Title

Interactions among sea anemones, their algal endosymbionts, and associated communities on California rocky intertidal shores

Author

Bedgood, Samuel Alexander

Publication Date

2021

UNIVERSITY OF CALIFORNIA,
IRVINE

Interactions among sea anemones, their algal endosymbionts, and associated communities on California rocky intertidal shores

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Ecology and Evolutionary Biology

by

Samuel Alexander Bedgood

Dissertation Committee:
Professor Matthew Bracken, Chair
Associate Professor Donovan German
Professor Stephen Weller

2021

DEDICATION

I dedicate this dissertation to my wife and partner Samantha.

Without her constant advice and support this would not have been possible.

“Do. Or do not. There is no try. - Yoda”

- Samantha Levell

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

I thank Dr. Matthew Bracken, a fellow sea anemone lover, for his guidance, support, and advice over the last five years. Dr. Bracken encouraged and guided me to be an independent researcher, even when my research was tangential to the rest of the group. He was always available to help in any way he could, and I always looked forward to discussing science and life with him in our weekly meetings. I could not have asked for a better advisor, and I am the researcher I am today thanks to him.

I also thank Dr. Donovan German and Dr. Stephen Weller for their unique perspectives on my work. They both challenged me to be a better researcher and had excellent suggestions for experimental design modifications during our meetings. I appreciate their engagement with my work; this dissertation would not have been completed without them.

Portions of this dissertation were reviewed by Bracken and Sorte Laboratory members past and present including Genevieve Bernatchez, Laura Elsberry, Nyssa Silbiger, Amy Henry, Sarah Mastroni, Sam Mahanes, Piper Wallingford, Racine Rangel, Heidi Waite, Lauren Pandori, Lauren Lees, and Raechel Hill. They were all mentors to me, helping me learn laboratory methods, field methods, and statistical analyses. My presentations would be boring, and my data would be uncollected without their constant advice and generous help.

I have had the privilege to mentor eight undergraduate researchers during my time at UCI: James Alex Caramagno, Calvin Huang, Misha Wahie, Courtney Moulton, Abe Razzak, Alan Yue, Queenie Baetiong, and Matthew Barna. They have served in so many roles including volunteering during field and lab work, analyzing large datasets, and conducting their own independent research. Each one had their own expertise, and they always had unique and

awesome perspectives on each project. Their contributions to this dissertation in both hours and intellectual contributions are immense.

I thank all my mentors that came before graduate school, especially Dr. Eddy Stringer, Carol Zimmerman, Dr. Janie Wulff, and Dr. Will Ryan. Each one had an enormous impact on my worldview and the way I approach science. They were all so passionate about science and mentorship, and they passed it on to me.

The person who deserves the most credit is Samantha Lavell, my wife and scientific colleague. She has supported and taught me so much during the past five years. She has kept me focused on what is most important in life, even when my research seems all-consuming, and she has shown me time and again that perfection is the enemy of completion. I would not have even made it to graduate school let alone completed a dissertation without her advice and encouragement.

My family played a large role in cultivating my love of science from an early age, and they have encouraged me at every step in the process. My mom and dad (Jana and Doug Bedgood) let me explore my passion for marine life from an early age, allowing their house to be flooded many times from aquaria gone awry. They took me to state parks as often as possible so I could explore seagrass meadows and salt marshes. They celebrated every step in the process, even when I moved 2,200 miles away from them to the west coast. I could not have accomplished this without them.

Lastly, I want to acknowledge the support my animals have given me. Our cats (Sashimi, Mochi, and Soba) served as constant stress-relief and writing buddies throughout my time here. More recently, our horses (Honor, Valor, and Eclipse) have helped me survive two years of a pandemic. I would have never guessed that I would own a horse, but here I am. They bring me joy every day, and I can honestly say this dissertation would not have been completed without them.

This work was funded by the following awards and fellowships:

University of California, Irvine OCEANS Initiative Graduate Fellowship

National Science Foundation Graduate Student Research Fellowship (GRFP)

Natural Reserves System Mildred E. Mathias Grant

Coal Oil Point Graduate Student Research Grant

Samuel A. Bedgood

Education

2016-present	*University of California Irvine*, Irvine, CA Department of Ecology and Evolutionary Biology, Ph.D. in progress *Ph.D. Thesis advised by Dr. Matthew Bracken* GPA: 4.0
2013-2016	*Florida State University*, Tallahassee, FL B. S. in Biological Sciences, with Honors in the Major, Minor in Chemistry *Senior thesis advised by Dr. Janie Wulff* GPA: 3.92
2010-2013	*Tallahassee Community College*, Tallahassee, FL Associate in Arts Degree, focus in sciences GPA: 3.96

Publications

Bedgood, S.A., J.A. Caramagno, M.E.S. Bracken. *In prep*. Are sea anemones picky eaters? Feeding preferences of temperate intertidal sea anemones and mechanisms of predation avoidance by their prey

Bedgood, S.A., S.T. Levell, and M.E.S. Bracken. *Submitted*. Microhabitats created by sea anemone-algal mutualisms enhance diversity of associated species on temperate rocky shores

Levell, S.T., **S.A. Bedgood**, J. Travis. *In review*. Maternal effects of social density on reproduction and fitness in the least killifish, *Heterandria formosa*. *Evolution*.

Bedgood, S.A., S.E. Mastroni, and M.E.S. Bracken. 2020. Flexibility of nutritional strategies within a mutualism: food availability affects algal symbiont productivity in two congeneric sea anemone species. *Proceedings of the Royal Society B* 287: 20201860.

Bedgood, S.A., M.E.S. Bracken, W.H. Ryan, S.T. Levell, and J.L. Wulff. 2020. Nutritional drivers of adult locomotion and asexual reproduction in a symbiont-hosting sea anemone *Exaiptasia diaphana*. *Marine Biology* 167: 1-12.

Wallingford, P.D., L.L.M. Pandori, **S.A. Bedgood**, M.E.S. Bracken, L.A. Elsberry, A.K. Henry, S.A. Mahanes, and C.J.B. Sorte. 2018. A guide to the relationships between marine spatial patterns and ecological processes. *Frontiers of Biogeography* 10: e39410.

Book Chapters

Reznick, D.N., **S.A. Bedgood**, S.T. Levell, K.M. Edwards, R.M. Conway, M.D. Green, J.G. Randall. 2019. Biology Laboratory 101 Series. *Journal of Visualized Experiments*.
First author chapter: Physiology and the Circulatory System

Reznick, D.N., **S.A. Bedgood**, S.T. Levell, K.M. Edwards, and R.M. Conway. 2019. Biology Laboratory 102 Series. *Journal of Visualized Experiments*.

First author chapters: Measuring Biodiversity, Plant Diversity & Evolution, Animal Diversity & Evolution, Eusociality & Division of Labor, and Community Diversity & Ecosystem Health

Oral Presentations

Bedgood S.A., S.T. Levell, M.E.S. Bracken. 2021. Sea anemone symbiosis enhances intertidal biodiversity by ameliorating abiotic stress. Ecological Society of America Annual Meeting, Virtual.

Bedgood S.A. and M.E.S. Bracken. 2020. Fire nematocysts first and ask questions later: sea anemones choose prey after ingestion. Western Society of Naturalists Annual Meeting, virtual meeting.

Bedgood S.A. and M.E.S. Bracken. 2020. Making it big and losing friends: algal symbiont contributions are shaped by sea anemone life history. Society for Integrative and Comparative Biology Annual Meeting, Austin, Texas.
Awarded Mary Rice Best Oral Student Presentation for the Division of Invertebrate Zoology

Bedgood S.A. and M.E.S. Bracken. 2019. Sea anemone mutualism leads to facilitation: complex interactions increase biodiversity in the rocky intertidal zone. Western Society of Naturalists Annual Meeting, Ensenada, Mexico.

Bedgood S.A. and M.E.S. Bracken. 2019. Spatial subsidies change the interaction between host sea anemones and their algal symbionts. Yosemite Symbiosis Workshop Annual Meeting, Wawona, California.

Bedgood S.A. and M.E.S. Bracken. 2019. Rare spatial subsidies and close friends: interactions between sea anemones and their algal symbionts. Society for Integrative and Comparative Biology Annual Meeting, Tampa, Florida.

Bedgood S.A. and M.E.S. Bracken. 2018. Rare spatial subsidies and close friends: interactions between sea anemones and their algal symbionts. Western Society of Naturalists Annual Meeting, Tacoma, Washington.

Bedgood S.A. and M.E.S. Bracken. 2018. Niche partitioning in three congeneric sea anemones. Winter Ecology and Evolutionary Biology Graduate Student Symposium, Irvine, California.

Bedgood S.A. and M.E.S. Bracken. 2018. Algal symbionts and niche partitioning in the sea anemone genus *Anthopleura*. University of California Natural Reserve System California Ecology Course, Cambria, California.
Invited course talk

Bedgood S.A. 2016. Context dependent benefits of symbiotic algae to their host anemone and associated modification of anemone behavior. Western Society of Naturalists Annual Meeting, Monterey, California.

Bedgood S.A. 2016. Why do anemones move? Context dependent benefits of symbiotic algae to their host anemones. Southeastern Ecology and Evolutionary Biology Conference Annual Meeting, Tallahassee, Florida.

Poster presentations

Bedgood S.A. and M.E.S. Bracken. 2018. Algal symbionts and niche partitioning in the sea anemone genus *Anthopleura.* Yosemite Symbiosis Workshop Annual Meeting, Wawona, California.

Bedgood S.A. and M.E.S. Bracken. 2017. Evidence for niche partitioning in three congeneric sea anemones. Western Society of Naturalists Annual Meeting, Pasadena, California.

Bedgood S.A. 2016. Why do anemones move? trade-offs between symbiotic algae and their host anemones. Florida State University Undergraduate Research Symposium, Tallahassee, Florida.

Bedgood S.A. 2015. Trade-offs between friends and food? phototactic response of the anemone *Aiptasia pallida*. President's Showcase of Undergraduate Research Excellence, Tallahassee, Florida.

Fellowships, scholarships, and Grants

2020	Mary Rice Best Oral Student Presentation Award ($200)
2019	Coal Oil Point Graduate Student Research Award ($2,000)
2018-2019	Mathias Graduate Student Research Fellowship ($3,000)
2018-2021	National Science Foundation Graduate Research Fellowship ($138,000)
2017-2018	UCI OCEANS Fellowship ($4,474)
2016-2017	Voth Fellowship ($16,707)
2016	UCI Biological Sciences Graduate Fellowship ($10,000)
2016	UCI Diversity Graduate Recruitment Fellowship ($5,000)
2015	FSU Mentored Research and Creative Endeavors Award ($1,000)
2015	John Mark Caffrey Endowed Scholarship ($1,500)
2015	Bess H. Ward Thesis Award ($750)
2012-2013	TCC STEM Star Scholarship

Outreach

2019	Worked at the UCI Reef Relief Earth Day Booth with approximately 150 visitors
2019	Worked at the Communicating the Impacts of Plastic on Marine Ecosystems Booth at UCI Homecoming event with approximately 1,000 visitors
2018, 2019	Gave presentation and lab tour to Mt SAC students in the STEM TP2 program who were searching for summer research opportunities
2017, 2018	Lead lessons and activities focusing on greenhouse gases at a local K-12 school as a member of the group Climate Literacy Empowerment and iNquiry
2016	Lead lab tours and educational sessions on ecosystem ecology in the Bracken Lab for local high school students
2015	Worked at the Coral Reef Exhibit at the FSU Coastal Marine Laboratory for an event with approximately 300 visitors
2015	Worked at the Wulff Laboratory invertebrate and research awareness exhibit at Gulf Specimen Marine Laboratory for an event with approximately 200 visitors

Media

2020	Featured on UCI School of Biological Sciences News, article titled: *Sea Anemones Find Sweet Arrangement with Under-skin Algae for Emergency Food*

2019	Interviewed on the NudiBrains Podcast by Emily Pierce focusing on my thesis research, episode titled: *40 Fish Tanks, Symbionts, and Anemone Diets*

Mentorship

James Alex Caramagno. 2019-2021. BIO SCI 199 undergraduate researcher. Independent research project focus: laboratory feeding choice of sea anemones in the genus *Anthopleura*.

Calvin Huang. 2019-2021 Undergraduate volunteer. Research project: laboratory feeding choice of sea anemones in the genus *Anthopleura*.

Misha Wahie. 2019-2021. BIO SCI 199 undergraduate researcher. Independent research project focus: asexual reproduction by fission in the sea anemone *Anthopleura elegantissima*.

Courtney Moulton. 2020. University of California Riverside undergraduate student. Independent research project focus: Seasonal impacts on the fat and population cycles of invasive western mosquitofish, *Gambusia affinis*.
Presented research as a poster at Society for Advancement of Chicanos/Hispanics and Native Americans in Science (SACNAS) Annual Meeting 2020 and at the Western Society of Naturalists (WSN) Annual Meeting 2020

Abe Razzak. 2019-2020. Undergraduate volunteer. Research project: asexual reproduction by fission in the sea anemone *Anthopleura elegantissima*.

Alan Yue. 2017-2020. Mt. San Antonio College STEM TP2 participant and volunteer. Independent research project focus: attachment of debris by sea anemones protects against photo-oxidative stress.
Presented research as a poster at the 2017 UCI Environmental Research Poster Symposium

Queenie Baetiong. 2018-2019. BIO SCI 199 undergraduate researcher. Independent research project focus: diet preference and selectivity of two sea anemone species in the rocky intertidal using time lapse videos in the field and laboratory.

Matthew Barna. 2018. BIO SCI 199 undergraduate researcher. Independent research project focus: sea anemones serve as protective microhabitat for mobile invertebrates in the rocky intertidal zone.

Ung Seop Jeon. Fall 2018. Science Writing Integrated Mentoring (SWIM) student mentee. Project title: broad-scale distribution and abundance patterns of the three species of *Anthopleura* across the Pacific intertidal coast of California.
Final project awarded 2019 UCI Writing Awards best essay for Excellent Academic Writing in Science and Technology

Annalie Ramos. Fall 2018. SWIM student mentee. Project title: photosynthesis by the symbionts in the *Anthopleura* species determines the intertidal location of this anemone.

Mai Le. Fall 2018. SWIM student mentee. Project title: positive trends in tidal and latitudinal gradient on the sea anemone *Anthopleura elegantissima* colonies in California.

Krishan Ferrer. Fall 2018. SWIM student mentee. Project title: niche overlap among three sea anemone species (*Anthopleura*) found along the rocky intertidal zone of the Pacific coast of California.

Teaching

Fall 2021	Teaching assistant for Ecology and Evolution (E106), UC Irvine
Winter 2018	Teaching assistant for Organisms to Ecosystems Biology (Bio Sci 94), UC Irvine
Winter 2017	Teaching assistant for Philosophy of Biology (Bio Sci E142W), UC Irvine
Fall 2016	Teaching assistant for Introduction to Life (Bio Sci 1a), UC Irvine
Spring 2016	Assistant instructor for General Biology Laboratory, Florida State University
Fall 2015	Assistant instructor for General Biology Laboratory, Florida State University
Spring 2015	Tutor and teaching assistant for Biological Science II, Florida State University
Fall 2014	Tutor and teaching assistant for Biological Science II, Florida State University

Workshops, training, skill development

2020-2021	Designed and created four marine aquarium systems for sea anemone research and teaching, Soka University of America
2021	Completed the UCI Graduate Division Mentoring Excellence Certificate Program
2018-2020	Served as a committee member and leader for UC Irvine Ecology and Evolutionary Biology graduate student recruitment
2019	Participated in Joshua Schimel Writing Workshop, UC Irvine
2016-2019	Participated in Ecology Group (career development and training)
2015-2016	Designed and created two marine aquarium systems (40 gallons each) for bryozoan research, Florida State University

Employment (excluding teaching and fellowships)

2018	Federal work study, UC Irvine (3 months)
2018	Graduate student researcher (GSR), UC Irvine (2 months)
2018	Contracted writer and actor for Journal of Visualized Experiments (6 months)
2016	Intern for the Guppy/Rivulus mark recapture project in Trinidad (3 months)

ABSTRACT OF THE DISSERTATION

Interactions among sea anemones their algal endosymbionts and associated communities on California rocky intertidal shores

by
Samuel Alexander Bedgood
Doctor of Philosophy in Ecology and Evolutionary Biology
University of California, Irvine, 2021
Professor Matthew Bracken, Chair

Sea anemones in the genus *Anthopleura* are conspicuous community members on California rocky shores. They live symbiotically with algae and exchange nitrogen for photosynthetically derived sugars and lipids. The mutualism between host and endosymbiont has been well-studied, but the impact of this mutualism in a larger context of community and ecosystem ecology has not. Here I investigate how sea anemone diets affect the mutualism between host and symbiont, which invertebrate prey are chosen and digested by sea anemones, and how favorable microhabitat created by sea anemones is a direct result of the mutualism between sea anemones and algal endosymbionts. In chapter 1, I found evidence for a trade-off between autotrophy of endosymbionts and heterotrophic diet. When food was increased, sea anemones down-regulated endosymbionts, and when it was decreased, they up-regulated endosymbionts. However, the direction of the response was sea anemone species specific. In chapter 2, I provide evidence for sea anemone prey choice and predation avoidance by common prey species. All snail species that we tested escaped sea anemones alive if they were undamaged, but crustaceans, while sometimes escaping ingestion, were always digested. We suggest that the snail species *Tegula funebralis* may release a chemical deterrent to predation after ingestion by the sea anemone. In chapter 3, I describe how the mutualism between sea anemone and algal endosymbionts creates favorable microhabitat that ameliorates abiotic stressors during low tides. Mobile invertebrates take advantage of this microhabitat and stay next to sea anemones during low tides. Temperature and relative desiccation were lower and mobile invertebrate richness and biomass were higher in sea anemone habitat as compared to adjacent rock habitat, suggesting a facilitative role for this sea anemone-algal mutualism. Taken together, these results reveal the complex trophic and non-trophic interactions caused by this temperate cnidarian-algal mutualism.

INTRODUCTION

Sea anemones are conspicuous members of marine ecosystems in many regions around the world. The first observations of these animals were written by Aristotle, who studied sea anemone behavior, likely in the rocky intertidal zone of Lesbos (Aristotle et al. 1862). Five congeneric species of sea anemone in the genus *Anthopleura* co-occur along the California shoreline (Pearse and Francis 2000; Vassallo-Avalos et al. 2020), and three of those are hosts to unicellular algal endosymbionts that live in the gastrodermal tissue of the sea anemone (Geller and Walton 2001; Koch et al. 2020). The sea anemone provides a safe environment and nitrogen to the algae, and the algae provide sugars and lipids produced through photosynthesis in return. This mutualistic relationship has been the focus of much work (Verde and McCloskey 1996; Saunders and MullerParker 1997; Schwarz et al. 2002; Bergschneider and Muller-Parker 2008; Levine and Muller-Parker 2012), but little has been accomplished in terms of understanding the role of these sea anemones and their algal endosymbionts in a broader community and ecosystem level perspective (but see Sebens 1981a, b). This dissertation considers sea anemones at multiple scales, from interactions between partners (sea anemone and algae) to consequences of the anemone-algal mutualism on local communities.

Here I focus on the three symbiotic species in the genus *Anthopleura*, but it is important to note for comparison the two non-symbiotic species: *Anthopleura artemisia* (moonglow anemone) and *Anthopleura mariae*. Both species are cryptic during the day, choosing to live under rocks or beneath the substrate (*pers. obs.*). They are relatively small, growing to only 0.5-1 cm across the oral disc. Conversely, the three symbiotic species, *Anthopleura elegantissima*, *Anthopleura sola*, and *Anthopleura xanthogrammica*, are found in open habitats where their algal endosymbionts can photosynthesize. The largest symbiotic species (*A. xanthogrammica*) can grow larger than 14 cm across the oral disc. I focus on the symbiotic species because they are the most abundant, and their mutualistic relationship with algal endosymbionts results in complex trophic and non-trophic interactions with other species.

Among the symbiotic species, *A. sola* and *A. xanthogrammica* are the largest species, and *A. elegantissima* is much smaller (Fig. i.1). The size difference is likely due to reproductive mode, *A. sola* and *A. xanthogrammica* reproduce only sexually while *A. elegantissima* reproduces both sexually and asexually (Pearse and Francis 2000). As a result, *A. sola* and *A. xanthogrammica* are usually found as solitary individuals that grow only as a whole individual while *A. elegantissima* reach a maximum individual size (~2.5 cm) before they divide by longitudinal fission (Sebens 1983; *pers. obs.*). *A. elegantissima* can form large clonal mats in the intertidal zone after many fissions (Sebens 1983). These colonies can contain thousands of individuals that are no larger than 1-2 cm in diameter. Observational work suggests that asexual reproduction occurs in only a portion of individuals once a year, suggesting each large colony takes tens of years to form (Sebens 1983). In fact, Sebens (1983) suggests that, once large, *Anthopleura xanthogrammica* (and likely the other two species) are long-lived with no senescence and very few whole-anemone predation events observed during a relatively long-term study (2 years).

To better understand these species, I surveyed nine sites along the coast of California from San Diego to Trinidad Head. I counted and measured the size of sea anemones in 0.25 m^2 circular quadrats along six belt transects at each site during low tides (average of 36 quadrats at each site). All figures presented in the introduction were generated from these data.

My work explores symbiotic sea anemone physiology and ecology along the California coast where all three species overlap in their ranges (Fig. i.2). *A. elegantissima* extends from Baja

California, Mexico to Alaska, USA. It is the most widespread species (Hand 1955). *A. sola* is in the southern region from Baja California to Northern California (Pearse and Francis 2000; Fig. i.3). There is evidence for temperature setting the range limit for *A. sola,* which has likely extended its northern range limit as temperatures have risen (Sanford et al. 2019). *A. xanthogrammica*'s distribution is complex, appearing in parts of Mexico and Southern California (Dana Point and Government Point), but it only exists in high densities beginning in central California (Francis 1979; Smith and Potts 1987, Fig. i.3). Its distribution is likely tied to upwelling and productivity of adjacent areas, since there is a close correlation between these factors and *A. xanthogrammica*'s presence at southern sites (Francis 1979; Smith and Potts 1987).

All three species can be found from the bottom of the intertidal zone up to the splash zone in tide pools, but there are some small differences in tidal distribution (Fig. i.4). In the southern region of California I surveyed, *A. elegantissima* tends to be higher in the intertidal zone than *A. sola* (Fig. i.4). This relationship has been described by previous authors and may suggest some differentiation in habitat use (Francis 1979). *A. sola*'s tidal distribution may be limited by protected habitat availability; they are found more frequently in protected habitat as tide height increases, but the same relationship is not observed in *A. elegantissima* (Fig. i.5). The coexistence of all three species in the central to northern California region may be partly explained by the size difference between *A. elegantissima* and the other two species, but the drivers of coexistence between *A. sola* and *A. xanthogrammica* are unclear given that both species occupy similar habitat, have similar diets, and maintain the same algal endosymbiont type (see below). Some evidence for autotrophic and heterotrophic diet differences between the two species are discussed in Chapter 1.

These three sea anemone species maintain a symbiosis with algal endosymbionts, which is physiologically similar to the relationship between tropical scleractinian corals and their dinoflagellate algal endosymbionts. In fact, *A. elegantissima* and its algae have been extensively studied as a model system for coral-algal symbiosis (Saunders and MullerParker 1997; Schwarz and Weis 2003). The main difference between *Anthopleura*-algal and coral-algal symbiosis is that algal endosymbionts are facultative (unnecessary for survival) to these sea anemones but obligate (necessary for survival) to most coral species (Weis and Reynolds 1999; Hiebert and Bingham 2012). This is partly because temperate sea anemones (*e.g. Anthopleura*) live in nutrient-rich systems where heterotrophic diet can compensate for the loss of algal endosymbionts while tropical coral live in nutrient-poor systems (see chapter 1). Unlike corals which host only a single family of algal endosymbiont (Symbiodiniaceae), at least two of the three sea anemone species (*A. elegantissima* and *A. xanthogrammica*) maintain a symbiosis with either a single or a combination of two algal endosymbionts from different phyla. One is from a dinoflagellate family called Symbiodiniaceae (*Breviolum 'muscatinei'*), the same family of endosymbionts found in tropical coral, and the other is a green alga chlorophyte, *Elliptochloris marina*, whose relatives are found in hydra (Secord and Augustine 2000; Lewis and Muller-Parker 2004).

I focus on the dinoflagellate endosymbiont because the chlorophyte is only present at the northernmost region of California in *A. xanthogrammica* and less frequently in *A. elegantissima* (Secord and Augustine 2000; *pers. obs.*). Although all three sea anemones host the same dinoflagellate species of algal endosymbiont, there is evidence for local adaptation of the endosymbiont *Breviolum 'muscatinei'* to different sites, tide heights, and sea anemone species (Cornwell 2020; Cornwell and Hernández 2021). Genetic variation within the algal endosymbiont is greater than the host sea anemones, and Cornwell and Hernández (2021) hypothesize that the algal endosymbiont may allow local adaptation by the holobiont (sea anemone and algal endosymbionts together).

The role of sea anemone holobionts in the California rocky shores is complex; they serve as primary producers (photosynthesizing algae), prey, scavengers, and opportunistic predators. Algal endosymbionts can provide the host sea anemone with a large portion of its dietary carbon (> 50%), increasing the algae's productivity with high concentrations of nitrogen produced from heterotrophic feeding (Bergschneider and Muller-Parker 2008). Predators and especially parasites of these sea anemones are abundant and include *Dermasterias imbricata* (seastar; Bachman and Muller-Parker 2007), *Aeolidia papillosa* (nudibranch; Edmunds et al. 1976), *Clinocottus globiceps* (sculpin; Augustine and Muller-Parker 1998), *Epitonium tinctum* (snail; Smith 1977), and pycnogonids (sea spiders; Burris 2011). Most of these are parasites that only consume part of the sea anemone or colony, and the larger sea anemones are likely less vulnerable to predation (*pers. obs.*). While there are many organisms that consume them, *Anthopleura* sea anemone diets are even more diverse than their predators. They will consume almost any animal that encounters their stinging tentacles and can fit in their mouths. Some extreme terrestrial-originating predation events include seabird nestlings (Guy et al. 2014), a garter snake (Harmer 2019), and a rabbit (*pers. obs.*), but more commonly their diets consist of mussels, crustaceans, and other sessile animals that break loose from the substrate and wash into tentacles (see chapter 2; Sebens 1981b).

Little work has investigated the role of *Anthopleura* spp. outside of their trophic interactions. However, many ecologists have observed mobile invertebrates like snails, limpets, and chitons congregating next to and under the edges of sea anemones during low tides, especially in the upper intertidal zone where sea anemones are one of the only biotic habitat providers (*pers. comm.*; *pers. obs.*). Previous research by Bingham et al. (2011) on *A. elegantissima* has shown that the sea anemone can ameliorate abiotic stressors like desiccation and high temperatures by releasing stored water during low tides. This suggests a mechanism for microhabitat creation by these sea anemones, potentially facilitating mobile invertebrates in this system.

In these three chapters, I aimed to investigate the impact of the holobiont sea anemones on the local rocky intertidal community and vice versa. Chapter 1 addresses how the heterotrophic diet of *A. sola* and *A. xanthogrammica* influences the autotrophic contribution of algal endosymbionts to the host, demonstrating a trade-off between external and internal dietary carbon sources. Chapter 2 demonstrates active feeding choice of all three sea anemone species and describes intertidal invertebrates that avoid predation by various means. Chapter 3 provides evidence for sea anemones as microhabitat providers, increasing the richness and biomass of mobile invertebrates in the mid to upper rocky intertidal zone. Taken together, these results contribute to our understanding of the trophic and non-trophic contributions of a well-studied mutualism viewed in a larger context.

