# Flexibility of nutritional strategies within a mutualism: food availability affects algal endosymbiont productivity in two congeneric sea anemone species

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**Abstract**

Mutualistic symbioses are common, especially in nutrient-poor environments where an association between hosts and symbionts can allow the symbiotic partners to persist and collectively out-compete non-symbiotic species. Usually these mutualisms are built on an intimate transfer of energy and nutrients (e.g., carbon and nitrogen) between host and symbiont. However, resource availability is not consistent, and the benefit of the symbiotic association can depend on the availability of resources to mutualists. We manipulated the diets of two temperate sea anemone species in the genus *Anthopleura* in the field and recorded the responses of sea anemones and algal symbionts in the family Symbiodiniaceae to our treatments. Algal symbiont density, symbiont volume, and photosynthetic efficiency of symbionts responded to changes in sea anemone diet, but the responses depended on the species of sea anemone. We suggest that temperate sea anemones and their symbionts can respond to changes in anemone diet, modifying the balance between heterotrophy and autotrophy in the symbiosis. Our data support the hypothesis that symbionts are upregulated or downregulated based on food availability, allowing for a flexible nutritional strategy based on external resources.

Key words: *Anthopleura*, context-dependent, eco-physiology, mutualistic symbiosis, sea anemone, Symbiodiniaceae

## 1. Introduction

In nutrient-poor environments, mutualistic symbioses are common [1–3]. In these symbioses a diverse set of nutrients are exchanged between partners, but the unifying theme is an exchange of carbon and nitrogen. For example, in relatively nutrient-poor environments, partnerships form

between legumes and rhizobia [4], fungi and algae (i.e., lichens) [5], and corals and algal endosymbionts [6]. However, these environments are not static, and as resources for hosts and symbionts fluctuate [7,8], the benefit to each partner may change, potentially disrupting the symbiosis. Legumes in nitrogen-enriched soil no longer benefit from their symbiotic rhizobia [8], lichens are impacted by nitrogen deposition [7], and coral-algal symbioses may break down as a result of human-induced nutrient fluctuations [9]. Most previous studies have focused on anthropogenic changes in nutrient availability; we know less about how natural fluctuations in resources affect mutualistic symbioses *in situ*. A species that can obtain external resources when they are plentiful and simultaneously maintain its association with symbionts could employ a flexible nutritional strategy that depends on resource availability.

Scleractinian coral and their algal endosymbionts have been described using an ecophysiological framework based on nutrient and energy exchange since these relationships were first described [10,11]. Studies of coral-algal symbioses have informed our understanding of metabolic exchange between symbiotic partners including autotrophic products from the algae and heterotrophic nutrients from zooplankton captured by the coral [12–14]. In recent years, a large body of research has focused on the breakdown between corals and their algal symbionts, highlighting the importance of symbionts in coral metabolism [15,16]. However, symbiotic coral species are obligate mutualists (with the exception of *Astrangia poculata*) where symbiont and host derived nutrition are balanced and critical for survival; flexibility between autotrophic and heterotrophic nutritional pathways is limited (but see [16–18]).

Some tropical and temperate sea anemone species are similar to corals in obligately associating with algal endosymbionts [19], but many symbiotic sea anemones, especially temperate species, are facultative mutualists [20]. In contrast to the nutrient-poor environments

where corals and some tropical sea anemones live, temperate anemones often benefit from nutrient-rich environments where prey are abundant [19,21], enhancing the potential for nutritional flexibility in these symbioses. Symbiont densities in natural populations can vary substantially, and these densities are affected by light intensity and temperature [22,23] [S. Bedgood, *unpubl. data*]. At the same time, sea anemones are opportunistic passive suspension feeders that rely on water currents, tides, waves, and chance to deliver potential prey, so food availability can be unpredictable and can vary among individuals and across time [24–26]. Whereas several studies have addressed how starvation affects the relationship between anemones and algal symbionts in lab manipulations of tropical [27–29] and temperate [30] species, the applicability of these studies to field conditions remains unknown, as little is known about how variation in food availability affects algal symbionts and their contribution to the host sea anemones in the field. If the relationship between the sea anemone and its algal symbionts is driven by the requirements of the anemone host, then symbionts would be downregulated when prey are readily available and upregulated when prey are scarce. Here we investigate if realistic, *in situ* changes in the food available to sea anemone hosts, based on naturally occurring fluctuations observed in previous studies [24] [S. Bedgood, *unpubl. data*], affect the abundance, photophysiology, and interactions between algal symbionts and their host sea anemone.

We studied *Anthopleura sola* and *Anthopleura xanthogrammica,* two sea anemone species that host algal symbionts. Both species coexist on California rocky shores [31,32] [S. Bedgood, *unpubl. data*], where light is abundant for photosynthesizing symbionts, and food is washed in from adjacent intertidal habitats and the ocean. Both species are similar in size, consume the same prey, and use similar habitat in the mid-intertidal zone [S. Bedgood, *unpub. data*; this study].

