

2010

# The effects of season and microhabitat on the distribution and nutritional contributions of two algal symbionts in the intertidal anemone *Anthopleura xanthogrammica*

Michael R. (Michael Rory) Levine  
*Western Washington University*

Follow this and additional works at: <https://cedar.wwu.edu/wwuet>



Part of the [Marine Biology Commons](#)

---

## Recommended Citation

Levine, Michael R. (Michael Rory), "The effects of season and microhabitat on the distribution and nutritional contributions of two algal symbionts in the intertidal anemone *Anthopleura xanthogrammica*" (2010). *WWU Graduate School Collection*. 35.  
<https://cedar.wwu.edu/wwuet/35>

This Masters Thesis is brought to you for free and open access by the WWU Graduate and Undergraduate Scholarship at Western CEDAR. It has been accepted for inclusion in WWU Graduate School Collection by an authorized administrator of Western CEDAR. For more information, please contact [westerncedar@wwu.edu](mailto:westerncedar@wwu.edu).

**THE EFFECTS OF SEASON AND MICROHABITAT ON THE DISTRIBUTION  
AND NUTRITIONAL CONTRIBUTIONS OF TWO ALGAL SYMBIONTS IN  
THE INTERTIDAL ANEMONE *ANTHOPLLEURA XANTHOGRAMMICA***

by

Michael R. Levine

Accepted in Partial Completion  
of the Requirements for the Degree  
Master of Science

---

Moheb A. Ghali, Dean of the Graduate School

ADVISORY COMMITTEE

---

Chair, Dr. Gisèle Muller-Parker

---

Dr. Brian Bingham

---

Dr. Deborah A. Donovan

## **MASTER'S THESIS**

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Western Washington University, I grant Western Washington University the non-exclusive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms, including electronic format, via and digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe or violate any rights of others. I warrant that I have obtained written permissions from the owner of any third party copyrighted material included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, non-commercial reproduction of this work for educational research purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without my written permission.

Signature \_\_\_\_\_  
Date \_\_\_\_\_

**THE EFFECTS OF SEASON AND MICROHABITAT ON THE DISTRIBUTION  
AND NUTRITIONAL CONTRIBUTIONS OF TWO ALGAL SYMBIONTS IN  
THE INTERTIDAL ANEMONE *ANTHOPLLEURA XANTHOGRAMMICA***

A Thesis  
Presented to  
The Faculty of  
Western Washington University

In Partial Fulfillment  
Of the Requirements for the Degree  
Master of Science

by  
Michael R. Levine  
January 2010

**Abstract.** The intertidal sea anemone *Anthopleura xanthogrammica* is distributed widely along the Pacific coast, from Baja California Mexico (~30°N) to southern Alaska (57°N). In much of its range, *A. xanthogrammica* has the ability to co-host algal symbionts from two distinct taxa known as zoochlorellae (the chlorophyte *Elliptochloris marina*) and zooxanthellae (brown dinophytes in the genus *Symbiodinium*). Laboratory studies and field distributions have demonstrated that zoochlorellae and zooxanthellae represent “cool” and “warm” symbionts respectively, based on their relative temperature tolerances and intertidal distributions.

This study examined the effects of two intertidal microhabitats on the seasonal distribution, density, and mitotic index of zoochlorellae and zooxanthellae in tentacles of *A. xanthogrammica* at Slip Point, Clallam Bay WA (48°N). Tentacles were sampled from anemones at both the lower and upper intertidal limits of the distribution of *A. xanthogrammica* in tidepools and surge channels in July 2008, November 2008, and April 2009. Temperatures in these microhabitats were recorded with data loggers from summer 2008 to summer 2009. The surge channel microhabitat at Slip Point experienced more extreme temperatures (both higher high temperatures and lower low temperatures) than the tidepool. The distribution and density of zoochlorellae (but not of zooxanthellae) in *A. xanthogrammica* tentacles differed in these microhabitats. Anemone tentacles containing dense zoochlorellae assemblages predominated in low intertidal tidepools and low surge channels as well as in the high tidepool microhabitat; zoochlorellae density was much lower in the high surge channel. The density of zooxanthellae was low in all microhabitats, and was often nearly one order of magnitude lower than that of zoochlorellae. Patterns in symbiont density in anemones between microhabitats were consistent in all seasons. Comparatively

high temperatures in the high surge channel are the likely cause of the reduced density of zoochlorellae (and of the higher proportion of zooxanthellae) in this microhabitat. The distribution of zoochlorellae was not related to differences in algal growth rates alone, as there were no significant differences in the mitotic index of zoochlorellae from the high tidepool and the high surge channel algal populations, despite large differences in algal density of tentacles between these two habitats. Symbiont composition was stable seasonally, with more than 80% of anemone tentacles containing predominantly zoochlorellae in all seasons.

The relative contributions of zoochlorellae, mixed algal assemblages composed primarily of zoochlorellae, and of *Mytilus californianus* mussels (the dominant heterotrophic food source) to the diet of *A. xanthogrammica* were estimated using stable isotope analysis in summer 2008 and spring 2009. The carbon contributions of symbionts (~62-70%) were greater than those of external food sources (~31-38%) in both seasons. Zoochlorellae contribute substantially to *A. xanthogrammica* diet, based on analysis of tentacle samples.

*A. xanthogrammica* is proposed as an ideal model intertidal organism for following long-term biological responses to climate change. Because it is long lived and may host both “cool” and “warm” symbionts that are stable in response to seasonal temperature patterns, shifts in symbiont populations (monitored using non-lethal sampling of tentacles) in high intertidal anemones may be used to indicate long-term biological responses to changing thermal conditions.

## **Acknowledgements**

I thank Dr. Gisèle Muller-Parker for her expert help in planning research, her assistance in my field and lab work, and her endless patience and guidance in the preparation of presentations, proposals, and this thesis. I also thank Dr. Brian Bingham for his frequent statistical advice as well as willingness to include me in fieldwork, laboratory activities, and trips to scientific conferences throughout my research. Dr. Deborah Donovan has thoughtfully commented on my research from its inception, for which I am also thankful.

Without the assistance of Nate Schwarck, Gene McKeen, and Jude Apple at Shannon Point Marine Center and the Biology stockroom staff (especially Peter Thut), my research would not have been possible. Finally, I would like to thank my numerous field assistants: Gisèle Muller-Parker, Gabe Headley, Jay Dimond, Julie Conrad, Iliana Freytes, Angela Fletcher, Rachel Allee, Tyler Ritchie, and Michael Parker worked enthusiastically in challenging conditions to help collect my samples. This research was supported by grants from the National Science Foundation (NSF IOS 0822179 to Drs. Muller-Parker and Bingham), Western Washington Fund for the Enhancement of Graduate Research, and Sigma Xi Grants-in-Aid of research.

## TABLE OF CONTENTS

|                       |      |
|-----------------------|------|
| ABSTRACT              | iv   |
| ACKNOWLEDGEMENTS      | vi   |
| LIST OF FIGURES       | viii |
| LIST OF TABLES        | x    |
| INTRODUCTION          | 1    |
| MATERIALS AND METHODS | 8    |
| RESULTS               | 29   |
| DISCUSSION            | 55   |
| LITERATURE CITED      | 70   |



## List of Figures

- Figure 1.** Study site. **a)** Slip Point is located on the outer Strait of Juan de Fuca. **b)** All sampling was completed at Submarine Rock, an outcrop near Slip Point. Submarine Rock is an approximately 0.75 km walk from the Coast Guard beach access visible in the bottom left of picture. Images from Google Earth. 9
- Figure 2.** Schematic of Submarine Rock. Gray= water; white= rocky shore. TP= tidepool; SC= surge channel. Only tidepools and surge channels used for the seasonal anemone sampling are indicated (more tidepools and surge channels were present at Submarine Rock than are indicated). Diamonds represent the location of the tidepool and surge channel data loggers. 11
- Figure 3.** Monthly average seawater temperature ( $\pm$ SE) at Slip Pt, Clallam Bay WA recorded using in-situ temperatures data loggers and air temperature ( $\pm$ SE) at NOAA station NEAW1 for August 2008 to June 2009. Standard errors are an estimate of within-month variation. 30
- Figure 4.** Monthly temperature averages ( $\pm$ SE) in a tidepool (+1.59 m MLLW) and a surge channel (+1.19 m MLLW) at Slip Pt, Clallam Bay, WA. Uppermost lines represent average monthly high, middle lines represent average, and low lines represent average monthly low temperature within each microhabitat. Standard errors are an estimate of within-month variation. Arrows indicate anemone tentacle sampling dates. 31
- Figure 5.** Difference above (positive values) and below (negative values) daily seawater temperature in a tidepool (+1.59 m MLLW) and a surge channel (+1.19 m MLLW) at Slip Pt, Clallam Bay WA from 8/31/08-11/12/08. 34
- Figure 6.** Boxplots of percent zoochlorellae in **a)** high tidepool **b)** high surge channel **c)** low tidepool and **d)** low surge channel microhabitats. Black lines within a boxplot represents the median percent zoochlorellae, gray areas below and above the median represent the 25<sup>th</sup> percentile and 75<sup>th</sup> percentiles, lines below and above boxes represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles, and circles represent outliers. The 10<sup>th</sup> and 90<sup>th</sup> percentiles are only calculated for  $n \geq 9$ . 36
- Figure 7.** Histogram of percent zoochlorellae in tentacles of anemones sampled in each season (across all microhabitats). Bins encompass a 10% range; number of anemones within a bin should be interpreted as having percent zoochlorellae composition within 10%. Summer and spring N=50; fall N=25. 37

**Figure 8.** Average ( $\pm$  SE) density of zoochlorellae (a-c) and zooxanthellae (d-f) in anemone tentacles from low tidepool (LTP), low surge channel (LSC), high tidepool (HTP) and high surge channel (HSC) microhabitats seasonally. Y-axis scales are one order of magnitude higher for zoochlorellae densities than for zooxanthellae densities. Numbers above bars indicate sample size; different letters within a season and symbiont type indicate significant difference between microhabitats at  $p < 0.05$ . NS= no significant difference in algal densities between any microhabitat within a season. 39

**Figure 9.** Mitotic index ( $\pm$  SE) of zoochlorellae (a-c) and zooxanthellae (d-f) in anemone tentacles from low tidepool (LTP), low surge channel (LSC), high tidepool (HTP) and high surge channel (HSC) microhabitats seasonally (a-c). Numbers above bars indicate sample size; different letters within a season and symbiont type indicate significant difference between microhabitats at  $p < 0.05$ . nd= no data; ns= no significant difference between any microhabitat within a season. 41

**Figure 10.** Average body column diameter ( $\pm$  SE) in anemones from low tidepool (LTP), low surge channel (LSC), high tidepool (HTP) and high surge channel (HSC) microhabitats seasonally (a-c) as well as body column diameter averaged across all microhabitats) between seasons (d). Numbers above bars indicate sample size; different letters within a season and symbiont type show significant differences at  $p < 0.05$ . nd= no data; NS= no significant difference in body column diameter between any microhabitat within a season. 43

**Figure 11.** Correlations of anemone size (body column diameter) vs. **a)** zoochlorellae and **b)** zooxanthellae densities. All samples across all seasons and microhabitats. All data are plotted logarithmically. 45

**Figure 12.** Correlations of sample size (tentacle wet weight) vs. **a)** zoochlorellae and **b)** zooxanthellae densities. All samples across all seasons and microhabitats. All data are plotted logarithmically. 46

**Figure 13.**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values ( $\pm$ SE) for zoochlorellate ( $\geq 90\%$  zoochlorellae) and mixed zoochlorellate (50-90% zoochlorellae) anemone tentacle tissue, isolated symbionts composed of zoochlorellae ( $\geq 90\%$  zoochlorellae) and mixed zoochlorellae (50-90% zoochlorellae) assemblages, and tissue from *M. californianus* mussels. Dashed lines connect summer values (dark symbols) to spring values (open symbols). Sample sizes are listed in Table 5. 49

**Figure 14.** **a)**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values ( $\pm$  SE) for zooxanthellate ( $\geq 90\%$  zooxanthellae) and mixed zooxanthellate (10-50% zooxanthellae) anemone tentacle tissue, isolated symbionts composed of zooxanthellae ( $\geq 90\%$  zooxanthellae) and mixed zooxanthellae (10-50% zooxanthellae) assemblages, and tissue from *M. californianus* mussels. **b)** An enlargement of the anemone and algal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values. Triangles represent anemones, circles represent symbionts. Dashed lines connect summer values (dark symbols) to spring values (open symbols). Sample sizes are listed in Table 5. 50

## List of Tables

- Table 1.** Number of *Anthopleura xanthogrammica* sampled in three seasons for algal parameters and stable isotope analysis. 15
- Table 2.** Physical characteristics of microhabitats and numbers of *A. xanthogrammica* sampled (*n*) from locations identified in Figure 2. nm= not measured. 16
- Table 3.** Number of individual *A. xanthogrammica* sampled in summer 2008, fall 2008, and spring 2009. Numbers in parentheses are the number of individual anemones re-sampled from summer sampling. 19
- Table 4.** Monthly average temperature variation ( $^{\circ}\text{C} \pm \text{SE}$ ) above (positive values) and below (negative values) ambient seawater temperature as well as monthly extreme high and low temperatures in a tidepool (MLLW +1.59 m) and a surge channel (MLLW +1.19 m) at Slip Pt, Clallam Bay WA. p-values refer to paired t-test comparisons of tidepool and surge channel temperatures when both were recorded concurrently. 32
- Table 5.** Nested ANOVA table of the variability in zoochlorellae density estimates from tentacles obtained from individual anemones (“between anemones”; n=10) as compared to variability within each anemone (“within an anemone”; n=29) . Each anemone was sampled three times, except for one anemone that was only sampled twice. 48
- Table 6.** Average ( $\pm \text{SE}$ )  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (‰) values for *A. xanthogrammica* tentacle tissue isolated from hosts of varying symbiont composition, grouped as zoochlorellate ( $\geq 90\%$  zoochlorellae/ mg anemone tentacle protein), mixed zoochlorellate (50-90% zoochlorellae), mixed zooxanthellate (10-50% zoochlorellae), and zooxanthellate ( $\leq 10\%$  zoochlorellae), isolated symbionts, and tissue from *M. californianus* mussels. 51
- Table 7.** Relative contribution (percent  $\pm \text{SE}$ ) of symbiotic zoochlorellae or mixed zoochlorellae and *M. californianus* to *A. xanthogrammica* diet. Carbon isotope fractionation is assumed to be 0.4 ‰ per trophic level (McCutchan *et al.* 2003). 53

## Introduction

The intertidal sea anemone *Anthopleura xanthogrammica* is distributed widely along the Pacific coast, from Baja California, Mexico (~30°N) to southern Alaska (57°N) (Hand 1955). *Anthopleura xanthogrammica* hosts members of two distinct algal taxa, known as zooxanthellae and zoochlorellae, throughout much of its range. Zooxanthellae are brown dinophytes in the genus *Symbiodinium* (Muscatine 1980a), and zoochlorellae are green algal chlorophytes recently shown to belong in the genus *Elliptochloris* and named *E. marina* (Letsch et al. 2009). *Anthopleura xanthogrammica* is found in wave-exposed intertidal and subtidal communities dominated by the mussel *Mytilus californianus* (Sebens 1983). These anemones show very little movement over annual scales (Batchelder & Gonor 1981, Sebens 1983) and they are exceptionally stable members of these communities with survival greater than 100 years (Sebens 1983). Because *A. xanthogrammica* has no significant predators (Dayton 1973), mortality of anemones may be restricted to extreme events such as large waves and log or rock scouring (as observed on the Washington coast by Dayton, in Sebens 1983).

The ability of *A. xanthogrammica* to host two distinct algal partners and persist over long temporal scales makes this anemone a useful model organism for studying the nature of symbiotic association over the range of seasonal conditions characterizing the Pacific Northwest intertidal zone. Seasonal temperature ranges experienced by intertidal anemones in this region are reflected in the body temperature of the congeneric anemone *A. elegantissima*, which experiences body temperatures ranging from 5°C (winter) to 28°C (summer) during low tide exposures (Dingman 1998). Despite this marked range, field studies indicate that the densities of zoochlorellae and zooxanthellae in *A.*

*elegantissima* remain constant seasonally (Dingman 1998, Bergschneider & Muller-Parker 2008), supporting suggestions that temperate algal-cnidarian symbioses may be remarkably stable (Muller-Parker & Davy 2001). The differences in life history traits between *A. xanthogrammica* and the more intensely studied *A. elegantissima* may be illustrative in further testing this stability. Although these congeners share symbiont species, *A. xanthogrammica* individuals are solitary (as opposed to the clonally aggregating *A. elegantissima*), reproduce exclusively sexually (as opposed to both sexually and through asexual longitudinal fission) and have a comparatively large body size (25 cm in *A. xanthogrammica* vs. 1-6 cm in *A. elegantissima*) (Hand 1955, Sebens 1981, 1983).

Zoochlorellae and zooxanthellae hosted by *A. xanthogrammica* (and *A. elegantissima*) represent “cool” and “warm” symbionts respectively. In the laboratory, densities of zoochlorellae increase at cool temperatures (13°C), but decline at warmer temperatures (20°C; O’Brien & Wytenbach 1980, Saunders & Muller-Parker 1997), while zooxanthellae do not decline in density until at least 26°C (Muller-Parker et al. 2007). The observed distribution of symbionts, both on intertidal and latitudinal scales, suggests that cooler regions are associated with more zoochlorellae in host anemones, while warmer regions are associated with more zooxanthellae. Studies of *A. xanthogrammica* on Vancouver Island (48°N) and in Coos Bay, OR (44°N) have shown that zooxanthellae are hosted at higher shore heights, mixed zoochlorellae and zooxanthellae populations are found at intermediate shore height, and zoochlorellae occur primarily at lower shore heights (Bates 2000, Kitaeff 2007). This pattern for *A. xanthogrammica* is also evident on a latitudinal scale, with zooxanthellate anemones

found at relatively warmer low latitudes, mixed anemones at intermediate latitudes, and zoochlorellate anemones at relatively cooler high latitudes (Secord & Augustine 2000, Kitaeff 2007).

In addition to shore height and latitude, the distribution of symbionts may also be influenced by “cool” and “warm” microhabitats within the intertidal zone. Because *A. xanthogrammica* is predominantly found in tidepools and surge channels (Sebens 1983), it is illustrative to examine these microhabitats. Bates (2000) observed zooxanthellate *A. xanthogrammica* closer to the surface of tidepools and zoochlorellate *A. xanthogrammica* lower in tidepools. In her study, cracks and crevices at a given shore height contained more zoochlorellate anemones than tidepools at the same shore height (Bates 2000). Thermal differences between tidepools and surge channels might change seasonally; tidepools may reach temperatures greater than 26°C in the Pacific Northwest during the summer (Bates 2000, Jensen & Muller-Parker 1994), and surge channels may provide “cool spots” during emersion if they provide shading or exposure to wave splash that is cooler than surrounding air temperature. In contrast, during wintertime low tides, anemones in exposed surge channels may experience the coldest thermal conditions annually.

The distribution of zoochlorellae and zooxanthellae within anemones in different microhabitats and seasons is of interest because the relative contributions of these two symbionts to host nutrition may differ. Based on mitotic index, zoochlorellae have growth rates from 4-20 times greater than zooxanthellae (Verde & McCloskey 1996, Saunders & Muller-Parker 1997, Bergschneider & Muller-Parker 2008). As a result, zoochlorellae may use more photosynthetically fixed carbon for their own growth than

zooxanthellae, potentially providing less carbon to their host. Carbon budgets (calculations of carbon contributions to host anemones using estimates of symbiont productivity, respiration, and growth) have estimated the potential contribution of algal carbon to *A. elegantissima* respiratory carbon consumption to be lower for zoochlorellae (9%) than zooxanthellae (48%) (Verde & McCloskey 1996b) or to be 30-50% higher for zoochlorellae than zooxanthellae (Bergschneider & Muller-Parker 2008).

