2011

The effects of symbiotic state on heterotrophic feeding in the temperate anemone Anthopleura elegantissima

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THE EFFECTS OF SYMBIOTIC STATE ON HETEROTROPHIC FEEDING IN THE TEMPERATE ANEMONE *ANTHOPLEURA ELEGANTISSIMA*

by

Terra Celeste Hiebert

Accepted in Partial Completion

of the Requirements for the Degree

Master of Science

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MASTER'S THESIS

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Terra Celeste Hiebert
June 2011

THE EFFECTS OF SYMBIOTIC STATE ON HETEROTROPHIC FEEDING IN THE TEMPERATE ANEMONE *ANTHOPLEURA ELEGANTISSIMA*

A Thesis

Presented to

The Faculty of

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ABSTRACT

The temperate sea anemone, *Anthopleura elegantissima* is facultatively symbiotic with at least two distinct algae: zooxanthellae (*Symbiodinium muscatinei*) and zoochlorellae (*Elliptochloris marina*). Symbiotic *A. elegantissima* potentially receive excess photosynthate from their algal partners, which supplements heterotrophic feeding. But asymbiotic individuals must rely solely on heterotrophic food sources. We predicted that asymbiotic *A. elegantissima*, due to their lack of algal symbionts, would have a more effective heterotrophic feeding strategy. Symbiotic and asymbiotic *A. elegantissima* were collected from the field and heterotrophic feeding features were measured (i.e., anemone morphology, tentacle adhesive force, nematocyte sensitivity, cnida size, cnida density, ingestion time, digestion time and absorption efficiency). The anemones were then exposed to natural sunlight or shaded conditions for three weeks and the feeding features were again compared. Few aspects of heterotrophic feeding in *A. elegantissima* were affected by symbiotic state. Asymbiotic anemones had the largest nematocysts immediately after collection, but were not more efficient predators. We found the greatest nematocyte sensitivity in anemones hosting zooxanthellae, suggesting a greater nutritional need for anemones in this symbiotic state. Though sunlight appeared to increase digestion rate in all anemones, irradiance also had negative effects. Anemones exposed to sunlight had lower cnida densities and smaller spirocysts. Sunlight also appeared to reduce cnidocyte function in asymbiotic individuals. Our results show that symbiotic state has little effect on heterotrophic feeding in *A. elegantissima*, suggesting that the symbiotic algae may contribute little to the host anemones' daily nutritional requirement and that nutrition in *A. elegantissima* may be obtained primarily through heterotrophy.

ACKNOWLEDGEMENTS

I would like to express my gratitude to many people who have helped shape and strengthen this thesis. First, I would like to thank my advisor, Brian Bingham. His knowledge, patience, and attention to detail were essential in writing this thesis. My committee members, Deborah Donovan and Suzanne Strom, offered insightful questions and expertise for my research and conclusions. Jay Dimond provided thoughtful and constructive advice in all aspects of this project, which was greatly appreciated. Expert advice and enthusiasm from Gisèle Muller-Parker helped to form the questions of my research. I am also thankful for the fruitful conversations with Liz Francis in all aspects of anemone cnida characteristics. Monica Ponce-McDermott and Amy Burgess provided fun, reliable assistance and thoughtful critique. I thank the entire staff at Shannon Point Marine Center, particularly Gene McKeen who constructed the micromanipulator used in all force measurements and was instrumental in the setup of all experiments. I also thank Nate Schwarck for helping in the collection of anemones and photographic advice. I thank my friends and family for their interest in this work and for asking important questions. Finally, I would like to thank George Ayres for his endless editing, overwhelming humor and fine food. I would not have been able to complete this work without funding from Research and Sponsored Programs, Huxley College and Shannon Point Marine Center at Western Washington University. This work was also funded by NSF grant IOS-0935820 (awarded to Brian Bingham).

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INTRODUCTION

Temperate anemones in the genus *Anthopleura* host algal symbionts representing two phyla. *Symbiodinium* spp. are brown dinoflagellates, commonly referred to as zooxanthellae, which are symbiotic with *Anthopleura* spp. along the coasts of California (*S. californium* and *S. muscatinei*), Oregon (*S. muscatinei*) and Washington (*S. muscatinei*) (LaJeunesse and Trench 2000). Chlorophytes called zoochlorellae, *Elliptochloris marina* (Letsch *et al.* 2009), have a symbiotic range that overlaps that of *S. muscatinei*, but extends farther north into Alaska (Kitaeff 2007).

Anthopleura elegantissima is facultatively symbiotic and can be found hosting one (zooxanthellate or zoochlorellate), both (mixed) or neither (asymbiotic) symbiont within its gastrodermal tissue. The distribution of the symbionts on geographic and intertidal scales is determined primarily by light and temperature (Saunders and Muller-Parker 1997; Verde and McCloskey 2001, 2002; Secord and Muller-Parker 2005). Secord and Muller-Parker (2005) found zooxanthellate anemones near the opening of an intertidal cave where irradiance was greatest while zoochlorellate anemones were in areas of reduced light. Asymbiotic anemones were only in the darkest portion of the cave. Symbiont distribution also differs vertically in intertidal zones. Zooxanthellate anemones occur throughout the intertidal, while zoochlorellate anemones are limited to lower intertidal areas (Secord and Augustine 2000; Bates 2000).

The nutritional relationship between *A. elegantissima* and its symbionts is not yet clearly understood. However, current research suggests that *A. elegantissima* receives a greater

nutritional benefit from hosting zooxanthellae than zoochlorellae (Verde and McCloskey 1996, 2001, 2002, 2007) and that both zooxanthellate and zoochlorellate anemones have a nutritional advantage over anemones that lack symbionts altogether. Both symbionts are equally productive through much of the year, but during summer months, isolated zooxanthellae fix more than twice as much carbon as zoochlorellae (Bergschneider and Muller-Parker 2008). Both Verde and McCloskey (2007) and Bergschneider and Muller-Parker (2008) found that zooxanthellate *A. elegantissima* potentially receive more carbon from their symbionts than do zoochlorellate individuals despite higher densities of zoochlorellae in host tissues. The estimated annual carbon contribution to host respiration from zooxanthellae is nearly twice the contribution of zoochlorellae (87% and 44% respectively, Verde and McCloskey 2007).

Intertidal zones of the Pacific Northwest can be highly productive and anemones there do not rely solely on their symbionts for nutrition. *Anthopleura elegantissima* are also effective heterotrophic feeders (Sebens 1981; Shick 1991; Muller-Parker and Davy 2001). While asymbiotic anemones feed exclusively heterotrophically, heterotrophy is supplemented by photosynthesis in symbiotic individuals. Bergschneider and Muller-Parker (2008) showed that zooxanthellate, zoochlorellate and asymbiotic *A. elegantissima* have distinct stable isotope ratios revealing their different diets. Asymbiotic anemones have ratios similar to epibenthic crustaceans, a common prey item for *A. elegantissima* (Sebens 1981), while symbiotic individuals have ratios closer to their respective algal symbionts, indicating that a portion of their nutrition derives from algal photosynthate.

Nutritional contribution of photosynthetic symbionts may be influenced by the habitat of the host. Anthony and Fabricius (2000) found that prolonged shading increased heterotrophic feeding in the symbiotic scleractinian coral, *Goniastrea retiformis*, presumably because photosynthesis of the symbionts was reduced by the lack of light. Irradiance and temperature both influence symbiont productivity and the quantity of nutrients translocated to *A. elegantissima* (Verde and McCloskey 2001, 2002, 2007). To obtain optimal irradiances, anemones position themselves toward or away from sunlight depending on light intensity and their symbiotic state (Pearse 1974). Heterotrophic feeding behavior may also be influenced by changes in light levels. Without sunlight, symbiotic anemones may be more dependent on heterotrophic feeding and may have to devote more energy to prey capture and thus behave more like asymbiotic individuals.

Heterotrophic feeding ability of *A. elegantissima* is affected by at least three features. The first is prey capture surface area. As suspension feeders, anemones rely on contact of prey with their tentacles and oral disc, and the probability of prey contact depends on the size of the prey capture surface area (Sebens 1981; Zamer 1986). Research on feeding and growth of *A. elegantissima* suggests that larger individuals do not eat larger prey, but have a larger prey capture surface area and greater probability of prey contact (Sebens 1981).

Sensitivity and strength of anemone cnidae can also influence feeding success. Cnidae are secretions of cells called cnidocytes which are located in the ectoderm of all cnidarians. The cnidocyte discharges the cnida which adhere to (spirocyst) or penetrate into (nematocyst) potential prey. Submerged anemones have a feeding response that involves both chemical

and mechanical stimulation (Mariscal, 1984; Shick 1991). Physical contact with tentacles induces firing of cnidae (Williams 1968). Chemicals released by the damaged prey then induce oral disc expansion, tentacle extension (Shick 1991) and heightened cnidocyte sensitivity (Williams 1972; Thorington and Hessinger 1988a, 1988b). This complete feeding response, however, is dependent on anemone nutritional need. Starved anemones show a purely tactile response, ingesting all prey items that contact their tentacles (Pantin and Pantin 1943; McFarlane 1970). Satiated anemones are less responsive to food, decreasing the frequency of cnida discharge and the force with which they hold prey (Sandberg *et al*. 1971; Zamer 1986; Thorington *et al.* 2010). Maximum feeding responses and cnida discharge in the anemone *Aiptasia pallida* are seen after 72 hr of starvation (Thorington *et al.* 2010).

The nematocysts found in *A. elegantissima* tentacles are called basitrichs that are 14-25 μm long and 2-3 μm wide (Hand 1955). Spirocysts have a coiling thread that is more adhesive than penetrant (Mariscal 1984) and are also found in the tentacles of *A. elegantissima*. Spirocysts are 9-29 μm long and 2-3 μm at their widest point (Hand 1955). Nematocyst and spirocyst sizes may be adaptive and dependent on anemone size, habitat and prey type (Francis 2004; Kramer and Francis 2004). Cnida characteristics could also be variable by symbiotic state. Asymbiotic anemones freshly collected from the field are reported to feel stickier than symbiotic anemones collected from the same location and habitat, suggesting larger or more numerous cnidae (B. Bingham, M. Levine, pers. com).

A final feature influencing heterotrophic feeding success is the time required to ingest, digest and absorb captured prey. Absorption efficiency is potentially variable and related to

nutritional need. High intertidal *A. elegantissima* have greater prey absorption efficiencies, possibly due to a reduction in time available for submerged heterotrophic feeding (Zamer 1986). Zamer (1986) suggests that high intertidal anemones are undernourished and capture more prey by discharging more nematocysts upon contact. He speculates that one might find similar differences among symbiotic states of *A. elegantissima* due to differential nutritional needs (Zamer 1986).

To investigate variation in heterotrophic feeding as influenced by symbiotic state, we compared feeding and prey capture abilities of zooxanthellate, zoochlorellate, and asymbiotic *A. elegantissima*. We measured tentacle number, prey capture surface area, cnida density and cnida size in anemones in each symbiotic state. Cnida sensitivity and strength were also measured as were ingestion rate, digestion rate and absorption efficiency. These measurements were repeated under natural sunlight and shade treatments to assess the effect of symbiont productivity on heterotrophic feeding. Our goal was to determine whether asymbiotic anemones are more efficient predators due to a greater nutritional need.

METHODS

Anemone collection

Anthopleura elegantissima were collected from Swirl Rocks, WA (48˚25'6" N, 122˚50'58" W) on 14 July 2010. This location was chosen because *A. elegantissima* exist in asymbiotic, zooxanthellate and zoochlorellate forms within 2 m of each other at approximately equal tidal heights. Asymbiotic anemones were found under a large boulder, while zooxanthellate and zoochlorellate anemones were plentiful on a nearby exposed rock surface. Anemones in

each symbiotic condition were gently detached with metal spatulas and placed in Ziploc bags with seawater. Forty-one anemones were transported to the Shannon Point Marine Center in Anacortes, WA and acclimated in a flow-through seawater table for one week before experiments began. From the collected anemones, nine similar-sized individuals of each symbiotic state were selected and placed on numbered 5 x 6.5 mm slate tiles. These 27 anemones were used in all experiments; their attachment to the tiles permitted easy transfer during experiments.

Photographs and anemone morphology

Digital photographs were taken of all 41 anemones five days following collection. Fully submerged individuals were given five minutes to expand before photographs were taken. Photographs were used to measure prey capture area of the 27 experimental anemones. The remaining 14 anemones were photographed, blotted on a paper towel and weighed on an analytical balance to generate a regression of anemone oral disc diameter to wet weight. We used that regression equation to estimate wet weights of the 27 anemones used in our feeding experiments. Using Image J software (National Institutes of Health), we determined oral disc diameter and tentacle crown diameter of the 27 experimental anemones as the mean of three measurements extending between the bases or tips of tentacles and directly across the oral disc (Fig. 1a, b). All visible tentacles were then counted and length and midpoint width were measured for one fully visible tentacle in each of three quadrants of the oral disc (Fig. 1c). Oral disc surface area was calculated as the area of a circle circumscribed within the oral disc diameters (Fig. 1a) and tentacle surface area was calculated as if the tentacles were perfect

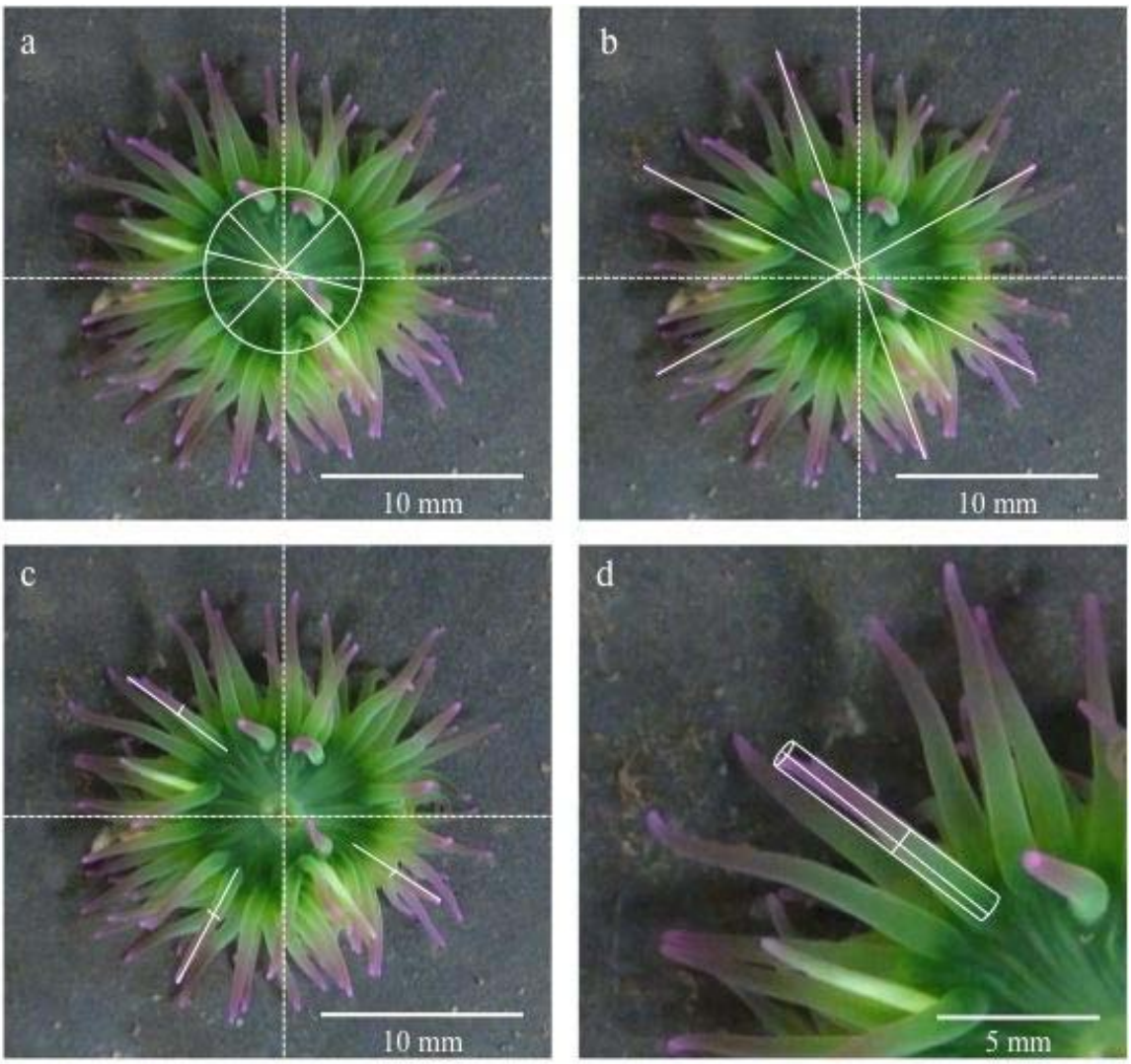

Figure 1. Anemone size data generated from three measurements of: a) oral disc diameter and oral disc surface area, b) tentacle crown diameter, c) tentacle length and width and d) tentacle surface area.

