carbon in some species and in these cases other symbiont-derived metabolites may be more
447 critical for host fitness. *Montipora capitata* showed a greater capacity for heterotrophic
448 supplementing of metabolism than two *Porites* spp. during lab-induced bleaching [84],
449 providing a possible explanation for the differences in symbiont to host carbon sharing
450 observed between *Montipora* and *Porites* in the present study. Ultimately, a greater
451 understanding of the metabolic interactions between coral hosts and symbionts is needed in
452 order to test their roles in fluctuating cost/benefit scenarios.

453 5. CONCLUSIONS

454 The major transitions approach, defined by Maynard Smith and Szathmáry [4], has
455 recently been proposed as a unifying conceptual framework for investigating how fully
456 integrated symbioses evolve from simple partnerships [3, 9]. We suggest that reef-building
457 corals meet the criteria for a major transition between species: the partnership is obligate,
458 vertical transmission has evolved repeatedly, and in some cases, this evolution has aligned
459 partner interests, resulting in increased holobiont fitness. Reef-building corals have the
460 potential to fill an empirical gap in the study of major transitions between species and new
461 investigations into the coral-algal symbiosis using this broader evolutionary framework will
462 increase understanding of one of the most ecologically important and critically endangered

463 symbioses on the planet. For example, changes in transmission mode are not the only means
464 of increasing cooperation between species [18]. Partner sanctions, in which hosts selectively
465 weed out underperforming symbionts or reward the most productive individuals, are known
466 to be important in other endo-symbioses [85, 86]. Corals are known to regularly expel viable
467 symbionts (reviewed in [78]); however, this expulsion has yet to be examined in the context
468 of cooperation. It is possible that this process is not random, but rather targets removal of
469 underperforming symbionts. An improved molecular tool-kit is needed in order to
470 manipulate *Symbiodinium* performance and track subsequent expulsion to test this
471 hypothesis. Emerging model systems, such as *Aiptasia* [87], will likely facilitate future work
472 in this area.

473 DATA ARCHIVING

474 All scripts and input files will be available on DRYAD upon manuscript acceptance.

475 COMPETING INTERESTS

476 We have no competing interests.

477 AUTHOR CONTRIBUTIONS

478 CDK designed research; CDK and LKB performed research; CDK analysed data; CDK wrote
479 the first draft of the manuscript and both authors contributed to revisions and gave final
480 approval for publication.

481 ACKNOWLEDGEMENTS

482 The authors gratefully acknowledge the efforts of A Bouriat in maintaining aquaria and
483 processing coral samples. M Salmon, G Milton, A Severati, C Humphrey implemented the
484 experimental aquaria design. Coral collection was accomplished with the help of S Noonan,

V Mocellin, A Severati and M Nayfa. P Muir provided advice on coral taxonomic identification. Comments from J Caley, B Schaffelke and 2 anonymous reviewers greatly improved this manuscript.

FUNDING

An NSF International Postdoctoral Research Fellowship, DBI-1401165, to CDK and funding from the Australian Institute of Marine Science to LKB, supported this work.

504 Table S1. Best BLASTn matches to the NCBI *nr* database [58] for the most abundant
505 operational taxonomic units (OTUs) detected across samples (>0.1% abundance). Value in %
506 column indicates mean percent abundance across all samples in which OTU was identified.
507 Origin column indicates which sample set OTU was identified in, species pools (P) or
508 individual *Galaxea* spp. (I).