The algal symbionts within *A. sola* and *A. xanthogrammica* at our study location are in the family Symbiodiniaceae, the same group that includes symbionts in tropical corals [33]. These symbionts are in the genus *Breviolum* (previously Clade B) [34–36] and provide a substantial portion of the anemones' dietary carbon as demonstrated by stable isotope analyses [37,38]. Genetic differences between symbionts in *A. sola* and *A. xanthogrammica* at the same site and tidal height are minimal in this region; genetically identical symbionts are found in both sea anemone species [36]. Therefore, differences in the responses of symbionts are likely due to differences between sea anemone species, not differences in symbiont identity.

The growth rate potential of symbiont cells is likely always higher than that of host cells in cnidarian-algal symbioses, so it is crucial that the host has some control of symbiont density [39]. Algal symbionts reproduce asexually within their anemone hosts resulting in higher densities [37] and can vary in volume likely based on productivity [37,40]. *Anthopleura elegantissima* (a congeneric co-occurring species) can exocytose and egest algal cells to control their densities [41,42]. There are costs to maintaining high symbiont densities in this species, most notably the production of oxygen radicals ($H_2O_2$) by photosynthesizing symbionts under intense light that damage host cells [43,44]. While the mechanisms underlying control of symbiont densities in *Anthopleura* spp. are not fully understood, symbiont densities are known to be maintained by nitrogen availability within the host anemone [45,46], by coregulation of host and symbiont cell cycles [47], and by symbiont degradation within the host in tropical cnidarian-algal symbioses [39]. While the algal symbionts may increase their densities by reproducing within the host, the anemone likely has substantial control of symbiont density.

If symbionts function as a partial substitute for captured prey, and there is a cost to the host of maintaining high densities of symbionts within the tissue, then we would expect to

observe reduced symbiont abundances when prey are abundant and/or higher abundances when prey are scarce (Fig. 1). We hypothesize that this symbiotic partnership is nutritionally flexible and therefore predict that realistic changes in host diet will influence three measures of symbiont productivity (see Fig. 1). (1) Symbiont density – which we hypothesize is controlled by the host - will increase when prey are removed and decrease when prey are added. (2) Individual symbiont cell volume will decrease when prey are removed (i.e., more photosynthetic products are given to the host and less is stored in the symbiont cell) and increase when prey are added (i.e., symbionts store photosynthetic products that are not translocated to the host, increasing cell volume). (3) Photosynthetic efficiency will be affected by nitrogen availability within the host (i.e., hosts with added prey may translocate more nitrogen to their symbionts). However, we do not predict any change in photosynthetic efficiency when prey are removed, as hosts in nutrient-rich environments are likely to retain nitrogen when prey are scarce.

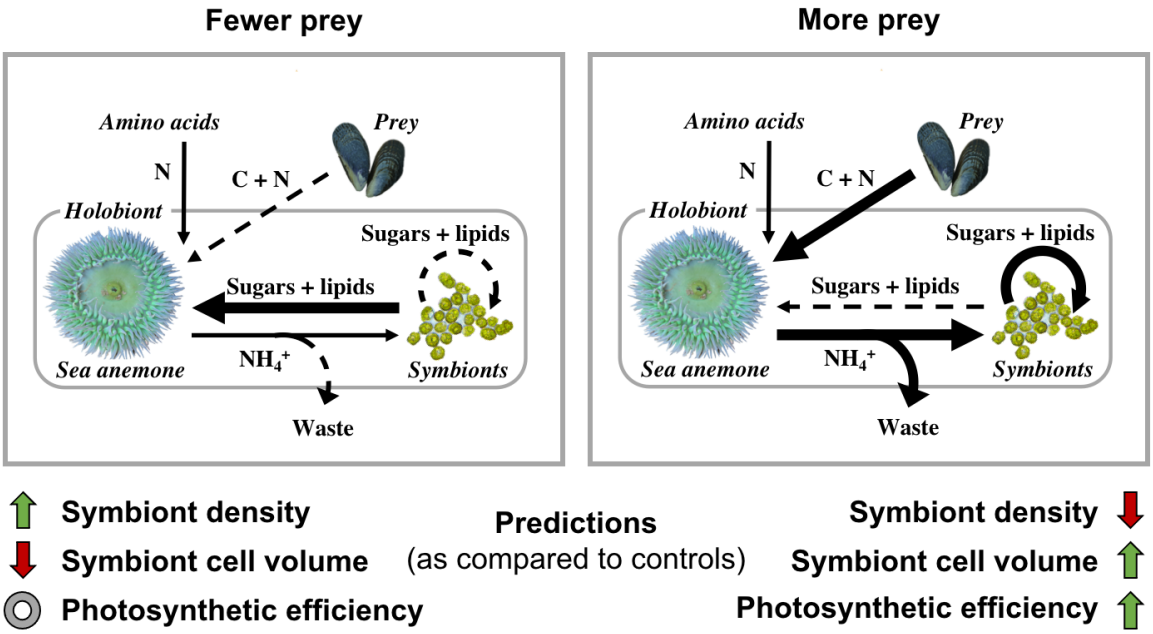


**Figure 1.** Predictions of algal symbiont contributions based on prey availability. Arrows represent the flow of carbon and nitrogen from one source to another. The thickness of each arrow represents the relative contribution and dashed lines represent a reduction in contribution. Predictions of algal symbiont responses to sea anemone dietary changes (increase, decrease, or no effect) are listed under each scenario.