cylinders (Sebens 1981, Fig. 1c, d). Following Zamer (1986), we calculated the total prey capture surface area (PCSA) for each anemone as the sum of the total tentacle surface area (the average tentacle surface area of three tentacles multiplied by the total number of tentacles) and the oral disc surface area. All data were individually analyzed with one-way ANCOVA to test for differences in tentacle number, tentacle crown diameter or PCSA between anemones of each symbiotic state. Anemone protein mass (mg protein anemone^{-1}, determined at conclusion of all experiments) was used as the covariate.

Feeding experiments

To determine whether symbiotic state affects ingestion time, digestion time or absorption efficiency, anemones on their tiles were cleared of debris and placed in 8-cm glass finger bowls in a flow-through seawater bath that maintained water temperature yet isolated each anemone and, ultimately, the egesta it produced. Seawater was added to each bowl (100 ml of 5 μm filtered seawater), and, ten minutes later, 1 ml of squid mantle homogenate (approximately 1 g of squid mantle in 40 ml of 5 μm filtered seawater) was added to elicit the pre-feeding response to a chemical stimulus (McFarlane 1970; Williams 1972; Shick 1991). Twenty minutes later, a pellet of squid mantle tissue was placed on each anemone's outer tentacle tips. The pellets were equivalent to approximately 5% of each anemone's body weight as estimated from oral disc diameters.

Digital photographs of each anemone were taken 0, 5, 10, 15, 25, 30 min and 1 hr after the experiment started and visual observations were made at 1-hr intervals thereafter. At each interval, we observed the anemones for ingestion of the squid and presence of egesta.

Ingestion time was measured as the time the squid ration was placed on the tentacles until it was completely consumed and no longer visible. Digestion time was from complete ingestion of the squid to the first appearance of egested material. Egesta produced over the following 24 hours was collected by pipette and placed in labeled microfuge tubes. These samples were then centrifuged and rinsed with 2 ml of Nanopure water onto pre-dried and weighed 25 mm GF/C filters with vacuum filtration. The filters were dried at 60˚C for 24 hours and weighed on a microbalance to determine absorption efficiency as in Zamer (1986).

$$\text{Absorption efficiency} = \frac{\text{mg ingested -mg egested}}{\text{mg ingested}} \times 100$$

Ingestion time, digestion time and absorption efficiencies were analyzed individually with one-way ANOVA with symbiotic state as the independent factor. Anemones that failed to ingest the squid ration within 4 hours of contact with tentacle were not used in the analyses.

Tentacle adhesive force

To determine the adhesive force of anemone tentacles and test the hypothesis that adhesive force varies by symbiotic state, we used a method modified from Thorington and Hessinger (1988b, 1990, 1996, 1998), Giebel *et al.* (1988) and Thorington *et al*. (2010). Anemones were placed in an 11.5 L Plexiglas tank filled with 5 μm filtered seawater. A 0.2 mm diameter steel wire (Ernie Ball) with a 1 mm diameter glass bead coated in 30% (w/v) Knox gelatin attached to the tip was lowered with a micromanipulator (1 cm s^{-1}) until it just touched the tip of the test anemone's tentacle. Upon contact, the wire was immediately raised at 1 cm s^{-1} until the upward force exceeded the tentacle's adhesive force and the test

probe broke free. This process was digitally recorded in side view. The maximum deflection of the wire was later determined using digital video software (iMovie) (Fig. 2). One probe was used for each measurement and two tentacles were measured on each of the 27 anemones (9 per symbiotic state). A calibration curve relating deflection of the wire to weight was determined using the same micromanipulator to press the wire on the surface of an analytical balance. As described by Thorington and Hessinger (1988b), acceleration in this experiment is minimal and the force required to bend the cantilevered test probe can be approximated by mass and is reported in hybrid units of milligram-force (mgf). Because adhesive force is related to the number of cnidae discharged, it was necessary to count discharged cnidae for every trial.

After an adhesion measurement was made, the bead from the tip of the wire was soaked for 4 hr in 10 μl of 2% Trizyme (Amway). This dissolved the gelatin leaving behind the fired cnidae (Thorington and Hessinger 1988b). These samples were frozen at −70˚C until cnida counts could be done. Discharged basitrich capsules and threads were clearly visible and could be counted with a microscope under 1000x (oil immersion). Each force measurement was normalized to the number of basitrichs fired (i.e. mgf basitrich^{-1}). Spirocysts, which consist of adhesive hollow tubules that entangle prey (Mariscal 1984), were also present, but were difficult to differentiate once fired and were not counted. Adhesive force per basitrich and the total number of fired basitrichs were analyzed with one-way ANOVA to determine if anemones in different symbiotic states exert different amounts of force upon contact with potential prey.

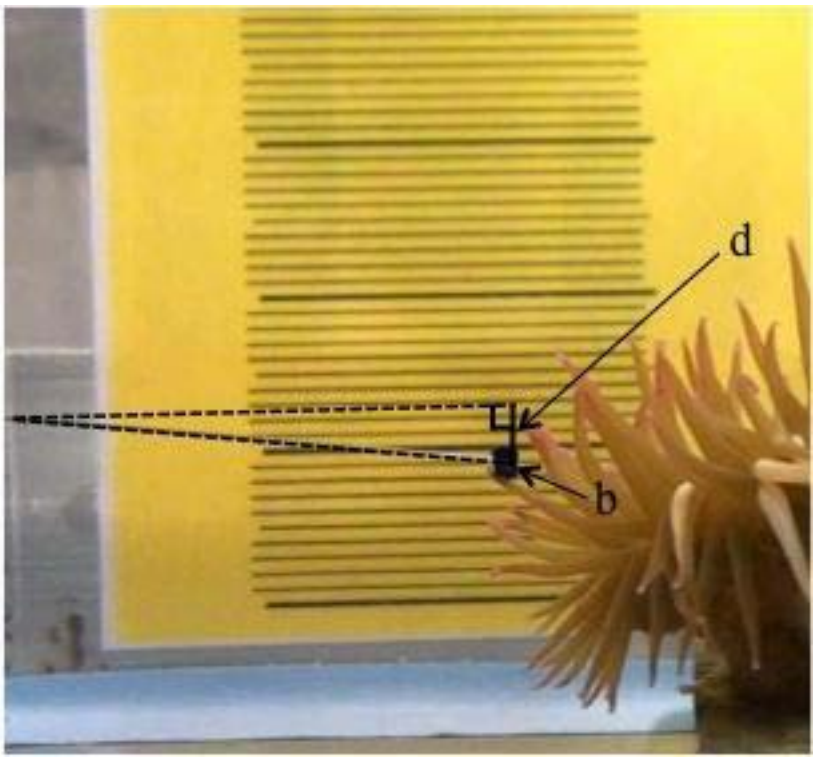

Figure 2. Measurement of tentacle adhesive force by maximum deflection distance (d). Gelatin coated glass bead (b) is indicated at the tip of the test probe.

Cnida measurements

To determine the effect of symbiotic state on cnida size and density, one arbitrarily chosen tentacle from each anemone was removed and frozen in a microfuge tube at –70˚C. These tentacles were later homogenized in 20 μl of 5 μm filtered seawater using a Teflon tissue grinder and motorized overhead stirrer (Wheaton Science Products, Millville NJ). The homogenates were examined on a hemocytometer and cnida density (basitrich and spirocyst) was determined. These densities were normalized to tentacle protein content measured with the Lowry method (Lowry *et al.* 1951). Bovine serum albumin (BSA) was used as a standard with absorbance read at 740 nm using an Agilent 8453A UV-VIS Diode Array spectrophotometer. Length and width of the first three unfired cnidae seen in each homogenate were measured using ImagePro Plus image analysis software. Basitrich density and spirocyst density were each analyzed with one-way ANOVA. Because multiple measurements were taken from each anemone, cnidae length and width data were analyzed with nested ANOVA with individual nested in symbiotic state and anemone body size as a covariate.

Light experiment

To test the effect of light exposure on the heterotrophic feeding abilities of anemones previously tested, we imposed a light treatment immediately following the experiments described above. The same nine anemones from each symbiotic state were randomly split between shade (n = 4) and sunlight (n = 5) treatments in a single outdoor flow-through seawater tank. In this tank, anemones experienced low tides that corresponded to daily low tides (< 0.3 m) at their collection site. All anemones were acclimated in the

light treatments for three weeks (5 Aug to 3 Sep 2010) and were fed a single squid pellet weekly. Seawater temperatures were measured continuously with Hobo data loggers and irradiance data were provided by the nearby Padilla Bay National Estuarine Research Reserve (D. Bulthuis, N. Burnett, and H. Bohlmann, unpublished data, Padilla Bay NERR Monitoring Program). Underwater irradiances were measured using a QSL-101 Light Meter (Biospherical Instruments, Inc.) to determine the proportion of ambient light experienced by anemones in sunlight and shaded treatments. Water temperature and irradiance in the treatments were compared with one-way ANOVA. Following the three-week period of exposure to sunlight or shade, all measurements of feeding efficiency, adhesive strength, cnida size and density were repeated.

Light experiment: Feeding experiments, tentacle force, and cnida measurements

Prey ingestion, digestion and absorption efficiency were measured as described above with three consecutive feeding trials at 72-hr intervals to investigate individual variability over time (25 Aug-2 Sep, 2010). Anemones remained in their respective light treatments during experiments. Two-way ANOVAR was used to test the effects of light (sunlight or shade), symbiotic state (zooxanthellate, zoochlorellate or asymbiotic) and trial (1-3) on anemone ingestion, digestion and absorption efficiency.

Seventy-two hours after the final feeding experiment, an adhesive force experiment was done as previously described. This interval was chosen to replicate the previous feeding trials and because cnida discharge rates are greatest 72-hr post feeding in *Aiptasia pallida*

(Thorington *et al.* 2010). Force measurements were analyzed as a two-way ANOVA with light treatment and symbiotic state as main effects.

Following the force measurements, a tentacle was removed from each anemone and cnida measurements were done as described above. Cnida length and width were individually analyzed with two-way nested ANCOVA and cnida density data were analyzed with a two-way ANOVA.

Algal density and protein analysis

To confirm symbiotic state and to measure anemone size, all anemones were homogenized in 5 μm filtered seawater at the conclusion of all experiments. Homogenates were frozen at -70˚C until they could be processed. The homogenates were later examined with a hemacytometer at 100x to determine symbiont complement and density. Four subsamples of homogenate were examined with at least 100 cells counted in each. Anemone protein content was determined using the method of Lowry *et al.* (1951), with bovine serum albumin (BSA) as a standard. Algal cell density was normalized to mg protein and used to confirm symbiotic state. Total anemone protein content was used as a measure of anemone size to determine if individual size affected our heterotrophic feeding measurements.

Statistical analysis

All data were analyzed using SPSS v 18. Analysis of variance data were checked for homogeneous variances using Levene's test. For covariate analyses, the interaction

between anemone size and treatment level was tested to confirm the assumption of parallel covariate lines. If assumptions were violated ($p < 0.05$), data were transformed. Count data were square root transformed, size data were log transformed and percentage values were arcsine transformed where appropriate. If variances of transformed data remained heterogeneous, a more conservative α was adopted ($\alpha = 0.025$, Underwood 1981). If a covariate interaction persisted despite transformation, the covariate was not included in the analysis. Pairwise comparisons (Tukey's HSD) were used to compare treatment means or covariate-adjusted treatment means where appropriate.

RESULTS

Algal density and anemone morphology

At the conclusion of all experiments, zooxanthellate and zoochlorellate individuals had at least 95% *Symbiodinium muscatinei* and *Elliptochloris marina,* respectively. Symbiont densities (cells mg protein^{-1}) were nearly 2x higher in zoochlorellate anemones as in zooxanthellate individuals (Table 1). Asymbiotic anemones hosted low densities of *S. muscatinei* and all anemones fell in a similar size range (Table 1). One anemone, initially assigned to the zoochlorellate group, was removed from all analyses because it hosted a mixed algal complement (55% zooxanthellae, 45% zoochlorellae).

Total number of tentacles was significantly related to anemone symbiotic state ($p = 0.02$) even with anemone size removed as a significant covariate ($p = 0.013$, Appendix A1). Asymbiotic anemones, on average, had 32% fewer tentacles than zoochlorellate individuals (Fig. 3). Both prey capture surface area ($p < 0.001$) and tentacle crown

Table 1. Algal cell density, algal composition, and anemone protein biomass for *A. elegantissima* used in experiments. Errors are standard errors.

