1   **Full title**: Symbiont type influences trophic plasticity of a model cnidarian-dinoflagellate

2   symbiosis

3

4   Miguel C. Leal[1*], Kenneth Hoadley[2], D. Tye Pettay[2], Alejandro Grajales[3], Ricardo Calado[1],

5   Mark E. Warner[2]

6

7   [1] Departamento de Biologia & CESAM, Universidade de Aveiro, Campus Universitário de

8   Santiago, 3810-193 Aveiro, Portugal

9   [2]College of Earth, Ocean, and Environment, University of Delaware, Lewes DE, 19958, USA

10 [3]Department of Invertebrate Zoology, American Museum of Natural History, Central Park

11 West at 79th Street, New York NY, New York 10024, USA

12

13 *Corresponding author. Telephone: +351-234270779; Fax: +351-234372587; E-mail:

14 miguelcleal@gmail.com

15

16

17 **Keywords**: *Symbiodinium*; functional diversity; nutrition; photosynthesis

18

19

Abstract

The association between cnidarians and photosynthetic dinoflagellates within the genus *Symbiodinium* is a prevalent relationship in tropical and subtropical marine environments. Although the diversity of *Symbiodinium* provides a possible axis for niche diversification, increased functional range and resilience to physical stressors such as elevated temperature, how such diversity relates to the physiological balance between autotrophy and heterotrophy of the host animal remains unknown. Here we experimentally show inter- and intraspecific variability of photosynthetic carbon fixation and subsequent translocation by *Symbiodinium* to the model cnidarian host *Aiptasia pallida*. By using a clonal anemone line harboring different species of *Symbiodinium*, we determined that symbiont identity influences trophic plasticity through its density, capacity to fix carbon, quantity of translocated carbon, and ultimately the host's capacity to ingest and digest prey. Symbiont carbon translocation and host prey ingestion were positively correlated across symbiont combinations that consisted of different isoclonal lines of *S. minutum*, while a combination with type D4-5 *Symbiodinium* displayed lower carbon translocation, and prey capture and digestion more similar to *Aiptasia* lacking symbionts. The absence of a shift toward greater heterotrophy when carbon translocation is low suggests that the metabolic demand of feeding and digestion may overwhelm nutritional stores when photosynthesis is reduced, and amends the possible role of animal feeding in resistance to or recovery from the effects of climate change in more obligate symbioses such as reef building corals.

Introduction

Several animals thrive in oligotrophic environments, such as coral reefs, by capitalizing on the photosynthetically-fixed carbon of endosymbiotic dinoflagellates (Venn et al., 2008). The association between coral reef cnidarians and dinoflagellates within the genus *Symbiodinium* represents one such key cosmopolitan symbiosis in the oceans (Coffroth and Santos, 2005; Weis et al., 2008). While the interspecific diversity and flexibility of *Symbiodinium* is well known (Coffroth and Santos, 2005; Sampayo et al., 2008), the intraspecific diversity as well as functional diversity of *Symbiodinium* and subsequent consequences to the nutritional balance of the cnidarian host remains unexplored. Symbiotic cnidarians rely on autotrophy, through the photosynthetically fixed carbon translocated by their symbionts, and heterotrophy, by feeding on planktonic organisms (Falkowski et al., 1984). In some cases, this trophic plasticity is critical for the resilience of these symbiotic associations (Grottoli et al., 2006). A shifting reliance from autotrophy to heterotrophy with light attenuation and depth is usually observed within different coral species (Muscatine et al., 1989; Anthony and Fabricius, 2000; Leal et al., 2014a). Heterotrophy may also affect the photosynthetic performance of the symbionts but have no consequence to the total amount of carbon translocated to the host (Davy and Cook, 2001). In addition, autotrophy may be inversely related to heterotrophic contribution in response to stress events, such as bleaching or notable changes in light regime (Grottoli et al., 2006; Hughes and Grottoli, 2013; Tremblay et al., 2014).

Symbiont genotypic diversity affects the quantity and quality of translocated material to the host (Loram et al., 2007; Starzak et al., 2014), which ultimately has physiological consequences for symbiotic cnidarians and may contribute to the selective loss of cnidarian diversity (Grottoli et al., 2014). However, how symbiont diversity directly relates to the trophic plasticity of the holobiont (used here simply as the animal and photosynthetic symbionts) remains unknown. Notably, no direct simultaneous comparisons exist between auto- and heterotrophic performance that account for symbiont identity in a genetically controlled host environment. Here we address the consequences of inter- and intraspecific variability of *Symbiodinium* and its relationship to the trophic plasticity of the association as defined by the interaction of autotrophic and heterotrophic performance We hypothesized that symbiotic associations with low carbon translocated to the animal counterbalance such nutritional loss by increasing feeding and digestion. We used the sea anemone *Aiptasia pallida* due to the considerable recent interest in using this model organism to understand the physiology and coevolution of cnidarian-*Symbiodinium* symbioses (Weis et al., 2008;

76   Thornhill et al., 2013). An isoclonal line of the symbiotic sea anemone *Aiptasia pallida* (line

77   CC7) was used while individually hosting two isoclonal lines (i.e. strains 1 and 2) of its

78   naturally occurring clade B symbiont (*Symbiodinium minutum*, ITS2 type B1), and a mixture

79   of one *S. minutum* with a different symbiont within the clade B lineage (*Symbiodinium*

80   *psygmophilum*, ITS2 type B2). A single isolate of *Symbiodinium* sp. from the putatively

81   thermally tolerant clade 'D' lineage (ITS2 type D4-5) (Rowan, 2004; Lajeunesse et al.,

82   2010a) was also used with the CC7 host. Additionally, we tested a different *A. pallida* line

83   originally collected from Bermuda that hosted an additional unique strain of *S. minutum*

84   (strain 3). Photosynthetic performance and carbon translocation by the symbionts to the host,

85   together with host prey capture and digestion, provided the proxies to score nutritional

86   plasticity of these symbioses. To gauge zooplankton capture (ingestion) and digestion alone,

