Contributions of photoautotrophy and heterotrophy to the carbon and nitrogen nutrition of *Anthopleura elegantissima* in three symbiotic states

by
Kathryn Anne Hampton-Wonder

A THESIS

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Virginia Weis

The Pacific coast sea anemone *Anthopleura elegantissima* is an excellent model organism for the study of temperate symbiosis due to its unique relationship with two microalgal symbionts, *Elliptochloris marina* and *Symbiodinium* spp. In addition to hosting one or both of these symbionts, *A. elegantissima* can live aposymbiotically, allowing for the comparison of nutritional compositions of hosts with different symbionts. This study used stable isotope analysis to quantify the photoautotrophic contributions by symbionts and heterotrophic contributions by hosts. Algal, anemone-only, and algae+anemone samples were isolated from *A. elegantissima* symbiotic with *E. marina*, *Symbiodinium* spp., or anemones lacking symbionts. Samples were analyzed for $\delta^{15}N$ and $\delta^{13}C$ in the OSU Stable Isotope Laboratory. Isotope analysis showed that symbiotic anemone-only and algae+anemone samples were very close to the high $\delta^{15}N$ and $\delta^{13}C$ values of aposymbiotic samples, while algal samples were much lower. These results indicate that symbiotic anemones are relying primarily on heterotrophic nutrition, and receiving little-to-no benefit from hosting either *E. marina* or *Symbiodinium* spp.

Key Words: *Anthopleura elegantissima, Elliptochoris marina, Symbiodinium,* stable isotope

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Kathryn Anne Hampton-Wonder, Author

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1. Introduction

1.1 Symbiosis

The relationship between corals and their microalgal symbionts, *Symbiodinium* spp., is instrumental in allowing corals to thrive in nutrient-poor environments (Bates et al. 2010; Muscatine and Porter 1977). However, this relationship is threatened by rising ocean temperatures and ocean acidification among other factors. The breakdown of this relationship, known as coral bleaching, has led to intensive studies of cnidarian-microalgal symbioses in hope of advancing our understanding of their ability to adapt to a changing environment.

While the majority of well-known cnidarian-microalgal symbioses are tropical, temperate symbioses are both abundant and ecologically important (Bates et al. 2010; Muller-Parker and Davy 2001). They are typically facultative and characterized by their ability to withstand large fluctuations in irradiance, temperature, and nutrients (Muller-Parker and Davy 2001; Bergschneider and Muller-Parker 2008).

Irradiance is the most important variable governing the productivity of these symbionts as it directly limits the rate of photosynthesis (Muller-Parker and Davy 2001; Bates et al. 2010). Temperate irradiance is highly variable—in the Pacific Northwest measured irradiance fluxes are up to 6.5X greater in the summer compared to the winter (Muller-Parker and Davy 2001). Thus, photosynthetic symbionts in temperate symbioses exhibit lower rates of photosynthesis than photosynthetic tropical symbionts (Muller-Parker and Davy 2001). Despite this, temperate systems do not behave as though they are light limited—one would expect these symbioses to exist only in high light areas close to the surface, yet many do not appear depth restricted (Muller-Parker and Davy 2001). This observation supports the idea that temperate hosts are not as reliant on their symbionts for nutrition as their tropical counterparts.

Coupled with differences in irradiance come differences in temperature and nutrient availability. Tropical symbioses thrive in a warm, temperature-stable environment, while their temperate counterparts cope with lower average ocean temperatures as well as seasonal and diurnal fluctuations in temperature (Muller-Parker and Davy 2001). In addition, tropical oceans are uniformly nutrient-poor, while

temperate seas experience seasonal fluctuations in organisms such as zooplankton, consumed by heterotrophic cnidarians (Harrison et al. 1983). Summertime peaks in sources of heterotrophic nutrition coincide with peaks in photoautotrophic contributions from symbionts, contrasting to wintertime conditions when both food sources fall to low levels (Muller-Parker and Davy 2001). Despite the variability inherent in temperate ecosystems, temperate symbioses appear well-able to handle the changing conditions without undue stress (Bates et al. 2010; Muller-Parker and Davy 2001). This makes temperate systems excellent for studying how environmental variation affects both the microbial symbionts and their cnidarian hosts (Engebretson and Muller-Parker 1999).

1.2 Anthopleura elegantissima

A key organism for studying temperate symbioses is the intertidal sea anemone *Anthopleura elegantissima*. *A. elegantissima* is a small, clonal anemone abundant in the Pacific Northwest. It occurs continuously from south-east Alaska to central California, and exists in patches into Baja California (Secord and Augustine 2000). In extremely low light environments, such as caves, *A. elegantissima* can be found living aposymbiotically and obtaining all nutrition from heterotrophic feeding (Secord and Augustine 2000).

While *A. elegantissima* can acquire photoautotrophic nutrition when in symbiosis, heterotrophic contributions by the anemone appear to be important sources of overall nutrition, especially during conditions that are unfavorable for symbiosis (Engebretson and Muller-Parker 1999; Bergschneider and Muller-Parker 2008; Muller-Parker and Davy 2001). Engbretson and Muller-Parker (1999) suggest that symbiotic anemones gain the majority of their nutrition heterotrophically—experimental symbiotic anemones gained weight based on whether or not they were fed regardless of the light conditions they were kept in. It is also hypothesized that when symbiotic cnidarians experience decreases in photosynthetically-derived nutrition, they will increase their rate of heterotrophy (Ferrier-Pagès et al. 2011).

