University of Massachusetts Boston
ScholarWorks at UMass Boston

Graduate Doctoral Dissertations | Doctoral Dissertations and Masters Theses

12-31-2015

Critical Forces that Structure Subtidal Ecological Communities in the Gulf of Maine, and the Integration of Invasive Species into these Communities

Martine C. Wagstaff
University of Massachusetts Boston

Follow this and additional works at: https://scholarworks.umb.edu/doctoral_dissertations

Part of the Biology Commons

Recommended Citation

Wagstaff, Martine C., "Critical Forces that Structure Subtidal Ecological Communities in the Gulf of Maine, and the Integration of Invasive Species into these Communities" (2015). *Graduate Doctoral Dissertations*. 230.
https://scholarworks.umb.edu/doctoral_dissertations/230

This Open Access Dissertation is brought to you for free and open access by the Doctoral Dissertations and Masters Theses at ScholarWorks at UMass Boston. It has been accepted for inclusion in Graduate Doctoral Dissertations by an authorized administrator of ScholarWorks at UMass Boston. For more information, please contact scholarworks@umb.edu.

CRITICAL FORCES THAT STRUCTURE SUBTIDAL ECOLOGIAL COMMUNITIES IN THE GULF OF MAINE, AND THE INTEGRATION OF INVASIVE SPECIES INTO THESE COMMUNITIES

A Dissertation Presented

by

MARTINE C. WAGSTAFF

Submitted to the Office of Graduate Studies,
University of Massachusetts Boston,
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2015

Environmental Biology Program

© 2015 by Martine C. Wagstaff
All rights reserved

CRITICAL FORCES THAT STRUCTURE SUBTIDAL ECOLOGICAL COMMUNITIES IN THE GULF OF MAINE, AND THE INTEGRATION OF INVASIVE SPECIES INTO THESE COMMUNITIES

A Dissertation Presented

by

MARTINE C. WAGSTAFF

Approved as to style and content by:

_____
Ron Etter, Professor
Chairperson of Committee

_____
Alan Christian, Associate Professor
Member

_____
Rick Kesseli, Professor
Member

_____
Robyn Hannigan, Professor, Dean of the School for the Environment
Member

_____
Linda Huang, Graduate Program Director
Biology Department

_____
Rick Kesseli, Chairperson
Biology Department

ABSTRACT

CRITICAL FORCES THAT STRUCTURE SUBTIDAL ECOLOGICAL COMMUNITIES IN THE GULF OF MAINE, AND THE INTEGRATION OF INVASIVE SPECIES INTO THESE COMMUNITIES

December 2015

Martine C. Wagstaff, B.Sc., University of Bristol, England
M.Sc., University of Nottingham, England
Ph.D., University of Massachusetts Boston, USA

Directed by Professor Ron Etter

Shallow subtidal epibenthic communities worldwide are under threat from exploitation, pollution, eutrophication, acidification, climate change, and invasive species, with implications for ecosystem diversity, productivity, function, and services. Subtidal ecosystems in the Gulf of Maine are particularly impacted, making it crucial to understand these habitats so that our impacts can be predicted and mitigated. I investigated the basic ecological forces that structure shallow subtidal epibenthic communities in this region, and how invasive species integrate themselves into these communities. I used community phylogenetic and functional trait analyses to investigate

if invertebrate communities in the rocky subtidal are assembled via deterministic or random forces, experimental manipulations to quantify how macroalgae might influence sessile invertebrates on subtidal surfaces, and measurements of life history traits of *Botrylloides violaceus,* an invasive colonial ascidian, to estimate whether growth of this species differs among man-made versus natural habitats. Based on community phylogenetic analyses, rocky subtidal invertebrate communities appear to be structured by deterministic forces, with evidence for both competitive exclusion and environmental filtering operating at different spatial scales. These findings support existing studies that show that competition structures communities at local scales, and also expand our knowledge of the processes that act regionally, i.e. environmental filtering. On shallow sunlit experimental surfaces suspended from floating docks, macroalgae had little effect on invertebrate abundance or diversity, contrary to findings from experiments in the rocky subtidal. Macroalgae did influence composition as well as enhance invertebrate colonization in the early stages of community assembly. Different factors appear to influence the balance between heterotrophs and autotrophs in floating dock and rocky subtidal systems with implications for community structure, function and productivity. In different habitats, colonies of the invasive ascidian *B. violaceus* exhibited differences in life history traits. It grew faster and attained larger sizes in man-made floating dock versus natural rocky subtidal and eelgrass bed habitats. Again, differences among habitats appear to influence invasion success. In conclusion, competitive exclusion, facilitation, and environmental filtering play key roles in controlling the structure, composition, and function of shallow subtidal communities. Invasive species have the

potential to disrupt these forces as they integrate themselves into man-made and subsequently natural habitats.

ACKNOWLEDGEMENTS

Thanks to my advisor Ron Etter for his patience, commitment, and hard work, and to my committee Rick Kesseli, Alan Christian, and Robyn Hannigan for their good humor and support. Jarrett Byrnes and Liam Revell patiently and unfalteringly answered many questions regarding statistical and phylogenetic analyses. Mike Rex was an early committee member and all around great help and greater guy throughout my years at UMass as was Rick Kesseli as Graduate Program Director and then Chairperson. Biology Department administrative staff was wonderful and so a huge thank you to Maria Mahoney, Alexa MacPherson, Charlie King, and Alana Boyle. Thanks to Beth Boyle, Rob Jennings, and Amanda Glazier for help in the lab. Scott Morello, those early days of field work when we had no idea what we were doing were so fun and then, when we did know, we got some fantastic undergraduates that actually wanted to come and help. I had the best times on early morning drives and dives with Zack Smith and Damien Katzmark, it was just what the doctor ordered mid-PhD. Edgar Franck helped throughout with lab, field, and machine shop work; the latter led by the ever kind and helpful Tom Goodkind. The latest additions to the lab, Casey McCabe and Aaron Honig, have provided moral support and I'm sorry that the crossing of our paths was so short. Thanks to my dad throughout and more recently, to my lovely boyfriend Dennis who has been a steady force over the last few years. Over the last few weeks Jess Torossian, Chris Desmond, and Kate Longley have been superb. Thanks for funding go to University of Massachusetts Boston for the Doctoral Dissertation Research Grant and the Craig R.

Bollinger Memorial Research Grant, to Ron Etter for the Research Trust Funds, to Sigma Xi and to the American Museum of Natural History for the Lerner-Gray Memorial Research Grant. Thanks to everyone, it was fun, but I'm glad it's over! Is it over?!

TABLE OF CONTENTS

ABSTRACT iv

ACKNOWLEDGMENTS vii

LIST OF TABLES xi

LIST OF FIGURES xiii

CHAPTER Page

1. INTRODUCTION 1
References 5

2. COMMUNITY ASSEMBLY IN SUBTIDAL EPIBENTHIC INVERETBRATE COMMUNITIES IN THE GULF OF MAINE - A COMMUNITY PHYLOGENETIC AND FUNCTIONAL TRAIT APPROACH
Abstract 9
Introduction 10
Methods 14
Results 27
Discussion 31
Conclusions 42
References 44
Tables 57
Figures 62

3. INTERKINGDOM COMPETITION FOR SPACE OR FACILITATION? MACROALGAE VERSUS SESSILE INVERTEBRATES IN A RESOURCE LIMITED ENVIRONMENT
Abstract 71
Introduction 72
Methods 75
Results 82
Discussion 84
Conclusions 93
References 95
Tables 105
Figures 112

4. THE RESPONSE OF AN INVASIVE SPECIES, *BOTRYLLOIDES VIOLACEUS*, TO NOVEL HABITATS IN GULF OF MAINE
Abstract 116
Introduction 117
Methods 119
Results 126
Discussion 128
Conclusions 134
References 136
Tables 147
Figures 150

5. FINAL CONCLUSIONS 158
References 162

APPENDIX

2A. SUMMARY OF TRAIT DATA 164
2B. R CODE FOR BETA DIVERSITY NULL MODELS 170
2C. RELATIONSHIP BETWEEN CHLOROPHYLL A CONCENTRATION AND COMPETITIVE EXCLUSION 173
3A. CHAPTER 3 RAW DATA 176
4A. SUMMARY OF LIFE HISTORY TRAIT DATA 180
4B. CHAPTER 4 RAW DATA 182

LIST OF TABLES

Table Page

2.1 Subtidal epibenthic invertebrate species and accession numbers used to generate the regional phylogeny for the Gulf of Maine 57

2.2 Functional traits for subtidal epibenthic invertebrate species in the Gulf of Maine. 58

2.3 Phylogenetic diversity of subtidal epibenthic invertebrate species at eight sites in the Gulf of Maine..... 59

2.4 Phylogenetic signal in functional traits for all subtidal, epibenthic invertebrate species in the regional phylogeny..... 60

2.5 Functional trait diversity for subtidal epibenthic invertebrate species at eight sites in the Gulf of Maine..... 61

3.1 Species list for all algae and sessile invertebrates found on settlement plates at Dorchester Yacht Club..... 105

3.2 Starting community manipulations for Displacement, Recruits, and Settlers experiments 106

3.3 Results of MANOVAs for Assembly, Displacement, Recruits, and Settlers experiments 107

3.4 Results of univariate ANOVAs for percent cover and diversity of primary space occupying sessile invertebrates for the Assembly, Displacement, Recruits, and Settlers experiments..... 108

3.5 Results of PERMANOVA, using the Bray-Curtis distance metric, for community composition of primary space occupying sessile invertebrates for the Assembly, Displacement, Recruits, and Settlers experiments 109

3.6 Percent cover of primary space occupying sessile invertebrate taxa (mean ± SE) in Assembly, Displacement, Recruits, and Settlers experiments by treatment 110

LIST OF TABLES

Table Page

4.1. Abiotic and biotic characteristics of floating dock, rocky subtidal, and eelgrass bed habitats..... 147

4.2. Results of ANOVA, testing for a difference in cumulative settlement density among habitats, with site as a random factor 148

4.3. Results of univariate, habitat X site ANOVAs for terminal age, terminal size, maximum growth rate, and colony regression 149

LIST OF FIGURES

Figure Page

2.1 Patterns of a community phylogenetic structure 62

2.2 Map of the Gulf of Maine showing the eight sites, Shag Rocks (SR), Wood Island (WI), Thrumcap (TC), Bunker Point (BP), Moose Cove (MC), Cutler (CR), Whale Cove (WC), and Pubnico (PU) 63

2.3 Bayesian tree based on 18S rRNA. Posterior probabilities are reported on branches..... 64

2.4 Maximum likelihood tree based on 18S rRNA 65

2.5 Species presence-absence by site, a filled circle representing species presence..... 66

2.6 Phylogenetic signal and functional trait diversity, different shades representing different values for each trait 67

2.7 Null model results for A) taxonomic, B) phylogenetic nearest neighbour, C) functional trait nearest neighbour, D) phylogenetic pairwise, and E) functional trait pairwise beta diversity between pairs of sites in the Gulf of Maine..... 68

2.8 Relationship between geographical distance and A) taxonomic, B) phylogenetic nearest neighbour, C) functional trait nearest neighbour, D) phylogenetic pairwise, and E) functional trait pairwise beta diversity 69

2.9 The relationship between chlorophyll a concentration and competitive exclusion..... 70

3.1 Starting community manipulations..... 112

3.2 Treatments in the Settlers experiment through time..... 113

LIST OF FIGURES

Figure Page

3.3 Centroid plots showing percent cover and diversity of primary space occupying sessile invertebrates, for all treatments in the A) Assembly, B) Displacement, C) Recruits, and D) Settlers experiments 114

3.4 NMDS ordination plots of community replicates separated by treatments using Bray-Curtis dissimilarities for communities of space occupying sessile invertebrates for the A) Assembly, B) Displacement, C) Recruits, and D) Barrier experiments 115

4.1. Map of Massachusetts Bay showing location of three replicate sites 150

4.2 Experimental set-up and example plates 151

4.3. Colony growth from settlement until terminal size (Colony ID is G_D_G_ES; see Appendix 4A)..... 152

4.4. Cumulative settlement of *B. violaceus* recruits in the 2012 season 153

4.5. Life history traits of *B. violaceus* colonies in floating dock, rocky subtidal, and eelgrass bed habitats for A) terminal age, B) terminal size, C) maximum growth rate, and D) duration of colony regression 154

4.6 Patterns of *B. violaceus* colony regression in floating dock colonies..... 155

4.7. Growth curves for *B. violaceus* colonies in all habitats at all sites 156

4.8. A) Daily settlement of *B. violaceus*, and B) growth curves for individual *B. violaceus* recruits colonies in floating dock habitats in Gloucester showing relationship between settlement and cohort dynamics 157

CHAPTER 1

INTRODUCTION

Around 3.2 billion people live within 200 km of the coast, that is about half of the world's population on just 10 percent of its surface (Hinrichsen 1998). As a result, shallow subtidal benthic ecosystems, such as eelgrass beds, kelp forests, and soft and rocky bottoms, are at risk from over exploitation, pollution, eutrophication, hypoxia, acidification, climate change, changes in sedimentation and salinity, and invasive species (Worm et al. 2006, Halpern et al. 2008, Crain et al. 2009). Coastal benthic communities in the Western Atlantic are particularly impacted (Jackson 2001) and the Gulf of Maine is one of the most highly altered areas worldwide (Halpern et al. 2008). Unfortunately, the Western Atlantic coastal region was transformed before modern ecological investigations began and thus we do not know what is natural (Jackson 2001). In the last 40 years, however, the combined stresses of overfishing, climate change and species invasions have left the Gulf of Maine considerably different from its earlier state (Harris and Tyrrell 2001).

Our understanding of marine ecosystems has typically lagged behind terrestrial counterparts due to an 'out of sight out of mind' attitude (Ray and Grassle 1991), a mistaken belief of oceans as extensive wilderness (Jackson 2001), and because

conducting research in the ocean realm is generally difficult, resource intensive, and expensive (Richardson and Poloczanska 2008). Between 1987 and 2004, less than 10 % of biodiversity research was conducted in the marine environment (Hendriks and Duarte 2008). This inferior knowledge and effort is unacceptable today because oceans are extremely diverse in terms of genetics, species, phyla and habitats (Gray 1997) and, while land transformation is the primary cause of biodiversity loss, degradation of the oceans also contributes substantially (Vitousek et al. 1997). In turn, loss of marine biodiversity degrades ecosystem functioning and services (Worm et al. 2006, Stachowicz et al. 2007, Gamfeldt et al. 2014) such as coastal protection, nutrient cycling, erosion control, water purification, carbon sequestration, and tourism and recreation (Barbier et al. 2011). With these unprecedented rates of anthropogenic change, it is critical that we increase our understanding of shallow, subtidal ecosystems, so that we can identify, predict, and mitigate our impacts (Vitousek et al. 1997, Hendriks and Duarte 2008).

Central to basic ecology is to understand how communities assemble from the regional species pool (Chase and Leibold 2003). Communities can assemble via competitive exclusion (Hardin 1960) or environmental filtering (Weiher and Keddy 1995), which are deterministic processes, or via neutral processes (Hubbell 2001), which are random. In terrestrial systems, methods in phylogenetic community ecology have been developed to address this question (Webb 2000, Webb et al. 2002) and, in chapter 2, these methods are applied to a marine setting to investigate if subtidal epibenthic invertebrate communities in rocky subtidal habitats in the Gulf of Maine are structured by deterministic or random processes. The use of these methods alone, however, has

been criticized (Losos 2008, Cavender-Bares et al. 2009), and so a functional trait approach (McGill et al. 2006) was also employed.