87   i.e. heterotrophic performance (Leal et al., 2014b), results of feeding assays were compared to

88   CC7 anemones lacking any symbionts (aposymbiotic).

89

90   **Results**

91   Overall, photosynthesis was a good predictor of carbon translocation to the animal

92   when grouping all animal-dinoflagellate combinations, and a significant positive correlation

93   was observed between the rate of carbon fixation (photosynthesis) and the carbon translocated

94   to the animal when normalized to *Symbiodinium* cell number (Fig. 1; Pearson correlation, $n =$

95   25, $r = 0.89$, $t = 9.368$, $df = 23$, $P<0.001$). However, when comparing this pattern among

96   different *Symbiodinium* genotypes, significant differences were noted, particularly in the CC7

97   anemone hosting the D4-5 symbiont where carbon translocated cell^{-1} remained high across the

98   range of photosynthesis ($P<0.05$; Table A1). Substantial differences were observed in

99   symbiont density (ANOVA, $n = 25$, $F = 13.16$, $P<0.001$), particularly between strain 3 and

100   the D4-5 symbiont which averaged 5057 and 1382 cells μg^{-1} protein, respectively (Table 1).

101   Nevertheless, photosynthetic performance of each animal-dinoflagellate combination together

102   with symbiont density resulted in greater carbon translocated to the host as *Symbiodinium*

103   density increased (Pearson correlation, $n = 25$, $r = 0.75$, $t = 5.4352$, $df = 23$, $P<0.001$). Carbon

104   translocation significantly varied among symbiont genotypes (ANOVA, $n = 25$, $F = 4.006$,

105   $P<0.05$), particularly between strain 2 and D4-5 symbionts which averaged 1.09×10^{-7} and

106   3.14×10^{-7} μg C cell^{-1} h^{-1}, respectively (Table 1). However, the positive correlation between

107   symbiont density and carbon translocated was observed among the two symbiont clades and

108   anemone lines (i.e. clade B vs. D, and strain 3; Table A1).

109   For all dinoflagellate-anemone combinations that contained *Symbiodinium* clade B,

110 including the other anemone line hosting *S. minutum* (strain 3), there was a positive

111 association between the rate of carbon translocation and prey ingestion (Fig. 2A). However,

112 the D4-5 symbiosis had considerably lower rates of carbon translocation relative to the high

113 rate of prey ingestion. Furthermore, significant inter- and intraspecific differences were

114 observed for prey ingestion and prey DNA breakdown (a proxy for prey degradation) among

115 the different dinoflagellate-anemone combinations (Figs 2B, 2C). In particular, a pattern of

116 high prey ingestion and slow prey digestion was observed for aposymbiotic individuals

117 (lacking symbionts) and anemones hosting the D4-5 symbiont (Table A2). This pattern was

118 also noted for the other anemone line hosting a different genotype of *S. minutum* (strain 3;

119 Figs 2B, 2C).

120

121 **Discussion**

122   This study shows that the genetic identity of *Symbiodinium* influences the nutritional

123 plasticity of a cnidarian-dinoflagellate symbiosis. The marked differences between host prey

124 feeding and digestion are associated with both photoautotrophic carbon translocation and

125 symbiont type, even at the symbiont sub-species level. Notable differences in symbiont

126 density and the resulting variability in carbon translocation among *Symbiodinium* strains

127 within *S. minutum* alone underscore the functional diversity within these dinoflagellates, the

128 intraspecific variability within a single *Symbiodinium* species, and that broad physiological

129 generalizations for entire clades of *Symbiodinium* (e.g. Fabina et al., 2013) are likely not

130 warranted.

131 We hypothesized that symbiotic associations with lower autotrophic contribution (i.e. less

132 carbon translocated to the animal) would compensate for such losses by increasing feeding

133 and digestion (Grottoli et al., 2006; Ferrier-Pagès et al., 2011; Hughes and Grottoli, 2013).

134 However, results suggest that higher autotrophy is strongly associated with increased

135 heterotrophy, apart from the D4-5 symbiont that did not fit this pattern (Fig. 2A). The

136 association with the D4-5 symbiont resulted in relatively low total carbon translocated but

137 high prey ingestion (Fig. 2A). Likewise, aposymbiotic anemones rely on prey capture and

138 ingestion efforts as their only source of nutrients (Grottoli et al., 2006; Leal et al., 2013).

139 Similar prey ingestion and digestion between aposymbiotic *Aiptasia* and anemones hosting

140 D4-5 symbionts (Figs. 2B, 2C) suggests that both groups of anemones increase their

141 heterotrophic efforts through prey capture and ingestion but subsequent prey breakdown and

142 digestion are relatively slow. While the high prey gut content of these anemones (Fig. 2B)

143　may justify their slow digestion rates (Table A2), the similarity between the aposymbiotic and

144　clade D-harboring anemones may also be explained by the reduced autotrophic contribution

145　of D4-5 symbionts to support host metabolism, and particularly physiological costs associated

146　with prey digestion may play a role (Sebens, 2002). These results are in contrast to those of

147　Hiebert and Bingham (2012) who noted no significant difference in the feeding ability of the

148　temperate anemone, *Anthopleura elegantissima,* while aposymbiotic or harboring

149　*Symbiodinium* or 'zoochlorellae.' While not measured here, it is also possible that symbiont

150　type in *Aiptasia* affects the anemone's capacity to capture prey through morphological

151　variations such as tentacle number, size, or cnida characteristics (Hiebert and Bingham, 2012).