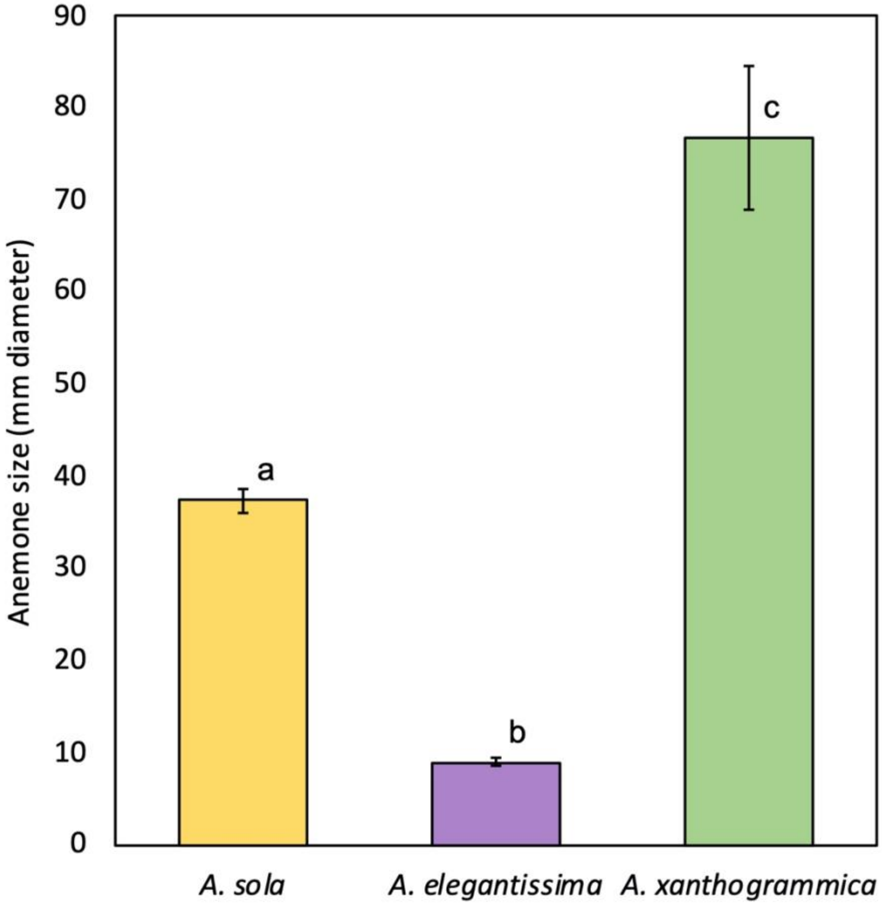


Figure i.1. Mean ± SEM sea anemone size of each species at Hazards Canyon Reef. Letters indicate significance from post hoc analysis.

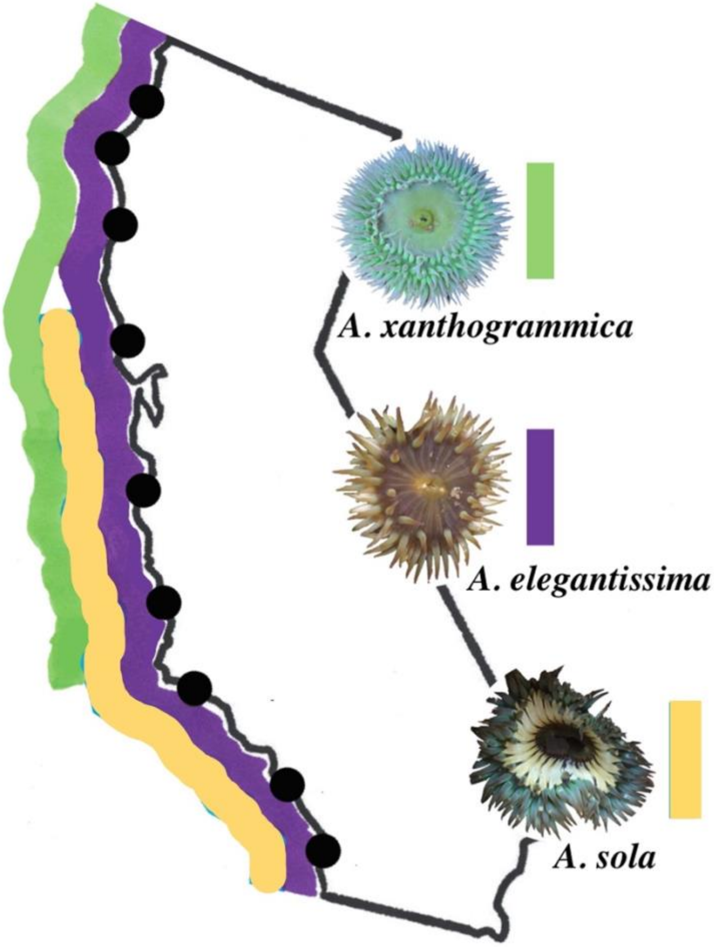


Figure i.2. Map of California with the coastal distribution of three species of sea anemone. Note that all three species are found in the same rocky intertidal area. Lines are offset for clarity. Black dots indicate field sites where data were collected. Note that *A. xanthogrammica*'s range extends south of Point Conception, but its abundance is rare and infrequent along the Southern California coastline.

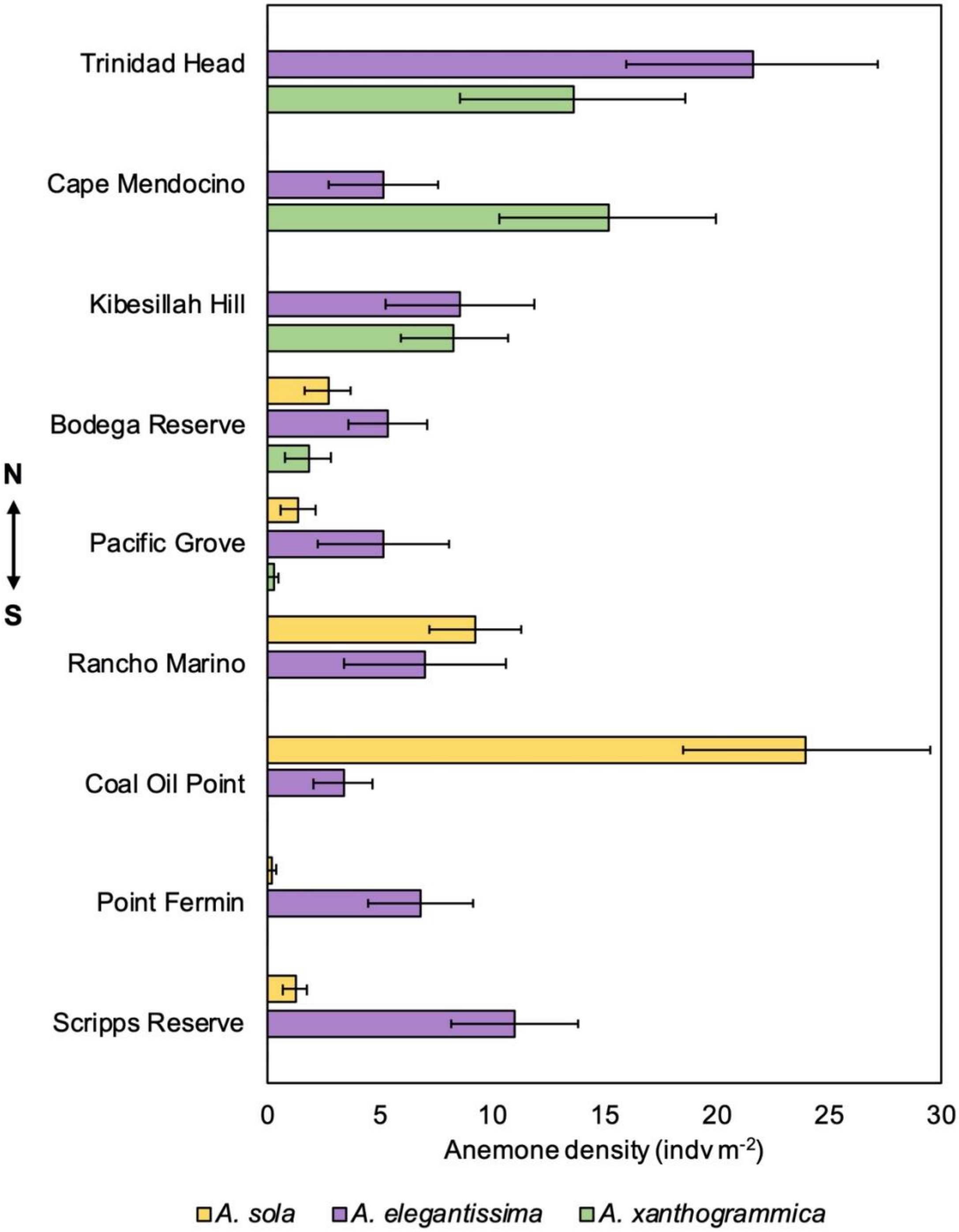


Figure i.3. Mean ± SEM sea anemone density at nine sites surveyed along the coast of California. *A. elegantissima* density is based on colony count, not individuals within each colony.

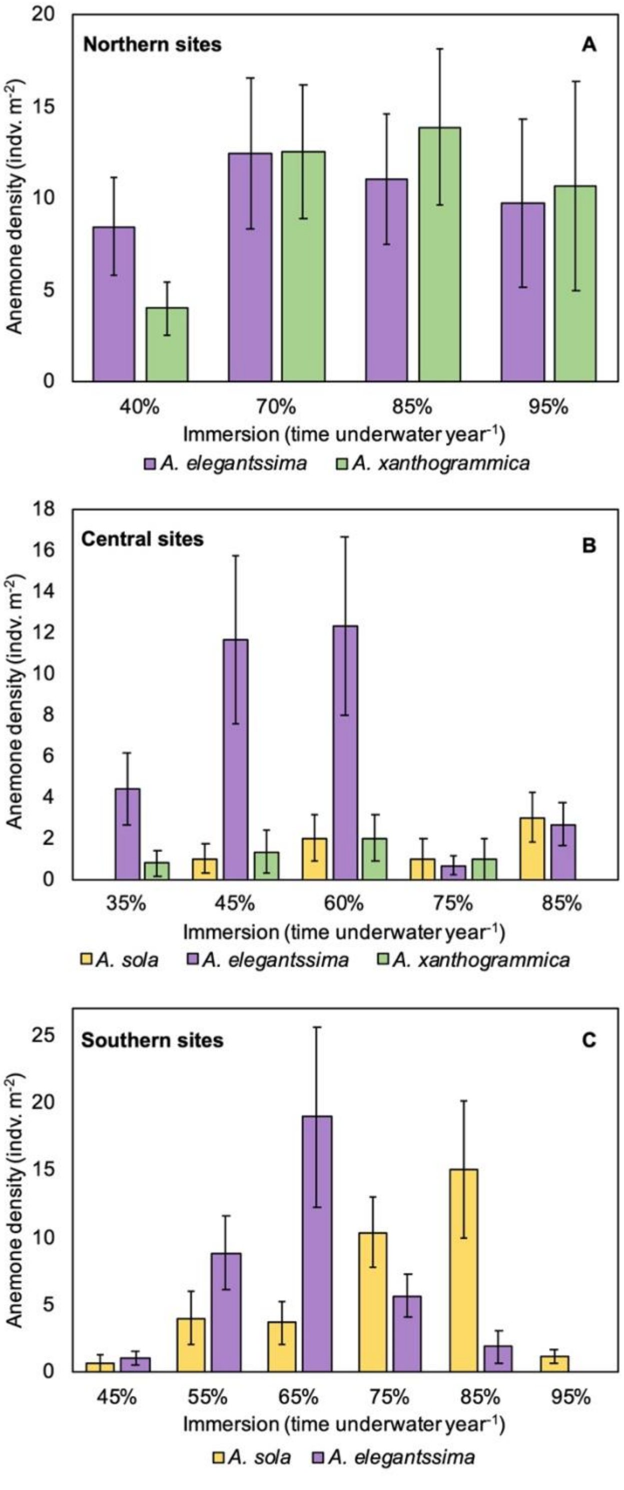


Figure i.4. Mean ± SEM sea anemone density at different tide heights based on immersion time per year. Larger immersion values indicate lower tide heights and vice versa. Tide height categories were chosen based on the available areas in the intertidal zone. *A. elegantissima* density is based on colony count, not individuals. (A) Northern sites include Kibesillah Hill, Cape Mendcino, and Trinidad Head. (B) Central sites include Pacific Grove and Bodega Reserve. (C) Southern sites include Scripps Reserve, Point Fermin, Coal Oil Point, and Rancho Marino. The sites were split into these categories based on the presence or absence of each anemone species.

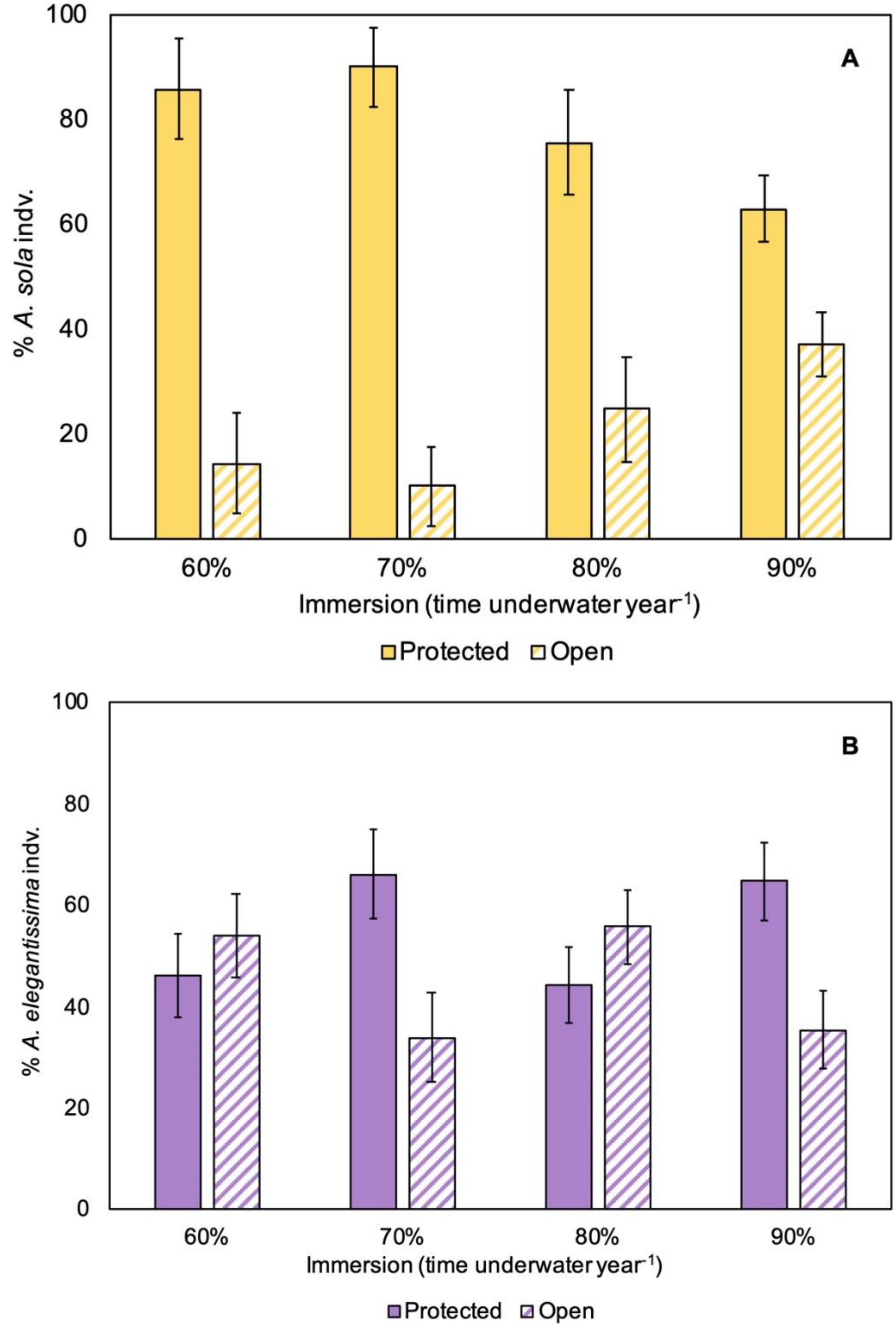


Figure i.5. Mean ± SEM percent sea anemones in protected or open habitat versus immersion per year. Protected habitat includes crevices and pockets in the rock, and open habitat is exposed rock. (A) *A. sola* distribution between habitat types. (B) *A. elegantissima* distribution between habitat types.

References

Augustine L, Muller-Parker G (1998) Selective predation by the mosshead sculpin *Clinocottus globiceps* on the sea anemone *Anthopleura elegantissima* and its two algal symbionts. Limnol Oceanogr 43:711–715. doi: 10.4319/lo.1998.43.4.0711

Aristotle, Cresswell, R., & Schneider, J. G. (1862). Aristotle's History of animals: In ten books. London: H.G. Bohn.

Bachman S, Muller-Parker G (2007) Viable algae released by the seastar *Dermasterias imbricata* feeding on the symbiotic sea anemone *Anthopleura elegantissima*. Mar Biol 150:369–375. doi: 10.1007/s00227-006-0344-y

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

Bingham B. L., Freytes I, Emery M, Dimond J, Muller-Parker G (2011) Aerial exposure and body temperature of the intertidal sea anemone *Anthopleura elegantissima*. Invertebr Biol 130:291–301. doi: 10.1111/j.1744-7410.2011.00241.x

Burris ZP (2011) Larval morphologies and potential developmental modes of eight sea spider species (Arthropoda: Pycnogonida) from the southern Oregon coast. J Mar Biol Assoc U K 91:845–855. doi: 10.1017/S0025315410001554

Cornwell BH (2020) Gene flow in the anemone *Anthopleura elegantissima* limits signatures of local adaptation across an extensive geographic range. Mol Ecol 29:2550–2566. doi: 10.1111/mec.15506

Cornwell BH, Hernández L (2021) Genetic structure in the endosymbiont *Breviolum 'muscatinei'* is correlated with geographical location, environment and host species. Proc R Soc B Biol Sci 288:20202896. doi: 10.1098/rspb.2020.2896

Edmunds M, Potts GW, Swinfen RC, Waters VL (1976) Defensive behaviour of sea anemones in response to predation by the opisthobranch mollusc *Aeolidia papillosa* (L.). J Mar Biol Assoc U K 56:65–83. doi: 10.1017/S0025315400020440

Francis L (1979) Contrast between solitary and clonal lifestyles in the sea anemone *Anthopleura elegantissima*. Integr Comp Biol 19:669–681. doi: 10.1093/icb/19.3.669

Geller JB, Walton ED (2001) Breaking up and getting together: evolution of symbiosis and cloning by fission in sea anemones (genus *Anthopleura*). Evolution 55:1781–1794. doi: 10.1111/j.0014-3820.2001.tb00827.x

Guy LS, Habecker LB, Oxwang G (2014) Giant Green Anemones consume seabird nestlings on the Oregon coast. Mar Ornithol 42:1–2.

Hand C (1955) The sea anemones of central california part II the Endomyarian and Mesomyarian anemones. https://digitalcollections.usfca.edu/digital/collection/p15129coll11/id/223/. Accessed 13 Aug 2020

Harmer, A. C. (2019). *Thamnophis elegans terrestris* (coast gartersnake). Predation. Herpetological Review 50(4)

Hiebert TC, Bingham BL (2012) The effects of symbiotic state on heterotrophic feeding in the temperate sea anemone *Anthopleura elegantissima*. Mar Biol 159:939–950. doi: 10.1007/s00227-011-1871-8

Koch JC, Verde EA, Weis VM (2020) Carbonic anhydrases are influenced by the size and symbiont identity of the aggregating sea anemone *Anthopleura elegantissima*. J Exp Biol. doi: 10.1242/jeb.221424

Levine M, Muller-Parker G (2012) Distribution patterns and nutritional contributions of algal symbionts in the sea anemone *Anthopleura xanthogrammica*. Mar Ecol Prog Ser 453:79–94. doi: 10.3354/meps09602

Lewis LA, Muller-Parker G (2004) Phylogenetic placement of "zoochlorellae" (chlorophyta), algal symbiont of the temperate sea anemone *Anthopleura elegantissima*. Biol Bull 207:87–92. doi: 10.2307/1543583

Pearse V, Francis L (2000) *Anthopleura sola*, a new species, solitary sibling species to the aggregating sea anemone, *A. elegantissima* (Cnidaria : Anthozoa : Actiniaria : Actiniidae). Proc Biol Soc Wash 113:596–608.

Sanford E, Sones JL, García-Reyes M, Goddard JHR, Largier JL (2019) Widespread shifts in the coastal biota of northern California during the 2014–2016 marine heatwaves. Sci Rep 9:4216. doi: 10.1038/s41598-019-40784-3

Saunders BK, MullerParker G (1997) The effects of temperature and light on two algal populations in the temperate sea anemone *Anthopleura elegantissima* (Brandt, 1835). J Exp Mar Biol Ecol 211:213–224. doi: 10.1016/S0022-0981(96)02723-2

Schwarz J, Weis V, Potts D (2002) Feeding behavior and acquisition of zooxanthellae by planula larvae of the sea anemone *Anthopleura elegantissima*. Mar Biol 140:471–478. doi: 10.1007/s00227-001-0736-y

Schwarz JA, Weis VM (2003) Localization of a symbiosis-related protein, Sym32, in the *Anthopleura elegantissima–Symbiodinium muscatinei* association. Biol Bull 205:339–350. doi: 10.2307/1543297

Sebens KP (1981a) Recruitment in a sea anemone population: juvenile substrate becomes adult prey. Science 213:785–787. doi: 10.1126/science.213.4509.785

Sebens KP (1981b) The allometry of feeding, energetics, and body size in three sea anemone species. Biol Bull 161:152–171. doi: 10.2307/1541115

Sebens KP (1983) Population dynamics and habitat suitability of the intertidal sea anemones *Anthopleura elegantissima* and *A. xanthogrammica*. Ecol Monogr 53:405–433. doi: 10.2307/1942646

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*. Invertebr Biol 119:139–146. doi: 10.1111/j.1744-7410.2000.tb00002.x

Smith BL, Potts DC (1987) Clonal and solitary anemones (*Anthopleura*) of western North America: population genetics and systematics. Mar Biol 94:537–546. doi: 10.1007/BF00431400

Smith C (1977) Chemical recognition of prey by the gastropod *Epitonium tinctum* (Carpenter, 1864).

Vassallo-Avalos A, González-Muñoz R, Acuña FH, Rivas G (2020) *Anthopleura mariae* sp. nov. (Cnidaria: Actiniaria: Actiniidae), a new species of sea anemone from the Mexican Pacific. Pac Sci 74:159–170. doi: 10.2984/74.2.5

Verde EA, McCloskey LR (1996) Photosynthesis and respiration of two species of algal symbionts in the anemone *Anthopleura elegantissima* (Brandt) (Cnidaria; Anthozoa). J Exp Mar Biol Ecol 195:187–202. doi: 10.1016/0022-0981(95)00080-1

Weis VM, Reynolds WS (1999) Carbonic anhydrase expression and synthesis in the sea anemone *Anthopleura elegantissima* are enhanced by the presence of dinoflagellate symbionts. Physiol Biochem Zool 72:307–316. doi: 10.1086/316674

CHAPTER 1

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

Originally published (along with supplemental materials) in Proceedings of the Royal Society B (2020). 287: 20201860 doi: 10.1098/rspb.2020.1860

Abstract

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

Introduction

In nutrient-poor environments, mutualistic symbioses are common (Muscatine and Porter 1977; Boucher et al. 1982; Odum and Biever 1984). In these symbioses a diverse set of nutrients are exchanged between partners, but the unifying theme is an exchange of carbon and nitrogen. For example, in relatively nutrient-poor environments, partnerships form between legumes and rhizobia (Masson-Boivin and Sachs 2018), fungi and algae (i.e., lichens; Nash 1996), and corals and algal endosymbionts (Yellowlees et al. 2008). However, these environments are not static, and as resources for hosts and symbionts fluctuate (Herk et al. 2003; Regus et al. 2017), the benefit to each partner may change, potentially disrupting the symbiosis. Legumes in nitrogen-enriched soil no longer benefit from their symbiotic rhizobia (Regus et al. 2017), lichens are impacted by nitrogen deposition (Herk et al. 2003), and coral-algal symbioses may break down as a result of human-induced nutrient fluctuations (Muller-Parker 2015). Most previous studies have focused on anthropogenic changes in nutrient availability; we know less about how natural fluctuations in resources affect mutualistic symbioses *in situ*. A species that can obtain external resources when they are plentiful and simultaneously maintain its association with symbionts could employ a flexible nutritional strategy that depends on resource availability.

Scleractinian coral and their algal endosymbionts have been described using an ecophysiological framework based on nutrient and energy exchange since these relationships were first described (Yonge 1931; Johannes et al. 1970). Studies of coral-algal symbioses have informed our understanding of metabolic exchange between symbiotic partners including autotrophic products from the algae and heterotrophic nutrients from zooplankton captured by the coral (Davies 1984; Davies 1991; Houlbrèque and Ferrier-Pagès 2009). In recent years, a large body of research has focused on the breakdown between corals and their algal symbionts, highlighting the importance of symbionts in coral metabolism (Matthews et al. 2017; Morris et al. 2019). However, symbiotic coral species are obligate mutualists (with the exception of *Astrangia poculata*) where symbiont and host derived nutrition are balanced and critical for survival; flexibility between autotrophic and heterotrophic nutritional pathways is limited (but see Morris et al. 2017; Szmant-Froelich and Pilson 1980; Dimond and Carrington 2007).

Some tropical and temperate sea anemone species are similar to corals in obligately associating with algal endosymbionts (Muller-Parker and Davy 2001), but many symbiotic sea anemones, especially temperate species, are facultative mutualists (Hiebert and Bingham 2012). In contrast to the nutrient-poor environments where corals and some tropical sea anemones live, temperate anemones often benefit from nutrient-rich environments where prey are abundant (Muller-Parker and Davy 2001; Hiebert and Bingham 2012; Davy et al. 1996), enhancing the potential for nutritional flexibility in these symbioses. Symbiont densities in natural populations can vary substantially, and these densities are affected by light intensity and temperature (Saunders and Muller-Parker 1997; Secord and Muller-Parker 2005; S. Bedgood, *unpubl. data*).

At the same time, sea anemones are opportunistic passive suspension feeders that rely on water currents, tides, waves, and chance to deliver potential prey, so food availability can be unpredictable and can vary among individuals and across time (Sebens 1981; Shick 2012; Wells 2019). Whereas several studies have addressed how starvation affects the relationship between anemones and algal symbionts in lab manipulations of tropical (Muller-Parker et al. 1996; Cook et al. 1992; Muller-Parker 1985) and temperate (Fitt and Pardy 1981) species, the applicability of these studies to field conditions remains unknown, as little is known about how variation in food availability affects algal symbionts and their contribution to the host sea anemones in the field. If the relationship between the sea anemone and its algal symbionts is driven by the requirements of the anemone host, then symbionts would be downregulated when prey are readily available and upregulated when prey are scarce. Here we investigate if realistic, *in situ* changes in the food available to sea anemone hosts, based on naturally occurring fluctuations observed in previous studies (Sebens 1981, S. Bedgood, *unpubl. data*), affect the abundance, photophysiology, and interactions between algal symbionts and their host sea anemone.