In order to acquire photoautotrophic nutrition, *A. elegantissima* forms a stable symbiosis with one or both of two algal symbionts. The first symbiont, *Symbiodinium* spp., is a photosynthetic dinoflagellate found widely throughout marine symbioses. The second symbiont is the recently-identified chlorophyte *Elliptochloris marina* (Letsch et al. 2009). *A. elegantissima* can thus live in four unique states: as aposymbiotic individuals, or symbiotic with *E. marina*, *Symbiodinium*, or both. This makes *A. elegantissima* an ideal model organism for studying temperate symbioses, as the ability to host multiple symbionts allows for comparisons of how symbiont identity may affect the nature of the symbiosis (Bates et al. 2010). Additionally, it has been suggested that hosting a combination of symbionts with different physiologies may confer a benefit to temperate hosts living in variable environments (Bates et al. 2010).

1.3 Symbionts: Elliptochloris marina *and* Symbiodinium *spp*.

The most common microalgal symbiont of *A. elegantissima* are the dinoflagellate *Symbiodinium* spp. (Bates et al. 2010). *A. elegantissima* containing *Symbiodinium* spp. are abundant in areas of high irradiance and temperature, and *Symbiodinium* spp. are the exclusive symbionts in *A. elegantissima* populations south of 43° (Engebretson and Muller-Parker 1999; Secord and Augustine 2000). In contrast, *E. marina*-containing anemones predominate in the northern portion of *A. elegantissima*'s range, increasing in low intertidal habitats where irradiance is lower (Secord and Augustine 2000; Engebretson and Muller-Parker 1999). This distribution mirrors the conditions that favor each symbiont –*Symbiodinium* spp. has been shown to increase its photosynthetic efficiency under conditions of high light and temperature, while *E. marina* flourishes in lower light and temperature (Saunders and Muller-Parker 1997; Muller-Parker and Davy 2001; Verde and McCloskey 2002; Bergschneider and Muller-Parker 2008)

Analysis of photosynthetic efficiency using carbon flux models indicate that *Symbiodinium* spp. can transfer more photosynthetic product, typically as glycerol, than *E. marina* under all conditions—making *Symbiodinium* spp. the more favorable symbiont in carbon limited organisms (Verde and McCloskey2007; Bates et al. 2010; Engebretson

and Muller-Parker 1999; Trench 1971). Multiple studies have shown a variety of non-glycerol products transferred from symbiont to host, including lipids and various amino acids. (Maier et al. 2010; Engebretson and Muller-Parker 1999). Factors other than nutrition may promote the fitness of *E. marina* containing anemones—sculpins, for example, appear to selectively prey on *Symbiodinium*-containing anemones, which would give *E. marina*-containing anemones an advantage (Augustine and Muller-Parker 1998).

1.4 Sources of Nutrients

Since *Symbiodinium* spp. and *E. marina* differ in their physiologies, it is of interest to assess the nutritional contributions of each symbiont type to its host. Photoautotrophic contributions of carbon require that carbon dioxide from the surrounding seawater be first transferred to symbionts for use in photosynthesis. Photosynthate is then used for symbiont metabolism or transferred to *A. elegantissima*. Inorganic nitrogen acquisition is less well-understood. Cnidarians are known to take up dissolved inorganic nitrogen (DIN) from seawater, which can then be converted into organic forms by the symbiont (Maier, Weinbauer, and Patzold 2010; Pernice et al. 2012). It is also believed that many cnidarians will "recycle" used metabolic nitrogen rather than excreting it, which would be useful in waters that are very low in DIN (Ferrier-Pagès et al. 2011; Wilkerson and Muscatine 1984). *A. elegantissima* thus has multiple sources for acquiring carbon and nitrogen.

1.5 Stable Isotopes

Stable isotope analysis is an excellent tool for determining the contributions of heterotrophy and photoautotrophy to overall host nutrition in different symbiotic states, by comparing carbon and nitrogen isotope ratios from the symbionts and host. Fractionation, the separation of isotopes based on their differing weights, occurs through the biochemical pathways used to transform molecules and varies between organisms depending on their physiologies (Fry 2006). The fractionation of isotopes

serves to enrich tissues in either lighter or heavier isotopes and leads to distinctive signatures which can be used to assess nutritional sources in organisms (Maier, Weinbauer, and Patzold 2010; Fry 2006). Stable isotopes are measured through the use of a mass spectrometer. Values are reported using δ notation, which uses the ratio of heavy to light isotope in the sample divided by the ratio of heavy to light isotope in an international standard (Fry 2006). A lower or more negative δ value means that the sample is enriched in the lighter isotope and a high δ value indicates an isotopically heavy sample.

In pathways that are predominated by kinetic reactions, such as photosynthesis, lighter isotopes react faster than heavier ones. Overall fractionation by ribulose bisphosphate carboxylase/oxygenase (Rubsico) in photosynthesis results in lower $\delta^{13}C$ values in the products compared to the source molecules (Bergschneider and Muller-Parker 2008; Kürten et al. 2014). The degree of enrichment depends on the precise enzymes used in the reactions—for example, Bergschneider and Muller-Parker (2008) showed that Form II Rubisco, found in *Symbiodinium* spp., is less discriminatory towards ^{13}C than Form I Rubisco, found in *E. marina*. Thus *Symbiodinium* spp. is expected to have a higher $\delta^{13}C$ value than *E. marina*.