152　　　　　Carbon fixation and translocation varied among *Symbiodinium* genotypes (Table 1).

153　However, the similar trends observed across genotypes between photosynthesis and carbon

154　translocation, and between *Symbiodinium* density and carbon translocation suggest a similar

155　autotrophic mechanism among strains of the same species (*S. minutum*) and regardless of

156　anemone line. Results also suggest that differences in *Symbiodinium* density affect the

157　congruence between high carbon fixation and translocation symbiont^{-1} and total translocation

158　to the host, as was observed in the anemones harboring the D symbiont (Fig. 1, Tables 1 and

159　A1). As a consequence of the low cell density of the D4-5 symbiont, increased photosynthetic

160　rates are needed to translocate the same amount of carbon (Fig. 1). Symbiont cell density is

161　determined, in part, by the photosynthetic carbon flux and individual metabolic requirements

162　of symbionts, which varies with symbiont type (Dubinsky and Berman-Frank, 2001; Starzak

163　et al., 2014). Although Davy and Cook (2001) have suggested that low symbiont density

164　promotes high photosynthetic rates due to decreased competition for CO_2, results here show

165　that symbioses supporting high *Symbiodinium* densities may also display relatively high

166　photosynthesis, such as strain 3 (Table 1). Moreover, notably different photosynthetic rates

167　were observed among closely related symbionts displaying similar cell density (strain 1 and 2;

168　Table 1). It is, therefore, likely that a combination of the effects of symbiont type, symbiont

169　density and anemone line affect photosynthetic rates. Further, possible space constraints or

170　other as yet uncharacterized control elements of the animal and cellular recognition signals

171　between the partners may play a defining role here (Schoenberg and Trench, 1980; Smith and

172　Muscatine, 1999). The effect of symbiont type and density may be of particular importance in

173　D4-5 symbionts, as low cell density is probably affecting total carbon translocation to the host.

174　While the symbiosis with the D4-5 symbiont was stable, it may represent a suboptimal

175　symbiosis between host and symbiont lineages that have not coevolved together. This may

176　cause the strong relationship between autotrophy and heterotrophy noted in the other

177 holobionts and across *S. minutum* genotypes to break down in this particular symbiosis (Fig.

178 2A). If true, this symbiont may be an inefficient competitor against homologous symbionts of

179 *Aiptasia* such as *S. minutum*. While not measured in this study, a difference in the quality of

180 translocated material between different symbiont types, as already noted in another tropical

181 anemone harboring different clades of *Symbiodinium* (Loram et al., 2007), could also

182 contribute to this trend and certainly warrants further study.

183   The positive association between autotrophy and heterotrophy for the different *S.*

184 *minutum* strains and the anemone harboring the mixed clade B assemblage may be due to two

185 non-mutually exclusive reasons: 1) increased feeding is the result of autotrophic energy

186 supply that is available to support metabolically demanding heterotrophic processes, and 2)

187 increased heterotrophic contribution is critical to the assimilation of photosynthetic carbon

188 into host biomass. Both explanations are likely associated with respiratory demand. In

189 particular, prey digestion is an energetic-demanding process that increases metabolism

190 (Sebens, 2002) and requires photosynthetically-fixed carbon to support animal respiration

191 (Falkowski et al., 1984). If autotrophic energy exceeds respiratory demand and

192 heterotrophically-derived carbon is available, then autotrophic carbon may be combined with

193 heterotrophic energy and elements such as nitrogen and phosphorous to build host biomass

194 (Dubinsky and Jokiel, 1994; Hoogenboom et al., 2010). This mechanism driven by respiratory

195 demand is supported by the differential fate of carbon in symbiotic cnidarians:

196 photosynthetically fixed carbon is quickly respired by the animal host, whereas

197 heterotrophically-derived carbon is incorporated more into animal biomass such as

198 phospholipids, proteins and nucleic acids (Bachar et al., 2007). The increased prey ingestion

199 observed in strain 3 thus follows the positive association between autotrophy and

200 heterotrophy (Fig. 2A). However, the reduced prey breakdown observed for this same strain

201 differs from anemones hosting *S. minutum*, i.e. strain 1, 2 and mixture, and is similar to results

202 observed in aposymbiotic and D4-5 anemones (Fig. 2C). This may be a consequence of

203 physiological differences among different host genetic lines. Different lines of anemones may

204 have physiological differences that slow down digestion or different morphological features,

205 as previously detailed, that increase prey capture rate (Hiebert and Bingham, 2012). In

206 contrast, as the same host line was used in all anemones apart from strain 3, the increased

207 prey ingestion with slower digestion in aposymbiotic and D4-5 anemones is associated with

208 the absence of symbionts or type of symbiont, respectively. This suggests that heterotrophy is

209 a partial energetic sink in itself due to absent or insufficient carbon from autotrophy to

210 support animal metabolism. Such quantitative and qualitative differences may have

211 implications for energy budgets of the symbiosis. Further, widely used models quantifying

212 autotrophic and heterotrophic contributions to animal respiration, known as CZAR

213 (Muscatine et al., 1981) and CHAR (Grottoli et al., 2006) respectively, may need to be

214 adjusted to account for different *Symbiodinium* types and the important metabolic costs of

215 feeding and digestion. Likewise, as isolating the respiratory contribution from the algal and

216 animal components within intact symbioses is impossible, such models and their underlying

217 assumptions may not provide the necessary detail to tease apart such differences noted here.

218   In conclusion, heterotrophy is positively associated with autotrophy, and the latter is

219 significantly correlated with the symbionts' capacity to fix and translocate carbon in a density

220 dependent manner. Physiological variability within a single *Symbiodinium* species thus results

221 in a change in the nutritional status of the cnidarian host where both auto- and heterotrophy

222 play a pivotal role. The influence of symbiont type in contributing to this trade-off in trophic

223 patterns is especially important in the context of coral bleaching events, as not all stable

224 dinoflagellate/animal symbioses are equally nutritionally advantageous to the animal.

225 Bleached corals may increase their feeding rates (Grottoli et al., 2006; Hughes and Grottoli,

226 2013; Leal et al., 2014a) but may not take advantage of such opportunities if host metabolic

227 processes associated with prey digestion are affected by symbiont competency and

228 consequent autotrophic capacity. *Symbiodinium* functional diversity that extends below the

229 species-level is thus critical to the nutritional plasticity of cnidarian-dinoflagellate symbioses,

230 influencing their resilience and acclimation, and having ecological implications for the

231 success of these symbioses in marine environments.

232

233 Material and methods

234 Animal models

235   Anemones consisted of a monoclonal line of aposymbiotic (Burriesci et al., 2012)

236 *Aiptasia pallida* (clone CC7) (provided by the J. Pringle Stanford University, USA).