$^{14}\text{C}$  carbon translocation experiments (in which photosynthetic carbon flow from symbionts to hosts is measured directly using radioactive carbon isotopes) suggest that zoochlorellae and zooxanthellae do not differ greatly in their contributions of carbon to their host. Engebretson & Muller-Parker (1999), using freshly collected anemones, found that zoochlorellae, zooxanthellae, and mixed assemblages of these each translocate about 30% of photosynthetically fixed carbon to *A. elegantissima*. Seasonal  $^{14}\text{C}$  translocation experiments demonstrate that zoochlorellae translocate slightly more carbon than zooxanthellae to *A. elegantissima* in both summer and winter (Bergschneider & Muller-Parker 2008). Finally, use of stable  $^{13}\text{C}$  and  $^{15}\text{N}$  isotopes (in which carbon and nitrogen flow are estimated by exploiting the fact that  $^{13}\text{C}$  and  $^{15}\text{N}$  signatures in consumers vary predictably from those of their food sources, as in Peterson and Fry 1987) suggests that heterotrophic food sources are more important to anemone nutrition than the contributions of either symbiont. The  $^{13}\text{C}$  and  $^{15}\text{N}$  signatures of symbiotic anemones more closely match those of non-symbiotic anemones than those of the symbionts themselves, indicating similar (and heterotrophic) carbon and nitrogen sources for symbiotic and aposymbiotic anemones (Bergschneider & Muller-Parker 2008).

Nutritional studies in *A. elegantissima* suggest that both zoochlorellae and zooxanthellae provide anemones with less of their energetic needs than heterotrophic feeding, but extrapolating to *A. xanthogrammica* is problematic. Most significantly, the life history traits of *A. xanthogrammica* suggest that it may even be more reliant than *A. elegantissima* on a heterotrophic diet. *Anthopleura xanthogrammica* consumes *M. californianus* mussels that are detached from the rocks by *Pisaster ochraceus* foraging and by wave action (Sebens 1983). Sebens (1983) freed mussels prior to high tide on Tatoosh Island, WA, and measured a 33% capture rate by anemones within 2 m from the mussel release point. Dayton (1973) found that the coelenteron contents of *A. xanthogrammica* on Washington's outer coast were composed of nearly 70% *M. californianus*. With a reliable and constantly available prey source, algal contributions may be insignificant for *A. xanthogrammica*. Greater reliance on heterotrophic food sources may indicate that symbiont type may not actually "matter" energetically from a host perspective for this anemone.

The symbionts in *A. xanthogrammica* may also be useful biological indicators of projected climate change. Because the upper limits of intertidal species distribution are generally determined by physical factors, including thermal tolerance (Connell 1972, Somero 2002, Davenport & Davenport 2005), many organisms may already be living near their physiological limits. With 2 to 4°C of global surface warming predicted over the next century (IPCC 2007), intertidal species may shift their distributions in response to elevated temperatures. Hypothesized ecological responses include poleward shifts in the latitudinal ranges of species (Barry et al. 1995, Southward et al. 1995, Sagarin et al. 1999, Parmesan et al. 2005) and localized extinction in "hotspots" caused by the



interaction of mid-day summer low tides and increasingly extreme temperatures (Helmuth et al. 2002). The symbionts contained within *A. xanthogrammica* could potentially indicate responses to both of these dynamics. Because *A. xanthogrammica* individuals have an exceptionally long lifespan and can host both a “cool” symbiont and a “warm” symbiont, it may be possible to follow long-term shifts in the symbiont composition of a single “hot” or “cool” spot or a larger latitudinal gradient via non-lethal tentacle sampling. Baseline data on the distribution of symbionts as well as their responses to seasonal thermal variation are needed to test the utility of this symbiosis as a biological indicator.

This study determined temperature differences between tidepool and surge channel microhabitats at one site on the outer Strait of Juan de Fuca, WA (48°N). The seasonal distribution, density, and growth of zoochloellae, a thermally “cool” symbiont were compared to that of zooxanthellae, a thermally “warm” symbiont co-occurring in *A. xanthogrammica* in these microhabitats. Finally, the relative contributions of these symbionts and heterotrophic feeding on mussels to *A. xanthogrammica* diet were investigated using stable  $^{13}\text{C}$  and  $^{15}\text{N}$  analysis. These parameters were obtained from seasonal samples between summer 2008 and spring 2009.

The following hypotheses were tested:

1. Spatially close microhabitats (tidepools and surge channels) experiencing similar emersion times will differ thermally; tidepools will be the warmer microhabitat on an annual basis.

2. Zooxanthellae will predominate in anemones in tidepools and zoochlorellae will predominate in anemones in surge channels. Symbiont distribution, density, and growth within each microhabitat will not change seasonally.
3. Heterotrophic food sources will be the most significant source of carbon to *A. xanthogrammica* regardless of season, microhabitat, and symbiont type.

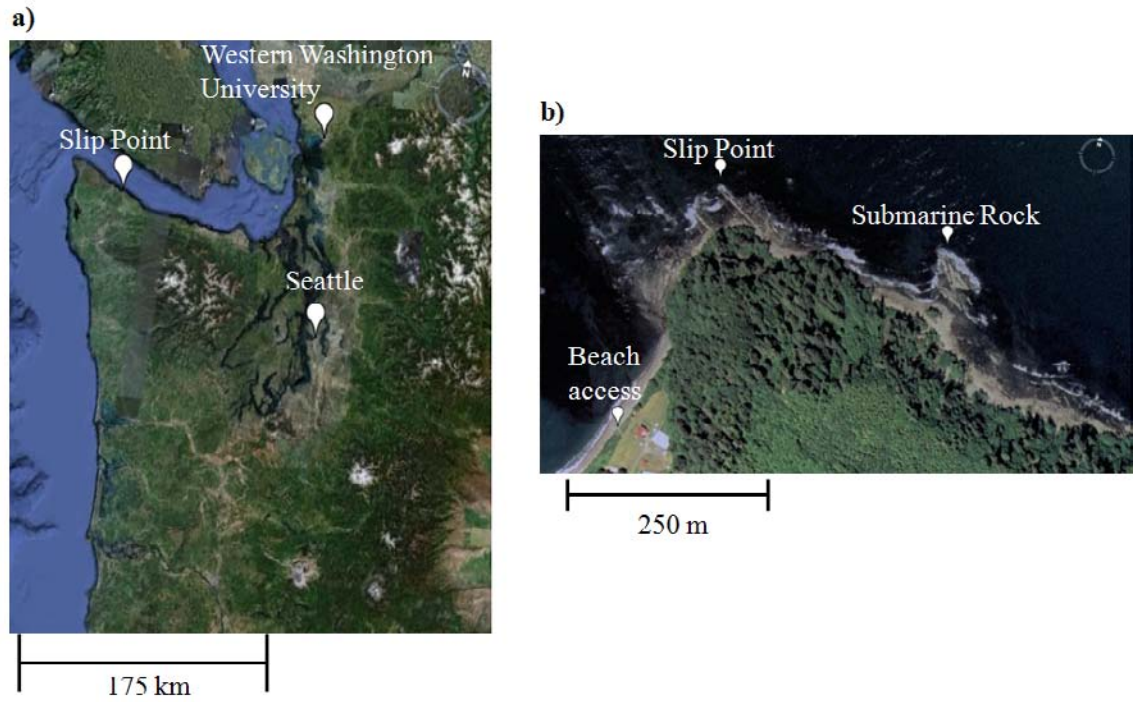
## Materials and Methods

### Overview

This study examined the effects of two intertidal microhabitats on zoochlorellae and zooxanthellae contained in tentacles of *A. xanthogrammica* on the outer Strait of Juan de Fuca, Washington. Temperature data loggers in tidepools and surge channels were deployed between summer 2008-summer 2009 in order to determine if these microhabitats provided anemones with differing thermal regimes. Anemone tentacles were sampled in summer and fall 2008 and in spring 2009 to examine seasonal patterns in symbiont distribution, density, and mitotic index in anemones in relation to these microhabitats. Finally, this study estimated the relative contributions of symbionts and heterotrophic feeding on mussels to *A. xanthogrammica* nutrition in summer 2008 and spring 2009 using stable isotope analysis.

### Field site description

All sampling occurred at Submarine Rock on Slip Point, a rocky outcrop in Clallam Bay on the outer Strait of Juan de Fuca, Washington with exposures ranging from northwest to northeast (Figure 1). Submarine Rock (48°15'50"N; 124°14'143"W) is a north facing outcrop located a 0.75 km walk northeast of the Clallam Bay Coast Guard beach access. This site was selected for its relative inaccessibility, diversity of microhabitats, and abundance of *Anthopleura xanthogrammica*. Named for its “submarine-like” appearance during tidal changes, access to Submarine Rock requires a 20 minute walk during low tide over benches and boulder fields that are largely submerged during high tide. Submarine Rock extends seaward from a rock shelf for



**Figure 1.** Study site. **a)** Slip Point is located on the outer Strait of Juan de Fuca. **b)** All sampling was completed at Submarine Rock, an outcrop near Slip Point. Submarine Rock is an approximately 0.75 km walk from the Coast Guard beach access visible in the bottom left of picture. Images from Google Earth.

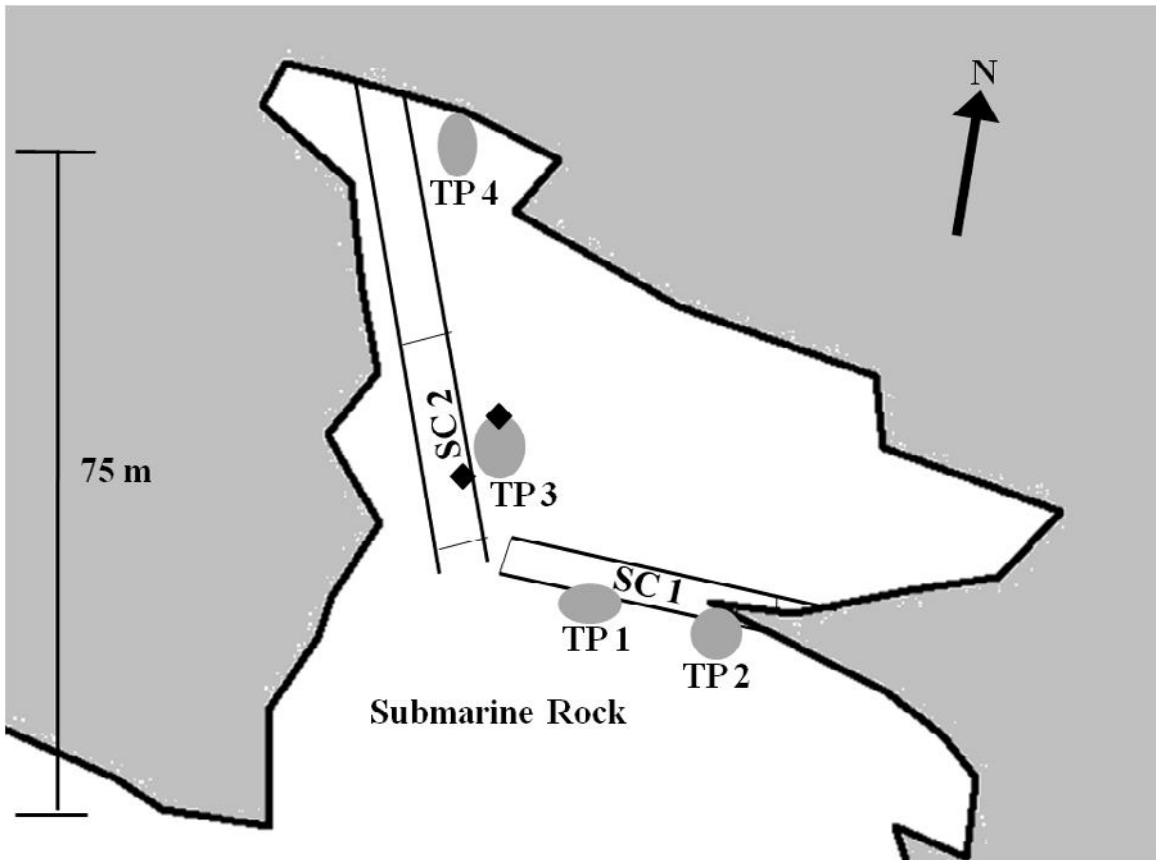
approximately 75 m and is 75 m wide at its widest point (Figure 1). It consists of a flat shelf with numerous tidepools, surge channels extending inward from the northwest and east edges of the shelf, and vertical walls dropping into the subtidal zone on three sides (Figure 2). Extensive beds of the mussel *Mytilus californianus* and gooseneck barnacle *Pollicipes polymerus* are present, indicative of considerable wave exposure at the site.

*Anthopleura xanthogrammica* is abundant in surge channels, tidepools, and along sloping rock surfaces at Submarine Rock. The distribution of *A. xanthogrammica* has an upper vertical tide limit of +1.59 m in relation to Mean Lower Low Water (MLLW) in tidepools along the upper shelf (all tide heights are also reported in reference to MLLW). The anemones' lower limit is subtidal, extending well below the lowest extent of sampling at -0.61 m. The congeneric anemone *A. elegantissima* is commonly found in small cracks and around mussel beds, predominantly clustered around +0.91 m.

Tide heights were measured using a stadia rod sighted with an engineer's transit. Heights measured on the stadia rod were referenced to known tide levels using a Clallam Bay tide chart with 15-minute tide intervals (Nobeltec Tides and Currents software, 2006).

#### Microhabitat temperature monitoring

HOBO temperature data loggers ( $\pm 0.2^\circ\text{C}$  accuracy; Onset Computer Corporation U22 Water Temp Pro model, Bourne MA) were anchored in a high tidepool (+1.59 m) and a high surge channel (+1.19 m) to continuously record temperatures in microhabitats experiencing similar tidal emersion times. Plastic zip-ties were used to attach data loggers



**Figure 2.** Schematic of Submarine Rock. Gray= water; white= rocky shore. TP= tidepool; SC= surge channel. Only tidepools and surge channels used for the seasonal anemone sampling are indicated (more tidepools and surge channels were present at Submarine Rock than are indicated). Diamonds represent the location of the tidepool and surge channel data loggers.

to stainless steel eyebolts embedded in ZSPAR splash zone epoxy (RPM Inc., Medina OH). Data loggers recorded temperatures every 10 minutes. The tidepool data logger recorded from 7/31/08-4/10/09. The surge channel logger recorded from 8/30/08-11/13/08 and from 4/13/09-6/19/09.

HOBO loggers provided 144 daily temperature measurements for each microhabitat (except for days on which loggers were downloaded or deployed, which provided at least 66 daily temperature measurements). Each day was summarized by calculating the average temperature as well as noting the recorded daily maximum and minimum temperature. Daily average, maximum, and minimum temperatures were used to calculate monthly average, average daily high and average daily low temperatures. Standard errors of all monthly averages were calculated as an estimate of daily variability, as in Helmuth and Hoffmann (2001).

Data loggers also provided a record of seawater temperatures during immersion. A Clallam Bay tide chart was used to determine times when the seawater level was greater than +1.83 m (except for 7 days when the tide did not reach +1.83 m and times when the seawater level was greater than +1.52 m were included), and only temperatures recorded during these times were used to measure seawater temperature. This tide height (+1.83 m) represents a conservative estimate of immersion, as at this height seawater was at least 0.24 m higher than the location of the tidepool data logger and 0.59 m higher than the location of the surge channel data logger. Because seawater temperature varied considerably less than air temperature, maximum and minimum temperatures were not considered, and only daily average water temperatures were calculated. Monthly averages ( $\pm$  SE) were created from daily averages. Daily seawater temperature during immersion

was also used as a baseline for comparison of the temperatures recorded in tidepools and surge channels, with the range above or below daily seawater temperature indicating the relative amount of thermal change in each microhabitat during low tide exposures to air.

Regional-scale air temperature trends were derived from temperatures collected at the NOAA Neah Bay weather station (Station NEAW1: [http://www.ndbc.noaa.gov/station\\_page.php?station=new1](http://www.ndbc.noaa.gov/station_page.php?station=new1)). This station is located approximately 30 km NW of Slip Point in the protected harbor of Neah Bay. Temperatures recorded every 6 minutes from 7/31/08-6/18/09 were used to calculate daily average temperatures. Monthly air temperature averages ( $\pm$ SE) were obtained from daily averages.

#### Anemone selection and tentacle sampling

*A. xanthogrammica* individuals at Slip Point were clustered in tidepools and on the floors of surge channels in close proximity to *M. californianus* beds. Anemones in these two microhabitats were compared because tidepools provide constant immersion while surge channels subject anemones to periods of aerial exposure. Sampling sites consisted of paired tidepool and surge channel microhabitats in close proximity and at similar shore height to minimize differences in emersion time, irradiance and wave exposure regimes. Based on the observed distribution of anemones, individuals located at tide heights above +0.91 m were designated high intertidal, and those below +0.91 m were considered low intertidal. Separation into high and low tide height habitats was more appropriate than the high, medium, and low tide height designations used in other studies (as in Kitaeff 2007) because most anemones were at the “low” and “high”



extremes (45 out of 50 anemones sampled were located below +0.61 m or above +1.07 m).

Four tidepools and two surge channels were used for seasonal anemone tentacle sampling (see Table 1 for sample sizes, Figure 2 for locations, and Table 2 for microhabitat characteristics). Tidepools #1 and #2 contained exclusively low intertidal anemones, and were located near and above large seaweed assemblages consisting predominantly of *Egregia menziesii*, *Costaria costata* and *Laminaria setchelli*. These two tidepools were paired with east-facing surge channel #1, which spanned a vertical height of -0.6 m to +1.33 m over a distance of approximately 25 m. Anemones sampled from this surge channel were in both the high and low intertidal categories, with approximately 70% in the low intertidal category. Tidepools #3 and #4 contained exclusively high intertidal individuals and were located along a broad shelf covered in dense *M. californianus* beds. These high tidepools were paired with northwest-facing surge channel #2, a parallel channel containing exclusively high intertidal anemones. Anemones were sampled along a 24 m long section of the channel, which continued seaward to the edge of the Submarine Rock shelf.

Anemones were selected randomly or haphazardly for sampling. To select anemones in tidepools #2, #3, #4 and surge channel #1, a transect tape was placed along the longest axis and a random number table was used to generate points along the tape. The anemone located closest to a point was sampled. As tidepool #1 contained only 6 individuals, all were sampled. A transect tape was laid along the longest axis of surge channel #2, and anemones falling along the tape were sampled haphazardly.

**Table 1.** Number of *Anthopleura xanthogrammica* sampled in three seasons for algal parameters and stable isotope analysis.

| Season | Sampling dates   | Anemones | Algal parameters           |                     | Stable isotope analysis |       |         |
|--------|------------------|----------|----------------------------|---------------------|-------------------------|-------|---------|
|        |                  |          | Algal biomass <sup>a</sup> | Algal mitotic index | Anemones                | Algae | Mussels |
| Summer | 7/30-/08-7/31/08 | 50       | 50                         | 48                  | 36                      | 19    | 8       |
| Fall   | 11/13/2008       | 25       | 25                         | 23                  | 0                       | 0     | 0       |
| Spring | 4/10/09-4/11/09  | 50       | 50                         | 48                  | 42                      | 22    | 10      |

<sup>a</sup>Algal biomass includes symbiont enumeration and anemone tentacle protein measurement.

**Table 2.** Physical characteristics of microhabitats and numbers of *A. xanthogrammica* sampled (*n*) from locations identified in Figure 2. nm= not measured.

| <b>Location</b>  | Vertical distance from MLLW (m) | High or low designation | Maximum length, Maximum width (m) | Tidepool Maximum Depth (cm) | <i>n</i> Summer, Fall, Spring |
|------------------|---------------------------------|-------------------------|-----------------------------------|-----------------------------|-------------------------------|
| Tidepool #1      | +0.51                           | Low                     | 6.8 , 2.6                         | 41                          | 6, 6, 6                       |
| Tidepool #2      | -0.03                           | Low                     | 2.4 , 2.0                         | 38                          | 4, 4, 4                       |
| Tidepool #3      | +1.59                           | High                    | 6.6 , 1.6                         | 38                          | 10, 3 ,10                     |
| Tidepool #4      | +1.53                           | High                    | 2.9 , 0.7                         | 20                          | 10, 0, 10                     |
| Surge channel #1 | -0.6 - +1.29                    | Both                    | 25, nm                            |                             | 10, 9, 10                     |
| Surge channel #2 | +1.06 - +1.33                   | High                    | 24, nm                            |                             | 10, 3 ,10                     |

Small scissors were used to remove approximately 6 tentacles from each anemone. Tentacles were sampled because their removal is non-lethal and repeatable. In addition, tentacles contain the highest density of algae within *A. xanthogrammica* (Kitaeff 2007). Tentacles were immediately placed into a 1.5 ml microfuge tube and stored in a cooler. After sampling tentacles, plastic Vernier calipers ( $\pm 0.1$  mm precision) were used to measure the body column diameter at the base of an anemone to the nearest mm. The exact position of the anemone along the transect tape was noted, as was the submergence depth of anemones in tidepools. Tentacles were stored in a standard freezer as soon as possible (within three hours of sampling) and transported back to Shannon Point Marine Center in Anacortes, WA in an ice-filled cooler. Tentacles were then stored at  $-70^{\circ}\text{C}$  until analysis.