OTU (name)	**%**	**Origin**	**Description**	**E-value**	**Accession**
OTU1 (C1)	49	P, I	Symbiodinium sp. C1	0.0	JN558041.1
OTU2 (D1)	31	P, I	Symbiodinium sp. D1	3e-174	JN558075.1
OTU3 (C_BH34)	13	P, I	Symbiodinium sp. C	4e-168	JN711493.1
OTU4 (D1a)	7	P, I	Symbiodinium sp. D1a	2e-170	JN558080.1
OTU5 (D1a-2)	6	P, I	Symbiodinium sp. D1a	2e-170	JN558080.1
OTU6 (D1a-3)	3	P, I	Symbiodinium sp. D1a	2e-168	JN558080.1
OTU7 (C_BH39)	1	P, I	Symbiodinium sp. C	5e-172	JN711498.1
OTU8 (C15)	2	P, I	Symbiodinium sp. C15	1e-173	JN558044.1
OTU9 (D1a-4)	0.7	P, I	Symbiodinium sp. D1a	1e-168	JN558080.1
OTU10 (C1_1178)	0.7	P, I	Symbiodinium sp. C1	4e-168	EU074889.1
OTU11 (D1-2)	1	P, I	Symbiodinium sp. D1	2e-155	AB778759.1
OTU12 (C1-2)	0.4	P, I	Symbiodinium sp.	4e-167	EU786053.1
OTU13 (C1-3)	0.4	P	Symbiodinium sp.	4e-167	EU118163.1
OTU14 (C1-4)	0.2	P, I	Symbiodinium sp.	4e-167	EU786015.1
OTU15 (C1-5)	0.2	P, I	Symbiodinium sp.	3e-164	KP134434.1
OTU16 (C_BH39-2)	0.2	P, I	Symbiodinium sp. C	2e-165	JN711498.1
OTU17 (C_BH39-3)	0.5	P, I	Symbiodinium sp. C	2e-165	JN711498.1
OTU18 (D1-3)	0.2	I	Symbiodinium sp. D1	1e-163	JN558075.1
OTU19 (C1-6)	0.2	P, I	Symbiodinium sp. C1	1e-163	JN558041.1
OTU20 (C_BH34-2)	0.2	P, I	Symbiodinium sp. C	4e-158	JN711493.1
OTU21 (D1a-5)	0.3	I	Symbiodinium sp. D1a	5e-162	JN558080.1
OTU22 (C_BH34-3)	0.3	I	Symbiodinium sp. C	8e-160	JN711493.1
OTU23 (C1-7)	0.1	I	Symbiodinium sp.	1e-163	KP134433.1
OTU24 (D1-4)	0.1	I	Symbiodinium sp. D1	3e-159	JN558075.1
OTU25 (C_BH34-4)	0.3	P	Symbiodinium sp. C	2e-146	JN711493.1
OTU26 (C_BH39-4)	0.1	I	Symbiodinium sp. C	2e-165	JN711498.1
OTU27 (D1a-6)	0.1	I	Symbiodinium sp. D1a	3e-159	JN558080.1
OTU28 (C1_1142)	0.1	I	Symbiodinium sp. C1	2e-165	EU074884.1
OTU30 (C_BH39-5)	0.7	P	Symbiodinium sp. C	1e-163	JN711498.1
OTU31 (C1-9)	0.1	P	Symbiodinium sp. C1	1e-163	JN558041.1
OTU34 (C1v8)	0.1	P	Symbiodinium sp. C1	1e-147	HG942434.1
OTU35 (C1-10)	0.1	P	Symbiodinium sp. C1	3e-154	JN558041.1
OTU38 (D1-5)	0.1	I	Symbiodinium sp. D1	1e-163	JN558075.1

509

510

511

512

513

514

Figure S1. Daily measures of effective quantum yield (EQY±SEM, note that range of error bars is extremely small, in most cases barely exceeding points) of *Symbiodinium* photosystem II by temperature treatment. Bars show the difference in mean EQY between treatments through time (n=720 per bar).

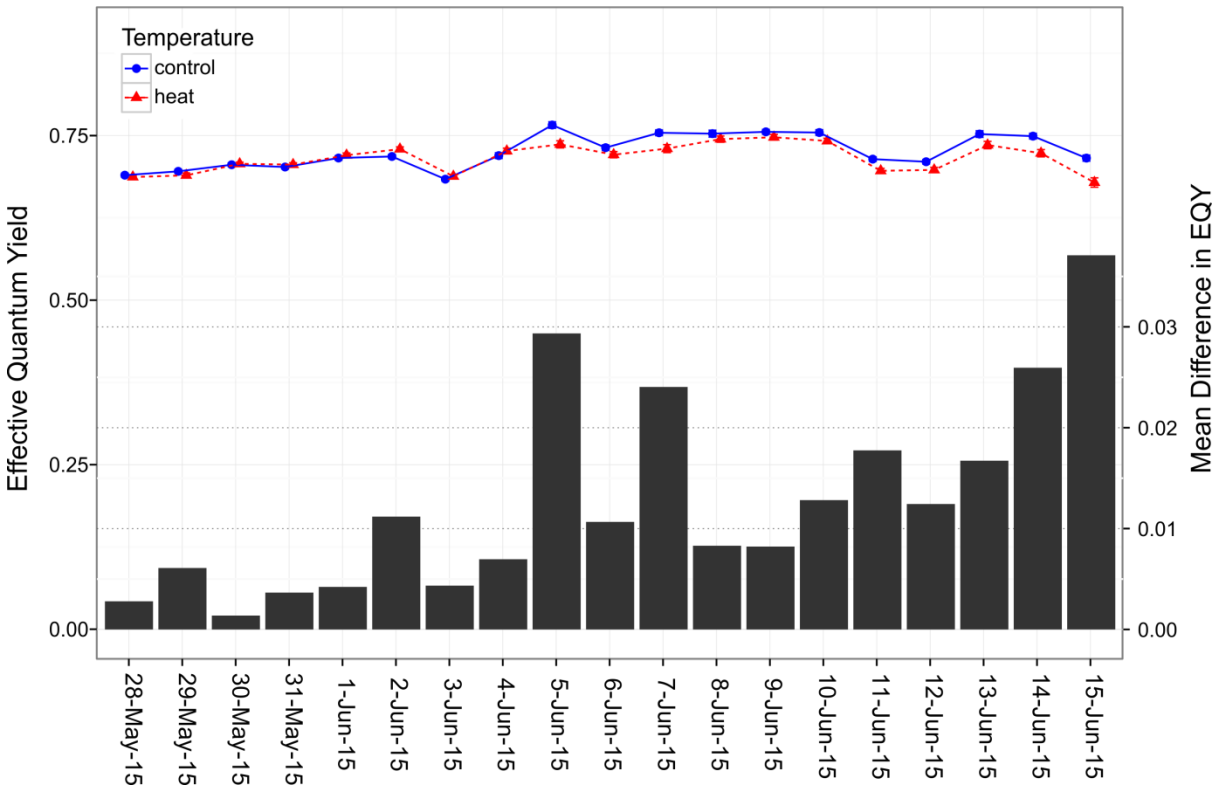


Figure S2. Temperature profile of experimental treatment tanks (±SD) by date. Bars indicate time of sampling.