We studied *Anthopleura sola* and *Anthopleura xanthogrammica,* two sea anemone species that host algal symbionts. Both species coexist on California rocky shores (Hand 1955;

Francis 1973; S. Bedgood, *unpubl. data*), where light is abundant for photosynthesizing symbionts, and food is washed in from adjacent intertidal habitats and the ocean. Both species are similar in size, consume the same prey, and use similar habitat in the mid-intertidal zone (S. Bedgood, *unpub. data*; this study).

The algal symbionts within *A. sola* and *A. xanthogrammica* at our study location are in the family Symbiodiniaceae, the same group that includes symbionts in tropical corals (LaJeunesse et al. 2018). These symbionts are in the genus *Breviolum* (previously Clade B; LaJeunesse et al. 2018; LaJeunesse and Trench 2000; Sanders and Palumbi 2011) and provide a substantial portion of the anemones' dietary carbon as demonstrated by stable isotope analyses (Bergschneider and Muller-Parker 2008; Levine and Muller-Parker 2012). Genetic differences between symbionts in *A. sola* and *A. xanthogrammica* at the same site and tidal height are minimal in this region; genetically identical symbionts are found in both sea anemone species (Cornwell 2018). Therefore, differences in the responses of symbionts are likely due to differences between sea anemone species, not differences in symbiont identity.

The growth rate potential of symbiont cells is likely always higher than that of host cells in cnidarian-algal symbioses, so it is crucial that the host has some control of symbiont density (Davy et al. 2012). Algal symbionts reproduce asexually within their anemone hosts resulting in higher densities (Bergschneider and Muller-Parker 2008) and can vary in volume likely based on productivity (Bergschneider and Muller-Parker 2008; McBride et al. 2009). *Anthopleura elegantissima* (a congeneric co-occurring species) can exocytose and egest algal cells to control their densities (McCloskey et al. 1996; Muller-Parker et al. 2007). There are costs to maintaining high symbiont densities in this species, most notably the production of oxygen radicals (H_2O_2) by photosynthesizing symbionts under intense light that damage host cells (Dykens et al. 1992;

Dimond et al. 2017). While the mechanisms underlying control of symbiont densities in *Anthopleura* spp. are not fully understood, symbiont densities are known to be maintained by nitrogen availability within the host anemone (Smith and Muscatine 1999; Xiang et al. 2020), by coregulation of host and symbiont cell cycles (Tivey et al. 2020), and by symbiont degradation within the host in tropical cnidarian-algal symbioses (Davy 2012). While the algal symbionts may increase their densities by reproducing within the host, the anemone likely has substantial control of symbiont density.

If symbionts function as a partial substitute for captured prey, and there is a cost to the host of maintaining high densities of symbionts within the tissue, then we would expect to observe reduced symbiont abundances when prey are abundant and/or higher abundances when prey are scarce (Fig. 1.1). We hypothesize that this symbiotic partnership is nutritionally flexible and therefore predict that realistic changes in host diet will influence three measures of symbiont productivity (see Fig. 1.1). (1) Symbiont density – which we hypothesize is controlled by the host - will increase when prey are removed and decrease when prey are added. (2) Individual symbiont cell volume will decrease when prey are removed (i.e., more photosynthetic products are given to the host and less is stored in the symbiont cell) and increase when prey are added (i.e., symbionts store photosynthetic products that are not translocated to the host, increasing cell volume). (3) Photosynthetic efficiency will be affected by nitrogen availability within the host (i.e., hosts with added prey may translocate more nitrogen to their symbionts). However, we do not predict any change in photosynthetic efficiency when prey are removed, as hosts in nutrient-rich environments are likely to retain nitrogen when prey are scarce.

Methods

Site description and experimental treatments

Individuals of both sea anemone species (*A. sola* and *A. xanthogrammica*; *n* = 28 each) were located in the intertidal zone at Kenneth S. Norris Rancho Marino Reserve (35°32'24.32"N, 121° 5'34.12"W). Sea anemones were excluded if their largest closed crown diameter was less than 40 mm because anemones smaller than this had distinctly different diets (*i.e.* no mussels or sea urchins, S. Bedgood, *pers. obs.*). We used the length and width of the closed crown to calculate the area (using an ellipse shape) as a measure of anemone size at the beginning and end of the experiment. All sea anemones were located between +0.4 m and +1.1 m above mean lower-low water. Each *A. sola* was paired with a nearby *A. xanthogrammica* within the same habitat. We used a blocked design consisting of 8 sea anemones (4 *A. sola* and 4 *A. xanthogrammica*) in close proximity (e.g., within the same tide pool) that matched all four feeding and species treatments (*n* = 7 blocks).

Four treatments were maintained for three weeks in both species, beginning in June 2018 under a California Department of Fish and Wildlife Scientific Collecting Permit (to S.A.B., ID # SC-13728). Treatments included supplement, control, reduction, and probe. “Supplement” anemones were fed either squid or mussel tissue once daily during the daytime low tide. These are representative of the types of food items that *Anthopleura* spp. consume at this site (S. Bedgood, *unpub. data*). The size of the prey items offered to each anemone was proportional to the anemone’s size and ranged between 3 and 4 g wet mass. “Supplement” anemones likely captured additional prey, so the added food supplemented their natural diet. We did not manipulate the anemones in the control treatments, allowing them to capture prey as usual. We touched the tentacles of “reduction” anemones, waited for their mouths to open, and reached in with a probe or fingers to remove any prey that we found in the gastrovascular cavity. If possible, the prey items were identified prior to being disposed of. We did this once daily during low tide. Since anemones may digest prey within a few hours (Hiebert and Bingham 2012), this treatment likely represented a reduction in food availability instead of complete removal. We treated the "probe" anemones the same way as the "removal" individuals but did not remove any prey.

Symbiont density and cell volume

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

An aliquot of the homogenate was placed on a Brightline hemocytometer (Hausser Scientific, Horsham, Pennsylvania, USA), and photos of each sample were taken on a microscope at 200X magnification. To count the number of symbionts in each square (1 mm^2, $n = 10$), we loaded photos into FIJI (Schindelin et al. 2012), where we batch processed images with a custom macro using the particle analysis function (see Appendix A supplemental). To standardize the symbiont density, we measured animal protein from the same homogenate using the Lowry Method (Lowry et al. 1951) for protein estimation with Bovine Serum as a standard (Hiebert and Bingham 2012; Bergschneider and Muller-Parker 2008).

We calculated symbiont volume using the same photos taken for symbiont density. We batch-processed photos with the particle analysis function (see Appendix A supplemental) using an ellipse-shape fit of particles. Using the length and width output, we calculated the volume based on Hillebrand et al. (Hillebrand 1999), assuming a prolate spheroid shape as described for Symbiodiniaceae.

Chlorophyll a

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

Photosynthetic efficiency

We quantified the symbionts' photosynthetic efficiency (F_v/F_m of dark-adapted Photosystem II) using a Pulse Amplitude Modulation (PAM) fluorometer (Heinz Walz GmbH, Effeltrich, Germany) to determine the effect of host feeding on photosynthetic electron transport. Chlorophyll *a* concentrations give an estimate of photosynthetic activity potential, but combining those data with measurements of the photosynthetic efficiency of chlorophyll provides further insights into photosynthetic productivity responses. PAM measurements of sea anemones were taken in the dark, between 04:00 and 05:00, on the same days we collected tissue samples. Most anemones were closed when measurements were taken, so the sensor was placed at the top of the anemone column, where symbionts are present but at a lower density than in the tentacle tissue

(Dykens and Shick 1984; and see Appendix A supplemental). If the anemone was open, we disturbed it and waited for it to close. We took the average of three measurements of each anemone.

δ^{13}C analysis

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

Statistical analyses

We conducted all analyses in R 3.6.2 and RStudio 1.2.5003 (R Core Team 2019) using the packages lme4 to create general linear mixed models (GLMMs) and emmeans for post hoc analyses. We checked the diet composition data for normality with a Shapiro-Wilk Test, and then used a paired t-test to compare anemone diets. We used GLMMs paired with ANOVA and Tukey post hoc analyses to analyze δ^{13}C, symbiont density, symbiont cell volume, and photosynthetic efficiency. Data from the two anemone species were typically analyzed separately. δ^{13}C values were analyzed using GLMMs with the main effects of treatment and tissue type (anemone and symbiont) and a random effect of anemone. Symbiont density, symbiont cell volume, and photosynthetic efficiency were measured over time with two control groups, so we compared treatment groups in pairs through time: control/supplement and probe/reduction. These data were analyzed with GLMMs with main effects of treatment and time and a random effect of anemone.

Results

Composition of diets

Prey were found in the gastrovascular cavity of *A. xanthogrammica* almost twice as frequently as in *A. sola* (paired t-test: t = -3.56, p = 0.003). Prey were found within *A. sola* during 12.92 ± 2.31% (mean ± SE) of daily checks, while prey were found within *A. xanthogrammica* during 23.47 ± 3.18% of checks. The greatest proportion of both species' diets (40% of observations) was composed of the California sandcastle worm, *Phragmatopoma californica*. Other prey items included limpets, hermit crabs, and sea urchins, but each of these comprised less than 10% of diets. There was no apparent difference in the diet composition of the two anemone species. The frequency of prey was 0.90 ± 0.22 items per week for *A. sola* and 1.64 ± 0.15 items per week for *A. xanthogrammica*. We removed an average of 2-6 items from each anemone in the "reduction" treatment over the course of the experiment. The diet supplement treatments received an additional prey item daily, which represented a substantial increase from ambient prey capture rates. However, this frequency of food availability is not uncommon during periods of high wave exposure, when all anemones surveyed had at least one prey item on consecutive days.

Stable isotope analysis

Anemone diet affected δ^{13}C values (GLMM ANOVA: *A. s.* = *A. sola* treat*portion - $F = 5.73$, $p = 0.025$; *A. x.* = *A. xanthogrammica* treat - $F = 9.74$, $p = 0.007$), but this result was largely associated with the algal symbiont portion for both species (Fig. 1.2). Symbionts from "supplemented" anemones had δ^{13}C signatures that were 2-5‰ lower than the controls (GLMM Tukey HSD: *A. s.* - $t = -4.89$, $p = 0.001$; *A. x.* - $t = -4.1$, $p = 0.004$), but reduction of diet had no effect (*A. s.* - $t = 1.96$, $p = 0.165$; *A. x.* - $t = -0.84$, $p = 0.684$). δ^{13}C values did not differ between anemones and their algae within a treatment, except in the supplement treatment where the symbionts had a lower δ^{13}C (*A. s.* - $t = 3.0$, $p = 0.015$; A. x. - $t = 2.57$, $p = 0.033$).

Symbiont density and chlorophyll a

The symbiont density was affected by treatment (GLMM ANOVA: *A. s.* - $F = 5.06$, $p = 0.044$) and the effect of treatment changed over time (*A. x.* - $F = 7.69$, $p = 0.003$), but the effect was observed in different treatment groups in each anemone species. In *A. sola*, supplementing food resulted in decreased symbiont densities after one week of treatment (GLMM Tukey HSD: $t = 2.74$, $p = 0.01$), but symbiont density did not increase when food was reduced ($t = 1.39$, $p = 0.173$). In *A. xanthogrammica*, supplementing food did not affect symbiont density ($t = -0.17$, $p = 0.869$), but reducing food increased symbiont density after one week of treatment ($t = -4.23$, $p < 0.001$). All symbiont density measurements changed over time (Figs. 1.3, 1.4) due to an increase in symbiont density after one week. Chl *a* per symbiont was not affected by treatment (GLMM ANOVA: *A. s.* reduction - $F = 0.83$, $p = 0.378$; *A. s.* supplement - $F = 0.63$, $p = 0.444$; *A. x.* reduction - $F = 0.17$, $p = 0.684$; *A. x.* supplement - $F = 1.37$, $p = 0.264$), so Chl *a* concentrations tracked symbiont density measurements closely throughout the experiment (Figs. 1.3 and 1.4). However, while there was no effect of supplementation on symbiont density in *A. xanthogrammica*, the Chl *a* concentration in the supplement treatment was lower than the control at week three (Fig. 1.4; GLMM Tukey HSD: $t = 2.55$, $p = 0.017$). Anemone growth (final size – initial size / initial size) was not different among treatment groups at the final time point (ANOVA: *A. s.* reduction - $F = 0.28$, $p = 0.607$; *A. s.* supplement - $F = 0.29$, $p = 0.603$; *A. x.* reduction - $F = 1.97$, $p = 0.186$; *A. x.* supplement - $F = 2.95$, $p = 0.112$), so anemone growth did not affect symbiont density measurements asymmetrically among groups.

Symbiont cell volume

Both sea anemone species had larger symbionts in the supplement treatment (GLMM Tukey HSD: *A. s.* - $t = -4.69$, $p < 0.001$; *A. x.* - $t = -2.26$, $p = 0.033$; Figs. 1.3, 1.4) and symbionts were marginally smaller in *A. xanthogrammica* where food was reduced ($t = 2.05$, $p = 0.051$). There was a main effect of time in both species and treatment comparisons where symbiont volume generally decreased over the course of the experiment (*A. s.* reduction - $F = 25.1$, $p < 0.001$; *A. s.* supplement - $F = 17.2$, $p < 0.001$; *A. x.* reduction - $F = 11.5$, $p < 0.001$; *A. x.* supplement - $F = 4.20$, $p = 0.028$).

Photosynthetic efficiency

The photosynthetic efficiency of algal symbionts was higher in *A. sola* than in *A. xanthogrammica* at the start of the experiment (paired t-test: $t = 5.72$, $p < 0.001$). This difference persisted throughout the experiment, except when food was supplemented. Then, photosynthetic efficiency in *A. xanthogrammica* increased from 0.56 ± 0.05 (mean ± SE) to 0.71 ± 0.01 F_v/F_m (GLMM Tukey HSD: $t = -2.91$, $p = 0.006$) and did not differ from the mean photosynthetic efficiency of the control treatment *A. sola* symbionts (0.67 ± 0.01 F_v/F_m) by the end of the experiment (paired t-test: $t = -1.35$, $p = 0.225$). Photosynthetic efficiency generally increased through time for all groups (Figs. 1.3, 1.4; GLMM ANOVA: *A. s.* supplement - $F = 5.61$, $p = 0.01$; *A. x.* reduction - $F = 5.41$, $p = 0.012$; *A. x.* supplement - $F = 8.07$, $p = 0.002$) except the *A. sola* reduction pairing ($F = 1.74$, $p = 0.198$).

Discussion

Algal symbionts within two species of sea anemone responded to changes in anemone diet, but the responses differed between the anemone species and changed over the course of the experiment. Our framework for dietary carbon-source switching (Fig. 1.1) was supported by our results, but support for our predictions depended on the anemone species. Symbionts within *A. sola* responded to diet supplementation, and symbionts within *A. xanthogrammica* responded to both reduction and supplementation. This may be associated with the fact that *A. xanthogrammica* captured twice as many prey items as *A. sola*, so the reduction treatment had a larger impact on *A. xanthogrammica* than on *A. sola*. Supplementation affected both species, resulting in reduced $\delta^{13}C$ values in symbionts. Furthermore, $\delta^{13}C$ did not differ between anemones and their symbionts, except where food was added. Lower $\delta^{13}C$ values have previously been associated with an increase in heterotrophy in corals (Alamaru et al. 2009) and in *Anthopleura* anemones (Bergschneider and Muller-Parker 2008; Levine and Muller-Parker 2012). A lower $\delta^{13}C$ signature (supplement treatment) occurs when algae selectively incorporate the lighter carbon isotope (^{12}C) over the heavier isotope (^{13}C). Highly productive algal symbionts at high densities cannot choose the lighter carbon isotope because CO_2 is limited within the host tissue, resulting in a heavier carbon isotope signature (reduction and control treatments; Muscatine et al. 1989).

Symbiont densities were affected by host dietary changes, but underlying mechanisms are not well-understood. It is likely that the sea anemone host benefits from a reduction in symbiont density when they are unnecessary (supplement treatment) as they can cause damage to tissue via oxygen radicals (Dykens et al. 1992; Dimond et al. 2017). The host would also benefit from an increase in symbiont density or chlorophyll when heterotrophic diet decreases (reduction treatment) to compensate for lost dietary carbon as an increase in either would allow for increased translocation of photosynthetic products from the symbionts to the host (Fig. 1.1).

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

Reduction of host diet had an effect on *A. xanthogrammica* and its symbionts but not on *A. sola*. Symbiont density increased and symbiont volume decreased when food was reduced in *A. xanthogrammica*, suggesting that the anemone host maintained a higher symbiont density to compensate for the loss of dietary carbon by either retaining symbionts that would otherwise be egested or by increasing the reproduction of symbionts. *A. xanthogrammica* anemones that received fewer external resources had higher autotrophic potential (symbiont density and chlorophyll), but the effect was short-lived and disappeared after three weeks of treatment. Our results suggest there is a trade-off between sources of nutrition – external and symbiont-mediated – in this mutualism. Similar previous work that involved starving sea anemones under laboratory conditions provided conflicting perspectives on the effect of host diet on symbiont density (Muller-Parker 1985; Clayton and Lasker 1984; Cook et al. 1988), but we show here that realistic, *in situ* changes in sea anemone diet reveal ecologically relevant trade-offs in symbiont-host nutrition that were previously unexplored.

Not all algae-hosting cnidarians can switch carbon sources. Tropical corals tend to lose symbionts when starved (Titlyanov et al. 2001; Towle et al. 2015), suggesting that symbionts do not serve as a comparable nutritional pathway in the absence of heterotrophy (but see Grottoli et al. 2006). This is likely because most tropical corals are obligate mutualists, whereas *Anthopleura* anemones are facultative. A better comparison may be to a freshwater hydra where algal symbiont density decreases immediately after predatory feeding (Fishman et al. 2008) and increases with starvation (Douglas and Smith 1984).

Analogous partner interactions exist in terrestrial mutualisms where legumes host fewer rhizobium (via nodules) when external sources of nitrogen are available in the soil (Arrese-Igor et al. 1997; Voisin et al. 2002) and the benefit and cost of arbuscular mycorrhizal fungi to plants is dependent on environmental resources (Johnson et al. 1997). Holobionts with flexible nutritional strategies – like the ones we describe here – may be able to withstand periods of resource limitation, allowing species to persist in an otherwise inhospitable environment. Interactions between hosts and symbionts are dependent on external resource availability in normally nutrient-poor environments. Some mutualisms may break down as a result of perturbations (Wiedenmann et al. 2013; Regus et al. 2017), but others are flexible, requiring more from symbionts when nutrients are scarce or less from them when nutrients are abundant (Fishman et al. 2008; Douglas and Smith 1984; Mariotte et al. 2017). Future research on flexible mutualisms should focus on how realistic fluctuations of external resources affect the production and storage of resources by symbiotic partners.

Our results suggest that even modest changes in resource availability have the potential to alter the interaction between partners in a mutualistic symbiosis, but those changes are species-specific even in congeneric species sharing the same symbiont. We found evidence for a trade-off between autotrophic and heterotrophic nutritional pathways within an algal-symbiont hosting sea anemone, but these pathways are not equal. We propose that autotrophy allows for persistence, but growth likely requires heterotrophy as evidenced in this and other studies on cnidarians (Douglas and Smith 1984; Bedgood 2020). Anemone hosts and algal symbionts respond to changes in heterotrophic diet by altering their interactions with each other, compensating for externally derived nutrition. The potential for flexible nutritional strategy in other mutualistic symbioses is largely unexplored, especially in systems where environmental resources are naturally stochastic.

Acknowledgments

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

Funding

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

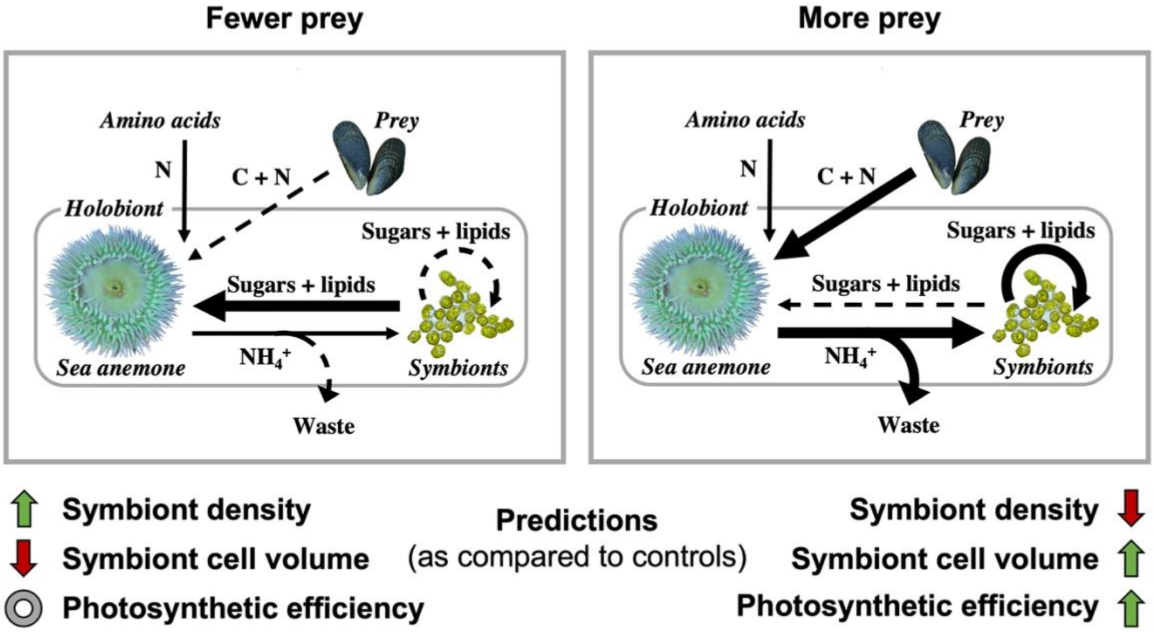


Figure 1.1. Predictions of algal symbiont contributions based on prey availability. Arrows represent the flow of carbon and nitrogen from one source to another. The thickness of each arrow represents the relative contribution and dashed lines represent a reduction in contribution. Predictions of algal symbiont responses to sea anemone dietary changes (increase, decrease, or no effect) are listed under each scenario.

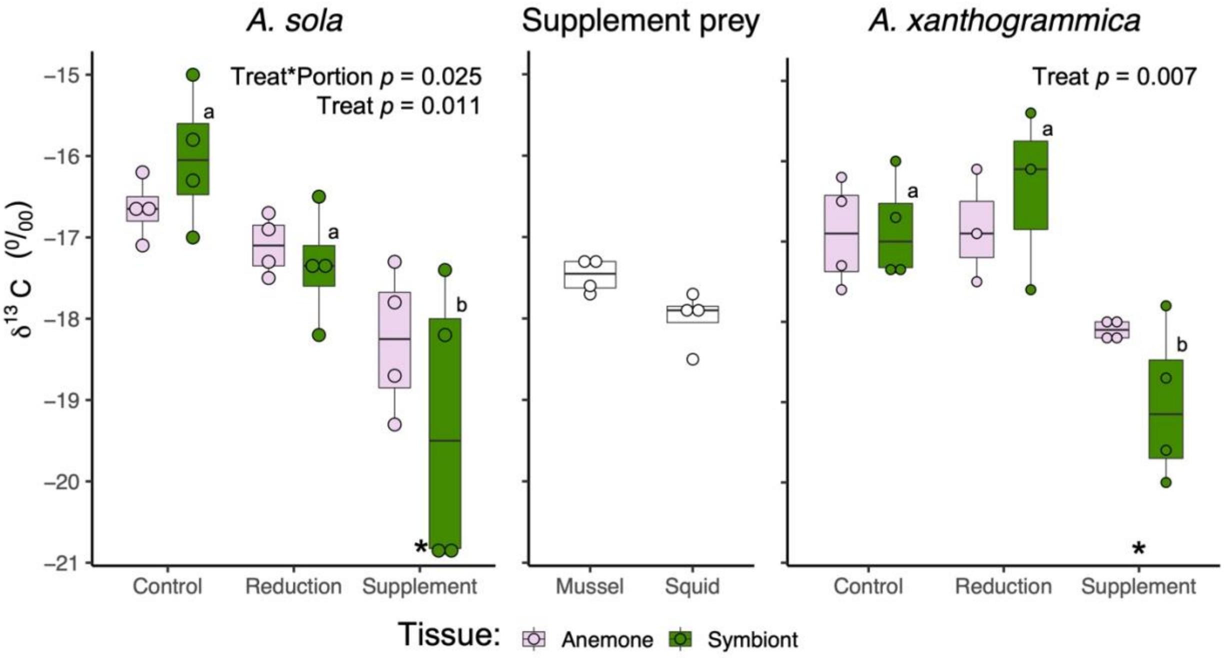


Figure 1.2. Boxplots with overlaid data points (n = 3 or 4) showing δ13C ($^{0}/_{00}$) values for *A. sola*, *A. xanthogrammica*, and supplemented prey items. Tissue samples from anemones were separated into anemone and algal symbiont portions before analysis. Comparisons are made among the control, reduction, and supplement treatments. Significant main effects and interactions from GLMMs are listed in the upper right-hand corner of each graph with p-values. Asterisks above the x-axis signify significant differences between portions within a treatment from a Tukey post hoc analysis. Lowercase letters represent significant differences among treatment groups within the algal symbiont portion.