Analysis of $\delta^{13}C$ is especially useful in determining the sources of metabolic energy for symbiont and host. Heterotrophic feeding by the host typically results in less kinetic fractionation than in photosynthesis, and causes a $\delta^{13}C$ value similar to that of food sources (Muscatine, Porter, and Kaplan 1989). Heikoop et al (2000) showed that this relationship holds true in corals, with heterotrophic corals having $\delta^{13}C$ values close to those of food sources while autotrophic corals reflected the $\delta^{13}C$ of their symbionts.

In contrast to carbon metabolism, nitrogen metabolism is dominated by exchange reactions. Lighter isotopes of nitrogen form weaker bonds causing amine groups containing ^{14}N to be favored in transamination and deamination during amino acid synthesis (Adams and Sterner 2000; Gannes, del Rio, and Koch 1998). This results in the organism having a higher $\delta^{15}N$ value than its nitrogen source. $\delta^{15}N$ values have been shown to increase by approximately 3.4‰ as trophic level increases (Adams and Sterner

2000; Minagawa and Wada 1984). However, limitations in source nitrogen can lower this value—if an organism is highly nitrogen-limited all nitrogen in the source will be converted to the product, resulting in a net increase of 0‰ (Maier, Weinbauer, and Patzold 2010; Adams and Sterner 2000).

1.6 Purpose and Predictions

The goal of this study was to examine the contributions of *Symbiodinium* spp. and *E. marina* symbionts to *A. elegantissima* nutrition. By analyzing the stable isotope signatures of *Symbiodinium*, *E. marina*, and anemone tissue from *A. elegantissima* in each symbiotic state I compared the relative contributions of each symbiont to its host. It was expected that *Symbiodinium*-containing anemones would have low $\delta^{13}C$ values close to that of *Symbiodinium*-algal samples, while *E. marina*-containing anemones would have higher $\delta^{13}C$ *values. E. marina*-algal $\delta^{13}C$ values were expected to be the lowest, followed by *Symbiodinium*-algal values, since both symbionts acquire their carbon photosynthetically. Aposymbiotic anemone tissue was predicted to have the highest $\delta^{13}C$ of all the samples since these anemones feed entirely heterotrophically. Since $\delta^{15}N$ tends to reflect trophic level, it was expected that all anemone samples will have high values, while both symbionts would show much lower values. Analysis of the differing contributions of symbiont to host gave a better understanding of how symbiont identity affects overall host nutrition, and allowed for predictions of how these relationships will change in the future.

2. Methods

2.1 Anemone Collection and Preparation

Anthopleura elegantissima were collected on October 25th, 2014 from Boiler Bay, Oregon (44°49’ N, 124°03’ W). Symbiont identity was assessed visually—*Elliptochloris marina*-containing anemones were green, *Symbiodinium*-containing anemones brown, and aposymbiotic anemones white. Anemones were brought back to Oregon State University and cut in half from oral to aboral end, labeled, and frozen at -80°C. The three

largest halves of three aposymbiotic, three *E. marina*-containing, and three *Symbiodinium*-containing anemones were chosen for this experiment.

2.2 Symbiont Density Quantification

A diagonal slice from the middle of the oral cavity to the outer trunk column was taken from each anemone for algal cell counts and protein analysis. Slices were homogenized in 100-400µl cold DH_2O using plastic pestles. Cells were counted on a Brightline hemocytometer in triplicate. Cell density was then calculated by dividing the average cell counts by the volume of solution counted in one replication on the hemocytometer (0.4 µl). Remaining homogenate was analyzed for anemone protein content using a Bradford Protein Assay. Anemone protein was isolated by centrifugation at 1600 RPM for one minute. Cell density was indexed to anemone protein to obtain the number of symbionts per milligram protein.

2.3 Anemone Preparation for Stable Isotope Analysis

Each anemone was finely chopped with a razor-blade, large particles of grit were removed, and anemones were homogenized in 6ml cold DH_2O using a 30 ml glass tissue grinder and Teflon pestle (Bergschneider and Muller-Parker 2008). The homogenate was poured into a 15 ml tube, and the tissue grinder and pestle were rinsed with 3 ml cold DH_2O. The rinsate was added to the 15 ml tube to give a total homogenate volume of 9ml. 3 ml of algae+anemone homogenate was aliquoted and set aside.

Separation of the algae and anemone fractions from the homogenate was achieved through repeated centrifugation. The homogenate was centrifuged at 1600 RPM for one minute. The resulting supernatant contained anemone homogenate only and was decanted into a 50 ml tube and reserved. The pellet was then resuspended in 2 ml cold DH_2O and centrifuged at 1600 RPM for one minute. The supernatant was decanted into the anemone-only tube. This re-suspension process was repeated three times for a total of four washes. After the final wash, the pellet was assessed to see if a visible white anemone layer remained beneath the algal layer. If so, the pellet was

resuspended in 2 ml cold DH_2O and centrifuged at 100 RPM for three minutes to separate the anemone and algae. The resulting supernatant was largely free of host material and was pipetted out without disturbing the loose anemone pellet. This process was repeated for each of the nine anemones.

Each sample was visually inspected under a compound microscope and photographed to verify and document the separation of anemone and algal samples compared to their algae+anemone combined counterpart. Samples showed good separation between algae-only and anemone-only fractions compared to the algae+anemone fraction (Fig. 1).

Samples were vacuum-filtered onto Pall 47mm glass fiber filter type A/E, 1µm particle retention (Inga Conti-Jerpe 2014). Filters were pre-combusted at 550°C for 24 hours and pre-weighed (Kürten et al. 2014). Sample filters were allowed to dry on the vacuum filter for 10 minutes, then re-weighed in order to determine sample mass. Samples were then wrapped in tinfoil and placed into a 50°C chamber to dry further. Approximate weight of carbon in the samples was calculated as half of the total weight.