During the initial summer 2008 sampling, field reference marks and site descriptions were taken to aid in identifying anemones for later sampling in fall 2008 and spring 2009. Reference marks of ZSPAR splash zone epoxy (RPM Inc., Medina OH) were used to re-position tapes in surge channel transects. Photographs of transect tape placements in tidepools were used to reestablish these transects in other seasons. Finally, numbered photographs were taken of each sampled anemone. Individual anemones were relocated successfully, even during nighttime sampling, using a combination of transect locations, body column diameter measurements, and photographs. If anemones were missing or individuals could not be identified with confidence, new individuals were selected for sampling.

Because tentacle samples were removed from the most accessible area of the tentacle crown and the size of individual tentacles sampled was variable, it was possible

that individual samples were not representative of all tentacles of a given anemone. To estimate the variability in algal density among tentacles of an individual anemone, a second set of anemones was used to sample tentacles repeatedly from each anemone and compare values obtained. Tentacles were removed from different locations of the tentacle crown of individuals sampled in spring 2009. Anemones selected for this sampling (N=10) were located in small tidepools near tidepools #3 and #4 (Figure 2). All tidepool surfaces were at approximately +1.5 m (approximately the same vertical height as tidepools #3 and #4), and no more than two anemones were sampled from each tidepool. All sampling was completed on 4/11/2009. An anemone was selected haphazardly if it was in a completely expanded body position, with all tentacles accessible for clipping. The anemone tentacle crown was visually divided into three equal sections, with between one and three tentacles clipped from each one. Samples were placed in individual 1.5 ml microfuge tubes and kept on ice. Upon return to Shannon Point Marine Center, samples were stored at -70°C.

#### Seasonal sampling of *A. xanthogrammica* tentacles

Tentacle samples were taken from selected anemones in summer (7/30-7/31/2008), fall (11/13/2008) and spring (4/10-4/11/2009) seasons. Fifty anemones were initially sampled in summer 2008. Due to anemone contraction behavior during low tides at night when air temperatures were very low, only 25 anemones were sampled in fall 2008, and winter sampling was not attempted. Twenty-two (88%) of the anemones sampled in fall were confirmed as re-samples from the July sampling. In spring 2009, 50 anemones were again sampled, with 30 (60%) confirmed as re-samples from July. Of

**Table 3.** Number of individual *A. xanthogrammica* sampled in summer 2008, fall 2008, and spring 2009. Numbers in parentheses are the number of individual anemones re-sampled from summer sampling.

| <b>Location</b>     | Summer    | Fall           | Spring         | Resampled in both Fall and Spring |
|---------------------|-----------|----------------|----------------|-----------------------------------|
| Low tidepool        | 10        | 10 (10)        | 10 (6)         | 6                                 |
| Low surge           | 7         | 6 (5)          | 7 (3)          | 3                                 |
| High tidepool       | 20        | 3(2)           | 20 (12)        | 2                                 |
| High surge          | 13        | 6 (5)          | 13 (9)         | 5                                 |
| <b>Total number</b> | <b>50</b> | <b>25 (22)</b> | <b>50 (30)</b> | <b>16</b>                         |

these, 16 anemones were sampled repeatedly in July, November, and April (Table 3).

### Mussel sampling

To estimate the contribution of *M. californianus* to anemone nutrition, mussels were haphazardly collected for stable isotope analysis from the same microhabitats used in the seasonal study. Individuals were collected in summer (7/31/08) and spring (4/11/09). In both samplings, two mussels were taken from the edges of each of three tidepools (tidepools #2, #3, and #4), and two individuals were taken from each of surge channel #1 and #2 (total N=10 in each season) (Figure 2, Table 2). Mussels were combined and placed in a bucket filled with approximately 5 L of 5- $\mu$ m filtered seawater for 24 hours to evacuate their gut contents/ They were then stored on ice until return to Shannon Point Marine Center, at which point individuals were stored at -70°C until processing.

### Analysis of tentacle samples

Except for the fall samples, each tentacle sample from an individual anemone was divided into two approximately equal halves; one was analyzed for biomass parameters (algal cell density, algal mitotic index, and anemone protein content), and the other was analyzed for  $^{13}\text{C}$  and  $^{15}\text{N}$  stable isotopic signatures. Tentacle samples collected in the fall season were analyzed only for biomass parameters and all available tentacles were homogenized (Table 1). To split samples, half of the tentacles were haphazardly selected and removed from each microfuge tube with metal spatulas immediately prior to biomass parameter analysis, and the remainder were immediately re-frozen. To ensure that

tentacles were free of body column debris and food items, tentacles were placed on parafilm squares, visually inspected, and any contaminants removed using tweezers before processing.

Tentacle samples analyzed for biomass parameters were weighed on an analytical balance (Mettler Toledo, Columbus OH) to 0.0001 g precision prior to homogenization in a small amount of 5- $\mu$ m filtered sea water using a 3-ml tapered glass tissue homogenizer and Teflon pestle (Wheaton Science Products, Millville NJ) attached to a motorized overhead stirrer (Wheaton Science Products, Millville NJ). Samples were cooled on ice during homogenization. The average wet weight of tentacle samples was  $0.026 \text{ g} \pm 0.002 \text{ g SE}$  (n=125), and the average homogenate volume was  $1.73 \text{ ml} \pm 0.02 \text{ ml SE}$  (n=125). The homogenate was well mixed by vortexing and dispensed into two 1.5 ml microfuge tubes. One tube was used for protein analysis, and the second for algal density and mitotic index counts. Homogenates were stored at  $-70^{\circ}\text{C}$  prior to analysis.

#### Algal cell density

Thawed homogenate samples were mixed by vortexing prior to being loaded on a hemacytometer slide (Brightline, Reichert Scientific Instruments, Buffalo NY). Squares containing  $1 \times 10^{-4}$  ml of homogenate were counted until at least one hundred cells of the dominant algal type (zooxanthellae or zoochlorellae) were enumerated. Zooxanthellae and zoochlorellae were counted concurrently using a compound microscope at 400x magnification, or 100x in the case of especially dilute samples. Four replicate counts were made for each sample, and average densities of zooxanthellae and zoochlorellae (in  $\text{cells} \cdot \text{ml}^{-1}$ ) were calculated.



### Algal mitotic index

Mitotic index, a measure of the number of algal cells dividing at the time of sampling, was calculated for the dominant symbiont, defined as the one that comprised over half of the algae within a sample. Cells with a complete cleavage furrow were scored as dividing, as judged using a compound microscope at 400x magnification. One thousand zooxanthellae or zoochlorellae were counted, and mitotic index expressed as the percent of zooxanthellae or zoochlorellae dividing. In two very low algal density samples, only 500 algae were counted.

### Protein content of tentacles

Expressing density as the number of zooxanthellae or zoochlorellae per unit weight of homogenate protein allows direct comparisons of algal densities among samples. Although total homogenate samples (containing both algae and anemone tissue) were used to measure anemone protein, intact algae do not contribute significantly to the protein values obtained (Verde and McCloskey 2001, Muller-Parker *pers. comm.*) and homogenate protein concentrations are considered representative of the anemone (animal) component. Protein concentration was measured using the Lowry method (Lowry et al. 1951), as modified by Muller-Parker (Muller-Parker 2008). Bovine serum albumin standard (BSA) (Pierce, Rockford IL) was used to create a standard curve from 25µg to 200µg. Homogenate samples were thawed, thoroughly mixed by vortexing, and duplicate 100-µl subsamples taken for analysis. 200 µl of 1 N NaOH were added to all samples and standards to solubilize protein, and total volume was brought up to 1 ml with

Nanopure® water. Samples and standards were then solubilized for 30 minutes at approximately 40°C. After protein color development, samples were centrifuged at ~3000 rpm for 5 minutes to precipitate sea water residues and the absorbance of the supernatant was measured spectrophotometrically at 660 nm (Spectronic 20 D +, Thermo Fisher Scientific, Waltham, MA). Duplicate samples with absorbance values within 10% agreement were averaged and related to the standard curve to determine protein content. Duplicates that differed by more than 10% were rejected and new duplicate samples were analyzed. Algal densities were calculated by dividing the total concentration of cells (zooxanthellae and zoochlorellae · ml<sup>-1</sup>) by the concentration of homogenate protein (mg protein · ml<sup>-1</sup> homogenate).

#### Statistical analysis of algal density, algal mitotic index, and anemone size

All data were checked for homogeneity of variances prior to analysis. Data were square-root transformed in cases where homogeneity of variances was violated (judged with Levene's test). All percentage values were arcsine transformed prior to analysis (Sokal & Rohlf 1969). A significance criterion of  $\alpha < 0.05$  was used for all comparisons meeting the assumption of homogeneity of variances. In cases where this assumption was not met, significance was adjusted to  $\alpha < 0.025$  (Underwood 1981). All statistical analyses were performed using SPSS v.15.0 (SPSS Inc., Chicago IL).

Microhabitats (4 levels: low tidepool, low surge channel, high tidepool, and high surge channel) were compared using a multivariate analysis of variance (MANOVA) with density of zooxanthellae and density of zoochlorellae as the dependent variables. Because the goal of this analysis was to describe the distribution of zoochlorellae and

zooxanthellae in relation to microhabitat, it was assumed that anemones can host a continuum of symbionts from 100% zoochlorellae to 100% zooxanthellae and both symbionts were treated as continuous variables (i.e., anemones were grouped by microhabitat as opposed to symbiotic state prior to analysis). If MANOVA indicated a significant effect of microhabitat on algal densities, the density of zooxanthellae and zoochlorellae between microhabitats was examined with one-way ANOVA tests. Significant ANOVA tests were followed by pairwise comparisons among microhabitats using Tukey's HSD tests.

One-way ANOVA tests were used to determine if microhabitat affected biomass parameters including the mitotic index of zooxanthellae and zoochlorellae, the percentage of zoochlorellae and zooxanthellae in tentacles, and anemone size. Significant ANOVA tests were followed by pairwise comparisons between microhabitats using Tukey's HSD tests (mitotic index and anemone size) or contrasts between the high surge channel and high tidepool microhabitats (percent zoochlorellae within an individual). The potential relationships between anemone size and tentacle sample size and algal density were analyzed using correlations, and the variability in algal densities between tentacles within an individual as compared to the variability between individuals was analyzed using a nested ANOVA.

Although this study originally intended to repeatedly sample all anemones (allowing for the use of repeated-measures ANOVA to compare seasonal change in algal composition, density, and mitotic index) only 16 anemones were sampled in all seasons and repeated-measures ANOVA was not used to compare this subset of anemones. In addition, the presence of repeatedly sampled individuals in all seasons violated the

ANOVA assumption of independence, and ANOVA was not used to compare parameters between seasons. As a result, only trends between seasons, not statistical differences between seasons, were estimated in this study.

#### Preparation of tentacle samples for stable isotope analysis

The second half of the tentacle samples collected in summer 2008 and spring 2009 was prepared for  $^{13}\text{C}$  and  $^{15}\text{N}$  stable isotope analysis. Because the high salt content of sea water can influence sample weights (Pitt *et al.* 2009), tentacles were homogenized, as previously described, in Nanopure® water. Due to the high sensitivity of isotopic analysis to trace organic contamination, all equipment in contact with samples (homogenizer, pestle and pointed metal spatulas) was cleaned with 3 rinses of methanol followed by 3 tap water rinses and 3 nanopure water rinses before use, and between each sample. Homogenate samples were mixed by vortexing, placed into two 1.5-ml microfuge tubes and stored at  $-70^{\circ}\text{C}$  prior to further processing.

Homogenates were centrifuged in microfuge tubes at  $\sim 5000$  rpm for 4 minutes (Eppendorf microfuge, Hamburg Germany) to separate algae from anemone animal fraction. The anemone supernatant was decanted into a 20-ml glass scintillation vial, and the algal pellet was resuspended in 1 ml of nanopure water. Three centrifugation and resuspension cycles were completed. The final algal suspension was filtered sequentially through 63- $\mu\text{m}$  and 30- $\mu\text{m}$  Nitex mesh screens to remove clumps of anemone tissue, and the algal suspension was stored in 1.5-ml microfuge tubes. Anemone and algae components were stored at  $-70^{\circ}\text{C}$  prior to being freeze-dried for 24 hours in scintillation vials or microfuge tubes using a VirTis Freezmobile freeze dryer (VirTis, Gardiner NY).

Posterior adductor muscle tissue of *M. californianus* was dissected from 8 mussels collected in summer and 10 collected in spring. Posterior adductor muscle integrates the carbon and nitrogen isotopic signatures of mussel food sources over relatively long time scales (Gorokhova & Hansson 1999), making it well suited for use as a mussel isotopic signature. Thawed adductor muscle was cleaned of any attached viscera, rinsed with nanopure water, placed in a 20-ml glass scintillation vial, and stored at -70°C prior to 24 hour freeze drying. All tools used in dissection (scalpel, scissors, forceps, and dissecting pan) were cleaned using the protocol described above for tentacle homogenization.

After freeze drying, anemone samples were placed into microfuge tubes and homogenized by grinding into a fine powder using pointed metal spatulas. Algae samples were homogenized in the microfuge tubes they were dried in. Mussel tissue was ground in a methanol-rinsed mortar and pestle until a fine powder was obtained. All samples were encapsulated in Costech tin capsules (Costech Analytical Technologies, Valencia CA) and shipped to the UC Davis Stable Isotope Facility for flow-through mass spectrometric analysis. Values are reported using  $\delta$  notation, which is the parts per thousand (‰) deviation of the sample  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratio from that of an accepted standard (PeeDee Belemnite for carbon and air for nitrogen, respectively).

During separation of symbionts from host anemone tissue, the homogenized host tissue forms a supernatant after centrifugation that is unlikely to contain heavier algal cells. However, the algal pellet may contain remnant host tissues in addition to symbionts, resulting in a contaminated symbiont  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signature. Furthermore, to trace energy flow between dietary sources and consumers isotopic signals must be

distinguishable between them (Phillips & Gregg 2001, Pitt et al. 2009). Therefore,  $\delta$  values of symbionts were inspected initially to see if they comprised a “clean” sample containing largely algae.  $\delta^{15}\text{N}$  gets progressively “heavier” as it moves up trophic levels, increasing by about 2.3‰ per level (McCutchan et al. 2003). Because of this predictable trend, any mean isotopic  $\delta^{15}\text{N}$  symbiont values that are equal to or higher than host tissue values are very unlikely, especially when allowing for trophic fractionation. The values for algae that were equal to or higher than host tissue values for  $\delta^{15}\text{N}$  were interpreted as evidence of anemone tissue contamination and neither  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values for these samples were included in the dietary analysis (see below).

To examine the relative contributions of symbiotic algae and *M. californianus* to host anemone carbon composition, the partitioning of carbon isotopes was examined using the Isoerror two-source mixing model (Phillips and Gregg 2001). This mass-balance model was used to calculate the proportion of host carbon derived from each source (symbiotic algae and *M. californianus*) using mean  $\delta^{13}\text{C}$  values of each component. With  $\delta^{13}\text{C}$  values from a consumer (C, represented by anemone tentacle tissue) and two dietary sources (A and B, represented by symbionts and mussels, respectively), the relative contributions of each source to the consumer were calculated using the following formulas:

$$f_A = \frac{\delta C - \delta B}{\delta A - \delta B} \text{ and } f_B = \frac{\delta C - \delta A}{\delta B - \delta A},$$

where  $f_A$  and  $f_B$  represent the proportions of source A and source B to the mixture. Standard errors of the source  $\delta^{13}\text{C}$  values were used to calculate standard errors of

estimated proportions using the following equations (presented for one source, but solved for both sources with substitution):

$$se^2_{f_A} = \frac{1}{(\delta_A - \delta_B)^2} (se_C^2 + f_A^2 \cdot se_A^2 + (1 - f_A)^2 \cdot se_B^2),$$

$$se_{f_A} = \sqrt{se^2_{f_A}}$$

where  $se^2_C$ ,  $se^2_A$ , and  $se^2_B$  are the standard errors of the mean  $\delta^{13}C$  signatures for the consumer (C) and its sources (A and B) (all equations, and accompanying Microsoft Excel spreadsheet, from Phillips and Gregg 2001).

Anemone tentacle tissue was classified as zoochlorellate (containing  $\geq 90\%$  zoochlorellae prior to separation), mixed zoochlorellate (containing 50-90% zoochlorellae), mixed zooxanthellate (containing 50-90% zooxanthellae) and zooxanthellate (containing  $\geq 90\%$  zooxanthellae); the same categorization was applied to the separated algal assemblages. Prior to addition to the model, dietary sources were corrected for carbon fractionation of 0.4‰, per trophic level, which is the average for aquatic systems provided by McCutchan *et al.* (2003).

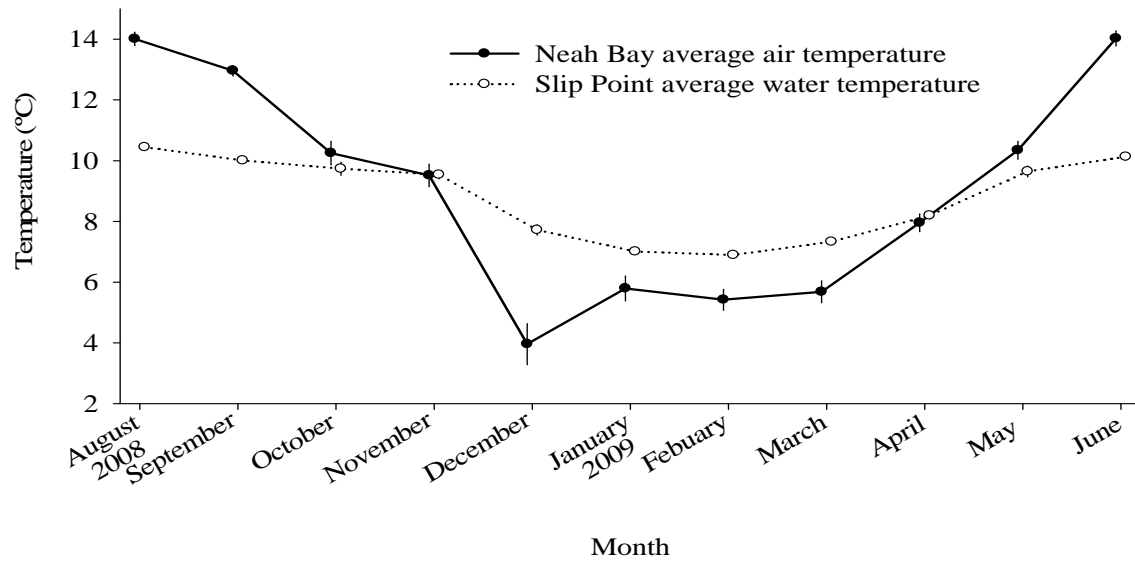
## Results

### Temperature monitoring

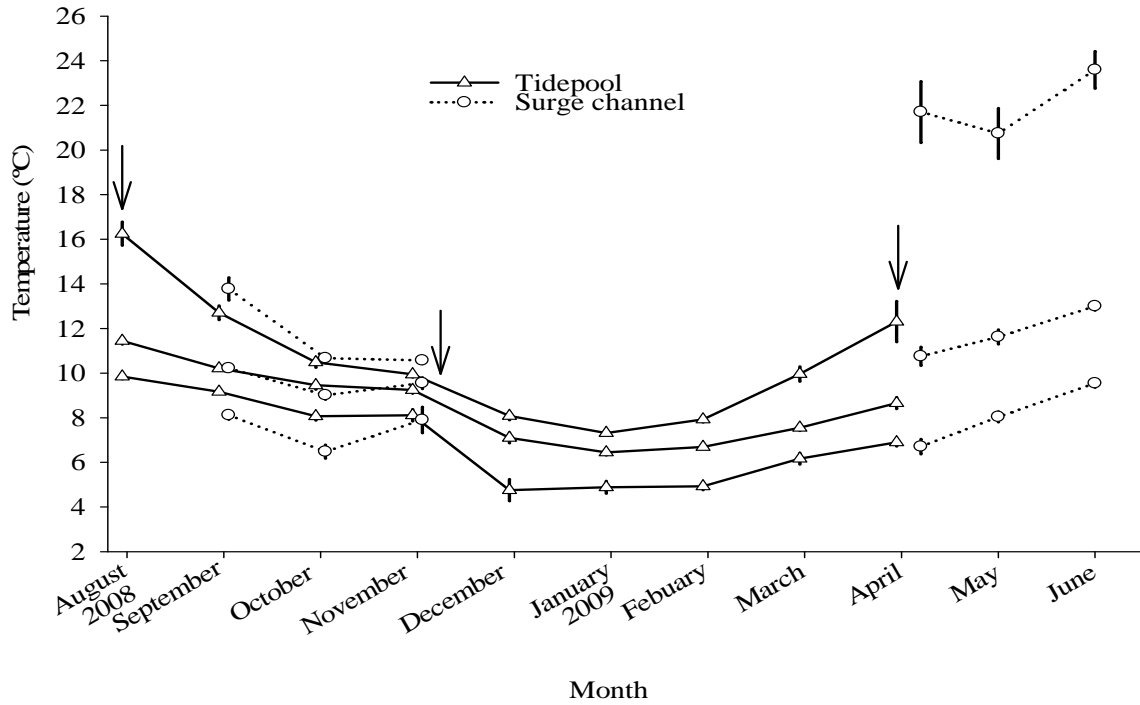
Air temperatures between July 2008 and June 2009 along the outer Strait of Juan de Fuca indicate a cool climate, with the warmest monthly average air temperatures at Neah Bay reaching a high of 14°C in August 2008 ( $14.0 \pm 0.22^\circ\text{C}$ ,  $n=31$ ; all values are reported as mean  $\pm$  SE) and June 2009 ( $14.0 \pm 0.24^\circ\text{C}$ ,  $n=18$ ; Figure 3). The coolest monthly air temperature at Neah Bay,  $4.0 \pm 0.68^\circ\text{C}$  ( $n=31$ ), was recorded in December 2008. Water temperatures at Slip Point were also relatively cool, ranging from a monthly high of  $10.5 \pm 0.11^\circ\text{C}$  ( $N=31$ ) in August 2008 and to a monthly low of  $6.9 \pm 0.04^\circ\text{C}$  ( $n=28$ ) in February 2009, a range of  $3.6^\circ\text{C}$  (Figure 3).