## 2. Methods

### (a) Site description and experimental treatments

Individuals of both sea anemone species (*A. sola* and *A. xanthogrammica*; $n = 28$ each) were located in the intertidal zone at Kenneth S. Norris Rancho Marino Reserve (35°32'24.32"N, 121° 5'34.12"W). Sea anemones were excluded if their largest closed crown diameter was less than 40 mm because anemones smaller than this had distinctly different diets [i.e. no mussels or sea urchins, S. Bedgood, *pers. obs.*]. We used the length and width of the closed crown to calculate the area (using an ellipse shape) as a measure of anemone size at the beginning and end of the experiment. All sea anemones were located between +0.4 m and +1.1 m above mean lower-low water. Each *A. sola* was paired with a nearby *A. xanthogrammica* within the same habitat. We used a blocked design consisting of 8 sea anemones (4 *A. sola* and 4 *A.*

142 *xanthogrammica*) in close proximity (e.g., within the same tide pool) that matched all four
143 feeding and species treatments ($n$ = 7 blocks).

144 Four treatments were maintained for three weeks in both species, beginning in June 2018.
145 Treatments included supplement, control, reduction, and probe. "Supplement" anemones were
146 fed either squid or mussel tissue once daily during the daytime low tide. These are representative
147 of the types of food items that *Anthopleura* spp. consume at this site [S. Bedgood*, unpub. data*].
148 The size of the prey items offered to each anemone was proportional to the anemone's size and
149 ranged between 3 and 4 g wet mass. "Supplement" anemones likely captured additional prey, so
150 the added food supplemented their natural diet. We did not manipulate the anemones in the
151 control treatments, allowing them to capture prey as usual. We touched the tentacles of
152 "reduction" anemones, waited for their mouths to open, and reached in with a probe or fingers to
153 remove any prey that we found in the gastrovascular cavity. If possible, the prey items were
154 identified prior to being disposed of. We did this once daily during low tide. Since anemones
155 may digest prey within a few hours [20], this treatment likely represented a reduction in food
156 availability instead of complete removal. We treated the "probe" anemones the same way as the
157 "removal" individuals but did not remove any prey.

158 **(b) Symbiont density and cell volume**

159 We collected 2-3 tentacles with dissecting scissors from each sea anemone one week
160 before treatments began, one week after treatments were initiated, and three weeks after
161 treatments began. We immediately placed samples on ice and transported them to a -25 °C
162 freezer for storage within 24 hours of collection. Samples were thawed in the lab, and we then
163 separated the gastrodermal tissue layer from the epidermal layer by squashing samples between
164 two microscope slides until the clear, tough epidermal layer was devoid of any algal symbionts

165 or gastrodermal anemone cells. We removed the epidermal tissue, added the remaining tissue to
166 1.5 mL of deionized water, and homogenized the tissue and water at 30 beats/sec for 5 min. This
167 method produced well-homogenized samples without breaking algal cells.
168 An aliquot of the homogenate was placed on a Brightline hemocytometer (Hausser
169 Scientific, Horsham, Pennsylvania, USA), and photos of each sample were taken on a
170 microscope at 200X magnification. To count the number of symbionts in each square (1 $mm^2$, *n*
171 = 10), we loaded photos into FIJI [48], where we batch processed images with a custom macro
172 using the particle analysis function (see supplemental). To standardize the symbiont density, we
173 measured animal protein from the same homogenate using the Lowry Method [49] for protein
174 estimation with Bovine Serum as a standard [20,37].
175 We calculated symbiont volume using the same photos taken for symbiont density. We
176 batch-processed photos with the particle analysis function (see supplemental) using an ellipse-
177 shape fit of particles. Using the length and width output, we calculated the volume based on
178 Hillebrand et al [50], assuming a prolate spheroid shape as described for Symbiodiniaceae.

179 **(c) Chlorophyll *a***

180 We took a 1 mL aliquot from the homogenate for chlorophyll *a* (Chl *a*) analysis. The
181 homogenate was centrifuged at 2000g for 5 minutes to create an algal pellet. The supernatant
182 was discarded, and we added 5 mL 90% acetone to each sample. Samples were stored at -25°C
183 overnight before being read on a Turner Design Trilogy Fluorometer.

184 **(d) Photosynthetic efficiency**

185 We quantified the symbionts' photosynthetic efficiency ($F_v/F_m$ of dark-adapted
186 Photosystem II) using a Pulse Amplitude Modulation (PAM) fluorometer (Heinz Walz GmbH,

187 Effeltrich, Germany) to determine the effect of host feeding on photosynthetic electron transport.
188 Chlorophyll *a* concentrations give an estimate of photosynthetic activity potential, but combining
189 those data with measurements of the photosynthetic efficiency of chlorophyll provides further
190 insights into photosynthetic productivity responses. PAM measurements of sea anemones were
191 taken in the dark, between 04:00 and 05:00, on the same days we collected tissue samples. Most
192 anemones were closed when measurements were taken, so the sensor was placed at the top of the
193 anemone column, where symbionts are present but at a lower density than in the tentacle tissue
194 [51] (and see supplemental). If the anemone was open, we disturbed it and waited for it to close.
195 We took the average of three measurements of each anemone.