A typical pattern found in rocky subtidal habitats is that shallow horizontal surfaces are primarily covered with various species of macroalgae while on deeper vertical surfaces invertebrates are the dominant forms (Miller and Etter 2011). The ecological and evolutionary forces that control this balance between heterotrophs and autotrophs have far-reaching implications for ecosystem productivity, function, and services (Mineur et al. 2014, Strong et al. 2015). Recent experiments in the rocky subtidal revealed that algae appear to exclude invertebrates on shallow sunlit horizontal surfaces (Miller and Etter 2008) and, in Chapter 3, manipulative experiments were used to explore if algae influence invertebrate recruitment or survivorship. Potential mechanisms of exclusion were also investigated via the use of plastic algal mimics which allowed algae structure to be kept intact while eliminating any chemical characteristics.

In the Gulf of Maine, invasive colonial ascidians are becoming an increasing problem (Pederson et al. 2005, Dijkstra et al. 2007). They are transported around the world on boat hulls (Lambert and Lambert 2003) from which they can colonize man-made floating docks (Lambert 2007) and subsequently expand into natural systems (Lambert 2005). Their presence alters the structure and function of these communities (Dijkstra and Harris 2009, Dijkstra et al. 2013) and the ascidians themselves likely change ecologically and evolutionarily in response to new habitats and ranges (Hanfling and Kollmann 2002). In Chapter 4, the life history traits of *Botrylloides violaceus*, a

colonial ascidian, were estimated in three different habitats, floating docks, rocky subtidal, and eelgrass beds. Quantifying differences in life history traits is the first step in identifying an evolutionary aspect to invasions. This is necessary because changes in invasive species have the potential to feedback to native species and ecosystems (Ghalambor 2007).

This work is important because we know much less about marine systems in general (Steneck and Carlton 2001, Hendriks and Duarte 2008), and of invasions in coastal, estuarine, and marine environments in particular (Grosholz 2002). Furthermore, the highly degraded nature of the Gulf of Maine (Halpern et al. 2008) makes research in this region a priority. As anthropogenic impacts and species invasions are only likely to increase, it is important that we understand the processes governing subtidal ecosystems before they are disrupted, and ecosystems changed beyond recognition. The results from this research will identify critical forces that influence the structure, composition, and function of shallow subtidal communities in the Gulf of Maine, and how invasive species might disrupt these by integrating into natural communities. It will provide much needed information on how we can identify, predict, and mitigate our impacts.

References

Barbier, E. B., S. D. Hacker, C. Kennedy, E. W. Koch, A. C. Stier, and B. Silliman. 2011. The value of estuarine and coastal ecosystem services. Ecological Monographs 81:169–193.

Cavender-Bares, J., K. H. Kozak, P. V. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. Ecology Letters 12:693–715.

Chase, J. M., and M. A. Leibold. 2003. Ecological niches: linking classical and contemporary approaches. University of Chicago Press, Chicago, USA.

Crain, C. M., B. S. Halpern, M. W. Beck, and C. V. Kappel. 2009. Understanding and Managing Human Threats to the Coastal Marine Environment. Annals of the New York Academy of Sciences 1162:39–62.

Dijkstra, J. A. and L. G. Harris. 2009. Maintenance of diversity altered by a shift in dominant species: implications for species coexistence. Marine Ecology Progress Series 387:71–80.

Dijkstra J., L. G. Harris, and E. Westerman. 2007. Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. Journal of Experimental Marine Biology and Ecology 342:61–68.

Dijkstra, J. A., W. J. Lambert, and L. G. Harris. 2013. Introduced species provide a novel temporal resource that facilitates native predator population growth. Biological Invasions 15:911–919.

Gamfeldt, L., J. S. Lefcheck, J. E. Byrnes, B. J. Cardinale, J. E. Duffy, and J. N. Griffin. 2014. Marine biodiversity and ecosystem functioning: what's known and what's next? Oikos 124:252–265.

Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology 21:394–407.

Gray, J. S. 1997. Marine biodiversity: patterns, threats and conservation needs. Biodiversity and Conservation 6:153–175.

Grosholz, E. 2002. Ecological and evolutionary consequences of coastal invasions. Trends in Ecology and Evolution 17:22–27.

Halpern, B. S., S. Walbridge, K. A. Selkoe, C. V. Kappel, F. Micheli, C. D'Agrosa, J. F. Bruno, K. S. Casey, C. Ebert, H. E. Fox, R. Fujita, D. Heinemann, H. S. Lenihan, E. M. P. Madin, M. T. Perry, E. R. Selig, M. S. Spalding, R. Steneck, and R. Watson. 2008. A Global Map of Human Impact on Marine Ecosystems. Science 319:948–952.

Hanfling, B. and K. Kollman. 2002. An evolutionary perspective of biological invasions. Trends in Ecology and Evolution 17:545–546.

Hardin, G. 1960. The competitive exclusion principle. Science 131:1292–1297.

Harris, L. G, and M. C. Tyrrell. 2001. Changing community states in the Gulf of Maine: synergism between invaders, overfishing and climate change. Biological Invasions 3:9–21.

Hendriks, I. E., and C. M. Duarte. 2008. Allocation of effort and imbalances in biodiversity research. Journal of Experimental Marine Biology and Ecology 360:15–20.

Hinrichsen, D. 1998. Coastal Waters of the World: Trends, Threats, and Strategies. Island Press, Washington D.C., USA.

Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, New Jersey, USA.

Jackson, J. B. C. 2001. What was natural in the coastal oceans? Proceedings of the National Academy of Sciences USA 98:5411–5418.

Lambert, G. 2005. Ecology and natural history of the protochordates. Canadian Journal of Zoology 83:34–50.

Lambert, G. 2007. Invasive sea squirts: a growing global problem. Journal of Experimental Marine Biology and Ecology 342:3–4.

Lambert, C., and G. Lambert. 2003. Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Marine Ecology Progress Series 259:145–161.

Losos, J. B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecology Letters 11: 995–1003.

McGill, B. J., B. J. Enquist, E. Weiher, E., and M. Westoby. 2006. Rebuilding community ecology from functional traits. Trends in Ecology and Evolution 21:178–185.

Miller, R. J., and R. J. Etter. 2008. Shading facilitates sessile invertebrate dominance in the rocky subtidal Gulf of Maine. Ecology 89:452–462.

Miller, R. J., and R. J. Etter. 2011. Rock walls: small-scale diversity hotspots in the subtidal Gulf of Maine. Marine Ecology Progress Series 425:153–165.

Mineur, F., J. Assis, A. J. Davies, A. H. Engelen, F. Fernandes, E. Malta, T. Thibaut, T. Van Nguyen, F. Vaz-Pinto, S. Vranken, E. A. Serrão, and O. De Clerck. 2014. European seaweeds under pressure: Consequences for communities and ecosystem functioning. Journal of Sea Research: http://dx.doi.org/10.1016/j.seares.2014.11.004

Pederson, J. P., R. Bullock, J. Carlton, J. Dijkstra, N. Dobroski, P. Dyrynda, R. Fisher, L. Harris, N. Hobbs, G Lambert, E. Lazo-Wasem, A. Mathieson, M. Miglietta, J. Smith, J. Smith III, and M. Tyrrell. 2005. Marine Invaders in the Northeast: Rapid Assessment Survey of Non–native and native Marine Species of Floating Dock Communities. MIT Sea Grant College Program, Cambridge, Massachusetts, USA.

Ray, G. C. and J. F. Grassle. 1991. Marine biological diversity: a scientific program to help conserve marine biological diversity is urgently required. BioScience 41:453–458.

Richardson, A. J., and E. S. Poloczanska. 2008. Under-resourced, under threat. Science 320:1294–1295

Stachowicz, J. J., J. F. Bruno, and J. E. Duffy. 2007. Understanding the effects of marine biodiversity on communities and ecosystems. Annual Review of Ecology, Evolution, and Systematics 38:739–766.

Steneck, R. S. and J. T. Carlton. 2001. Human Alterations of Marine Communities: Students Beware! Pages 445-468 in M. D. Bertness, S. Gaines, and M. E. Hay, editors. Marine Community Ecology. Sinauer Associates, Sunderland, Massachusetts, USA.

Strong, J. A., E. Andonegi, K. Can Bizsel, R. Danovaro, M. Elliott, A. Franco, E. Garces, S. Little, K. Mazik, S. Moncheva, N. Papadopoulou, J. Patrício, A. M. Queirós, C. Smith, K. Stefanova, and O. Solaun. 2015. Marine biodiversity and ecosystem function relationships: the potential for practical monitoring applications. Estuarine, Coastal and Shelf Science 161:46–64.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science 277:494–499.

Webb, C. O. 2000. Exploring the Phylogenetic Structure of Ecological Communities: An Example for Rain Forest Trees. American Naturalist 156:145–155.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and Community Ecology. Annual Review of Ecological Systematics 33:475–505.

Weiher, E., and P. A. Keddy. 1995. The assembly of experimental wetland plant communities. Oikos 73:323–335.

Worm, B., E. B. Barbier, N. Beaumont, J. E. Duffy, C. Folke, B. S. Halpern, J. B. C. Jackson, H. K. Lotze, F. Micheli, S. R. Palumbi, E. Sala, K. A. Selkoe, J. J. Stachowicz, and R. Watson. 2006. Impacts of biodiversity loss on ocean ecosystem services. Science 314:787–790.

CHAPTER 2

COMMUNITY ASSEMBLY IN SUBTIDAL EPIBENTHIC INVERETBRATE COMMUNITIES IN THE GULF OF MAINE — A COMMUNITY PHYLOGENETIC AND FUNCTIONAL TRAIT APPROACH

Abstract

How organisms assemble to form a community is a central question in ecology, with commonly inferred mechanisms including environmental filtering, competitive exclusion, and random processes. Community phylogenetic and functional trait analyses have been used to identify the relative importance of these processes, most often in terrestrial angiosperm systems consisting of a single taxon (e.g. class). Here, community phylogenetic and functional trait analyses are applied to multiphyletic subtidal epibenthic invertebrate assemblages at eight sites in the Gulf of Maine, to investigate the relative importance of deterministic or stochastic forces in structuring these communities. At local sites, some communities were phylogenetically overdispersed, suggesting community assembly via competitive exclusion. Five traits exhibited phylogenetic signal and for these traits, phylogeny can be used as a proxy for ecological similarity. Functional trait diversity was overdispersed for one site and clustered for another, indicative of assembly via competitive exclusion and environmental filtering respectively. Regionally, taxonomic beta diversity was less than expected by chance but

phylogenetic and functional trait beta diversity were greater than expected, indicating that species are not dispersal limited and that different environmental conditions among sites select for different species. These results suggest that subtidal epibenthic invertebrate assemblages in the Gulf of Maine are structured by deterministic forces and that different assembly processes operate at different spatial scales: locally, competitive exclusion appears to be important whereas regionally, environmental filtering plays a role.

Introduction

A central goal of ecology is to explain species coexistence in terms of community assembly (Weiher and Keddy 1999, Chase and Leibold 2003), which is critical for understanding the forces that generate and maintain biological diversity at a variety of spatial scales. Within a trophic level, mechanisms invoked to explain assembly processes include competitive exclusion (Hardin 1960), environmental filtering (Weiher and Keddy 1995), and neutral processes (Hubbell 2001). Historical factors also play a role (Ricklefs 1987, Ricklefs and Schluter 1993) but traditionally, ecologists have neglected these, focusing instead on contemporary abiotic and biotic interactions (Webb et al 2002). Recent advances in phylogenetic data and computational ability have bridged this gap between ecology and evolution (Webb et al. 2002, Cavender-Bares et al. 2009, Vamosi et al. 2009), and phylogenetic community ecology now serves as "glue" sticking these two disciplines together (Webb et al. 2002).

Phylogenetic community ecology investigates if species in an assemblage are more or less phylogenetically related than expected by chance (Webb 2000). Under the

assumption of phylogenetic signal, inferences are then made about whether communities are structured by deterministic (competitive exclusion and abiotic filtering) or random (neutral) forces (Webb 2000). This sub-discipline has advanced our understanding of the processes driving community assembly (Webb 2000, Cavender-Bares et al. 2004) and how this varies with spatial scale (Cavender-Bares et al. 2006, Kembel and Hubbel 2006, Swenson et al. 2006, 2007) and environmental gradients (Swenson 2011, Graham et al. 2012, Qian et al. 2013, Weinstein et al. 2014). It has also demonstrated the importance of evolution in the assembly process, essentially integrating the investigations of ecological and evolutionary processes to understand the causes, maintenance, and consequences of biodiversity (Cavender-Bares et al. 2009).

To date, most phylogenetic community analyses have been conducted in tropical terrestrial angiosperm assemblages with few studies conducted in other systems (Vamosi et al. 2009). Similar to terrestrial trees, taxa in epifaunal marine communities are sessile, and occupy and compete for primary space. Between these two systems, however, organisms differ in body size by orders of magnitude, as do the size of the sampling units. Perhaps the most striking differences between these two systems are taxonomic and phyletic diversity. While angiosperms are taxonomically diverse, these are representative of a single taxonomic class. In contrast, in subtidal epifaunal marine communities, many phyla coexist (Witman et al. 2004, Miller and Etter 2011) but communities can be species poor. Can community phylogenetic analyses be applied to such systems and what do they reveal about community structure in subtidal epibenthic invertebrate communities in the Gulf of Maine?

Phylogenetic community ecology is based on the premise that species persist in ecological communities because they 1) occur in the regional species pool, 2) possess traits that interact with other species as well as the abiotic environment, and 3) possess a history contingent on chance events and deterministic interactions with other species in historical communities (Webb et al. 2006). Once a regional phylogeny has been created, species in local communities are mapped onto the tips of the phylogeny and any patterns of randomness, clustering, or overdispersal can be identified (Webb 2000, Webb et al. 2002) (Fig. 2.1). Interpretations of patterns are based on the assumption of phylogenetic signal, which is the tendency of closely related species to resemble each other (Blomberg and Garland 2002), i.e. species traits are conserved within lineages and a positive relationship exists between species phylogeny and ecological similarity (Webb 2000). If for example, species in local communities are clustered when mapped on to the regional phylogeny, then the taxa in the local community are more related than expected by chance (Fig. 2.1). This is suggestive of environmental filtering and assumes that closely related species are ecologically similar (Webb et al. 2002). If the opposite is found, and local species are overdispersed within the regional phylogeny, this is suggestive of competitive exclusion and minimum niche overlap between species (Webb et al. 2002) (Fig. 2.1). Neutral structuring of the community would lead to a random distribution of taxa within the phylogeny (Kraft et al. 2007). Phylogeny, however, does not necessarily serve as a perfect proxy for ecological similarity and clustering can also result from ecological divergence of closely related species facilitating coexistence, and overdispersion from distantly related but ecologically similar species being filtered by the

environment (Webb et al. 2002, Losos 2008). Patterns of phylogenetic community structure therefore need to be interpreted cautiously (Cavender-Bares et al. 2009) and trait information is required to tease out processes behind the patterns.

Functional traits are those that define species in terms of their ecological roles, i.e. how they interact with the environment and with other species (Diaz and Cabido 2001, Westoby et al 2002, Petchley and Gaston 2006). By adding trait data to species in a phylogeny, processes behind patterns can be more easily inferred. While functional traits are well characterized for terrestrial plants with globally accepted protocols for their measurement (Westoby et al. 2002, Cornelissen et al. 2003), similar information in the marine realm is not available. Some groups, however, are working towards applying trait-based analyses to subtidal marine systems (Bremner et al 2003, Bremner et al. 2006, Frid et al. 2008).