Although regional air and seawater temperatures were generally moderate, anemones at Slip Point were frequently exposed to air temperatures exceeding  $20^\circ\text{C}$  in the high surge channel microhabitat and sporadically exposed to water temperatures greater than  $20^\circ\text{C}$  in the high tidepool (Figure 4, Table 4). Average daily tidepool temperatures were highest in August 2008 ( $16.3 \pm 0.54^\circ\text{C}$ ,  $n=31$ ) and lowest in December 2008 ( $4.8 \pm 0.48^\circ\text{C}$ ,  $n=31$ ; Figure 4). Recorded temperatures in the tidepool ranged  $25^\circ\text{C}$ , from a low of  $-0.7^\circ\text{C}$  on December 20, 2008 to a high of  $24.4^\circ\text{C}$  on August 11, 2008 (Table 4). Average daily temperatures in the high surge channel were highest in June 2009 ( $23.6 \pm 0.82^\circ\text{C}$ ,  $n=18$ ) and lowest in October 2008 ( $6.5 \pm 0.31^\circ\text{C}$ ,  $n=31$ ; Figure 4). Recorded temperatures in the surge channel ranged  $27^\circ\text{C}$ , from a low of  $3.5^\circ\text{C}$  on November 15, 2008 to a high of  $30.6^\circ\text{C}$  on May 16, 2009 (Table 4). All extreme temperature events occurred during low tide exposure to air, as indicated by the





**Figure 3.** Monthly average seawater temperature ( $\pm$ SE) at Slip Pt, Clallam Bay WA recorded using in-situ temperatures data loggers and air temperature ( $\pm$ SE) at NOAA station NEAW1 for August 2008 to June 2009. Standard errors are an estimate of within-month variation.



**Figure 4.** Monthly temperature averages ( $\pm$ SE) in a tidepool (+1.59 m MLLW) and a surge channel (+1.19 m MLLW) at Slip Pt, Clallam Bay, WA. Uppermost lines represent average monthly high, middle lines represent average, and low lines represent average monthly low temperature within each microhabitat. Standard errors are an estimate of within-month variation. Arrows indicate anemone tentacle sampling dates.

**Table 4.** Monthly average temperature variation ( $^{\circ}\text{C} \pm \text{SE}$ ) above (positive values) and below (negative values) ambient seawater temperature as well as monthly extreme high and low temperatures in a tidepool (MLLW +1.59 m) and a surge channel (MLLW +1.19 m) at Slip Pt, Clallam Bay WA. p-values refer to paired t-test comparisons of tidepool and surge channel temperatures when both were recorded concurrently.

| Month                 | Tidepool                                       |          | Surge channel                                  |          |          |
|-----------------------|--|----------|--|----------|----------|
|                       | Variation from seawater ( $^{\circ}\text{C}$ ) | Extremes | Variation from seawater ( $^{\circ}\text{C}$ ) | p-value  | Extremes |
| August 2008           | 5.80 $\pm$ 0.53                                | 24.36    |  |          |          |
|                       | -0.60 $\pm$ 0.07                               | 8.64     |  |          |          |
| September             | 2.70 $\pm$ 0.29                                | 16.75    | 3.76 $\pm$ 0.49                                | p= 0.001 | 20.67    |
|                       | -0.84 $\pm$ 0.10                               | 7.47     | -1.89 $\pm$ 0.25                               | p<0.001  | 5.92     |
| October               | 0.75 $\pm$ 0.11                                | 12.90    | 0.93 $\pm$ 0.13                                | p=0.078  | 12.85    |
|                       | -1.67 $\pm$ 0.20                               | 6.33     | -3.26 $\pm$ 0.36                               | p<0.001  | 3.75     |
| November <sup>a</sup> | 0.44 $\pm$ 0.07                                | 11.35    | 0.72 $\pm$ 0.20                                | p=0.154  | 11.20    |
|                       | -1.43 $\pm$ 0.20                               | 5.61     | -1.94 $\pm$ 0.51                               | p=0.003  | 3.51     |
| December              | 0.37 $\pm$ 0.07                                | 10.12    |  |          |          |
|                       | -2.96 $\pm$ 0.37                               | -0.73    |  |          |          |
| January 2009          | 0.31 $\pm$ 0.05                                | 8.40     |  |          |          |
|                       | -2.13 $\pm$ 0.23                               | 0.74     |  |          |          |
| February              | 1.03 $\pm$ 0.14                                | 9.56     |  |          |          |
|                       | -1.96 $\pm$ 0.16                               | 3.43     |  |          |          |
| March                 | 2.63 $\pm$ 0.32                                | 13.86    |  |          |          |
|                       | -1.17 $\pm$ 0.22                               | 2.29     |  |          |          |
| April <sup>b</sup>    | 4.63 $\pm$ 0.90                                | 15.68    | 13.22 $\pm$ 1.37                               |          | 28.99    |
|                       | -0.77 $\pm$ 0.26                               | 5.87     | -1.78 $\pm$ 0.39                               |          | 3.54     |
| May                   |  |          | 11.09 $\pm$ 1.12                               |          | 30.60    |
|                       |  |          | -1.61 $\pm$ 0.25                               |          | 5.59     |
| June <sup>c</sup>     |  |          | 13.46 $\pm$ 0.85                               |          | 30.44    |
|                       |  |          | -0.59 $\pm$ 0.12                               |          | 7.37     |

Upper number in a box represents difference above average seawater; lower number represents difference below average seawater.

<sup>a</sup>Surge channel temperatures from 11/01/08-11/12/08

<sup>b</sup>Tidepool temperatures from 04/01/09- 04/10/09; surge channel temperatures from 4/13/09-4/30/09

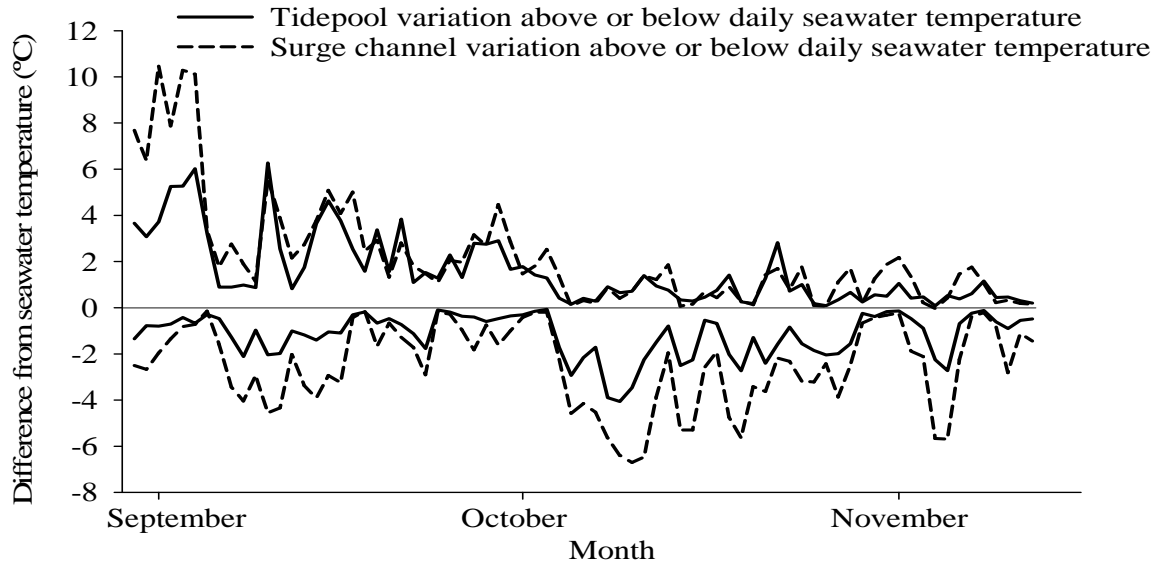
<sup>c</sup>Surge channel temperatures from 06/01/09- 06/18/09

comparatively small variation in seawater temperatures at Slip Point (Figure 3).

When data loggers were recording concurrently, the surge channel microhabitat exposed anemones to temperatures that tended toward higher highs and lower lows than those in the tidepool (Figure 5). In October 2008, temperatures in the surge channel were  $3.3 \pm 0.36^{\circ}\text{C}$  ( $n=31$ ) below that of ambient sea water and  $1.6^{\circ}\text{C}$  lower than the mean daily departure from seawater temperature in the tidepool (paired t-test,  $t=8.00$ ,  $df=30$ ,  $p<0.001$ ). In September 2008, temperatures in the surge channel ( $n=30$ ) averaged  $1.0^{\circ}\text{C}$  cooler than the tidepool in relation to ambient seawater temperature (paired t-test,  $t=6.97$ ,  $df=30$ ,  $p<0.001$ ). The surge channel also experienced significantly higher excursions in temperature in September than the tidepool, averaging  $1.1^{\circ}\text{C}$  higher daily (paired t-test,  $t=-3.67$ ,  $df=30$ ,  $p=0.001$ ). Extreme high and low temperatures were much greater in the surge channel (Table 3). For example, on a relatively warm day ( $18.3^{\circ}\text{C}$  at Neah Bay, September 1, 2008), the surge channel reached  $10.5^{\circ}\text{C}$  above the daily seawater temperature, while the tidepool temperature increased only by  $3.7^{\circ}\text{C}$  relative to seawater. On a relatively cold day ( $2.3^{\circ}\text{C}$  at Neah Bay, October 11, 2008), temperatures in the surge channel dropped  $6.5^{\circ}\text{C}$  relative to seawater, while tidepool temperatures only dropped  $2.3^{\circ}\text{C}$ .

#### Distribution of symbionts in tentacles of *A. xanthogrammica*

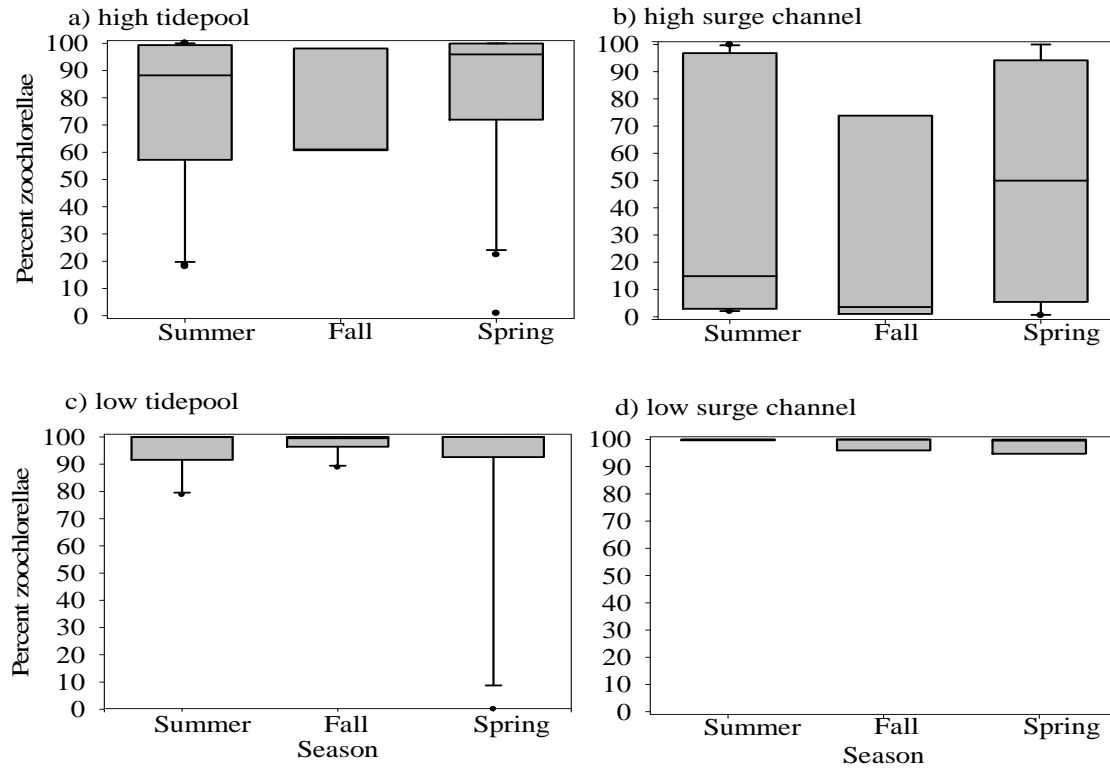
Zoochlorellate anemone tentacles were predominantly found in the low tidepool and low surge channel habitats, mixed tentacles containing primarily zoochlorellae were



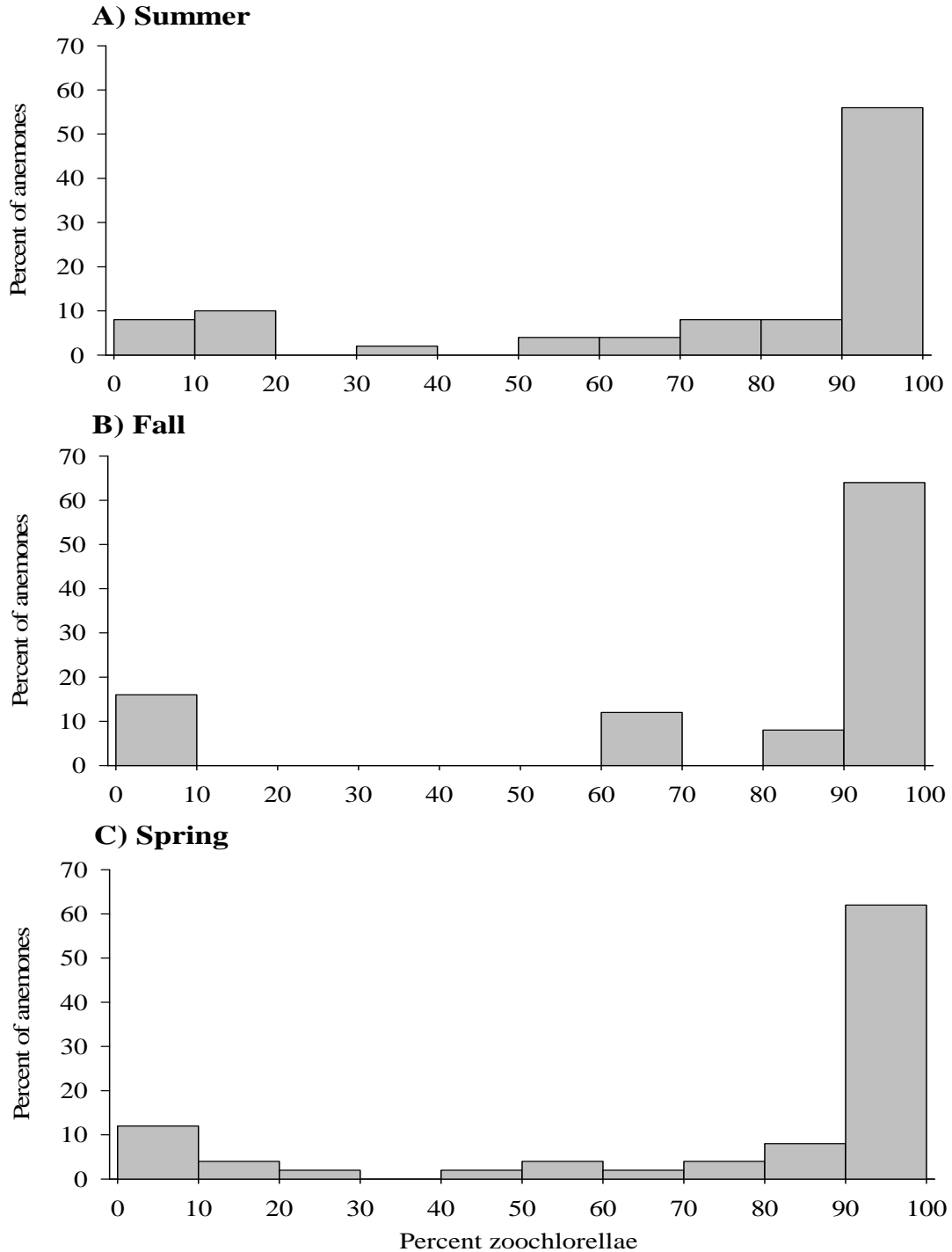
**Figure 5.** Difference above (positive values) and below (negative values) daily seawater temperature in a tidepool (+1.59 m MLLW) and a surge channel (+1.19 m MLLW) at Slip Pt, Clallam Bay WA from 8/31/08-11/12/08.

found in high tidepool microhabitats, and mixed tentacles containing a comparatively high proportion of zooxanthellae were found in the surge channel at high tide heights (Figure 6a-d). This pattern did not change seasonally, with the exception of one low tidepool anemone that demonstrated a dramatic shift from 93% zoochlorellate in summer 2008 and 89% zoochlorellate in fall 2008 to 100% zooxanthellate in spring 2009. High tidepools yielded anemone tentacles containing a significantly higher proportion of zoochlorellae than the high surge channels in all seasons (contrasts between the high tidepool and surge channel: summer  $p=0.007$ , fall  $p=0.011$ , spring  $p=0.011$ ;  $\alpha$  adjusted to 0.025). Despite containing a higher proportion of zooxanthellae than any other microhabitat, high surge channel anemones yielded tentacles ranging from nearly 100% zoochlorellae to nearly 100% zooxanthellae (Figure 6b). Interestingly, nearly all of these high surge channel anemone tentacles contained some degree of algal mixing, with only 3 anemones sampled across seasons (9% of the total in this microhabitat) containing tentacles with pure unialgal populations, two of which were composed of zoochlorellae. This is similar to high tidepool anemone tentacles, where 16% of tentacles contained unialgal zoochlorellae populations, but in contrast to the low tidepool and low surge channel, where ~50% of tentacles contained unialgal populations of zoochlorellae.