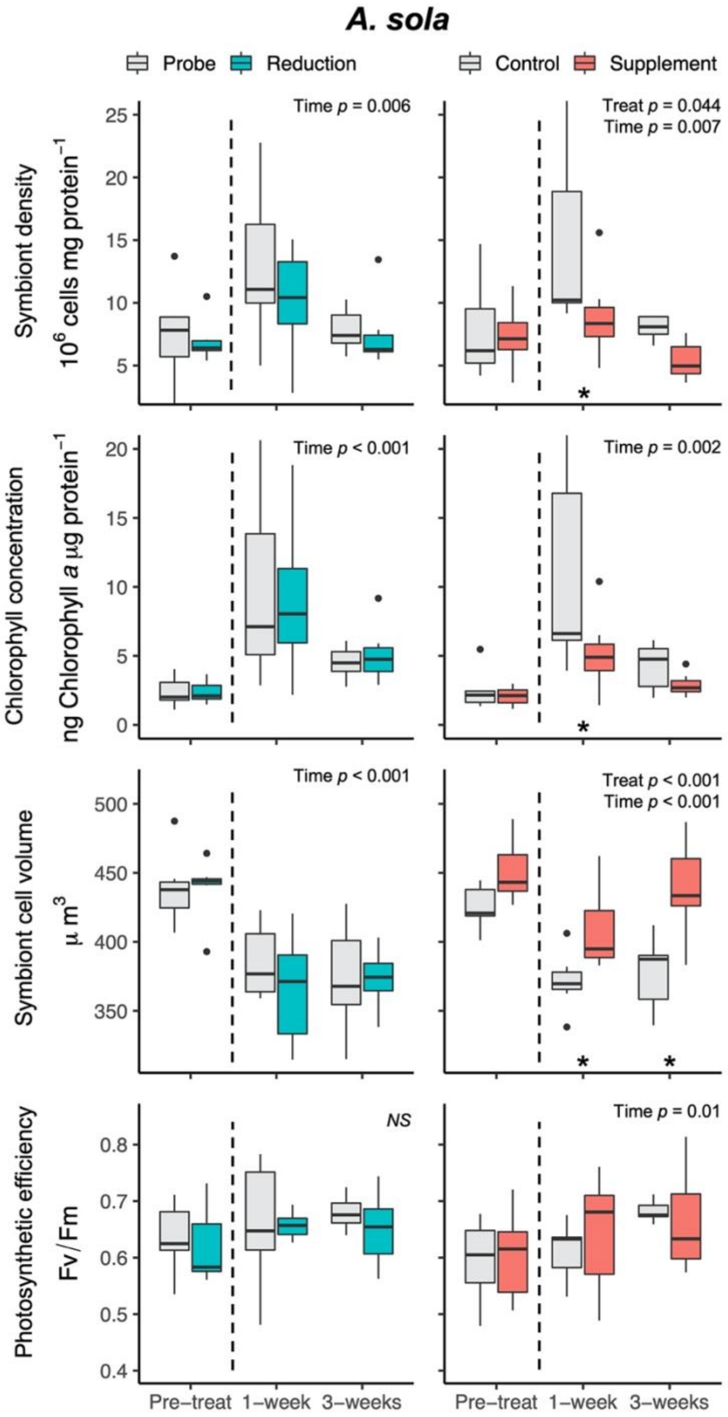


Figure 1.3. Boxplots showing symbiont density, chlorophyll concentration, symbiont cell volume, and photosynthetic efficiency of symbionts within *A. sola* throughout the experiment. Comparisons are made between the supplement and reduction treatments and their respective control treatments. The vertical dashed line represents the start of treatments. Significant main effects and interactions from GLMMs are listed in the upper right-hand corner of each graph with p-values. Asterisks above the x-axis signify significant differences between controls and treatments at a given time-point from a Tukey post hoc analysis. n = 7 for each treatment at each time point.

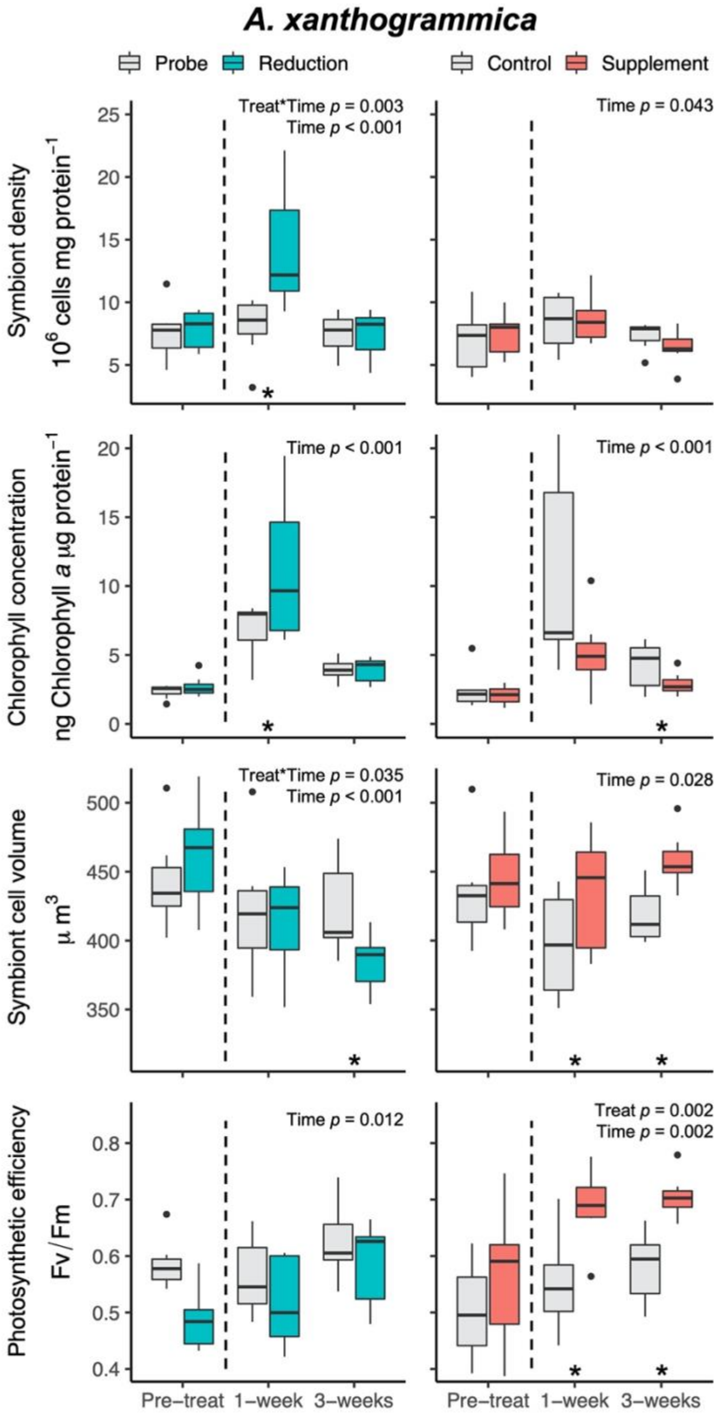


Figure 1.4. Boxplots showing symbiont density, chlorophyll concentration, symbiont cell volume, and photosynthetic efficiency of symbionts within *A. xanthogrammica* throughout the experiment. Comparisons are made between the supplement and reduction treatments and their respective control treatments. The vertical dashed line represents the start of treatments. Significant main effects and interactions from GLMMs are listed in the upper right-hand corner of each graph with p-values. Asterisks above the x-axis signify significant differences between controls and treatments at a given time-point from a Tukey post hoc analysis. n = 7 for each treatment at each time point.

References

Alamaru A, Loya Y, Brokovich E, Yam R, Shemesh A. 2009 Carbon and nitrogen utilization in two species of Red Sea corals along a depth gradient: Insights from stable isotope analysis of total organic material and lipids. *Geochim. Cosmochim. Acta* 73, 5333–5342. (doi:10.1016/j.gca.2009.06.018)

Arrese-Igor C, Minchin FR, Gordon AJ, Nath AK. 1997 Possible causes of the physiological decline in soybean nitrogen fixation in the presence of nitrate. *J. Exp. Bot.* 48, 905–913. (doi:10.1093/jxb/48.4.905)

Bedgood SA, Bracken MES, Ryan WH, Levell ST, Wulff J. 2020 Nutritional drivers of adult locomotion and asexual reproduction in a symbiont-hosting sea anemone *Exaiptasia diaphana*. *Mar. Biol.* 167, 39. (doi:10.1007/s00227-020-3649-3)

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

Boucher DH, James S, Keeler KH. 1982 The ecology of mutualism. *Annu. Rev. Ecol. Syst.* 13, 315–347. (doi:10.1146/annurev.es.13.110182.001531)

Clayton WS, Lasker HR. 1984 Host feeding regime and zooxanthellal photosynthesis in the anemone, *aiptasia pallida* (verrill). *Biol. Bull.* 167, 590–600. (doi:10.2307/1541412)

Cook CB, D'Elia CF, Muller-Parker G. 1988 Host feeding and nutrient sufficiency for zooxanthellae in the sea anemone *Aiptasia pallida*. *Mar. Biol.* 98, 253–262. (doi:10.1007/BF00391203)

Cook CB, Muller-Parker G, D’Elia CF. 1992 Ammonium enhancement of dark carbon fixation and nitrogen limitation in symbiotic zooxanthellae: Effects of feeding and starvation of the sea anemone *Aiptasia pallida*. *Limnol. Oceanogr.* 37, 131–139. (doi:10.4319/lo.1992.37.1.0131)

Cornwell BH. 2018 Environmental variation across nested spatial scales differentially shapes patterns of host and symbiont population genetic structure in a marine symbiotic relationship. Ph.D., University of California, Davis, United States -- California. See https://search.proquest.com/dissertations/docview/2088559165/10E76F14556D4C3DPQ/1.

Davies PS. 1984 The role of zooxanthellae in the nutritional energy requirements of Pocillopora eydouxi. *Coral Reefs* 2, 181–186.

Davies PS. 1991 Effect of daylight variations on the energy budgets of shallow-water corals. *Mar. Biol.* 108, 137–144. (doi:10.1007/BF01313481)

Davy SK, Allemand D, Weis VM. 2012 Cell Biology of Cnidarian-Dinoflagellate Symbiosis. *Microbiol. Mol. Biol. Rev.* 76, 229–261. (doi:10.1128/MMBR.05014-11)

Davy SK, Lucas IAN, Turner JR. 1996 Carbon budgets in temperate anthozoan-dinoflagellate symbioses. *Mar. Biol.* 126, 773–783. (doi:10.1007/BF00351344)

Dimond J, Carrington E. 2007 Temporal variation in the symbiosis and growth of the temperate scleractinian coral *Astrangia poculata*. *Mar. Ecol. Prog. Ser.* 348, 161–172. (doi:10.3354/meps07050)

Dimond JL, Orechovesky S, Oppenheimer J, Rodríguez-Ramos J, Bingham BL. 2017 Photophysiology and hydrogen peroxide generation of the dinoflagellate and chlorophyte symbionts of the sea anemone *Anthopleura elegantissima*. *J. Exp. Mar. Biol. Ecol.* 489, 43–47. (doi:10.1016/j.jembe.2017.01.008)

Douglas A, Smith DC. 1984 The green hydra symbiosis. VIII. Mechanisms in symbiont regulation. *Proc. R. Soc. Lond. B Biol. Sci.* 221, 291–319. (doi:10.1098/rspb.1984.0035)

Dykens JA, Shick JM. 1984 Photobiology of the symbiotic sea anemone, *Anthopleura elegantissima*: defenses against photodynamic effects, and seasonal photoacclimatization. *Biol. Bull.* 167, 683–697. (doi:10.2307/1541419)

Dykens J, Shick J, Benoit C, Buettner G, Winston G. 1992 Oxygen radical production in the sea anemone *Anthopleura elegantissima* and its endosymbiotic algae. *J. Exp. Biol.* 168, 219–241.

Fishman Y, Zlotkin E, Sher D. 2008 Expulsion of symbiotic algae during feeding by the green hydra – a mechanism for regulating symbiont density? *PLOS ONE* 3, e2603. (doi:10.1371/journal.pone.0002603)

Fitt WK, Pardy RL. 1981 Effects of starvation, and light and dark on the energy metabolism of symbiotic and aposymbiotic sea anemones, *Anthopleura elegantissima*. *Mar. Biol.* 61, 199–205. (doi:10.1007/BF00386660)

Francis L. 1973 Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. *Biol. Bull.* 144, 73–92. (doi:10.2307/1540148)

Grottoli AG, Rodrigues LJ, Palardy JE. 2006 Heterotrophic plasticity and resilience in bleached corals. *Nature* 440, 1186–1189. (doi:10.1038/nature04565)

Hand C. 1955 The sea anemones of central California part II the endomyarian and mesomyarian anemones. *Wasmann J. Bio.* 13, 37-100

Herk CM van, Mathijssen-Spiekman E a. M, Zwart D de. 2003 Long distance nitrogen air pollution effects on lichens in Europe. *The Lichenologist* 35, 347–359. (doi:10.1016/S0024-2829(03)00036-7)

Hiebert TC, Bingham BL. 2012 The effects of symbiotic state on heterotrophic feeding in the temperate sea anemone *Anthopleura elegantissima*. *Mar. Biol.* 159, 939–950. (doi:10.1007/s00227-011-1871-8)

Hillebrand H, Dürselen C-D, Kirschtel D, Pollingher U, Zohary T. 1999 Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* 35, 403–424. (doi:10.1046/j.1529-8817.1999.3520403.x)

Houlbrèque F, Ferrier-Pagès C. 2009 Heterotrophy in tropical scleractinian corals. *Biol. Rev.* 84, 1–17. (doi:10.1111/j.1469-185X.2008.00058.x)

Johannes RE, Coles SL, Kuenzel NT. 1970 The role of zooplankton in the nutrition of some scleractinian corals. *Limnol. Oceanogr.* 15, 579–586. (doi:10.4319/lo.1970.15.4.0579)

Johnson NC, Graham JH, Smith FA. 1997 Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytol.* 135, 575–585. (doi:10.1046/j.1469-8137.1997.00729.x)

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR. 2018 Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr. Biol.* 28, 2570-2580.e6. (doi:10.1016/j.cub.2018.07.008)

LaJeunesse TC, Trench RK. 2000 Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biol. Bull.* 199, 126–134. (doi:10.2307/1542872)

Levine M, Muller-Parker G. 2012 Distribution patterns and nutritional contributions of algal symbionts in the sea anemone *Anthopleura xanthogrammica*. *Mar. Ecol. Prog. Ser.* 453, 79–94. (doi:10.3354/meps09602)

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951 Lowry: protein measurement with the folin phenol reagent - Google Scholar. *J. Biol. Chem.* 193, 265–275.

Mariotte P, Canarini A, Dijkstra FA. 2017 Stoichiometric N:P flexibility and mycorrhizal symbiosis favour plant resistance against drought. *J. Ecol.* 105, 958–967. (doi:10.1111/1365-2745.12731)

Masson-Boivin C, Sachs JL. 2018 Symbiotic nitrogen fixation by rhizobia—the roots of a success story. *Curr. Opin. Plant Biol.* 44, 7–15. (doi:10.1016/j.pbi.2017.12.001)

Matthews JL, Crowder CM, Oakley CA, Lutz A, Roessner U, Meyer E, Grossman AR, Weis VM, Davy SK. 2017 Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis. *Proc. Natl. Acad. Sci.* 114, 13194–13199. (doi:10.1073/pnas.1710733114)

McBride BB, Muller-Parker G, Jakobsen HH. 2009 Low thermal limit of growth rate of *Symbiodinium californium* (dinophyta) in culture may restrict the symbiont to southern populations of its host anemones (*Anthopleura* spp.; Anthozoa, Cnidaria)1. *J. Phycol.* 45, 855–863. (doi:10.1111/j.1529-8817.2009.00716.x)

McCloskey LR, Cove TG, Verde EA. 1996 Symbiont expulsion from the anemone *Anthopleura elegantissima* (Brandt) (Cnidaria; Anthozoa). *J. Exp. Mar. Biol. Ecol.* 195, 173–186. (doi:10.1016/0022-0981(95)00079-8)

Morris LA, Voolstra CR, Quigley KM, Bourne DG, Bay LK. 2019 Nutrient availability and metabolism affect the stability of coral–symbiodiniaceae symbioses. *Trends Microbiol.* 27, 678–689. (doi:10.1016/j.tim.2019.03.004)

Muller-Parker G. 1985 Effect of feeding regime and irradiance on the photophysiology of the symbiotic sea anemone *Aiptasia pulchella*. *Mar. Biol.* 90, 65–74. (doi:10.1007/BF00428216)

Muller-Parker G, Davy SK. 2001 Temperate and tropical algal-sea anemone symbioses. *Invertebr. Biol.* 120, 104–123.

Muller-Parker G, D'Elia CF, Cook CB. 2015 Interactions between corals and their symbiotic algae. In *Coral Reefs in the Anthropocene* (ed C Birkeland), pp. 99–116. Dordrecht: Springer Netherlands. (doi:10.1007/978-94-017-7249-5_5)

Muller-Parker G, Lee KW, Cook CB. 1996 Changes in the ultrastructure of symbiotic Zooxanthellae (*Symbiodinium* sp., Dinophyceae) in fed and starved sea anemones maintained under high and low light. *J. Phycol.* 32, 987–994. (doi:10.1111/j.0022-3646.1996.00987.x)

Muller-Parker G, Pierce-Cravens J, Bingham BL. 2007 Broad thermal tolerance of the symbiotic dinoflagellate *Symbiodinium muscatinei* (dinophyta) in the sea anemone *Anthopleura elegantissima* (cnidaria) from northern latitudes. *J. Phycol.* 43, 25–31. (doi:10.1111/j.1529-8817.2006.00302.x)

Muscatine L, Porter JW. 1977 Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *BioScience* 27, 454–460. (doi:10.2307/1297526)

Muscatine L, Porter JW, Kaplan IR. 1989 Resource partitioning by reef corals as determined from stable isotope composition. *Mar. Biol.* 100, 185–193. (doi:10.1007/BF00391957)

Nash TH. 1996 *Lichen Biology*. Cambridge University Press.

Odum EP, Biever LJ. 1984 Resource quality, mutualism, and energy partitioning in food chains. *Am. Nat.* 124, 360–376. (doi:10.1086/284279)

R Core Team. 2019 *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. See https://www.R-project.org/.

Regus JU, Wendlandt CE, Bantay RM, Gano-Cohen KA, Gleason NJ, Hollowell AC, O'Neill MR, Shahin KK, Sachs JL. 2017 Nitrogen deposition decreases the benefits of symbiosis in a native legume. *Plant Soil* 414, 159–170. (doi:10.1007/s11104-016-3114-8)

Saunders BK, Muller-Parker G. 1997 The effects of temperature and light on two algal populations in the temperate sea anemone *Anthopleura elegantissima* (Brandt, 1835). *J. Exp. Mar. Biol. Ecol.* 211, 213–224. (doi:10.1016/S0022-0981(96)02723-2)

Sanders JG, Palumbi SR. 2011 Populations of *Symbiodinium muscatinei* show strong biogeographic structuring in the intertidal anemone *Anthopleura elegantissima*. *Biol. Bull.* 220, 199–208. (doi:10.1086/BBLv220n3p199)

Schindelin J *et al.* 2012 Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9, 676–682. (doi:10.1038/nmeth.2019)

Sebens KP. 1981 Recruitment in a sea anemone population: juvenile substrate becomes adult prey. *Science* 213, 785–787. (doi:10.1126/science.213.4509.785)

Secord D, Muller-Parker G. 2005 Symbiont distribution along a light gradient within an intertidal cave. *Limnol. Oceanogr.* 50, 272–278. (doi:10.4319/lo.2005.50.1.0272)

Shick JM. 2012 *A Functional Biology of Sea Anemones*. Springer Science & Business Media.

Smith GJ, Muscatine L. 1999 Cell cycle of symbiotic dinoflagellates: variation in G1 phase-duration with anemone nutritional status and macronutrient supply in the *Aiptasia pulchella–Symbiodinium pulchrorum* symbiosis. *Mar. Biol.* 134, 405–418. (doi:10.1007/s002270050557)

Szmant-Froelich A, Pilson MEQ. 1980 The effects of feeding frequency and symbiosis with zooxanthellae on the biochemical composition of *Astrangia Danae* Milne Edwards & Haime 1849. *J. Exp. Mar. Biol. Ecol.* 48, 85–97. (doi:10.1016/0022-0981(80)90009-X)

Titlyanov EA, Titlyanova TV, Yamazato K, van Woesik R. 2001 Photo-acclimation of the hermatypic coral *Stylophora pistillata* while subjected to either starvation or food provisioning. *J. Exp. Mar. Biol. Ecol.* 257, 163–181. (doi:10.1016/S0022-0981(00)00308-7)

Tivey TR, Parkinson JE, Weis VM. 2020 Host and symbiont cell cycle coordination is mediated by symbiotic state, nutrition, and partner identity in a model cnidarian-dinoflagellate symbiosis. *mBio* 11. (doi:10.1128/mBio.02626-19)

Towle EK, Enochs IC, Langdon C. 2015 Threatened Caribbean coral is able to mitigate the adverse effects of ocean acidification on calcification by increasing feeding rate. *PLOS ONE* 10, e0123394. (doi:10.1371/journal.pone.0123394)

Voisin A-S, Salon C, Munier-Jolain NG, Ney B. 2002 Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.). *Plant Soil* 242, 251–262. (doi:10.1023/A:1016214223900)

Wells CD. 2019 Biology and ecology of hexacorallians in the San Juan archipelago. Thesis. See https://digital.lib.washington.edu:443/researchworks/handle/1773/44063.

Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret F-E, Postle AD, Achterberg EP. 2013 Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nat. Clim. Change* 3, 160–164. (doi:10.1038/nclimate1661)

Xiang T, Lehnert E, Jinkerson RE, Clowez S, Kim RG, DeNofrio JC, Pringle JR, Grossman AR. 2020 Symbiont population control by host-symbiont metabolic interaction in

Symbiodiniaceae-cnidarian associations. *Nat. Commun.* 11, 108. (doi:10.1038/s41467-019-13963-z)

Yellowlees D, Rees TAV, Leggat W. 2008 Metabolic interactions between algal symbionts and invertebrate hosts. *Plant Cell Environ.* 31, 679–694. (doi:10.1111/j.1365-3040.2008.01802.x)

Yonge CM. 1931 The significance of the relationship between corals and zooxanthellae. *Nature* 128, 309–311. (doi:10.1038/128309a0)

CHAPTER 2

Are sea anemones picky eaters? Feeding preferences of temperate intertidal sea anemones and mechanisms of predation avoidance by their prey

Abstract

Sea anemones are conspicuous members of many marine communities, but their diets and prey choice are often understudied. They are opportunistic passive suspension feeders, consuming most animals that encounter their tentacles, but there are appreciable differences between surrounding animal communities and the contents of the sea anemones' gastrovascular cavities, suggesting that sea anemones may be actively choosing their prey. Here we test the preference of three congeneric sea anemones in the genus *Anthopleura* for several ecologically relevant prey items in laboratory feeding trials. We controlled for time between feeding, choice before and after ingestion, and the state of prey (living, dead, mimic). We describe a tri-phasic feeding response by sea anemones: (1) tentacle wrapping, (2) ingestion, and a newly described response, (3) egestion of undigested prey after ingestion. Sea anemones were unsuccessful in digesting any living snail species offered, but they readily digested dead snails. Our results suggest a possible chemical defense against predation by sea anemones associated with the turban snail *T. funebralis*. Two crustacean species were readily digested by sea anemones, but all items were more likely to be retained when they were offered dead. Algae offered to sea anemones were ingested at a higher rate if the algae had bryozoan epiphytes on their surfaces. Taken together, our results suggest a complex role of these three sea anemone species as scavengers and active predators in temperate intertidal food webs.

Introduction

Sea anemones are conspicuous members of marine communities, but their roles remain understudied relative to other organisms. They are typically viewed as opportunistic consumers of suspended detritus or animals (Erralde and Acuña 2020; Wells et al. 2021) and as food for some specialized predators and parasites (Edmunds et al. 1976; Elliott et al. 1989; Augustine and Muller-Parker 1998). Most sea anemones are considered passive suspension feeders, extending tentacles with nematocysts to capture prey (Sebens 1981; Lesser et al. 1994). They are usually considered indiscriminate in their choice of prey, consuming any animal that comes in contact with their tentacles (Chintiroglou 1992; Quesada et al. 2014), but their ability to capture different animals may vary (Pantin and Pantin 1943; Nagai and Nagai 1973). Because of this, some animals and zooplankton that are common in the surrounding water can be consistently absent from gastrovascular cavities (Sebens 1981; Sebens and Koehl 1984; Chintiroglou 1992; Wells et al. 2021). However, few studies have considered the potential for active prey choice by sea anemones (but see Nagai and Nagai 1973).

Lindstedt (1971) described a biphasic feeding response by the sea anemone *Anthopleura elegantissima* where (1) tentacles react to prey and bring food to the mouth and (2) the mouth opens and ingests prey. Each of these behaviors is driven by chemical feeding activators and mechanical cues. Asparagine induces tentacle wrapping, and reduced glutathione on the mouth induces ingestion in *A. elegantissima* (Lindstedt 1971) and in many other cnidarians (Loomis 1955). The first feeding response of sea anemones (tentacle wrapping) is easily induced by mechanical or chemical stimuli, but ingestion of prey does not always follow if reduced glutathione or other chemical activators from prey are not present (Pantin and Pantin 1943; Williams 1972; Bursey and Guanciale 1977). This may partially explain how sea anemones and other cnidarians can distinguish between animal and other non-nutritive items (*e.g.* algae, plant, inorganic).

Time since their last meal can also impact the behavior of sea anemones. The discharge of nematocysts by tentacles is negatively correlated with the amount of prey they consume and can even be reduced by non-nutritive glass beads filling their gastrovascular cavities (Sandberg et al. 1971). *Anthopleura elegantissima* sea anemones from the upper intertidal zone have a higher prey capture rate as compared to anemones from the lower intertidal zone, likely because higher intertidal sea anemones have a shorter feeding period than those in the lower intertidal zone (Zamer 1986). Sea anemones are less discerning of potential prey when food is rare (Zamer 1986).

Here we focus on three congeneric species in the genus *Anthopleura* to determine if sea anemones display feeding preferences for a variety of ecologically relevant prey. *Anthopleura elegantissima*, *Anthopleura sola*, and *Anthopleura xanthogrammica* overlap in their ranges along the central coast of California (Hand 1955); all three species can be found in high abundance at sites in this region (S. Bedgood, *pers. obs.*). All are found in similar habitat on rocky intertidal shores and are exposed to a wide variety of potential prey that are carried across the intertidal zone by waves (S. Bedgood, *unpubl. data*). While other mostly subtidal sea anemone species like *Metridium senile* and *Metridium farcimen* capture zooplankton and other small prey (Sebens 1981; Wells et al. 2021), *Anthopleura* sea anemones can consume items as large as or sometimes larger than their oral disc (S. Bedgood, *pers. obs.*; Guy et al. 2014). This ability is useful in an environment where wave disturbance is frequent, and organisms break loose and are carried across the intertidal zone to opportunistic sea anemones.

Sebens (1981) described three methods of feeding for this group: capture of suspended zooplankton, capture of sessile prey that are dislodged from the substrate by wave action or by foraging predators, and capture of motile prey that run into sea anemone tentacles. Since these sea anemones are reliant on adjacent communities and disturbance to provide prey, their diets are highly variable in composition and change over time (Sebens 1981; Bedgood et al. 2020). Contrary to predictions, algae, plant materials, and sometimes rocks are found in the gastrovascular cavity of these three species during surveys, while some very common rocky intertidal animals are not found in the gastrovascular cavity (S. Bedgood; *pers. obs*.; Sebens 1981; Bedgood et al. 2020).

There are several limitations to gastrovascular cavity sampling *in-situ*. First, gastrovascular cavity sampling is usually lethal to the sea anemones, and finding prey items in the gastrovascular cavity is rare (< 10% of samples; S. Bedgood, *unpubl. data*), so sampling can have a large impact on populations. Second, time between feeding events cannot be controlled and therefore prey capture effort by sea anemones is not considered. Third, the presence of an item in the gastrovascular cavity does not mean that the item will be digested. Fourth, there is no distinction between scavenging events and live prey capture in this type of sampling. The role of sea anemones as either scavengers or active predators in food webs has not been previously determined in this system. To avoid these issues, we used laboratory mesocosms to test feeding preference on common prey items where we controlled time between feedings, followed the fate of each prey individual, and manipulated the state (living, dead, mimic) of each prey species.