After drying overnight, filters were trimmed to remove excess filter without losing sample material. Samples with estimated weights of carbon less than 2.7 mg were prepared as follows: The percentage of the filter area needed to obtain approximately 0.8-0.9 mg of sample material was calculated and cut from the remainder. Filters were then wrapped into tin capsules. Samples with estimated carbon weight greater than 2.7 mg were scraped with a metal spatula to remove material from the filter. The resulting material was weighed on a microbalance to have a mass of approximately 0.8-0.9 mg, then wrapped in a tin capsule.

Wrapped samples were sent to the OSU Stable Isotope Laboratory for ^{13}C and ^{15}N analysis using an EA Delta Plus Isotope Ratio Mass Spectrometer. Samples were standardized using the international standard IAEA-600 (caffeine) against Vienna PeeDee Belemnite (VPDB). Results were calibrated against VPDB using two international references and one internal lab reference (USGS40, IAEA-N2, and SIL Sucrose, respectively) included in each run. Repeat analysis of references shows precision at

±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$. Isotope ratios were expressed using standard δ notation of the ratio of heavy to light isotope, following the equation:

$$\delta X‰ = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 10^3$$

X: ^{13}C or ^{15}N

R: Ratio of ($^{13}C/^{12}C$) or ($^{15}N/^{14}N$) of the sample or the standard

2.4 Statistical Analysis

Stable Isotope data were compared to individual anemone ratios of algal cells to anemone protein using calculated cells/mg protein. Data was then analyzed using two-way ANOVAs and post hoc Tukey tests on $\delta^{13}C$ and $\delta^{15}N$ to compare the effects of sample types. No outliers were present and the normality condition was well met for both carbon and nitrogen data. Fitted residuals of both carbon and nitrogen data showed variations in variance that are attributed to the small sample sizes. All analysis was completed using R statistical software.

3. Results

3.1 Symbiont Density across Symbiotic States

Comparisons of each anemone's algal cell to protein ratios showed that anemones symbiotic with *E. marina* had on average more algal cells/mg compared to anemones symbiotic with *Symbiodinium* spp. However, this is influenced greatly by anemone BB2g13, which had 5,711 more cells/mg protein than the next highest *Symbiodinium*-containing anemone (Fig. 2). Aposymbiotic anemones had close to zero cells/mg protein in all cases (Fig. 2).

3.2 Stable Isotope $\delta^{13}C$ and $\delta^{15}N$

The distribution of average $\delta^{13}C$ and $\delta^{15}N$ for separated algal, anemone-only, and algae+anemone fractions of *E. marina*, *Symbiodinium*, and aposymbiotic samples, as well as an outside comparison from a heterotrophic mussel, *Mytilus* sp. (supplied by Weis Lab), is shown in Figure 3. $\delta^{13}C$ varied across samples, with *E. marina* algal samples

being much lower than their anemone-only and algae+anemone counterparts. *E. marina* showed the lowest $\delta^{13}C$ at –23.8‰, followed by an *E. marina* algae+anemone sample at -20.1‰ and *Symbiodinium* algae (-19.84‰; Table 1). The *E. marina* algal samples also had the greatest variability of $\delta^{13}C$ values, with a difference of 2.21‰ between the highest and lowest values (Table 1). Aposymbiotic algal samples had the highest carbon signatures (-17.6‰), and there were no significant differences between any aposymbiotic fractions or symbiotic anemone-only and algae+anemone fractions ($p=0.61$, Table 1). $\delta^{15}N$ values also varied across samples, with algal samples being uniformly lower than their anemone-only and algae+anemone counterparts (Fig. 3). *Symbiodinium* algal $\delta^{15}N$ values were the lowest at 4.09‰, significantly lower than the next lowest *E. marina* algal values at 6.49‰ ($p=1.23x10^{-4}$, Table 1). As expected, the aposymbiotic samples had the highest $\delta^{15}N$ values, but there were only small differences between the aposymbiotic, *Symbiodinium* anemone-only, *E. marina* anemone-only, and *E. marina* algae+anemone samples (Table 1). The *Symbiodinium* algae+anemone sample did show a lowered nitrogen signature compared to its anemone-only counterpart (Table 1; Fig 3).

4. Discussion

4.1 Contribution of Heterotrophy to Host Nutrition

It has been shown that *A. elegantissima* hosts *E. marina* at higher densities than *Symbiodinium* spp., potentially due to the smaller size of *E. marina* and its lower photosynthetic productivity compared to *Symbiodinium* spp. (Verde and McCloskey 1996; Engebretson and Muller-Parker 1999). Average cells per milligram anemone protein reflected this, but the average for *E. marina*- containing anemones was greatly increased by the outlier BBg13. With the exception of this individual, both *Symbiodinium*-containing anemones and *E. marina*-containing anemones had similar densities of algal cells per milligram anemone protein (Fig. 2). Comparisons of cell counts confirmed that the classification of anemones as aposymbiotic, *Symbiodinium*-

containing, or *E. marina*-containing based on visual inspection was reflected in the type and amount of symbionts counted.