Symbiotic State	Algal Cell Density (cells mg protein^{-1})	Composition	Protein Content (mg protein anemone^{-1})
Zooxanthellate	52,468 ± 6,846	> 99% *S. muscatinei*	27.98 ± 3.17
Zoochlorellate	100,111 ± 24,505	> 95% *E. marina*	24.14 ± 4.63
Asymbiotic	8,334 ± 2,478	> 99% *S. muscatinei*	21.61 ± 2.98

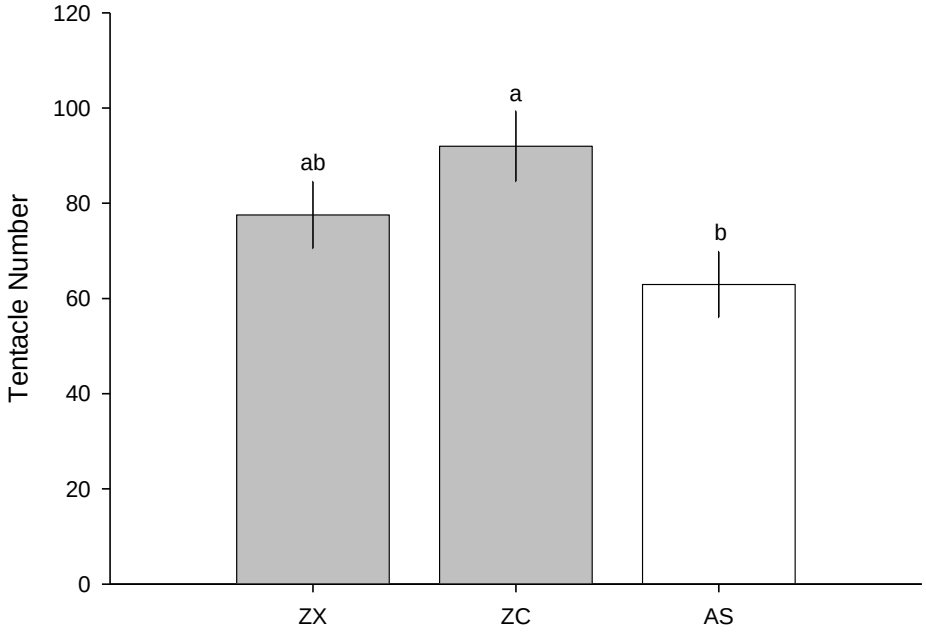

Figure 3. Covariate-adjusted tentacle number (±SE) for zooxanthellate (ZX), zoochlorellate (ZC), and asymbiotic (AS) anemones. Different letters show differences among treatments as indicated by Tukey's HSD pairwise comparisons of covariate-adjusted means. Standard errors are shown.

diameter ($p < 0.001$) increased with anemone size (Fig. 4), but neither feature was related to symbiotic state (Appendix A1). Anemone body weights, used to determine feeding rations, were related to measured oral disc diameter according to the following equation ($r^2 = 0.71$): body weight (mg) = 116.15 (oral disc diameter (mm)) – 511.16.

Feeding experiments

Neither ingestion time, digestion time, nor absorption efficiency was affected by symbiotic state (Appendix A2). Mean ingestion times appeared nearly twice as long for zoochlorellate and asymbiotic anemones as for zooxanthellate anemones, but the difference was not statistically significant (Fig. 5a). Mean digestion times ranged from 7 to 9 hrs and again showed no significant treatment effect (Fig. 5b). Absorption efficiencies were greater than 75% for all anemones, regardless of symbiotic state (Fig. 5c). Though none of the patterns were significant, there was a trend toward faster ingestion, shorter digestion time and greater absorption efficiency in zooxanthellate *A. elegantissima*.

Tentacle adhesive force

On average, asymbiotic anemones discharged the fewest basitrichs upon contact with the test probe (Fig. 6a), but exerted the greatest adhesive force per basitrich (Fig. 6b). However, effect sizes were low due to high variance and the patterns were not statistically significant (Appendix A3). Symbiotic anemones exerted near equal forces and created that force by firing similar numbers of basitrichs. The average total adhesive force exerted by a single tentacle touch on all anemones was 19.6 ± 3.5 mgf.

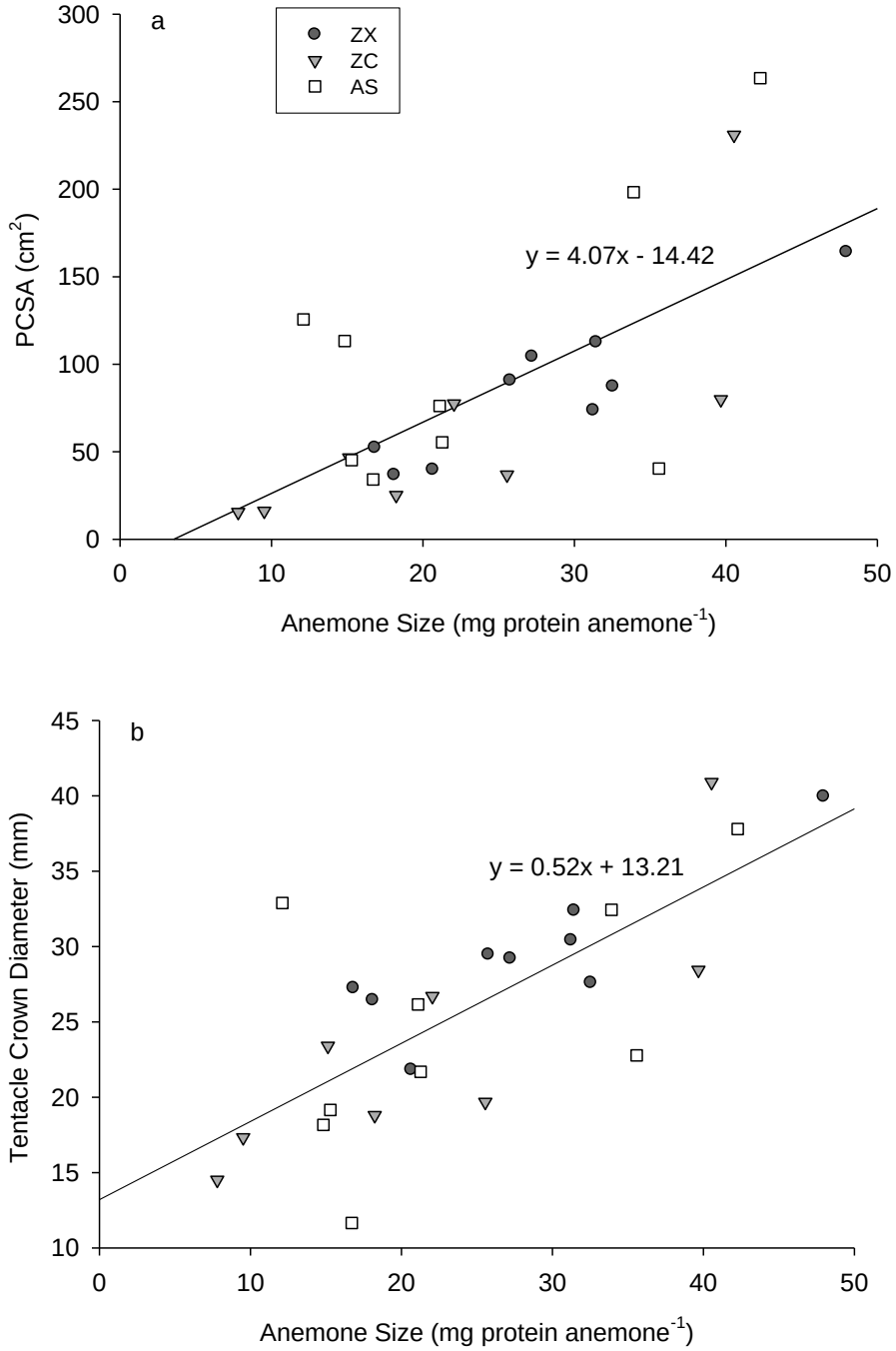

Figure 4. a) Prey capture surface area (PCSA, r^2=0.45) and b) tentacle crown diameter (r^2=0.56) as a function of anemone biomass for zooxanthellate (ZX), zoochlorellate (ZC) and asymbiotic anemones (AS). Regression line shows the relationship for pooled data since there were no significant differences between treatments.

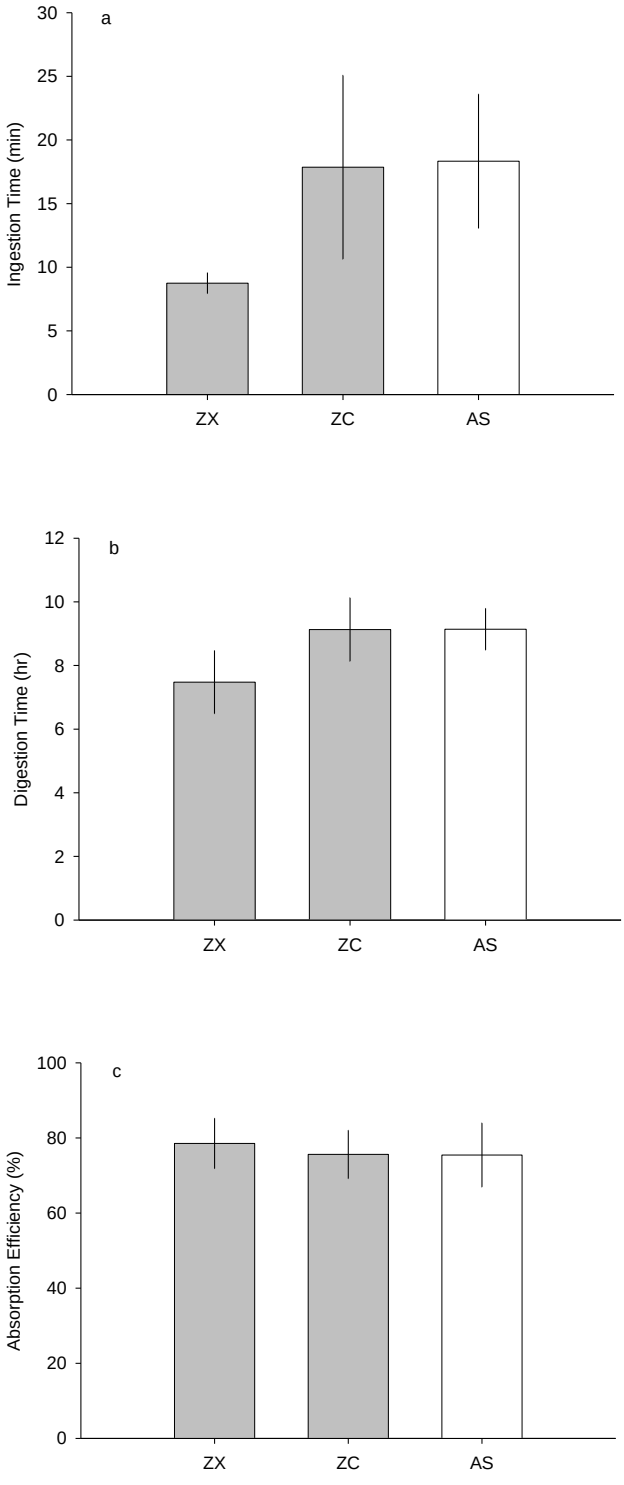

Figure 5. a) Ingestion time, b) digestion time, and c) absorption efficiency in zooxanthellate (ZX), zoochlorellate (ZC) and asymbiotic (AS) *A. elegantissima*. Standard errors are shown.

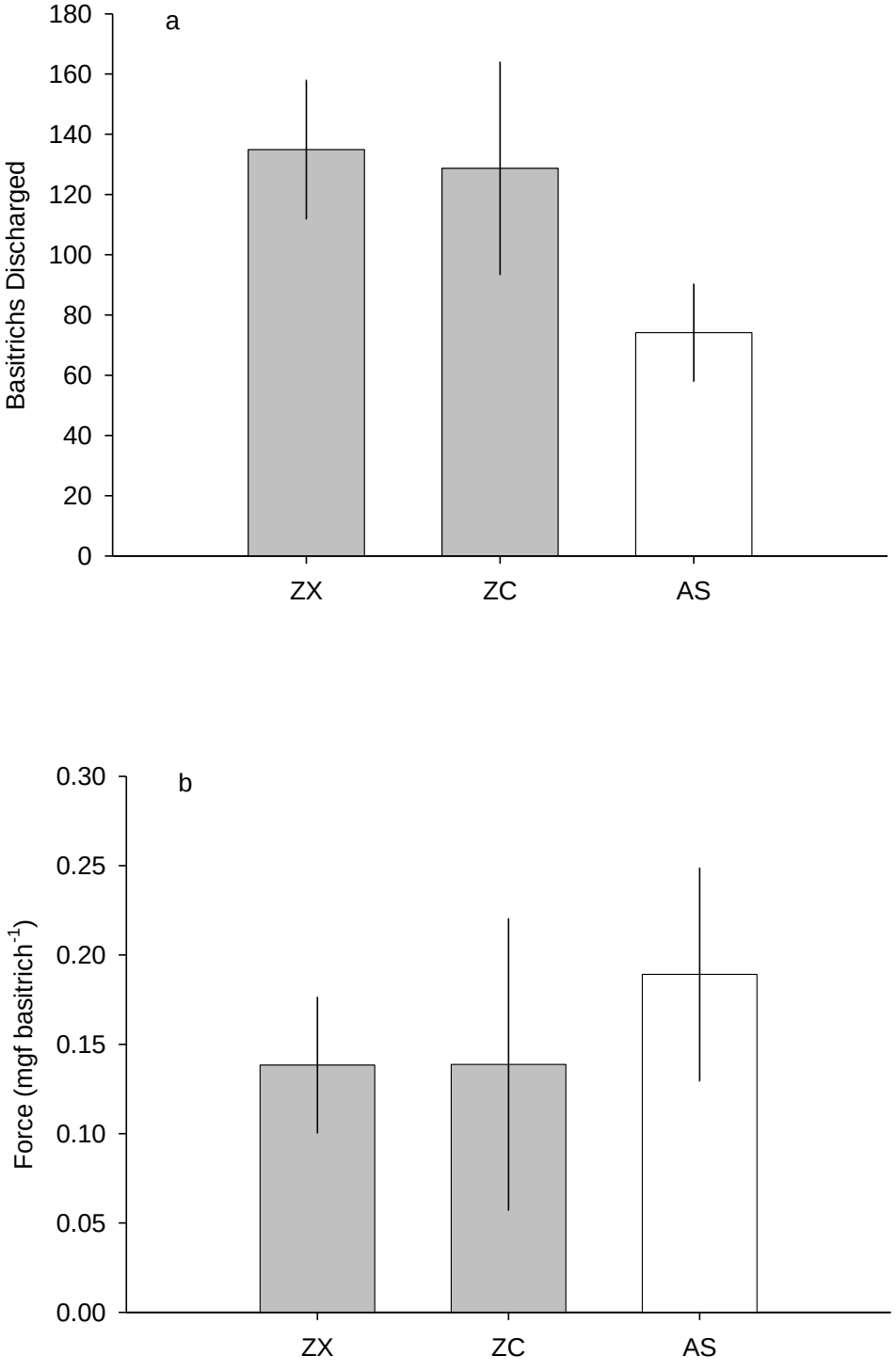

Figure 6. a) Number of basitrichs discharged and b) force per basitrich for zooxanthellate (ZX), zoochlorellate (ZC) and asymbiotic (AS) *A. elegantissima*. Standard errors are shown.

Cnida size

Basitrich length was related to symbiotic state when anemone size (measured as total protein per anemone) was removed as a covariate (Appendix A4). At a given body size, asymbiotic anemones had longer basitrichs than did zoochlorellate anemones (Fig. 7a). Mean basitrich widths were similar in all anemones (Fig. 7b, Appendix A4). Spirocyst length and width were not significantly different in any symbiotic state of *A. elegantissima* (Fig. 8, Appendix A4), though there was again a trend toward longer cnidae in the asymbiotic individuals.

