Title

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Journal

Proceedings of the Royal Society B: Biological Sciences, 287(1940)

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Publication Date

2020-12-09

DOI

10.1098/rspb.2020.1860

Peer reviewed

PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Bedgood SA, Mastroni SE, Bracken MES. 2020 Flexibility of nutritional strategies within a mutualism: food availability affects algal symbiont productivity in two congeneric sea anemone species. *Proc. R. Soc. B* **287**: 20201860.
https://doi.org/10.1098/rspb.2020.1860

Received: 2 August 2020
Accepted: 16 November 2020

Subject Category:
Ecology

Subject Areas:
ecology, physiology

Keywords:
Anthopleura, context-dependent, ecophysiology, mutualistic symbiosis, sea anemone, Symbiodiniaceae

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Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5227819.

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

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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.

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 corals 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.A.B. 2018, unpublished 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 laboratory 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.A.B. 2018, unpublished 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.A.B. 2017, unpublished 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.A.B. 2017, unpublished 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 to be due to differences between sea anemone species, not differences in symbiont identity.

The growth rate potential of symbiont cells is probably 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, probably 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 probably 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 (figure 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 (figure 1). (i) Symbiont density—which we hypothesize is controlled by the host—will increase when prey are removed and decrease when prey are added. (ii) 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). (iii) 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.

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.A.B. 2018, personal observation). 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. xanthogrammica*) in close proximity (e.g. within the same tide pool) that matched all four feeding and species treatments ($n = 7$ blocks).

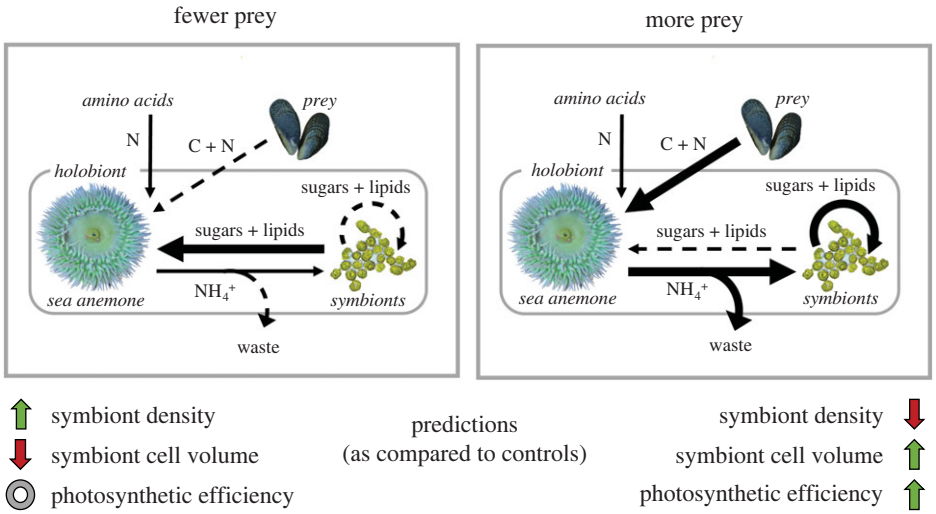

Figure 1. Predictions of algal symbiont contributions based on prey availability. Arrows represent the flow of carbon and nitrogen from one source to another. The thickness of each arrow represents the relative contribution and dashed lines represent a reduction in contribution. Predictions of algal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

Four treatments were maintained for three weeks in both species, beginning in June 2018 under a California Department of Fish and Wildlife Scientific Collecting Permit (to S.A.B., ID # SC-13728). Treatments included supplement, control, reduction and probe. 'Supplement' anemones were fed either squid or mussel tissue once daily during the daytime low tide. These are representative of the types of food items that *Anthopleura* spp. consume at this site (S.A.B. 2018, unpublished data). The size of the prey items offered to each anemone was proportional to the anemone's size and ranged between 3 and 4 g wet mass. 'Supplement' anemones probably captured additional prey, so the added food supplemented their natural diet. We did not manipulate the anemones in the control treatments, allowing them to capture prey as usual. We touched the tentacles of 'reduction' anemones, waited for their mouths to open, and reached in with a probe or fingers to remove any prey that we found in the gastrovascular cavity. If possible, the prey items were identified prior to being disposed of. We did this once daily during low tide. Since anemones may digest prey within a few hours [20], this treatment probably represented a reduction in food availability instead of complete removal. We treated the 'probe' anemones the same way as the 'removal' individuals but did not remove any prey.