Stable isotope analysis of ^{13}C showed distinct differences in values for isolated *E. marina* and *Symbiodinium* spp. algal samples. Both symbionts showed very negative values, as is expected from the photoautotrophic fractionation of carbon (Fry 2006). The carbon values obtained here closely matched carbon values obtained by Bergschneider and Muller-Parker (2008) during their October sampling for *E. marina* and *Symbiodinium* spp. isolated from *A. elegantissima* in Washington. *E. marina* was significantly more negative than *Symbiodinium spp.*, which can be explained in part by the differences between Form I and Form II Rubisco. Form I Rubisco, which is present in *E. marina*, is much more discriminatory towards ^{13}C than *Symbiodinium*'s Form II Rubisco (Bergschneider and Muller-Parker 2008). This difference results in *E. marina* having greater fractionation of carbon during the photosynthesis. In addition, the difference in carbon signatures between these two algae could lie in their different carbon needs. *Symbiodinium* spp. has been shown to be much more productive than *E. marina*, and thus may be carbon limited (Engebretson and Muller-Parker 1999; Verde and McCloskey 1996). Carbon limitations lower carbon fractionation, causing carbon values to be more positive. Compared to *Symbiodinium* spp., *E. marina* is less productive, and so has a relative abundance of carbon, making its $\delta^{13}C$ more negative.

While algal carbon signatures were very negative, the symbiotic anemone-only and algae+anemone carbon values were higher and much closer to the values of the pure heterotrophs: the aposymbiotic anemones and *Mytilus* sp. The lack of variation between symbiotic anemone-only and aposymbiotic anemone-only signatures supports the idea that these animals, whether symbiotic or not, are deriving the vast majority of their nutrition through heterotrophic feeding. The *Symbiodinium* algae+anemone samples were slightly more negative than their counterpart anemone-only samples, indicating that *Symbiodinium* spp. may be contributing a small amount of carbon to the host. This contrasts with the findings of Bergschneider and Muller-Parker (2008), who found differences between their symbiotic anemones carbon values and those of their

aposymbiotic anemones. The discrepancy between the data found here and the Bergschneider and Muller-Parker findings could be due to the fact that Bergschneider and Muller-Parker compared combined algae+anemone signatures to aposymbiotic signatures. My methodology isolated both the algal fraction and the anemone fraction, allowing me to compare algae, algae+anemone, and anemone-only to aposymbiotic anemones.

Nitrogen isotope analysis of symbiotic and aposymbiotic samples show uniformly high $\delta^{15}N$ values across symbiotic anemone and algae+anemone samples, aposymbiotic samples, and the mussels. This indicates that all of these animals are receiving sources of nitrogen with similar $\delta^{15}N$ signatures—in this case, all are likely obtaining nitrogen heterotrophically from benthic invertebrates and zooplankton (Sebens 1981). In contrast, the $\delta^{15}N$ nitrogen values were markedly lower for both algal types, with *E. marina* averaging 4.34‰ below its anemone-only value and *Symbiodinium* at 7.18‰ below its anemone-only value (Table 1). It was expected that algal nitrogen values would be lower than their anemone and algae+anemone counterparts because $\delta^{15}N$ values rise as trophic level increases (Minagawa and Wada 1984; Adams and Sterner 2000). Both *E. marina* and *Symbiodinium* are primary producers, while *A. elegantissima* is a heterotroph. However, *Symbiodinium* had a significantly lower $\delta^{15}N$ value than *E. marina* (3.41‰; Table 1). The average *Symbiodinium* algae value was 4.23‰, very close to 5‰, the average $\delta^{15}N$ of seawater (Table 1; Dahnke and Thamdrup 2013). Since it is believed that the DIN in seawater is a major nitrogen source for photoautotrophic symbionts, the low algal signature of *Symbiodinium* could indicate that it is extremely nitrogen limited and is taking up not only DIN but isotopically light waste nitrogen excreted from the host (Pernice et al. 2012; Wilkerson and Muscatine 1984). Conversely, the heightened signature of *E. marina* algal samples (7.64‰) indicates that this symbiont is not nitrogen limited, allowing fractionation to readily occur (Table 1).

The stable isotope data from this study confirm that *A. elegantissima* is acquiring the majority of its nutrition heterotrophically and is not dependent upon its symbionts for nutrition. The differences between anemone and algae nitrogen and carbon for the

symbiont types suggest that *Symbiodinium* spp. confer some nutritional benefit to the host, while *E. marina* does not appear to benefit the host. The trends in isotopic signatures we see in this study fit with the findings of Bergschneider and Muller-Parker (2008). The similarity of the data from anemones in Washington and Oregon suggests that these trends may be present in multiple populations of *A. elegantissima*. Future studies of *A. elegantissima* populations elsewhere will be required to confirm this hypothesis, and will lead to a greater understanding of whether *A. elegantissima* receives any benefit from hosting *Symbiodinium* spp. or *E. marina*.

References

Adams, T. S., and R. W. Sterner. 2000. The effect of dietary nitrogen content on trophic level 15N enrichment. *Limnology and Oceanography* 45 (3): 601–7. doi:10.4319/lo.2000.45.3.0601.

Augustine, L., and G. Muller-Parker. 1998. Selective predation by the mosshead sculpin *Clinocottus globiceps* on the sea anemone *Anthopleura elegantissima* and its two algal symbionts. *Limnology and Oceanography* 43 (4): 711–15. doi:10.4319/lo.1998.43.4.0711.

Bates, A. E., L. Mclean, P. Laing, L. A. Raeburn, and C. Hare. 2010. Distribution patterns of zoochlorellae and zooxanthellae hosted by two Pacific northeast anemones, *Anthopleura elegantissima* and *A. xanthogrammica*. *The Biological Bulletin* 218 (3): 237–47.