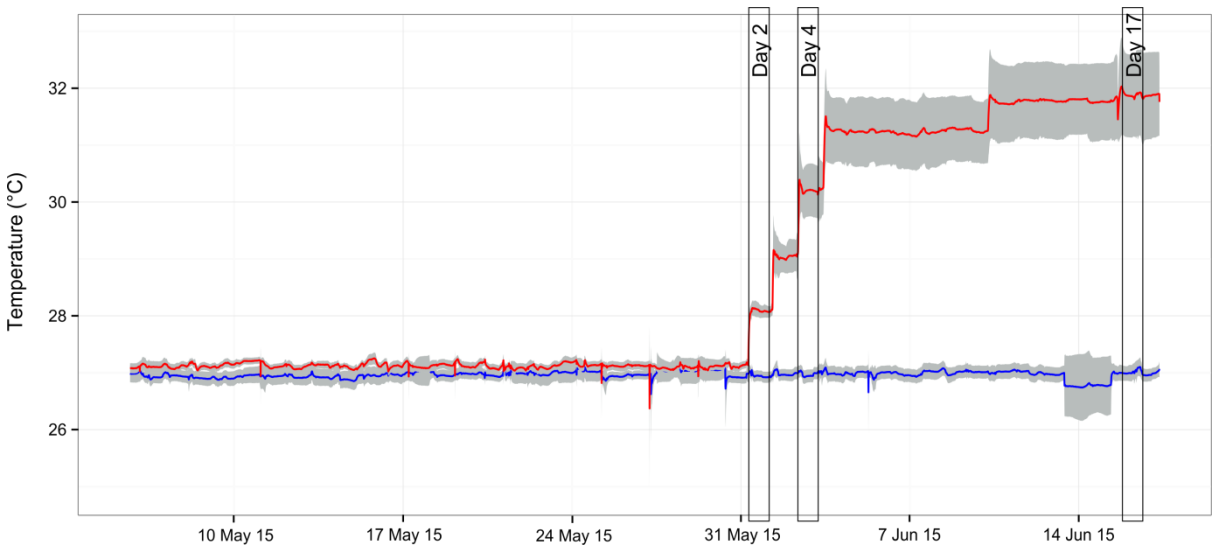


Figure S3. Taq1 digest of *Symbiodinium* LSU rRNA. Samples are grouped by species pairs. Standard shows expected banding pattern for *Symbiodinium* genotypes in clades C and D.