Cnida density

Density of basitrichs was not significantly different between symbiotic states (Appendix A5), but there was a trend toward asymbiotic anemones having slightly higher densities (Fig. 9a). Spirocyst densities were not significantly different among symbiotic states (Fig. 9b, Appendix A5), but the trend was toward lower densities in zoochlorellate individuals (Fig. 9b).

Light experiment

During the three-week acclimation period, our sunlight and shade treatments were equal to 84% and 2% ambient light, respectively. Anemones in the full sunlight treatment experienced a wide range of irradiances (mid-day mean = 1210.81 ± 40.90 μmol photons m^{-2} s^{-1}), while irradiances in the shade treatment was relatively constant (mid-day mean = 25.49 ± 0.86 μmol photons m^{-2} s^{-1}). During this three-week period, one zoochlorellate anemone in the sunlight treatment died and was removed from the experiment. One

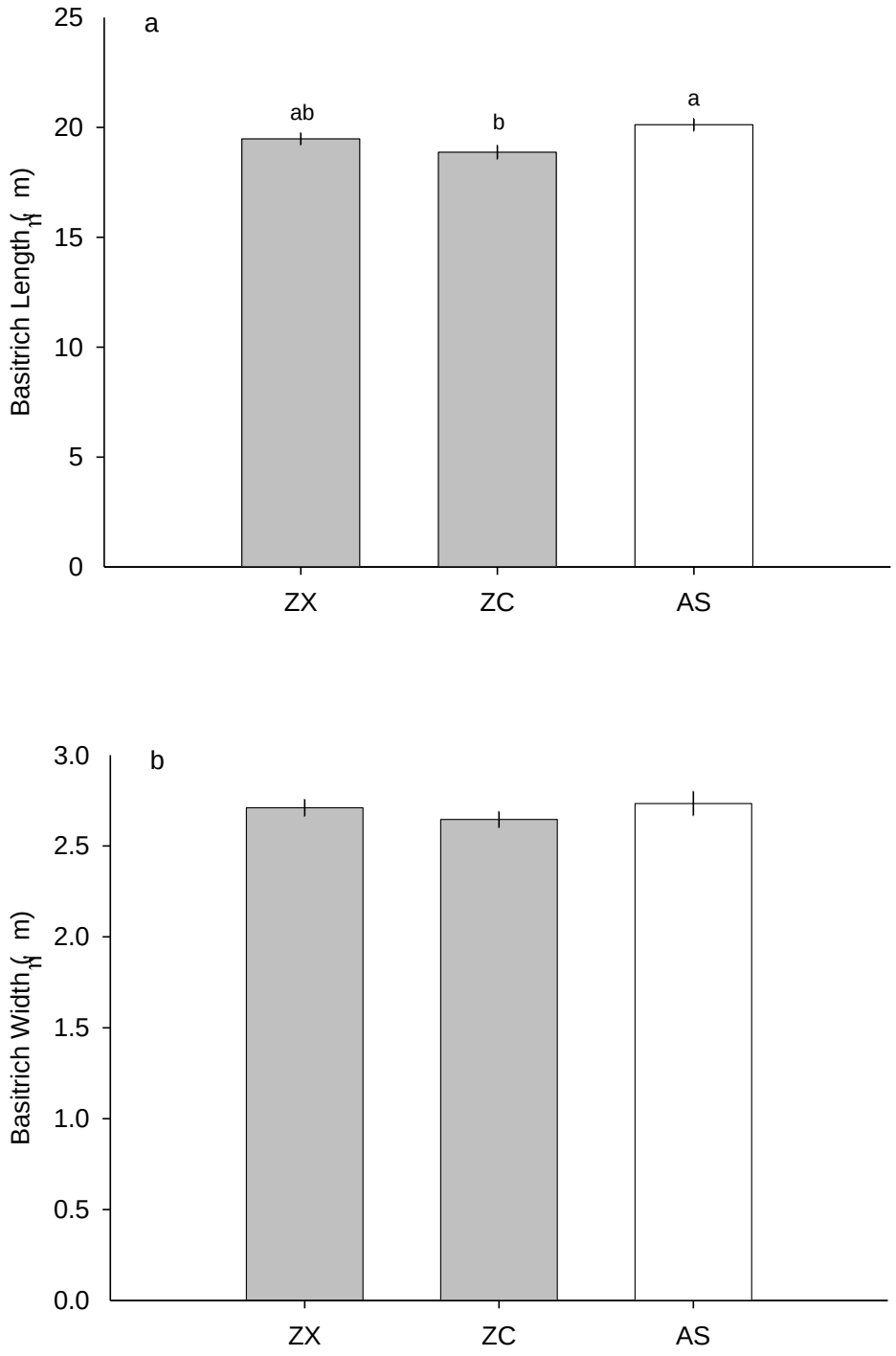

Figure 7. a) Covariate adjusted basitrich length and b) basitrich width for zooxanthellate (ZX), zoochlorellate (ZC) and asymbiotic (AS) *A. elegantissima*. Different letters represent differences between treatments as indicated by Tukey's HSD pairwise comparisons. Standard errors are shown.

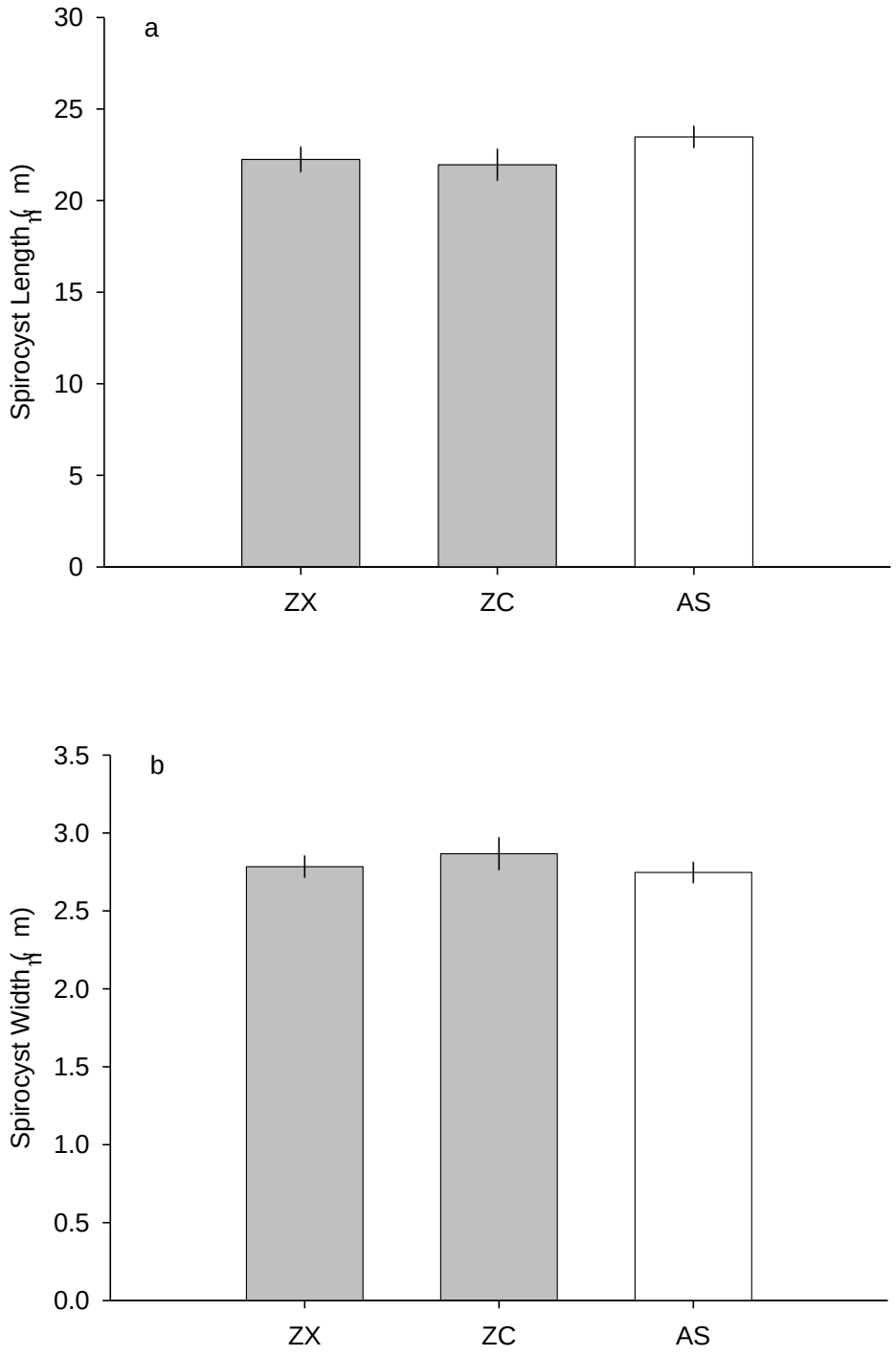

Figure 8. a) Spirocyst length and b) spirocyst width for zooxanthellate (ZX), zoochlorellate (ZC) and asymbiotic (AS) *A. elegantissima*. Standard errors are shown.

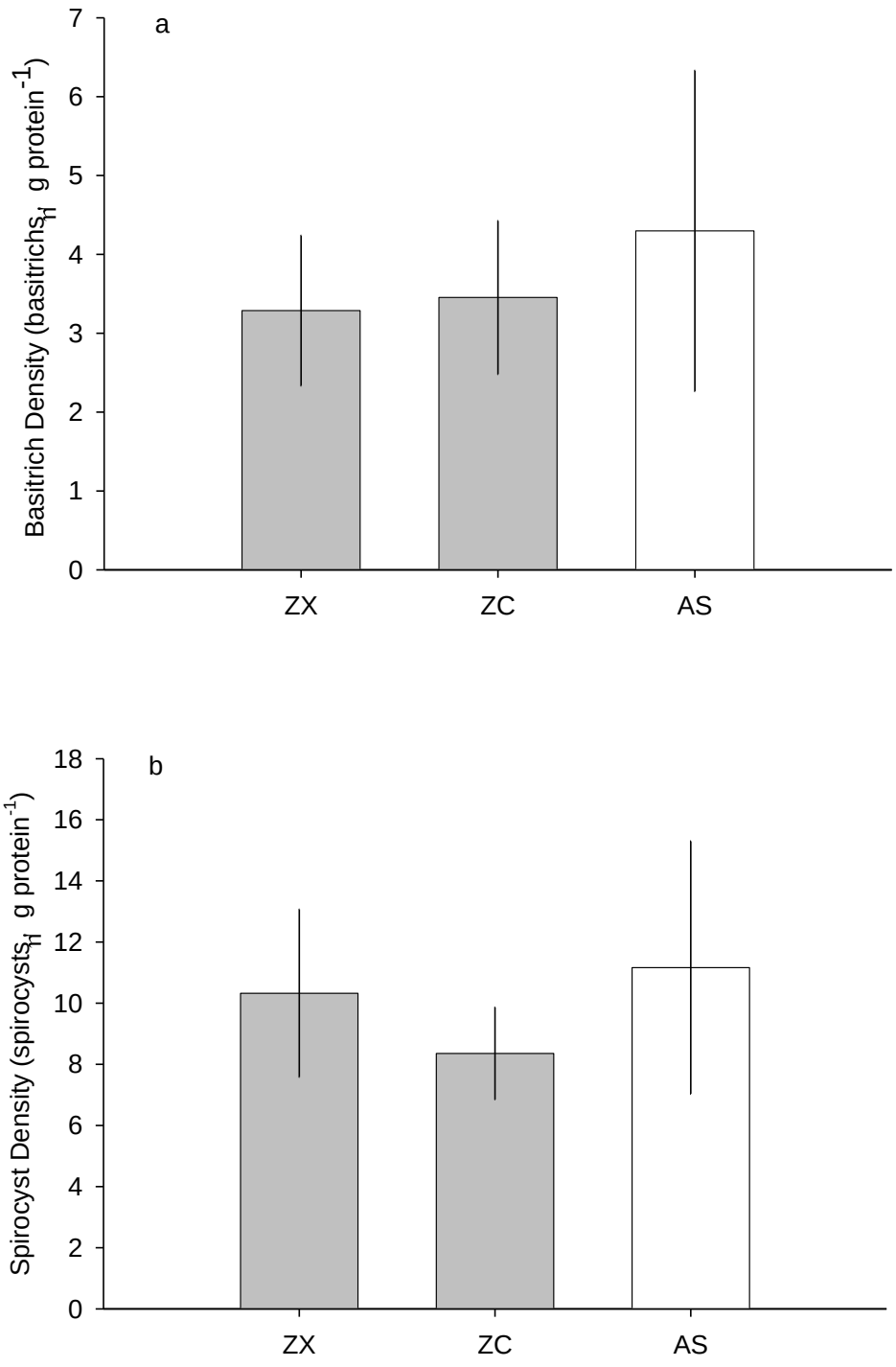

Figure 9. a) Basitrich and b) spirocyst densities for zooxanthellate (ZX), zoochlorellate (ZC) and asymbiotic (AS) *A. elegantissima*. Standard errors are shown.

asymbiotic anemone in the shade treatment divided into two individuals and both were included in the remaining experiments. Therefore, the sample sizes for our light experiment included four zoochlorellate anemones and five zooxanthellate and asymbiotic anemones in the sunlight treatment and four zooxanthellate and zoochlorellate anemones and five asymbiotic anemones in the shaded treatment.

Temperatures in the sunlight and shade treatments were similar except near mid-day in trials 1 and 3 when temperature in the sunlight treatment exceeded the shaded treatment by almost 2ºC for less than 2 hr (Fig. 10). Statistical comparison of temperatures in the sunlight and shaded treatments showed that temperatures were significantly higher in the sunlight treatment only during trial 1, and the mean difference was only 0.2ºC. The average daily irradiances for the sunlight and shade treatments were significantly different on all trial days. The third trial day had the greatest range of irradiances and the warmest temperatures (Fig. 10).