Bergschneider, H., and G. Muller-Parker. 2008. Nutritional role of two algal symbionts in the temperate sea anemone *Anthopleura elegantissima* Brandt. *Biological Bulletin* 215 (1): 73–88. doi:10.2307/25470685.

Conti-Jerpe, I. 2014. Whipping up dinner: The heterotrophic feeding of 3 temperate Atlantic gorgonian octocorals. Masters Thesis, University of North Carolina Wilmington.

Dahnke, K. and B. Thamdrup. 2013. Nitrogen isotope dynamics and fractionation during sedimentary denitrification in Boknis Eck, Baltic Sea. *Biogeosciences* 10: 3079-3088.

Engebretson, H. P. and G. Muller-Parker. 1999. Translocation of photosynthetic carbon from two algal symbionts to the sea anemone *Anthopleura elegantissima*. *Biological Bulletin* 197 (1): 72–81. doi:10.2307/1542998.

Ferrier-Pagès, C., A. Peirano, M. Abbate, S. Cocito, A. Negri, C. Rottier, P. Riera, R. Rodolfo-Metalpa, and S. Reynaud. 2011. Summer autotrophy and winter heterotrophy in the temperate symbiotic coral *Cladocora caespitosa*. *Limnology and Oceanography* 56 (4): 1429–38. doi:10.4319/lo.2011.56.4.1429.

Fry, B. 2006. *Stable Isotope Ecology*. New York, NY: Springer Science & Buisness Media, LLC.

Gannes, L. Z., C. M. del Rio, and P. Koch. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 119 (3): 725–37. doi:10.1016/S1095-6433(98)01016-2.

Harrison, P. J., J. D. Fulton, F. J. R. Taylor, and T. R. Parsons. 1983. Review of the biological oceanography of the Strait of Georgia: pelagic environment. *Canadian Journal of Fisheries and Aquatic Sciences* 40 (7): 1064–94. doi:10.1139/f83-129.

Heikoop, J. M., J. J. Dunn, M. J. Risk, T. Tomascik, H. P. Schwarcz, I. M. Sandeman, and P. W. Sammarco. 2000. δ15 N and δ13 C of coral tissue show significant inter-reef variation. *Coral Reefs* 19 (2): 189–93. doi:10.1007/s003380000092.

Kürten, B., A. M. Al-Aidaroos, U. Struck, H. S. Khomayis, W. Y. Gharbawi, and U. Sommer. 2014. Influence of environmental gradients on C and N stable isotope ratios in

coral reef biota of the Red Sea, Saudi Arabia. *Journal of Sea Research* 85 (January): 379–94. doi:10.1016/j.seares.2013.07.008.

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) 1. *Journal of Phycology* 45 (5): 1127–35. doi:10.1111/j.1529-8817.2009.00727.x.

Maier, C., M. Weinbauer, and J. Patzold. 2010. Stable isotopes reveal limitations in C and N assimilation in the Caribbean reef corals *Madracis auretenra, M. carmabi* and *M. Formosa. Marine Ecology Progress Series* 412: 103–12.

Minagawa, M. and E. Wada. 1984. Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ 15N and animal age. *Geochimica et Cosmochimica Acta* 48 (May): 1135–40. doi:10.1016/0016-7037(84)90204-7.

Muller-Parker, G. and S. K. Davy. 2001. Temperate and tropical algal-sea anemone symbioses. *Invertebrate Biology* 120 (2): 104–23.

Muscatine, L. and J. W. Porter. 1977. Reef corals: Mutualistic symbioses adapted to nutrient-poor environments. *BioScience* 27 (7): 454–60. doi:10.2307/1297526.

Muscatine, L., J. W. Porter, and I. R. Kaplan. 1989. Resource partitioning by reef corals as determined from stable isotope composition. *Marine Biology* 100 (2): 185–93. doi:10.1007/BF00391957.

Pernice, M., A. Meibom, A. Van Den Heuvel, C. Kopp, I. Domart-Coulon, O. Hoegh-Guldberg, and S. Dove. 2012. A single-cell view of ammonium assimilation in coral–dinoflagellate symbiosis. *The ISME Journal* 6 (7): 1314–24. doi:10.1038/ismej.2011.196.

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). *Journal of Experimental Marine Biology and Ecology* 211 (2): 213–24. doi:10.1016/S0022-0981(96)02723-2.

Sebens, K. P. 1981. The allometry of feeding, energetics, and body size in three sea anemone species. *The Biological Bulletin* 161 (1): 152–71.

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*. *Invertebrate Biology* 119 (2): 139–46.

Trench, R. K. 1971. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. I. The assimilation of photosynthetic products of zooxanthellae by two marine coelenterates. *Proceedings of the Royal Society of London B: Biological Sciences* 177 (1047): 225–35. doi:10.1098/rspb.1971.0024.

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). *Journal of Experimental Marine Biology and Ecology* 195 (2): 187–202. doi:10.1016/0022-0981(95)00080-1.

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). *Marine Biology* 141 (2): 225–39. doi:10.1007/s00227-002-0824-7.

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. *Marine Biology* 152 (4): 775–92. doi:10.1007/s00227-007-0737-6.

Wilkerson, F. P., and L. Muscatine. 1984. Uptake and assimilation of dissolved inorganic nitrogen by a symbiotic sea anemone. *Proceedings of the Royal Society of London B: Biological Sciences* 221 (1222): 71–86. doi:10.1098/rspb.1984.0023.