Another key challenge in community ecology is to integrate data from different spatial scales (Graham and Fine 2008). Beta diversity, the change in species composition across geographic space, provides a link between local alpha diversity and regional gamma diversity (Whittaker 1960, Tuomisto 2010). While taxonomic beta diversity describes the turnover of species between sites, it does not provide information about phylogenetic turnover (Graham and Fine 2008). Thus a measure of phylogenetic beta diversity, defined as the phylogenetic distance between samples of individual organisms between any two sites, is required to provide different insights about the ecological, historical, and evolutionary processes that structure communities (Hardy and Senterre 2007). Comparing taxonomic, phylogenetic, and functional trait beta diversity can also

provide new insight into the origin and maintenance of biodiversity (Weinstein et al. 2014). For example, high taxonomic, high phylogenetic, and low trait beta diversity is suggestive of similar ecological roles of species in an environment and convergent adaptation (Weinstein et al. 2014). Combined with geographical and environmental data, this approach has the potential to unravel the relative importance of processes acting across spatial scales (Swenson et al. 2011, Weinstein et al. 2014).

To investigate if community phylogenetic analyses can provide novel insights about the assembly of subtidal epibenthic invertebrate communities in the Gulf of Maine at a variety of spatial scales, a molecular phylogeny was created for the regional species pool. Local communities at eight sites were then mapped onto the regional phylogeny, and patterns of community structure identified. Functional traits were developed for all taxa and functional diversity was compared to phylogenetic diversity. Patterns in taxonomic, phylogenetic, and functional trait beta diversity were also investigated, and interpreted under community assembly theory.

Methods

Study system, study sites, and data collection

Subtidal epifaunal communities on rock walls are dominated by sessile suspension feeding invertebrates such as sponges, cnidarians, bryozoans and ascidians (for review see Witman and Dayton 2001). As many as 125 different species can coexist in a small, 0.25 m^2 quadrat in diverse tropical systems with as many as 300 species found at a given site (Witman et al. 2004). Quadrats in this study contained a more modest 15

species, representing 8 phyla, and the most diverse site harbored 31 species, representing 9 phyla. The multiphyletic nature of these marine communities is a sharp contrast to terrestrial plant systems, as are the size of organisms and sampling units.

This study used data collected by Miller and Etter in August and September of 2003 (Miller and Etter 2011). Eight sites in the Gulf of Maine were sampled, all on exposed rocky coasts (Fig. 2.2). Sampling involved taking photoquadrats (0.25 m^2) of epifaunal communities on vertical granitic rock substrate at a depth of 10 to 12 m below mean low tide and identifying organisms to species level (for more details see Miller and Etter 2011). Approximately 18 phtotoquadrats per site were sufficient to approximate the asymptote of the species accumulation curve and generate species presence-absence data for each site.

In total, the regional species pool consisted of 49 species of sessile invertebrates (Table 2.1). For all species where an entry existed, 18S nucleotide sequence data was downloaded from the National Center for Biotechnology Information database (NCBI, Bethesda, Maryland, USA). The remaining species were collected for sequencing from Gloucester, Massachusetts (42.571525°N, 70.710407°W), using SCUBA. Animals were put in vials in the field and placed on ice in chilled seawater until sorting in the laboratory. Approximately 125 mm^3 of tissue for each specimen was then dissected using sterile techniques and frozen until further processing. For the species that did not yield DNA, appropriate congeners (and in one instance a confamilial) were selected from GenBank and their sequences downloaded. An additional sequence was downloaded for an outgroup (Table 2.1).

Sequencing

To sequence species in the regional phylogeny, DNA was extracted with the DNEasy DNA Extraction Kit (Qiagen) following the manufacturer's protocol. PCR amplification of the 18S gene initially employed primers18e and 18L (Hillis and Dixon, 1991). This primer did not work well with bryozoans, tending to amplify small organisms living in zooecia instead. Bryozoan specific primers were therefore designed for use with template DNA. The new bryozoan-specific forward primer was Bryo18Sf (5'-CACCCGAGTAATTGCCGCC-3') and the reverse Bryo18Sr (5'-GGAGAAACACGCTGATGCAAA-3'). 50 uL PCRs consisted of 27.2 ul d2H_2O, 10.5 ul GoTaq Flexi Buffer (Promega, Madison, WI), 5 ul $MgCl_2$, 2.5 ul BSA, 1 ul dNTPs, 1 ul forward primer, 1 ul reverse primer, 0.3 ul GoTaq Flexi DNA Polymerase (Promega), and 2 ul of extracted DNA template. The PCR protocol was as follows: initial denaturation at 94ºC for 3 minutes; 35 cycles at 94ºC for 30 seconds, 55ºC for 45 seconds, and 72ºC for 90 seconds; and final extension at 72ºC for 3 minutes. Negative and positive controls were included with each round of reactions. PCR products were checked for single bands through gel electrophoresis and then outsourced to Agencourt (a Beckman-Coulter company, Beverly, MA) for bi-directional sequencing. Forward and reverse sequences were edited, aligned using Sequencher 5.0.1 (Gene Corp. Ann Arbor, MI), and compared to the GenBank database using BLAST to check that DNA for the correct taxa had been amplified. Sequences generated were deposited in the Nucleotide section of GenBank (Accession Numbers KF699106 – KF699115 (see Table 2.1). The final dataset consisted of 18S sequences for 50 species (31 sequences for species from

GenBank, nine sequences generated by PCR, eight sequences for congeners, one for a confamilial and one outgroup) (Table 2.1).

Phylogenetic tree generation

To investigate the evolutionary history of subtidal epibenthic invertebrate species in the Gulf of Maine, 18S sequences from all individuals were aligned in MUSCLE (Edgar 2004) and the alignment was checked by eye in MacClade 4.08 (Maddison and Maddison 2005) to ensure correct base calling. The original raw chromatograms were also referred to where necessary with appropriate edits made. Sequences were trimmed at the beginning and end of the alignments to minimize gaps and saved in Nexus format for tree generation. The function modelTest, which computes log-likelihood, Akaike's Information Criterion (AIC), and Bayesian Information Criterion (BIC), from the Phangorn v 1.70 package (Schliep 2011) in the R version 3.0.2 (R Core Team 2013) was used to identify the best model of DNA evolution. The general time-reversible model (GTR) was selected, with an estimated proportion of DNA sites invariant (I), and mutation rates among sites following a gamma distribution (G). The GTR+I+G model was then used to estimate a maximum likelihood (ML) and a Bayesian gene tree. The maximum likelihood tree was estimated using the PhyML web server (Dereeper et al. 2008, 2010). Default settings were used except for 'Substitution Model' where GTR+I+G was selected as well as the 'Bootstrapping procedure' (100 times) to assess support for tree nodes. The Bayesian tree was obtained using BEAST v 1.7.5 (Drummond et al. 2012). Priors were the GTR+I+G model of DNA substitution, an

uncorrelated lognormal relaxed clock, random starting tree and a birth-death model for speciation. The Markov chain Monte Carlo (MCMC) chain ran for 80 million steps, logging every 1000 trees. Trace plots were visualized in Tracer (Rambaut et al. 2014) to ensure sufficient burnin, which was determined by the MCMC chain reaching a stationary distribution with a relatively constant mean and variance and a sufficient ESS of greater than 200. The posterior distribution of species trees was summarized into a point estimate of topology using the default 'Maximum Clade Credibility Tree' option in TreeAnnotator v1.7.5 (Drummond et al. 2012). This option scores clades within a tree based on the fraction of times they appear in the entire set of sampled posterior trees, and gives each tree an overall score. The tree with the highest score is the maximum clade credibility tree. This single target tree is then annotated with the posterior support for each branch summarized from all the other trees. The two barnacle species did not group out as expected which might be due to long-branch attraction as they are the only arthropods in the tree. To ensure proper placement of the barnacles three more arthropod species (*Gammarus* sp. *Artemia* sp. and *Carcinus maenas*) were added to the data set and the analyses repeated as above. These additional taxa were then deleted using the "Manage taxa" option in Mesquite v 2.75 (Maddison and Maddison 2011) before community phylogenetic analyses.

Community Phylogenetic analysis

Using the Bayesian tree (which was very similar to the maximum likelihood tree (see results)), metrics of phylogenetic diversity were calculated and used to make

inferences about community assembly. A matrix of phylogenetic distances between all species was first generated using the cophenetic function in R version 3.0.2 (R Core Team 2013). This was then used in the calculation of two commonly used metrics, the mean nearest neighbor distance (MNND) and mean phylogenetic distance (MPD) (Webb 2000, Webb et al. 2002). MNND is a measure of the mean phylogenetic distance from each species to its closest relative in the focal set, where a focal set here is all species at a given site. MNND measures whether the most closely related co-occurring species in a community are more or less closely related than expected by chance. It is sensitive to patterns of clustering and overdispersal close to the tips of the phylogeny and is good for assessing fine scale relatedness. It is calculated as:

$$MNND = \frac{\sum_i^n \min\ d_{i,j}}{n}$$

where min $d_{i,j}$ is the minimum phylogenetic distance between species i and its nearest neighbor species j, and n is the number of species in the community. MPD is a measure of the mean phylogenetic distance between every possible pair of species in the focal set. It is sensitive to deep relationships and quantifies overall, tree-wide patterns of phylogenetic clustering (Webb 2000, Webb et al. 2002). It is calculated as:

$$MPD = \frac{\sum_i^n \sum_j^n\ d_{i,j}}{n}$$

where $d_{i,j}$ is the phylogenetic distance between species i and j, and n is the number of taxa. Both metrics are used in this analysis as they can influence the patterns observed (Hardy 2008, Vellend et al. 2011) with nearest neighbor tests having greater power to

detect patterns due to competition, while pairwise tests perform better with habitat filtering (Kraft et al. 2007).

Because adding species to a tree alters the topology, most raw metrics correlate to species richness. Thus standardized metrics were used to enable comparisons among samples of different species richness. Measures of standardized effect sizes (SES) compare the difference between phylogenetic distances separating taxa in the observed communities versus null communities generated with a randomization method, divided by the standard deviation of phylogenetic distances in the null data (Gotelli and Graves 1996, Gotelli and Rohde 2002). The standard effect size of the mean nearest neighbor distance (SES MNND) is:

$$SES\ MNND = \frac{MNND_{obs} - \ \mathrm{mean}(MNND_{null})}{\mathrm{sd}(MNND_{null})}$$

where $MNND_{obs}$ is the observed mean nearest neighbor distance between species in the community and $MNND_{null}$ is the mean nearest neighbor distance between species in the null model. The null model asks what distribution of values would be expected if there was no phylogenetic structure when other factors, such as species richness, are held constant (Gotelli and Graves 1996, Gotelli and Rohde 2002). To create the null distribution for a community with S species, a focal set of S species is drawn 999 times from the regional phylogeny, which is equivalent to shuffling species randomly among the tips of the tree (Kembel 2009). Standardized effect sizes and quantiles can then be calculated. Positive SES values and high quantiles (> 0.95) indicate phylogenetic overdispersal, or a greater phylogenetic distance among co-occurring species than

expected. Negative SES values and low quantiles (< 0.05) indicate phylogenetic clustering, or smaller phylogenetic distances among co-occurring species than expected (Kembel 2009). SES MNND and SES MPD were calculated for each local community using the Picante package (Kembel et al. 2010) in R version 3.0.2 (R Core Team 2013) and inferences about phylogenetic community structure made.

Phylogenetic signal

To investigate phylogenetic signal in traits, species traits relevant to the study system were first selected. In assemblages composed of single trophic levels, theoretical models assume that functional diversity equates to resource use complementarity i.e. differences in how species gain resources (Díaz and Cabido 2001). In epibenthic marine systems, the limiting resource is usually space (Sebens 1985, 1986) and, by acquiring it, sessile species can acquire other resources such as food (Sebens 1986). Six traits were selected to cover a range of life history, morphological, and behavioral characteristics relating to space and food acquisition (Table 2.2). Additional traits were eliminated from the analysis because of redundancy. All traits were divided into discrete categories (Table 2.2), and species were placed in categories based on the their characteristics. For the one continuous trait, categories were assigned based on the species average. Trait information was obtained from a variety of sources including personal knowledge, on-line databases, natural history texts, and primary literature. To summarize, the trait data comprises five nominal traits (coloniality, reproduction, growth form, food capture, and

defense), and one continuous trait (body size) divided into nominal categories. Trait data are provided in Appendix 2A.

To investigate if the phylogenetic relatedness of species reflects ecological similarity, i.e. phylogenetic conservatism, Pagel's lambda (Pagel 1999) was used to quantify the regional, tree-wide phylogenetic signal in each of the six traits. Pagel's lambda is a tree transformation model where internal branch lengths are multiplied by the lambda parameter, the lambda parameter specifying the degree of phylogenetic signal in the data. When lambda is 0, the phylogenetic tree collapses to a star phylogeny, traits evolve independent of the phylogeny, and there is no correlation between species. As lambda increases from 0 to 1, the influence of phylogeny increases, and species become more correlated. A lambda of 1 indicates complete phylogenetic signal with the structure of the phylogeny alone explaining changes in traits. The value of lambda that best fits our phylogenetic tree was estimated by maximum likelihood using the fitDiscrete function in geiger (Harmon et al. 2008) in R version 3.0.2 (R Core Team 2013). The negative log likelihood for the original tree was then compared to the negative log likelihood for a transformed tree with no phylogenetic signal, i.e. with lambda = 0. This essentially tests the hypothesis of phylogenetic signal, lambda > 0, against the null hypothesis of no phylogenetic signal, lambda = 0. Negative log likelihoods were compared using a likelihood ratio test, which was approximated by a chi-squared distribution to generate p-values.

Functional trait diversity

To investigate if functional trait diversity is suggestive of deterministic or neutral community assembly processes, methods analogous to those used to calculate phylogenetic diversity were employed (Webb 2000). As well as being used to calculate phylogenetic distance between species in an assemblage, the mean nearest neighbor distance (MNND) and the mean pairwise distance (MPD) metrics can also be used to calculate functional trait diversity (Swenson 2014). A matrix of trait distances between species was first generated using the gowdis function in the R (version 3.0.2 (R Core Team 2013)) package FD (Laliberté et al. 2014), which calculates the Gower dissimilarity (Gower 1971, Podani and Schmera 2006) between variables. The Gower dissimilarity splits dichotomous and polychotomous nominal variables into as many dummy variables as there are categories. It then assigns a 0 or 1 to the dummy variable depending on the original value. For example, the trait growth form would be split into three dummy variables, encrusting, mound, and erect. The encrusting sponge *Halichondria panacea* would be assigned a 1 for the encrusting dummy variable, and 0s for the remaining dummy variables. Dissimilarity is then calculated using:

$$D_{jk} = \frac{\sum_{i=1}^{n} W_{ijk}\, S_{ijk}}{\sum_{i=1}^{n} W_{ijk}}$$

where n is the number of functional traits, W_{ijk} is the weight of the comparison between species j and k and is 0 if the comparison is not allowed for variable i because of missing data but is 1 otherwise, and S_{ijk} is a measure of disagreement between species j and k for variable i. For nominal variables, $S_{ijk} = 0$ if $X_{ij} \neq X_{ik}$, and $S_{ijk} = 1$ if $X_{ij} = X_{ik}$ where X_{ik} is

the raw data score for variable *i* and species *j*. The distance matrix was then used to calculate trait MNND and MPD (see equations above). The standard effect size (SES) of MNND and MPD was calculated in the same way as for phylogenetic diversity (see equation above) (Gotelli and Graves 1996, Gotelli and Rohde 2002), and a similar null model employed whereby species names were shuffled in the trait distance matrix (Kembell 2009). Positive SES values and high quantiles (> 0.95) indicate higher trait diversity than expected with co-occurring species being less similar than expected. Negative SES values and low quantiles (< 0.05) indicate lower trait diversity than expected with co-occurring species being more similar than expected by chance.

Under the assumption of phylogenetic signal (Webb 2000), sites with higher phylogenetic diversity should also display higher functional trait diversity, suggestive of trait divergence and community assembly by competitive exclusion. An inverse relationship, whereby sites with higher phylogenetic diversity display lower functional trait diversity, would suggest convergence of traits and community assembly by environmental filtering. To investigate if phylogenetic diversity and functional trait diversity are positively correlated, i.e. whether sites with high phylogenetic MNND also have high functional trait MNND, Pearson product-moment correlation coefficient was calculated. This was also repeated for phylogenetic MPD and functional trait MPD.