Light experiment: Feeding experiments

Ingestion times were not statistically different among sunlight or shade adapted zooxanthellate, zoochlorellate and asymbiotic *A. elegantissima* (Appendix B1). All asymbiotic anemones in the shade treatment and all symbiotic anemones in both treatments ingested their squid ration. However, only 73% of the asymbiotic anemones maintained in the sunlight treatment ingested their squid ration. Anemones that failed to ingest their ration were removed from the analysis, reducing the sample size for

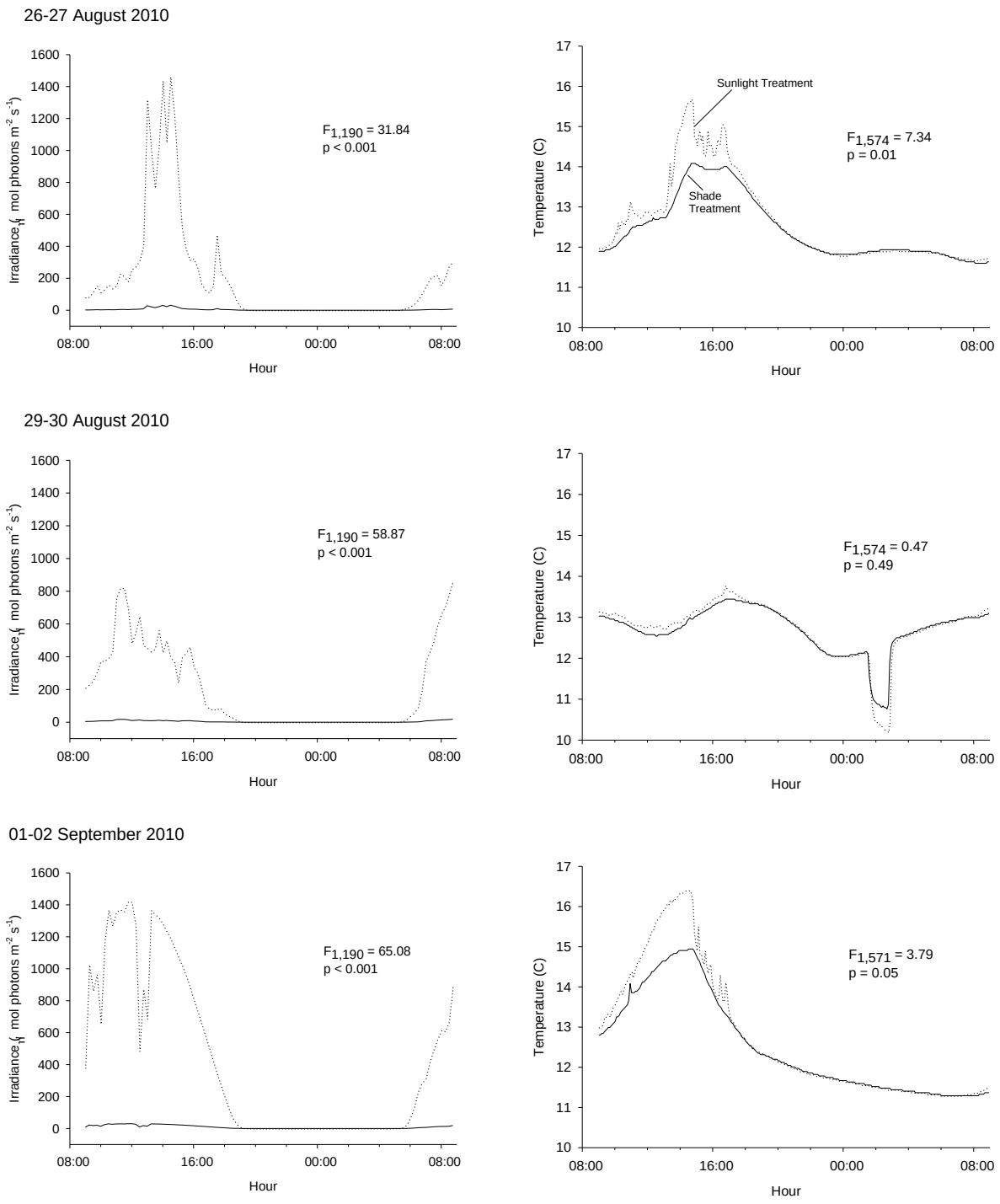

Figure 10. Irradiance (measured at 15 min intervals) and temperature (measured at 5 min intervals) for the sunlight and shade treatments over three trial days. For each day, feeding experiments began at 9:00 and concluded 24 hr later.

asymbiotic anemones in the sunlight treatment to 2. Ingestion times were significantly different on the trial dates, with a trend toward faster ingestion times with each day (Fig 11a-c). Ingestion times were not affected by anemone size or by symbiotic condition (Appendix B1).

Digestion time was the only factor significantly affected by light exposure ($p < 0.001$, Appendix B1). All anemones digested faster in the sunlight than in the shade treatment (Fig. 11d-f). Digestion times were also significantly different on trial days, with the fastest digestion times on the third day (Fig. 11f, Appendix B1). Digestion times were not affected by symbiotic state or anemone size (Appendix B1).

Absorption efficiencies exceeded 70% in all anemones, and were not affected by light treatment, symbiotic state or anemone size (Fig. 11g-i, Appendix B1). On the first trial day, there was a trend toward higher absorption efficiencies in the sunlight treatment, but it was not significant (Fig. 11g).

Light experiment: Tentacle adhesive force

After all feeding trials had been completed we measured the adhesive force per basitrich and the total number of basitrichs that fired into the test probe. We found that zooxanthellate anemones overall fired significantly more basitrichs upon contact than did anemones in the other symbiotic states ($p = 0.015$, Fig. 12a, Appendix B2). All anemones fired more basitrichs following the light experiment than immediately after collection (compare Fig. 6a). However, there were also no significant differences in the

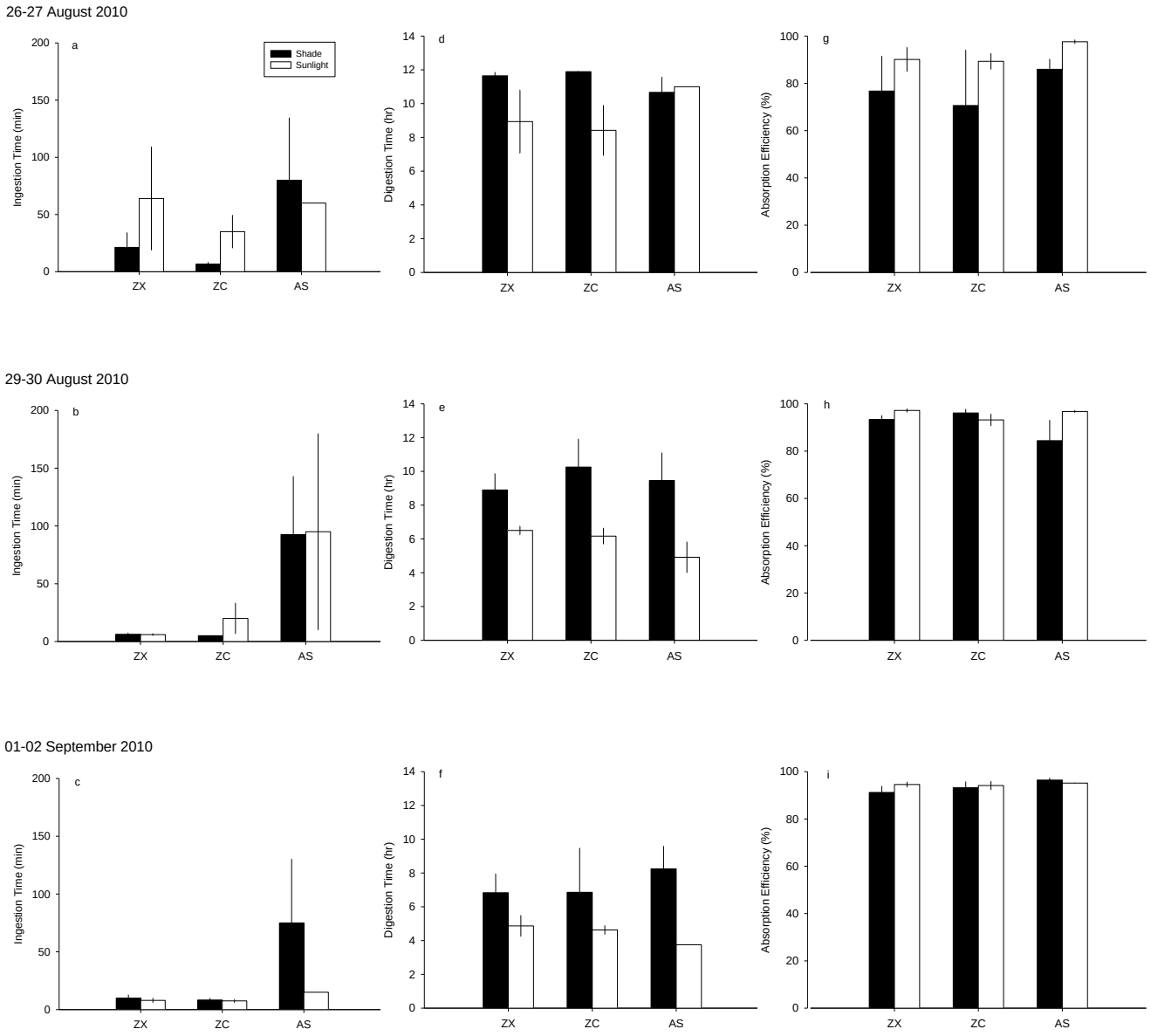

Figure 11. (a-c) Ingestion time, (d-f) digestion time and (g-i) absorption efficiency over three trial days for zooxanthellate (ZX), zoochlorellate (ZC) and asymbiotic (AS) *A. elegantissima*. Standard errors are shown.

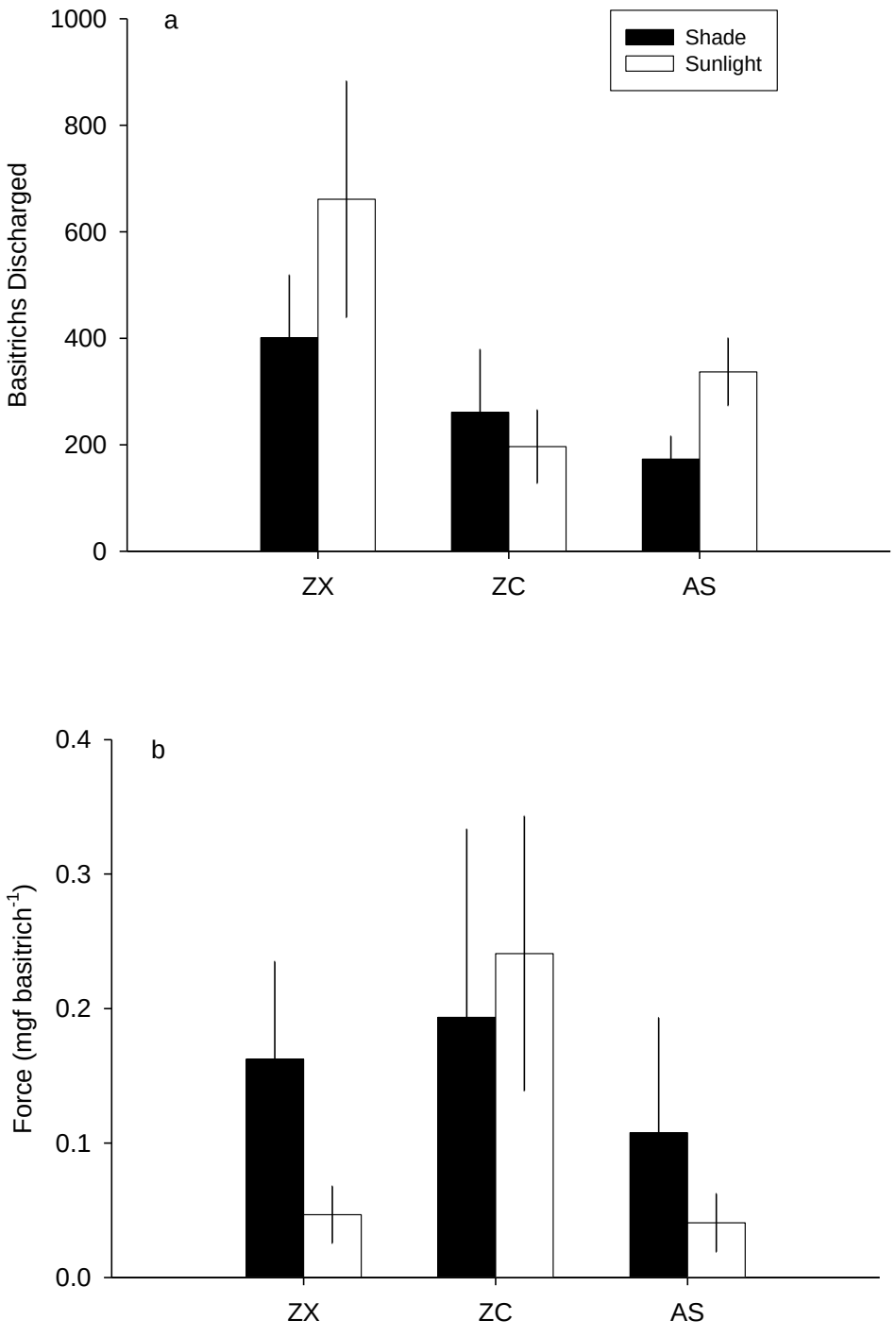

Figure 12. a) Number of basitrichs discharged and b) force per basitrich for zooxanthellate (ZX), zoochlorellate (ZC) and asymbiotic (AS) *A. elegantissima* in shade and sunlight treatments. Standard errors are shown.

force per basitrich (Fig. 12b). There was a trend toward zoochlorellate anemones exhibiting a greater force after exposure to the sunlight treatment than the shade treatment and this was opposite the trend seen for zooxanthellate and asymbiotic individuals (Fig. 12b).

Light experiment: Cnida size

Neither basitrich length nor width was affected by light treatment or symbiotic state (Fig. 13, Appendix B3). However, light had a significant effect on spirocyst length (Appendix B3). For all anemones, spirocysts were longer after exposure to the shade treatment (Fig. 14a). Spirocyst width was not affected by light or symbiotic state (Fig. 14b, Appendix B3). Anemone size was included as a covariate in the 2 way nested ANCOVA, but was not significant.

Light experiment: cnida density

Basitrich (Fig. 15a) and spirocyst densities (Fig. 15b) were greater for all anemones following exposure to the shade treatment with no differences among symbiotic states (Appendix B4). Spirocyst densities overall were approximately two times greater than basitrich densities (Fig. 15). Both basitrich and spirocyst density nearly quadrupled over what we had measured three weeks earlier in the same anemones (compare Fig. 9).