196 **(e) $\delta^{13}C$ analysis**

197 We collected a 1 $cm^2$ piece of tissue that included both tentacles and column from 4
198 random sea anemones in the control, supplement, and reduction treatments to estimate the
199 contribution of symbiont photosynthate and prey to the anemone's dietary carbon budget.
200 Because this sampling method harms (but does not kill) the animals and could compromise
201 further measurements, these samples were collected at the end of the experiment. Samples were
202 homogenized as described previously. The homogenate was then centrifuged at 2000g for 5 min
203 to separate the anemone cells from the algal symbiont cells. The top layer of anemone cells was
204 then agitated, and the supernatant with suspended anemone cells was removed. Both the algae
205 portion and anemone portion (supernatant) were re-homogenized and centrifuged 2-3 more times
206 to remove any non-target cells. Both the symbiont and anemone portions were placed on separate
207 microscope slides and dried (60 °C for > 48 hr) before analysis at the UCI Stable Isotope Ratio
208 Mass Spectrometry Facility.

209 **(f) Statistical analyses**

We conducted all analyses in R 3.6.2 and RStudio 1.2.5003 [52] using the packages lme4 to create general linear mixed models (GLMMs) and emmeans for post hoc analyses. We checked the diet composition data for normality with a Shapiro-Wilk Test, and then used a paired t-test to compare anemone diets. We used GLMMs paired with ANOVA and Tukey post hoc analyses to analyze $\delta^{13}C$, symbiont density, symbiont cell volume, and photosynthetic efficiency. Data from the two anemone species were typically analyzed separately. $\delta^{13}C$ values were analyzed using GLMMs with the main effects of treatment and tissue type (anemone and symbiont) and a random effect of anemone. Symbiont density, symbiont cell volume, and photosynthetic efficiency were measured over time with two control groups, so we compared treatment groups in pairs through time: control/supplement and probe/reduction. These data were analyzed with GLMMs with main effects of treatment and time and a random effect of anemone.

## (3) Results

### (a) Composition of diets

Prey were found in the gastrovascular cavity of *A. xanthogrammica* almost twice as frequently as in *A. sola* (paired t-test: $t = -3.56$, $p = 0.003$). Prey were found within *A. sola* during 12.92 ± 2.31% (mean ± SE) of daily checks, while prey were found within *A. xanthogrammica* during 23.47 ± 3.18% of checks. The greatest proportion of both species' diets (40% of observations) was composed of the California sandcastle worm, *Phragmatopoma californica*. Other prey items included limpets, hermit crabs, and sea urchins, but each of these comprised less than 10% of diets. There was no apparent difference in the diet composition of the two anemone species. The frequency of prey was 0.90 ± 0.22 items per week for *A. sola* and 1.64 ± 0.15 items per week for *A. xanthogrammica*. We removed an average of 2-6 items from each anemone in the "reduction" treatment over the course of the experiment. The diet supplement

233 treatments received an additional prey item daily, which represented a substantial increase from
234 ambient prey capture rates. However, this frequency of food availability is not uncommon during
235 periods of high wave exposure, when all anemones surveyed had at least one prey item on
236 consecutive days.

237 **(b) Stable isotope analysis**

238 Anemone diet affected $\delta^{13}C$ values (GLMM ANOVA: *A. s.* = *A. sola* treat*portion - $F = 5.73$, $p$
239 = 0.025; *A. x.* = *A. xanthogrammica* treat - $F = 9.74$, $p = 0.007$), but this result was largely
240 associated with the algal symbiont portion for both species (Fig. 2). Symbionts from
241 "supplemented" anemones had $\delta^{13}C$ signatures that were 2-5‰ lower than the controls (GLMM
242 Tukey HSD: *A. s.* - $t = -4.89$, $p = 0.001$; *A. x.* - $t = -4.1$, $p = 0.004$), but reduction of diet had no
243 effect (*A. s.* - $t = 1.96$, $p = 0.165$; *A. x.* - $t = -0.84$, $p = 0.684$). $\delta^{13}C$ values did not differ between
244 anemones and their algae within a treatment, except in the supplement treatment where the
245 symbionts had a lower $\delta^{13}C$ (*A. s.* - $t = 3.0$, $p = 0.015$; A. x. - $t = 2.57$, $p = 0.033$).