Taxonomic, phylogenetic, and functional trait beta diversity

To further investigate the processes influencing community structure, the relationships between taxonomic, phylogenetic, and functional trait beta diversity were

explored. Quantifying changes in phylogenetic patterns between sites extends research on phylogenetic community assembly, and can provide insights into community assembly with respect to environmental gradients and geographic barriers (Graham and Fine 2008). Taxonomic beta diversity was calculated using the Bray-Curtis dissimilarity metric. Phylogenetic beta diversity was calculated using the comdistnt and comdist functions in Picante (Kembel et al. 2010) in R version 3.0.2 (R Core Team 2013). Comdistnt is the among-community equivalent of MNND and detects subtle turnover in composition from site to site that may not be detected with pairwise metrics. Its output is the average nearest neighbor phylogenetic distance between sites. It is calculated as:

$$D_{nn} = \frac{\sum_{i}^{nk_1} \min\ d_{ik_2} + \sum_{j}^{nk_2} \min\ d_{jk_1}}{nk_1 + nk_2}$$

where min d_{ik_2} is in the minimum phylogenetic distance between species *i* in community k_1 and all species in community k_2, min d_{jk_1} is in the minimum phylogenetic distance between species *j* in community k_2 and all species in community k_1, and *n* is the number of species in the respective communities (Webb et al. 2008a, Swenson 2011). Comdist is the among-community equivalent of MPD: for each taxon in a site it finds the average distance to all taxa in the other site, and calculates the mean. This pairwise metric is good for detecting major compositional turnover from community to community. It is calculated as:

$$D_{pw} = \frac{\sum_{i}^{nk_1} \sum_{j}^{nk_2}\ d_{ij}}{nk_1 + nk_2}$$

where d_{ij} is in the phylogenetic distance between species i in community k_1 to all species in community k_2, nk_1 is the number of species in community k_1,and nk_2 is the number of species in community k_2 (Webb et al. 2008a, Swenson 2011). Functional trait beta diversity was also calculated using the comsidnt and comdist functions in Picante (Kembel et al. 2010).

As taxonomic, phylogenetic, and functional trait beta diversity between all pairs of sites are likely to correlate with each other on average, an alternative approach is to use null models to investigate if the different facets of beta diversity are greater than, or less than, expected by chance (Swenson et al. 2012b). A null distribution for taxonomic beta diversity was generated by randomizing species from the regional pool within sites while keeping site species richness the same (i.e. by shuffling the presences and absences within the matrix while maintaining row totals), for phylogenetic beta diversity by shuffling species on the tips of the phylogeny, and for functional trait beta diversity by shuffling the species names in the trait distance matrix (see Appendix 2B for the R code used to implement these). As before, this was repeated 999 times to calculate standard effect sizes (SES) (Gotelli and Graves 1996, Gotelli and Rohde 2002). Again, positive SES values and high quantiles (> 0.95) indicate that beta diversity is higher than expected by chance whereas negative SES values and low quantiles (< 0.05) indicate that beta diversity is lower than expected by chance.

To test whether geographical distance among sites influences community assembly, I calculated the relationships between geographical distance and taxonomic, phylogenetic, and functional trait beta diversity. The geographical distance between sites

was calculated using the Haversine formula, with the distHaversine function in the geosphere package (Hijmans 2014) in R version 3.0.2 (R Core Team 2013). The Haversine distance, also known as the 'Haversine' great circle distance, measures the shortest distance between two points on a sphere from their latitudes and longitudes. Multiple regressions on distance matrices (MRM) (Legendre et al. 1994, Lichstein 2007) were used to determine if the great circle distance between pairs of sites influenced taxonomic, phylogenetic, and functional trait beta diversity. MRM involves regression of a response matrix on an explanatory matrix, where each matrix contains distances or similarities between all pair-wise combinations of objects. Statistical significance is determined by permuting the response matrix while holding the explanatory matrix constant and calculating R^2 for each permutation to generate a null distribution. MRM is advantageous over Mantel tests as it allows the exploration of linear and nonlinear models. Regressions were calculated using the MRM function in the ecodist package (Goslee and Urban 2007) in R version 3.0.2 (R Core Team 2013).

Results

Phylogenetic trees

Estimates of phylogenetic relatedness among the regional species pool using either Bayesian (Fig. 2.3) or Maximum Likelihood (Fig. 2.4) inferences were very similar and agreed well with other published phylogenies (sponges — Borchiellini et al. 2001, Manuel 2006; cnidarians — Collins 2009; bryozoans — Fuchs et al. 2009, Tsyganov-Bodounov et al. 2009; arthropods — Regier et al. 2010; ascidians — Stach and

Turbeville 2002; metazoans — Bourlat 2008, Dunn et al. 2008, Edgecombe 2011, Nosenko 2013, Dunn et al. 2014).

Community phylogenetic structuring

Some subtidal epibenthic invertebrate communities in the Gulf of Maine were phylogenetically overdispersed, i.e. they exhibited a greater phylogenetic diversity among co-occurring species than expected by chance (Table 2.3; Fig. 2.5). Phylogenetic MNND was significant for Thrumcap, Bunker Point, and Moose Cove, suggesting that communities at these sites show fine-scale overdispersal (Table 2.3; Fig. 2.5). Phylogenetic MPD was significant for Thrumcap and Bunker Point, suggesting that communities at these sites also show evidence of tree-wide overdispersal (Table 2.3; Fig. 2.5). In general, for seven out of eight sites for both MNND and MPD, standard effect sizes were positive and quantiles high suggesting a trend of overdispersion. Traditionally, under the assumption that phylogeny is a proxy for ecological similarity, phylogenetic overdispersal would be suggestive of community assembly via competitive exclusion (Webb 2000).

Phylogenetic signal

Phylogenetic signal was found in five traits, coloniality, reproduction, food capture, growth form, and defense (Table 2.4; Fig. 2.6), suggesting that phylogeny for these traits can be used as a proxy for ecological similarity. The maximum likelihood estimate for lambda for these traits was significantly different from lambda = 0, i.e. when

the tree was transformed to star phylogeny with no phylogenetic signal (Table 2.4). There was no phylogenetic signal in body size (Table 2.4; Fig. 2.6).

Functional trait diversity

Functional trait diversity was greater than expected by chance at one site and lower than expected at another, suggesting that local communities at these two sites were structured by deterministic forces (Table 2.5). At Whale Cove, trait MNND was less than expected (Table 2.5) suggesting that nearest neighbors are more functionally similar than expected, indicative of environmental filtering. Trait MPD was greater than expected by chance at Shag Rocks (Table 2.5), suggesting that communities at this site are assembled via competitive exclusion. Again, the general trend for MPD was for positive standard effect sizes and high quantiles suggesting overdispersion. For MNND this pattern was equivocal: four sites had negative standard effect sizes and low quantiles and four sites had positive standard effect sizes and high quantiles. Phylogenetic MNND was positively correlated with functional trait MNND (Pearson product-moment correlation coefficient, $r = 0.87$, $p = 0.005$). Thus, at the nearest neighbor level, sites that are more phylogenetically diverse also show greater diversity of functional traits, consistent with community assembly via competitive exclusion at the nearest neighbor level, under the assumption of phylogenetic signal. There was no relationship between phylogenetic MPD and functional trait MPD (Pearson product-moment correlation coefficient, $r = 0.01$, $p = 0.973$). The lack of relationship between phylogenetic and functional trait pairwise metrics may be because their respective distance matrices are

generated using different techniques, magnifying the incongruence between them when using pairwise instead of nearest neighbor metrics (Swenson 2011).

Taxonomic, phylogenetic, and functional trait beta diversity

Taxonomic beta diversity was, on average, lower than expected by chance (Fig. 2.7A). Wood Island was similar taxonomically to all other sites, with the exception of Cutler. Pubnico was similar taxonomically to Wood Island, Moose Cove, and Cutler, Whale Cove was similar to Wood Island and Bunker Point, and Shag Rocks was similar to Wood Island and Thrumcap. For nearest neighbor phylogenetic and functional trait beta diversity, there was no clear directional trend in turnover suggesting that beta diversity was random for both phylogeny and functional traits (Fig. 2.7B,C). Only four of 28 pairs of sites were more similar phylogenetically than expected by chance at the nearest neighbor level. These were Shag Rocks and Moose Cove, and Shag Rocks, Wood Island and Whale Cove with Cutler. Only one site pair was more similar functionally at the nearest neighbor level and this was Wood Island and Bunker Point. Four pairs of sites were functionally different at the nearest neighbor level and these were Shag Rocks with Wood Island, Bunker Point and Whale Cove, and Pubnico and Wood Island. The pairwise metrics showed a directional trend and both phylogenetic and functional trait pairwise beta diversity were higher than expected by chance (Fig. 2.7D,E). This was particularly pronounced for phylogenetic beta diversity where almost 50% of site pairs (13 out of 28) were more phylogenetically different than expected (Fig. 2.7D). Both Thrumcap and Bunker Point were phylogenetically different from all other

sites at the basal clade level. Pairwise functional trait beta diversity was different between the following pairs of sites: Shag Rocks and Bunker Point, Whale Cove and Punico Bunker Point and Moose Cove; and Pubnico and Cutler. Only two pairs of sites that were phylogenetically different at the pairwise level were also different at the functional trait pairwise level. Thus, there is a mismatch between the pairs of sites that exhibit greater phylogenetic beta diversity than expected by chance at the clade level and those that exhibit greater functional trait diversity than expected by chance. There was no relationship between geographic distance and any beta diversity metric (Fig. 2.8).

Discussion

Understanding how organisms assemble from a regional species pool to form a local community is an important question in ecology and helps us to identify factors that structure ecological communities at a variety of spatial scales. Here, community phylogenetic and functional trait analyses revealed some evidence for community assembly by competitive exclusion at a local scale and for environmental filtering at a regional one.

Competitive exclusion at local scales

At some local sites, communities were phylogenetically overdispersed, suggestive of competitive exclusion under the assumption of phylogenetic signal, i.e. distantly related species are less ecologically similar than closely related species and can thus co-occur (Webb 2000). Indeed, phylogenetic signal was found in five out of six traits (coloniality, reproduction, food capture, growth form, and defense) supporting the

hypothesis of community assembly by competitive exclusion. This agrees with the findings of others, that interspecific competition for space structures subtidal epibenthic invertebrate communities in the Gulf of Maine (Sebens 1982, 1985, 1986). Here, competition is usually mediated by direct overgrowth (Sebens 1982, 1985, 1986) with species organized in a competitive hierarchy (Sebens 1986). By occupying space, organisms gain access to food resources (Sebens 1982, 1985, 1986), which they can then compete for (Buss 1979, Buss and Jackson 1981). With more food, organisms can grow faster and occupy more space (Svensson and Marshall 2015), becoming more likely to come into contact and interact via competition. Competition for space also structures similar communities in the Caribbean (Buss and Jackson 1979, Jackson 1979, Suchanek and Green 1981), Puget Sound (Bruno and Witman 1996), Great Barrier Reef, Australia (Connell et al. 2004), and the Arctic and Subarctic (Barnes and Kuklinski 2004).

Competition has a long history in the ecological literature (Gause 1934, Hardin 1960, Connell 1961, MacArthur 1972, Jackson 1977, Buss and Jackson 1981) and, although the extent to which competitive exclusion structures ecological communities has been questioned (Weins 1977, Connor and Simberloff 1979, Schoener 1982), generally there is a consensus that it is an important biotic interaction (Roughgarden 1983, Schoener 1983, Connell 1983, Gurevitch et al. 1992). Indeed, competition has an even longer history in Geologic time and evidence of overgrowth competition for space can be found in the fossil record (Liddell and Brett 1982, Taylor 1984, Taylor and Wison 2003). The evolution of important traits can also be observed, e.g. there has been an increase in skeletalization of bryozoans from the Paleozoic into the Mesozoic and Cenozoic, which

may be a response to increasing levels of predation (Taylor and Wilson 2003). By resisting predation organisms can better hold onto space.

Here we add a phylogenetic perspective to competition, and suggest that local communities comprise species that are distantly related to each other phylogenetically, and that species that are closely related phylogenetically, i.e. that are ecologically similar, competitively exclude one another. We also suggest functional traits that might mediate this interaction, and provide some evidence for species coexistence whereby species that are distantly related functionally co-occur in local communities. Patterns of functional trait dissimilarity were not as pronounced as for phylogenetic dissimilarity, however, which suggests two things: 1) there is environmental filtering to some degree for the traits selected, and/or 2) that competition might be mediated by unmeasured traits that show phylogenetic signal (see also Swenson 2012b). Such traits could be position in a competitive hierarchy, (Sebens 1986), particle retention size (Gili and Coma 1998), filtration rates (Gili and Coma 1998) and degree of skeletalization. These traits were not included in the analyses as this data does not exist for all species in the regional species pool and collecting it would have required considerable resources beyond the scope of this study. While data exists for common well studied species such as *Mytilus edulis* (Riisgård and Randløv 1981, Riisgård 1988) similar information is lacking for rare species.

Interestingly, along the coastline of the Gulf of Maine, the sites that show evidence for community assembly by completive exclusion, Moose cove, Bunker Point, and Thrumcap, are in the middle of the coast (Fig 2.9A). The location of these sites

coincides with the Eastern Maine Coastal Current (Fig 2.9B), which is associated with a plume of nutrient-rich cold water (Townsend et al. 1987, Pettigrew et al. 1998, Townsend 2006). Although the spring bloom is earlier in the year in the western Gulf of Maine, the fall bloom begins earlier in the east (Song et al. 2010) and, throughout most of the year, chlorophyll concentration (Song et al. 2010) and various phytoplankton functional groups such as diatoms, cryptophytes, and haptophytes are higher in the eastern Gulf of Maine (Pan et al. 2011), coinciding with the circulation pattern of this current. Elevated flow and particle concentration can increase feeding rates (Leichter and Witman 1997, Witman and Dayton 2001), which can, in turn, lead to faster growth rates and greater competition for space in sessile invertebrates (Buss and Jackson 1981, Sebens 1984). Indeed, when food levels were manipulated in benthic invertebrate communities off southeast Australia, it was found that increased levels of food lead to greater species richness, higher growth of organisms, and less available space (Svensson and Marshall 2015). Regressions of SES for phylogenetic and functional trait MNND and MPD for local sites against remotely sensed SeaWiFS chlorophyll a concentrations (Northeast Ocean Data Portal, 2015) (see Appendix 2C) at these sites (Fig 2.9C) showed a clear directional trend whereby the greater the chlorophyll a concentration, the higher the SES (Fig 2.9D, Appendix 2C), where high SES is indicative of community assembly by competitive exclusion. Regressions were not significant when alpha was Bonferroni corrected but nonetheless this pattern is interesting and should be investigated further.

Our finding of phylogenetic overdispersal and community assembly by competitive exclusion contrasts to the general trend found in other studies that used

community phylogenetics to investigate mechanisms of community assembly. Based on a recent review, 59% of similar investigations found the opposite pattern, i.e. phylogenetic clustering, with only 33% finding overdispersal (Vamosi et al.2009). The majority of these studies, 62%, however, were conducted on land plants (Vamosi et al. 2009). As already discussed marine and terrestrial systems differ in terms of taxonomic and phyletic diversity: in this study we had high phyletic diversity and found evidence for phylogenetic overdispersal. Interestingly, in two angiosperm systems, when taxonomic breadth was widened, assemblages became increasingly clustered (Cavender-Bares et al. 2006, Swenson et al. 2006). For example, when examining a single phylogenetic lineage in state parks in Florida, such as *Quercus*, *Pinus*, or *Ilex*, communities tended to be overdispersed but, when communities were defined more broadly to include all seed plants, communities became clustered (Cavender-Bares et al. 2006). These contrasting findings between marine and terrestrial realms are interesting and should be investigated further. It is possible, however, that if a similar study to this one was conducted in a different oceanographic region, then a different pattern of phylogenetic dispersal might be found: strong abiotic differences between sites could, for example, cause environmental filtering. To date, the only other community phylogenetic analysis conducted in the marine environment investigated if the phylogenetic relatedness of amphipods could predict species coexistence (Best et al. 2013), which was not found to be the case. Finally, this study represents only a single snapshot of local Gulf of Maine communities in time and space. Repeating the study in a different season or year, or with different local sites, might yield different results. Again, different sites, seasons, or years

could have differing abiotic regimes, which could result in stronger or weaker signals of environmental filtering and/or competition. In addition, regional species pools are themselves dynamic in space and time and models of community assembly have started to include evolutionary changes (Pigot and Etienne 2014, Mittelbach and Schemske 2015).