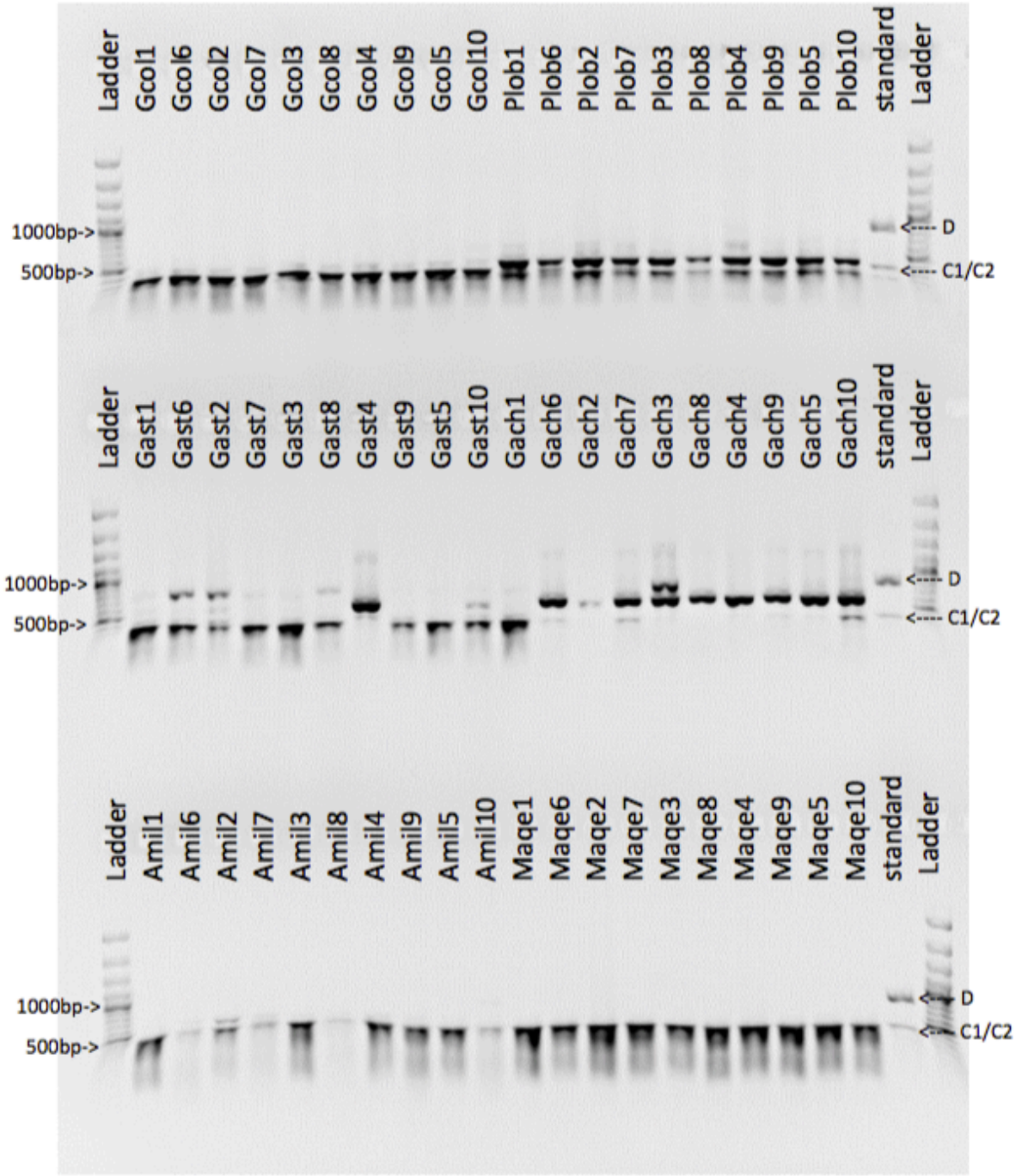


Figure S4. Mean net photosynthesis (±SEM, n=10 per bar) in ambient conditions (27°C) by sampling day and species.

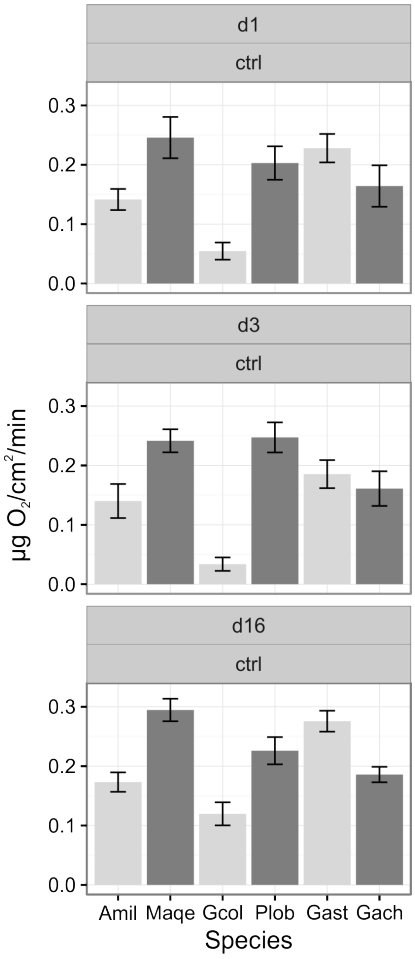


Figure S5. Mean calcification (±SEM, n=10 per bar) in ambient conditions (27°C) by sampling day and species.

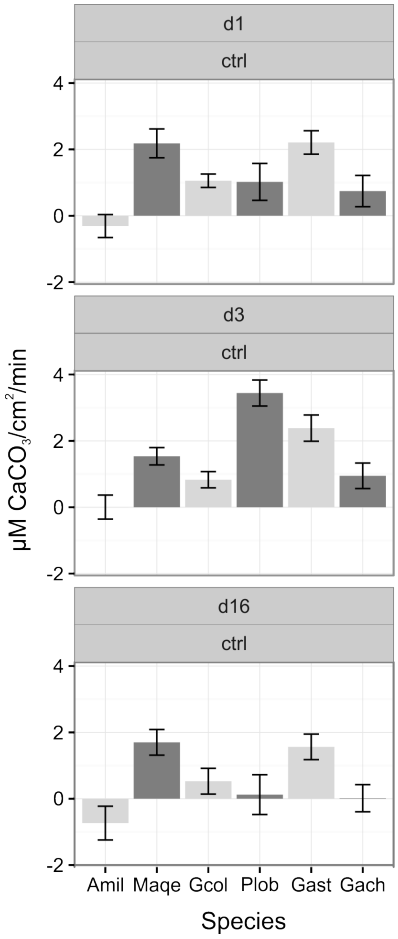


Figure S6. Mean carbon transfer from host to symbiont (±SEM, n=5 per bar) in ambient conditions (27°C) by sampling day and species.