Methods

Collections and maintenance

We collected sea anemones and prey items twice, once in November 2018 and again in January 2020. We included three of the most conspicuous sea anemone species in the rocky intertidal zone of California, *Anthopleura elegantissima, Anthopleura sola,* and *Anthopleura xanthogrammica.* A total of 72 sea anemones were collected (n = 12 per species per trip) from Hazards Canyon Reef (35°17'22.7"N, 120°53'00.6"W) with a metal scoopula, carefully separating the pedal disc of sea anemones from the rocky substrate. All individuals were from similar tide heights, between +0.3 and +0.4 m above mean-lower low water. *A. sola* and *A. xanthogrammica* were ≥ 4 cm in diameter, measured across the crown of the sea anemone when it was closed, and *A. elegantissima* were > 1 cm in diameter. We transported sea anemones to a closed seawater system at the University of California Irvine (UCI) where they were maintained in two 110-liter aquaria at 17 °C with a 12/12 light/dark photoperiod under t5 high output fluorescent lights that provided > 300 mmol photons m^{-2} s^{-1} of photosynthetically active radiation. Illumination is necessary for maintenance of these species, as they host symbiotic unicellular algae. To reduce aggression among sea anemones and easily identifying individuals, we kept each sea anemone in a cage, which consisted of a plastic dish, travertine tile, and gutter guard. The tops of the cages were open, and the sides were porous to allow maximum water flow. Water flow is important to the health of these sea anemone species (S. Bedgood, *pers. obs.*), so we maintained a 30X per hour turnover rate of water flow with aquarium powerhead pumps. All sea anemones were maintained in this environment, except during feeding trials.

We collected prey items from Hazards Canyon Reef and Rancho Marino Reserve (35°32'01.2"N 121°05'06.5"W) at the same time as the sea anemone collections. We chose prey species based on previous research investigating the gastrovascular cavity contents of these sea anemone species (Sebens 1981; Bedgood et al. 2020) and on species that are found near sea anemones but not in the gastrovascular cavity of these sea anemones. Collections included *Mytilus californianus* (California mussels), *Pagurus samuelis* (blueband hermit crab), *Pachygrapsus crassipes* (striped shore crab), *Tegula funebralis* (black turban snail), *Acanthinucella punctulata* (spotted thorn drupe snail), and *Paciocinebrina circumtexta* (circled rock snail). We collected two representative size classes of prey species to standardize prey size by sea anemone biomass, smaller for *A. elegantissima* and larger for *A. sola* and *A. xanthogrammica*. We were unable to collect the smaller size class of *A. punctulata*, *P. circumtexta*, and *P. crassipes*, so these prey species were only offered to *A. sola* and *A. xanthogrammica*. All prey items were transported to UCI where they were either frozen at -25 °C or maintained in a recirculating seawater system, where each species was kept in a separate 30-liter aquarium. These prey items were fed daily with appropriate food for each species (wrack, chopped krill, or littorine snails).

A common observation made by researchers studying the diets of *Anthopleura* sea anemones is the presence of macroalgae in the gastrovascular cavity (Sebens 1981; S. Bedgood, *pers. obs.*). It has been proposed that sea anemones cannot digest the algae, but may be consuming the animals and epiphytes associated with the algae (*e.g.* bryozoans and small crustaceans). To test whether sea anemones choose prey based on epiphytes, we collected two size classes of *Macrocytis pyrifera* (giant kelp) both with and without *Membranipora villosa* (lacy crust bryozoan), one of the most common epiphytes found on kelp that washes into the rocky intertidal zone at our sites. Collections were made in the same habitat where sea anemones were collected. Because they were washed up as wrack, very few of the bryozoan animals were alive upon collection. Therefore, we did not try to maintain living kelp and bryozoans in the lab; they were stored at -25 °C until the feeding trials began.

In addition to live and frozen prey items, we created mimic and manipulated prey. Mimic *T. funebralis* and *M. californianus* were created from the shells of these species by freezing, thoroughly cleaning, drying, and finally filling the shells with 100% silicone, affixing the halves together for *M. californianus* and the operculum for *T. funebralis*. No silicone was exposed on the outside of these mimics; it was used only to approximate the buoyant weight of these species. Silicone was allowed to cure for a minimum of 48 hours, and then all mimics were placed in the same aquaria as live prey items so that their surface biofilms would be as similar to live items as possible. We created three additional manipulated *T. funebralis* prey types to progressively increase the damage to living snails. *T. funebralis* without operculums were created by carefully removing the operculum with a scalpel and allowing recovery for a minimum of 24 hours before they were used in feeding trials. *T. funebralis* with cracked shells were created immediately before feeding trials by tapping the apex of the shell with a hammer until a crack was created in the shell extending from the apex to the base, exposing tissue that would normally be covered with the shell. *T. funebralis* without shells were created immediately before trials in the same way as those with cracked shells, but the shell was carefully removed in this manipulation, leaving a maximum of 20% of the shell where the snail tissue attached. These manipulations were created based on results from a pilot study that revealed that the condition of *T. funebralis* was critical for feeding outcomes with *Anthopleura* sea anemones.

Feeding trials

Feeding trials began after a minimum two-week adjustment period for sea anemones. Two sets of feeding trials were conducted because we could not maintain all the potential prey items during a single experimental period. The first experiment ran from March to April 2019 using sea anemones and prey collected during November 2018, and the second ran from February to March 2020 using collections from January 2020. The experimental methods for both were identical, but the individual sea anemones and prey items offered were different. Sea anemones were taken out of their cages in the seawater system and housed individually in 1.5-liter containers for feeding trials with water from the same seawater system. Each container had an air stone to maintain a high flow rate during trials, and all were illuminated from above by LED daylight (5500K) flood lamps. GoPro cameras (Hero Session and Hero 3) were mounted above these containers and below the lamps to record sea anemone behavior during trials (see Appendix B Supp. Fig. 1).

Sea anemones were tested in groups of 12 (four of each sea anemone species) every four days with a single prey item on each trial day. Individual sea anemones were offered all possible prey items within the experimental period, and the order of prey items offered was randomized for each sea anemone. Sea anemones usually opened within a few minutes of transfer to individual trial containers, but if they did not, we poured seawater over them repeatedly to induce opening before offering the prey item. Live or mimic prey were taken from their tanks, and dead prey were thawed in seawater before being offered to sea anemones with metal forceps where the sea anemone’s tentacles and oral disc met. Feeding trials lasted 2.5 hours from first contact with prey to the end of video recording. We chose 2.5 hours because pilot studies showed that most feeding behaviors and decisions were completed within two hours after introduction of prey. After trials, sea anemone cages were checked daily for egested prey or prey parts (shells or carapaces) to determine digestion success. If a sea anemone rejected an offered prey item before the end of the trial or if the prey item was non-nutritive (*i.e.* kelp, snail mimic), we fed it krill (San Francisco Bay Brand Sally’s frozen krill) proportional to the size of the sea anemone. By doing this we controlled for time between feeding (four days).

Following the conclusion of feeding trials, we analyzed videos for behavioral time points. Behaviors included reaction time, handling time, and gastrovascular cavity time. We defined reaction time as the time it took for sea anemones to cover 50% of the prey item with tentacles after initial contact. Handling time was defined as the time between contact and fully ingesting prey (closed mouth). We defined gastrovascular cavity time as the total time prey spent in the gastrovascular cavity before being egested by the sea anemone. The sample size of this last behavior was smaller than other behavioral times because this behavior only applied to items that were rejected within the 2.5 hours of recording.

Sea anemone size

We attempted to control for sea anemone size, but there was significant variation in size even within sea anemone species. Size plays a large role in prey capture of other species (Anthony 1997), so we included sea anemone size as a covariate in our analyses. We collected size data with images from feeding trials before sea anemones opened, measuring length and width of the base of sea anemones with FIJI (Schindelin et al. 2012). Size was estimated by calculating the area of an ellipse form these values, a well-established method for non-destructively measuring sea anemone size (Bedgood et al. 2020). Size data were collected for each individual anemone from both experiments (n=72) at three time points, once within the first week of the feeding trials, once in the middle of the trials, and once within the last week. From there, size data for all remaining trials were estimated using a linear regression based on the three known data points for each anemone.

Statistical analyses

All analyses were conducted in R version 4.0.5 (R Core Team 2021). Models were created using the package lme4 (Bates et al. 2015), these were analyzed with the package car (Fox and Weisberg 2019) and emmeans (Lenth 2021), and subsequent figures were assembled with the packages tidyverse and ggplot2 (Wickham et al. 2019). For each analysis, we checked the assumptions and modified our approach accordingly (*e.g.* data distributions). The binomial data (retain/reject and digest) were analyzed with generalized linear models with a binomial family distribution and brglmFit method (Kosmidis et al. 2020) to avoid complete separation. To test for preference in binomial data, we used binomial tests comparing to 0.5 (no preference in either direction). Behavioral data (GVC and handling times) were analyzed with generalized linear mixed effects models with gamma distributions and sea anemone individual as a random effect.

Results

Sea anemones responded with tentacles (feeding phase 1; [Lindstedt 1971]) to all items that were offered, potentially as a result of short-term starvation between trials (4 days), but ingestion varied with offered prey. Virtually all dead prey items (*T. funebralis*, *M. californianus*, *P. samuelis*) offered to sea anemones were retained (98.89%), but only 72.33% of living prey items were ingested. It quickly became clear during the trials that individuals were also choosing to reject prey after ingestion. 16.59% of prey items that were first ingested were rejected undigested during the 2.5-hour trial period. Here we describe retention of prey items - termed "retained" - only if the sea anemone kept the prey item for the full trial period. Some prey species were frequently retained but were found alive and not digested, even after several days in the gastrovascular cavity. This was especially true of the snail species tested; many were retained but none were digested (Fig. 2.1). The proportion digested was out of the total offered not of the total retained, so proportion digested in figure 2.1 does not exceed the proportion retained. If they are equal, then all retained prey were digested.

Prey species affected whether sea anemones retained or rejected items ($\chi^2 = 58.10$, $P < 0.001$), and a post hoc analysis found differences between *T. funebralis* and all other prey species (example: *T. funebralis-M. californianus* $z = 4.94$, $P < 0.001$). Sea anemone species also affected prey retention ($\chi^2 = 9.82$, $P = 0.007$); *A. sola* retained more prey items than the other two species (*A. sola-A. elegantissima*: $z = -2.57$, $P = 0.028$; *A. sola-A. xanthogrammica*: $z = 2.54$, $P = 0.030$). Sea anemones usually retained *M. californianus* (27 of 36, $P = 0.004$) and *P. crassipes* (21 of 24, $P < 0.001$), but almost always rejected *T. funebralis* (3 of 36, $P < 0.001$). *P. samuelis* was not different from no preference (22 of 36, $P = 0.243$), but this may have been driven by a strong escape behavior from *P. samuelis*, which even left their shell to escape in one trial. Kelp (*M. pyrifera*) with encrusting bryozoans (*M. villosa*) was usually retained (29 of 36, $P < 0.001$), but kelp without bryozoans was not different from no preference (13 of 36, $P = 0.133$). Sea anemones retained a larger proportion of kelp with bryozoans than without ($\chi^2 = 8.55$, $P = 0.003$) regardless of sea anemone species ($\chi^2 = 1.89$, $P = 0.389$).

Prey species also affected digestion of those prey that were retained ($\chi^2 = 140.61$, $P < 0.001$), and *A. sola* sea anemones digested more prey than the other two species (*A. sola-A. elegantissima*: $z = -3.23$, $P = 0.004$; *A. sola-A. xanthogrammica*: $z = 2.50$, $P = 0.033$). The three snail species, *T. funebralis, A. punctulata, P. circumtexta,* were never digested, appearing alive in the sea anemone cages a day to several days after they were ingested. 14.82% of *M. californianus* that were retained by sea anemones were later found alive. Both crab species, *P. samuelis* and *P. crassipes* were always digested if they were retained (Fig. 2.1).

To evaluate mechanisms underlying retention of prey items, we offered sea anemones manipulated *T. funebralis* (snail) and *M. californianus* (mussel; Fig. 2.2). There was an effect of prey type ($\chi^2 = 174.99$, $P < 0.001$) and sea anemone species ($\chi^2 = 7.08$, $P = 0.029$), but no significant interaction ($\chi^2 = 11.27$, $P = 0.337$) for *T. funebralis* manipulations. Sea anemones usually rejected live *T. funebralis* if they were unmanipulated or had no operculum, but they always retained *T. funebralis* if the snails lacked a shell or if they were dead with the shell (Fig. 2.2). Those snails without an operculum that were retained were digested by the sea anemone, while the two snails that were ingested with intact operculum survived and were found a day later. Sea anemone species influenced the responses to mimic and cracked shell snails. *A. sola* and *A. elegantissima* sea anemones had no preference for *T. funebralis* mimics (*A. sola*: 7 of 12, $P = 0.774$; *A. elegantissima*: 5 of 12, $P = 0.774$), but *A. xanthogrammica* always rejected mimics (0 of 12). Similarly, *A. sola* and *A. xanthogrammica* had no preference for *T. funebralis* with cracked shells (*A. sola*: 8 of 12, $P = 0.388$; *A. xanthogrammica*: 9 of 12, $P = 0.146$), but *A. elegantissima* always retained them (12 of 12). Manipulation of *M. californianus* ($\chi^2 = 37.48$, $P < 0.001$) and sea anemone species affected retention ($\chi^2 = 7.01$, $P = 0.030$). *A. sola* was the only sea anemone species that significantly retained unmanipulated *M. californianus* (11 of 12, $P = 0.006$). Sea anemones had no preference for mussel mimics, but they always retained dead mussels (Fig. 2.2).

Living *T. funebralis* were almost never rejected outright, they were usually ingested and then subsequently egested within our 2.5-hour trial period. Cracked-shell and dead *T. funebralis* are not included here because all items were retained, and only rejected items had gastrovascular cavity (GVC) times. The amount of time *T. funebralis* spent in the GVC was affected by our manipulations ($\chi^2 = 94.85$, $P < 0.001$), but sea anemone species had no effect ($\chi^2 = 1.49$, $P = 0.475$). Unmanipulated snails were in the GVC for only 16.92 ± 4.02 (mean ± SEM) minutes while snail mimics were kept in the GVC for over 5 times as long, 84.95 ± 12.87 minutes ($z =$

7.39, $P < 0.001$). Those snails without an operculum were in the GVC for even less time (8.48 ± 2.31 minutes) than unmanipulated snails ($z = 3.76$, $P = 0.001$).

Handling times of prey (contact to ingestion) were affected by prey species or type ($\chi^2 = 36.58$, $P < 0.001$), but they were not affected by sea anemone species ($\chi^2 = 2.22$, $P = 0.330$). *T. funebralis* had the shortest handling time of 5.26 ± 0.65 minutes, and *M. pyrifera* with *M. villosa* (bryozoa) had the longest handling time of 27.94 ± 6.58 minutes (Fig. 2.4). *T. funebralis* handling times were significantly shorter than the three other animal species tested (*T. funebralis*-*M. californianus*: $z = -3.62$, $P = 0.004$; -*P. samuelis*: $z = -4.53$, $P < 0.001$; -*P. crassipes*: $z = 4.33$, $P < 0.001$). Even though *M. pyrifera* with bryozoans were retained more often than *M. pyrifera* alone (Fig. 2.1), sea anemones took a longer time deciding to retain *M. pyrifera* with bryozoans than kelp without ($z = 3.36$, $P = 0.010$).

Sea anemone species differences found in our behavior data were not a product of size differences among groups even though size varied considerably within each species. Sea anemone size had no effect on retention ($\chi^2 = 0.18$, $P = 0.670$), digestion ($\chi^2 = -3.38$, $P = 1$), GVC times ($\chi^2 = 0.744$, $P = 0.389$), or handling times ($\chi^2 = 1.63$, $P = 0.202$).

Discussion

Our results confirm that these sea anemone species do have a feeding preference during the second part of their described biphasic feeding response – when the mouth opens and ingests prey (Lindstedt 1971) – but they also demonstrate a choice after items are ingested, egesting some prey undigested within a few hours. Based on our results, we suggest that these sea anemones exhibit a triphasic feeding response: (1) response by tentacles to potential prey by wrapping and moving the item towards the mouth, (2) opening of the mouth and ingestion of the item, (3) either retention or rejection of prey items in the gastrovascular cavity within several hours of ingestion. Each step may be important to sea anemones choosing to retain or reject items in an environment that is highly stochastic.

The two crab species tested here (*P. samuelis* and *P. crassipes*) were not always retained by sea anemones. This was a result of the crabs' escape response from the oral disc and not sea anemone choice. *P. samuelis* was especially good at this escape behavior, letting tentacles grab the shell and not their legs. They would crawl across the oral disc before dismounting the sea anemone, even leaving behind their shell to escape in at least one trial. If a crab was ingested, it was always digested.

None of the intact snails tested here were digested, suggesting that this group is safe from predation by these sea anemone species. It is still unclear what mechanism allows for this survival, but we suggest that snails may be able to close their opercula and wait for sea anemones to egest them within a few days. This is especially true for *P. circumtexta* and *A. punctulata*, which were usually retained within the 2.5-hour trial period and were found sometimes several days later egested and alive. This mechanism may also explain why not all *M. californianus* were digested. It may be a waiting game between the sea anemone and its living prey within the gastrovascular cavity, where prey must stay closed for hours or even days to avoid digestion.

The explanation for *T. funebralis* survival seems to be more complex than the other species. Most of these snails were ingested and handling times were faster for this species than any other animal, but very few (2 of 12) were retained. Manipulations of *T. funebralis* to determine mechanisms for survival had a large impact on the retention of individuals. Removing the operculum had no effect on retention, but those few that were retained did not survive, supporting our hypothesis that staying shut may stop digestion. *A. elegantissima* and *A. sola* had no preference in either direction for our mimic snails, which approximated tightly closed living snails. This hinted at a role of the living snail in rejection by sea anemones. Any other manipulations to the shell with a living snail inside usually ended in retention and digestion, but *T. funebralis* with a cracked shell were not always retained by *A. sola* and *A. xanthogrammica*.

Gastrovascular cavity times of manipulated *T. funebralis* reveal that living snails spend much less time in the sea anemone than the mimics. Our results demonstrate that sea anemones will usually retain and digest this species if the shell is compromised or the snail is dead. If snails are simply staying closed while in the gastrovascular cavity, then they would be egested at a similar time as the mimics or other two snail species tested here. We hypothesize that this snail species may release some aversive chemical when in the gastrovascular cavity of these sea anemones. Those snails without an operculum were in the gastrovascular cavity for even less time than the intact snails, suggesting a faster release of the potential chemical defense. There are at least two described examples of anti-predator defenses by sea anemone prey. Bryan et. al. (1998) found that the nudibranch *Tritoniella belli* was captured and frequently ingested by the sea anemone *Isotealia antarctica*, but 70% of the nudibranchs were released alive minutes to hours after ingestion, likely due to chemical defenses. Kicklighter and Derby (2006) describe an anti-predatory response of the sea hare *Aplysia californica* to predation by *A. sola*, where the sea hare releases ink that is aversive to the sea anemone before ingestion occurs, causing the anemone to drop the prey. More work is needed to test whether *T. funebralis* releases any aversive chemicals when ingested by *Anthopleura* or if some other mechanism can explain this pattern. Several studies (Tomanek and Somero 2000; Kelly et al. 2010; Gleason and Burton 2013) have addressed the population genetics and genes associated with heat shock proteins in *T.*

funebralis. We suggest that a genome or metabolome approach may be able to identify candidates for aversive chemical production in this snail species.

Finding algae or plants in the gastrovascular cavity of sea anemones is not uncommon, but they likely serve no nutritive role (S. Bedgood, *unpubl. data*; Sebens 1981). We have observed that algae and surfgrass in the gastrovascular cavity are almost always covered in epiphytes, suggesting the target may be the animal epiphyte and not the algae themselves. In our experiments, kelp with bryozoan epiphytes was retained more often than kelp without. However, handling times were longer for kelp with bryozoans than kelp without. One explanation may be that sea anemones are indecisive about ingesting kelp when epiphytes are present. It remains unclear why a significant number of sea anemones in our trials quickly ingested kelp with no epiphytes and egested it days later undigested.

Sea anemone species only played a minor role in the response to prey items, and chemical cues were not necessary for ingestion. *A. sola* tended to retain and digest more items than the other two species, but there were no significant interactive effects between sea anemone species and prey items. *A. xanthogrammica* was more likely to reject mimic prey items, potentially suggesting that this species relies on chemical cues more than the other two species, but overall, chemical cues did not seem to influence the ingestion of prey. Living and mimic prey were ingested at a similar rate, but dead animals that were previously frozen were ingested more often. We assume there would be more chemical cues associated with the previously frozen prey items, which likely had broken cell walls and would produce more glutathione.

These sea anemones likely serve as both predators and scavengers; they are more likely to retain and digest dead prey than living prey. Crustaceans seem to be the most vulnerable if they are captured, and the three snail species tested could not be digested if they were alive, suggesting only a scavenger role of sea anemones for this group. This explains some of the patterns observed in Sebens' (1981) *in-situ* gastrovascular cavity sampling of *A. elegantissima* and *A. xanthogrammica*, where mussels and crustaceans made up the majority of prey items found. Sebens observed a few snail species present, including *Littorina* spp. and one *T. funebralis*, but these made up only 7.8% of items found in *A. elegantissima* and 2.3% of items found in *A. xanthogrammica*. New work suggests that, instead of a consumptive role, *Anthopleura* sea anemones may provide favorable microhabitat during low tides to these snail species (Bedgood et. al., *submitted*).

Acknowledgements

We would like to thank Alan Yue, Queenie Baetiong, and Calvin Huang for their help in collecting animals in the field, caring for them in the laboratory, and quantifying behavior from video assays. We also thank the University of California Natural Reserve System for access to Kenneth S. Norris Rancho Marino Reserve (doi: 10.21973/N37943). This research was supported by the National Science Foundation (Graduate Research Fellowship to S.A.B. and OCE-1736891 to M.E.S.B. and A. Martiny), the Mildred E. Mathias Graduate Student Research Grant (to S.A.B.).

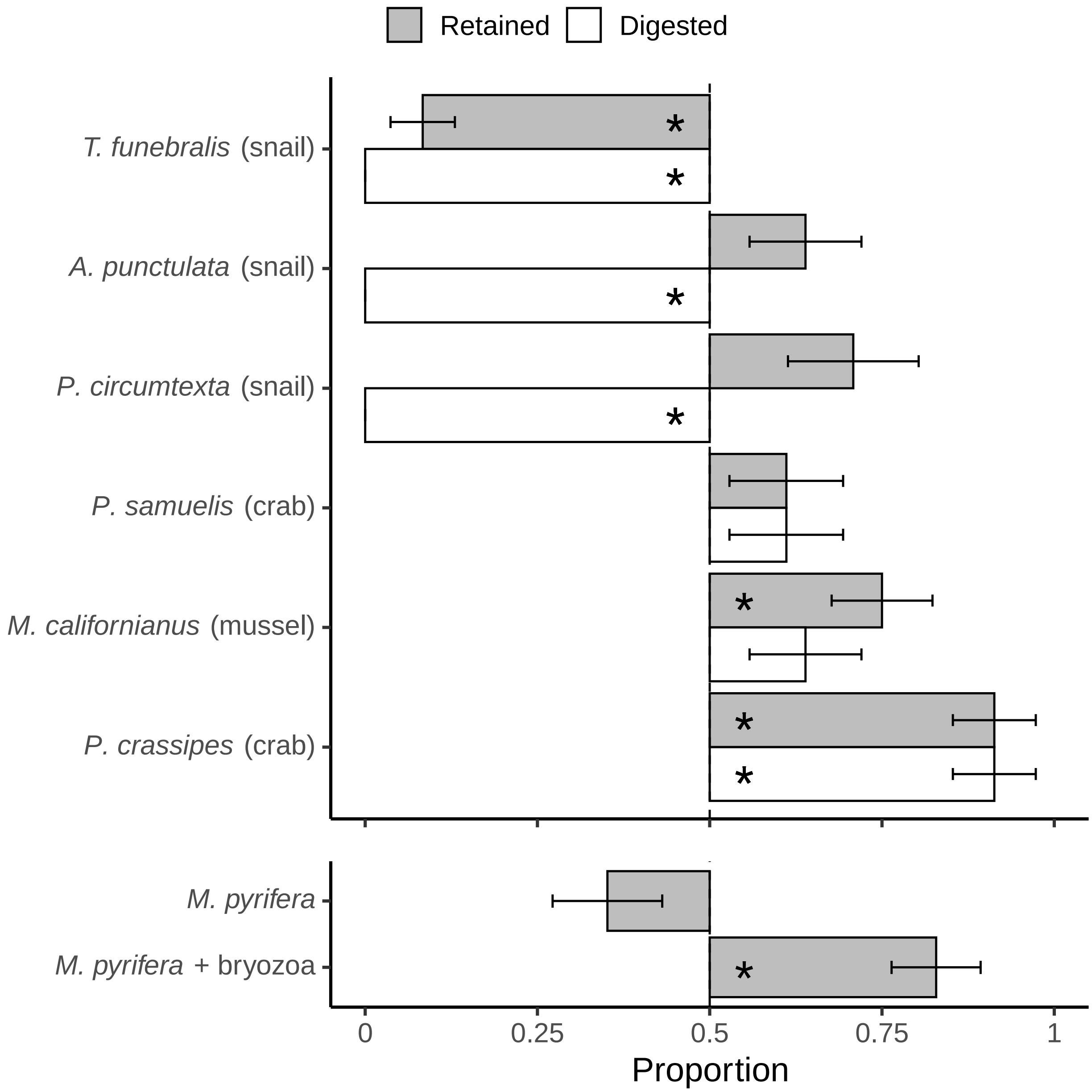


Figure 2.1. Proportion of items retained (filled) and digested (empty) of each prey species. Sea anemone species were pooled. Asterisks indicate significant differences from no preference (0.5). Each bar has n = 36 observations for all species except *P. crassipes* and *P. circumtexta*, which had n = 24 observations (no data for *A. elegantissima*). Bars are mean ± SEM taken from binary data.