Environmental filtering at the regional scale

At the regional scale, there was evidence for non-random community assembly processes suggestive of environmental filtering — both pairwise phylogenetic and functional trait beta diversity were greater than expected by chance between some site pairs, indicating that different environmental conditions select for different species in terms of phylogeny and functional traits. In coral reef systems, different abiotic conditions such as depth, visibility, temperature, current, salinity, and sedimentation are hypothesized to filter sponges in Sulawesi, Indonesia (Becking et al. 2006, Cleary et al. 2007), and exposure and micro-substrate type are hypothesized to influence coral assemblages in the same region (Becking et al. 2006). In similar systems in Eastern Australia, differences in some of these environmental conditions — temperature, visibility, and velocity — have been shown to select for coral species on the basis of ecological traits (Sommer et al. 2014). At higher latitudes, in the northwest Mediterranean, beta-diversity of subtidal hard substrate algae and invertebrate assemblages are influenced by sedimentation (Balata et al. 2007). Only one of these

studies (Sommer et al. 2014), however, used traits data to identify if the environment is filtering for species with similar traits.

A major limitation to this study is the lack of environmental data to identify variables that might sort species into different sites but the obvious trend for pairwise phylogenetic and functional trait beta diversity metrics to be greater than expected by chance suggests that environmental filtering is occurring. Between-site abiotic factors that influence rocky subtidal community structure in the Gulf of Maine include light (Miller and Etter 2008), temperature (Witman and Dayton 2001), nutrient availability (Leichter and Witman 1997), sedimentation (Witman and Dayton 2001), flow (Leichter and Witman 1997, Genovese and Witman 1999), internal waves (Witman et al. 2004), and disturbance (Witman and Cooper 1983, Witman 1985, Sebens 1985, 1986). Indeed, within the Gulf of Maine some environmental factors vary in space and time such as sea surface temperature (Townsend et al. 2001, 2014, Luerssen et al. 2005), flow (Townsend et al. 1987, Pettigrew et al. 1998, Townsend 2006), chlorophyll a concentration (Thomas et al. 2003, Song et al. 2010, Pan et al. 2011) and nutrients (Townsend et al. 2014). In terrestrial studies that employed similar methodology, environmental distances, such as annual precipitation (Swenson 2011, Qian et al. 2013) and mean annual temperature (Qian et al. 2013), accounted for turnover in tree assemblages. Furthermore, these environmental distances were more important than geographical distance in explaining turnover. In this study, geographical distance did not predict any beta-diversity metric.

An alternative explanation to environmental filtering at the regional scale is that species are dispersal limited within the region, which would also account for high

phylogenetic and functional trait turnover between sites. However, taxonomic beta-diversity was less than expected by chance, i.e. there is little species turnover between sites and the region is taxonomically homogenous as suggested by others (Miller and Etter 2011). Thus these contrasting directions patterns, that taxonomic beta diversity is less than expected while phylogenetic and functional trait MPD are greater than expected, are likely because all species can get to all sites but do not persist at all locations because of differing environmental conditions. This highlights the importance of adding phylogenetic and functional trait data when assessing beta diversity (Swenson et al. 2012b). Taken alone, the taxonomic similarity among sites could suggest that between-site processes do not lead to significant differences in the structure of shallow subtidal epifaunal communities in the Gulf of Maine (Miller and Etter 2011) whereas the addition of phylogenetic and functional trait data suggest otherwise.

As at the local site scale, beta-diversity patterns were more pronounced for phylogenetic than functional trait beta diversity further suggesting that unmeasured traits may be important (Swenson et al. 2012b). This may account for the mismatch between sites that show evidence for phylogenetic overdispersion and those that show functional trait overdispersion. That the pairwise metrics revealed deterministic community assembly processes while the nearest neighbor metrics did not, suggests that phylogenetic turnover is at the clade level. This is consistent with entire clades missing from, or being poorly represented at, some sites (Fig. 2.5). At Thrumcap, for example, sponges and bryozoans are represented by just one species each, and at Bunker Point, cnidarian are entirely absent. Unsurprisingly, these are the two sites that were phylogenetically

different from all other sites at the basal clade level. In tropical tree assemblages, patterns of pairwise phylogenetic and functional trait beta diversity were opposite to each other i.e. phylogenetic beta diversity was greater than expected by chance and functional trait beta diversity was less than expected by chance (Swenson et al. 2002a), suggesting trait convergence and environmental filtering of traits, and turnover of similar, but dispersal limited, species among subplots.

For the first time we show how, in the Gulf of Maine, subtidal epibenthic species assemble from a regional pool to form local communities. Communities appear to be structured by both competitive exclusion and environmental filtering, with these processes operating at different spatial scales. Indeed, biotic processes such as competitive exclusion tend to operate locally, and abiotic processes often resulting in environmental filtering tend to operate regionally (Ricklefs 1987, Webb et al. 2002). This supports a hierarchical model of community assembly whereby environmental filtering will sort ecologically similar species to sites with similar abiotic conditions and then, within a relatively homogeneous abiotic patch, biotic interactions will structure communities (Webb et al. 2002, Webb et al. 2008b, Swenson et al. 2012a). However, because competitive exclusion and environmental filtering can operate simultaneously, they can also obscure and cancel each other out (Helmus et al. 2007). In bird assemblages, these processes can be better teased out by using metrics for a single niche axis, or functional trait, whereas metrics that measured multiple niche axes, such as MNND and MPD, obscured the nuances of community assembly (Trisos et al. 2014). Certainly, when quantifying patterns of functional trait MNND and MPD, there were

signals of both assembly processes and, exploring different metrics of single and multiple niche axes might be reveal a more comprehensive picture of community assembly.

Generally, our findings agree with those of others, that at high latitudes the regional species pool is well represented in local communities (taxonomic beta diversity was less than expected by chance) (Witman et al. 2004, Freestone and Inouye 2015) and thus local communities are likely to be influenced by processes operating at larger scales (Witman et al. 2004). Interestingly, it has been suggested that regional-scale processes are more important in marine than terrestrial communities possibly because of differences in propagule dispersal ability due to the differences in the media in which propagules are suspended (Cornell and Harrison 2013).

Caveats

The results of any community phylogenetic and functional trait analyses are dependent upon methodological choices. First, the correctness of the phylogeny depends upon gene selection, estimation of evolutionary models, and calibration of branch lengths (Cadotte et al. 2013). Tree topology influences the detection of patterns of community structure, with simulations showing that balanced trees make it easier to detect clustering and imbalanced trees overdispersal (Vellend et al. 2011), where balanced trees have taxa that are evenly distributed among the clades, and unbalanced trees have more taxa in some clades than in others of a similar rank. Here the regional phylogenetic tree for subtidal epibenthic invertebrates in the Gulf of Maine appears unbalanced, which may explain the observation of phylogenetically overdispersed communities. The choice of

metric, as discussed in the methods section, can also influence patterns observed (Hardy 2008, Vellend et al. 2011). Power to detect community structure is affected by the number of species in a local community relative to the number in the regional species pool (Kraft et al. 2007). Power is greatest when local communities comprise around 30 to 60% of the regional pool. The number of species at a site in this study as a percentage of the regional species pool ranged from 33 to 61%.

While uncertainty exists when measuring phylogenetic diversity, uncertainty is greater when measuring functional diversity, with the degree of similarity or difference among species strongly dependent on the choice of traits (Vellend et al. 2011). As well as being discrete or continuous (Chapin et al. 1996), functional traits can also be soft or hard (Cornelissen et al. 2003). Soft traits are easier to quantify and are often used as a proxy for hard traits, which are directly related to function (Weiher et al. 1999, Cornelissen et al. 2003). For example, in tree assemblages, an easy to measure soft trait would be seed mass, which is used as a proxy for seed persistence, which would be more resource intensive to measure (Cornelissen et al. 2003). Soft traits, however, may not be good analogues of hard ones. In terrestrial plant systems, key traits have been identified that are good indicators of ecological strategy and can be measured relatively easily (Westoby et al. 2002, Swenson et al. 2012a). Unfortunately this is not the case in the marine realm, and some of the traits used in this study are soft traits. Food capture, for example, was used to represent food acquisition, but a better choice may have been filtration rates or particle retention size. In this study, the continuous trait body size was also forced into discrete categories and is thus likely to suffer from discretization error.

Furthermore, body size was not measured for every organism and instead, was placed in a category based on information for the species as a whole. Aside from the problem that organisms within a species could fit into more than one category due to interspecific variation, trait means may also differ significantly from place to place as a result of physiological responses to environmental conditions (Swenson et al. 2011). Species will also differ in size based on ontogeny.

The choice of traits, and their measurements, reflects the difficultly in obtaining measurements in a marine setting. It is hard to imagine, for example, how in situ measurements of carbon uptake could be taken for every organism in the study system. Without experiments, it is also difficult to know which traits are more important at resolving important processes, such as competition, and which traits are influenced by environmental factors. Essentially, the traits selected may not capture the mechanisms of community assembly. Thus, the functional trait analysis here suffers from the same problems as those in better-studied systems, such as overlooking important traits and including uninformative ones (Cadotte et al. 2013). Petchley and Gaston (2006) caution that there is no perfect measure of functional diversity, nor is there likely to be, and that functional classifications should be treated at best as hypotheses that need to be tested.

Conclusions

Community assembly in the Gulf of Maine appears to follow a hierarchical model: firstly, under environmental filtering, species from the regional pool are sorted into patches that are abiotically homogeneous and secondly, within these patches, at a

local scale, communities assemble by competitive exclusion. Understanding how communities assemble at a variety of spatial scales is important in understanding the organizing forces that structure communities and how these might respond to anthropogenic stressors that differ in the geographical extent of their effects. The transfer of techniques developed in terrestrial systems to the marine environment also enables cross-ecosystem comparisons which is necessary for identifying common ecological processes and further predicting the response of communities to environmental change (Webb 2012). The potential thus exists to apply phylogenetic and functional traits analyses to investigate community assembly along broad environmental gradients (Swenson et al. 2012a) and in different study systems. The next step is to experimentally evaluate the hypotheses presented by such analyses.

Supplementary information

Appendix 2A. Summary of trait data.

Appendix 2B. R code for beta diversity null models.

References

Balata, D., L. Piazzi, and L. Benedetti-Cecchi. 2007. Sediment disturbance and loss of beta diversity on subtidal rocky reefs. Ecology 88:2455–2461.

Barnes, D. K., and P. Kuklinski. 2004. Scale-dependent variation in competitive ability among encrusting Arctic species. Marine Ecology Progress Series 275:21–32.

Becking, L. E., D. F. Cleary, N. J. Voogd, W. Renema, M. Beer, R. W. Soest, and B. W. Hoeksema. 2006. Beta diversity of tropical marine benthic assemblages in the Spermonde Archipelago, Indonesia. Marine Ecology 27:76–88.

Best, R. J., N. C. Caulk, and J. J. Stachowicz. 2013. Trait vs. phylogenetic diversity as predictors of competition and community composition in herbivorous marine amphipods. Ecology Letters 16:72–80.

Blomberg, S. P., and T. Garland. 2002. Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. Journal of Evolutionary Biology 15:899–910.

Borchiellini, C., M. Manuel, E. Alivon, N. Boury-Esnault, J. Vacelet, and Y. Le Parco. 2001. Sponge paraphyly and the origin of Metazoa. Journal of Evolutionary Biology 14:171–179.

Bourlat, S. J., C. Nielsen, A. D. Economou, and M. J. Telford. 2008. Testing the new animal phylogeny: a phylum level molecular analysis of the animal kingdom. Molecular Phylogenetics and Evolution 49:23–31.

Bremner, J. 2008. Species traits and ecological functioning in marine conservation and management. Journal of Experimental Marine Biology and Ecology 366:37–47.

Bremner, J., O. A. L. Paramor, and C. L. J Frid. 2006. Developing a methodology for incorporating ecological structure and functioning into designation of Special Areas of Conservation SAC in the 0–12 nautical mile zone. A report to English Nature from University of Liverpool, Liverpool, UK.

Bremner, J., S. I. Rogers, and C. L. J. Frid. 2003. Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. Marine Ecology Progress Series 254:11–25.

Bruno, J. F., and J. D. Witman. 1996. Defense mechanisms of scleractinian cup corals against overgrowth by colonial invertebrates. Journal of Experimental Marine Biology and Ecology 207:229–241.

Brusca, R. C., and G. J. Brusca. 2003. Invertebrates. Sinauer Associates, Sunderland, Massachusetts, USA.

Buss, L. W. 1979. Bryozoan overgrowth interactions — the interdependence of competition for space and food. Nature 281:475–477.

Buss, L. W., and J. B. Jackson. 1981. Planktonic food availability and suspension-feeder abundance: evidence of in situ depletion. Journal of Experimental Marine Biology and Ecology 49:151–161.

Buss, L. W., and J. B. C Jackson. 1979. Competitive networks: nontransitive competitive relationships in cryptic coral reef environments. American Naturalist 113:223–234.

Cadotte, M., C. H. Albert, and S. C. Walker. 2013. The ecology of differences: assessing community assembly with trait and evolutionary distances. Ecology Letters 16:1234–1244.

Cavender-Bares, J., D. D. Ackerly, D. A. Baum, and F. A. Bazzaz. 2004. Phylogenetic overdispersion in Floridian oak communities. American Naturalist 163:823–843.

Cavender-Bares, J., A. Keen, and B. Miles. 2006. Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. Ecology 87:S109–S122.

Cavender-Bares, J., K. H. Kozak, P. V. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. Ecology Letters 12:693–715.

Chapin, F. S., M. S. Bret-Harte, S. E. Hobbie, and H. Zhong. 1996. Plant functional types as predictors of transient responses of arctic vegetation to global change. Journal of Vegetation Science 7:347–358.

Chase, J. M., and M. A. Leibold. 2003. Ecological niches: linking classical and contemporary approaches. University of Chicago Press, Chicago, Illinois, USA.

Cleary, D. F., and N. J. De Voogd. 2007. Environmental associations of sponges in the Spermonde Archipelago, Indonesia. Journal of the Marine Biological Association of the United Kingdom 87:1669–1676.

Collins, A. G. 2009. Recent insights into cnidarian phylogeny. Smithsonian Contributions to Marine Sciences 38:139–149.

Connell, J. H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology 42:710–723.

Connell, J. H. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. American Naturalist 122:661–696.

Connell, J. H., T. P. Hughes, C. C. Wallace, J. E. Tanner, K. E. Harms, and A. M. Kerr. 2004. A long-term study of competition and diversity of corals. Ecological Monographs 74:179–210.

Connor, E. F., and D. Simberloff. 1979. The assembly of species communities: chance or competition? Ecology 60:1132–1140.

Cornelissen J. H. C., S. Lavorel, E. Garnier, S. Díaz, N. Buchmann, D. E. Gurvich, P. B. Reich, H. ter Steege, H. D. Morgan, M. G. A. van der Heijden, J. G. Pausas and H. Poorter. 2003. A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. Australian Journal of Botany 51:335–380.