237 Aposymbiotic anemones were kept in constant darkness over two years in a closed system

238 containing filtered seawater (1 μm; FSW) and fed brine shrimp weekly. Different

239 combinations of *A. pallida* symbioses were generated by infecting aposymbiotic anemones

240 (similar to Schoenberg and Trench (1980), minus the seawater extract of *Artemia*) with

241 genetically distinct cultures of *Symbiodinium* originating from various symbiotic cnidarians

242 (Table A3). Two of the symbionts used are recently described species of clade B

243 *Symbiodinium*, *S. minutum* and *S. psygmophilum* (Lajeunesse et al., 2012), while the third,

244 D4-5, represents a stress tolerant clade of symbionts (Glynn et al., 2001; Lajeunesse et al.,

245 2010b). Three strains (designated as strains 1, 2 & 3) of the species *S. minutum* (ITS2 type

246 B1) were chosen because they are the dominant symbiont of wild *Aiptasia pallida* (Thornhill

247 et al., 2013). A mixture of *S. minutum* (ITS2 type B1) with *S. psygmophilum* (ITS2 type B2)

248 was also included to investigate the effects of a mixed symbiont infection between two

249 species within the same clade. Another monoclonal line of *A. pallida*, originally collected in

250 Bermuda, was used as well and hosted one of the unique strains of *S. minutum* (strain 3). All

251 associations of *A. pallida* symbiosis were stable for over 1 year and kept in flow-through

252 systems using FSW under normal growth conditions (26ºC, ~33 ‰, 150 µmol photons m^{-2} s^{-1}

253 photosynthetic photon flux, 12-h light-dark cycle) and fed brine shrimp weekly (Leal et al.,

254 2012). Associations with each symbiont(s) genotype were confirmed before experiments (see

255 below). Aposymbiotic anemones were confirmed to be free of dinoflagellates by periodic

256 microscopic examination as well as after shifting several anemones into lighted incubators

257 over several months.

258

259 **Genetic identification of *Symbiodinium* types**

260   One representative anemone from each of the five *Symbiodinium* combinations was

261 sampled from batch isoclonal cultures prior to the experiment to confirm the genetic identity

262 of the symbionts. Following the heterotrophic feeding (see below), symbiont identity was

263 again confirmed for all five replicates per host/symbiont combination by extracting total DNA

264 from 100 µl of the anemone homogenate (LaJeunesse, 2002). The partial 5.8S and ITS2

265 region of each sample was fingerprinted using PCR-DGGE and the fingerprints characterized

266 by Sanger sequencing of the fingerprint (LaJeunesse, 2002). The symbionts, and their

267 corresponding ITS2 fingerprints, used in this study are detailed in Table A3. Addition

268 diversity and resolution within the B1 ITS2 lineage that better approximates species-level

269 diversity can be gained by sequencing the microsatellite locus B7Sym15 (Finney et al., 2010;

270 Lajeunesse et al., 2012). Therefore, the three strains used were characterized based on flanker

271 region sequence of this locus to definitively place them within the species *S. minutum*

272 (matching genbank# JX263427). To further elucidate the genetic diversity within *S. minutum*

273 and characterize each strain (i.e., multilocus genotype) used in this study, fragment analysis

274 was conducted on five microsatellite loci (B7Sym15, B7Sym34, B7Sym36, CA4.86 and

275 CA6.38) for the samples prior to the experiment (Table A4) (Santos and Coffroth, 2003;

276 Pettay and LaJeunesse, 2007). Two of these loci (B7Sym15 and B7Sym36) were further used

277 at the conclusion of the feeding experiment (see below) to confirm the identity of the

278 genetically distinct *S. minutum* strains and verify that the symbioses had remained stable at

279   both the ITS2 and strain level. Lastly, to verify that the *Aiptasia* clones were genetically

280   distinct, fragment analysis was conducted on six microsatellite loci (AIPT6, AIPT8, AIPT14,

281   AIPT15, AIPT17 and AIPT20) developed for *Aiptasia* spp. Briefly, microsatellite loci were

282   developed from EST sequences of *Aiptasia pallida* (Sunagawa et al., 2009) that were vectored

283   screened (using VecScreen & the UniVec NCBI vector library) and assembled (CAP3 (Huang

284   and Madan, 1999)). Contigs and singlent sequences were screened for SSR of di, tri, tetra,

285   penta and hexanucleotides with more than 6 repeats (WebSat (Martins et al., 2009)). Primers

286   were designed for candidate loci (Primer3 (Rozen and Skaletsky, 2000)) and the loci screened

287   on *A. pallida* samples from the Florida Keys and Bermuda collected in 2011. The two clones

288   used in this study differed by at least one allele at all loci except AIPT14, proving that the

289   clonal lines are genetically different (Table A5).

290

291   **Carbon uptake and translocation**

292   Starved anemones of each *Symbiodinium* combination genotype were placed in

293   separate 7 ml scintillation vials containing 2 ml of seawater spiked with 15 μl of ^{14}C

294   bicarbonate (specific activity 17 μCi μmol^{-1}) following previously described procedures

295   (Davy and Cook, 2001). Five anemones were used for each *Symbiodinium* combination and

296   treatment. All vials were then placed on a LED light-table (Cool White Cree XPG-R5; 150

297   μmol photons m^{-2} s^{-1}; 28°C) for 90 minutes. Two additional anemones from each

298   *Symbiodinium* combination were placed in vials with spiked seawater and maintained in the

299   dark for 90 minutes to account for carbon uptake in the dark. Three additional vials containing

300   only the spiked seawater were also included for measurement of total activity. After

301   incubations each anemone was ground up in 1 ml of seawater with a 1.5 ml Ten Broeck tissue

302   grinder. A 100 μl sample of the resulting homogenate was removed and fixed with 10 μl of

303   1% glutaraldehyde (Acros Organics) and utilized for symbiont cell counts (see below). The

304   remaining homogenate was centrifuged at 5000 g for 5 minutes to separate the host and

305   symbiont portions. A 500 μl sample of the host supernatant (H_s) was removed and the

306   remaining supernatant was stored for downstream calculation of protein content. The

307   symbiont pellet (S) was then resuspended in 400 μl of FSW. Dissolved organic carbon (DOC)

308   released from the organism was also calculated by removing 200 μl of seawater from each

309   anemone-containing vial. All samples were acidified using an equal volume of 2N HCL for

310   24 hours, then dosed with 5ml of scintillation cocktail. After an additional 24-hour period,

311   radioactivity was determined with a liquid scintillation counter (Beckman LS-6500).