(b) Symbiont density and cell volume

We collected 2–3 tentacles with dissecting scissors from each sea anemone one week before treatments began, one week after treatments were initiated and three weeks after treatments began. We immediately placed samples on ice and transported them to a −25°C freezer for storage within 24 h of collection. Samples were thawed in the laboratory, and we then separated the gastrodermal tissue layer from the epidermal layer by squashing samples between two microscope slides until the clear, tough epidermal layer was devoid of any algal symbionts or gastrodermal anemone cells. We removed the epidermal tissue, added the remaining tissue to 1.5 ml of deionized water and homogenized the tissue and water at 30 beats s^{-1} for 5 min. This method produced well-homogenized samples without breaking algal cells.

An aliquot of the homogenate was placed on a Brightline hemocytometer (Hausser Scientific, Horsham, Pennsylvania, USA), and photos of each sample were taken on a microscope at 200× magnification. To count the number of symbionts in each square (1 mm^2, $n = 10$), we loaded photos into FIJI [48], where we batch processed images with a custom macro using the particle analysis function (see electronic supplementary material). To standardize the symbiont density, we measured animal protein from the same homogenate using the Lowry method [49] for protein estimation with bovine serum as a standard [20,37].

We calculated symbiont volume using the same photos taken for symbiont density. We batch-processed photos with the particle analysis function (see electronic supplementary material) using an ellipse-shape fit of particles. Using the length and width output, we calculated the volume based on Hillebrand *et al*. [50], assuming a prolate spheroid shape as described for Symbiodiniaceae.

(c) Chlorophyll *a*

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

(d) Photosynthetic efficiency

We quantified the symbionts' photosynthetic efficiency (F_v/F_m of dark-adapted Photosystem II) using a pulse amplitude modulation (PAM) fluorometer (Heinz Walz GmbH, Effeltrich, Germany) to determine the effect of host feeding on photosynthetic electron transport. Chlorophyll *a* concentrations give an estimate of photosynthetic activity potential, but combining those data with measurements of the photosynthetic efficiency of chlorophyll provides further insights into photosynthetic productivity responses. PAM measurements of sea anemones were taken in the dark, between 04.00 and 05.00, on the same days we collected tissue samples. Most anemones were closed when measurements were taken, so the sensor was placed at the top of the anemone column, where symbionts are present but at a lower density than in the tentacle tissue [51] (and see electronic supplementary material). If the anemone was open, we disturbed it and waited for it to close. We took the average of three measurements of each anemone.

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

We collected a 1 cm^2 piece of tissue that included both tentacles and column from 4 random sea anemones in the control, supplement, and reduction treatments to estimate the contribution of symbiont photosynthate and prey to the anemone's dietary carbon budget. Because this sampling method harms (but does not kill) the animals and could compromise further measurements,