Cornell, H. V., and S. P. Harrison. 2013. Regional effects as important determinants of local diversity in both marine and terrestrial systems. Oikos 122:288–297.

Dereeper, A., S. Audic, J.M. Claverie, and G. Blanc. 2010. BLAST-EXPLORER helps you building datasets for phylogenetic analysis. BMC Evolutionary Biology 10:8.

Dereeper, A., V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J. F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J. M. Claverie, and O. Gascuel. 2008. Phylogeny.fr: robust phylogenetic analysis for the non–specialist. Nucleic Acids Research 36:W465–W469.

Díaz, S., and M. Cabido. 2001. Vive la difference: plant functional diversity matters to ecosystem processes. Trends in Ecology and Evolution 16:646–655.

Drummond, A.J., M.A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology And Evolution 29:1969–1973.

Dunn, C. W., G. Giribet, G. D. Edgecombe, and A. Hejnol. 2014. Animal Phylogeny and Its Evolutionary Implications. Annual Review of Ecology, Evolution, and Systematics 45:371–395.

Dunn, C. W., A. Hejnol, D. Q. Matus, K. Pang, W. E. Browne, S. A. Smith, E. Seaver, G. W. Rouse, M. Obst, G. D. Edgecombe, M. V. Sørensen, S. H. D. Haddock, A. Schmidt–Rhaesa, A. Okusu, R. Møbjerg Kristensen, W. C. Wheeler, M. Q. Martindale, and G. Giribet. 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. Nature 452:745–749.

Dyrynda, P. E. J. 1986. Defensive strategies of modular organisms. Philosophical Transactions of the Royal Society of London B: Biological Sciences 313:227–243.

Edgar, R. C. 2004, MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–97.

Edgecombe, G. D., G. Giribet, C.W. Dunn, A. Hejnol, R. M. Kristensen, R. C. Neves, G. W. Rouse, K. Worsaae, and M. V. Sørensen. 2011. Higher-level metazoan relationships: recent progress and remaining questions. Organisms Diversity and Evolution 11:151–172.

Freestone, A. L., and B. D. Inouye. 2015. Nonrandom community assembly and high temporal turnover promote regional coexistence in tropics but not temperate zone. Ecology 96:264–273.

Frid, C. L. J., O. A. L. Paramor, S. Brockington, and J. Bremner. 2008. Incorporating ecological functioning into the designation and management of marine protected areas. Hydrobiologia 606:69–79.

Fuchs, J., M. Obst, and P. Sundberg. 2009. The first comprehensive molecular phylogeny of Bryozoa (Ectoprocta) based on combined analyses of nuclear and mitochondrial genes. Molecular Phylogenetics and Evolution 52:225–233.

Gause, G. F. 1934. The struggle for existence Williams and Wilkins. Baltimore, Maryland, USA.

Gili, J. M., and R. Coma. 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology and Evolution 13:316–321.

Goslee, S.C. and D. L. Urban. 2007. The ecodist package for dissimilarity-based analysis of ecological data. Journal of Statistical Software 22:1–19.

Gotelli, N. J., and G. R Graves. 1996. Null models in ecology. Smithsonian Institution Press, Washington, D.C., USA.

Gotelli, N. J., and K. Rohde. 2002. Co-occurrence of ectoparasites of marine fishes: a null model analysis. Ecology Letters 5:86–94.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27:857–871.

Graham, C. H., and P. V. Fine. 2008. Phylogenetic beta diversity: linking ecological and evolutionary processes across space in time. Ecology Letters 11:1265–1277.

Graham, C. H., J. L. Parra, B. A. Tinoco, F. G. Stiles, and J. A. McGuire. 2012. Untangling the influence of ecological and evolutionary factors on trait variation across hummingbird assemblages. Ecology 93:S99–S111.

Gurevitch, J., L. L. Morrow, A. Wallace, and J. S. Walsh. 1992. A meta-analysis of competition in field experiments. American Naturalist 140:539–572.

Hardin, G. 1960. The competitive exclusion principle. Science 131:1292–1297.

Hardy, O. J. 2008. Testing the spatial phylogenetic structure of local communities: statistical performances of different null models and test statistics on a locally neutral community. Journal of Ecology 96:914–926.

Hardy, O.J. and B. Senterre. 2007. Characterizing the phylogenetic structure of communities by an additive partitioning of phylogenetic diversity. Journal of Ecology 95:493–506.

Harmon L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129–131.

Helmus, M. R., K. Savage, M. W. Diebel, J. T. Maxted, and A. R. Ives. 2007. Separating the determinants of phylogenetic community structure. Ecology Letters 10:917–925.

Hijmans, R. J. 2014 . geosphere: Spherical Trigonometry. R package version 1.3–11. http://CRAN.R–project.org/package=geosphere

Hillis, D.M., and M.T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Quarterly Review of Biology 66:411–453.

Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, New Jersey, USA.

Jackson, J. B. C. 1977. Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. American Naturalist 111:743–767.

Jackson, J. B. C. 1979. Overgrowth competition between encrusting cheilostome ectoprocts in a Jamaican cryptic reef environment. The Journal of Animal Ecology 48:805–823.

Jackson, J. B. C. 1986. Modes of dispersal of clonal benthic invertebrates: consequences for species' distributions and genetic structure of local populations. Bulletin of Marine Science 39:588–606.

Kembel, S. W. 2009. Disentangling niche and neutral influences on community assembly: assessing the performance of community phylogenetic structure tests. Ecology Letters 12:949–960.

Kembel, S.W., P.D. Cowan, M.R. Helmus, W.K. Cornwell, H. Morlon, D.D. Ackerly, S.P. Blomberg, and C.O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463–1464.

Kembel, S.W. and S. P. Hubbell. 2006. The phylogenetic structure of a neotropical forest tree community. Ecology 87:S86–S99.

Kraft, N.J.B., W. K. Cornwell, C.O. Webb, and D. D. Ackerly. 2007. Trait evolution, community assembly, and the phylogenetic structure of ecological communities. American Naturalist 170:271–283.

Laliberté, E., P. Legendre, and B. Shipley. 2014. FD: measuring functional diversity from multiple traits, and other tools for functional ecology. R package version 1.0-11. http://CRAN. R-project.org/package=FD

Legendre, P., F. J. Lapointe, and P. Casgrain. 1994. Modeling brain evolution from behavior: a permutational regression approach. Evolution 48:1487–1499.

Leichter, J. J., and J. D. Witman. 1997. Water flow over subtidal rock walls: relation to distributions and growth rates of sessile suspension feeders in the Gulf of Maine Water flow and growth rates. Journal of Experimental Marine Biology and Ecology 209:293–307.

Lichstein, J. W. 2007. Multiple regression on distance matrices: a multivariate spatial analysis tool. Plant Ecology 188:117–131.

Liddell, W. D., and C. E. Brett. 1982. Skeletal overgrowths among epizoans from the Silurian Wenlockian Waldron Shale. Paleobiology 8:67–78.

Losos, J. B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecology Letters 11:995–1003.

Luerssen, R. M., A. C. Thomas, and J. Hurst. 2005. Relationships between satellite-measured thermal features and *Alexandrium*-imposed toxicity in the Gulf of Maine. Deep Sea Research Part II: Topical Studies in Oceanography 52:2656–2673.

MacArthur, R. H. 1972. Geographical ecology: patterns in the distribution of species. Princeton University Press, Princeton, New Jersey, USA.

Maddison, D. R., and W. P. Maddison. 2005. MacClade 4: Analysis of phylogeny and character evolution. Version 4.08a.

Maddison, W. P., and D. R. Maddison. 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75.

Manuel, M. 2006 . Phylogeny and evolution of calcareous sponges. Canadian Journal of Zoology 84:225–241.

Miller, R. J., and R. J. Etter. 2011. Rock walls: small–scale diversity hotspots in the subtidal Gulf of Maine. Marine Ecology Progress Series 425:153–165.

Mittelbach, G. G., and D. W. Schemske. 2015. Ecological and evolutionary perspectives on community assembly. Trends in Ecology and Evolution 30:241–247.

Northeast Ocean Data Portal. http://www.northeastoceandata.org/files/metadata/Biology/ChlorophyllAsummer.pdf (Accessed September 11, 2015).

Nosenko, T., F. Schreiber, M. Adamska, M. Adamski, M. Eitel, J. Hammel, M Maldonado, W. E. G. Müller, M. Nickel, B. Schierwater, J. Vacelet, M. Wiens, and G. Wörheide. 2013. Deep metazoan phylogeny: when different genes tell different stories. Molecular Phylogenetics and Evolution 67:223–233.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401:877–884.

Pan, X., A. Mannino, M. E. Russ, S. B. Hooker, and L. W. Harding. 2010. Remote sensing of phytoplankton pigment distribution in the United States northeast coast. Remote Sensing of Environment 114:2403–2416.

Parsons, T. R., M. Takahashi, and B. Hargrave. 1979. Biological Oceanographic Processes. Elsevier, Amsterdam, Netherlands.

Petchey, O. L., and K. J. Gaston. 2006. Functional diversity: back to basics and looking forward. Ecology Letters 9:741–758.

Pettigrew, N. R., D. W. Townsend, H. Xue, J. P. Wallinga, P. J. Brickley, and R. D. Hetland. 1998. Observations of the Eastern Maine Coastal Current and its offshore extensions in 1994. Journal of Geophysical Research: Oceans 103:30623–30639.

Pigot, A.L. and R. S. Etienne. 2014. A new dynamic null model for phylogenetic community structure. Ecology Letters 18:153–163

Podani, J., and D Schmera. 2006. On dendrogram-based measures of functional diversity. Oikos 115:179–185.

Qian, H., N. G. Swenson, and J. Zhang. 2013 . Phylogenetic beta diversity of angiosperms in North America. Global Ecology and Biogeography 22:1152–1161.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rambaut, A., M. A. Suchard, D. Xie, and A. J. Drummond. 2014. Tracer v1.6.

Regier, J. C., J. W Shultz, A. Zwick, A. Hussey, B. Ball, R. Wetzer, J. W. Martin, and C. W. Cunningham. 2010. Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. Nature 463:1079–1083.

Ricklefs, R. E. 1987. Community diversity: relative roles of local and regional processes. Science 235:167–171.

Ricklefs, R. E., and D. Schluter. 1993. Species diversity in ecological communities: historical and geographical perspectives. University of Chicago Press, Chicago, Illinois, USA.

Riisgård, H. U. 1988. Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. Marine Ecology Progress Series 45:217–223.

Riisgård, H. U., and A Randløv. 1981. Energy budgets, growth and filtration rates in *Mytilus edulis* at different algal concentrations. Marine Biology 61:227–34.

Roughgarden, J. 1983. Competition and theory in community ecology. American Naturalist 122:583–601.

Schliep, K.P. 2011. phangorn: phylogenetic analysis in R. Bioinformatics 27:592–593.

Schoener, T. W. 1982. The controversy over interspecific competition: despite spirited criticism, competition continues to occupy a major domain in ecological thought. American Scientist 70:586–595.

Schoener, T. W. 1983. Field experiments on interspecific competition. American Naturalist 122:240–285.

Sebens, K. P. 1982. Competition for space: growth rate, reproductive output, and escape in size. American Naturalist 120:189–197.

Sebens, K. P. 1984. Water flow and coral colony size: interhabitat comparisons of the octocoral *Alcyonium siderium*. Proceedings of the National Academy of Sciences 81:5473–5477.

Sebens, K. P. 1985. The Ecology of the Rocky Subtidal Zone: The subtidal rock surfaces in New England support a diversity of encrusting species that compete for space and that recolonize patches cleared through predation. American Scientist 73:548–557.

Sebens, K. P. 1986 . Spatial relationships among encrusting marine organisms in the New England subtidal zone. Ecological Monographs 56:73–96.

Sommer, B., P. L. Harrison, M. Beger, and J. M. Pandolfi. 2014. Trait-mediated environmental filtering drives assembly at biogeographic transition zones. Ecology 95:1000–1009.

Song, H., R. Ji, C. Stock, and Z. Wang. 2010. Phenology of phytoplankton blooms in the Nova Scotian Shelf–Gulf of Maine region: remote sensing and modeling analysis. Journal of Plankton Research 32:1485–1499.

Stach, T., and J. M. Turbeville. 2002. Phylogeny of Tunicata inferred from molecular and morphological characters. Molecular Phylogenetics and Evolution 25:408–428.

Suchanek, T. H., and D. J. Green. 1981, May. Interspecific competition between *Palythoa caribaeorum* and other sessile invertebrates on St. Croix reefs, US Virgin Islands. Proceedings of the Fourth International Coral Reef Symposium, Manilla 2:679–684.

Svensson, J. R., and D. J. Marshall. 2015. Limiting resources in sessile systems: food enhances diversity and growth of suspension feeders despite available space. Ecology 96:819–827.

Swenson, N. G. 2011. Phylogenetic beta diversity metrics, trait evolution and inferring the functional beta diversity of communities. PLoS ONE 6:e21264.

Swenson, N.G. 2014. Functional and Phylogenetic Ecology in R. Springer UseR! Series, Springer, New York, New York, U.S.A.

Swenson, N. G., P. Anglada-Cordero, and J. A. Barone. 2011. Deterministic tropical tree community turnover: evidence from patterns of functional beta diversity along an elevational gradient. Proceedings of the Royal Society of London B: Biological Sciences 278:877–884.

Swenson, N. G., B. J. Enquist, J. Pither, J. Thompson, and J. K. Zimmerman. 2006. The problem and promise of scale dependency in community phylogenetics. Ecology 87: 2418–2424.

Swenson, N. G., B. J. Enquist, J. Thompson, and J. K. Zimmerman. 2007. The influence of spatial and size scale on phylogenetic relatedness in tropical forest communities. Ecology 88:1770–1780.

Swenson, N. G., D. L. Erickson, X. Mi, N. A. Bourg, J. Forero-Montaña, X. Ge, R. Howe, J. K. Lake, X. Liu, K. Ma, N. Pei, J. Thompson, M. Uriarte, A. Wolf, S. J. Wright, W. Ye, J.Zhang, J. K. Zimmerman, and W. J. Kress. 2012a. Phylogenetic and functional alpha and beta diversity in temperate and tropical tree communities. Ecology 93:S112–S125.

Swenson, N. G., J. C. Stegen, S. J. Davies, D. L. Erickson, J. Forero–Montaña, A. H. Hurlbert, W. J. Kress, J. Thompson, M. A. Uriarte, S. J. Wright and J. K. Zimmerman. 2012b. Temporal turnover in the composition of tropical tree communities: functional determinism and phylogenetic stochasticity. Ecology 93:490–499.

Taylor, P.D. 1984. Adaptations for spatial competition and utilization in Silurian encrusting bryozoans. Special Papers in Palaeontology 32:197–210.

Taylor, P. D., and M. A. Wilson. 2003. Palaeoecology and evolution of marine hard substrate communities. Earth-Science Reviews 62:1–103.

Thomas, A. C., D. W. Townsend, and R. Weatherbee. 2003. Satellite-measured phytoplankton variability in the Gulf of Maine. Continental Shelf Research 23:971–989.

Townsend, D. W., J. P. Christensen, D. K. Stevenson, J. J. Graham, and S. B. Chenoweth. 1987. The importance of a plume of tidally-mixed water to the biological oceanography of the Gulf of Maine. Journal of Marine Research 45:699–728.

Townsend, D. W., D. J. McGillicuddy, M. A. Thomas, and N. D. Rebuck. 2014. Nutrients and water masses in the Gulf of Maine–Georges Bank region: Variability and importance to blooms of the toxic dinoflagellate *Alexandrium fundyense*. Deep Sea Research Part II: Topical Studies in Oceanography 103: 238–263.

Townsend, D. W., N. R. Pettigrew, and A. C. Thomas. 2001. Offshore blooms of the red tide dinoflagellate, *Alexandrium* sp., in the Gulf of Maine. Continental Shelf Research 21:347–369.