312   Calculations were made using the mean specific activity (gC dpm^{-1}), which was estimated

313 from the carbon content of 0.024 g C L^{-1} of seawater (Davy and Cook, 2001). All samples

314 were background and dark uptake corrected. Translocation (T) was calculated as (DOC+H$_s$)

315 normalized to host protein (see below). Photosynthesis (P$_{net}$) was calculated as (DOC+H$_s$+S)

316 normalized to total symbiont cells per anemone (see below). The fraction of photosynthate

317 translocated to the host was calculated as (DOC+H$_s$)/(DOC+H$_s$+S).

318

319 **Heterotrophic feeding**

320   *A. pallida* individuals starved over one week were placed in 1.2 l Plexiglas chambers

321 with unidirectional flow (0.1 m s^{-1}) (Leal et al., 2014b) and each replicate consisted of a

322 separate feeding chamber with one individual each for each symbiont genotype tested and

323 aposymbiotic anemones. Newly hatched *Artemia* nauplii (24 h) were added at a final

324 concentration of 2 nauplii ml^{-1} to each chamber once the polyps were expanded. After 15 min

325 feeding time, anemones were sampled and thoroughly rinsed three times in FSW to detach

326 any prey that were captured but not ingested. Five individuals were immediately flash-frozen

327 and stored at -80 °C for molecular assessment of prey ingestion, and the other five individuals

328 were transferred to a prey-free environment where they were allowed to digest ingested prey

329 over 24 h before sampling as previously described. Unfed *A. pallida* from each *Symbiodinium*

330 combination were also sampled prior to feeding incubations as negative controls. Samples (*n*

331 = 5) of a known number of *Artemia* nauplii (varying from 5 to 100 individuals) used in the

332 experiment were also flash-frozen and stored at -80 °C for gDNA extraction.

333 Each individual stored during feeding incubations was thawed at room temperature, ground

334 with ultra turrax (IKA, Staufen, Germany) in 1 ml of FSW, and 300 μL transferred for

335 genomic DNA (gDNA) extraction. The remaining 700 μl were centrifuged (5 min, 4 °C, 8000

336 g) to pellet the symbionts. The supernatant composed of the cnidarian host fraction was

337 transferred to a new tube and stored at -80 °C for later protein quantification. The pellet was

338 re-suspended in 400 μl of FSW and 100 μl was aliquoted to a new tube with 10 μl of

339 glutaraldehyde (1%, Acros Organics) and stored at 4 °C for determination of symbiont cell

340 densities.

341   Total animal protein was assessed using Pierce BCA Protein Assay Kit (Thermo

342 Scientific, Rockford, IL) following the manufacturer's instructions for microplate readings.

343 Absorbance (562 nm) was measured using a FluoStar Omega reader (BMG Labtech GmbH,

344 Ortenberg, Germany). Symbiont cell density was recorded by fluorescence microscopy. Six

345 independent replicate counts were performed for each sample on a haemocytometer. Samples

346 were photographed using a Nikon microphot-FXA epifluorescent microscope (100x

347 magnification). Photographs were then analyzed by computer software ImageJ (Schneider et

348 al., 2012) with the analyze particles function.

349   Extraction of genomic DNA (gDNA) was performed using the MoBio UltraClean

350 Tissue and Cells DNA isolation Kit (Carlsbad, Ca, USA) following the manufacturer's

351 instructions, apart from the bead beating step that used ~100 μl of 0.5 mm glass beads for

352 each extraction. After extraction, gDNA was quantified using a Nanodrop 2000c

353 spectrophotometer (Thermo Scientific, New York, NY, USA). Total gDNA from *Artemia*

354 nauplii samples was used to estimate the gDNA content per individual prey item (Leal et al.,

355 2014b). Prey DNA content in each anemone sample was estimated through qPCR

356 amplification of *Artemia* DNA using the primers Af18s-1298F and Af18S-1373R (Leal et al.,

357 2014b). For each qPCR reaction a dilution series of extracted gDNA from *Artemia* nauplii

358 was run as a quantitative standard. The appropriate amount of template DNA in all assays was

359 achieved using 1 μl of gDNA extract. qPCR reactions were performed using a 7500 RealTime

360 PCR System (Applied Biosystems, Foster City, Ca, USA) in 96-well plates with each reaction

361 well containing 5 μl of SensiMix SYBR Hi-ROX reaction mix (Bioline, UK), 400 nM of

362 primers and template gDNA. Amplification conditions included an initial denaturation step

363 (10 min, 95 °C) followed by 40 amplification cycles (15s, 94 °C; 30s 60 °C

364 annealing/extension temperature). Each reaction was followed by a melt-curve thermal profile

365 from 60 °C to 95 °C to evaluate specificity of the primers. All reactions were run in triplicate

366 and PCR-grade water was used as a template for negative control, as well as gDNA from

367 unfed anemones. Mean primer efficiency in all qPCR reactions was 99.2% ± 1.0%. To

368 estimate prey DNA break down, all samples were also run in qPCR reactions using primers

369 Af18s-1298F and Af18S-1422R (Leal et al., 2014b). As prey are digested there is decreasing

370 gDNA quantity with increasing amplicon size (Troedsson et al., 2009). Thus, the ratio

371 between prey DNA content obtained with the two primer sets amplifying amplicon sizes of 73

372 and 112 bp will be 0 if all prey DNA is degraded (no prey DNA detected using the primer set

373 amplifying the 112 bp amplicon) and 100 if no prey DNA is broken down (equal amount of

374 prey DNA detected using both primer sets) (Leal et al., 2014b). Percentage digestion rate was

375 assessed through the ratio between prey DNA content detected in samples collected after 24 h

376 digestion and immediately after ingestion. A digestion rate of 0% indicates that no prey DNA

377 was detected after 24h digestion, whereas 100% means identical prey DNA contents are

378 observed immediately after ingestion and after 24h digestion. Prey DNA content was

379 normalized to animal protein.