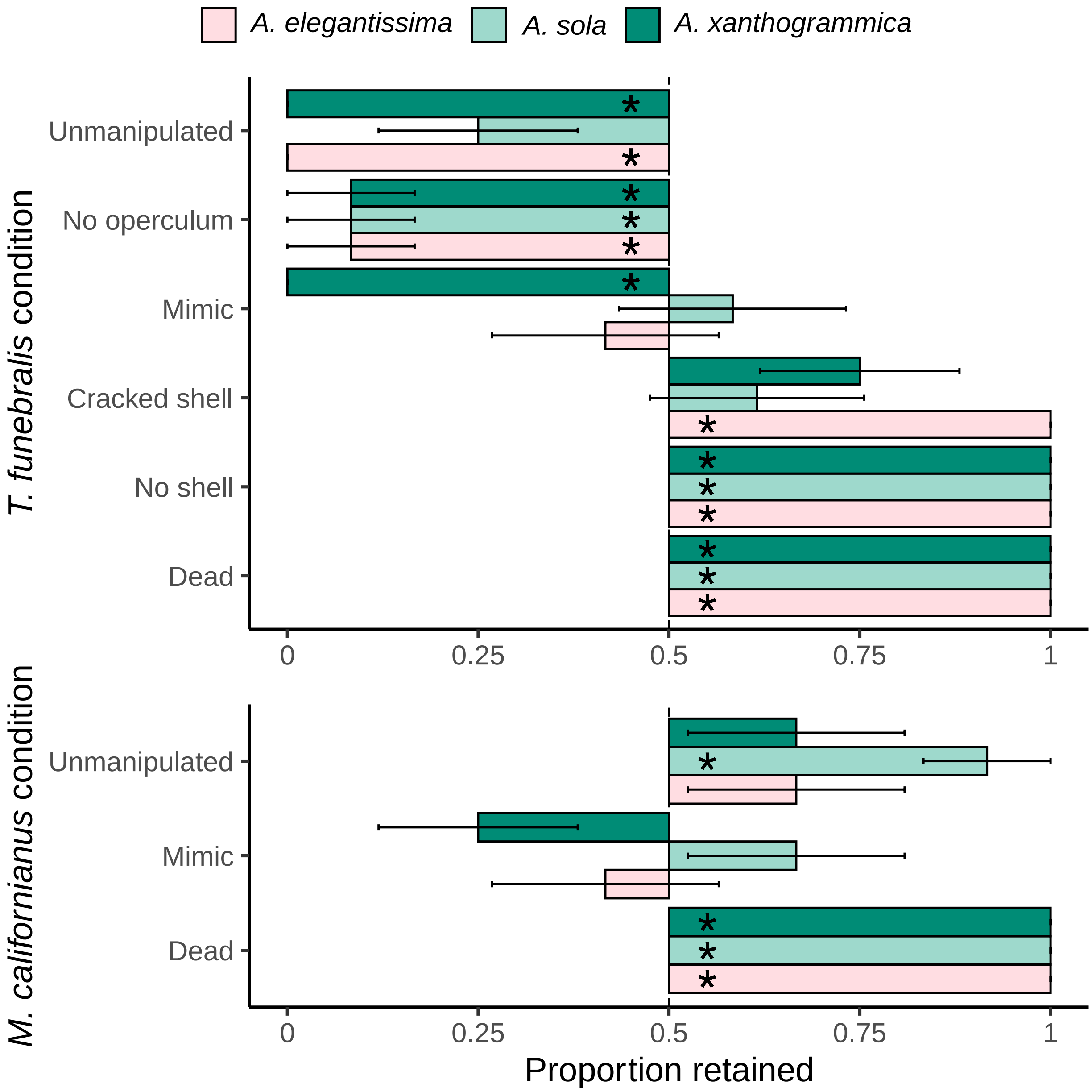


Figure 2.2. Proportion of items retained of intact and manipulated *T. funebralis* and *M. californianus* prey species by sea anemone species (colors). Asterisks indicate significant differences from no preference (0.5). Each bar has n = 12 observations. Bars are mean ± SEM taken from binary data.

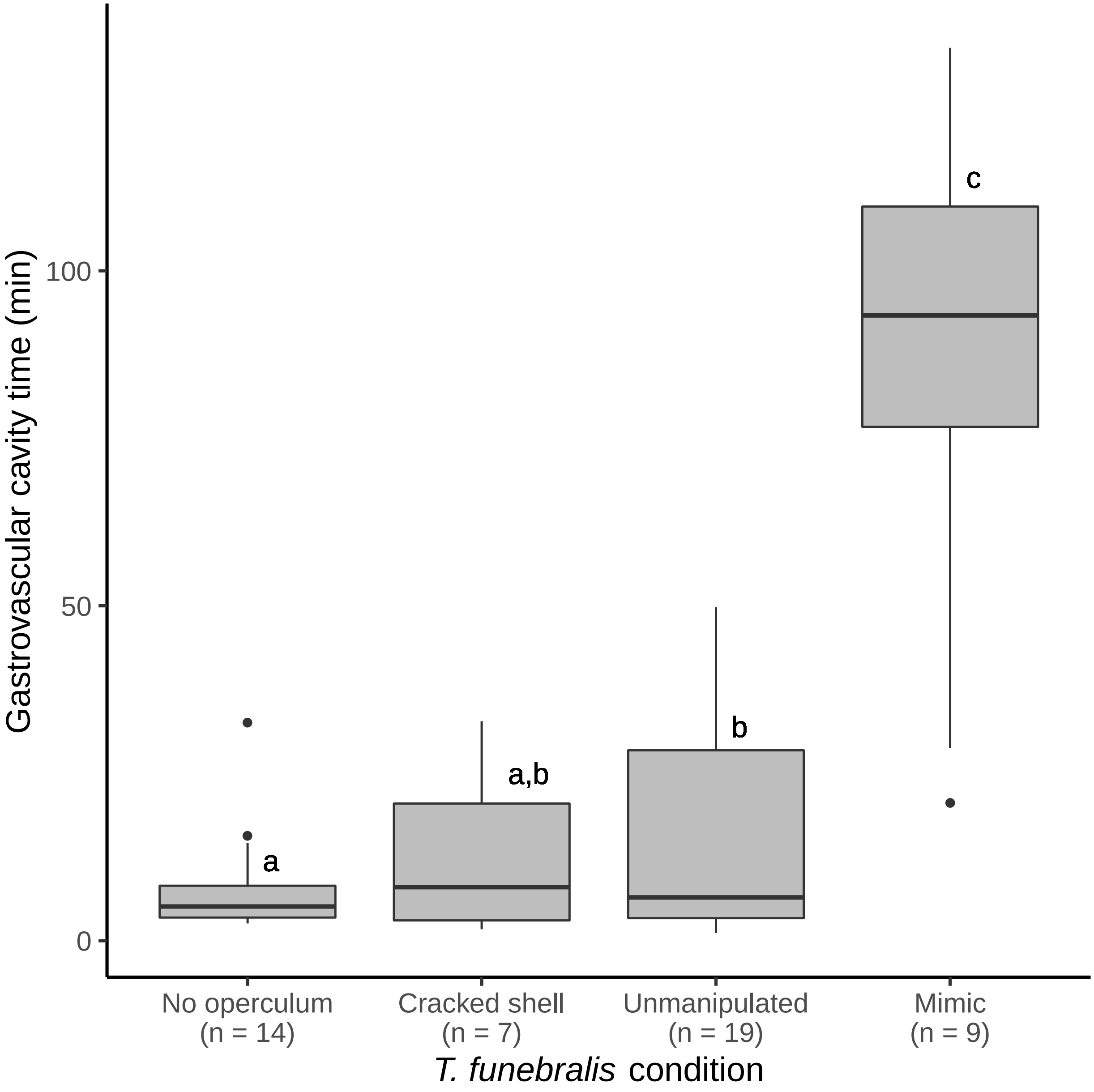


Figure 2.3. Boxplots of the time spent in the gastrovascular cavity of sea anemones by intact and manipulated *T. funebralis*. Letters above boxes indicate significant differences between groups based on a post hoc analysis. Sample sizes for each type are listed below the x-axis.

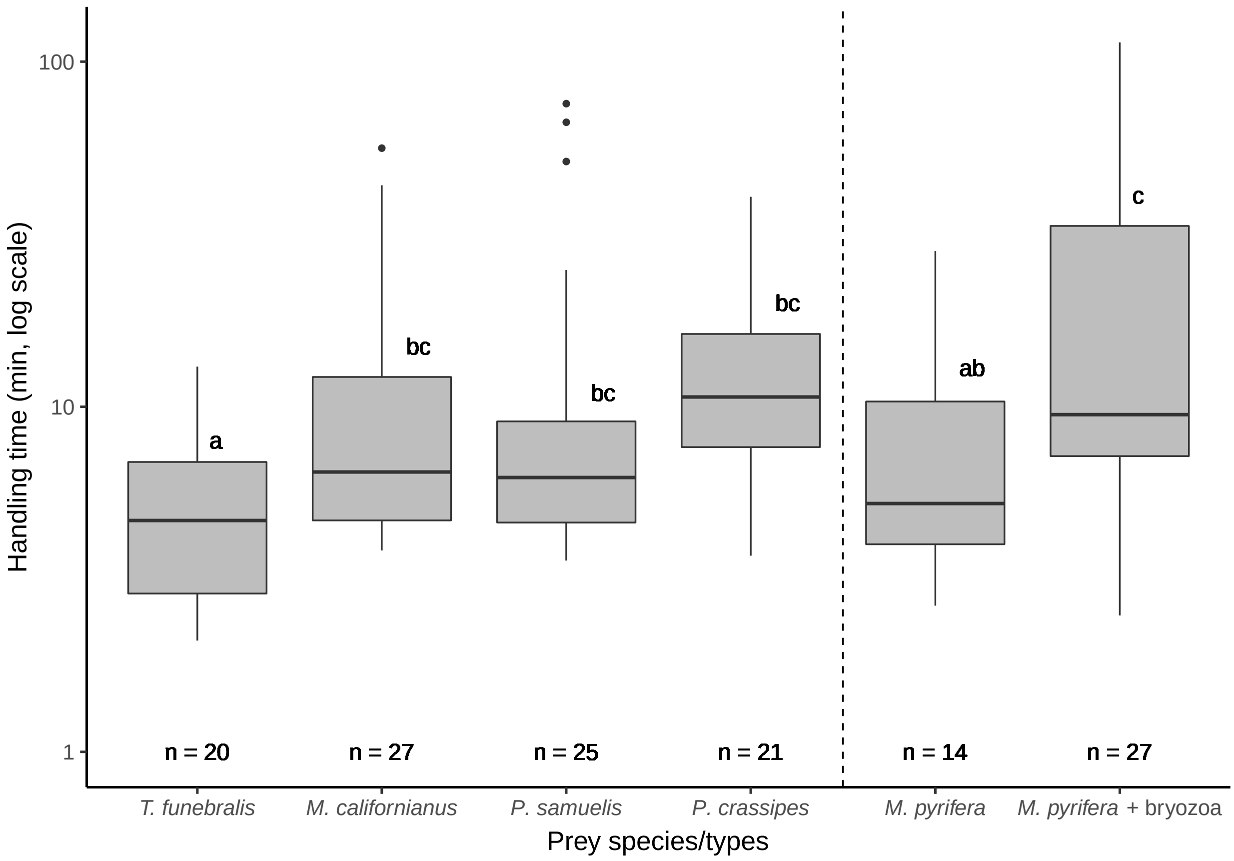


Figure 2.4. Boxplots of the handling time by sea anemones on a log scale (time between contact and ingestion) of six prey types including animals (left) and algae (right) separated by a vertical dashed line. Letters above boxes indicate significant differences between groups based on a post hoc analysis. Sample sizes for each type are listed above the x-axis.

References

Anthony KRN (1997) Prey capture by the sea anemone *Metridium senile* (L.): effects of body size, flow regime, and upstream neighbors. Biol Bull 192:73–86. doi: 10.2307/1542577

Augustine L, Muller-Parker G (1998) Selective predation by the mosshead sculpin *Clinocottus globiceps* on the sea anemone *Anthopleura elegantissima* and its two algal symbionts. Limnol Oceanogr 43:711–715. doi: 10.4319/lo.1998.43.4.0711

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw 67:1–48. doi: 10.18637/jss.v067.i01

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

Bryan PJ, McClintock JB, Baker BJ (1998) Population biology and antipredator defenses of the shallow-water Antarctic nudibranch *Tritoniella belli*. Mar Biol 132:259–265. doi: 10.1007/s002270050391

Bursey C, Guanciale JM (1977) Feeding behavior of the sea anemone *Condylactis gigantea*. Comp Biochem Physiol 57:115–117. doi: 10.1016/0300-9629(77)90359-0

Chintiroglou C (1992) The feeding habits of three Mediterranean sea anemone species, *Anemonia viridis* (ForskM), *Actinia equina* (Linnaeus) and *Cereuspedunculatus* (Pennant). 16.

Edmunds M, Potts GW, Swinfen RC, Waters VL (1976) Defensive behaviour of sea anemones in response to predation by the opisthobranch mollusc *Aeolidia papillosa* (L.). J Mar Biol Assoc U K 56:65–83. doi: 10.1017/S0025315400020440

Elliott JK, Ross DM, Pathirana C, Miao S, Andersen RJ, Singer P, Kokke WCMC, Ayer WA (1989) Induction of Swimming in *Stomphia* (Anthozoa: Actiniaria) by Imbricatine, a Metabolite of the Asteroid *Dermasterias imbricata*. Biol Bull 176:73–78. doi: 10.2307/1541574

Erralde SM, Acuña FH (2020) Trophic ecology of the intertidal sea anemone *Bunodosoma zamponii* (Cnidaria, Actiniaria): diet composition, seasonal variation and trophic parameters. An Acad Bras Ciênc. doi: 10.1590/0001-3765202020190520

Fox J, Weisberg S (2019) An R Companion to Applied Regression, Third. Sage, Thousand Oaks CA

Gleason LU, Burton RS (2013) Phenotypic evidence for local adaptation to heat stress in the marine snail *Chlorostoma* (formerly *Tegula*) *funebralis*. J Exp Mar Biol Ecol 448:360–366. doi: 10.1016/j.jembe.2013.08.008

Guy LS, Habecker LB, Oxwang G (2014) Giant Green Anemones consume seabird nestlings on the Oregon coast. Mar Ornithol 42:1–2.

Hand C (1955) The Sea Anemones of Central California Part II the Endomyarian and Mesomyarian Anemones. https://digitalcollections.usfca.edu/digital/collection/p15129coll11/id/223/. Accessed 13 Aug 2020

Kelly RP, Oliver TA, Sivasundar A, Palumbi SR (2010) A method for detecting population genetic structure in diverse, high gene-flow species. J Hered 101:423–436. doi: 10.1093/jhered/esq022

Kicklighter CE, Derby CD (2006) Multiple components in ink of the sea hare *Aplysia californica* are aversive to the sea anemone *Anthopleura sola*. J Exp Mar Biol Ecol 334:256–268. doi: 10.1016/j.jembe.2006.02.002

Kosmidis I, Pagui ECK, Sartori N (2020) Mean and median bias reduction in generalized linear models. Stat Comput 30:43–59.

Lenth RV (2021) emmeans: Estimated Marginal Means, aka Least-Squares Means.

Lesser MP, Witman JD, Sebnens KP (1994) Effects of flow and seston availability on scope for growth of benthic suspension-feeding invertebrates from the Gulf of Maine. Biol Bull 187:319–335. doi: 10.2307/1542289

Lindstedt KJ (1971) Biphasic feeding response in a sea anemone: control by asparagine and glutathione. Science 173:333–334. doi: 10.1126/science.173.3994.333

Loomis WF (1955) Glutathione control of the specific feeding reactions of hydra. Ann N Y Acad Sci 62:211–227. doi: 10.1111/j.1749-6632.1955.tb35372.x

Nagai Y, Nagai S (1973) Feeding factors for the sea anemone *Anthopleura midorii*. Mar Biol 18:55–60. doi: 10.1007/BF00347921

Pantin CFA, Pantin AMP (1943) The Stimulus to Feeding in *Anemonia Sulcata*. J Exp Biol 20:6–13. doi: 10.1242/jeb.20.1.6

Quesada AJ, Acuña FH, Cortés J (2014) Diet of the sea anemone *Anthopleura nigrescens*: composition and variation between daytime and nighttime high tides. Zool Stud 53:26. doi: 10.1186/s40555-014-0026-2

R Core Team (2021) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Sandberg DM, Kanciruk P, Mariscal RN (1971) Inhibition of nematocyst discharge correlated with feeding in a sea anemone, *Calliactis tricolor* (Leseur). Nature 232:263–265. doi: 10.1038/232263a0

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682. doi: 10.1038/nmeth.2019

Sebens KP (1981) The allometry of feeding, energetics, and body size in three sea anemone species. Biol Bull 161:152–171. doi: 10.2307/1541115

Sebens KP, Koehl MAR (1984) Predation on zooplankton by the benthic anthozoans *Alcyonium siderium* (Alcyonacea) and *Metridium senile* (Actiniaria) in the New England subtidal. Mar Biol 81:255–271. doi: 10.1007/BF00393220

Tomanek L, Somero GN (2000) Time course and magnitude of synthesis of heat-shock proteins in congeneric marine snails (genus *Tegula*) from different tidal heights. Physiol Biochem Zool 73:249–256. doi: 10.1086/316740

Wells CD, Paulay G, Nguyen BN, Leray M (2021) DNA metabarcoding provides insights into the diverse diet of a dominant suspension feeder, the giant plumose anemone *Metridium farcimen*. Environ DNA. doi: 10.1002/edn3.225

Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019) Welcome to the tidyverse. J Open Source Softw 4:1686. doi: 10.21105/joss.01686

Williams RB (1972) Chemical control of feeding behaviour in the sea anemone *Diadumene luciae* (Verrill). Comp Biochem Physiol A Physiol 41:361–371. doi: 10.1016/0300-9629(72)90067-9

Zamer WE (1986) Physiological energetics of the intertidal sea anemone *Anthopleura elegantissima*. Mar Biol 92:299–314. doi: 10.1007/BF00392670

CHAPTER 3

Microhabitats created by sea anemone-algal mutualisms enhance diversity of associated species on temperate rocky shores

Submitted to the journal Ecology as a Report

Abstract

We provide evidence for a previously undescribed mechanism of facilitation associated with a cnidarian-algal mutualism on temperate rocky shores. Sea anemones in the genus *Anthopleura* slowly release stored water at low tide to maintain ideal temperature conditions for their algal symbionts via evaporative cooling. This behavior results in leakage of water to surrounding intertidal habitats, producing unique microhabitats in the upper intertidal zone. We hypothesized that mobile invertebrate communities would use this habitat during low tides to ameliorate abiotic stress. An *in-situ* experiment spanning three sites along the coast of California, USA, found that maximum temperatures and relative desiccation rates were lower in sea-anemone-associated microhabitats as compared to adjacent rock habitats. Observational surveys revealed higher richness and biomass of mobile invertebrates in sea anemone microhabitats, and these differences were supported by manipulative experiments that included both adding and removing sea anemones. Our results support a novel community-wide facilitation driven by an adaptation to maintain a mutualistic relationship. The effects of sea anemone microhabitat on upper-shore biodiversity were associated with site and sea anemone species and highlight the role of mutualisms in mitigating stress and enhancing biodiversity.

Introduction

Positive interactions, including mutualism and facilitation, are an integral part of many ecosystems (Bertness and Callaway 1994, Stachowicz 2001). Central to many of these findings is a connection between mutualistic foundation species and the communities that they support. Examples include scleractinian corals and their dinoflagellate symbionts that provide structure for some of the most biodiverse ecosystems on earth (Bergsma 2012, Roth 2014), ribbed mussels and cordgrass in salt marshes that provide a hard substrate for a diverse set of other species (Bertness 1984, Daleo et al. 2007), and many plants and mycorrhizal fungi that allow persistence in otherwise nutrient poor environments, paving the way for other species (Van Der Heijden and Horton 2009, Chen et al. 2020).

Rocky intertidal habitats can be harsh environments for marine organisms, and species' upper distributional limits are typically set by abiotic stressors such as temperature and desiccation (Connell 1972). Some well-established groups that provide refuge to other species in the rocky intertidal zone include mussels (Witman 1985, Seed 1996), rockweeds (Schmidt et al. 2011, Elsberry and Bracken 2021), and surfgrass (Crouch 1991, Moulton and Hacker 2011). These groups increase biodiversity largely by providing habitat – and ameliorating stress – for mobile invertebrates. Here we propose that another well-known group of temperate rocky intertidal organisms, sea anemones, may also provide favorable microhabitats during low tides as a direct result of their mutualism with algal symbionts.

Sea anemones in the genus *Anthopleura* are prominent members of rocky intertidal zone communities in the northeastern Pacific Ocean, with members of the genus extending from Baja California, Mexico to Alaska, USA (Hand 1955). Three species in this genus, *A. elegantissima*, *A. sola*, and *A. xanthogrammica*, maintain mutualistic symbioses with algae (Secord and Augustine 2000). Their tidal range extends from the subtidal to upper intertidal zone, where they are usually found in habitats exposed to high levels of solar irradiance (S. Bedgood, *unpubl. data*). These sea anemones seek out this harsh environment because of the requirements of their algal symbionts. Sea anemones are not completely sessile (Bedgood et al. 2020), and symbiotic species move towards light to maximize the photosynthesis of their algal symbionts, choosing high-light habitats (Pearse 1974, Secord and Muller-Parker 2005). However, the high light intensities required to maximize algal photosynthesis also result in high temperatures during daytime low tides that can result in a break-down of the anemone-algal mutualism (dysbiosis; Muller-Parker et al. 2007). To decrease the risk of dysbiosis and desiccation, sea anemones slowly release water stored within their gastrovascular cavity at low tide (Shick and Dykens 1984; Appendix C Supp. Fig. 1), reducing their temperature by evaporative cooling. Bingham et. al. (2011) found that *A. elegantissima* lost up to 91% of its wet weight via release and evaporation of water over nine hours in a wind tunnel, maintaining body temperatures as far as 6 °C below ambient temperatures. This behavior has the potential to produce a unique microhabitat by ameliorating extreme temperatures and reducing local desiccation stress in the vicinity of sea anemones during low tides, especially in the upper intertidal zone where sea anemones are one of the only biotic habitat providers (S. Bedgood, *pers. obs.*).

In this study we investigate whether *A. elegantissima* and *A. sola* sea anemones provide favorable microhabitats at low tide to a diverse group of mobile invertebrates along the California coastline. To accomplish this, we compared sea anemone habitat (areas surrounding sea anemones) to adjacent rock habitat (Fig. 3.1A). We hypothesized that sea anemones would ameliorate temperature and desiccation stress, enhancing mobile invertebrate diversity and abundance and predicted that (1) maximum temperatures and rates of desiccation during low tides would be lower in sea anemone microhabitat, (2) mobile invertebrate richness and biomass would be higher in sea anemone microhabitat, and (3) manipulating sea anemone microhabitat (removal or addition) would change the richness and/or biomass of mobile invertebrates.

Methods

Sites and habitat

We chose three rocky intertidal sites along the California coast with unique mobile invertebrate community assemblages, including (from north to south): Kenneth S. Norris Rancho Marino Reserve (hereafter RMR, 35.5392°N, 121.0152°W), Coal Oil Point Reserve (hereafter COP, 34.4072°N, 119.8785°W), and Corona del Mar State Beach (hereafter CDM, 33.5882°N, 117.8677°W). In the upper intertidal zone at these three sites, sea anemones and occasionally rockweed algae are the only conspicuous biotic habitat providers. Only *A. elegantissima* can be found in the exposed upper intertidal zone at CDM, but our other two sites included both *A. elegantissima* and *A. sola*. We established paired survey plots with sea anemone habitat and adjacent rock habitat. The number of sea anemones in *A. sola* plots was always one because they are solitary, but *A. elegantissima* plots ranged in colony size between five and 18 individuals. Sea anemone habitat was defined as the area immediately surrounding an anemone (*A. sola*) or anemone colony (*A. elegantissima*) from contact with the anemone(s) to 5 cm away from the anemone(s), creating a band along the perimeter (Appendix C Supp. Fig. 2). We established paired adjacent rock habitats between 10 cm and 30 cm from anemone habitat. These survey areas were chosen to match the substrate immediately surrounding the paired anemone habitat and surveyed with a fixed circular quadrat (81 cm^2).

We used effective tidal elevations associated with the distributions of characteristic intertidal organisms (Benson 2002, Harley and Helmuth 2003) to identify a similar location for our sea anemone microhabitat plots in the mid to upper intertidal zone at all three locations. This region varied in tide height among sites: between +0.55 and +1.02 m above mean lower-low water (MLLW) at CDM (n = 19 pairs), between +0.37 and +1.02 m above MLLW at COP (n =

36 pairs), and between +0.31 and +1.28 m above MLLW at RMR (n = 44 pairs). We measured the elevation (above MLLW) of each plot pair because mobile invertebrate communities are strongly influenced by elevation, even over a small range.

Abiotic measurements

We deployed 20 temperature loggers (HOBO Pendant® MX Temperature/Light) in anemone habitat and adjacent rock habitat across all three sites from July to August 2019, but data from COP were excluded because sand covered the loggers shortly after they were deployed (n = 4 pairs). We filtered data to include the 10 lowest tides that fell within daylight hours (9:00-17:00 [HH:MM]) and took the highest temperature from each interval at RMR and CDM (Fig. 3.1). To measure relative desiccation rates, we soaked 40 chipboard (a type of cardboard) discs that were 1.5 mm thick and 2 cm in diameter in saltwater before placing them in paired habitat plots for two hours (during a daytime low tide) at RMR during August of 2019. After collecting, we calculated the difference in weight of the discs between the start and end of exposure.

Community surveys

We surveyed mobile invertebrates in paired observational plots two times at our three sites (June 2019), recording the abundance of each species. We recorded and calculated three community metrics: richness, biomass, and habitat preference. Mobile invertebrate species that are parasites of sea anemones (pycnogonids and wentletrap snails) were not included in our richness and biomass data, but they were included in our habitat preference data. To accurately calculate biomass without disturbing communities in our plots, we collected a representative sample (n = 8-13) of the 14 most common species found in our survey plots at CDM and RMR, which made up 95% of the total species surveyed. Any uncommon species (7 total) were not included in the biomass calculation and were found exclusively in sea anemone habitat. To determine an average biomass for each species, we determined ash-free dry mass by drying individuals in a 60° C oven to constant mass (≥ 48 hours), weighing them, then combusting them in a muffle furnace at 450° C for 4 hours. The ash-free dry mass was the difference between the dried and combusted masses.

To confirm that effects of sea anemones on mobile invertebrate communities were causal, we manipulated anemone presence experimentally. Using the same plots as the observational surveys and randomly assigning treatment pairs, we created four treatments: anemone control, rock control, anemone removal, and anemone addition. The control treatments were paired and unmanipulated. We created the anemone removal treatment by slowly lifting the anemone's pedal disc off the substrate with a metal scoopula, being careful not to disturb nearby mobile invertebrates. To create the anemone addition treatment, the unattached anemone was moved to the paired adjacent rock habitat where it was held against the substrate with nylon mesh secured to the rock with four anchored screws (Appendix C Supp. Fig. 3). After approximately 48 hours when the anemone had reattached in the new location, we removed the mesh. These manipulated and control plots were left for one month before surveying the mobile invertebrate communities as described previously.

Statistical analyses

All analyses were conducted in R version 4.0.5 (R Core Team 2021) with general and generalized linear mixed models with post hoc analyses (Bates et al. 2015 p. 4; Wickham et al. 2019). We filtered the logger temperature data before analysis by excluding data outside of the peak daytime hours 9:00-17:00 [HH:MM] when solar radiation would affect temperatures the most, then removed all remaining data except maximum temperatures for each habitat during the

10 lowest low tides during the data collection period. We treated multiple measurements from a single logger as repeated measures and logger identification as a random effect. We analyzed the desiccation data in a similar way but did not include site or a random effect because measurements were taken at one site (RMR) and were not repeated. The richness data met our assumptions for data distribution and were analyzed with general linear models, while the biomass data did not meet assumptions. We used generalized linear models with a Gamma distribution and log link to analyze all biomass data. We analyzed habitat preference data in each mobile invertebrate genera or species separately with two-sided t-tests, using the proportion of individuals in each habitat (bounded -1 to 1) as a response variable and a null hypothesis of no preference (0).

Results

Abiotic factors

Maximum temperatures during daytime low tides were substantially lower in sea anemone habitat as compared to adjacent rock habitat ($\chi^2 = 7.38$, $P = 0.007$; Fig. 3.1B). CDM experienced higher temperatures than RMR ($\chi^2 = 13.21$, $P < 0.001$). Sea anemone species and maximum temperatures were significantly related ($\chi^2 = 7.14$, $P = 0.008$) because *A. elegantissima* habitat was higher than *A. sola* habitat in the intertidal zone ($F_{1,94} = 52.98$, $P < 0.001$). Relative desiccation rates were lower in sea anemone habitat than adjacent rock habitat ($F_{1,33} = 61.00$, $P < 0.001$; Fig. 3.1C), and there was no effect of sea anemone species on desiccation ($F_{1,33} = 0.98$, $P = 0.330$).