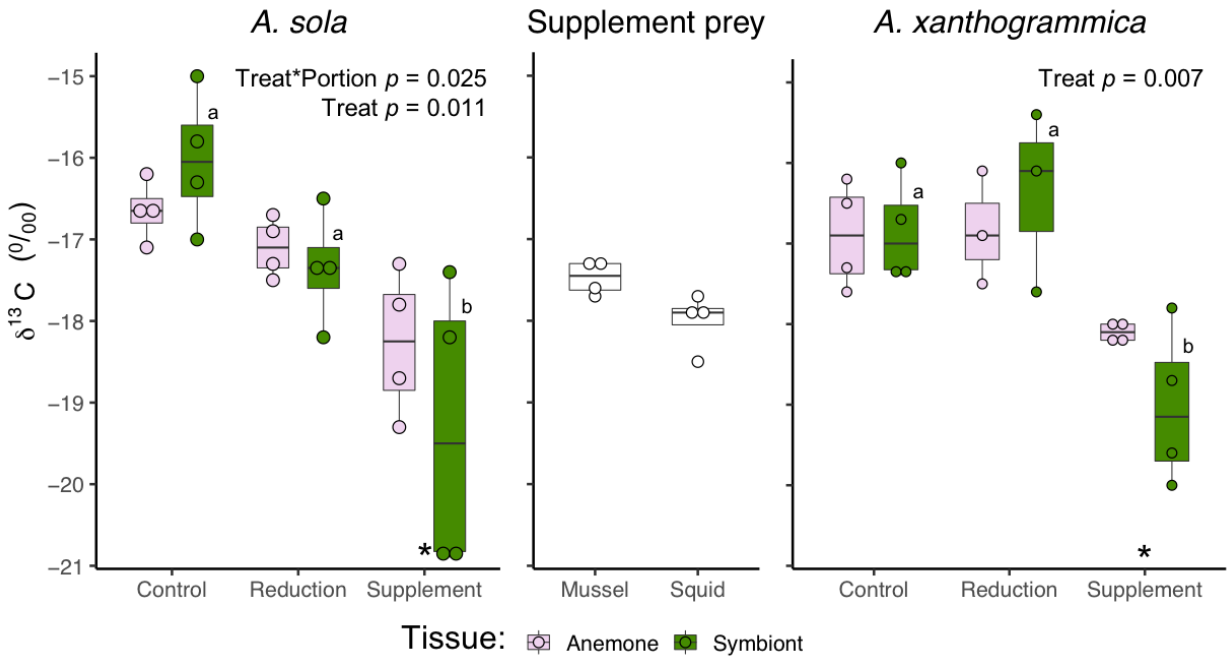


**Figure 2.** Boxplots with overlaid data points (n = 3 or 4) showing δ13C ($^0/_{00}$) values for *A. sola*, *A. xanthogrammica*, and supplemented prey items. Tissue samples from anemones were separated into anemone and algal symbiont portions before analysis. Comparisons are made among the control, reduction, and supplement treatments. Significant main effects and interactions from GLMMs are listed in the upper right-hand corner of each graph with p-values. Asterisks above the x-axis signify significant differences between portions within a treatment from a Tukey post hoc analysis. Lowercase letters represent significant differences among treatment groups within the algal symbiont portion.

**(c) Symbiont density and chlorophyll *a***

The symbiont density was affected by treatment (GLMM ANOVA: *A. s.* - $F = 5.06$, $p = 0.044$) and the effect of treatment changed over time (*A. x.* - $F = 7.69$, $p = 0.003$), but the effect was observed in different treatment groups in each anemone species. In *A. sola*, supplementing food resulted in decreased symbiont densities after one week of treatment (GLMM Tukey HSD: $t = 2.74$, $p = 0.01$), but symbiont density did not increase when food was reduced ($t = 1.39$, $p = 0.173$). In *A. xanthogrammica*, supplementing food did not affect symbiont density ($t = -0.17$, $p = 0.869$), but reducing food increased symbiont density after one week of treatment ($t = -4.23$, $p < 0.001$). All symbiont density measurements changed over time (Figs. 3, 4) due to an increase in symbiont density after one week. Chl *a* per symbiont was not affected by treatment (GLMM ANOVA: *A. s.* reduction - $F = 0.83$, $p = 0.378$; *A. s.* supplement - $F = 0.63$, $p = 0.444$; *A. x.*

reduction - $F = 0.17$, $p = 0.684$; *A. x.* supplement - $F = 1.37$, $p = 0.264$), so Chl *a* concentrations tracked symbiont density measurements closely throughout the experiment (Figs. 3 and 4). However, while there was no effect of supplementation on symbiont density in *A. xanthogrammica*, the Chl *a* concentration in the supplement treatment was lower than the control at week three (Fig. 4; GLMM Tukey HSD: $t = 2.55$, $p = 0.017$). Anemone growth (final size – initial size / initial size) was not different among treatment groups at the final time point (ANOVA: *A. s.* reduction - $F = 0.28$, $p = 0.607$; *A. s.* supplement - $F = 0.29$, $p = 0.603$; *A. x.* reduction - $F = 1.97$, $p = 0.186$; *A. x.* supplement - $F = 2.95$, $p = 0.112$), so anemone growth did not affect symbiont density measurements asymmetrically among groups.