380

Statistics

381

382   All measured parameters were compared among host/dinoflagellate combinations with

383 a one-way ANOVA. Tukey's HSD post-hoc test was used when statistical differences were

384 observed ($P<0.05$). Pearson's correlation was used to assess the relationship between different

385 parameters measured in the same *Aiptasia* individuals. Due to the necessarily destructive

386 nature of the methods, carbon translocation and prey ingestion were measured on different

387 individuals; hence some statistical comparisons were not possible. Patterns of symbiont

388 genotype and photosynthesis or symbiont cell density on carbon translocation per cell or per

389 unit host protein, respectively, were tested using ANCOVA. Model residuals were checked to

390 verify assumptions of normality and homogeneity of variance and transformations were not

391 required (Zuur et al., 2009). Statistical analyses were performed with R (R Development Core

392 Team, 2013)

393

394

398

399   **Competing interests**

400 The authors declare no competing interests.

401

402   **Author contributions**

403 M.C.L., K.H., D.T.P., A.G. and M.E.W. designed research, M.C.L., K.H., D.T.P. performed

404 research and analyzed data, A.G. and M.E.W. contributed new reagents/analytic tools, M.C.L.,

405 K.H., D.T.P., R.C and M.E.W wrote the manuscript.

406

413

414   **Supplementary Material**

415   Five tables are included as supplementary material.

416

417

418   **References**

419   **Anthony, K. R. N. and Fabricius, K. E.** (2000). Shifting roles of heterotrophy and
420   autotrophy in coral energetics under varying turbidity. *J. Exp. Mar. Biol. Ecol.* **252**, 221-253.
421   **Bachar, A., Achituv, Y., Pasternak, Z. and Dubinsky, Z.** (2007). Autotrophy versus
422   heterotrophy: The origin of carbon determines its fate in a symbiotic sea anemone. *J. Exp.*
423   *Mar. Biol. Ecol.* **349**, 295-298.
424   **Burriesci, M. S., Raab, T. K. and Pringle, J. R.** (2012). Evidence that glucose is the major
425   transferred metabolite in dinoflagellate-cnidarian symbiosis. *The Journal of Experimental*
426   *Biology* **215**, 3467-3477.
427   **Coffroth, M. A. and Santos, S. R.** (2005). Genetic diversity of symbiotic dinoflagellates in
428   the genus *Symbiodinium. Protist* **156**, 19-34.
429   **Davy, S. and Cook, C.** (2001). The relationship between nutritional status and carbon flux in
430   the zooxanthellate sea anemone *Aiptasia pallida. Mar. Biol.* **139**, 999-1005.
431   **Dubinsky, Z. and Jokiel, P. L.** (1994). Ratio of energy and nutrient fluxes regulates
432   symbiosis between zooxanthellae and corals. *Pacific Sci* **48**, 313-324.
433   **Dubinsky, Z. and Berman-Frank, I.** (2001). Uncoupling primary production from
434   population growth in photosynthesizing organisms in aquatic ecosystems. *Aquat. Sci.* **63**, 4-17.
435   **Fabina, N. S., Putnam, H. M., Franklin, E. C., Stat, M. and Gates, R. D.** (2013).
436   Symbiotic specificity, association patterns, and function determine community responses to
437   global changes: defining critical research areas for coral-*Symbiodinium* symbiosis. *Global*
438   *Change Biol.* **19**, 3306-3316.
439   **Falkowski, P., Dubinsky, Z., Muscatine, L. and Porter, J.** (1984). Light and the
440   bioenergetics of a symbiotic coral. *BioScience* **34**, 705-709.
441   **Ferrier-Pagès, C., Peirano, A., Abbate, M., Cocito, S., Negri, A., Rottier, C., Riera, P.,**
442   **Rodolfo-Metalpa, R. and Reynaud, S.** (2011). Summer autotrophy and winter heterotrophy
443   in the temperate symbiotic coral *Cladocora caespitosa. Limnol. Oceanogr.* **56**, 1429-1438.
444   **Finney, J. C., Pettay, D. T., Sampayo, E. M., Warner, M. E., Oxenford, H. A. and**
445   **LaJeunesse, T. C.** (2010). The relative significance of host-habitat, depth, and geography on
446   the ecology, endemism, and speciation of coral endosymbionts in the genus *Symbiodinium.*
447   *Microbial Ecology* **60**, 250-263.
448   **Glynn, P. W., Maté, J. L., Baker, A. C. and Calderón, M. O.** (2001). Coral bleaching and
449   mortality in Panama and Ecuador during the 1997-1998 El Niño-southern oscilation event:
450   spatial/temporal patterns and comparisons with the 1982-1983 event. *Bull. Mar. Sci.* **69**, 79-
451   109.
452   **Grottoli, A. G., Rodrigues, L. J. and Palardy, J. E.** (2006). Heterotrophic plasticity and
453   resilience in bleached corals. *Nature* **440**, 1186-1189.
454   **Grottoli, A. G., Warner, M. E., Levas, S. J., Aschaffenburg, M. D., Schoepf, V.,**
455   **McGinley, M., Baumann, J. and Matsui, Y.** (2014). The cumulative impact of annual coral
456   bleaching can turn some coral species winners into losers. *Global Change Biol.* **20**, 3823-
457   3833.
458   **Hiebert, T. C. and Bingham, B. L.** (2012). The effects of symbiotic state on heterotrophic
459   feeding in the temperate sea anemone *Anthopleura elegantissima. Mar. Biol.* **159**, 939-950.
460   **Hoogenboom, M., Rodolfo-Metalpa, R. and Ferrier-Pages, C.** (2010). Co-variation
461   between autotrophy and heterotrophy in the Mediterranean coral *Cladocora caespitosa. J.*
462   *Exp. Biol.* **213**, 2399-2409.