Overall, zoochlorellae were the dominant symbiont at Slip Point, with 81% of all sampled anemone tentacles containing algal assemblages comprised of  $\geq 50\%$  zoochlorellae. In all samplings, 56% to 64% of anemones contained  $\geq 90\%$  zoochlorellae, and 8% to 16% contained  $\geq 90\%$  zooxanthellae (Figure 7). Mixed algal assemblages of between 10% and 90% of one alga were found in 36% of anemone tentacles in summer 2008, and 20% and 26% in fall 2008 and spring 2009, respectively.



**Figure 6.** Boxplots of percent zoochlorellae in **a)** high tidepool **b)** high surge channel **c)** low tidepool and **d)** low surge channel microhabitats. Black lines within a boxplot represents the median percent zoochlorellae, gray areas below and above the median represent the 25<sup>th</sup> percentile and 75<sup>th</sup> percentiles, lines below and above boxes represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles, and circles represent outliers. The 10<sup>th</sup> and 90<sup>th</sup> percentiles are only calculated for  $n \geq 9$ .

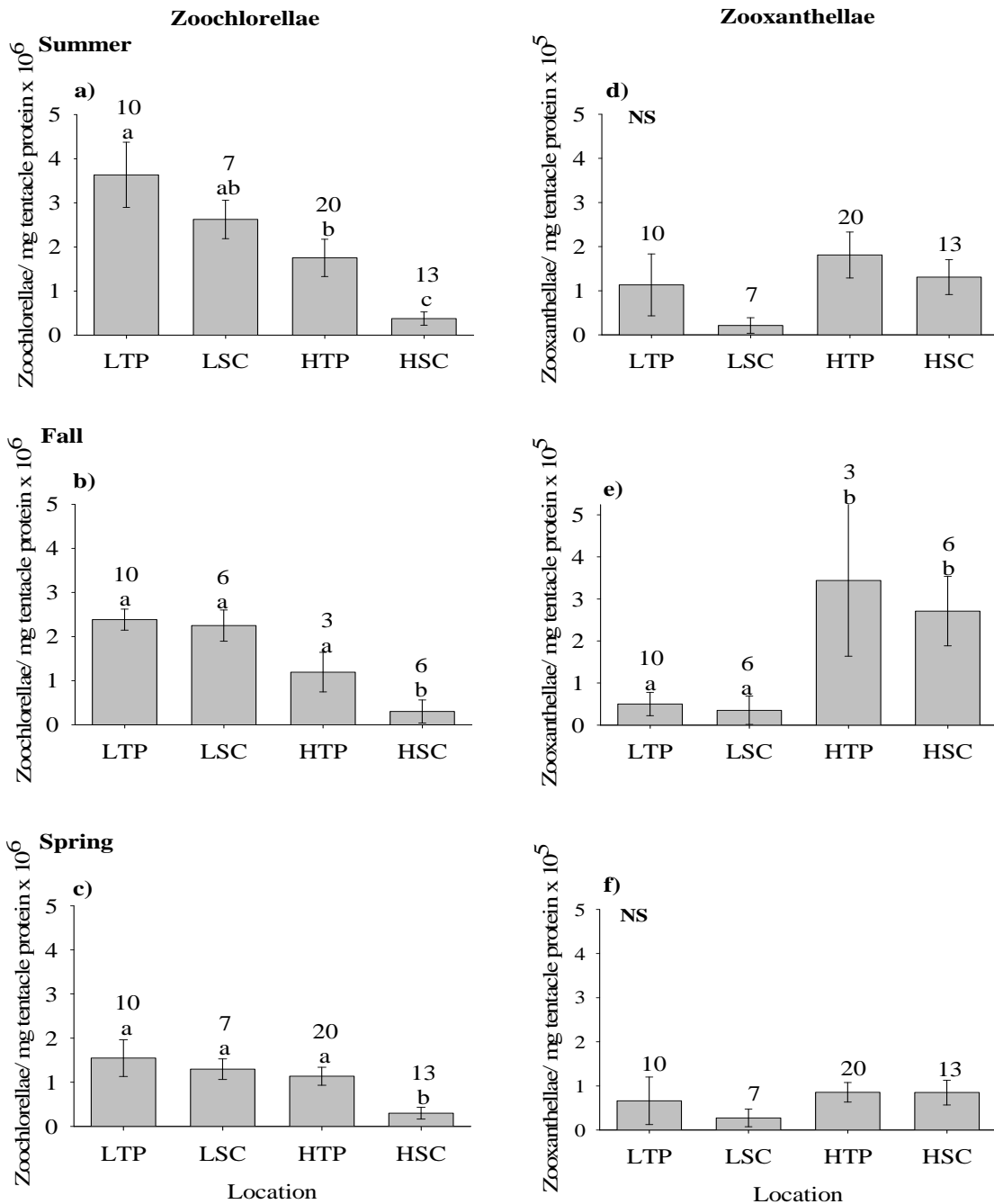


**Figure 7.** Histogram of percent zoochlorellae in tentacles of anemones sampled in each season (across all microhabitats). Bins encompass a 10% range; number of anemones within a bin should be interpreted as having percent zoochlorellae composition within 10%. Summer and spring N=50; fall N=25.



### Algal density

The patterns evident in algal proportions between microhabitats are also evident in the abundance (= density) of both algae between microhabitats. In all seasons, anemone tentacles from the high surge channel microhabitat contained significantly lower densities of zoochlorellae than those from all other microhabitats (Figure 8a-c). In summer, anemone tentacles in the high surge channel contained  $3.75 \times 10^5 \pm 1.04 \times 10^5$  zoochlorellae · mg<sup>-1</sup> tentacle protein, a significantly lower density of zoochlorellae than in anemone tentacles from the high tidepool (p=0.009), low surge channel (p=0.001) and low tidepool (p<0.001) microhabitats. In fall 2008, anemone tentacles in the high surge channel had a significantly lower density of zoochlorellae ( $3.02 \times 10^5 \pm 2.59 \times 10^5$  zoochlorellae · mg<sup>-1</sup> tentacle protein) than those in the high tidepool (p=0.034), low surge channel (p<0.001) and low tidepool (p<0.001) microhabitats. Densities in the latter three microhabitats did not differ significantly from each other (Tukey pairwise comparisons, all p>0.05). Spring 2009 showed a similar pattern to fall 2008, with anemone tentacles in the high surge channel containing a significantly lower density of zoochlorellae than anemone tentacles in the high tidepool (p=0.04), low surge channel (p=0.007) and low tidepool (p=0.002) microhabitats, which did not differ significantly from each other in algal density (Tukey pairwise comparisons, all p>0.05). There was a trend toward decreasing density of zoochlorellae between summer 2008 and spring 2009 in low tidepool and low surge channel anemone tentacles. Tentacles from these microhabitats showed decreases of approximately 57% and 50% respectively in this time period (Figure 8a-c).



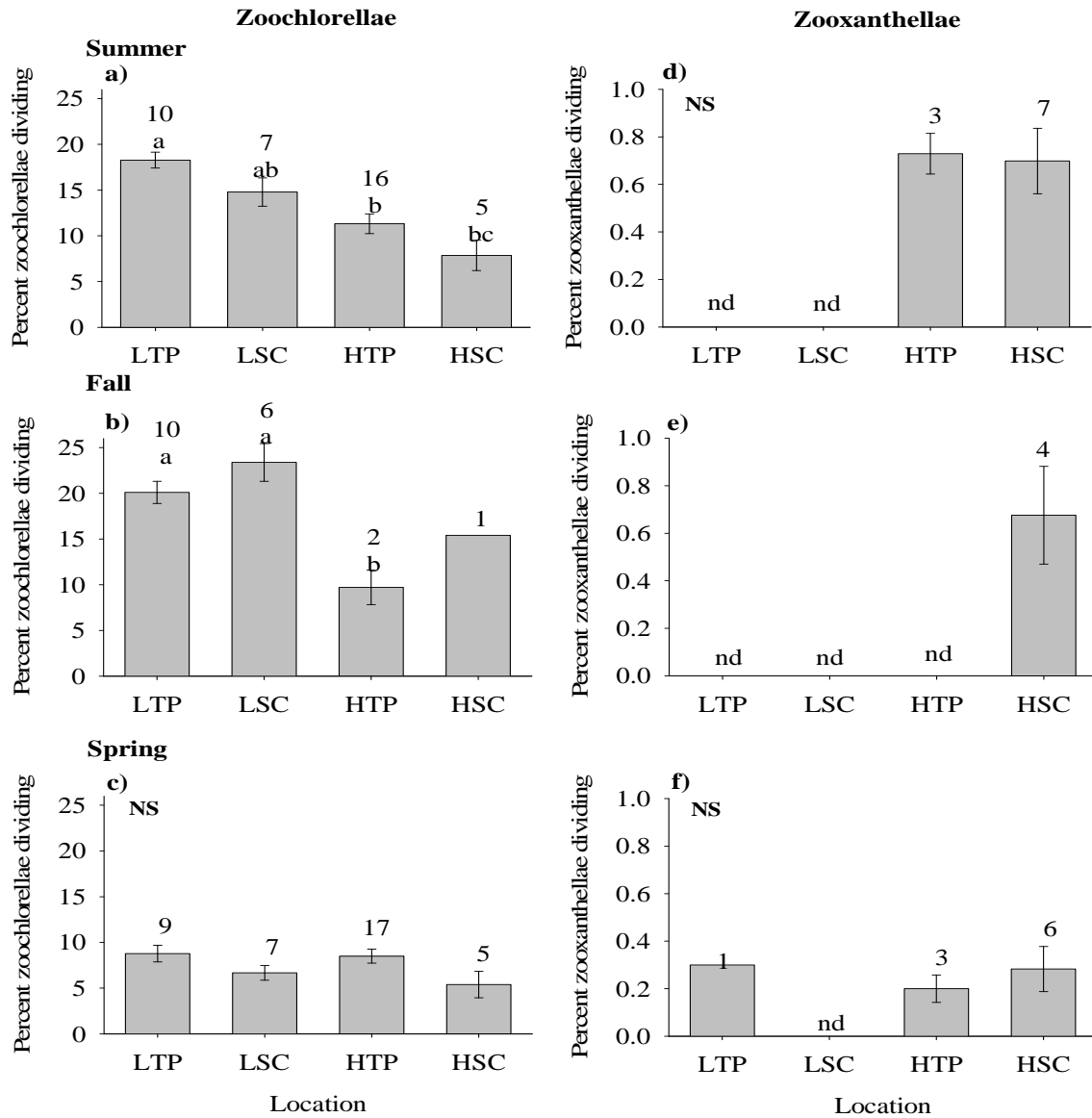
**Figure 8.** Average ( $\pm$  SE) density of zoochlorellae (a-c) and zooxanthellae (d-f) in anemone tentacles from low tidepool (LTP), low surge channel (LSC), high tidepool (HTP) and high surge channel (HSC) microhabitats seasonally. Y-axis scales are one order of magnitude higher for zoochlorellae densities than for zooxanthellae densities. Numbers above bars indicate sample size; different letters within a season and symbiont type indicate significant difference between microhabitats at  $p < 0.05$ . NS= no significant difference in algal densities between any microhabitat within a season.

Density of zooxanthellae was constantly low in all microhabitats, in contrast to the distinct patterns observed in density of zoochlorellae between microhabitats. Densities of zooxanthellae in tentacles of anemones at Slip Point were approximately an order of magnitude lower than densities of zoochlorellae in all seasons (Figure 8d-f). The density of zooxanthellae remained constant across microhabitats in all seasons except for fall 2008 (one-way ANOVA,  $n=25$ ,  $p=0.002$ ), when anemone tentacles in the high tidepool and high surge channel contained significantly more zooxanthellae than those in the low tidepool and low surge channel (Tukey HSD pairwise comparisons, all  $p<0.05$ ).

The goal to repeatedly sample individuals yielded information about anemone population characteristics. Nearly all anemones resampled in fall 2008 and spring 2009 (Table 3) did not move from their location in summer 2008, as indicated by photographs and transect markings. Four anemones disappeared between July 2008 and April 2009. Three of these were in the high surge channel microhabitat, resulting in 23% loss of anemones over approximately 8.5 months in this exposed environment. One individual from a high tidepool disappeared, resulting in a 5% reduction of the anemone population in this microhabitat.

#### Mitotic index

In contrast to the marked decline in the proportions and density of zoochlorellae between the high surge channel and high tidepool microhabitats, mitotic index (MI) did not differ between these microhabitats (Figure 9a-c). In summer 2008, the MI of zoochlorellae in tentacles of anemones in the high surge channel was  $7.85 \pm 1.66\%$ , less than half that of those in the low surge channel ( $14.78 \pm 1.55\%$ ;  $p=0.006$ ) and the

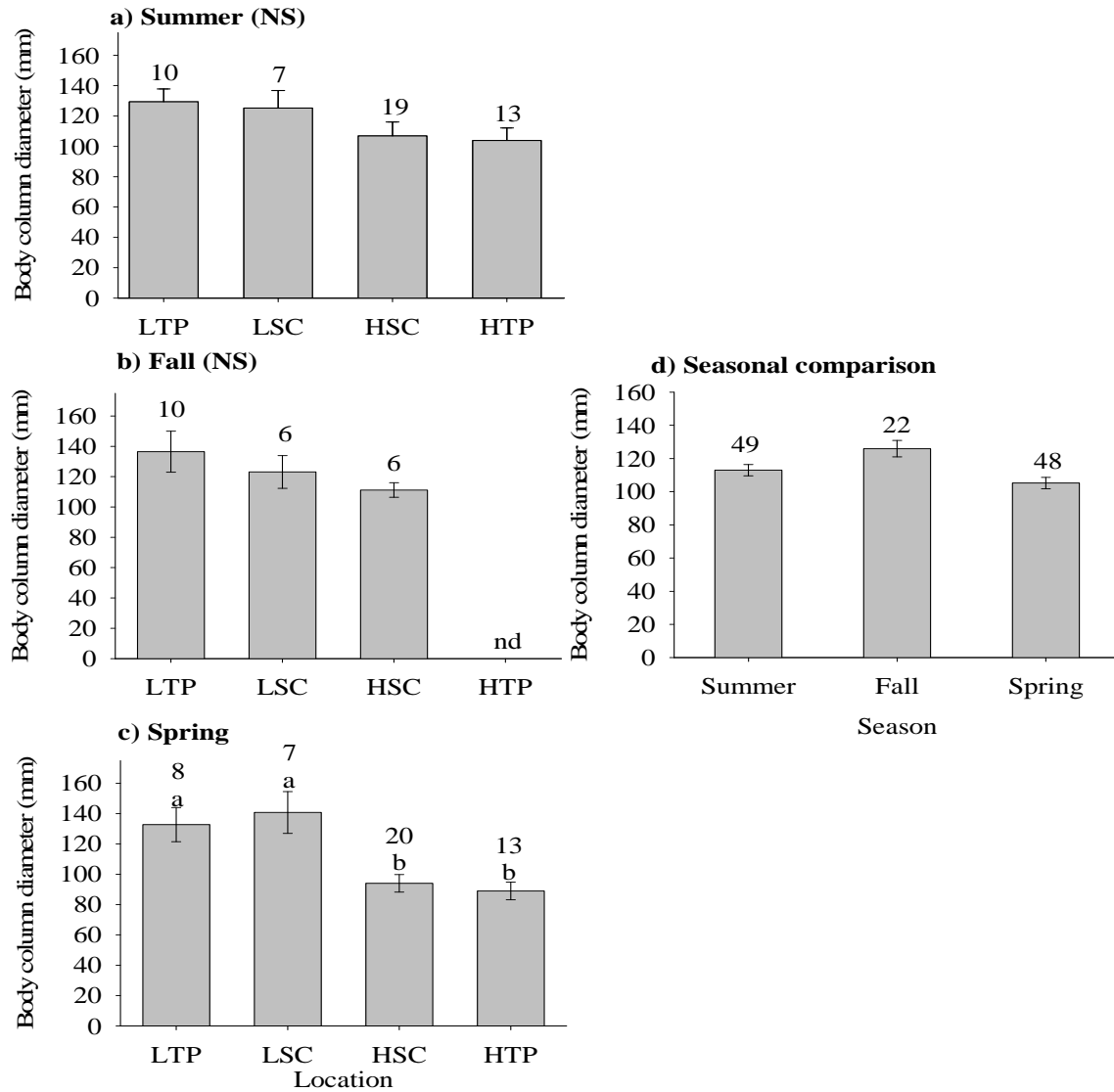


**Figure 9.** Mitotic index ( $\pm$  SE) of zoochlorellae (a-c) and zooxanthellae (d-f) in anemone tentacles from low tidepool (LTP), low surge channel (LSC), high tidepool (HTP) and high surge channel (HSC) microhabitats seasonally (a-c). Numbers above bars indicate sample size; different letters within a season and symbiont type indicate significant difference between microhabitats at  $p < 0.05$ . nd= no data; ns= no significant difference between any microhabitat within a season.

low tidepool ( $18.28 \pm 0.87\%$ ;  $p < 0.001$ ) microhabitats, but not significantly different from those in the high tidepool ( $12.02 \pm 0.86\%$ ;  $p = 0.068$ ). In fall 2008, zoochlorellae in high tidepool anemone tentacles had a MI of  $9.72 \pm 1.88\%$ , significantly lower than the MI of zoochlorellae in tentacles from the low tidepool and low surge channel (contrast of high tidepool vs. low tidepool and low surge channel,  $p = 0.001$ ). The only high surge channel anemone with zoochlorellate tentacles sampled in fall 2008 had a mitotic index of  $15.40\%$ ; this small sample did not allow trends in the high surge channel to be examined. There were no significant differences in MI between microhabitats in spring 2009 (one-way ANOVA,  $p = 0.079$ ,  $n = 38$ ). Seasonally, the MI of zoochlorellae across all microhabitats was highest in fall 2008 ( $19.80 \pm 1.29\%$ ,  $n = 19$ ), intermediate in summer 2008 ( $13.63 \pm 0.77\%$ ,  $n = 38$ ), and lowest in spring 2009 ( $7.82 \pm 0.49\%$ ,  $n = 38$ ). The MI of zooxanthellae was below 1% in all seasons, with no significant patterns evident among microhabitats (Figure 9d-f). However, sample sizes were low, allowing for only limited power to examine patterns in summer 2008 and spring 2009 ( $n = 10$  in each season), and no comparisons were possible in fall 2008 ( $n = 4$ , all in the high surge channel).