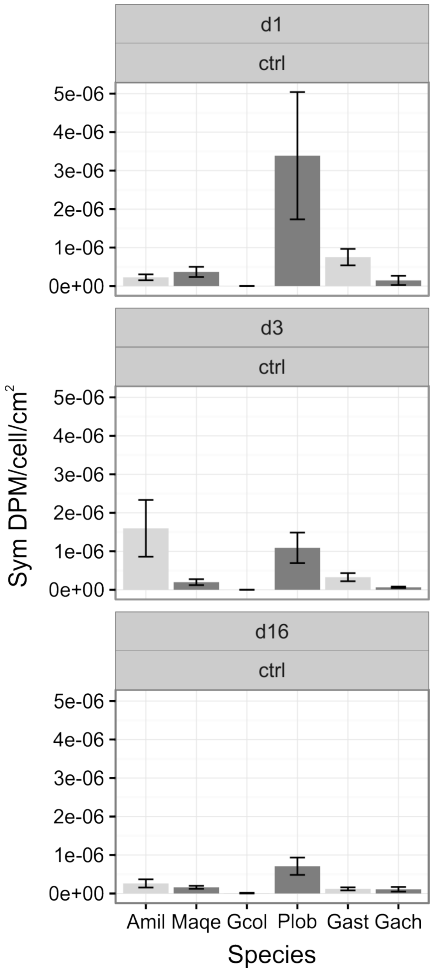
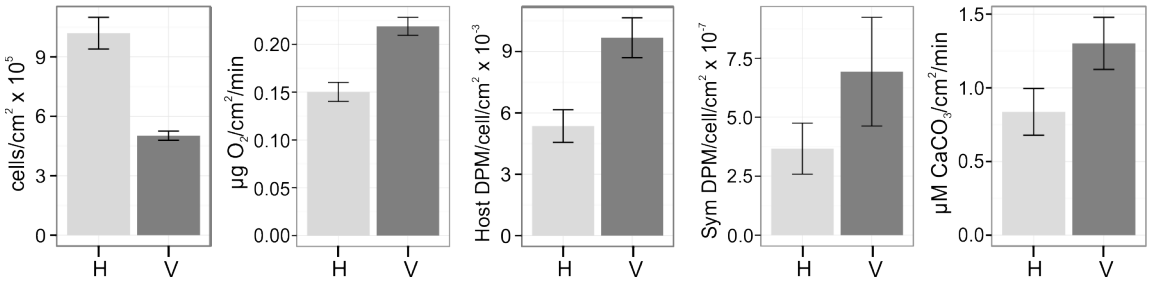


Figure S7. Average effect of transmission mode in ambient conditions (27°C) on mean *Symbiodinium* cell density (±SEM, n=90 per mode), mean net photosynthesis (±SEM, n=90 per mode), mean carbon transfer from symbiont to host (±SEM, n=45 per mode), mean carbon transfer from host to symbiont (±SEM, n=45 per mode) and mean calcification (±SEM, n=45 per mode).



REFERENCES

1. Moran N.A. 2006 Symbiosis. *Current Biology* **16**, R866-R871.
2. Friesen M.L., Jones E.I. 2013 Modelling the evolution of mutualistic symbioses. In *Bacterial Molecular Networks: Methods and Protocols* (eds. van Helden J., Toussaint A., Thieffry D.), pp. 481-499, Springer Science+Business Media.
3. Kiers T.E., West S.A. 2015 Evolving new organisms via symbiosis. *Science* **348**(6233), 392-394.
4. Maynard Smith J., Szathmary E. 1995 *The Major Transitions in Evolution*. Oxford, Freeman.
5. Bshary R. 2001 The cleaner fish market. In *Economics in Nature: Social Dilemmas, Mate Choice and Biological Markets* (ed. Noe R.), Cambridge University Press.
6. Oldroyd G.E.D., Murray J.D., Poole P.S., Downie J.A. 2011 The rules of engagement in the Legume-Rhizobial symbiosis. *Annual Review of Genetics* **45**, 119-144.
7. Dyall S.D., Brown M.T., Johnson P.J. 2004 Ancient invasions: From endosymbionts to organelles. *Science* **304**.
8. Damore J.A., Gore J. 2011 A slowly evolving host moves first in symbiotic interactions. *Evolution* **65**(8), 2391-2398. (doi:10.1111/j.1558-5646.2011.01299.x).
9. West S.A., Fisher R.M., Gardner A., Kiers T.E. 2015 Major evolutionary transitions in individuality. *Proceedings of the National Academy of Sciences of the United States of America*.
10. Nowak M.A., Bonhoeffer S., May R.M. 1994 Spatial games and the maintenance of cooperation. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 4877-4881.
11. Doebeli M., Knowlton N. 1998 The evolution of interspecific mutualisms. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 8676-8680.
12. Lesser M.P., Stat M., Gates R.D. 2013 The endosymbiotic dinoflagellates (Symbiodinium sp.) of corals are parasites and mutualists. *Coral Reefs* **32**, 603-611.
13. Sachs J.L., Essenberg C.J., Turcotte M.M. 2011 New paradigms for the evolution of beneficial infections. *Trends in Ecology & Evolution* **26**, 202-209.
14. Anderson R.M., May R.M. 1982 Coevolution of hosts and parasites. *Parasitology* **85**, 411-426.
15. Ebert D., Bull J.J. 2003 Challenging the trade-off model for the evolution of virulence: is virulence management feasible? *Trends in Microbiology* **11**, 15-20.
16. Bright M., Bulgheresi S. 2010 A complex journey: transmission of microbial symbionts. *Nature Reviews Microbiology* **8**, 218-230.
17. Bull J.J. 1994 Perspective: virulence. *Evolution* **48**, 1423-1437.
18. Sachs J.L., Mueller U.G., Wilcox T.P., Bull J.J. 2004 The evolution of cooperation. *Quarterly Review of Biology* **79**(2), 135-160.
19. Frank S.A. 1994 Genetics of mutualism: the evolution of altruism between species. *Journal of Theoretical Biology* **170**, 393-400.
20. Herre E.A., Knowlton N., Mueller U.G., Rehner S.A. 1999 The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology & Evolution* **14**.
21. Ebert D. 2013 The epidemiology and evolution of symbionts with mixed-mode transmission. *Annual Review of Ecology, Evolution, and Systematics* **44**, 7.1-7.21.
22. Bull J.J., Molineux I.J., Rice W.R. 1991 Selection of benevolence in a host-parasite system. *Evolution* **45**, 857-882.
23. Sachs J.L., Wilcox T.P. 2006 A shift to parasitism in the jellyfish symbiont Symbiodinium microadriaticum. *Proceedings of the Royal Society B-Biological Sciences* **273**, 425-429.
24. Stewart A.D., Logsdon J.M., Kelley S.E. 2005 An empirical study of the evolution of virulece under both horizontal and vertical transmission. *Evolution* **59**, 730-739.
25. Dusi E., Gougat-Barbera C., Berendonk T.U., Kaltz O. 2015 Long-term selection experiment produces breakdown of horizontal transmissibility in parasite with mixd transmission mode. *Evolution* **69**(4), 1069-1076.