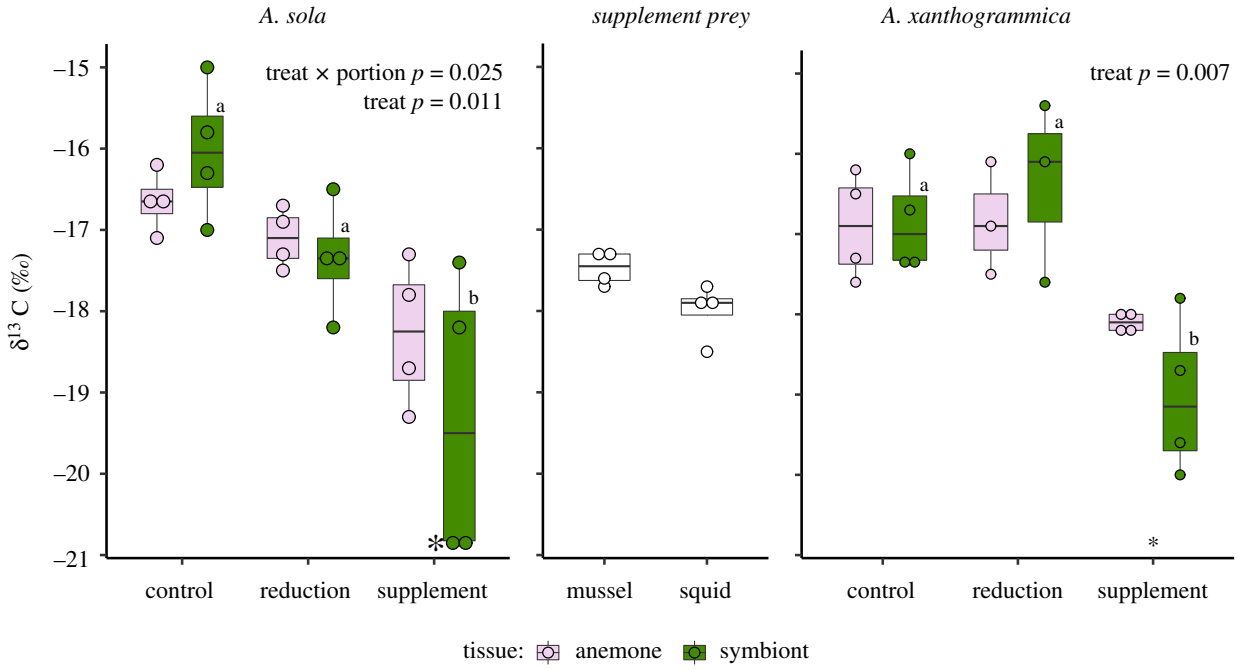

Figure 2. Boxplots with overlaid data points ($n = 3$ or 4) showing $\delta^{13}C$ (‰) values for *A. sola*, *A. xanthogrammica* and supplemented prey items. Tissue samples from anemones were separated into anemone and algal symbiont portions before analysis. Comparisons are made among the control, reduction 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. Lower case letters represent significant differences among treatment groups within the algal symbiont portion. (Online version in colour.)

these samples were collected at the end of the experiment. Samples were homogenized as described previously. The homogenate was then centrifuged at 2000*g* for 5 min to separate the anemone cells from the algal symbiont cells. The top layer of anemone cells was then agitated, and the supernatant with suspended anemone cells was removed. Both the algae portion and anemone portion (supernatant) were re-homogenized and centrifuged 2–3 more times to remove any non-target cells. Both the symbiont and anemone portions were placed on separate microscope slides and dried (60°C for greater than 48 h) before analysis at the UCI Stable Isotope Ratio Mass Spectrometry Facility.

(f) Statistical analyses

We conducted all analyses in R v. 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 analyse $\delta^{13}C$, symbiont density, symbiont cell volume and photosynthetic efficiency. Data from the two anemone species were typically analysed separately. $\delta^{13}C$ values were analysed 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 analysed 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 $12.92 \pm 2.31\%$ (mean ± s.e.) of daily checks, while prey were found within *A. xanthogrammica* during $23.47 \pm 3.18\%$ 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 treatments received an additional prey item daily, which represented a substantial increase from ambient prey capture rates. However, this frequency of food availability is not uncommon during periods of high wave exposure, when all anemones surveyed had at least one prey item on consecutive days.

(b) Stable isotope analysis

Anemone diet affected $\delta^{13}C$ values (GLMM ANOVA: *A. s.* = *A. sola* treat × portion, $F = 5.73$, $p = 0.025$; *A. x.* = *A. xanthogrammica* treat, $F = 9.74$, $p = 0.007$), but this result was largely associated with the algal symbiont portion for both species (figure 2). Symbionts from 'supplemented' anemones had $\delta^{13}C$ signatures that were 2–5‰ lower than the controls (GLMM Tukey HSD: *A. s.*, $t = -4.89$, $p = 0.001$; *A. x.*, $t = -4.1$, $p = 0.004$), but reduction of diet had no 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 anemones and their algae within a treatment, except in the supplement treatment where the symbionts had a lower $\delta^{13}C$ (*A. s.*, $t = 3.0$, $p = 0.015$; *A. x.*, $t = 2.57$, $p = 0.033$).