Tables and Figures

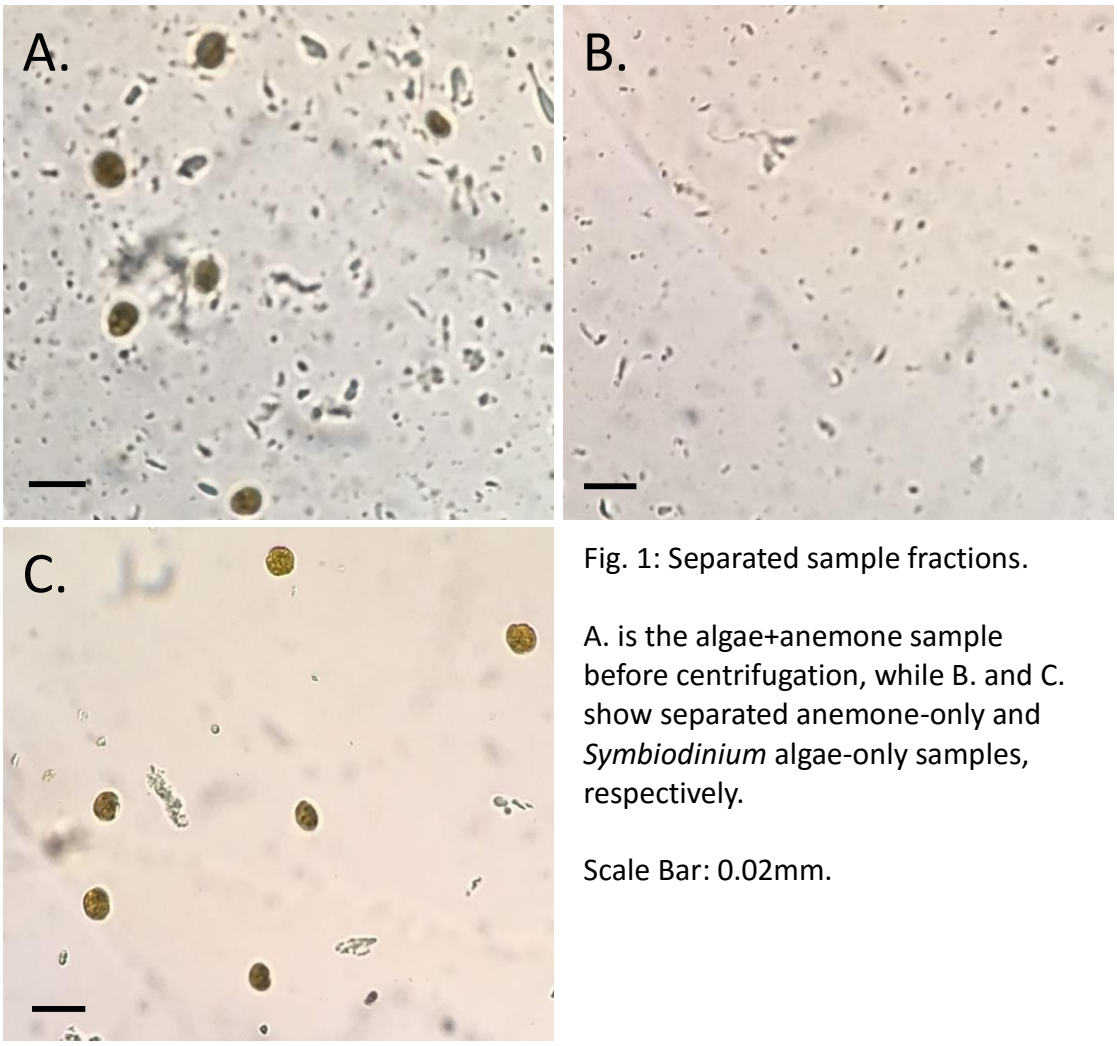

Fig. 1: Separated sample fractions.

A. is the algae+anemone sample before centrifugation, while B. and C. show separated anemone-only and *Symbiodinium* algae-only samples, respectively.

Scale Bar: 0.02mm.