26. Herre E.A. 1993 Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* **259**, 1442-1445.
27. Brown B.E. 1997 Coral bleaching: causes and consequences. *Coral Reefs* **16**, S129-S138.
28. Stanley G.D., Swart P.K. 1995 Evolution of the coral-zooxanthellae symbiosis during the Triassic: a geochemical approach. *Paleobiology* **21**(2), 179-199.
29. Daly M., Brugler M.R., Cartwright P., Collines A.G., Dawson M.N., Fautin D.G., France S.C., McFadden C.S., Opresko D.M., Rodriguez E., et al. 2007 The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. In *Linnaeus Tercentenary: Progress in Invertebrate Taxonomy* (eds. Zhang Z.Q., Shear W.A.), pp. 127-182, Zootaxa.
30. Harrison P.L., Wallace C.C. 1990 Reproduction, dispersal and recruitment of scleractinian corals. In *Ecosystems of the World: Coral Reefs* (ed. Dubinsky Z.), pp. 133-207. New York, Elsevier.
31. Kerr A.M., Baird A.H., Hughes T.P. 2011 Correlated evolution of sex and reproductive mode in corals (Anthozoa: Scleractinia). *Proceedings of the Royal Society B-Biological Sciences* **278**, 75-81.
32. van Oppen M.J.H., Baker A.C., Coffroth M.A., Willis B.L. 2009 Bleaching resistance and the role of algal endosymbionts. In *Coral Bleaching: Patterns, Processes, Causes and Consequences (Ecological Studies 205)* (eds. van Oppen M.J.H., Lough J.M.). Berlin Heidelberg, Springer-Verlag.
33. Baird A.H., Guest J.R., Willis B.L. 2009 Systematic and Biogeographical Patterns in the Reproductive Biology of Scleractinian Corals. *Annual Review of Ecology Evolution and Systematics* **40**, 551-571. (doi:10.1146/annurev.ecolsys.110308.120220).
34. Hughes T.P., Baird A.H., Bellwood D.R., Card M., Connolly S.R., Folke C., Grosberg R., Hoegh-Guldberg O., Jackson J.B.C., Kleypas J., et al. 2003 Climate change, human impacts, and the resilience of coral reefs. *Science* **301**(5635), 929-933.
35. Hoegh-Guldberg O., Mumby P.J., Hooten A.J., Steneck R.S., Greenfield P., Gomez E., Harvell C.D., Sale P.F., Edwards A.J., Caldeira K., et al. 2007 Coral reefs under rapid climate change and ocean acidification. *Science* **318**(5857), 1737-1742. (doi:10.1126/science.1152509).
36. Marshall P.A., Baird A.H. 2000 Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. *Coral Reefs* **19**(2), 155-163.
37. Edmunds P.J., Gates R.D. 2003 Has coral bleaching delayed our understanding of fundamental aspects of coral-dinoflagellate symbioses? *BioScience* **53**(10), 976-980.
38. Weis V.M. 2008 Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *The Journal of Experimental Biology* **211**, 3059-3066.
39. Kerr A.M. 2005 Molecular and morphological supertree of stony corals (Anthozoa: Scleractinia) using matrix representation parsimony. *Biological Reviews* **80**, 1-16.
40. Kitahara M.V., Cairns S.D., Stolarski J., Blair D., Miller D.J. 2010 A comprehensive phylogenetic analysis of the Scleractinia (Cnidaria, Anthozoa) based on mitochondrial CO1 sequence data. *PLoS ONE* **5**(7), e11490. (doi:10.1371/journal.pone.0011490).
41. Tonk L., Bongaerts P., Sampayo E.M., Hoegh-Guldberg O. 2013 SymbioGBR: a web-based database of Symbiodinium associated with cnidarian hosts on the Great Barrier Reef. *BMC Ecology* **13**(7).
42. Franklin E.C., Stat M., Pochon X., Putnam H.M., Gates R.D. 2012 GeoSymbio: a hybrid, cloud-based web application of global geospatial bioinformatics and ecoinformatics for Symbiodinium-host symbioses. *Molecular Ecology Resources* **12**, 369-373.
43. Ralph P.J., Larkum A.W.D., Kuhl M. 2005 Temporal patterns in effective quantum yield of individual zooxanthellae expelled during bleaching. *Journal of Experimental Marine Biology and Ecology* **316**, 17-28.
44. Strahl J., Stolz I., Uthicke S., Vogen N., Noonan S.H.C., Fabricius K.E. 2015 Physiological and ecological performance differs in four coral taxa at a volcanic carbon dioxide seep. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **184**, 179-186.
45. Chisolm J.R.M., Gattuso J.P. 1991 Validation of the alkalinity anomaly technique for investigating calcification and photosynthesis in coral-reef communities. *Limnology and Oceanography* **36**, 1232-1239.