DISCUSSION

The density and productivity of symbiotic algae can affect the heterotrophic feeding of tropical (Anthony and Fabricius 2000; Grottoli *et al.* 2006) and temperate (Hoogenboom

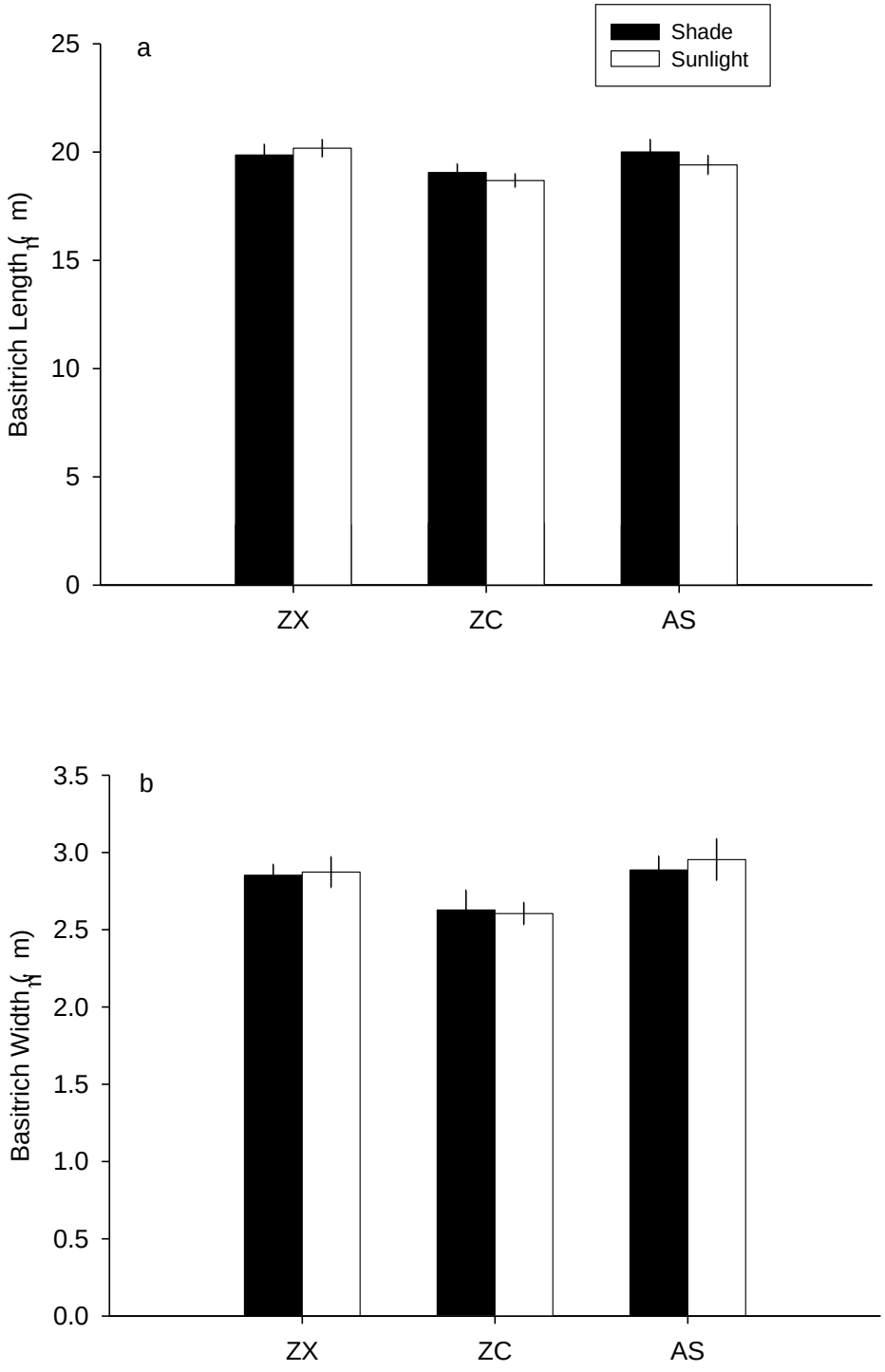

Figure 13. a) Basitrich length and b) width for zooxanthellate (ZX), zoochlorellate (ZC), and asymbiotic (AS) *A. elegantissima* in shade and sunlight treatments. Standard errors are shown.

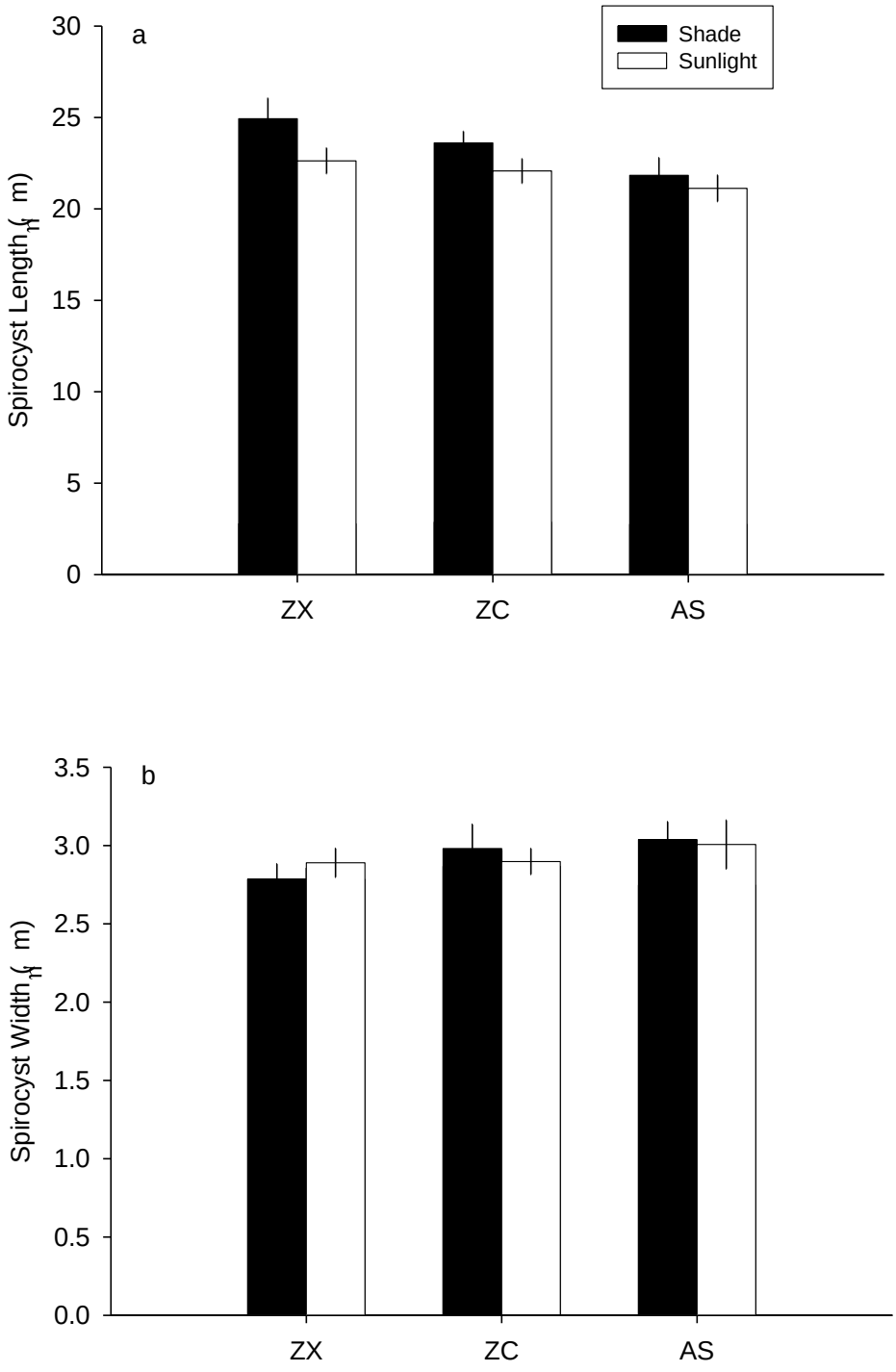

Figure 14. a) Spirocyst length and b) width for zooxanthellate (ZX), zoochlorellate (ZC), and asymbiotic (AS) *A. elegantissima* in shade and sunlight treatments. Standard errors are shown.

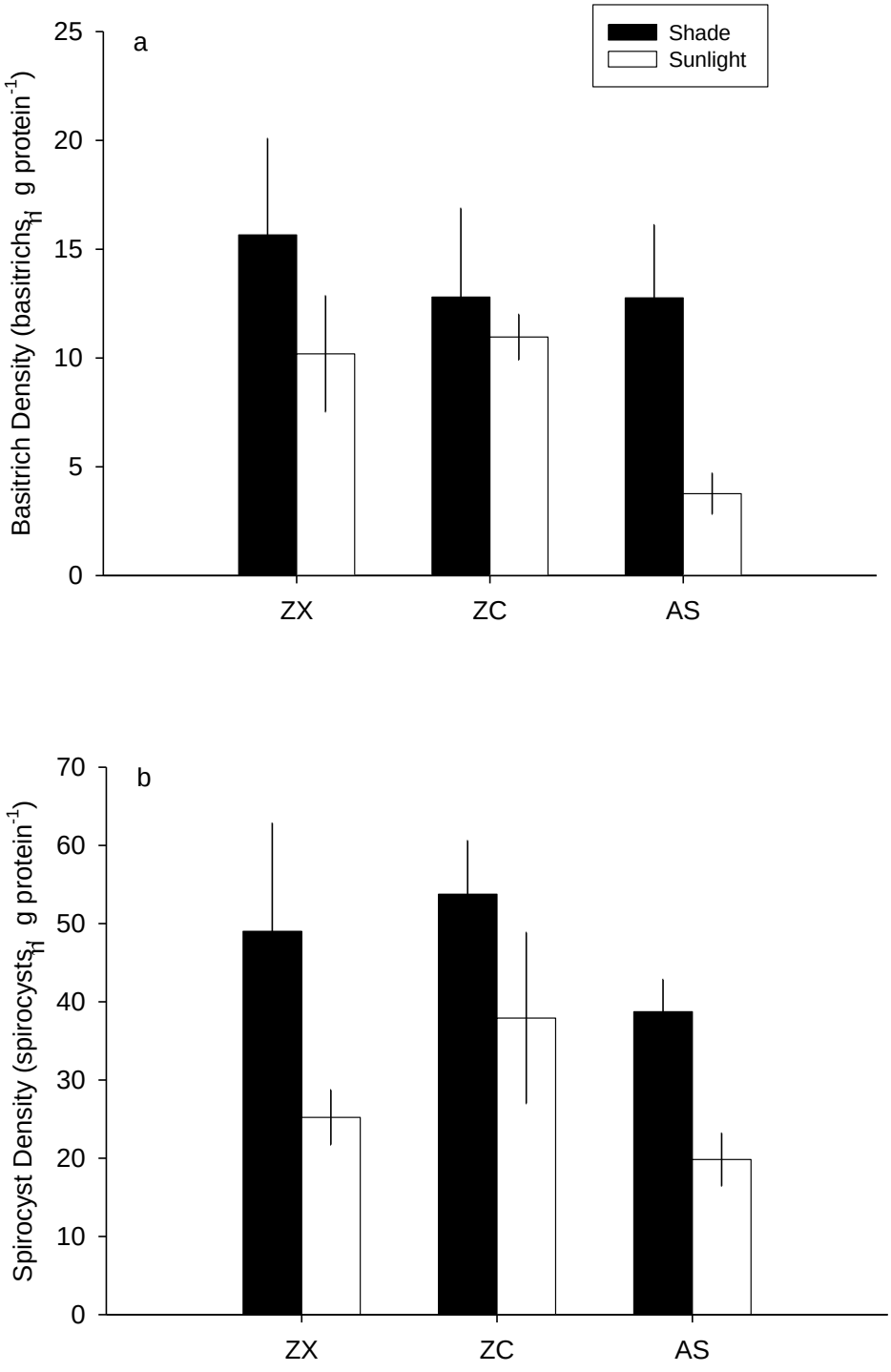

Figure 15. Basitrich (a) and spirocyst (b) densities for zooxanthellate (ZX), zoochlorellate (ZC), and asymbiotic (AS) *A. elegantissima* in shade and sunlight treatments. Standard errors are shown.

et al. 2010) cnidarian hosts. The goal of our research was to determine whether such influences could also be documented in the temperate anemone, *Anthopleura elegantissima*. We speculated that the identity and productivity of algal symbionts would influence the host anemones' heterotrophic nutritional need, resulting in differences in anemone morphology or feeding behavior. Specifically, we hypothesized that asymbiotic anemones, lacking nutritional input from the photosynthetic activities of algal symbionts, would be more effective heterotrophic feeders.

The greatest potential algal contribution in *A. elegantissima* symbiosis occurs during summer months when higher light and temperature increase algal productivity (Verde and McCloskey 2001, 2002, 2007; Bergschneider and Muller-Parker 2008). However, algae also absorb nutrients from the host anemone (Steen 1986), and during less productive seasons, carbon contribution from the algae could be negligible, turning this symbiotic relationship toward parasitism (Bergschneider and Muller-Parker 2008). Heterotrophic feeding in *A. elegantissima* also varies seasonally. The anemones shrink and may slow metabolism during winter months (Sebens 1980) when temperature, light level and food availability are reduced. Anemones in the current study were observed during summer months (June-Sep 2010) when anemone metabolic rates are high and symbiotic individuals should have the greatest nutritional advantage over individuals lacking symbionts. Even under these most favorable conditions, we saw only subtle differences in heterotrophic feeding, suggesting that symbiotic state has limited effects on *A. elegantissima* heterotrophy.

Anthopleura elegantissima feed on planktonic or benthic prey that contact their tentacles or oral disc, and prey capture increases with feeding surface area (Sebens 1981). Since asymbiotic individuals must rely solely on heterotrophic feeding, they might be expected to produce more tentacles, a larger prey capture surface area and higher numbers of cnidae for more efficient prey capture. Surprisingly, asymbiotic anemones actually had significantly fewer tentacles than zoochlorellate anemones (Fig. 3) though there was no resulting difference in prey-capture surface area. Feeding anemones stretch out their tentacles and actively move them, increasing the probability of prey contact (Sandberg *et al.* 1971; Shick 1991). We expect, therefore, that fewer tentacles would actually place asymbiotic anemones at a relative structural disadvantage when feeding heterotrophically.

The density of tentacle cnidae affects cnidarian feeding efficiency (Thomason 1988); low nematocyst densities in gorgonians result in the escape of potential prey (Lasker 1981; Coma *et al.* 1994). Starvation increases the number of nematocytes produced by *Hydra* spp. for up to fifteen days of starvation (Flechtner and Lesh-Laurie 1984). We found that freshly collected *A. elegantissima* had similar densities of cnidae regardless of symbiotic state (Fig. 9), but that cnida density changed in the laboratory. Anemones placed in both light and shade treatments increased their cnidae densities during the three-week acclimation period, suggesting that our feeding regime, prey type or tank conditions changed the feeding behavior of the anemones. The density increase was affected differently by light treatment. Both spirocyst and basitrich densities increased 3 – 6x in all anemones held in the shade; the increase was smaller among anemones in the sunlight. We predicted that moving anemones with symbionts into the shade would influence their nutritional need causing them to produce

additional cnidae while cnida densities in asymbiotic anemones would remain relatively unchanged. In fact, asymbiotic individuals showed the greatest change in basitrich densities (Fig. 15a). Since there is no reason to suppose that the nutritional need of asymbiotic individuals would differ in the light and shade, we believe that sunlight directly reduced production of cnidae and that those effects were most pronounced in asymbiotic anemones that lacked protective pigments. Anemones exposed to sunlight invest energy in pigments and enzymatic repair mechanisms as a defense against light (Shick and Dykens 1984; Dykens and Shick 1984) and temperature (Snyder and Rossi 2004) damage. This energetic cost may have been more substantial for the asymbiotic individuals, resulting in less energy available for other activities (e.g., production of cnidae).