Community surveys

Richness and biomass were both higher in sea anemone habitat than adjacent rock habitat (Fig. 3.2). Richness was affected by both the habitat ($\chi^2 = 136.67$, $P < 0.001$) and by tide height ($\chi^2 = 44.17$, $P < 0.001$), increasing with height regardless of habitat ($R^2 = 0.113$, $P < 0.001$). There was no effect of sea anemone species ($\chi^2 = 1.81$, $P = 0.178$) or site ($\chi^2 = 4.30$, $P = 0.117$) on richness. Biomass was not affected by tide height ($\chi^2 = 0.10$, $P = 0.753$) but was affected by habitat ($\chi^2 = 230.87$, $P < 0.001$), sea anemone species ($\chi^2 = 19.86$, $P < 0.001$), site ($\chi^2 = 75.64$, $P < 0.001$), and an interaction between habitat and site ($\chi^2 = 94.12$, $P < 0.001$). Biomass was higher in sea anemone habitat than adjacent rock at all three sites (CDM: $z = -4.87$, $P < 0.001$; COP: $z = -3.56$, $P < 0.001$; RMR: $z = -17.01$, $P < 0.001$), but biomass was lower overall, and the effect of habitat was weaker at COP than our other two sites (COP-CDM: $z = 4.40$, $P < 0.001$; COP-RMR: $z = -8.68$, $P < 0.001$; Appendix C Supp. Fig. 4). The effect of sea anemone species was driven by the community associated with *A. sola* at RMR, which included two large herbivorous mollusk species with high biomass, *Mopalia muscosa* and *Tegula funebralis*.

After the experimental manipulation, there was an interaction between sea anemone species and treatment for the addition comparison ($F_{1,51} = 8.40$, $P = 0.006$): there was no effect of *A. elegantissima* additions ($t = 0.42$, $P = 0.680$), but adding *A. sola* enhanced mobile richness ($t = 3.68$, $P < 0.001$). The effect of sea anemone removal depended on site (site*treatment: $F_{1,83} = 3.14$, $P = 0.48$). Treatments differed at CDM ($t = 2.78$, $P = 0.007$) and RMR ($t = 2.18$, $P = 0.032$) but not at COP ($t = -0.36$, $P = 0.723$). Overall, mobile invertebrate richness was higher in the sea anemone addition treatments than in the adjacent rock controls ($F_{1,51} = 5.28$, $P = 0.026$) and lower in sea anemone removal treatments than in the sea anemone controls ($F_{1,83} = 6.31$, $P = 0.014$; Fig. 3.2).

The effect of additions and removals on biomass depended on sea anemone species (addition: $\chi^2 = 7.38$, $P = 0.007$; removal: $\chi^2 = 5.69$, $P = 0.017$; Appendix C Supp. Fig. 5-6); additions and removals of *A. sola* affected biomass (addition: $z = 3.23$, $P = 0.001$; removal: $z = 3.75$, $P < 0.001$), whereas manipulations of *A. elegantissima* did not (addition: $z = 0.26$, $P = 0.792$; removal: $z = 0.80$, $P = 0.426$). There was also an effect of site (addition: $\chi^2 = 8.19$, $P = 0.017$; removal: $\chi^2 = 40.28$, $P < 0.001$), which was driven by the higher biomass of *A. sola* associated species at RMR. Overall, biomass was higher in the sea anemone addition treatment as compared to the adjacent rock control ($\chi^2 = 5.64$, $P = 0.018$) and lower in sea anemone removal treatment as compared to the sea anemone control ($\chi^2 = 8.41$, $P = 0.004$; Fig. 3.2).

Out of the 11 most common species found in our plots, seven preferred sea anemone habitat, two preferred adjacent rock habitat, and two had no preference (Appendix C Supp. Table 1; Fig. 3.3). Two species, the chiton *Mopalia muscosa* and the predatory snail *Acanthinucella punctulata*, were exclusively found in sea anemone habitat (mostly *A. sola*) during our surveys.

Discussion

Our results support our hypothesis that symbiotic sea anemones ameliorate temperature and desiccation stress, enhancing mobile invertebrate diversity and abundance and highlighting a novel, community-wide role for cnidarian-algal symbioses on temperate rocky shores. We found that sea anemone-adjacent habitats had lower maximum temperatures and relative desiccation rates compared to the adjacent rock habitat, and that these more benign intertidal habitats were favored by mobile invertebrates. We suggest that this results in higher richness and biomass of the mobile invertebrate communities in sea anemone habitat as compared to adjacent rock habitat, and our experimental manipulations demonstrate that the relationship is causal.

Most mobile invertebrate species were found more frequently in sea anemone habitat than in adjacent rock habitat. *Epitonium tinctum*, a parasite of *Anthopleura* sea anemones (Smith 1977), was almost always found in sea anemone habitat, where they likely used the anemones as both food and as a refuge during low tides. All other species that preferred sea anemone habitat during low tide likely use this habitat as a temporary refuge, moving away from the sea anemone during high tides to forage. The two species that preferred adjacent rock habitat, *Lottia austrodigitalis* and *Lottia scrabra*, are well-adapted to survive in the upper intertidal zone, extending above our survey plots; they move to established areas during low tides that are not associated with sea anemone habitat (Frank 1965, Haven 1973).

The effect of site on richness and biomass was expected because each site had a unique community of mobile invertebrates, but the effect of sea anemone species on biomass was unexpected. Sea anemone size plays an important role in determining which invertebrates can take refuge in the microhabitat. *A. elegantissima* reproduces asexually, creating large but low-profile clonal mats, whereas *A. sola* reproduces only sexually and grows more vertically (Pearse and Francis 2000). Larger invertebrate species like the chiton *Mopalia muscosa* and the turban snail *Tegula funebralis* may only be able to take refuge next to *A. sola*, where they would benefit from increased contact with the sea anemone. Our experimental results suggest that the habitat provided by *A. sola* may attract a wider range of larger mobile invertebrates.

The importance of the similar mutualism between tropical corals (Cnidaria: Scleractinia) and symbiotic algae has been well-established, as the alga-cnidarian partnership is essential for providing habitat and enhancing biodiversity in tropical low-nutrient ecosystems (Hatcher 1990, Urbina-Barreto et al. 2021). However, the broader effects of the mutualism between sea anemones (Cnidaria: Actinaria) and symbiotic algae has not been studied similarly in high-nutrient temperate systems. This may be because coral reefs depend on the coral-algal mutualism to provide resources in an otherwise nutrient-poor environment, whereas anemone-algal mutualisms on nutrient-rich temperate rocky shores, where primary producers are diverse and abundant (Muller-Parker and Davy 2001), are less obviously important. However, we show that symbiotic sea anemones on temperate shores help to mitigate physical stress, allowing the persistence of a variety of taxa during stressful low-tide conditions. We demonstrate the community-wide importance of a temperate cnidarian-algal mutualism, highlighting the role of mutualisms in ameliorating stress and structuring communities (Stachowicz 2001).

Acknowledgements

We thank A. Henry, K. Edwards, J. Caramagno, J. Bedgood, and E. Bedgood for assistance with fieldwork and the University of California Natural Reserve System for access to Kenneth S. Norris Rancho Marino (doi: 10.21973/N37943) and Coal Oil Point (doi: 10.21973/N3Z07N) Reserves. This research was supported by the National Science Foundation (Graduate Research Fellowship to S.A.B. and OCE-1736891 to M.E.S.B. and A. Martiny), the Mildred E. Mathias Graduate Student Research Grant (to S.A.B.), and the Coal Oil Point Graduate Student Research Award (to S.A.B.).

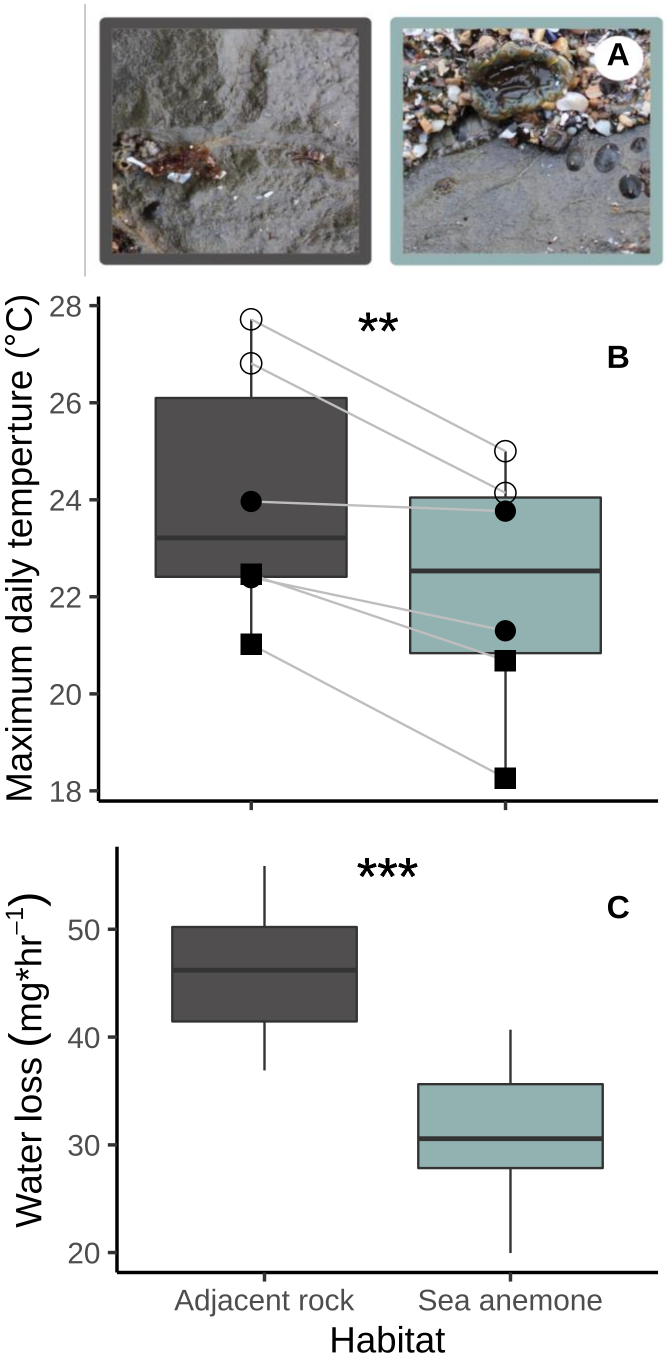


Figure 3.1. Abiotic factors measured in sea anemone and adjacent rock habitats. **(A)** An example of paired habitat plots from CDM. **(B)** Maximum daily temperatures in each habitat. Points represent the mean temperature (n = 10) for each logger with gray lines connecting paired loggers. Open circles represent *A. elegantissima* habitat at CDM, filled circles represent *A. elegantissima* habitat at RMR, and filled squares represent *A. sola* habitat at RMR. **(C)** Relative desiccation (water loss) rates in each habitat. Data were paired, but individual data points and connecting lines are not shown for simplicity (n = 18 pairs). Asterisks indicate significant differences between habitats.

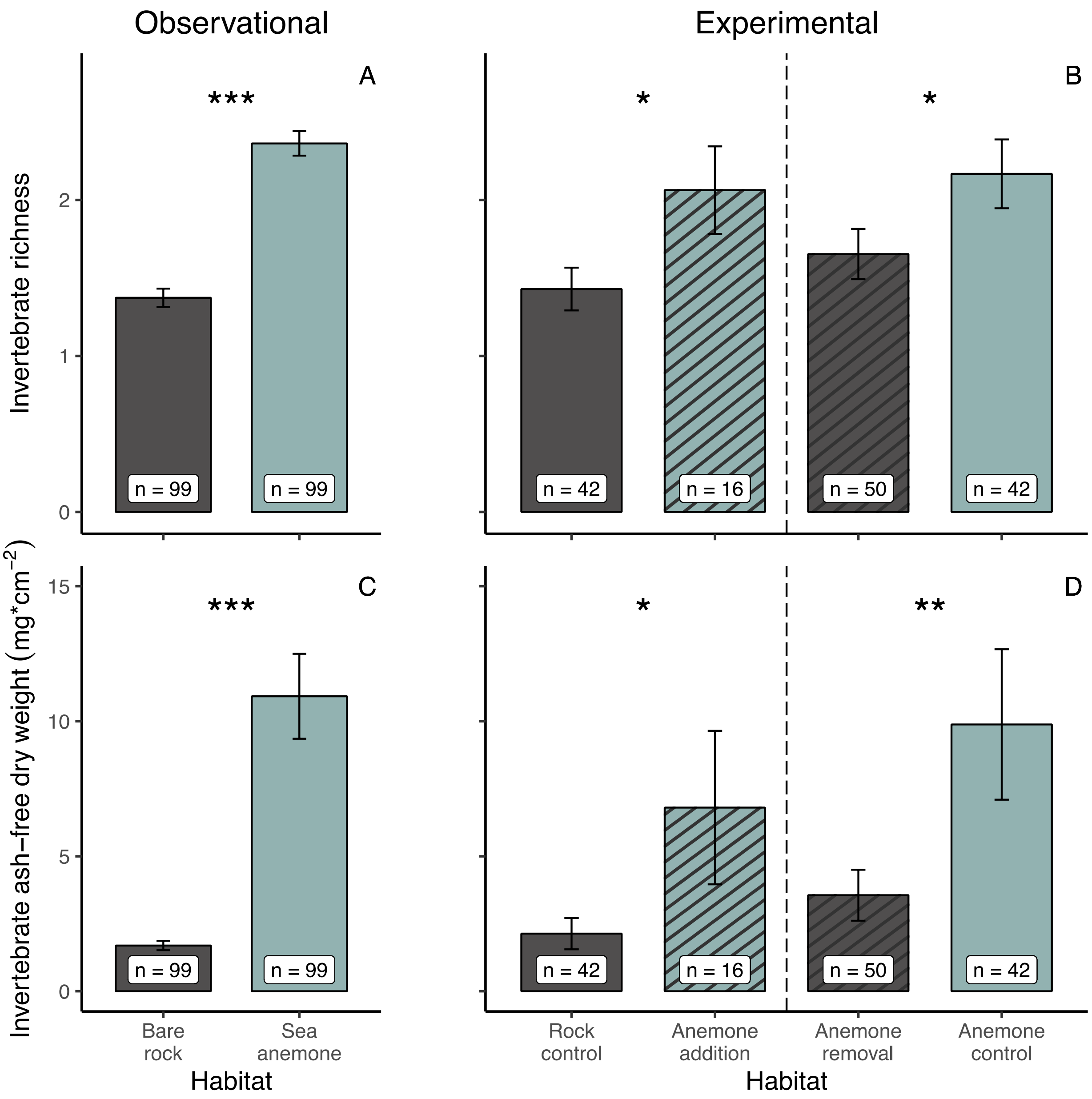


Figure 3.2. Mobile invertebrate richness and biomass where sea anemones are present and absent. **(A)** Invertebrate richness within each habitat plot during observational surveys and **(B)** one month after the manipulation of habitats during the experimental surveys. The vertical dashed line separates paired comparisons. **(C)** Mobile invertebrate ash-free dry weight during the observational surveys and **(D)** one month after the manipulation during the experimental surveys. Sample sizes are listed at the bottom of each bar, and significant differences between habitat pairs are indicated with asterisks. Values are means ± SEMs.

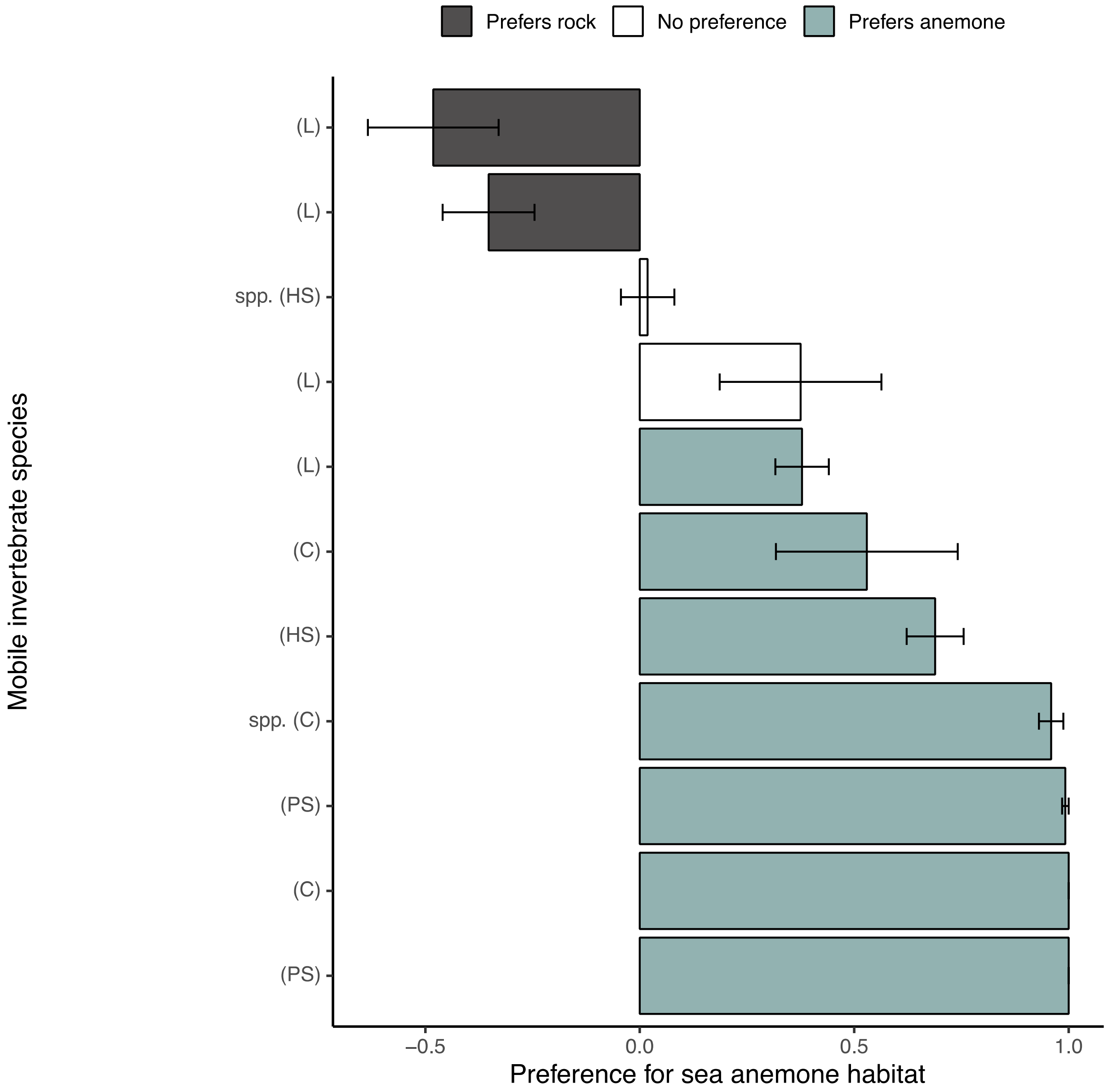


Figure 3.3. Habitat preferences of the most common mobile invertebrate species found in our plots. A value of 1 indicates that all individuals were found in sea anemone habitat, 0 indicates an equal number found in each habitat, and -1 indicates that all individuals were found in adjacent rock habitat. Bar color illustrates preference (or lack of) for either habitat type. Abbreviations in parentheses next to scientific names provide general taxonomic groupings: (L) is limpet, (C) is chiton, (HS) is herbivorous snail, and (PS) is predatory snail. Categories containing multiple species are indicated (spp.). Values are means ± SEMs.

References

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Bedgood, S. A., M. E. S. Bracken, W. H. Ryan, S. T. Levell, and J. Wulff. 2020. Nutritional drivers of adult locomotion and asexual reproduction in a symbiont-hosting sea anemone *Exaiptasia diaphana*. Marine Biology 167:39.

Benson, K. R. 2002. The study of vertical zonation on rocky intertidal shores—a historical perspective. Integrative and Comparative Biology 42:776–779.

Bergsma, G. S. 2012. Coral mutualists enhance fish abundance and diversity through a morphology-mediated facilitation cascade. Marine Ecology Progress Series 451:151–161.

Bertness, M. D. 1984. Ribbed mussels and *Spartina alterniflora* production in a New England salt marsh. Ecology 65:1794–1807.

Bertness, M. D., and R. Callaway. 1994. Positive interactions in communities. Trends in Ecology & Evolution 9:191–193.

Bingham, B. L., I. Freytes, M. Emery, J. Dimond, and G. Muller-Parker. 2011. Aerial exposure and body temperature of the intertidal sea anemone *Anthopleura elegantissima*. Invertebrate Biology 130:291–301.

Chen, J., Y. Yang, S. Wang, H. Sun, and C. Schöb. 2020. Shrub facilitation promotes selective tree establishment beyond the climatic treeline. Science of The Total Environment 708:134618.

Connell, J. H. 1972. Community interactions on marine rocky intertidal shores. Annual Review of Ecology and Systematics 3:169–192.

Crouch, C. A. 1991. Infaunal polychaetes of a rocky intertidal surfgrass bed in southern California. Bulletin of Marine Science 48:386–394.

Daleo, P., E. Fanjul, A. M. Casariego, B. R. Silliman, M. D. Bertness, and O. Iribarne. 2007. Ecosystem engineers activate mycorrhizal mutualism in salt marshes. Ecology Letters 10:902–908.

Elsberry, L. A., and M. E. S. Bracken. 2021. Functional redundancy buffers mobile invertebrates against the loss of foundation species on rocky shores. Marine Ecology Progress Series 673:43–54.

Frank, P. W. 1965. The Biodemography of an Intertidal Snail Population. Ecology 46:831–844.

Hand, C. 1955. The sea anemones of central California. Part II. The Endomyarian and Mesomyarian Anemones. The Wasmann Journal of Biology 13:37-99.

Harley, C. D. G., and B. S. T. Helmuth. 2003. Local- and regional-scale effects of wave exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal zonation. Limnology and Oceanography 48:1498–1508.

Hatcher, B. G. 1990. Coral reef primary productivity. A hierarchy of pattern and process. Trends in Ecology & Evolution 5:149–155.

Haven, S. B. 1973. Competition for food between the intertidal gastropods *Acmaea scabra* and *Acmaea digitalis*. Ecology 54:143–151.

Moulton, O. M., and S. D. Hacker. 2011. Congeneric variation in surfgrasses and ocean conditions influence macroinvertebrate community structure. Marine Ecology Progress Series 433:53–63.

Muller-Parker, G., and S. K. Davy. 2001. Temperate and tropical algal-sea anemone symbioses. Invertebrate Biology 120:104–123.

Muller-Parker, G., J. Pierce-Cravens, and B. L. Bingham. 2007. Broad thermal tolerance of the symbiotic dinoflagellate *Symbiodinium muscatinei* (Dinophyta) in the sea anemone *Anthopleura elegantissima* (Cnidaria) from northern latitudes. Journal of Phycology 43:25–31.

Pearse, V. B. 1974. Modification of sea anemone behavior by symbiotic zooxanthellae: phototaxis. The Biological Bulletin 147:630–640.

Pearse, V., and L. Francis. 2000. *Anthopleura sola*, a new species, solitary sibling species to the aggregating sea anemone, *A. elegantissima* (Cnidaria : Anthozoa : Actiniaria : Actiniidae). Proceedings of the Biological Society of Washington 113:596–608.

R Core Team. 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Roth, M. S. 2014. The engine of the reef: photobiology of the coral–algal symbiosis. Frontiers in Microbiology 5:422.

Schmidt, A. L., M. Coll, T. N. Romanuk, and H. K. Lotze. 2011. Ecosystem structure and services in eelgrass *Zostera marina* and rockweed *Ascophyllum nodosum* habitats. Marine Ecology Progress Series 437:51–68.

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

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

Seed, R. 1996. Patterns of biodiversity in the macro-invertebrate fauna associated with mussel patches on rocky shores. Journal of the Marine Biological Association of the United Kingdom 76:203–210.

Shick, J. M., and J. A. Dykens. 1984. Photobiology of the symbiotic sea anemone *Anthopleura elegantissima*: photosynthesis, respiration, and behavior under intertidal conditions. The Biological Bulletin 166:608–619.

Smith, C. 1977. Chemical recognition of prey by the gastropod *Epitonium tinctum* (Carpenter, 1864). Veliger 19:331-340.

Stachowicz, J. J. 2001. Mutualism, facilitation, and the structure of ecological communities. BioScience 51:235–246.

Urbina-Barreto, I., F. Chiroleu, R. Pinel, L. Fréchon, V. Mahamadaly, S. Elise, M. Kulbicki, J.-P. Quod, E. Dutrieux, R. Garnier, J. Henrich Bruggemann, L. Penin, and M. Adjeroud. 2021. Quantifying the shelter capacity of coral reefs using photogrammetric 3D modeling: From colonies to reefscapes. Ecological Indicators 121:107151.

Van Der Heijden, M. G. A., and T. R. Horton. 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. Journal of Ecology 97:1139–1150.

Wickham, H., M. Averick, J. Bryan, W. Chang, L. D. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. L. Pedersen, E. Miller, S. M. Bache, K. Müller, J. Ooms, D. Robinson, D. P. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, and H. Yutani. 2019. Welcome to the tidyverse. Journal of Open Source Software 4:1686.

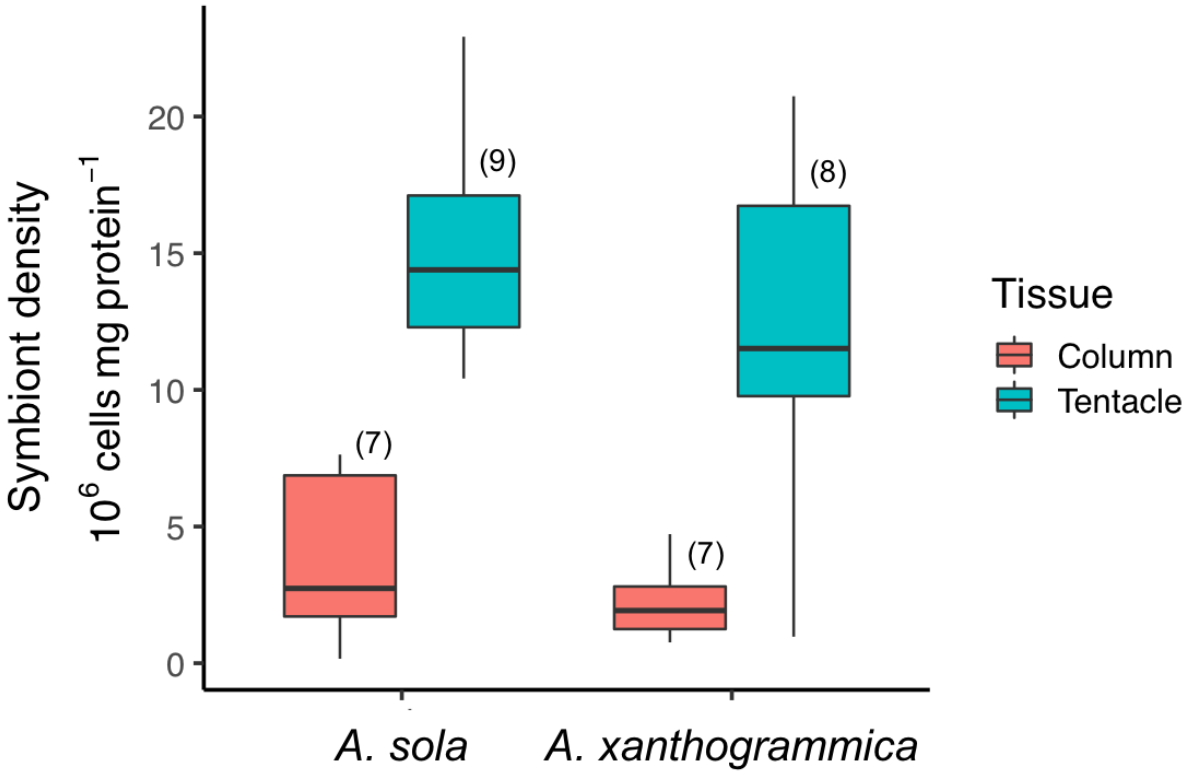
Witman, J. D. 1985. Refuges, biological disturbance, and rocky subtidal community structure in New England. Ecological Monographs 55:421–445.