Townsend, D. W., A. C. Thomas, L. M. Mayer, M. A. Thomas, and J. A. Quinlan. 2006. Oceanography of the northwest Atlantic continental shelf (1, W). The sea: the global coastal ocean: interdisciplinary regional studies and syntheses 14:119–168.

Trisos, C. H., O. L. Petchey, and J. A. Tobias. 2014. Unraveling the interplay of community assembly processes acting on multiple niche axes across spatial scales. The American Naturalist 184:593–608.

Tsyganov-Bodounov, A., P. J. Hayward, J. S. Porter, and D. O. Skibinski. 2009. Bayesian phylogenetics of Bryozoa. Molecular Phylogenetics and Evolution 52:904–910.

Tuomisto, H. 2010. A diversity of beta diversities: straightening up a concept gone awry. Part 1. Defining beta diversity as a function of alpha and gamma diversity. Ecography 33:2–22.

Vamosi, S. M., S. B. Heard, J. C. Vamosi, and C. O. Webb. 2009. Emerging patterns in the comparative analysis of phylogenetic community structure. Molecular Ecology 18: 572–592

Vellend, M., W. K. Cornwell, K. Magnuson-Ford, K., and A. Ø. Mooers. 2011. Measuring phylogenetic biodiversity. Pages 194–207 in A. E. Magurran, and B. J. McGill, editors. Biological diversity: frontiers in measurement and assessment. Oxford University Press, Oxford, UK.

Webb, C. O. 2000 . Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. American Naturalist 156:145–155.

Webb, C. O., D. D. Ackerly, and S. W. Kembel. 2008a. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics 24: 2098–2100.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics 33:475–505.

Webb, C. O., C. H. Cannon, and S. J. Davies. 2008b. Ecological organization, biogeography, and the phylogenetic structure of tropical forest communities. Pages 79–97 in W. P. Carson and S. S. Schnitzer, editors. Tropical Forest Community Ecology. Blackwell, Hoboken, New Jersey, USA.

Webb, C. O., J. B. Losos, and A. A. Agrawal. 2006. Integrating phylogenies into community. Ecology 87:S1–S2.

Webb, T. J. 2012. Marine and terrestrial ecology: unifying concepts, revealing
Weiher, E., and P. A. Keddy. 1995. The assembly of experimental wetland plant communities. Oikos 73:323–335.

Weiher, E., A. Werf, K. Thompson, M. Roderick, E. Garnier, and O. Eriksson. 1999. Challenging Theophrastus: a common core list of plant traits for functional ecology. Journal of Vegetation Science 10:609–620.

Weinstein, B. G., B. Tinoco, J. L. Parra, L. M. Brown, J. A. McGuire, F. G. Stiles, and C. H. Graham. 2014. Taxonomic, phylogenetic, and trait beta diversity in South American hummingbirds. American Naturalist 184:211–224.

Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant ecological strategies: some leading dimensions of variation between species. Annual Review of Ecology and Systematics 33:125–159.

Whittaker, R. H. 1960. Vegetation of the Siskiyou mountains, Oregon and California. Ecological Monographs 30:279–338.

Wiens, J. A. 1977. On Competition and Variable Environments: Populations may experience "ecological crunches" in variable climates, nullifying the assumptions of competition theory and limiting the usefulness of short-term studies of population patterns. American Scientist 65:590–597.

Witman, J. D. 1998. Natural disturbance and colonization on subtidal hard substrates in the Gulf of Maine. Pages 30–37 in E. M. Dorsey and J. Pederson, editors. Effects of fishing gear on the sea floor of New England. MIT Sea Grant Publication 98-4, Cambridge, Massachusetts, USA.

Witman, J. D., and P. K. Dayton. 2001. Rocky subtidal communities. Pages 339–366 in M. D. Bertness, S. Gaines, and M. E. Hay, editors. Marine Community Ecology. Sinauer Associates, Sunderland, Massachusetts, USA.

Witman, J. D., J. C. Ellis, and W. B. Anderson WB. 2004. The influence of physical processes, organisms, and permeability on cross-ecosystem fluxes. Pages 225–358 in G. A. Polis, M. E. Power, and G. R. Huxel, editors. Food webs at the landscape level. University of Chicago Press, Chicago, Illinois, USA.

Woodward, G., B. Ebenman, M. Emmerson, J. M. Montoya, J. M. Olesen, A. Valido, and P. H. Warren. 2005. Body size in ecological networks. Trends in Ecology and Evolution 20:402–409.

Table 2.1. Subtidal epibenthic invertebrate species and accession numbers used to generate the regional phylogeny for the Gulf of Maine. When sequences could not be obtained, congeners were used instead. * denotes sequence data generated in this study.

Sessile invertebrate taxa in the Gulf of Maine	Alternative species used in tree generation	Accession number
Porifera		
Leucosolenia botryoides	*Leucosolenia sp.*	AF100945.1
Sycon ciliatum		AJ627187.1
Halisarca dujardini		EU702418.1
Haliclona oculata		DQ927307.1
Haliclona cinerea		DQ927306.1
Halichondria panicea		KF699110*
Cliona celata		KC902383.1
Isodictya palmata		KF699109*
Iophon nigricans		KC901987.1
Hymedesmia similis	*Hymedesmia paupertas*	KC902073.1
Myxilla fimbriata		KF699108*
Plocamionida ambigua		KC902218.1
Cnidaria		
Ectopleura crocea		KF699111*
Obelia geniculata		FJ550548.1
Halecium sessile	*Halecium pusillum*	FJ550580.1
Alcyonium siderium	*Alcyonium gracillimum*	Q688007.1
Metridium senile		JF832982.1
Edwardsiella lineata		KF155691.1
Urticina crassicornis	*Urticina coriacea*	EU190877.1
Ascidiacea		
Aplidium glabrum		KF699115*
Didemnum vexillum		JF738071.1
Didemnum albidum		EU337058.1
Trididemnum tenerum	*Trididemnum paracyclops*	AB211077.1
Diplosoma listerianum		HM641906.1
Molgula manhattensis		L12426.2
Molgula citrina		HM807351.1
Halocynthia pyriformis		FM897327.1
Boltenia ovifera		FM883163.1
Boltenia echinata		KF699113*
Dendrodoa carnea	*Dendrodoa grossularia*	DQ345912.1
Botryllus schlosseri		FM244858.1
Botrylloides violaceus		AY903927.1
Annelida		
Myxicola infundibulum	*Myxicola sp.*	AY611450.1
Spirorbis sp.	*Spirorbis spirorbis*	AY527060.1
Filograna implexa		DQ317116.1
Brachiopoda		
Terebratulina septentrionalis	*Terebratulina retusa*	U08324.1
Mollusca		
Heteranomia squamula	*Anomia ephippium*	AF120535.1
Modiolus modiolus		AF124210.1
Mytilus edulis		L24489.1
Arthropoda		
Semibalanus balanoides		AY520626.1
Balanus balanus		AY520628.1
Bryozoans		
Tubulipora liliacea		FJ409622.1
Crisia eburnea		FJ196132.1
Caberea ellisii	*Caberea boryi*	AF119082.1
Parasmittina jeffreysi		KF699107*
Membranipora membranacea		JN680943.1
Schizomavella auriculata		KF699106*
Bugula turrita		AY210443.1
Dendrobeania murrayana		KF699112*

Table 2.2. Functional traits for subtidal epibenthic invertebrate species in the Gulf of Maine.

Trait	Ecological significance
LIFE HISTORY	
Coloniality	
Colonial	Acquisition of space and food (Jackson 1977)
Solitary	
Reproduction	
Asexual and sexual, vivipary	Asexual organisms can quickly occupy resources whereas sexual reproduction maintains genetic diversity (Brusca and Brusca 2003); dispersal should be greater in oviparous than viviparous species (Jackson 1986)
Asexual and sexual, ovipary	
Sexual, vivipary	
Sexual, ovipary	
Body size	
Very small < 0.5 cm	Affects the structure and dynamics of food webs (Woodward et al. 2005)
Small 0.5–2 cm	
Medium 2–5 cm	
Large 5–10 cm	
Very large > 10 cm	
MORPHOLOGY	
Growth Form	
Encrusting	Acquisition of space and food (Jackson 1977)
Mound	
Erect	
BEHAVIOUR	
Food capture	
Collar sieving	Partitioning of food resources; benthic suspension feeding has an important role in energy flow from the plankton to the benthos (Parsons et al. 1979, Gill and Coma 1998)
Prey capture	
Mucus nets	
Ciliary downstream collecting	
Ciliary sieving	
Cirri trapping	
Filter setae	
Defense	
Spicules	Survival, defense of space (Jackson 1977, Dyrynda 1986)
Nematocysts	
Chemicals	
Cellulose tunic	
Refuge	
Hard shell	
Avicularia	

Table 2.3. Phylogenetic diversity of subtidal epibenthic invertebrate species at eight sites in the Gulf of Maine. Mean nearest neighbor distance (MNND) and mean phylogenetic distance (MPD) are two measures of community phylogenetic structure. The table shows standard effect sizes for these metrics. Positive SES values and high quantiles (> 0.95) indicate phylogenetic overdispersion, or a greater phylogenetic distance among co-occurring species than expected. Quantiles that are greater than or less than expected by chance are shown in bold.

		Phylogenetic MNND		Phylogenetic MPD	
Site	n taxa	SES	Quantile	SES	Quantile
Shag Rocks	28	0.93	0.823	0.88	0.816
Wood Island	16	0.47	0.675	1.01	0.862
Thrumcap	16	1.89	**0.978**	1.70	**0.998**
Bunker Point	18	1.87	**0.974**	1.74	**0.998**
Moose Cove	25	1.68	**0.961**	0.81	0.778
Cutler	26	1.44	0.935	0.14	0.504
Whale Cove	30	0.40	0.641	0.24	0.541
Pubnico	28	-0.28	0.385	0.00	0.458

Table 2.4. Phylogenetic signal in functional traits for all subtidal epibenthic invertebrate species in the regional phylogeny. Phylogenetic signal in each trait was quantified using Pagel's lambda (Pagel 1999), which is a scaling parameter that transforms branch lengths. The negative log likelihood for the maximum likelihood estimate of lambda was compared to the negative log likelihood of a transformed phylogeny with lambda = 0, i.e. no phylogenetic signal. The likelihood ratio test was approximated by a chi-squared distribution to generate p-values. Significant p-values are shown in bold.

Trait	λ	p
Coloniality	1.00	**< 0.001**
Reproduction	1.00	**< 0.001**
Body size	0.00	0.896
Growth form	0.44	**0.015**
Food capture	1.00	**< 0.001**
Defense	1.00	**< 0.001**

Table 2.5. Functional trait diversity for subtidal epibenthic invertebrate species at eight sites in the Gulf of Maine. Positive SES values and high quantiles (> 0.95) indicate higher trait diversity than expected with co-occurring species being less similar than expected by chance. Negative SES values and low quantiles (< 0.05) indicate lower trait diversity than expected with co-occurring species being more similar than expected by chance. Quantiles that are greater than or less than expected by chance are shown in bold.

		Trait MNND		Trait MPD	
Site	n taxa	SES	Quantile	SES	Quantile
Shag Rocks	28	0.42	0.658	1.49	**0.960**
Wood Island	16	-1.37	0.089	0.69	0.735
Thrumcap	16	1.00	0.830	0.20	0.518
Bunker Point	18	0.18	0.581	0.85	0.800
Moose Cove	25	-0.72	0.242	0.89	0.808
Cutler	26	-0.26	0.409	0.63	0.705
Whale Cove	30	-2.29	**0.013**	1.38	0.933
Pubnico	28	0.13	0.552	0.88	0.798

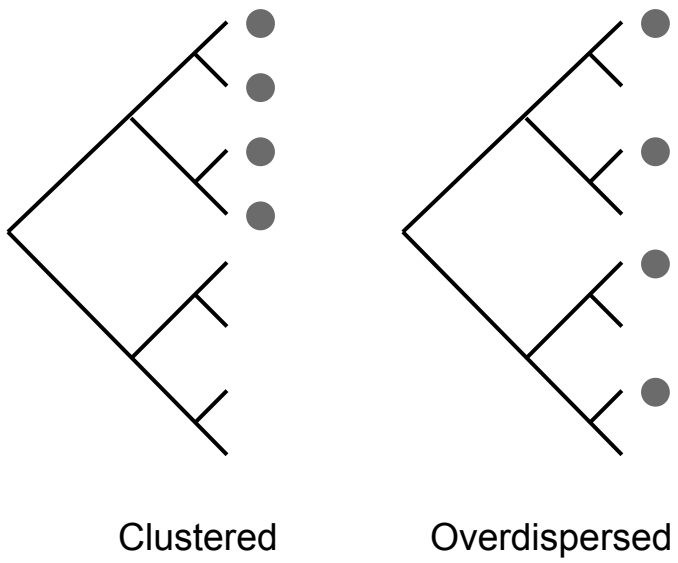

Figure 2.1. Patterns of a community phylogenetic structure. Assuming phylogenetic signal, i.e. phylogeny is a proxy for ecological similarity, a clustered pattern suggests environmental filtering and conservation of traits in sympatric species, and overdispersal suggests competitive exclusion. However, clustering can also result from ecological divergence of closely related species facilitating coexistence, and overdispersion from distantly related but ecologically similar species being filtered by the environment.

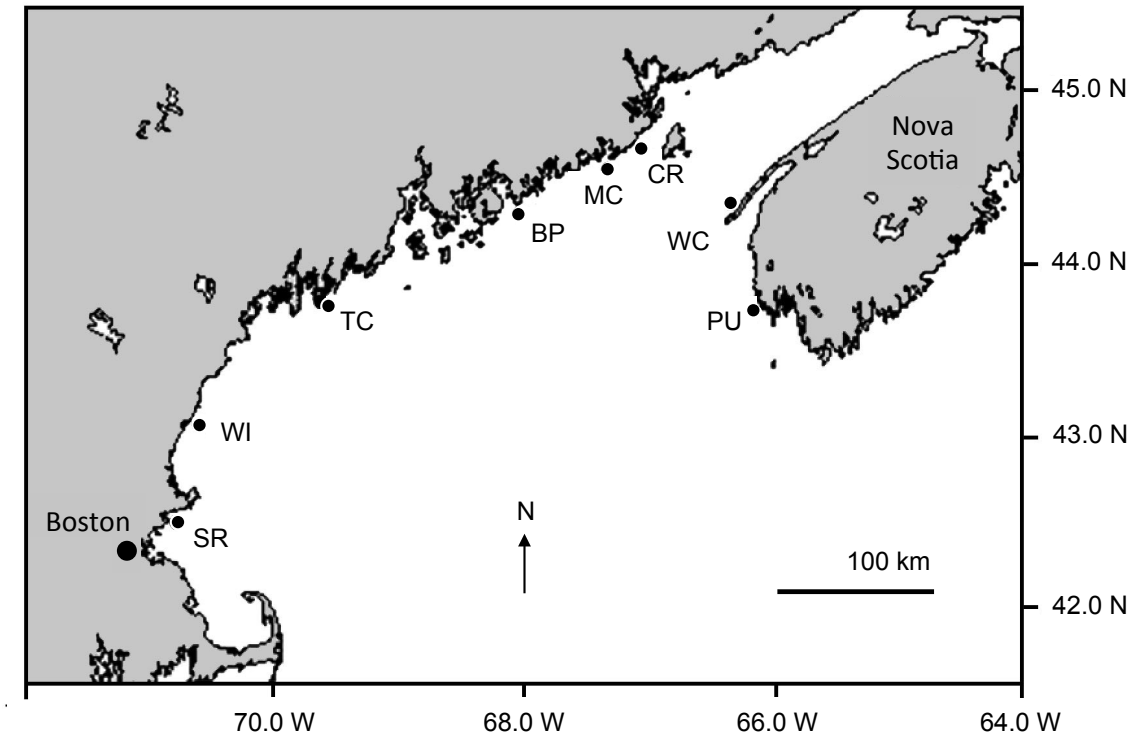

Figure 2.2. Map of the Gulf of Maine showing the eight study sites, Shag Rocks (SR), Wood Island (WI), Thrumcap (TC), Bunker Point (BP), Moose Cove (MC), Cutler (CR), Whale Cove (WC), and Pubnico (PU).