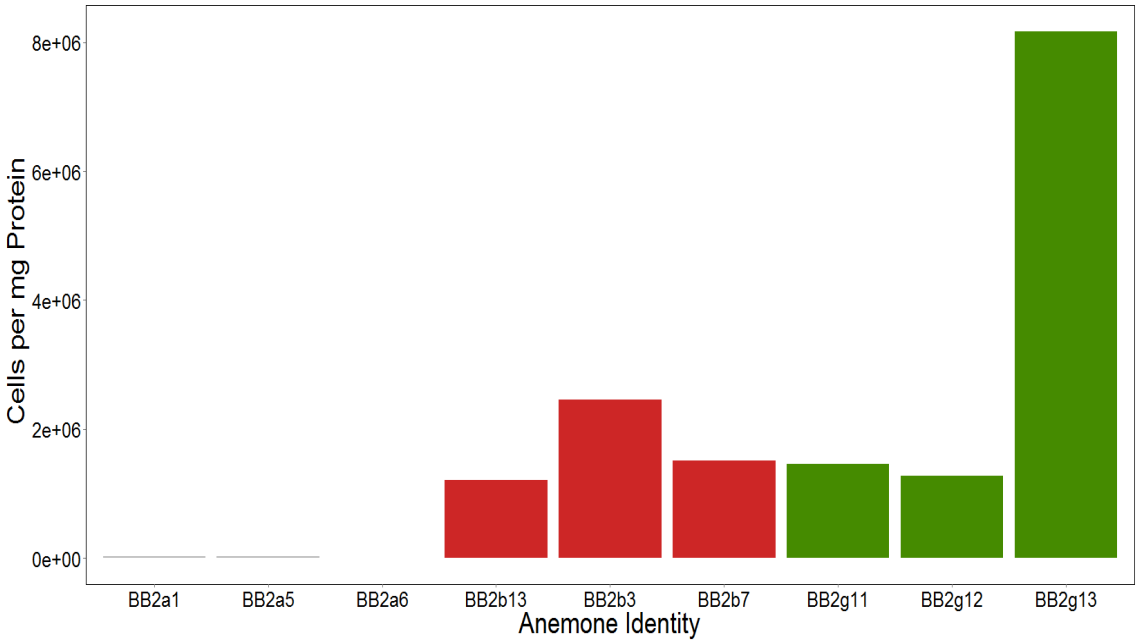

Fig. 2: Symbiont density in *A. elegantissima* with various symbionts. Grey, red and green bars indicate symbiont counts per mg anemone protein for aposymbiotic, *Symbiodinium*- containing, and *E. marina*-containing anemones respectively.

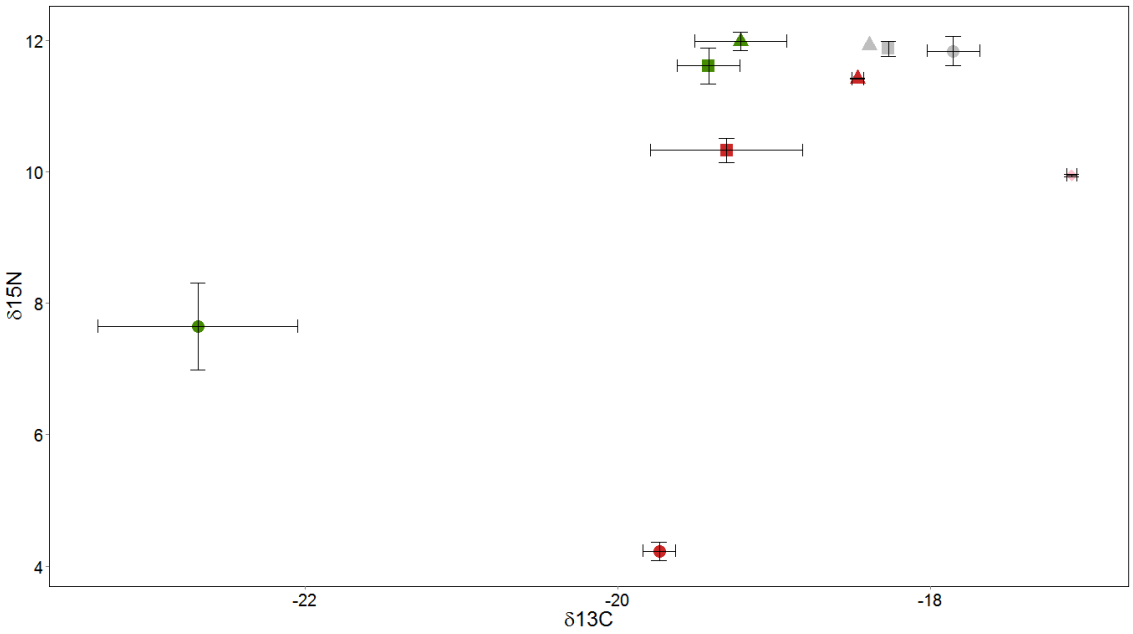

Fig. 3: Average $\delta^{15}N$ and $\delta^{13}C$ of algae, algae+anemone, and anemone samples from aposymbiotic, *E. marina* containing, and *Symbiodinium* containing anemones, as well as heterotrophic mussels *Mytilus spp*. Triangular points show anemone-only samples, while squares show algae+anemone samples and circles show algal samples. Colors show the sample type, with grey as aposymbiotic, green as *E. marina*, and red as *Symbiodinium*.

Table 1: Average $\delta^{13}C$ and $\delta^{15}N$ (±SE) and range for isolated algal samples, anemone-only samples, and algae+anemone samples from *Symbiodinium*-containing, *E. marina*-containing, and aposymbiotic anemones.

	$\delta^{13}C$			$\delta^{15}N$		
	Average	Range	n	Average	Range	n
Algae						
Aposymbiotic	−17.86±0.2264	−18.17-−17.6	3	11.84±0.2266	11.47-12.25	3
Symbiodinium spp.	−19.73±0.105	−19.84-−19.63	2	4.23±0.14	4.09-4.37	3
E. marina	−22.68±0.6381	−23.8-−21.59	3	7.64±0.6583	6.49-8.77	3
Anemone						
Aposymbiotic	−18.39±NA	NA	1	11.92±NA	NA	1
Symbiodinium-containing	−18.46±0.0377	−18.54-−18.43	3	11.41±0.0067	11.40-11.42	3
E. marina-containing	−19.22±0.295	−19.51-−18.92	2	11.98±0.14	11.84-12.12	2
Algae+anemone						
Aposymbiotic	−18.27±NA	NA	1	11.87±0.11	NA	1
Symbiodinium-containing	−19.06±0.3721	−19.79-−18.57	3	10.35±0.1105	10.14-10.41	3
E. marina-containing	−19.65±0.2544	−20.1-−19.22	3	11.50±0.1889	11.29-11.88	3

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