463 **Huang, X. and Madan, A.** (1999). CAP3: A DNA sequence assembly program. *Genome*
464 *Research* **9**, 868-877.
465 **Hughes, A. D. and Grottoli, A. G.** (2013). Heterotrophic compensation: a possible
466 mechanism for resilience of coral reefs to global warming or a sign of prolonged stress? *PLoS*
467 *One* **8**, e81172.
468 **LaJeunesse, T.** (2002). Diversity and community structure of symbiotic dinoflagellates from
469 Caribbean coral reefs. *Mar. Biol.* **141**, 387-400.
470 **Lajeunesse, T. C., Pettay, D. T., Sampayo, E. M., Phongsuwan, N., Brown, B., Obura, D.,**
471 **Hoegh-Guldberg, O. and Fitt, W. K.** (2010a). Long-standing environmental conditions,
472 geographic isolation and host-symbiont specificity influence the relative ecological
473 dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium*.
474 *Journal of Biogeography* **37**, 785-800.
475 **Lajeunesse, T. C., Smith, R., Walther, M., Pinzon, J., Pettay, D. T., McGinley, M.,**
476 **Aschaffenburg, M., Medina-Rosas, P., Cupul-Magana, A. L., Perez, A. L. et al.** (2010b).
477 Host-symbiont recombination versus natural selection in the response of coral-dinoflagellate
478 symbioses to environmental disturbance. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* **277**, 2925-
479 2934.
480 **Lajeunesse, T. C., Parkinson, J. E. and Reimer, J. D.** (2012). A genetics-based description
481 of *Symbiodinium minutum* sp. nov. and *S. psygmophilum* sp. nov. (Dinophyceae), two
482 dinoflagellates symbiotic with cnidaria. *J. Phycol.* **48**, 1380-1391.
483 **Leal, M. C., Nunes, C., Engrola, S., Dinis, M. and Calado, R.** (2012). Optimization of
484 monoclonal production of the glass anemone *Aiptasia pallida* (Agassiz in Verrill, 1864).
485 *Aquaculture* **354-355**, 91-96.
486 **Leal, M. C., Nunes, C., Kempf, S. C., Reis, A., Silva, T. L., Serôdio, J., Cleary, D. F. R.**
487 **and Calado, R.** (2013). Effect of light, temperature and diet on the fatty acid profile of the
488 tropical sea anemone *Aiptasia pallida*. *Aquacult. Nutr.* **19**, 818-826.
489 **Leal, M. C., Ferrier-Pagès, C., Calado, R., Brandes, J. A., Frischer, M. E. and**
490 **Nejstgaard, J. C.** (2014a). Trophic ecology of the facultative symbiotic coral *Oculina*
491 *arbuscula*. *Mar Ecol Prog Ser* **504**, 171-179.
492 **Leal, M. C., Nejstgaard, J. C., Calado, R., Thompson, M. E. and Frischer, M. E.** (2014b).
493 Molecular assessment of heterotrophy and prey digeston in zooxanthellate cnidarians. *Mol.*
494 *Ecol.* **23**, 3838-3848.
495 **Loram, J. E., Trapido-Rosenthal, H. G. and Douglas, A. E.** (2007). Functional significance
496 of genetically different symbiotic algae *Symbiodinium* in a coral reef symbiosis. *Mol. Ecol.* **16**,
497 4849-4857.
498 **Martins, W. S., Lucas, D. C. S., Souza Neves, K. F. and Bertioli, D. J.** (2009). WebSat: A
499 web software for microsatellite marker development. *Bioinformation* **3**, 282.
500 **Muscatine, L., McCloskey, L. and Marian, R.** (1981). Estimating the daily contribution of
501 carbon from zooxanthellae to coral animal respiration. *Limnol. Oceanogr.* **26**, 601-611.
502 **Muscatine, L., Porter, J. W. and Kaplan, I. R.** (1989). Resource partitioning by reef corals
503 as determined from stable isotope composition. I. δ13C of zooxanthellae and animal tissue vs
504 depth. *Mar. Biol.* **100**, 185-193.
505 **Pettay, D. T. and LaJeunesse, T. C.** (2007). Microsatellites from clade B *Symbiodinium* spp.
506 specialized for Caribbean corals in the genus *Madracis*. *Mol. Ecol. Notes* **7**, 1271-1274.
507 **R Development Core Team.** (2013). R: A language and environment for statistical
508 computing, (ed. R Foundation for Statistical Computing). Vienna, Austria (http://www.R-
509 project.org): R Foundation for Statistical Computing.
510 **Rowan, R.** (2004). Coral bleaching Thermal adaptation in reef coral symbionts. *Nature* **430**,
511 742.