(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 (figures 3 and 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 (figures 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 (figure 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.*, $t = -2.26$, $p = 0.033$; figures 3 and 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 ± s.e.) 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 efficiency of the control treatment *A. sola* symbionts (0.67 ± 0.01 F_v/F_m) by the end of the experiment (paired *t*-test: $t = -1.35$, $p = 0.225$). Photosynthetic efficiency generally increased through time for all groups (figures 3 and 4; GLMM ANOVA: *A. s.* supplement, $F = 5.61$, $p = 0.01$; *A. x.* reduction, $F = 5.41$, $p = 0.012$; *A. x.* supplement, $F = 8.07$, $p = 0.002$) except the *A. sola* reduction pairing ($F = 1.74$, $p = 0.198$).

4. Discussion

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

Symbiont densities were affected by host dietary changes, but underlying mechanisms are not well understood. It is likely that the sea anemone host benefits from a reduction in symbiont density when they are unnecessary (supplement treatment) as they can cause damage to tissue via oxygen radicals [43,44]. The host would also benefit from an increase in symbiont density or chlorophyll when heterotrophic diet decreases (reduction treatment) to compensate for lost dietary carbon as an increase in either would allow for increased translocation of photosynthetic products from the symbionts to the host (figure 1).

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

Reduction of host diet had an effect on *A. xanthogrammica* and its symbionts but not on *A. sola*. Symbiont density increased and symbiont volume decreased when food was reduced in *A. xanthogrammica*, suggesting that the anemone host maintained a higher symbiont density to compensate for the loss of dietary carbon by either retaining symbionts that would otherwise be egested or by increasing the reproduction of symbionts. *A. xanthogrammica* anemones that received fewer external resources had higher autotrophic potential (symbiont density and chlorophyll), but the effect was short-lived and disappeared after three weeks of treatment.