CONCLUSIONS

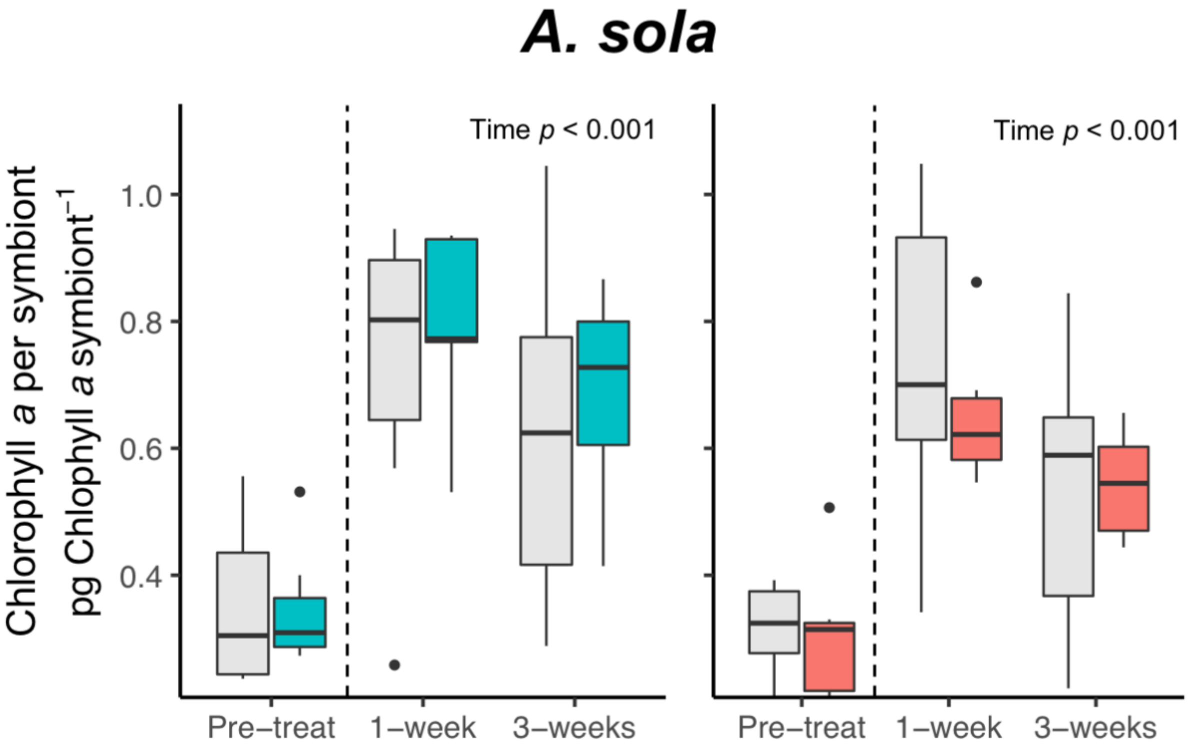
The impacts of *A. elegantissima*, *A. sola*, and *A. xanthogrammica* holobionts studied here contribute to our growing understanding of mutualisms in communities and ecosystems. Interactions between host sea anemone and algal endosymbionts have cascading effects on this system, increasing richness and biomass of the community due to the creation of microhabitat (Chapter 3). Those invertebrates that do not benefit from the microhabitat that sea anemones provide may be consumed by them if they get too close or are broken free from the substrate, but we hypothesize that *Anthopleura* spp. mostly serve to capture dead or dying animals and plankton or detritus that is passing through the system, serving as a carbon fixer in the rocky intertidal food web (Chapter 2). Holobionts also produce their own source of carbon from photosynthesizing algal endosymbionts that may serve as an alternative dietary carbon source during times when prey is scarce, buffering the holobiont from this stochastic system (Chapter 1). There are still many questions to be answered about the role of these charismatic sea anemone holobionts in a larger context, but these studies have begun to fill that gap.

APPENDIX A

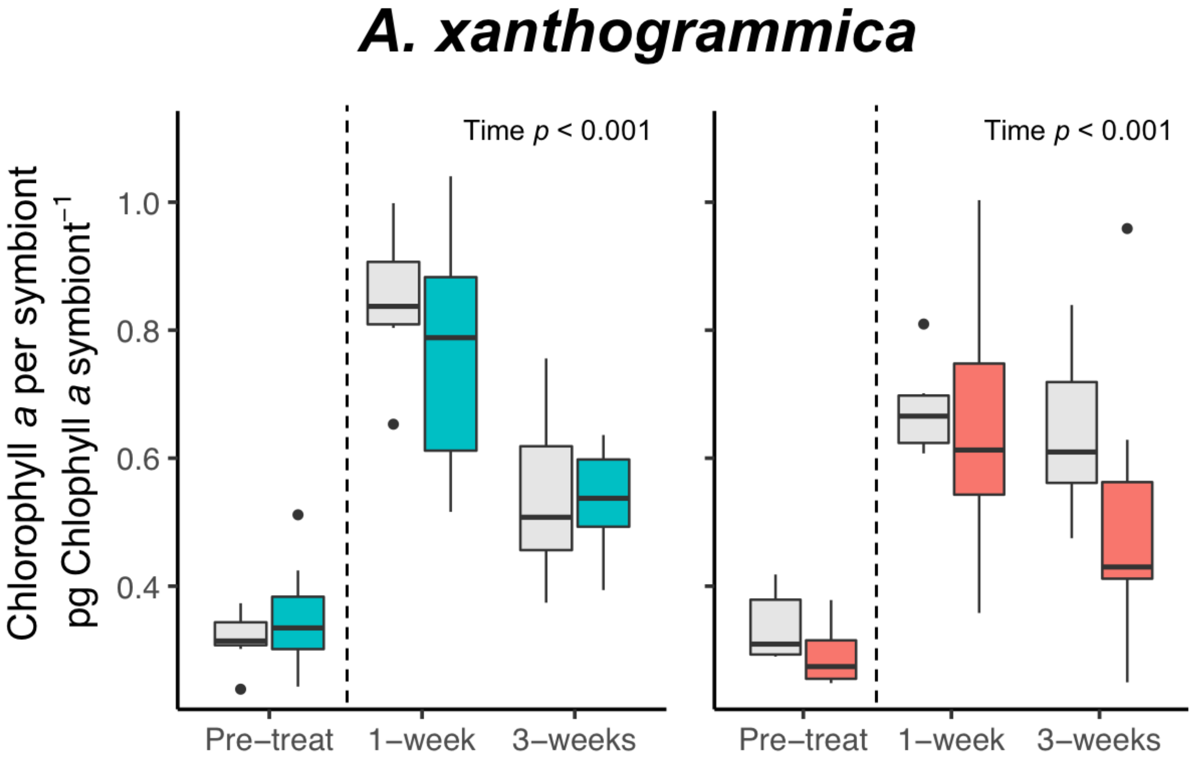
Supplementary materials for Chapter 1: Flexibility of nutritional strategies within a mutualism: food availability affects algal symbiont productivity in two congeneric sea anemone species



Supplementary Figure 1. Boxplots showing symbiont density of *A. sola* and *A. xanthogrammica* anemones collected from Bodega Marine Reserve October 2017. Tissue samples were taken from the column and tentacles of the anemones. A two-way ANOVA found a main effect of tissue ($p < 0.001$) but not species ($p = 0.071$). Sample sizes are listed above each bar.



Supplementary Figure 2. Boxplots showing Chlorophyll *a* per symbiont within *A. sola* throughout the experiment. Comparisons are made between supplement and reduction treatments and their respective control treatments. The vertical dashed line represents the start of treatments. Significant main effects and interactions from GLMMs are listed in the upper right-hand corner of each graph with p-values. There were no significant differences between controls and treatments at a given time-point from a Tukey post hoc analysis. n = 7 for each treatment at each time point.



Supplementary Figure 3. Boxplots showing Chlorophyll *a* per symbiont within *A. xanthogrammica* throughout the experiment. Comparisons are made between supplement and reduction treatments and their respective control treatments. The vertical dashed line represents the start of treatments. Significant main effects and interactions from GLMMs are listed in the upper right-hand corner of each graph with p-values. There were no significant differences between controls and treatments at a given time-point from a Tukey post hoc analysis. n = 7 for each treatment at each time point.

Batch process macro code for Fiji

This counts the symbionts in each photo

```
//run("Brightness/Contrast...");
setMinAndMax(91, 93);
run("8-bit");
setAutoThreshold("Default");
```

```
//run("Threshold...");
setThreshold(0, 88);
//setThreshold(0, 88);
setOption("BlackBackground", false);
run("Make Binary", "thresholded remaining black");
run("Fill Holes");
run("Watershed");
run("Analyze Particles...", "size=35-130 circularity=0.55-1.00 clear summarize add");
roiManager("Show All without labels");
```

This measures the length and width of each algal cell

```
//run("Brightness/Contrast...");
setMinAndMax(55, 163);
run("8-bit");
setAutoThreshold("Default");
//run("Threshold...");
setThreshold(0, 152);
//setThreshold(0, 152);
setOption("BlackBackground", false);
run("Make Binary", "thresholded remaining black");
run("Fill Holes");
run("Watershed");
run("Set Measurements...", "area fit redirect=None decimal=3");
run("Analyze Particles...", "size=30-150 circularity=0.50-1.00 clear summarize add");
```

APPENDIX B

Supplementary materials for Chapter 2: Are sea anemones picky eaters? Feeding preferences of temperate intertidal sea anemones and mechanisms of predation avoidance by their prey

Supplementary Figure 1. Photo of the laboratory mesocosms where sea anemones were recorded for 2.5 hours during feeding during trials.

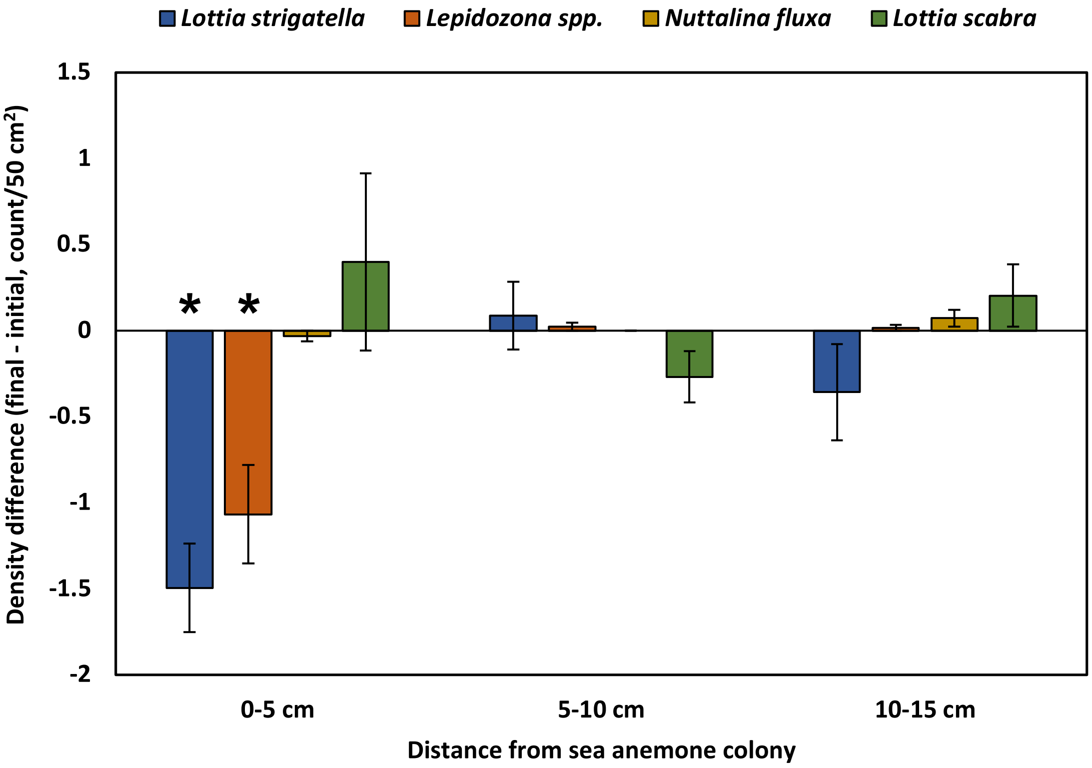
APPENDIX C

Supplementary materials for Chapter 3: Microhabitats created by sea anemone-algal mutualisms enhance diversity of associated species on temperate rocky shores

Table 1. One-sample two-sided t-test results for Figure 3.3. Values were compared against 0 (no preference). Sample sizes are listed (plot pairs). Species without p-values were constant, no variation in preference where 100% were found in sea anemone habitat.

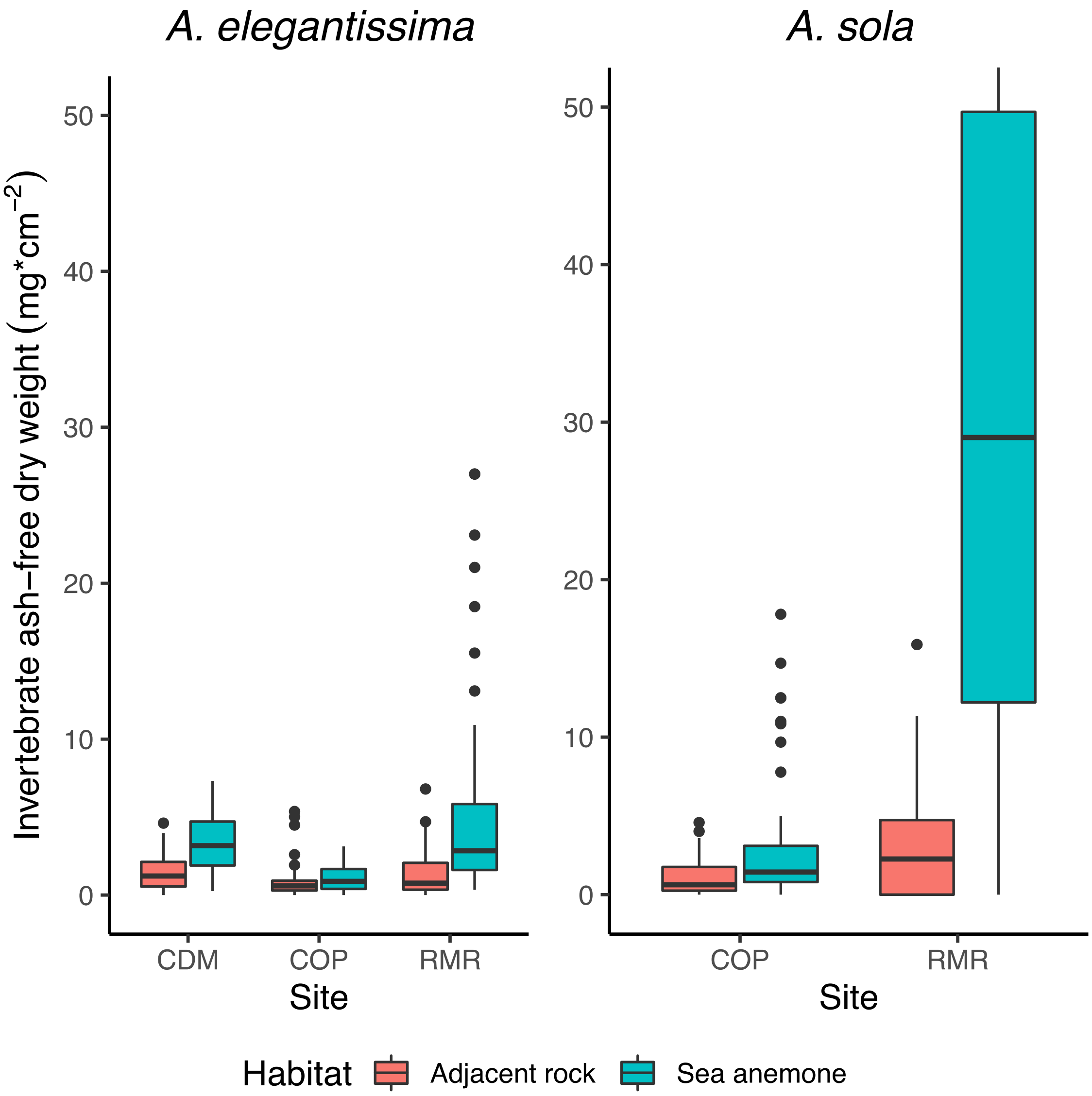
Species	**n**	***t*-value**	***p*-value**	**comment**
Lottia austrodigitalis	28	-3.16	**0.004**	
Lottia scabra	57	-3.29	**0.002**	
Littorina spp.	125	0.29	0.768	
Lottia limatula	24	1.99	0.059	
Lottia strigatella	132	6.08	**< 0.001**	
Nuttallina californica	17	2.5	**0.024**	
Tegula funebralis	73	10.38	**< 0.001**	
Lepidozona spp.	49	33.59	**< 0.001**	
Epitonium tinctum	66	131	**< 0.001**	
Mopalia muscosa	23	NA	NA	Constant, no variation in preference
Acanthinucella punctulata	11	NA	NA	Constant, no variation in preference

Supplementary Figure 1. A photo taken at CDM several hours after exposure at low tide illustrating the slow release of water from sea anemones. The *A. elegantissima* colonies present in this image were on a steep rock face (approximately 60°). The dark areas surrounding and below the colonies were produced by the release of water.

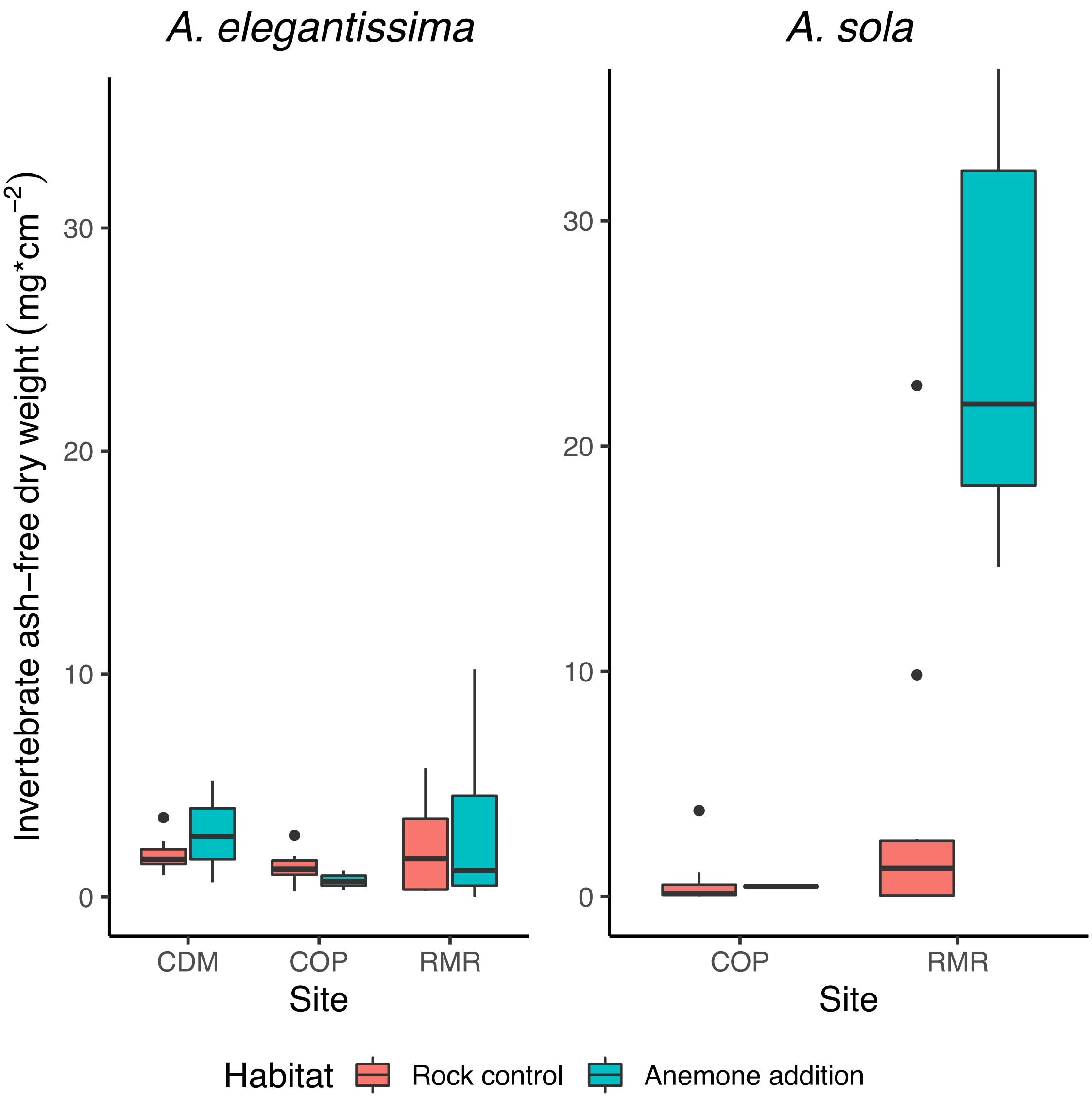


Supplementary Figure 2. The difference in density before and after sea anemone removal of four mobile invertebrate species surveyed in three distinct bands extending away from sea anemone (*A. elegantissima*) habitat. All data were collected from Point Fermin, California in May (initial) and June (final) of 2018, n = 12 plots surveyed. Asterisks indicate significant differences as compared to zero (no change) with a one sample t-test.

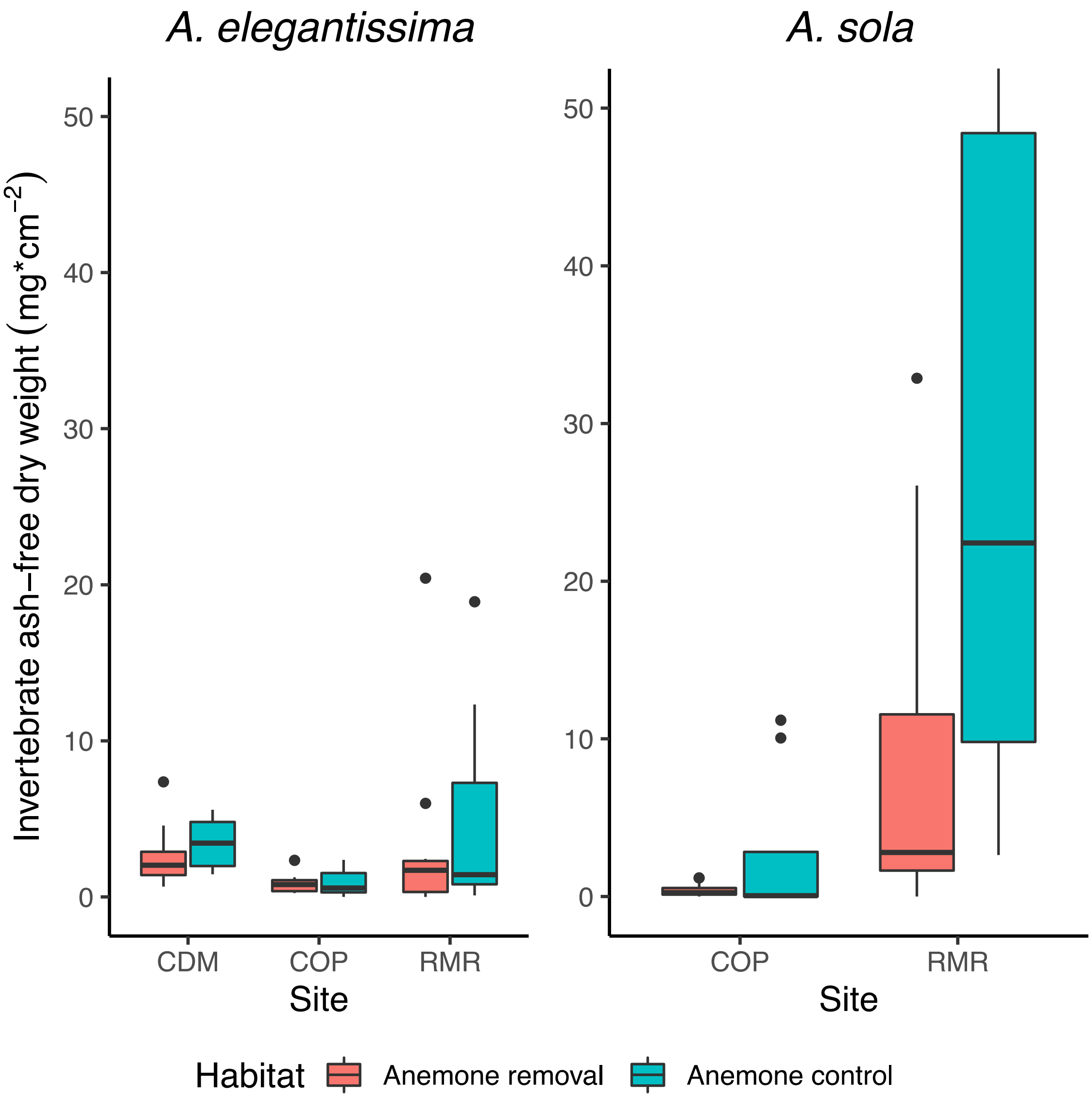
Supplementary Figure 3. A sea anemone (*A. sola*) addition treatment at Coal Oil Point immediately after manipulation and before removal of the nylon mesh. The sea anemone is held against the substrate (center) under nylon mesh secured with stainless steel screws at four corners. Mesh and screws were removed approximately 48 hours after deployment.



Supplementary Figure 4. Mobile invertebrate ash-free dry mass during the observational period of all distinct groupings: sea anemone species and sites. The y-axis was limited to 50 mg*cm^{-2} (losing the upper whisker of *A. sola* at RMR) so that differences between habitats in each of the other groups could be visualized.



Supplementary Figure 5. Boxplots of the mobile invertebrate ash-free dry mass in the sea anemone addition and rock control comparison with all distinct groupings: sea anemone species and sites. The y-axis was limited to 35 mg*cm^{-2} (losing the upper whisker of *A. sola* at RMR) so that differences between habitats in each of the other groups could be visualized.



Supplementary Figure 6. Boxplots of the mobile invertebrate ash-free dry mass in the sea anemone removal and sea anemone control comparison with all distinct groupings: sea anemone species and sites. The y-axis was limited to 50 mg*cm^{-2} (losing the upper whisker of *A. sola* at RMR) so that differences between habitats in each of the other groups could be visualized.