512 **Rozen, S. and Skaletsky, H.** (2000). Primer 3 on the WWW for general users and for
513 biologist programmers. *Methods Mol. Biol.* **132**, 365-386.
514 **Sampayo, E., Ridgway, T., Bongaerts, P. and Hoegh-Guldberg, O.** (2008). Bleaching
515 susceptibility and mortality of corals are determined by fine-scale differences in symbiont
516 type. *Proceedings of the National Academy of Sciences* **105**, 10444.
517 **Santos, S. R. and Coffroth, M. A.** (2003). Molecular genetic evidence that dinoflagellates
518 belonging to the genus *Symbiodinium* Freudenthal are haploid. *The Biological Bulletin* **204**,
519 10-20.
520 **Schneider, C., Rasband, W. and Eliceiri, K.** (2012). NIH Image to ImageJ: 25 years of
521 image analysis. *Nat. Methods* **9**, 671-675.
522 **Schoenberg, D. A. and Trench, R. K.** (1980). Genetic variation in *Symbiodinium*
523 (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine
524 invertebrates: III. Specificity and infectivity of *Symbiodinium microadriaticum*. *Proceedings*
525 *of the National Academy of Sciences* **207**, 445-460.
526 **Sebens, K. P.** (2002). Energetic contraints, size gradients, and size limits in benthic marine
527 invertebrates. *Integr. Comp. Biol.* **42**, 853-861.
528 **Smith, G. and Muscatine, L.** (1999). Cell cycle of symbiotic dinofagellates: variation in G1
529 phase-duration with anemone nutritional status and macronutrient supply in the *Aiptasia*
530 *pulchella-Symbiodinium pulchrorum* symbiosis. *Mar. Biol.*, 1-14.
531 **Starzak, D. E., Quinnell, R. G., Nitschke, M. R. and Davy, S. K.** (2014). The influence of
532 symbiont type on photosynthetic carbon flux in a model cnidarian-dinoflagellate symbiosis.
533 *Mar. Biol.* **161**, 711-724.
534 **Sunagawa, S., Wilson, E. C., Thaler, M., Smith, M. L., Caruso, C., Pringle, J. R., Weis,**
535 **V. M., Medina, M. and Schwarz, J. A.** (2009). Generation and analysis of transcriptomic
536 resources for a model system on the rise: the sea anemone *Aiptasia pallida* and its
537 dinoflagellate endosymbiont. *BMC genomics* **10**, 258.
538 **Thornhill, D. J., Xiang, Y., Pettay, D. T., Zhong, M. and Santos, S. R.** (2013). Population
539 genetic data of a model symbiotic cnidarian system reveal remarkable symbiotic specificity
540 and vectored introductions across ocean basins. *Mol. Ecol.* **22**, 4499-4515.
541 **Tremblay, P., Grover, R., Maguer, J. F., Hoogenboom, M. and Ferrier-Pagès, C.** (2014).
542 Carbon translocation from symbiont to host depends on irradiance and food availability in the
543 tropical coral *Stylophora pistillata*. *Coral Reefs* **33**, 1-13.
544 **Troedsson, C., Simonelli, P., Nägele, V., Nejstgaard, J. C. and Frischer, M. E.** (2009).
545 Quantification of copepod gut content by differential length amplification quantitative PCR
546 (dla-qPCR). *Mar. Biol.* **156**, 253-259.
547 **Venn, A., Loram, J. and Douglas, A.** (2008). Photosynthetic symbioses in animals. *J. Exp.*
548 *Bot.* **59**, 1069-1080.
549 **Weis, V., Davy, S., Hoegh-Guldberg, O., Rodriguez-Lanetty, M. and Pringle, J.** (2008).
550 Cell biology in model systems as the key to understanding corals. *Trends Ecol. Evol.* **23**, 369-
551 376.
552 **Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. and Smith, G. M.** (2009). Mixed
553 effects models and extensions in ecology with R. New York: Springer.

554

555

556 **Figure legends**

557

558 **Figure 1. Relationship between maximal photosynthesis and carbon translocation to the**

559 **host.** Photosynthesis and carbon translocation rates normalized to *Symbiodinium* cell number

560 in individual *Aiptasia pallida* hosting a single unique strain of *Symbiodinium minutum*

561 (denoted by strain 1, 2 or 3) or a 'mixture' of *S. minutum* strain 2 and *S. psygmophilum* or D4-

562 5 ($n = 5 \cdot Symbiodinium$ genotype combination^{-1}). Dashed lines correspond to a linear fit for

563 each strain. Symbioses with strains 1, 2, 'mixture,' and D4-5 were all with the same clonal

564 (CC7) anemone line.

565

566 **Figure 2. Autotrophy and heterotrophy.** Measured in individuals ($n = 5$) hosting a single

567 unique strain of *Symbiodinium minutum* (denoted by strain 1, 2 or 3), a mixture of *S. minutum*

568 (strain 2) and *S. psygmophilum*, or *Symbiodinium* D4-5. **A**, Total carbon translocation and

569 prey ingestion rates (mean ± s. e.). **B**, Prey ingestion rate (mean ± s. e.; estimated through

570 prey DNA content). **C**, Prey DNA breakdown at ingestion (mean ± s. e.; 100% indicates no

571 prey DNA breakdown and 0% indicates total prey DNA breakdown). *Apo* denotes

572 aposymbiotic anemones (mean: dashed line; s.e.: dotted line; **A**). Different superscript letters

573 (**B**, **C**) indicate significant differences among genotypes (Tukey's HSD, $P<0.05$). Symbioses

574 with strains 1, 2, 'mixture,' and D4-5 and the aposymbiotic animals were all with the same

575 clonal (CC7) anemone line.

576

17

577   **Table 1.** Photobiological characteristics (average ± st. deviation) of individual *Aiptasia*

578   *pallida* hosting a single unique strain of *Symbiodinium minutum* (denoted by strain 1, 2 or 3)

579   or a mixture of *S. minutum* strain 2 and *S. psygmophilum*, or D4-5 ($n = 5$ • *Symbiodinium*

580   genotype combination^{-1}). Different superscript letters in the same row denote significant

581   differences ($P<0.05$)

Parameter	Strain 1	Strain 2	Strain 3	Mixture	D4-5
Symbiodinium cell density (cells μg^{-1} protein)	2778±958 ^{A}	2544±328 ^{AB}	5057±827 ^{C}	1883±522 ^{AB}	1382±116 ^{B}
Photosynthesis (10^{-7} μg C cell^{-1} h^{-1})	7.92±1.17 ^{AB}	2.38±1.17 ^{C}	5.96±2.17 ^{BC}	5.60±4.04 ^{BC}	8.54±2.69 ^{B}
Carbon translocation (10^{-7} μg C cell^{-1} h^{-1})	2.49±0.38 ^{AB}	1.09±0.75 ^{B}	2.02±0.84 ^{AB}	2.31±1.31 ^{AB}	3.14±0.58 ^{A}
Total carbon translocation (10^{-4} μg C μg^{-1} protein h^{-1})	6.1±2.9 ^{AB}	3.5±1.8 ^{A}	11.0±4.4 ^{B}	4.4±2.5 ^{A}	5.1±4.1 ^{AC}

582