**(d) Symbiont cell volume**

Both sea anemone species had larger symbionts in the supplement treatment (GLMM Tukey HSD: *A. s.* - $t = -4.69$, $p < 0.001$; *A. x.* - $t = -2.26$, $p = 0.033$; Figs. 3, 4) and symbionts were marginally smaller in *A. xanthogrammica* where food was reduced ($t = 2.05$, $p = 0.051$). There was a main effect of time in both species and treatment comparisons where symbiont volume generally decreased over the course of the experiment (*A. s.* reduction - $F = 25.1$, $p < 0.001$; *A. s.* supplement - $F = 17.2$, $p < 0.001$; *A. x.* reduction - $F = 11.5$, $p < 0.001$; *A. x.* supplement - $F = 4.20$, $p = 0.028$).

**(e) Photosynthetic efficiency**

The photosynthetic efficiency of algal symbionts was higher in *A. sola* than in *A. xanthogrammica* at the start of the experiment (paired t-test: $t = 5.72$, $p < 0.001$). This difference persisted throughout the experiment, except when food was supplemented. Then, photosynthetic efficiency in *A. xanthogrammica* increased from 0.56 ± 0.05 (mean ± SE) to 0.71 ± 0.01 $F_v/F_m$ (GLMM Tukey HSD: $t = -2.91$, $p = 0.006$) and did not differ from the mean photosynthetic

281 efficiency of the control treatment *A. sola* symbionts (0.67 ± 0.01 $F_v/F_m$) by the end of the
282 experiment (paired t-test: $t = -1.35$, $p = 0.225$). Photosynthetic efficiency generally increased
283 through time for all groups (Figs. 3,4; GLMM ANOVA: *A. s.* supplement - $F = 5.61$, $p = 0.01$; *A.*
284 *x.* reduction - $F = 5.41$, $p = 0.012$; *A. x.* supplement - $F = 8.07$, $p = 0.002$) except the *A. sola*
285 reduction pairing ($F = 1.74$, $p = 0.198$).
286

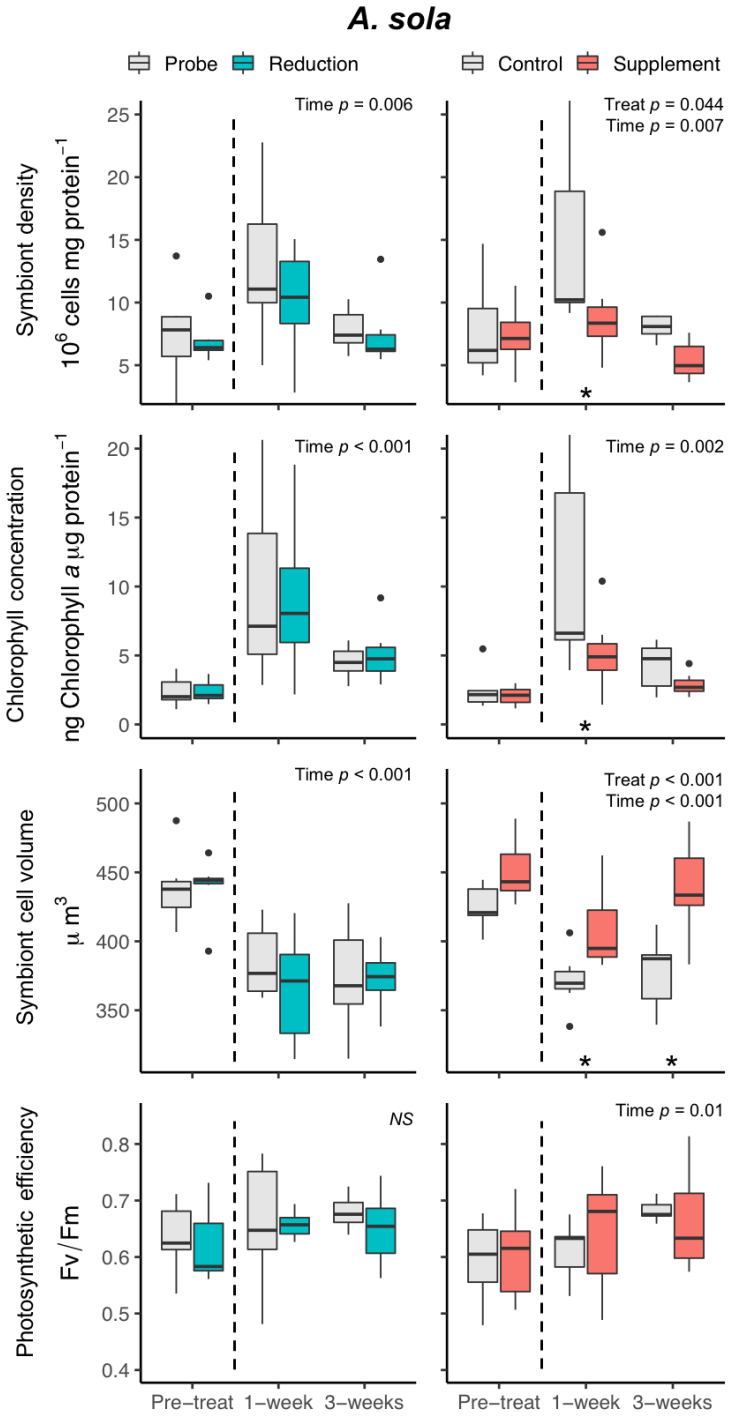


**Figure 4.** Boxplots showing symbiont density, chlorophyll concentration, symbiont cell volume, and photosynthetic efficiency of symbionts within *A. sola* throughout the experiment. Comparisons are made between the supplement and reduction treatments and their respective control treatments. The vertical dashed line represents the start of treatments. Significant main effects and interactions from GLMMs are listed in the upper right-hand corner of each graph with p-values. Asterisks above the x-axis signify significant differences between controls and treatments at a given time-point from a Tukey post hoc analysis. n = 7 for each treatment at each time point.