#### Anemone size

Anemones were the same size in all microhabitats in summer and fall 2008, averaging  $113 \pm 5$  mm and  $126 \pm 7$  mm body column diameter, respectively (Figure 10a-b; one-way ANOVA: summer 2008  $p = 0.157$ ,  $n = 49$ ; fall 2008  $p = 0.344$ ,  $n = 22$ ). There was a non-significant trend towards larger individuals in the low tide height microhabitats as compared to the high microhabitats in summer (Figure 10a; Tukey HSD pairwise comparisons, all  $p \geq 0.203$ ). In spring 2009, anemones differed in size among



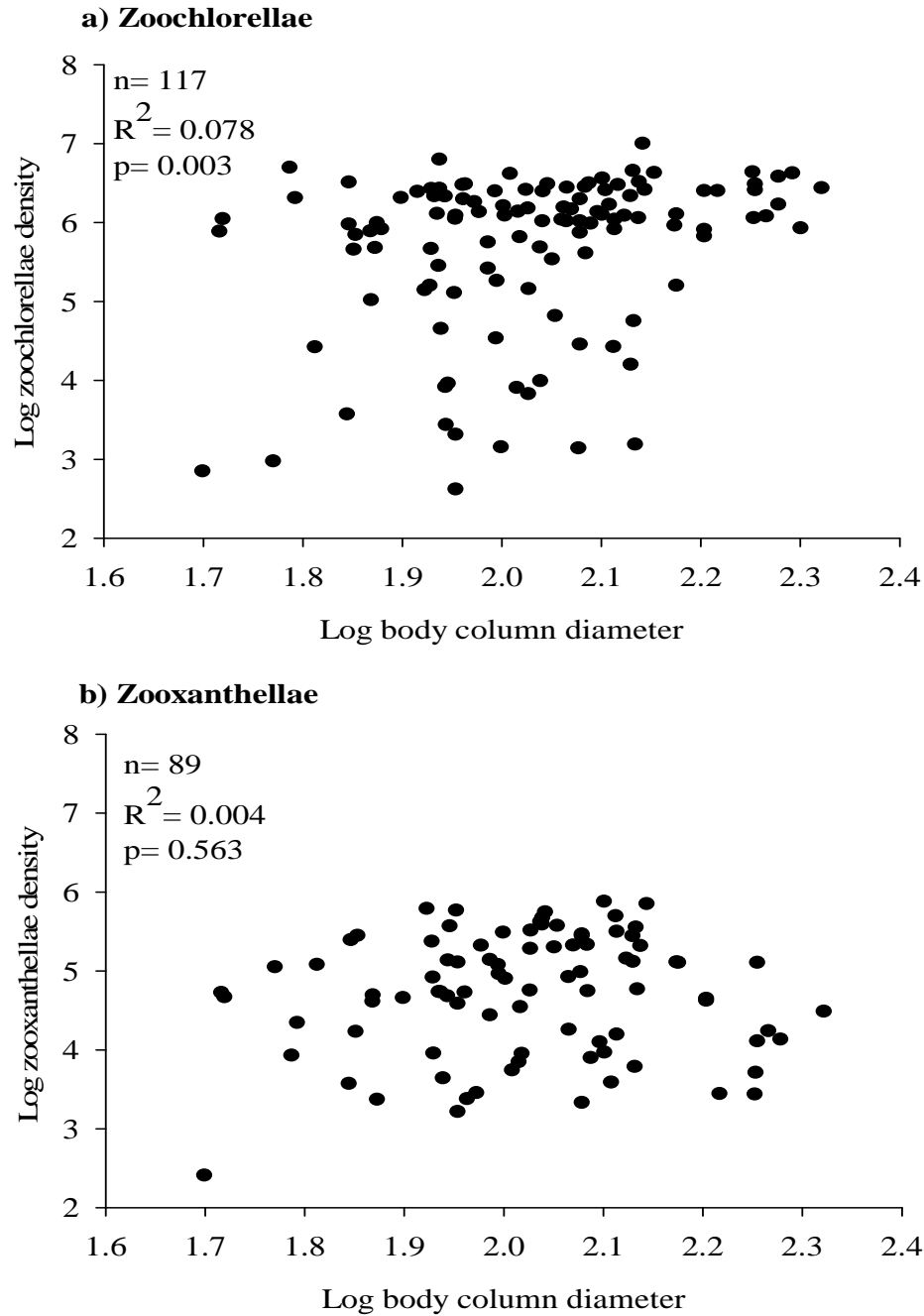
**Figure 10.** Average body column diameter ( $\pm$  SE) in anemones from low tidepool (LTP), low surge channel (LSC), high tidepool (HTP) and high surge channel (HSC) microhabitats seasonally (a-c) as well as body column diameter averaged across all microhabitats) between seasons (d). Numbers above bars indicate sample size; different letters within a season and symbiont type show significant differences at  $p < 0.05$ . nd= no data; NS= no significant difference in body column diameter between any microhabitat within a season.

microhabitats (Figure 10c, one-way ANOVA,  $n=48$ ,  $p<0.001$ ). Anemones from the high tidepool ( $89 \pm 6$  mm) and high surge channel ( $94 \pm 6$  mm) were significantly smaller than those in the low tidepool ( $141 \pm 14$  mm) and low surge channel ( $133 \pm 11$  mm) (Tukey HSD pairwise comparisons, all  $p \leq 0.015$ ). Anemones showed a trend towards increasing size in fall 2008 ( $126 \pm 7$  mm,  $n=22$ ) as compared to summer 2008 ( $113 \pm 5$  SE,  $n=49$ ) and spring 2009 ( $105 \pm 5$  mm,  $n=48$ ) (Figure 10d).

#### Potential effects of anemone size and tentacle sampling on algal density

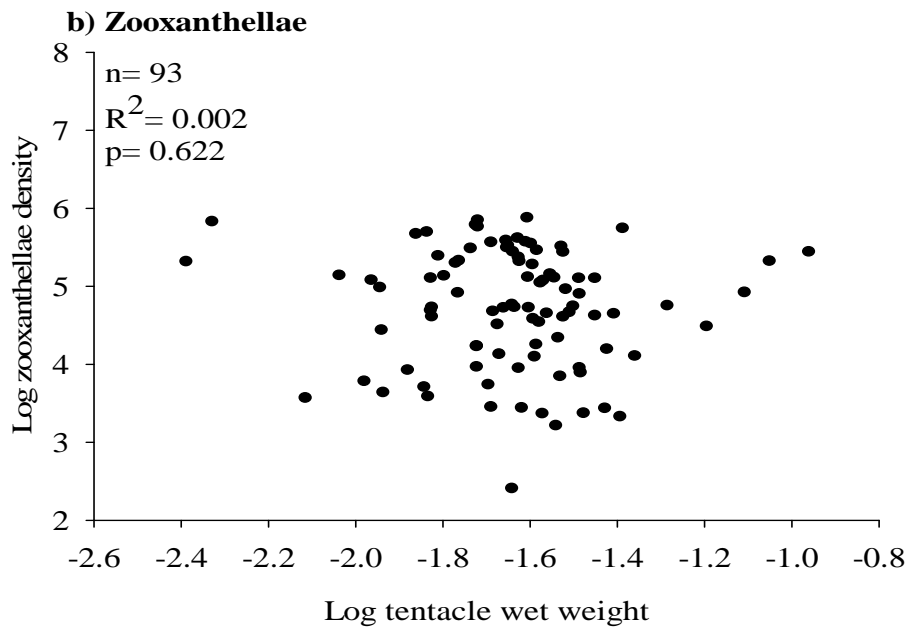
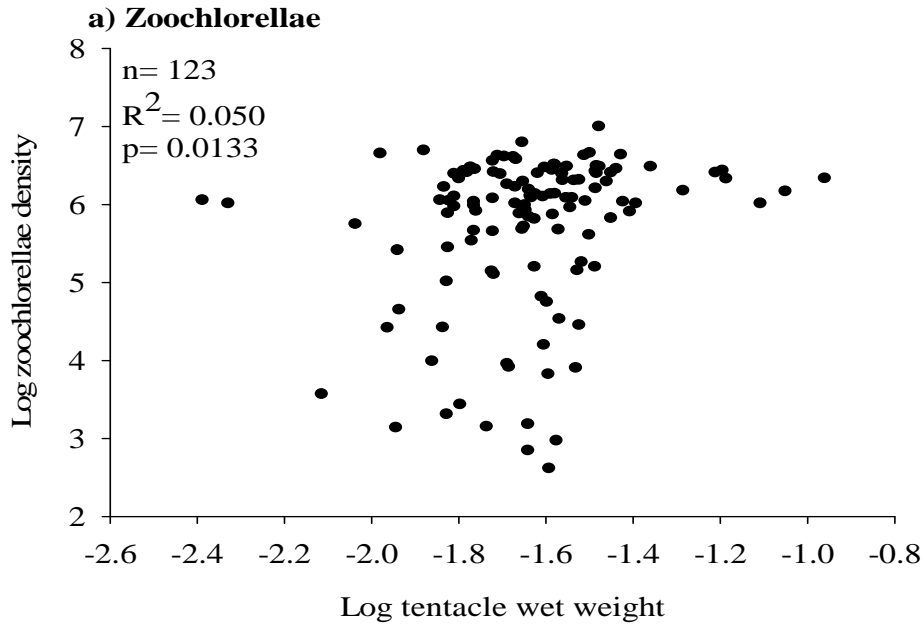
Although the goal of this study was to examine the relationship between microhabitat and the distribution and density of zooxanthellae and zoochlorellae in *A. xanthogrammica* tentacles, other factors may contribute to observed patterns. Anemone size and tentacle sample size were examined to determine their relationship with algal density (Figure 11, Figure 12). Anemone size was not correlated with the density of zooxanthellae in tentacles (log-scale correlation,  $R^2 = 0.004$ ,  $p=0.563$ ; Figure 11). Although a correlation between anemone size and density of zoochlorellae was obtained, it explains less than 8% of the variation and may be driven by the large sample size (log-scale correlation,  $R^2=0.078$ ,  $p=0.003$ ; Figure 11). Tentacle sample size (the wet weight of tentacles used to obtain biomass measures) was not correlated with zooxanthellae density (log-scale correlation,  $R^2 = 0.002$ ,  $p=0.622$ ; Figure 12). The effect of tentacle sample size on zoochlorellae density was small, explaining only 5% of variation in density (log-scale correlation,  $R^2=0.05$ ,  $p=0.013$ ; Figure 12).

Tentacles haphazardly collected from one section of an anemone's tentacle crown may yield different densities of zoochlorellae from those collected from another section



**Figure 11.** Correlations of anemone size (body column diameter) vs. **a)** zoochlorellae and **b)** zooxanthellae densities. All samples across all seasons and microhabitats. All data are plotted logarithmically.





**Figure 12.** Correlations of sample size (tentacle wet weight) vs. **a)** zoochlorellae and **b)** zooxanthellae densities. All samples across all seasons and microhabitats. All data are plotted logarithmically.

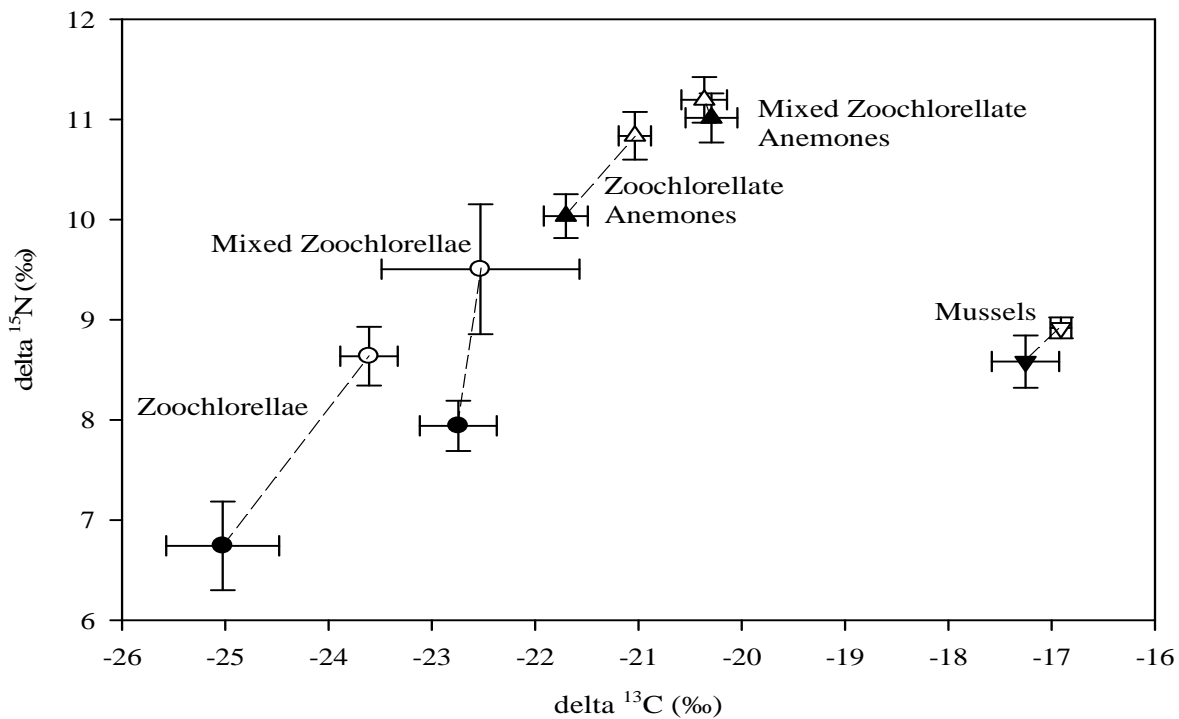
(nested ANOVA,  $p < 0.001$ ; Table 5). However, the extent of this difference is predicted to be considerably less than the difference in zoochlorellae density between any two anemones (nested ANOVA,  $p < 0.001$ , Table 5). The parameter partial  $\eta^2$ , an estimate of effects size (the proportion of the total (effects + error) variance in densities of zoochlorellae explained by the treatments “between anemones” and “within anemones”; Figure 5), is considerably larger between anemones than within anemones (0.889 vs. 0.589, respectively). Therefore, the variability resulting from haphazard tentacle clipping is not likely to be greater than the variability between any two randomly selected anemones.

#### Stable isotope analysis

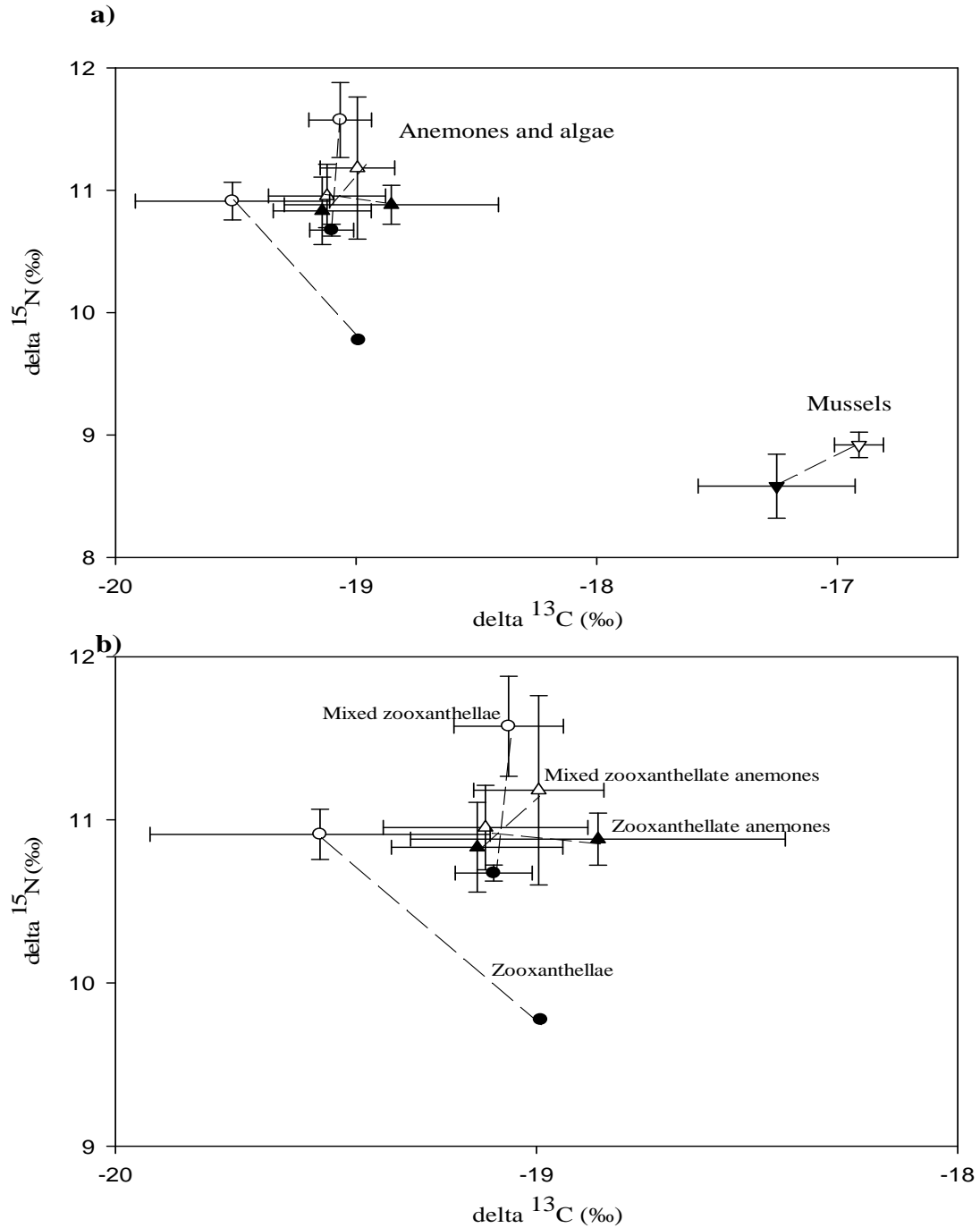
All components included in the dietary analysis of zoochlorellate and mixed zoochlorellate anemones had clearly distinguishable isotopic signatures (=  $\delta$  values, the parts per thousand (‰) deviation of the sample  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratio from that of an accepted standard) (Figure 13). Isolated symbionts had the lowest  $\delta^{13}\text{C}$  values, anemones were intermediate, and mussels were highest (Figure 13, Table 6). The  $\delta^{15}\text{N}$  values for anemone sources (symbiont assemblages and mussels) were lower than those from anemone tissue (Figure 13, Table 6). However,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of zooxanthellate and mixed zooxanthellate anemones were not distinguishable from isolated algae, as indicated by the considerable overlap between the  $\delta^{15}\text{N}$  of isolated symbionts and host tissue (Figure 14). Furthermore, microscopic inspection of isolated zoochlorellae and mixed zoochlorellae algal assemblages revealed only slight contamination with host tissue; zooxanthellae and mixed zooxanthellae were more heavily contaminated.

**Table 5.** Nested ANOVA table of the variability in zoochlorellae density estimates from tentacles obtained from individual anemones (“between anemones”; n=10) as compared to variability within each anemone (“within an anemone”; n=29) . Each anemone was sampled three times, except for one anemone that was only sampled twice.

| Source            | SS       | df | MS       | F                   | p      | partial $\eta^2$ |
|-------------------|----------|----|----------|---------------------|--------|------------------|
| between anemones  | 3.21E+14 | 9  | 3.97E+12 | $F_{9,19} = 288.49$ | <0.001 | 0.889            |
| tentacles         | 4.96E+12 | 19 | 1.37E+10 |                     |        |                  |
| within an anemone | 4.96E+12 | 19 | 1.37E+10 | $F_{19,87} = 42.9$  | <0.001 | 0.589            |
| tentacles         | 2.42E+12 | 87 | 3.20E+08 |                     |        |                  |



**Figure 13.**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values ( $\pm\text{SE}$ ) for zoochlorellate ( $\geq 90\%$  zoochlorellae) and mixed zoochlorellate (50-90% zoochlorellae) anemone tentacle tissue, isolated symbionts composed of zoochlorellae ( $\geq 90\%$  zoochlorellae) and mixed zoochlorellae (50-90% zoochlorellae) assemblages, and tissue from *M. californianus* mussels. Dashed lines connect summer values (dark symbols) to spring values (open symbols). Sample sizes are listed in Table 5.



**Figure 14. a)**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values ( $\pm$  SE) for zooxanthellate ( $\geq 90\%$  zooxanthellae) and mixed zooxanthellate (10-50% zooxanthellae) anemone tentacle tissue, isolated symbionts composed of zooxanthellae ( $\geq 90\%$  zooxanthellae) and mixed zooxanthellae (10-50% zooxanthellae) assemblages, and tissue from *M. californianus* mussels. **b)** An enlargement of the anemone and algal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values. Triangles represent anemones, circles represent symbionts. Dashed lines connect summer values (dark symbols) to spring values (open symbols). Sample sizes are listed in Table 5.