46. Veal C.J., Carmi M., Fine M., Hoegh-Guldberg O. 2010 Increasing the accuracy of surface area estimation using single wax dipping of coral fragments. *Coral Reefs* **29**, 893-897.
47. Thorington G., Margulis L. 1981 Hydra viridis: Transfer of metabolites between Hydra and symbiotic algae. *Biological Bulletin* **160**, 175-188.
48. Tonheim S.K., Koven W., Ronnestad I. 2000 Enrichment of Artemia with free methionine. *Aquaculture* **190**, 223-235.
49. Wilson K., Li Y., Whan V., Lehnert S., Byrne K., Moore S., Pongsomboon S., Tassanakajon A., Rosenberg G., Ballment E., et al. 2002 Genetic mapping of the black tiger shrimp Penaeus monodon with amplified fragment length polymorphism. *Aquaculture* **204**(3-4), 297-309.
50. Baker A.C., Rowan R. 1997 Diversity of symbiotic dinoflagellates (zooxanthellae) in scleractinian corals fo the Caribbean and Eastern Pacific. In *Proceedings of the 8th International Coral Reef Symposium* (pp. 1301-1306.
51. Palstra F. 2000 Host-endosymbiont specificity in Acropora corals of the Indo-Pacific? Townsville, Australia, James Cook University.
52. Team R.C. 2013 R: A language and environment for statistical computing. (Vienna, R Foundation for Statistical Computing.
53. Pinheiro J., Bates D., DebRoy S., Sarkar D., Team R.D.C. 2013 nlme: Linear and nonlinear mixed effects models. (R package version 3.1-113 ed.
54. Johnson P.C.D. 2014 Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. *Methods in Ecology and Evolution* **5**(9), 944-946.
55. Green E.A., Davies S.W., Matz M.V., Medina M. 2014 Quantifying cryptic Symbiodinium diversity with Orbicella faveolata and Orbicella franksi at the Flower Garden Banks, Gulf of Mexico. *PeerJ* **2**, e386. (doi:10.7717/peerj.386).
56. Fu L., Niu B., Zhu Z., Wu S., Li W. 2012 CD-HIT: accelerated for clustering the next generation sequencing data. *Bioinformatics* **28**, 3150-3152. (doi:10.1093/bioinformatics/bts565).
57. Quigley K.M., Davies S.W., Kenkel C.D., Willis B.L., Matz M.V., Bay L.K. 2014 Deep-Sequencing Method for Quantifying Background Abundances of Symbiodinium Types: Exploring the Rare Symbiodinium Biosphere in Reef-Building Corals. *PLoS ONE* **9**, e94297. (doi:doi:10.1371/journal.pone.0094297).
58. Pruitt K.D., Tatusova T., Browh G.R., Maglott D.R. 2012 NCBI Reference Sequences (RefSeq): current status, new features and genome annotation policy. *Nucleic Acids Research* **40**(D1), D130-D135.
59. LaJeunesse T.C. 2005 Species radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Molecular Biology and Evolution* **22**, 570-581.
60. Putnam H.M., Stat M., Pochon X., Gates R.D. 2012 Endosymbiotic flexibility associates with environmental sensitivity in scleractinian corals. *Proceedings of the Royal Society B: Biological Sciences* **279**, 4352-4361.
61. Jones A., Berkelmans R., van Oppen M.J.H., Mieog J.C., Sinclair W. 2008 A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B-Biological Sciences* **275**, 1359-1365.
62. Berkelmans R., van Oppen M.J.H. 2006 The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society B-Biological Sciences* **273**(1599), 2305-2312. (doi:10.1098/rspb.2006.3567).
63. Little A.F., van Oppen M.J.H., Willis B.L. 2004 Flexibility in algal endosymbioses shapes growth in reef corals. *Science* **304**(5676), 1492-1494.
64. Jones A., Berkelmans R. 2010 Potential Costs of Acclimatization to a Warmer Climate: Growth of a Reef Coral with Heat Tolerant vs. Sensitive Symbiont Types. *Plos One* **5**(5). (doi:10.1371/journal.pone.0010437).
65. Cantin N.E., van Oppen M.J.H., Willis B.L., Mieog J.C., Negri A.P. 2009 Juvenile corals can acquire more carbon from high-performance algal symbionts. *Coral Reefs* **28**(2), 405-414. (doi:10.1007/s00338-009-0478-8).