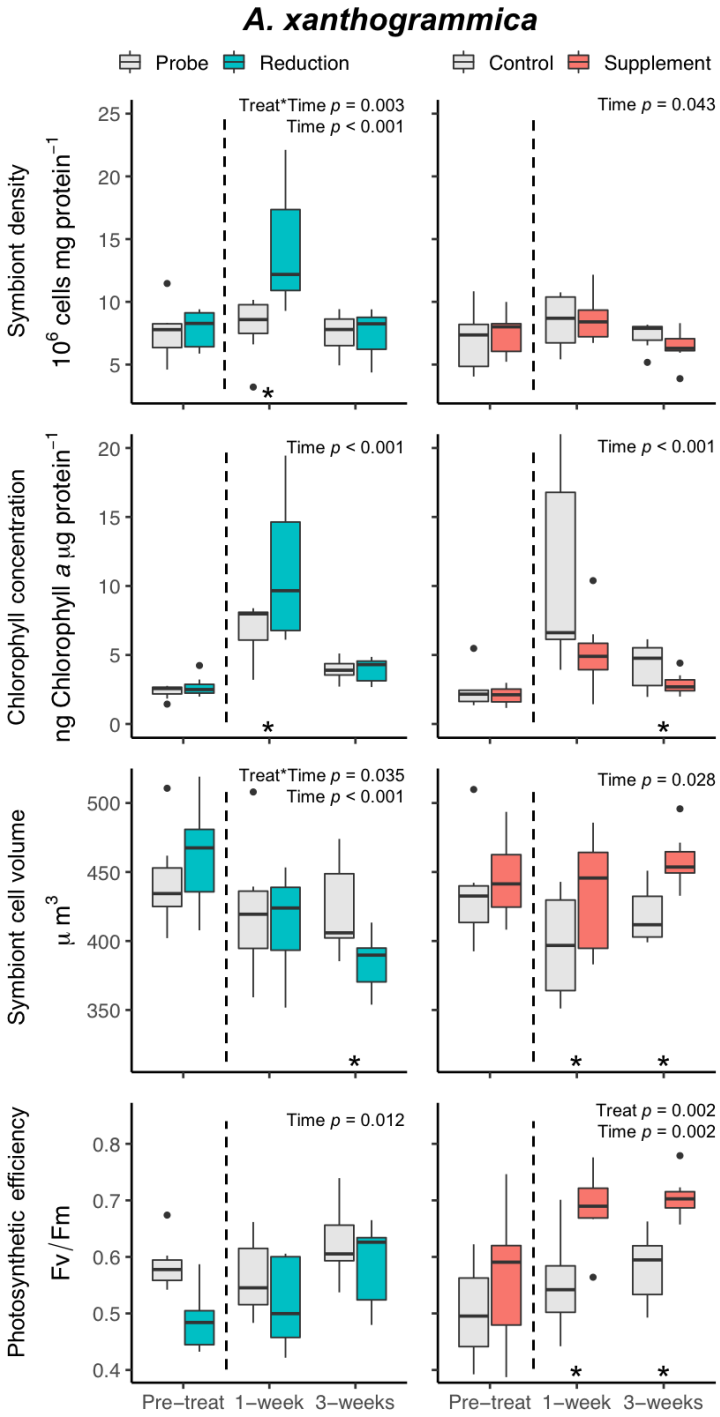


**Figure 4.** Boxplots showing symbiont density, chlorophyll concentration, symbiont cell volume, and photosynthetic efficiency of symbionts within *A. xanthogrammica* throughout the experiment. Comparisons are made between the supplement and reduction treatments and their respective control treatments. The vertical dashed line represents the start of treatments. Significant main effects and interactions from GLMMs are listed in the upper right-hand corner of each graph with p-values. Asterisks above the x-axis signify significant differences between controls and treatments at a given time-point from a Tukey post hoc analysis. n = 7 for each treatment at each time point.