**Table 6.** Average ( $\pm$  SE)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (‰) values for *A. xanthogrammica* tentacle tissue isolated from hosts of varying symbiont composition, grouped as zoochlorellate ( $\geq 90\%$  zoochlorellae/ mg anemone tentacle protein), mixed zoochlorellate (50-90% zoochlorellae), mixed zooxanthellate (10-50% zoochlorellae), and zooxanthellate ( $\leq 10\%$  zoochlorellae), isolated symbionts, and tissue from *M. californianus* mussels.

|                      | $\delta^{13}\text{C}$ (‰) |                   | $\delta^{15}\text{N}$ (‰) |                  | n      |        |
|----------------------|---------------------------|-------------------|---------------------------|------------------|--------|--------|
|                      | Summer                    | Spring            | Summer                    | Spring           | Summer | Spring |
| <b>Anemone</b>       |                           |                   |                           |                  |        |        |
| Zoochlorellate       | -21.70 $\pm$ 0.21         | -21.04 $\pm$ 0.16 | 10.03 $\pm$ 0.22          | 10.84 $\pm$ 0.24 | 20     | 26     |
| Mixed zoochlorellate | -20.29 $\pm$ 0.25         | -20.36 $\pm$ 0.22 | 11.02 $\pm$ 0.25          | 11.20 $\pm$ 0.23 | 7      | 7      |
| Mixed zooxanthellate | -19.14 $\pm$ 0.20         | -18.99 $\pm$ 0.16 | 10.83 $\pm$ 0.27          | 11.18 $\pm$ 0.58 | 6      | 4      |
| Zooxanthellate       | -18.85 $\pm$ 0.44         | -19.12 $\pm$ 0.24 | 10.88 $\pm$ 0.16          | 10.95 $\pm$ 0.26 | 3      | 5      |
| <b>Algae</b>         |                           |                   |                           |                  |        |        |
| Zoochlorellae        | -25.02 $\pm$ 0.55         | -23.61 $\pm$ 0.28 | 6.74 $\pm$ 0.44           | 8.64 $\pm$ 0.29  | 13     | 14     |
| Mixed zoochlorellae  | -22.74 $\pm$ 0.38         | -22.53 $\pm$ 0.96 | 7.94 $\pm$ 0.25           | 9.50 $\pm$ 0.65  | 3      | 3      |
| Mixed zooxanthellae  | -19.10 $\pm$ 0.09         | -19.07 $\pm$ 0.13 | 10.67 $\pm$ 0.05          | 11.57 $\pm$ 0.31 | 2      | 3      |
| Zooxanthellae        | -18.99                    | -19.51 $\pm$ 0.40 | 9.77                      | 10.91 $\pm$ 0.16 | 1      | 2      |
| <b>Mussel</b>        |                           |                   |                           |                  |        |        |
|                      | -17.25 $\pm$ 0.32         | -16.91 $\pm$ 0.10 | 8.58 $\pm$ 0.26           | 8.92 $\pm$ 0.10  | 8      | 10     |

Summer samples collected 7/30/08- 7/31/08; Spring samples collected 4/10/09-4/11/09.  
n= sample size.

Zooxanthellae appeared to “stick” to host tissue and were harder to separate by centrifugation.

Algae isolated from anemone tentacles showed more variation in  $\delta^{13}\text{C}$  than mussel or anemone tentacle tissue, both between summer 2008 and spring 2009 and within a season (indicated with standard error, Figure 13; Table 6). The  $\delta^{13}\text{C}$  values of zoochlorellate and mixed zoochlorellate anemones were intermediate between the  $\delta^{13}\text{C}$  values of their zoochlorellae and mixed zoochlorellae algal assemblages and of mussels (Figure 13). Zoochlorellae yielded the most negative  $\delta^{13}\text{C}$  values, averaging  $-25.02 \pm 0.55\text{‰}$  in summer and  $-23.61 \pm 0.28\text{‰}$  in spring (Table 6). Mixed zoochlorellae samples had more positive  $\delta^{13}\text{C}$  values of  $-22.74 \pm 0.38\text{‰}$  in summer and  $-22.53 \pm 0.96\text{‰}$  in spring. Mussel tissue  $\delta^{13}\text{C}$  was comparatively higher than both algal assemblages, averaging  $-17.25 \pm 0.32\text{‰}$  in summer and  $-16.91 \pm 0.10\text{‰}$  in spring.

In both summer 2008 and spring 2009, zoochlorellae and mixed zoochlorellae provided more carbon to the host than mussels, as indicated with the Isoerror dietary mixing model (Phillips and Gregg 2001). Zoochlorellae and mixed zoochlorellae provided  $\sim 62\%$  ( $\pm 5\text{-}7\%$  SE) of host carbon in summer 2008 and nearly  $70\%$  ( $\pm 4\text{-}12\%$  SE) of host carbon in spring 2009 (Table 7). The mixing model was not used to estimate algal contributions to zooxanthellate and mixed zooxanthellate anemones because the  $\delta^{15}\text{N}$  values were not greater for anemone tissue than symbionts (as would be expected following trophic transfer, McCutchan et al. 2003), implying symbiont contamination with anemone tissue (Figure 14a-b).

Although the hypotheses were structured for dietary comparisons of anemones between different microhabitats, it was only possible to compare between algal types

**Table 7.** Relative contribution (percent  $\pm$  SE) of symbiotic zoochlorellae or mixed zoochlorellae and *M. californianus* to *A. xanthogrammica* diet. Carbon isotope fractionation is assumed to be 0.4 ‰ per trophic level (McCutchan *et al.* 2003).

| <b>Anemone</b>       | Summer          |                         | Spring          |                         |
|----------------------|-----------------|-------------------------|-----------------|-------------------------|
|                      | symbiotic algae | <i>M. californianus</i> | symbiotic algae | <i>M. californianus</i> |
| Zoochlorellate       | 62 $\pm$ 5      | 38 $\pm$ 5              | 68 $\pm$ 4      | 32 $\pm$ 4              |
| Mixed Zoochlorellate | 63 $\pm$ 7      | 37 $\pm$ 7              | 69 $\pm$ 12     | 31 $\pm$ 12             |



(zoochlorellae and mixed zoochlorellae) due to small sample sizes for all anemone-algal combinations except for zoochlorellate anemones and zoochlorellae (Table 6). Even within this group, isotopic variation between anemones inhabiting different microhabitats was slight. In summer, there were small (but statistically significant) differences between  $\delta^{13}\text{C}$  values of zoochlorellate anemones in different microhabitats (one-way ANOVA,  $p=0.006$ ,  $n=20$ ). Only samples from the high surge channel ( $\delta^{13}\text{C}= -20.20 \pm 1.24$ ) and low tidepool ( $\delta^{13}\text{C}= -22.56 \pm 0.37$ ,  $n=5$ ) differed from each other (Tukey pairwise comparison,  $p=0.006$ ); the low surge channel ( $\delta^{13}\text{C}= -21.91 \pm 0.57$ ,  $n=5$ ) and high tidepool ( $\delta^{13}\text{C}= -21.41 \pm 0.83$ ,  $n=8$ ) samples did not differ in  $\delta^{13}\text{C}$  from either the low tidepool or high surge channel (Tukey pairwise comparisons, all  $p \geq 0.05$ ). In spring 2009, there were no significant differences between the  $\delta^{13}\text{C}$  values of zoochlorellate anemones from any microhabitat (one-way ANOVA,  $p=0.258$ ,  $n=26$ ).

## Discussion

The observed distribution of zoochlorellae and zooxanthellae in *A. xanthogrammica* tentacles corresponds with temperature patterns observed between microhabitats at Slip Point. In the high surge channel microhabitat, anemone tentacles contained lower zoochlorellae density and a higher proportion of zooxanthellae than tentacles in all other microhabitats across all seasons (Figure 10). Because zoochlorellae predominate at cooler temperatures relative to zooxanthellae (O'Brien & Wyttenbach 1980, Saunders & Muller-Parker 1997, Muller-Parker et al. 2007), higher zooxanthellae proportions in the high surge channel may result from the inability of zoochlorellae to maintain the same densities as in other microhabitats due to elevated temperatures.

The comparatively extreme thermal environment that high surge channel anemones experience is evident in the fact that the high surge microhabitat tended towards higher high temperatures than the high tidepool microhabitat (Figure 5). Any cooling shade or wave splash that surge channels provide during warm low tide exposures is less effective in moderating temperatures than the volume of seawater in tidepools. Although microhabitat temperatures were only recorded concurrently during fall 2008, the fact that differences between the tidepool and surge channel were most pronounced during exceptionally warm and cool exposures suggests that the moderating effects of tidepools (against both extreme high and low temperatures) would be even more pronounced in the summer and winter seasons (Figure 5).

Anemone tentacles in the low intertidal tidepool and low surge channel microhabitats contained mostly zoochlorellae, with nearly all individuals having >90% zoochlorellae in tentacles in all seasons (Figure 6). The overall lack of difference between

the low tidepool and low surge channel is consistent with the lack of thermal variation between these generally submerged microhabitats. Although temperatures were not recorded in low intertidal microhabitats, these are exposed to air for less than 20% of time on an annual basis at Slip Point (as estimated using Clallam Bay tide datum). Low intertidal tidepools and surge channels experience similar temperature conditions that do not differ as much as those in the high intertidal.

There are three possible explanations for how temperature might cause the reduced high surge channel density of zoochlorellae in *A. xanthogrammica*. First, the growth of zoochlorellae may be lower. Mitotic index (MI), a proxy for symbiont growth, may decrease with increasing temperature, resulting in a lower density of zoochlorellae. However, the fact that MI was never significantly lower in the high surge channel than the high tidepool (Figure 9) suggests that differences in MI between microhabitats are not sufficient to explain the relatively lower high surge channel densities. Laboratory studies have found different effects of temperature on the MI of zoochlorellae depending on duration of exposure. In both *A. xanthogrammica* and *A. elegantissima*, MI has been shown to decrease at 20°C over 20-25 days (O'Brien & Wytttenbach 1980, Saunders & Muller-Parker 1997), and to remain unchanged in *A. elegantissima* over a broad range of experimental temperatures up to 24°C over 1-4 days (Verde & McCloskey 2001, 2007). If sustained exposure to elevated temperature is required to decrease mitotic index (as in O'Brien & Wytttenbach 1980 and Saunders & Muller-Parker 1997), the duration of the daily low-tide exposures experienced at Slip Point may not be sufficient to prompt a decline in MI between the upper intertidal microhabitats.

Alternatively, *A. xanthogrammica* may preferentially expel zoochlorellae over zooxanthellae in the high surge channel microhabitat. In order to regulate symbiont densities, anemones may be able to preferentially expel dividing algal cells (Baghdasarian & Muscatine 2000). For example, the MI of expelled zoochlorellae and zooxanthellae of *A. elegantissima* was 4.2 x and 3.5 x greater than that of symbionts isolated from the host (McCloskey et al. 1996). In addition, the rate of expulsion of dividing cells may increase with temperature (Baghdasarian & Muscatine 2000). Because the mitotic index of zoochlorellae was approximately 10-20 times greater than that of zooxanthellae (Figure 9), any preferential expulsion of dividing algae would result in a greater loss of zoochlorellae than zooxanthellae. If expulsion was considerably more common in the warmer high surge channel environment than in the high tidepool, it may explain the reduced zoochlorellae densities observed here.

Finally, differences in anemone physiology and behavior in different microhabitats may influence the distribution of their symbionts. During daytime low tides, continuously submerged anemones tend to remain open and expanded (pers. obs.), a behavior seen in moderate (but not high) light with *A. elegantissima* that allows for higher rates of photosynthesis (Pearse 1974). During exposure to air, surge channel anemones were contracted to varying degrees, with far fewer tentacles exposed to the environment. Contraction lowers anemone oxygen demand and metabolic rate as compared to submerged individuals, and considerably reduces photosynthesis due to shading of algal symbionts (Shick & Dykens 1984, Zamer & Shick 1987). Surge channel anemones also covered their body column with small rocks and shell fragments, a behavior that also effectively lowers the irradiance received by symbionts (Pearse &

Muscatine 1971, Pearse 1974, Pierce 1974, Dykens & Shick 1984, Shick & Dykens 1984). Behaviors that both reduce anemone metabolism and exposure of symbionts to irradiance may result in the low densities of zoochlorellae and zooxanthellae in the high surge channel. Although comparatively high temperatures in the high surge channel are likely to be the main factor driving the lower density of zoochlorellae in this microhabitat, preferential expulsion of dividing cells and anemone behavior may also contribute to maintaining low densities across seasons.

The observed intertidal distribution of symbionts contained within *A. xanthogrammica* tentacles at Slip Point is consistent with the general trend towards increased proportions of zooxanthellae with increasing vertical shore height observed in Oregon and the outer coast of Vancouver Island (Kittaeff 2007, Bates 2000). However, Bates (2000) found an increasing proportion of zooxanthellae in tidepool anemone tentacles as compared with crevice anemone tentacles at the same shore height, in contrast to the dominance of zoochlorellate anemone tentacles in tidepools at Slip Point. Also in contrast to the current study, the high intertidal symbiont distribution at Tatoosh Island (48° N, approximately 30 km NW of Slip Point) was composed almost entirely of zoochlorellae (Secord & Augustine 2000, Kittaeff 2007). These differences may be explained by thermal conditions at these study sites. Just as relatively cool microhabitats at Slip Point support anemones containing a higher proportion of zoochlorellae, relatively cool study sites (including Tatoosh Island, as in Helmuth et al. 2002) may support zoochlorellate anemones to a greater extent than relatively warm sites. The observed distribution of zoochlorellae and zooxanthellae in anemones in the low intertidal zone is consistent with other studies. Kittaeff (2007) found that low intertidal anemone tentacles

in Coos Bay, Oregon (44°N) contained a much higher proportion of zoochlorellae than zooxanthellae. Similarly, in Bamfield, BC (48°N), low tidepools and crevices hosted predominately zoochlorellate anemone tentacles (Bates 2000).

Given the range of temperatures recorded between summer 2008 and summer 2009 (greater than 25°C), it is interesting that obvious seasonal variation in algal densities occurred only in anemone tentacles containing zoochlorellae in low intertidal microhabitats, where the density of zoochlorellae was lower in spring 2009 than in summer and fall 2008 (Figure 8). The densities of zooxanthellae were remarkably low in all seasons and across all microhabitats, and were generally at least an order of magnitude lower than the density of zoochlorellae (Figure 8), with little seasonal change. In addition, little seasonal change was evident in the relative proportions of zoochlorellae and zooxanthellae in anemone tentacles in any microhabitat (Figure 6).

The 50% decrease in zoochlorellae density in low tidepool and low surge channel anemone tentacles from summer 2008 to spring 2009 (Figure 10) was likely caused by the reduced MI observed in spring 2009 (Figure 11), assuming that symbionts are regularly expelled (McCloskey et al. 1996, Baghdasarian & Muscatine 2000) and that algal growth rates were not sufficient to replace the lost cells. The latter condition may be met during the Pacific Northwest winter. Although zoochlorellae are comparatively better suited to low irradiance conditions than zooxanthellae (Saunders & Muller-Parker 1997, Verde & McCloskey 2001, 2002, 2007), wintertime ambient irradiance in the low intertidal at Slip Point may not be sufficient to maintain zoochlorellae growth rates above algal expulsion rates, limiting the ability of *A. xanthogrammica* to maintain seasonally stable densities. In addition, one low tidepool anemone demonstrated a shift from ~90%

zoochlorellate in summer and fall 2008 to 100% zooxanthellate in spring 2009 (Figure 6).

This is unexpected given the overwhelmingly zoochlorellate composition of all other anemone tentacles sampled in this microhabitat, and suggests that variability between tentacles sampled from one segment of an anemone may occasionally be dramatically different from those sampled from another segment, in contrast to the limited variability generally observed in this study (Table 5). With these exceptions, the results of this study support the seasonal stability seen in *A. elegantissima* from Puget Sound, WA.

Bergschneider and Muller-Parker (2008) observed no differences in zooxanthellae or zoochlorellae density in *A. elegantissima* during summer (July) and winter (December and February).

It is important to note that temperature patterns in this study were measured in only two locations at Slip Point, and therefore the conclusion that the high surge channel is the more variable thermal microhabitat annually is based on limited observation. Despite this limitation, the temperature record obtained is likely a good approximation of microhabitat variability within Slip Point. The increased thermal variability recorded in the surge channel was not likely due to differences in emersion time between microhabitats. The vertical tidal height of the tidepool data logger was 0.4 m greater than that of the surge channel, so a temperature pattern driven purely by emersion time would instead favor more extreme tidepool temperatures. Although intertidal temperature differences may result from substratum angle (Helmuth & Hofmann 2001), this is also unlikely to be driving the observed pattern as both data loggers were placed on surfaces that were nearly horizontal. In addition, the nearby surge channel wall may have shaded the surge channel data logger. This would have led to a conservative estimate of air

temperatures in this microhabitat, minimizing observed differences. However, the tidepool selected for temperature monitoring may have provided more moderate temperatures than other nearby pools due to its comparatively large size. Although of similar depth and width many other tidepools located at the same shore height, it was of greater length (pers. obs.).

Although it is clear that the tidepool and surge channel microhabitats differ thermally, the actual temperatures experienced by anemones living in these environments may not be represented accurately by data loggers. Submerged ectothermic organisms have body temperatures that are very close to the surrounding water temperature (Helmuth 1998), while in air these organisms have body temperatures determined by environmental factors such as air temperature, wave splash, solar radiation, and wind speed, among other factors (Porter & Gates 1969, Helmuth 1998, Gilman et al. 2006, Helmuth et al. 2006). Behavioral factors are also important; anemones are able to evaporatively cool themselves by releasing water from their gastrovascular cavity (Shick 1991). It is also possible that actual anemone temperatures do not differ significantly from recorded surge channel temperatures. Kitaeff (2007) found that maximum *A. xanthogrammica* body temperatures generally were less than 3°C (and often less than 2°C) different from the maximum recorded air temperature during summer in Sitka, Alaska, with anemones recorded both above and below this maximum (Kitaeff 2007). Dingman (1998) also found that *A. elegantissima* maximum body temperatures were, on average, nearly 2°C higher than maximum recorded air temperature during summer in Anacortes, WA.



In view of the generally cool climate observed during the study period (Figure 3), it is not surprising that relatively low extreme temperatures were recorded in this study. Tidepool temperatures never exceeded 24.4°C and surge channel temperatures never exceeded 30.6°C (Table 4). In comparison, tidepools in the Pacific Northwest have been recorded to reach temperatures up to 31°C in the Puget Sound region (Jensen & Muller-Parker 1994) and 26°C along the outer coast of Vancouver Island (Bates 2000), suggesting that, on a regional basis, tidepools in the Northwest can experience extreme high temperature events. Although other studies have not measured surge channel temperatures, temperature loggers mimicking exposed rocks on Tatoosh Island (48°N, ~30km northwest of Slip Point) have recorded between 35°C-41.5°C during summer low tides (Harley & Helmuth 2003). This study suggests that surge channel temperatures will be much higher than those in the tidepool during extreme heat events at Slip Point, further restricting the persistence of zoochlorellae in this microhabitat.

With the exception of the high surge channel microhabitat, symbiont populations in *A. xanthogrammica* at Slip Point are primarily composed of dense assemblages of zoochlorellae in all seasons (Figure 6, Figure 7), so it is illustrative to know what nutritional benefits these symbionts provide to their host. Although it was hypothesized that *A. xanthogrammica* would derive most of its nutrition from *M. californianus*, the contribution of zoochlorellae (alone and in predominantly zoochlorellate assemblages) was greater than that of mussels (Table 7). The high observed contributions of zoochlorellae may be due to the use of tentacle tissue as opposed to whole anemone bodies in the analysis. Because zoochlorellae are at least twice as dense in the crown and tentacles as in the mid and base segments of *A. xanthogrammica* (Kitaeff 2007), the

contributions of symbionts are estimated for a body region where they are potentially greatest if most of the photosynthetically fixed carbon is utilized by nearby (tentacle) tissue and is not translocated into lower body sections. Contributions of photosynthetically derived carbon may be substantially lower (and contributions of heterotrophy much higher) in the column and base.

The contributions of algal carbon to host diet estimated in this study are considerably higher than those estimated by Bergschneider & Muller-Parker (2008). Using stable isotope analysis of whole *A. elegantissima* anemones, they found a greater reliance on heterotrophy than symbiotic algae, as judged by the close isotopic resemblance of symbiotic anemone tissue to that of aposymbiotic anemones (individuals which are entirely reliant on heterotrophy) (Bergschneider & Muller-Parker 2008). Densities of zoochlorellae were not remarkably different between studies despite the use of whole anemones (as compared to tentacles) by Bergschneider & Muller-Parker (2008). They observed  $\sim 1.0$  to  $1.6 \times 10^6$  zoochlorellae  $\cdot \text{mg}^{-1}$  anemone protein, as compared to  $1.9 \times 10^6$  and  $1.0 \times 10^6$  zoochlorellae  $\cdot \text{mg}^{-1}$  tentacle protein in summer 2008 and spring 2009, respectively, in *A. xanthogrammica* tentacles sampled in this study. Because the trend towards increased algal density in tentacles is even more pronounced in *A. elegantissima*, where density of zooxanthellae is 3-5 times greater than in the body column (Dingman 1998), Bergschneider and Muller-Parker (2008) may have reached similarly high estimates using only tentacles if most photosynthetically derived carbon is utilized by nearby tissue. However, their use of whole anemones is likely a more accurate estimate of the overall nutritional contributions of symbionts than the tentacle tissue used in the current study. Future study with whole *A. xanthogrammica* anemones is needed to

determine if tentacles overestimate the amount of algal carbon provided to the host and if symbiotic contributions vary with symbiont density.