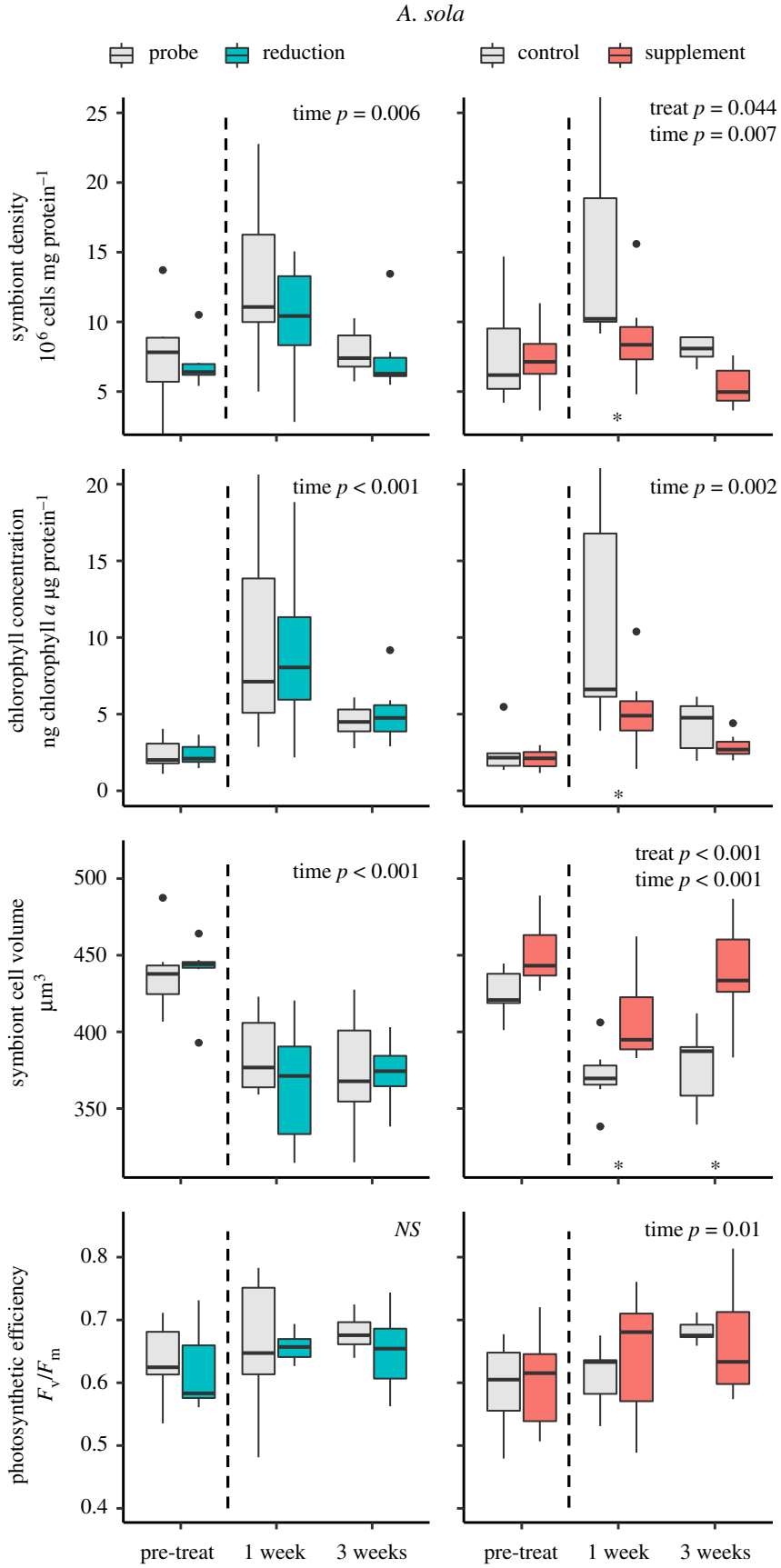

Figure 3. Boxplots showing symbiont density, chlorophyll concentration, symbiont cell volume and photosynthetic efficiency of symbionts within *A. sola* throughout the experiment. Comparisons are made between the supplement and reduction treatments and their respective control treatments. The vertical 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. (Online version in colour.)

Our results suggest there is a trade-off between sources of nutrition—external and symbiont-mediated—in this mutualism. Similar previous work that involved starving sea anemones under 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*