66. Abrego D., Ulstrup K.E., Willis B.L., van Oppen M.J.H. 2008 Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings of the Royal Society B-Biological Sciences* **275**(1648), 2273-2282. (doi:10.1098/rspb.2008.0180).
67. Baird A.H., Bhagooli R., Ralph P.J., Takahashi S. 2009 Coral bleaching: the role of the host. *Trends in Ecology & Evolution* **24**(1), 16-20. (doi:10.1016/j.tree.2008.09.005).
68. Kenkel C., Goodbody-Gringley G., Caillaud D., Davies S.W., Bartels E., Matz M. 2013 Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (Porites astreoides) from different reef environments. *Molecular Ecology* **22**(16), 4335-4348.
69. Richmond R.H., Hunter C.L. 1990 Reproduction and recruitment of corals - comparisons among the Caribbean, the tropical Pacific, and the Red-Sea. *Marine Ecology-Progress Series* **60**(1-2), 185-203.
70. Okubo N., Mezaki T., Nozawa Y., Nakano Y., Lien Y.-T., Fukami H., Hayward D.C., Ball E.E. 2013 Comparative Embryology of Eleven Species of Stony Corals (Scleractinia). *PLoS ONE* **8**(12), e84115. (doi:doi:10.1371/journal.pone.0084115).
71. Schwarz J.A., Krupp D.A., Weis V.M. 1999 Late larval development and onset of symbiosis in the scleractinian coral Fungia scutaria. *Biological Bulletin* **196**, 70-79.
72. Marlow H.Q., Martindale M.Q. 2007 Embryonic development in two species of scleractinian coral embryos: Symbiodinium localization and mode of gastrulation. *Evolution and Development* **9**(4), 355-367.
73. Cumbo V.R., Baird A.H., van Oppen M.J.H. 2013 The promiscuous larvae: flexibility in the establishment of symbiosis in corals. *Coral Reefs* **32**, 111-120.
74. Nitschke M.R., Davy S.K., Ward S. 2015 Horizontal transmission of Symbiodinium cells between adult and juvenile corals is aided by benthic sediment. *Coral Reefs*. (doi:doi:10.1007/s00338-015-1349-0).
75. Byler K.A., Carmi-Veal M., Fine M., Goulet T.L. 2013 Multiple symbiont acquisition strategies as an adaptive mechanism in the coral Stylophora pistillata. *PLoS ONE* **8**(3), e59596.
76. Boulotte N.M., Dalton S.J., Carroll A.G., Harrison P.L., Putnam H.M., Peplow L.M., van Oppen M.J.H. 2016 Exploring the Symbiodinium rare biosphere provides evidence for symbiont switching in reef-building corals. *The ISME Journal*, 1-9. (doi:10.1038/ismej.2016.54).
77. Fabina N.S., Putnam H.M., Franklin E.C., Stat M., Gates R.D. 2012 Transmission mode predicts specificity and interaction patterns in coral-Symbiodinium networks. *PLoS ONE* **7**(9), e44970. (doi:doi10.1371/journal.pone.0044970).
78. Davy S.K., Allemand D., Weis V.M. 2012 Cell biology of Cnidarian-dinoflagellate symbiosis. *Microbiology and Molecular Biology Reviews* **76**, 229-261.
79. Muscatine L. 1990 The role of symbiotic algae in carbon and energy flux in reef corals. In *Ecosystems of the world 25: Coral reefs* (ed. Dubinsky), pp. 75-87. New York, Elsiever.
80. Yellowlees D., Rees T., Leggat W. 2008 Metabolic interactions between algal symbionts and invertebrate hosts. *Plant, Cell & Environment* **31**(5), 679-694.
81. Gordon B.R., Leggat W. 2010 Symbiodinium-invertebrate symbioses and the role of metabolomics. *Marine Drugs* **8**(10), 2546-2568.
82. Von Holt C. 1968 Uptake of glycine and release of nucleoside-polyphosphates by zooxanthellae. *Comparative Biochemistry and Physiology* **26**(3), 1071-1079.
83. Lin S., Cheng S., Song B., Zhong X., Lin X., Li W., Li L., Zhang Y., Zhang H., Ji Z., et al. 2015 The Symbiodinium kawagutii genome illuminates dinoflagellate gene expression and coral symbiosis. *Science* **350**(6261), 691-694. (doi:10.1126/science.aad0408).
84. Grottoli A.G., Rodrigues L.J., Palardy J.E. 2006 Heterotrophic plasticity and resilience in bleached corals. *Nature* **440**, 1186-1189. (doi:10.1038/nature04565).
85. Koch E.J., Miyashiro T., McFall-Ngai M.J., Ruby E.G. 2014 Features governing symbiont persistence in the squid-vibrio association. *Molecular Ecology* **23**(6), 1624-1634.

86. Kiers T.E., Rosseau R.A., West S.A., Denison R.F. 2003 Host sanctions and the legume-rhizobium mutualism. *Nature* **425**(6953), 78-81.

87. Weis V.M., Davy S.K., Hoegh-Guldberg O., Rodriguez-Lanetty M., Pringle J.R. 2008 Cell biology in model systems as the key to understanding corals. *Trends in Ecology and Evolution* **23**(7), 369-376.