The observed  $\delta^{13}\text{C}$  values for zoochlorellae fall within the range of values obtained from zoochlorellae isolated from *A. elegantissima* (Bergschneider & Muller-Parker 2008). Mixed zoochlorellae (defined as 50-90% zoochlorellae) values in the current study are intermediate between the  $\delta^{13}\text{C}$  values of zoochlorellae and zooxanthellae isolated from *A. elegantissima* (Bergschneider & Muller-Parker 2008), likely due to the contributions of zooxanthellae to the assemblage.  $\delta^{15}\text{N}$  values for zoochlorellae and mixed zoochlorellae were more than 3‰ higher than those found by Bergschneider & Muller Parker (2008), however. The sources of  $\delta^{15}\text{N}$  variation have not been experimentally tested in zoochlorellae, but the  $\delta^{15}\text{N}$  of zooxanthellae in a variety of reef corals has been shown to decrease with irradiance (Muscatine & Kaplan 1994, Heikoop et al. 1998) and to vary with changes in nutrient source values, including nitrate (Heikoop et al. 2000). Finally, host tissue contamination could also lead to the higher observed  $\delta^{15}\text{N}$  values. While there was some remnant host tissue visible microscopically, it was in low amounts and the high densities of isolated symbiotic algae may mask much of its influence on observed values. It is likely that  $\delta^{15}\text{N}$  differences between studies reflect different nutrient  $\delta^{15}\text{N}$  values and/or lower irradiance at Slip Point, with a comparatively small effect of host tissue contamination.

The stable isotope results suggest that zoochlorellae are more “beneficial” to the host than has been estimated by algal productivity studies in *A. elegantissima* (Engebretson & Muller-Parker 1999, Verde & McCloskey 2001, 2002, 2007, Bergschneider & Muller-Parker 2008), but directly comparing these congeneric species is

problematic. Most obviously, no estimates of algal productivity are available for *A. xanthogrammica*, and extrapolating from studies in *A. elegantissima* may not be appropriate. Differences in anemone species morphology could also allow *A. xanthogrammica* to provide zoochlorellae with more favorable temperature and light regimes. The relatively larger size of *A. xanthogrammica* may allow for more symbiont shading and cooler body temperatures, creating a cooler, lower light microclimate. Kitaeff (2007) found mixed *A. xanthogrammica* can contain zooxanthellae in their tentacles and zoochlorellae (alone or mixed) in the middle and base sections, mirroring the overall “low temperature, low light” intertidal distribution of zoochlorellae (Bates 2000, Secord & Augustine 2000, Kitaeff 2007). If *A. xanthogrammica* is an intrinsically more suitable host for zoochlorellae, it may be that productivity estimates derived from *A. elegantissima* are too conservative and the relatively high contributions observed here are “normal” for *A. xanthogrammica* symbiosis. Future studies directly comparing the algal productivity of zoochlorellate *A. xanthogrammica* and *A. elegantissima* may resolve this question.

The time scale of carbon flow measured by stable isotopes is also much longer than that obtained from algal productivity studies.  $^{14}\text{C}$  tracer experiments to follow carbon translocation (as in Engebretson & Muller-Parker 1999) measure flux over a period of hours, while oxygen flux experiments (as in Verde & McCloskey 2001, 2002, and 2007) generally follow photosynthetic activity over a 24-hour period. Stable isotopes integrate longer-term carbon assimilation into tissues (Muscatine et al. 1989). While it is clear that algae, anemone, and mussel tissues show seasonal change in  $\delta^{13}\text{C}$  signatures (Figure 13), the actual time scales for these changes are not known. Furthermore, the

timescale of isotopic change is likely to differ for algae, anemone, and mussel tissues.

There are also a range of assumptions in stable isotope dietary analysis that may influence the observed values. Most fundamentally, the mixing model assumed that the entire carbon supply of *A. xanthogrammica* was represented. Assuming that the proportions of prey utilized at Slip Point are similar to those utilized on the outer coast, extrapolation of the heterotrophic prey items to the  $\delta^{13}\text{C}$  value of *M. californianus* is an acceptable assumption. While heterotrophic consumption is may be largely *Mytilus californianus* (approximately 70%, Dayton 1973, Sebens 1981), anemones at Slip Point may incorporate a higher proportion of other invertebrates into their diet than those along the comparatively more exposed outer coast of Washington, where dietary characterizations have been made (Dayton 1973, Sebens 1982). If these invertebrates resemble *M. californianus* isotopically, characterization of heterotrophy would not differ substantially. Dayton (1973) found the barnacle *Semibalanus cariosus* accounted for approximately 18% of *A. xanthogrammica* prey (together, the contribution of *M. californianus* and *S. cariosus* was nearly 90%), and a study on the Olympic Peninsula found little difference between the  $\delta^{13}\text{C}$  values of *M. californianus* and *S. cariosus* (-15.67 and -15.79, respectively) (Tallis 2009).

In addition to providing nutrients to their host, symbionts may also receive carbon and nitrogen from their host. This would lead to host and symbiont values becoming closer, resulting in overestimates of symbiont contributions in the mixing model. Nitrogen recycling may occur in which ammonium ( $\text{NH}_4^+$ ) waste from the host is passed back to symbionts, incorporated into organic compounds, and then translocated back to the host (Muscatine 1980b). Some experimental evidence exists for this in *A.*

*elegantissima*, where zooxanthellate anemones release less  $\text{NH}_4^+$  than aposymbiotic anemones and zooxanthellate anemones kept in the dark, implying zooxanthellae uptake nitrogen from host waste (Zamer & Shick 1987). However, the large amounts of  $\text{NH}_4^+$  released by *A. elegantissima* suggests that nitrogen recycling is limited (Jensen & Muller-Parker 1994). Carbon conservation may also occur, with symbionts receiving  $\text{CO}_2$  from host respiration or heterotrophic carbon for their own respiration (Bergschneider & Muller-Parker 2008). In this case, the  $\delta^{13}\text{C}$  of the host-derived  $\text{CO}_2$  will resemble host  $\delta^{13}\text{C}$  (Muscatine et al. 1989), shifting the observed symbiont  $\delta^{13}\text{C}$  values towards host values and overestimating the carbon contribution (=carbon isotope similarity) of symbionts to the host. Finally, the assumption of a carbon isotopic fractionation of 0.4‰ between host and diet may be incorrect. While this reflects the mean for aquatic animals in a recent review (McCutchan et al. 2003), small variations can have disproportionately large effects on dietary estimates. Shifting fractionation estimates by only  $\pm 0.4\text{‰}$  changes the estimates of zoochlorellae and mussel contributions by approximately 5%, for example.

Despite the range of uncertainty associated with applying stable isotope analysis to a diet with both symbiotic algal and heterotrophic contributions, the size of the estimated symbiotic contributions is great enough to establish zoochlorellae as an important nutritional source. An overestimate of even 20% would still allow the algal contributions to *A. xanthogrammica* to be greater than obtained for zoochlorellae in previous studies in *A. elegantissima*. This study adds to the knowledge of zoochlorellate anemone symbiosis by showing that zoochlorellae, when abundant in the favorable temperature and light regimes characterizing much of the study region, may be more

beneficial than has previously been estimated. However, further research in populations of *A. xanthogrammica* with a more even distribution of zoochlorellae and zooxanthellae than was observed at Slip Point is needed to test the relative contributions of these symbionts to host nutrition.

#### *A. xanthogrammica* as a model organism for the biological effects of climate change

Following the long-term effects of climate change on intertidal organisms is challenging, requiring investigations on multiple spatial and temporal scales. Long-term data sets are uncommon, but suggest that climate-related range shifts in species distribution are already underway. Resurveys of intertidal transects first sampled in the early 1930s at Hopkins Marine Station in Central California (~36°N) suggest that many “northern” species, including *A. xanthogrammica*, have declined in abundance due to higher average air and sea temperatures (Barry et al. 1995, Sagarin et al. 1999).

*Anthopleura xanthogrammica* individuals have an exceptionally long lifespan (greater than 100 years, Sebens 1983), and show minimal changes in the relative composition of “cool” and “warm” symbionts in response to seasonal temperature patterns. Distributions of these symbionts are instead related to thermal differences within the intertidal zone. As a result, the type of symbiont contained within *A. xanthogrammica* is a potentially valuable biological indicator for both range shifts and changes in local thermal environments. Long-term shifts in the distribution of zoochlorellae and zooxanthellae may be followed by sampling tentacles of *A. xanthogrammica*. With global climate change, an intertidal habitat with predominantly zoochlorellate anemones may be expected to shift over time towards one hosting predominantly zooxanthellate anemones.

Simple non-destructive repeated tentacle sampling of these long-lived anemones could provide evidence of biological response to climate change over time scales of years to decades. Where *A. xanthogrammica* populations contain both zooxanthellae and zoochlorellae (from ~38°N to at least 48°N; Bates 2000, Secord and Augustine 2000, Kitaeff 2007), monitoring anemone tentacles from populations in surge channels in the upper intertidal extent of anemone occurrence on an annual (or greater) scale may be sufficient to measure any changes in symbiont distribution. This study suggests that tentacle sampling can be limited to the upper intertidal extent of *A. xanthogrammica* occurrence because this region experiences greater temperature extremes and more often hosts anemones containing mixed symbiont assemblages as compared to the lower intertidal extent. Focused sampling to determine the relative distribution of zooxanthellae and zoochlorellae in surge channel anemones may be the most rapid way to judge biological response, as this microhabitat experiences comparatively greater temperature extremes than tidepools. Future monitoring of biological responses to climate change should account for, and take advantage of, the effects of microhabitat on intertidal organisms.



## Literature cited

- Baghdasarian G, Muscatine L (2000) Preferential expulsion of dividing algal cells as a mechanism for regulating algal-cnidarian symbiosis. *Biological Bulletin* 199:278-286
- Barry J, Baxter C, Sagarin R, Gilman S (1995) Climate-related, long term faunal changes in a California rocky intertidal community. *Science* 267:672-675
- Batchelder HP, Gonor JJ (1981) Population Characteristics of the Intertidal Green Sea Anemone, *Anthopleura xanthogrammica*, on the Oregon Coast. *Estuarine, Coastal and Shelf Science* 13:235-245
- Bates A (2000) The intertidal distribution of two algal symbionts hosted by *Anthopleura xanthogrammica* (Brandt 1835). *Journal of Experimental Marine Biology and Ecology* 249:249-262
- Bergschneider H, Muller-Parker G (2008) Nutritional role of two algal symbionts in the temperate sea anemone *Anthopleura elegantissima* Brandt. *Biological Bulletin* 215:73-88
- Connell J (1972) Community interactions on marine rocky intertidal shores. *Annual Review of Ecology and Systematics* 3:169-192
- Davenport J, Davenport J (2005) Effects of shore height, wave exposure, and geographical distance on thermal niche width of intertidal fauna. *Marine Ecology Progress Series* 292:41-50
- Dayton P (1973) Two cases of resource partitioning in an intertidal community: making the right prediction for the wrong reason. *The American Naturalist* 107:662-670
- Dingman H (1998) Environmental influence on algal symbiont populations in the sea anemone *Anthopleura elegantissima*. M.S. thesis, Western Washington University
- Dykens J, Shick J (1984) Photobiology of the symbiotic sea anemone, *Anthopleura elegantissima*: defenses against photodynamic effects, and seasonal photoacclimatization. *Biological Bulletin* 167:683-697
- Engebretson H, Muller-Parker G (1999) Translocation of photosynthetic carbon from two algal symbionts to the sea anemone *Anthopleura elegantissima*. *Biological Bulletin* 197:72-81
- Gilman S, Wetthey D, Helmuth B (2006) Variation in the sensitivity of organismal body temperature to climate change over local and geographic scales. *Proceedings of the National Academy of Sciences* 103:9560-9565
- Gorokhova E, Hansson S (1999) An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. *Canadian Journal of Fisheries and Aquatic Sciences* 56:2203-2210
- Hand C (1955) The sea anemones of Central California. Part II. The Endomyarian and Mesomyarian anemones. *Wasmann Journal of Biology* 13:37-97
- Harley C, Helmuth B (2003) Local- and regional-scale effects of wave exposure, thermal stress, and absolute vs. effective shore level on patterns of intertidal zonation. *Limnology and Oceanography* 48:1498-1508
- Heikoop J, Dunn J, Risk M, Sandeman I, Schwarz H, Waltho N (1998) Relationship between light and the  $\delta^{15}\text{N}$  of coral tissue: Examples from Jamaica and Zanzibar. *Limnology and Oceanography* 43:909-920

- Heikoop J, Dunn J, Risk M, Tomascik T, Schwarz H, Sandeman I, Sammarco P (2000)  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of coral tissues show significant inter-reef variation. *Coral Reefs* 19:189-193
- Helmuth B (1998) Intertidal mussel microclimates: predicting the body temperature of a sessile invertebrate. *Ecological Monographs* 68:51-74
- Helmuth B, Broitman B, Blanchette C, Gilman S, Halpin P, Harley C, O'Donnell J, Hofmann G, Menge B, Srickland D (2006) Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. *Ecological Monographs* 76:461-479
- Helmuth B, Harley C, Halpin P, O'Donnell M, Hofmann G, Blanchette C (2002) Climate change and latitudinal patterns of intertidal thermal stress. *Science* 298:1015-1017
- Helmuth B, Hofmann G (2001) Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biological Bulletin* 201:374-384
- IPCC (2007) Part 1: The physical basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change, 2007 Vol 1. Cambridge University Press, Cambridge, UK
- Jensen S, Muller-Parker G (1994) Inorganic nutrient fluxes in anemone-dominated tide pools. *Pacific Science* 48:32-43
- Kitaeff P (2007) Latitudinal differences in the distribution of two algal symbionts within the intertidal sea anemone, *Anthopleura xanthogrammica*., M.S. thesis, Western Washington University
- Letsch M, Muller-Parker G, Friedl T, Lewis L (2009) *Elliptochloris marina* sp. nov. (Trebouxiophyceae, Chlorophyta), symbiotic green alga of the temperate pacific sea anemones *Anthopleura xanthogrammica* and *A. elegantissima* (Anthozoa, Cnidaria). *Journal of Phycology* 45:1127-1135
- Lowry O, Rosenbrough N, Farr H, Randall R (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193:265-275
- McCloskey L, Cove T, Verde E (1996) Symbiont expulsion from the anemone *Anthopleura elegantissima* (Brandt) (Cnidaria: Anthozoa). *Journal of Experimental Marine Biology and Ecology* 195:173-186
- McCutchan JJ, Lewis WJ, Kendall C, McGrath C (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102:378-390
- Muller-Parker G (2008) Protein analysis of anemone samples. Protocol notes, unpublished
- Muller-Parker G, Davy S (2001) Temperate and tropical algal-sea anemone symbioses. *Invertebrate Biology* 120:104-123
- Muller-Parker G, Pierce-Cravens J, Bingham B (2007) Broad thermal tolerance of the symbiotic dinoflagellate *Symbiodinium muscatinei* (Dinophyta) in the sea anemone *Anthopleura elegantissima* (Cnidaria) from northern latitudes. *The Journal of Phycology* 43:25-31
- Muscantine L (1980a) Productivity of Zooxanthellae. In: Falkowski P (ed) *Primary Productivity in the Sea*. Plenum Press, New York
- Muscantine L (1980b) Uptake, retention, and release of dissolved inorganic nutrients by marine algae-invertebrate associations, Vol. Ohio State University, Columbus, OH

- Muscatine L, Kaplin I (1994) Resource partitioning by reef corals as determined by stable isotope composition. 2.  $\delta^{15}\text{N}$  of zooxanthellae and animal tissue versus depth. *Pacific Science* 48:304-312
- Muscatine L, Porter J, Kaplin I (1989) Resource partitioning by reef corals as determined from stable isotope composition. 1  $\delta^{13}\text{C}$  of zooxanthellae and animal tissue vs depth. *Marine Biology* 100:185-193
- O'Brien T, Wyttenbach C (1980) Some effects of temperature on the symbiotic association between zoochlorellae (Chlorophyceae) and the sea anemone *Anthopleura xanthogrammica*. *Transactions of the American Microscopical Society* 99:221-225
- Parmesan C, Gaines S, Gonzales L, Kaufman D, Kingsolver J, Townsend Peterson A, Sagarin R (2005) Empirical perspectives on species borders: from traditional biogeography to global change. *Oikos* 108:58-75
- Pearse V (1974) Modification of sea anemone behavior by symbiotic zooxanthellae: expansion and contraction. *Biological Bulletin* 147:641-651
- Pearse V, Muscatine L (1971) Role of symbiotic algae (zooxanthellae) in coral calcification. *Biological Bulletin* 141:350-363
- Phillips D, Gregg J (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171-179
- Pierce V (1974) Modification of sea anemone behavior by symbiotic zooxanthellae: expansion and contraction. *Biological Bulletin* 147:641-651
- Pitt K, Connolly R, Meziane T (2009) Stable isotope and fatty acid tracers in energy and nutrient studies of jellyfish: a review. *Hydrobiologia* 616:119-132
- Porter W, Gates D (1969) Thermodynamic equilibria of animals with environment. *Ecological Monographs* 39:245-270
- Sagarin R, Barry J, Gilman S, Baxter C (1999) Climate-related changes in an intertidal community over short and long time scales. *Ecological Monographs* 69:465-490
- Saunders B, Muller-Parker G (1997) The effects of temperature and light on two algal populations in the temperate sea anemone *Anthopleura elegantissima* (Brandt, 1835). *The Journal of Experimental Marine Biology and Ecology* 211:213-224
- Sebens K (1981) The allometry of feeding, energetics, and body size in three sea anemones. *Biological Bulletin* 161:152-171
- Sebens K (1982) The limits to indeterminate growth: an optimal size model applied to passive suspension feeders. *Ecology* 63:209-222
- Sebens K (1983) Population dynamics and habitat suitability of the intertidal sea anemones *Anthopleura elegantissima* and *A. xanthogrammica*. *Ecological Monographs* 53:405-433
- Secord D, Augustine L (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* 119:139-146
- Shick J (1991) A functional biology of sea anemones, Vol 1. Chapman & Hall, London
- Shick J, Dykens J (1984) Photobiology of the symbiotic sea anemone *Anthopleura elegantissima*: Photosynthesis, respiration and behavior under intertidal conditions. *Biological Bulletin* 166:608-619
- Sokal R, Rohlf J (1969) Introduction to biostatistics, Vol 1. W.H. Freeman and Company

- Somero G (2002) Thermal physiology and vertical zonation of intertidal animals: optima, limits and costs of living. *Integrative and Comparative Biology* 42:780-789
- Southward A, Hawkins S, Burrows M (1995) Seventy years' observations of changes in distribution and abundance of zooplankton and intertidal organisms in the western English Channel in relation to rising sea temperature *Journal of Thermal Biology* 20:127-155
- Tallis H (2009) Kelp and rivers subsidize rocky intertidal communities in the Pacific Northwest (USA). *Marine Ecology Progress Series* 389:85-96
- Underwood A (1981) Techniques of analysis of variance in experimental marine biology and ecology. *Oceanography and Marine Biology: An Annual Review* 19:513-605
- Verde E, McCloskey L (1996) Photosynthesis and respiration of two species of algal symbionts in the anemone *Anthopleura elegantissima* (Brandt) (Cnidaria; Anthozoa). *The Journal of Experimental Marine Biology and Ecology* 195:187-202
- Verde E, McCloskey L (1996b) Carbon budget studies of symbiotic cnidarian anemones- Evidence in support of some assumptions. *Journal of Experimental Marine Biology and Ecology* 195:161-171
- Verde E, McCloskey L (2001) A comparative analysis of the photobiology of zooxanthellae and zoochlorellae symbiotic with the temperate clonal anemone *Anthopleura elegantissima* (Brandt). I. Effect of temperature. *Marine Biology* 138:477-489
- Verde E, McCloskey L (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. *Marine Biology* 141:225-239
- Verde E, McCloskey L (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* 152:775-792
- Zamer W, Shick J (1987) Physiological energetics of the intertidal sea anemone *Anthopleura elegantissima*. II. Energy balance *Marine Biology* 93:481-491