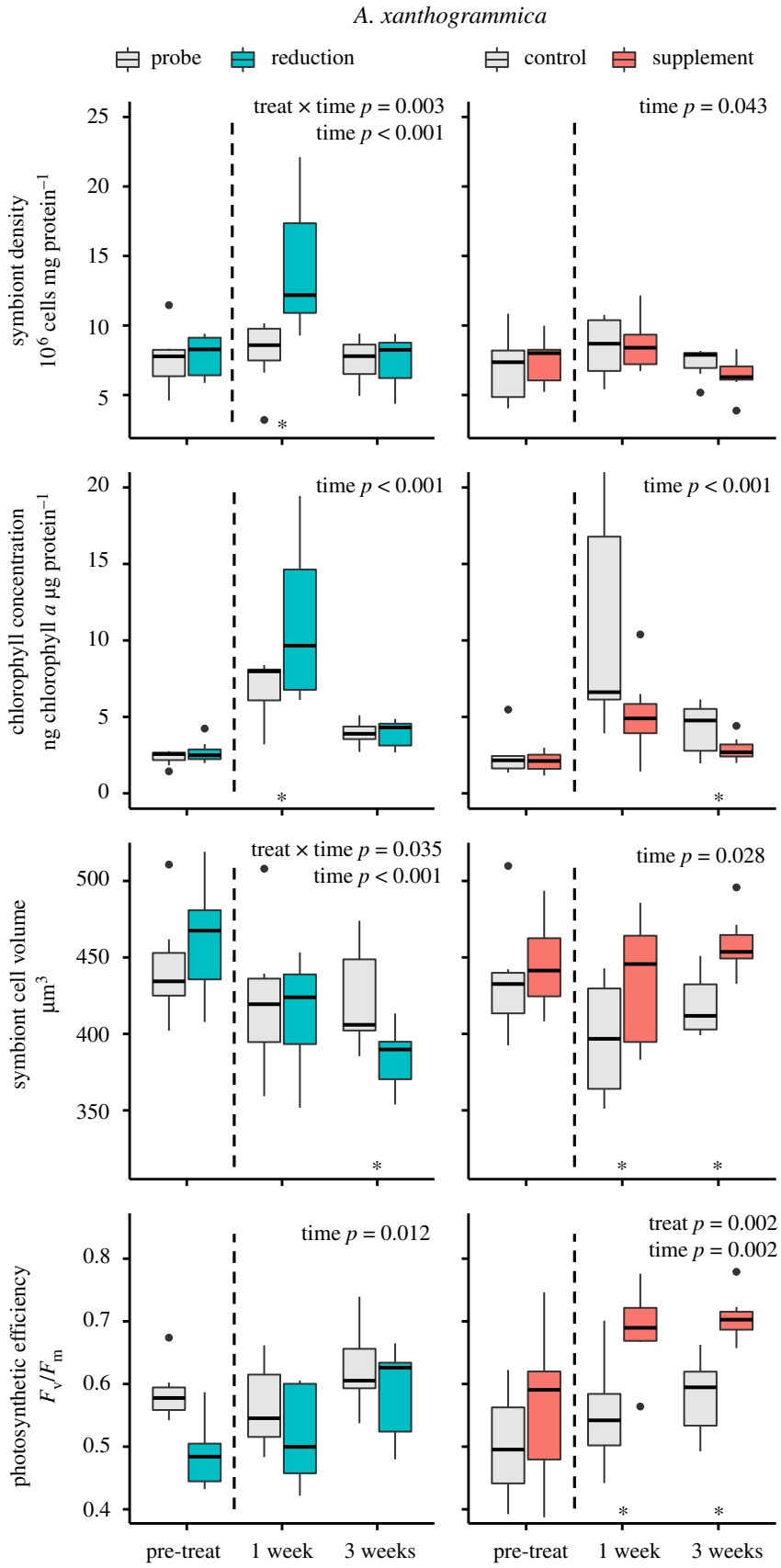

Figure 4. Boxplots showing symbiont density, chlorophyll concentration, symbiont cell volume and photosynthetic efficiency of symbionts within *A. xanthogrammica* throughout the experiment. Comparisons are made between the supplement and reduction treatments and their respective control treatments. The 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. (Online version in colour.)

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 probably 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 probably 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 strategies in other mutualistic symbioses is largely unexplored, especially in systems where environmental resources are naturally stochastic.

Ethics. All applicable international, national and/or institutional guidelines for the care and use of animals were followed. This work was conducted under a California Department of Fish and Wildlife Scientific Collecting Permit to S.A.B. (ID number SC-13728).

Data accessibility. Dataset files and R code for analyses and graphs are available from the Dryad Digital Repository: https://doi.org/10.7280/D1GD62 [68].

Authors' contributions. S.A.B. conceived of the study, designed the study, carried out the study, analysed the data and drafted the manuscript; S.E.M. carried out the study and made contributions to the manuscript; M.E.S.B. advised on experimental design, assisted in analysing the data and added major intellectual contributions to the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

Funding. This research was made possible through funding provided by the UC Irvine Oceans, Changing Environments, Arts, and Nearshore Societies (UCI OCEANS) Fellowship (to S.A.B.) and the National Science Foundation (Graduate Research Fellowship to S.A.B. and OCE-1736891 to M.E.S.B. and A. Martiny). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. All applicable state and institutional guidelines for the care and use of animals were followed.

Acknowledgements. We thank the late Don Canestro for the support he provided to us at Kenneth S. Norris Rancho Marino Reserve; this research would not have been possible without his assistance. We thank Joshua Goldberg and Nyssa Silbiger for their advice on analysis of our data, and Samantha Levell for her comments on early drafts of this manuscript. We also thank three anonymous reviewers who greatly improved the final version of this text.

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