Assessing the effects of sunlight required moving zooxanthellate, zoochlorellate and asymbiotic *A. elegantissima* to full sunlight or shade treatments. The zooxanthellate and zoochlorellate anemones had been collected from a rock face that received direct sunlight, but asymbiotic anemones came from a cavity shaded by a large boulder. Asymbiotic anemones placed in full sunlight, therefore, experienced a more dramatic change in irradiance. Shick and Dykens (1984) reported that anemones exposed to high irradiances close up to protect their tissues from damage. All anemones in our experiment, including asymbiotic individuals, remained 75-100% expanded even at maximum midday irradiances, suggesting that irradiance levels were below a threshold that would produce immediate negative effects. Although we saw no mortality among our asymbiotic individuals, and there was no obvious behavioral response to the light, it is possible that there were more subtle impacts.

Twenty-seven percent of asymbiotic anemones in the full sunlight dropped their squid rations, despite tentacle adhesive forces and cnida sizes that were similar to those of symbiotic anemones that all held onto and ingested their food. Basitrich densities tended to be lower for asymbiotic anemones in the sunlight and, although the pattern was not statistically significant, may explain this behavior. It is also possible that cnidocyte function was compromised in asymbiotic individuals. Satiation can reduce cnidocyte sensitivity, but 72 hr feeding intervals are sufficient for full cnida sensitivity in other anemones (Thorington *et al.* 2010). Extreme light levels (bright sunlight or complete darkness) can reduce or prevent prey capture by decreasing cnidocyte function in *Aiptasia pallida* (Thorington and Hessinger 1988a). The UV-absorbing ectodermal pigments in symbiotic anemones (Shick and Dykens 1984) may shade their cnidocytes, reducing the damaging effects of sunlight. Asymbiotic individuals, lacking those pigments, may be more vulnerable to cnidocyte damage reducing their prey capture ability in sunlight.

Among anemones in the sunlight, both zooxanthellate and zoochlorellate individuals tended to have higher basitrich densities than asymbiotic anemones, suggesting a positive relationship between autotrophy and heterotrophy (Fig. 15a). Hoogenboom *et al.* (2010) found that feeding rates were greatest in high light treatments for the scleractinian coral, *Cladocora caespitosa*. They propose that the demand of the symbionts for nutrients induces the host to feed more actively. Symbiotic anemones in our sunlight treatment may have increased their basitrich densities to feed more effectively. Asymbiotic anemones did not produce greater densities of cnidae than other symbiotic states, but the basitrichs they did

produced were significantly longer (Fig. 7a). Larger nematocysts may have longer threads or more spines for penetrating prey. Purcell (1984) found that siphonophores with larger nematocysts were able to capture larger prey than those with smaller nematocysts. The difference in basitrich size we saw may explain the observed stickiness of asymbiotic anemones collected from Tatoosh Island (B. Bingham, M. Levine pers. com.). In our study, the longer basitrichs present in freshly collected asymbiotic anemones suggests that, in their natural habitat, asymbiotic anemones may be better at capturing larger prey.

Despite having longer basitrichs, asymbiotic anemones did not show greater force per basitrich in laboratory experiments (Fig. 6b, Fig. 12b). Zooxanthellate anemones, with smaller basitrichs, showed greater nematocyte sensitivity (in terms of the number of discharged basitrichs) following our three-week light experiment (Fig. 12a). Nematocyte sensitivity is related to chemical or mechanical stimuli and to nutritional need (Mariscal 1984; Shick 1991); satiation reduces and starvation increases nematocyte sensitivity (Pantin and Pantin 1943; Sandberg *et al.* 1971; Thorington *et al.* 2010). Because all anemones in our study experienced similar chemical and mechanical stimuli the data suggest that zooxanthellate anemones may compensate for smaller basitrich size by firing more of them to achieve the same total adhesive force.

Zamer (1986) speculated that a lack of a photosynthetic partner in asymbiotic anemones could increase the anemones' heterotrophic feeding activities. If a significant portion of daily nutrition derives from algal photosynthesis, anemones that lack symbionts may compensate with faster ingestion, longer digestion or higher absorption efficiency. Contrary

to our expectations, we saw no differences in any of these among the three symbiotic states of *A. elegantissima* (Fig. 11); all anemones ingested, digested and absorbed prey similarly under laboratory conditions. These results suggest 1) that all anemones are feeding at or near their maximum capacity regardless of symbiotic state or 2) that the cost of maintaining the algae just balances their nutritional contribution so there is no net impact on the anemone and asymbiotic anemones are not at a nutritional deficit. It should be noted that none of the anemones used in our study were truly asymbiotic; even those we categorized as asymbiotic hosted small numbers of zooxanthellae (Table 1). However, densities were sufficiently different in our treatments that effects of symbionts should have been evident.

Digestion times in *A. elegantissima* decreased when anemones were placed in the sunlight. The first egesta were seen after only 4.5 hr in the sunlight compared to 7 hr in shaded individuals. Anemones in the sunlight experienced short periods of slightly warmer water temperatures than did anemones in the shade. Warmer water temperatures decrease digestion times in the jellyfish *Aurelia* spp. (Purcell 2009) and in the gorgonian, *Leptogorgia sarmentosa* (Rossi *et al.* 2004). However, the greatest differences in temperature between our sunlight and shade treatments were lower than those required to see effects in *Aurelia* spp. or *L. sarmentosa*. Furthermore, the differences were brief and it is more likely that the differences in digestion time were due to irradiance rather than temperature.

Strom (2001) showed that sunlight exposure enhances digestion in the protozoan *Noctiluca scintillans*, presumably through partial breakdown of ingested food by reactive oxygen species produced by irradiant energy. Reduction of oxygen (forming superoxide and

hydroxyl radicals and hydrogen peroxide) also occurs in *A. elegantissima* tissues (Dykens and Shick 1984), which may enhance digestive processes of individuals exposed to sunlight. Algal productivity increases the formation of reactive oxygen species so we may have expected symbiotic anemones to digest faster than asymbiotic anemones in the sunlight. However, to mitigate oxygen toxicity, symbiotic anemones produce enzymes that suppress the formation of reactive oxygen (Dykens and Shick 1984). These competing processes may explain why digestion was faster in the sunlight for all anemones, but was not further enhanced by the presence of symbiotic algae.

When held in the light, starved asymbiotic anemones lose body mass more quickly than symbiotic anemones, suggesting algal contribution to nutrition from symbiont productivity (Muscatine 1961; Fitt and Pardy 1981). A common way to quantify the potential benefit of the symbionts is as the percent contribution of zooxanthellae or zoochlorellae to host respiration (CZAR) as anemone respiratory requirements can be achieved with both heterotrophically and autrotrophically derived carbon (Verde and McCloskey 1996). Verde and McCloskey (1996) estimated that zooxanthellae contribute 48% of the daily respiratory needs of *A. elegantissima*, and that zoochlorellae contribute only 9%. These estimates are based on anemone respiration as well as algal respiration, photosynthesis and growth rates, which are extremely dependent on temperature, light and algal physiology. The effect of host environment and symbiont condition and density result in widely variable literature estimates for CZAR in *A. elegantissima*, ranging from 34-126% for zooxanthellate anemones and 9-82% for zoochlorellate anemones (Muller-Parker and Davy 2001). Davy *et al.* (1996) found algal contribution to decline to as little as 0.7% for temperate anemones on cloudy

days. When estimating CZAR, it is assumed that the host receives all photosynthetic carbon not used for algal growth or respiration (Engebretson and Muller-Parker 1999). However, this may not be the case. Verde and McCloskey (1996) calculated the amount of heterotrophically derived carbon required for respiration in zooxanthellate and zoochlorellate anemones based on their respective CZAR estimates. They suggest that zooxanthellate anemones require only half the heterotrophic carbon per day that zoochlorellate anemones do (702 μg C day^{-1} vs. 1214 μg C day^{-1} for zooxanthellate and zoochlorellate anemones). Despite this difference, we saw no variation in heterotrophic feeding abilities, suggesting that the actual algal contribution may be lower than predicted. Furthermore, algal symbionts acquire nutrients from their hosts and symbiotic anemones may actually have to feed more effectively to meet the needs of the symbionts (Hoogenboom *et al.* 2010), which would increase their daily heterotrophic requirement and decrease the total algal contribution to respiration.

Temperate cnidarians are less reliant on their algal symbionts for nutrition than are their tropical counterparts (Muller-Parker and Davy 2001). Piniak (2002) found that the presence of algal symbionts did not affect prey capture rates in the temperate scleractinian coral, *Oculina arbuscula* and concluded that *O. arbuscula* is primarily heterotrophic. Similarily, *A. elegantissima* growth (Tsuchida and Potts 1994) and diet (Bergschneider and Muller-Parker 2008) are more greatly affected by heterotrophic feeding than by symbiotic state. Results from our study further emphasize the primary role of heterotrophy in *A. elegantissima*. Although symbiotic algae can potentially translocate significant amounts of photosynthate to their hosts, it is possible that the algae, under natural field conditions, actually contribute

much less than they are capable. It is also possible that the symbiotic algae contribute photosynthetic products that benefit the host anemone in other ways. *A. elegantissima* may be able to meet daily nutritional requirements heterotrophically, but excess autotrophic energy may be used for growth or reproduction.

REFERENCES

Anthony, K. R. N. and K. E. Fabricius. 2000. Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *J. Exp. Mar. Biol. Ecol.* 252: 221-253.

Bates, A. 2000. The intertidal distribution of two algal symbionts hosted by *Anthopleura xanthogrammica* (Brandt 1835). *J. Exp. Mar. Biol. Ecol.* 249: 249–262.

Bergschneider, H. and G. Muller-Parker. 2008. Nutritional role of two algal symbionts in the temperate sea anemone *Anthopleura elegantissima* Brandt. *Biol. Bull.* 215: 73-88.

Coma, R., J. Gili, M. Zabala and T. Riera. 1994. Feeding and prey capture cycles in the aposymbiotic gorgonian *Paramuricea clavata*. *Mar. Ecol. Prog. Ser.* 115: 257-270.

Davy, S. K., I. A. N. Lucas and J. R. Turner. 1996. Carbon budgets in temperate anthozoan-dinoflagellate symbioses. *Mar. Biol.* 126: 773-783.

Dykens, J. A. and J. M. Shick. 1984. Photobiology of the symbiotic sea anemone, *Anthopleura elegantissima*: Defenses against photodynamic effects, and seasonal photoacclimatization. *Biol. Bull.* 167: 683-697.

Engebretson, H. P. and G. Muller-Parker. 1999. Translocation of photosynthetic carbon from two algal symbionts to the sea anemone *Anthopleura elegantissima*. *Biol. Bull.* 197: 72-81.

Fitt, W. K. and R. L. Pardy. 1981. Effects of starvation, and light and dark on the energy metabolism of symbiont and aposymbiotic sea anemones, *Anthopleura elegantissima*. *Mar. Biol.* 61: 199-205.

Flechtner, V. R. and G. E. Lesh-Laurie. 1984. Effects of starvation and resumption of feeding on the cell populations of *Hydra oligactis*. *Freshwater Invert. Biol*. 3: 12-20.

Francis, L. 2004. Microscaling: Why larger anemones have longer cnidae. *Biol. Bull.* 207: 116-129.

Giebel, G. E. M., G. U. Thorington, R. Y. Lim and D. A. Hessinger. 1988. Control of cnida discharge: II. Microbasic p-mastigophore nematocysts are regulated by two classes of chemoreceptors. *Biol. Bull.* 175: 132-136.

Grottoli, A. G., L. J. Rodrigues and J. E. Palardy. 2006. Heterotrophic plasticity and resilience in bleached corals. *Nature Letters*. 440: 1186-1189.

Hand, C. 1955. The sea anemones of central California part II. The endomyarian and mesomyarian anemones. *Wasmann J. Biol*. 13: 54-61.

Hoogenboom, M., R. Rodolfo-Metalpa and C. Ferrier-Pagès. 2010. Co-variation between autotrophy and heterotrophy in the Mediterranean coral *Cladocora caespitosa*. *J. Exp. Biol.* 213: 2399-2409.

Kramer, A. and L. Francis. 2004. Predation resistance and nematocyst scaling for *Metridium senile* and *M. farcimen*. *Biol. Bull.* 207: 130-140.

Kitaeff, R. R. N. 2007. Latitudinal differences in the distribution of two algal symbionts within the intertidal sea anemone *Anthopleura xanthogrammica*. Master's thesis, Western Washington University. Pp. 54.

LaJeunesse, T. C. and R. K. Trench. 2000. Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biol. Bull.* 199: 126-134.

Lasker, H. R. 1981. A comparison of the particulate feeding abilities of three species of gorgonian soft coral. *Mar. Ecol. Prog. Ser.* 5: 61-67.

Letsch, M.R., G. Muller-Parker, T. Friedl and L. A. Lewis. 2009. *Elliptochloris marina* sp. nov. (Trebouxiophyceae, Chlorophyta), symbiotic green alga of the temperate Pacific sea anemones *Anthopleura xanthogrammica* and *A. elegantissima* (Anthozoa, Cnidaria). *J. Phycol.* 45: 1127-1135.

Lowry, O. H., N. J. Rosenbrough, H. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Mariscal, R. N. 1984. Cnidaria: cnidae. Pp. 57-68 in *Biology of the Integument, Vol. 1, Invertebrates*, J. Bereiter-Hahn, A. G. Maltolsy and K. S. Richards, eds. Springer-Verlag, Berlin.

McFarlane, I. D. 1970. Control of preparatory feeding behavior in the sea anemone *Tealia felina*. *J. Exp. Biol.* 53: 211-220.

Muller-Parker, G. and S. K. Davy. 2001. Temperate and tropical algal-sea anemone symbioses. *Invert. Biol.* 120: 104-123.

Muscatine, L. 1961. Symbiosis in marine and fresh water coelenterates. Pp. 255-268 in *The Biology of Hydra and Other Coelenterates*, H. M. Lenhoff and W. F. Loomis, eds. University of Miami Press, Coral Gables, FL.

Pantin, C. F. A. and A. M. P. Pantin. 1943. The stimulus to feeding in *Anemonia sulcata*. *J. Exp. Biol.* 20: 6-13.

Pearse, V. B. 1974. Modification of sea anemone behavior by symbiotic zooxanthellate: Phototaxis. *Biol. Bull.* 147: 630-640.

Piniak, G. A. 2002. Effects of symbiotic status, flow speed, and prey type on prey capture by the facultatively symbiotic temperate coral *Oculina arbuscula*. *Mar. Biol.* 141: 449-455.

Rossi, M. R., R. Coma and J.-M. Gili. 2004. Temporal variability in zooplankton prey capture rate of the passive suspension feeder *Leptogorgia sarmentosa* (Cnidaria: Octocorallia). *Mar. Biol.* 144: 89-99.

Purcell, J. E. 1984. The functions of nematocysts in prey capture by epipelagic siphonophores (Coelenterata, Hydrozoa). *Biol. Bull.* 166: 310-327.

Purcell, J. E. 2009. Extension of methods for jellyfish and ctenophore trophic ecology to large-scale research. *Hydrobiologia.* 616: 23-50.

Ruppert, E. E., R. S. Fox and R. D. Barnes. 2004. *Invertebrate Zoology: A Functional Evolutionary Approach.* Brooks Cole, Belmont, CA. Pp. 119-122.