289

290 **(4) Discussion**

291 Algal symbionts within two species of sea anemone responded to changes in anemone
292 diet, but the responses differed between the anemone species and changed over the course of the
293 experiment. Our framework for dietary carbon-source switching (Fig. 1) was supported by our
294 results, but support for our predictions depended on the anemone species. Symbionts within *A.*
295 *sola* responded to diet supplementation, and symbionts within *A. xanthogrammica* responded to
296 both reduction and supplementation. This may be associated with the fact that *A.*
297 *xanthogrammica* captured twice as many prey items as *A. sola*, so the reduction treatment had a
298 larger impact on *A. xanthogrammica* than on *A. sola*. Supplementation affected both species,
299 resulting in reduced $\delta^{13}C$ values in symbionts. Furthermore, $\delta^{13}C$ did not differ between
300 anemones and their symbionts, except where food was added. Lower $\delta^{13}C$ values have
301 previously been associated with an increase in heterotrophy in corals [53] and in *Anthopleura*
302 anemones [37,38]. A lower $\delta^{13}C$ signature (supplement treatment) occurs when algae selectively
303 incorporate the lighter carbon isotope ($^{12}C$) over the heavier isotope ($^{13}C$). Highly productive
304 algal symbionts at high densities cannot choose the lighter carbon isotope because $CO_2$ is limited
305 within the host tissue, resulting in a heavier carbon isotope signature (reduction and control
306 treatments) [54].

307 Symbiont densities were affected by host dietary changes, but underlying mechanisms are
308 not well-understood. It is likely that the sea anemone host benefits from a reduction in symbiont
309 density when they are unnecessary (supplement treatment) as they can cause damage to tissue via
310 oxygen radicals [43,44]. The host would also benefit from an increase in symbiont density or
311 chlorophyll when heterotrophic diet decreases (reduction treatment) to compensate for lost

312 dietary carbon as an increase in either would allow for increased translocation of photosynthetic
313 products from the symbionts to the host (Fig. 1).

314 The anemone-algae holobiont responded to supplementation of the host diet largely by
315 decreasing symbiont density and/or chlorophyll while increasing symbiont cell volume. This
316 could have resulted from egestion of symbionts or by the slowing of symbiont reproduction
317 within the host. The remaining symbionts may have been larger because they were able to store
318 resources rather than translocate them to the host or because they did not asexually reproduce.
319 More research is needed to fully understand the mechanism(s) driving symbiont volume changes
320 in these anemones. Regardless of the mechanisms, those anemones that received more external
321 resources (prey) had lower autotrophic potential (fewer symbionts and/or lower chlorophyll).
322 However, symbionts within *A. xanthogrammica* may have compensated for the decrease in
323 chlorophyll by increasing photosynthetic efficiency.

324 Reduction of host diet had an effect on *A. xanthogrammica* and its symbionts but not on
325 *A. sola*. Symbiont density increased and symbiont volume decreased when food was reduced in
326 *A. xanthogrammica*, suggesting that the anemone host maintained a higher symbiont density to
327 compensate for the loss of dietary carbon by either retaining symbionts that would otherwise be
328 egested or by increasing the reproduction of symbionts. *A. xanthogrammica* anemones that
329 received fewer external resources had higher autotrophic potential (symbiont density and
330 chlorophyll), but the effect was short-lived and disappeared after three weeks of treatment.
331 Our results suggest there is a trade-off between sources of nutrition – external and symbiont-
332 mediated – in this mutualism. Similar previous work that involved starving sea anemones under
333 laboratory conditions provided conflicting perspectives on the effect of host diet on symbiont

density [29,55,56], but we show here that realistic, *in situ* changes in sea anemone diet reveal ecologically relevant trade-offs in symbiont-host nutrition that were previously unexplored.

Not all algae-hosting cnidarians can switch carbon sources. Tropical corals tend to lose symbionts when starved [57,58], suggesting that symbionts do not serve as a comparable nutritional pathway in the absence of heterotrophy (but see [59]). This is likely because most tropical corals are obligate mutualists, whereas *Anthopleura* anemones are facultative. A better comparison may be to a freshwater hydra where algal symbiont density decreases immediately after predatory feeding [60] and increases with starvation [61].

Analogous partner interactions exist in terrestrial mutualisms where legumes host fewer rhizobium (via nodules) when external sources of nitrogen are available in the soil [62,63] and the benefit and cost of arbuscular mycorrhizal fungi to plants is dependent on environmental resources [64]. Holobionts with flexible nutritional strategies – like the ones we describe here – may be able to withstand periods of resource limitation, allowing species to persist in an otherwise inhospitable environment. Interactions between hosts and symbionts are dependent on external resource availability in normally nutrient-poor environments. Some mutualisms may break down as a result of perturbations [8,65], but others are flexible, requiring more from symbionts when nutrients are scarce or less from them when nutrients are abundant [60,61,66]. Future research on flexible mutualisms should focus on how realistic fluctuations of external resources affect the production and storage of resources by symbiotic partners.

Our results suggest that even modest changes in resource availability have the potential to alter the interaction between partners in a mutualistic symbiosis, but those changes are species-specific even in congeneric species sharing the same symbiont. We found evidence for a trade-off between autotrophic and heterotrophic nutritional pathways within an algal-symbiont hosting

sea anemone, but these pathways are not equal. We propose that autotrophy allows for persistence, but growth likely requires heterotrophy as evidenced in this and other studies on cnidarians [61,67]. Anemone hosts and algal symbionts respond to changes in heterotrophic diet by altering their interactions with each other, compensating for externally derived nutrition. The potential for flexible nutritional strategy in other mutualistic symbioses is largely unexplored, especially in systems where environmental resources are naturally stochastic.

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