Sandberg, D. M., P. Kanciruk and R. N. Mariscal. 1971. Inhibition of nematocyst discharge correlated with feeding in the sea anemone, *Calliactis tricolor* (Leseur). *Nature*. 232: 263-264.

Saunders, B. K. and G. Muller-Parker. 1997. The effects of temperature and light on two algal populations in the temperate sea anemone *Anthopleura elegantissima* (Brandt, 1835). *J. Exp. Mar. Biol. Ecol.* 211: 213–224.

Sebens, K. P. 1980. The regulation of asexual reproduction and indeterminate body size in the sea anemone *Anthopleura elegantissima* (Brandt). *Biol. Bull*. 158: 370-382.

Sebens, K. P. 1981. The allometry of feeding, energetics, and body size in three sea anemone species. *Biol. Bull.* 161: 152-171.

Secord, D. and L. Augustine. 2000. Biogeography and microhabitat variation in temperate algal-invertebrate symbioses: Zooxanthellae and zoochlorellae in two Pacific intertidal sea anemones, *Anthopleura elegantissima* and *A. xanthogrammica. Invert. Biol.* 119: 139–146.

Secord, D. and G. Muller-Parker. 2005. Symbiont distribution along a light gradient within an intertidal cave. *Limnol. Oceanogr.* 50: 272-278.

Shick, J. M. 1991. *A Functional Biology of Sea Anemones.* Chapman & Hall, New York.

Shick, J. M. and J. A. Dykens. 1984. Photobiology of the symbiotic sea anemone *Anthopleura elegantissima*: Photosynthesis, respiration, and behavior under intertidal conditions. *Biol. Bull.* 166: 608-619.

Snyder, M. J. and S. Rossi. 2004. Stress protein (HSP70 family) expression in intertidal benthic organisms: The example of *Anthopleura elegantissima* (Cnidaria: Anthozoa). *Scientia Marina.* 68: 155-162.

Steen, R. G. 1986. Evidence for heterotrophy by zooxanthellae in symbiosis with *Aiptasia pulchella*. *Biol. Bull.* 170: 267-278.

Strom, S. L. 2001. Light-aided digestion, grazing and growth in herbivorous protists. *Aquat. Microb. Ecol.* 23: 253-261.

Thomason, J. C. 1988. The allometry of nematocysts. Pp 575-588 in *The Biology of Nematocysts*, D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego, CA.

Thorington, G. U. and D. A. Hessinger. 1988a. Control of discharge: Factors affecting discharge of cnidae. Pp. 233-253 in *The Biology of Nematocysts*, Hessinger, D. A. and H. M. Lenhoff, eds. Academic Press, San Diego, CA.

Thorington, G. U. and D. A. Hessinger. 1988b. Control of Cnida Discharge: I. Evidence for Two Classes of Chemoreceptor. *Biol. Bull.* 174: 163-171.

Thorington, G. U. and D. A. Hessinger. 1990. Control of cnida discharge: III. Spirocysts are regulated by three classes of chemoreceptors. *Biol. Bull.* 178: 74-83.

Thorington, G. U. and D. A. Hessinger. 1996. Efferent mechanisms of discharging cnidae: I. Measurements of intrinsic adherence of cnidae discharged from tentacles of the sea anemone, *Aiptasia pallida*. *Biol. Bull.* 190: 125-138.

Thorington, G. U. and D. A. Hessinger. 1998. Efferent mechanisms of discharging cnidae: II. A nematocyst release response in the sea anemone tentacle. *Biol. Bull*. 195: 145-155.

Thorington, G. U., V. McAuley and D. A. Hessinger. 2010. Effects of satiation and starvation on nematocyst discharge, prey killing, and ingestion in two species of sea anemone. *Biol. Bull.* 219: 122-131.

Tsuchida, C. B. and D. C. Potts. 1994. The effects of illumination, food and symbionts on growth of the sea anemone *Anthopleura elegantissima* (Brandt ,1835). I. Ramet growth. *J. Exp. Mar. Biol. Ecol.* 183: 227-242.

Underwood, A. J. 1981. Techniques of analysis of variance in experimental marine biology and ecology. *Oceanogr. Mar. Biol. Annu. Rev.* 19: 513-605.

Verde, E. A. and L. R. McCloskey. 1996. Photosynthesis and respiration of two species of algal symbionts in the anemone *Anthopleura elegantissima* (Brandt) (Cnidaria; Anthozoa). *J. Exp. Mar. Biol. Ecol.* 195: 187-202.

Verde, E. A. and L. R. McCloskey. 2001. A comparative analysis of the photobiology of zooxanthellae and zoochlorellae symbiotic with the temperate clonal anemone *Anthopleura elegantissima* (Brandt). I. Effect of temperature. *Mar. Biol.* 138: 477-489.

Verde, E. A. and L. R. McCloskey. 2002. A comparative analysis of the photobiology of zooxanthellae and zoochlorellae symbiotic with the temperate clonal anemone *Anthopleura elegantissima* (Brandt). II. Effect of light intensity. *Mar. Biol.* 141: 225-239.

Verde, E. A. and L. R. McCloskey. 2007. A comparative analysis of the photobiology of zooxanthellae and zoochlorellae symbiotic with the temperate clonal anemone *Anthopleura elegantissima* (Brandt). III. Seasonal effects of natural light and temperature on photosynthesis and respiration. *Mar. Biol.* 152: 775-792.

Williams, R. B. 1968. Control of the discharge of cnidae in *Diadumene luciae* (Verrill). *Nature.* 219: 959.

Williams, R. B. 1972a. Chemical control of feeding behaviour in the sea anemone *Diadumene luciae* (Verrill). *Comp. Biochem. Physiol.* 41A: 361-371.

Zamer, W. E. 1986. Physiological energetics of the intertidal sea anemone *Anthopleura elegantissima*. I. Prey capture, absorption efficiency and growth. *Mar. Biol.* 92: 299-31.

APPENDIX A
One-way analysis of variance tables for structural prey capture features and heterotrophic feeding ability as a function of anemone symbiotic state.

A1. One-way analysis of covariance for anemone morphological features including prey capture surface area (PCSA), total tentacle number and tentacle crown diameter. PCSA data were log transformed and tentacle number data were square root transformed.

Parameter	Sum of Squares	df	Mean Square	F	p	Partial η^2
Tentacle Number						
Symbiotic State	11.58	2	5.79	4.70	**0.020**	0.299
Total Protein	9.08	1	9.08	7.37	**0.013**	0.251
Error	27.11	22				
PCSA						
Symbiotic State	0.26	2	0.13	2.48	0.107	0.184
Total Protein	1.15	1	1.15	22.32	**<0.001**	0.504
Error	1.13	22	0.05			
Tentacle Crown Diameter						
Symbiotic State	41.44	2	20.72	0.78	0.470	0.066
Total Protein	661.32	1	661.32	24.94	**<0.001**	0.531
Error	583.49	22	26.52			

A2. One-way analysis of variance for features of anemone heterotrophic feeding including ingestion time, digestion time and absorption efficiency.

Parameter	Sum of Squares	df	Mean Square	F	p	Partial η^2
Ingestion time						
Symbiotic State	469.64	2	234.82	1.17	0.331	0.100
Error	4230.36	21	201.45			
Digestion time						
Symbiotic State	14.64	2	7.32	1.20	0.321	0.103
Error	128.04	21	6.10			
Absorption efficiency						
Symbiotic State	48.42	2	24.21	0.05	0.948	0.005
Error	9521.28	21	453.39			

A3. One-way analysis of variance for the number of basitrichs discharged and the adhesive force per basitrich.

Parameter	Sum of Squares	df	Mean Square	F	p	Partial η^2
Adhesive Force						
Symbiotic State	0.01	2	0.01	0.24	0.791	0.023
Error	0.56	20	0.03			
Basitrichs discharged						
Symbiotic State	17082	2	8541	1.81	0.190	0.160
Error	89513	19	4711			

A4. One-way nested analysis of covariance for cnida size including length and width of both basitrichs and spirocysts. Spirocyst length and basitrich width data require an adjusted alpha due to heterogeneous variance ($\alpha = 0.025$). Individual variances from multiple basitrich length measurements were not significant ($p > 0.05$) and were pooled.

Parameter	Sum of Squares	df	Mean Square	F	p	Partial η^2
Basitrich length						
Symbiotic State	18.30	2	9.15	4.46	**0.015**	0.112
Total Protein	9.12	1	9.12	4.44	**0.039**	0.059
Error	145.83	71	2.05			
Basitrich width						
Symbiotic State	0.15	2	0.08	0.70	0.508	0.061
Error	1.31	21	0.11			
Individual	2.28	21	0.11	1.76	0.051	0.420
Error	3.16	51	0.06			
Spirocyst length						
Symbiotic State	33.54	2	16.77	0.90	0.422	0.078
Error	397.70	21	18.64			
Individual	394.25	21	18.77	1.90	0.032	0.439
Error	504.78	51	9.90			
Spirocyst width						
Symbiotic State	0.25	2	0.13	0.46	0.635	0.042
Error	5.78	21	0.27			
Individual	5.76	21	0.27	2.54	**0.003**	0.511
Error	5.51	51	0.11			

A5. One-way analysis of variance for basitrich and spirocyst density mg protein anemone^{-1}.

Parameter	Sum of Squares	df	Mean Square	F	p	Partial η^2
Basitrich density						
Symbiotic State	5.18	2	2.59	0.14	0.87	0.013
Error	404.80	22	18.37			
Spirocyst density						
Symbiotic State	31.94	2	15.97	0.19	0.83	0.017
Error	1875.40	22	85.25			

APPENDIX B
Two-way analysis of variance results with anemone symbiotic state and light treatment as main effect factors.

B1. Repeated measures analysis of variance for ingestion time, digestion time and absorption efficiency over three trial days. Ingestion time and absorption efficiency data were square root and arcsine square root transformed, respectively.

Parameter	Sum of Squares	df	Mean Square	F	p	Partial η^2
Ingestion time						
Light (L)	2.65	1	2.65	0.10	0.752	0.006
Symbiotic State (S)	158.16	2	79.08	3.08	0.074	0.278
L x S	11.29	2	5.65	0.22	0.805	0.027
Error	411.30	16	25.71			
Trial (T)	34.89	2	17.45	3.33	**0.049**	0.172
T x L	23.13	2	11.57	2.21	0.127	0.121
T x S	23.73	4	5.93	1.13	0.359	0.124
T x L x S	4.95	4	1.24	0.24	0.916	0.029
Error	167.82	32	10.49			
Digestion time						
Light (L)	122.28	1	122.28	24.09	**<0.001**	0.601
Symbiotic State (S)	0.10	2	0.05	0.01	0.991	0.001
L x S	2.46	2	1.23	0.24	0.788	0.029
Error	81.20	16	5.08			
Trial (T)	212.60	2	106.30	19.03	**<0.001**	0.543
T x L	7.54	2	3.77	0.67	0.517	0.040
T x S	4.78	4	1.20	0.21	0.929	0.026
T x L x S	20.10	4	5.03	0.90	0.476	0.101
Error	178.77	32	5.59			
Absorption efficiency						
Light (L)	0.14	1	0.14	4.31	0.054	0.212
Symbiotic State (S)	0.02	2	0.01	0.32	0.732	0.038
L x S	0.01	2	0.01	0.22	0.804	0.027
Error	0.50	16	0.03			
Trial (T)	0.19	1.9	0.10	3.69	**0.039**	0.187
T x L	0.09	1.9	0.05	1.70	0.201	0.096
T x S	0.08	3.8	0.02	0.82	0.516	0.093
T x L x S	0.05	3.8	0.01	0.46	0.756	0.054
Error	0.80	30.4	0.03			

B2. Two-way analysis of variance for tentacle adhesive force basitrich^{-1} and number of basitrichs discharged.

Parameter	Sum of Squares	df	Mean Square	F	p	Partial η^2
Adhesive Force						
Light (L)	0.01	1	0.01	0.39	0.542	0.022
Symbiotic State (S)	0.09	2	0.04	1.59	0.234	0.157
S x L	0.23	2	0.01	0.41	0.668	0.046
Error	0.46	17	0.03			
Basitrichs discharged						
Light (L)	74731	1	74731	2.42	0.138	0.125
Symbiotic State (S)	335588	2	167794	5.44	**0.015**	0.390
S x L	89124	2	44562	1.44	0.264	0.145
Error	524835	17	30872			

B3: Two-way nested analysis of covariance for cnida size including length and width of both basitrichs and spirocysts. Basitrich length data were log transformed ($\alpha = 0.025$). Individual variances from multiple spirocyst length measurements were not significant ($p > 0.05$) and were pooled.

Parameter	Sum of Squares	df	Mean Square	F	p	Partial η^2
Basitrich length						
Light (L)	<0.001	1	<0.001	0.17	0.685	0.008
Error	0.045	20	0.002			
Symbiotic State (S)	0.008	2	0.004	1.70	0.208	0.145
Error	0.045	20	0.002			
L x S	0.001	2	0.001	0.32	0.734	0.031
Error	0.045	20	0.002			
Individual	0.045	20	0.002	2.01	**0.022**	0.437
Error	0.058	52	0.001			
Basitrich width						
Light (L)	0.100	1	0.100	0.04	0.846	0.002
Error	5.105	20	0.255			
Symbiotic State (S)	1.217	2	0.608	2.38	0.118	0.192
Error	5.105	20	0.255			
L x S	0.025	2	0.120	0.05	0.953	0.005
Error	5.105	20	0.255			
Individual	5.105	20	0.255	2.567	**0.003**	0.497
Error	5.170	52	0.099			
Spirocyst length						
Total Protein	18.15	1	18.15	1.97	0.165	0.027
Light (L)	58.10	1	58.10	6.31	**0.014**	0.082
Symbiotic State (S)	53.30	2	26.65	2.89	0.062	0.075
L x S	10.45	2	5.22	0.57	0.570	0.016
Error	654.03	71	9.21			
Spirocyst width						
Light (L)	0.000	1	0.000	0.000	0.983	0.000
Error	5.972	20	0.299			
Symbiotic State (S)	0.475	2	0.237	0.795	0.466	0.074
Error	5.972	20	0.299			
L x S	0.114	2	0.057	0.190	0.828	0.019
Error	5.972	20	0.299			
Individual	5.972	20	0.299	2.022	**0.022**	0.437
Error	7.680	52	0.148			

B4. Two-way analysis of variance for basitrich and spirocyst density mg protein anemone^{-1}. Spirocyst data were square root transformed ($\alpha = 0.025$).

Parameter	Sum of Squares	df	Mean Square	F	p	Partial η^2
Basitrich density						
Light (L)	185.40	1	185.40	5.03	**0.036**	0.201
Symbiotic State (S)	112.31	2	56.15	1.52	0.242	0.132
L x S	52.65	2	26.33	0.72	0.501	0.067
Error	736.77	20	36.84			
Spirocyst density						
Light (L)	16.94	1	16.94	10.32	**0.004**	0.340
Symbiotic State (S)	7.34	2	7.34	2.24	0.133	0.183
L x S	0.26	2	0.26	0.08	0.925	0.008
Error	32.82	20	32.82			