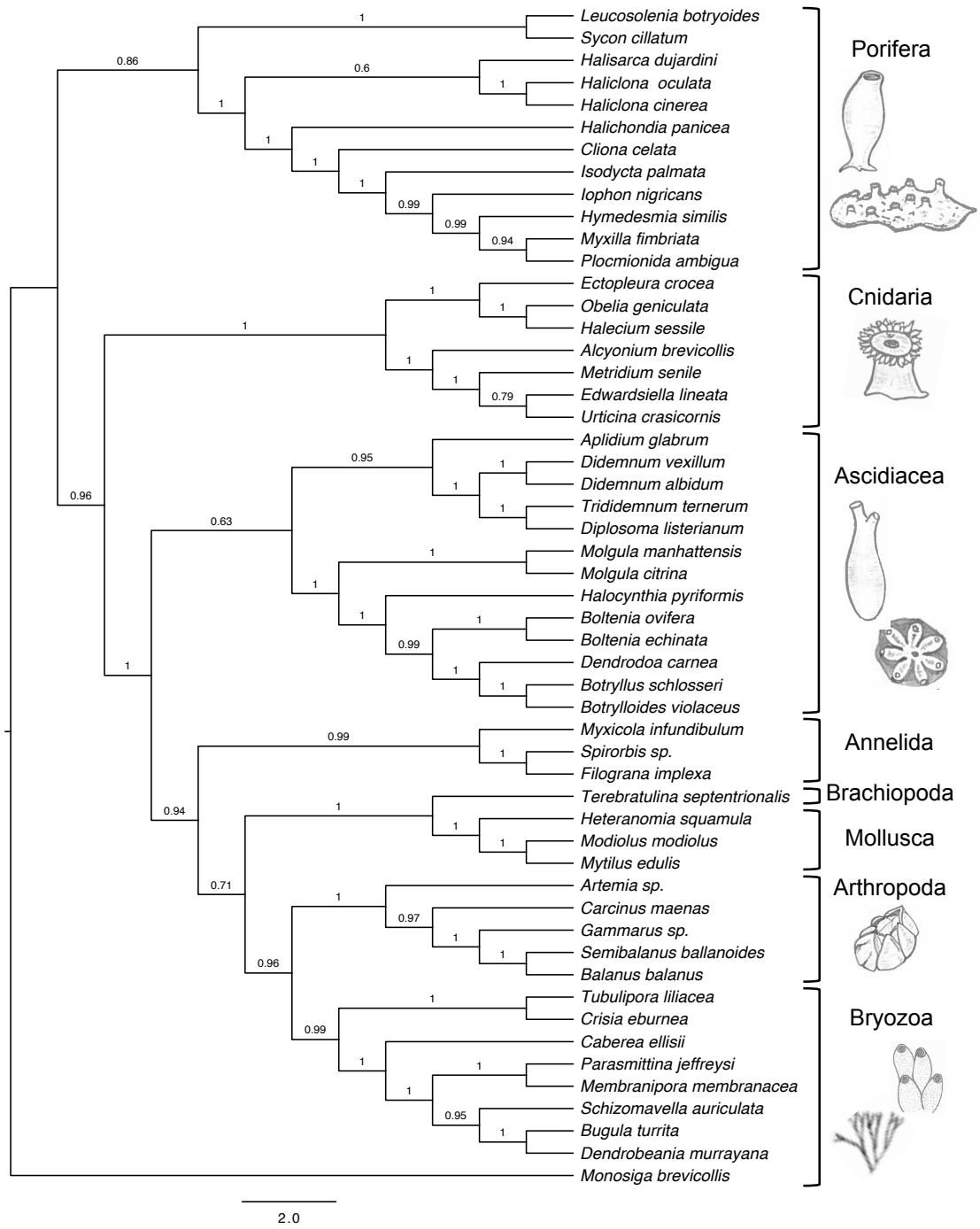

Figure 2.3. Bayesian tree based on 18S rRNA. Posterior probabilities are reported on branches. The scale bar indicates an evolutionary distance of 2.0 nucleotide substitutions per base pair.

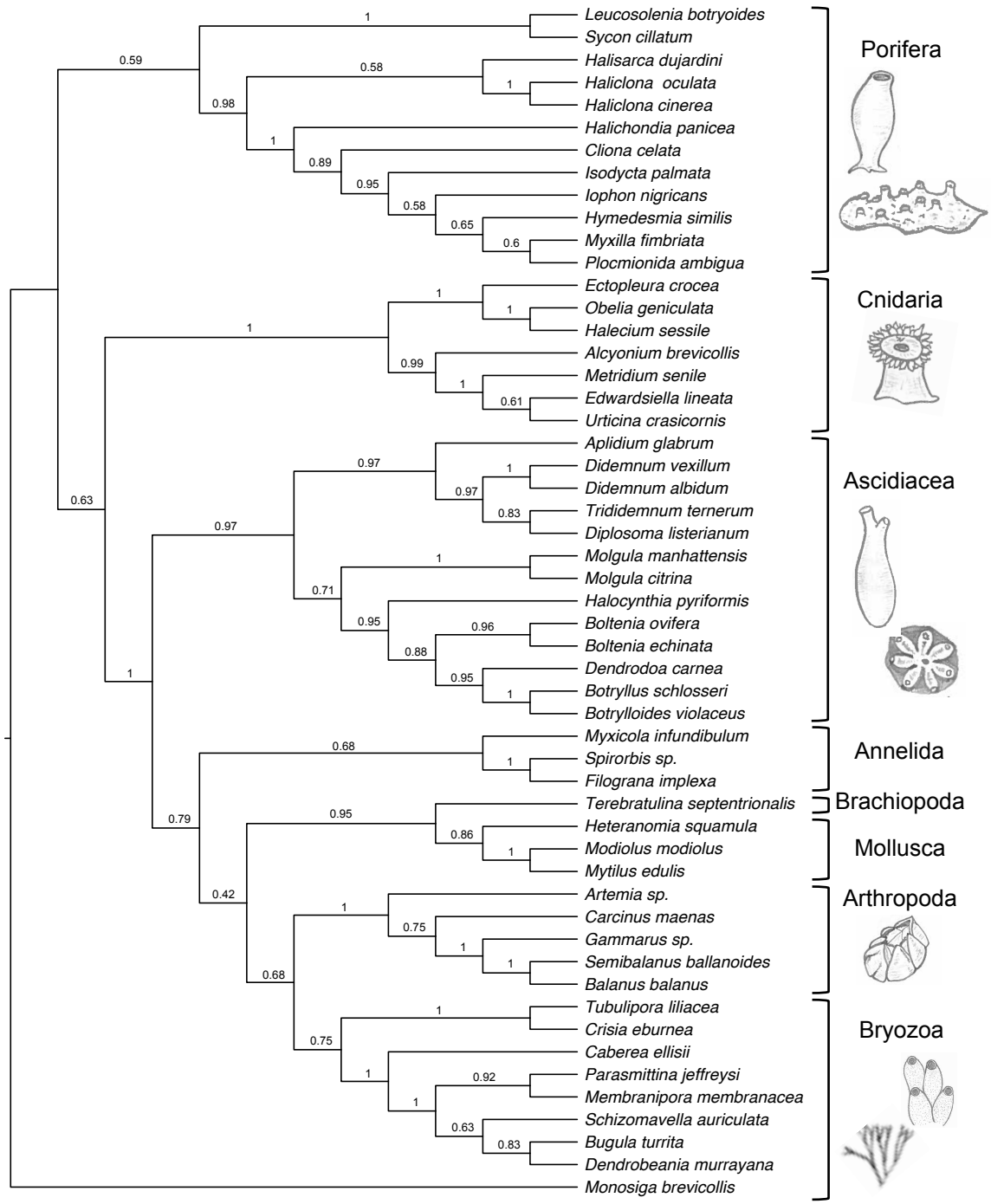

Figure 2.4. Maximum likelihood tree based on 18S rRNA. Bootstrap support values are represented on branches.

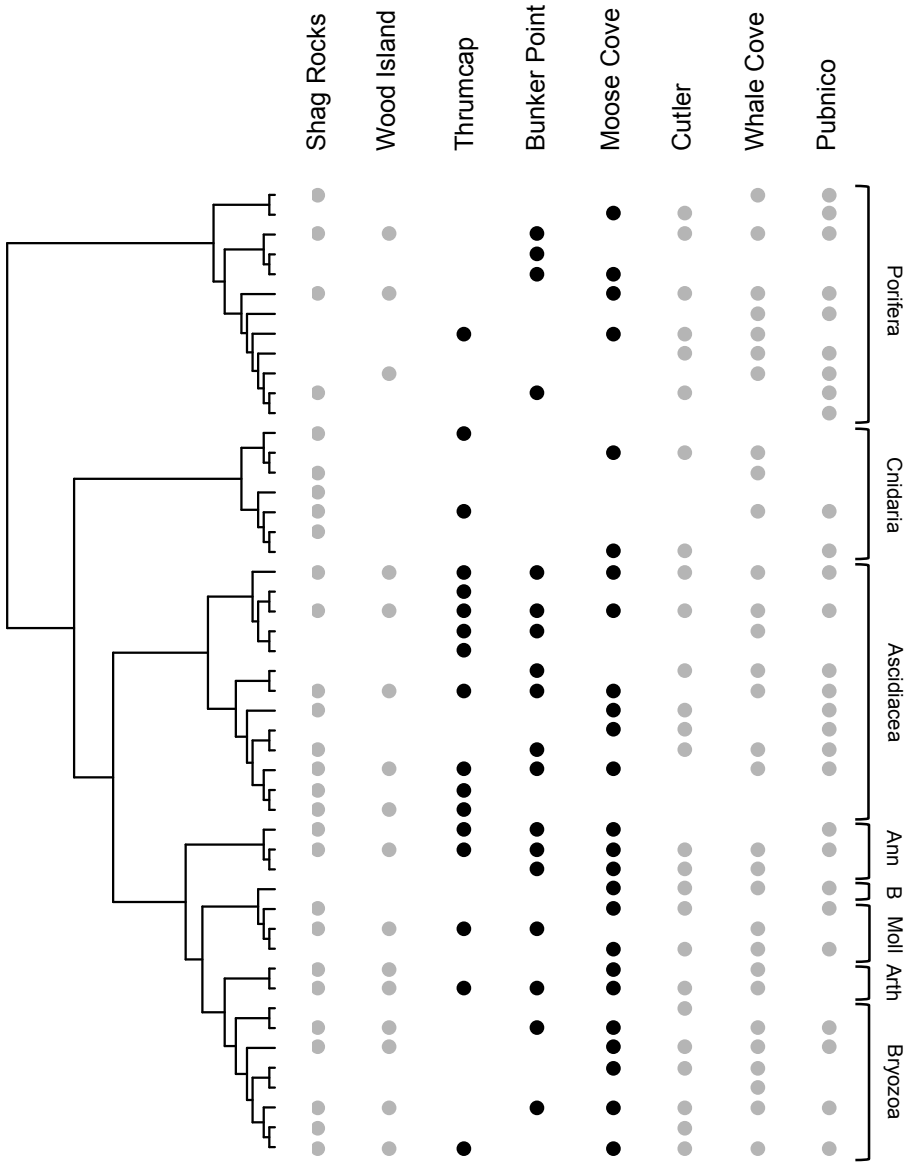

Figure 2.5. Species presence-absence by site, a filled circle representing species presence. Communities at all sites showed evidence of phylogenetic overdispersal. This was significant at three sites represented by the black circles (gray circles indicate that phylogenetic diversity was not significant). Thrumcap, Bunker Point, and Moose Cove were significantly overdispersed for MNND at the nearest-neighbour level and Thrumcap and Bunker Point were significantly overdispersed for MPD at the clade level. Ann = Annelida, B = Brachiopoda, Moll = Mollusca, and Arth = Arthropoda.

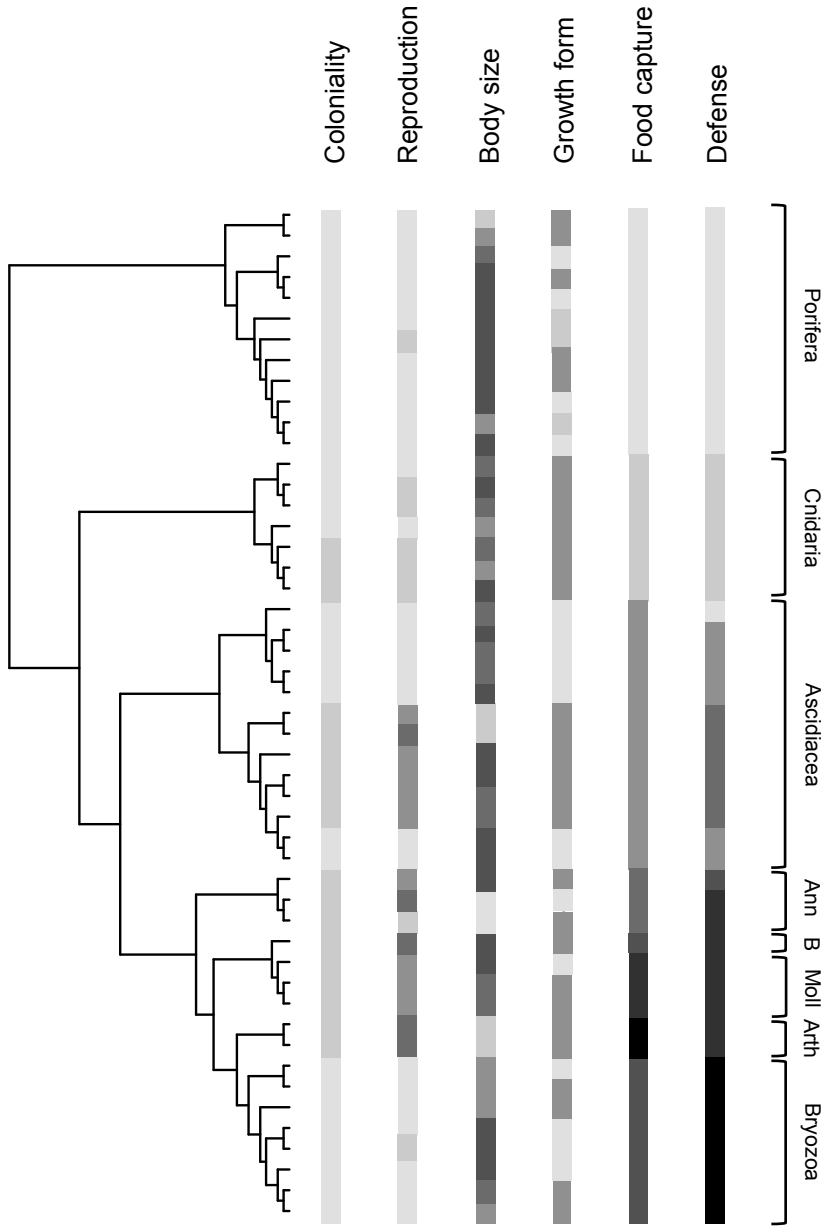

Figure 2.6. Phylogenetic signal and functional trait diversity, different shades representing different values for each trait. Coloniality, reproduction, food capture, growth form, and defense all showed evidence of phylogenetic signal. Ann = Annelida, B = Brachiopoda, Moll = Mollusca, and Arth = Arthropoda.

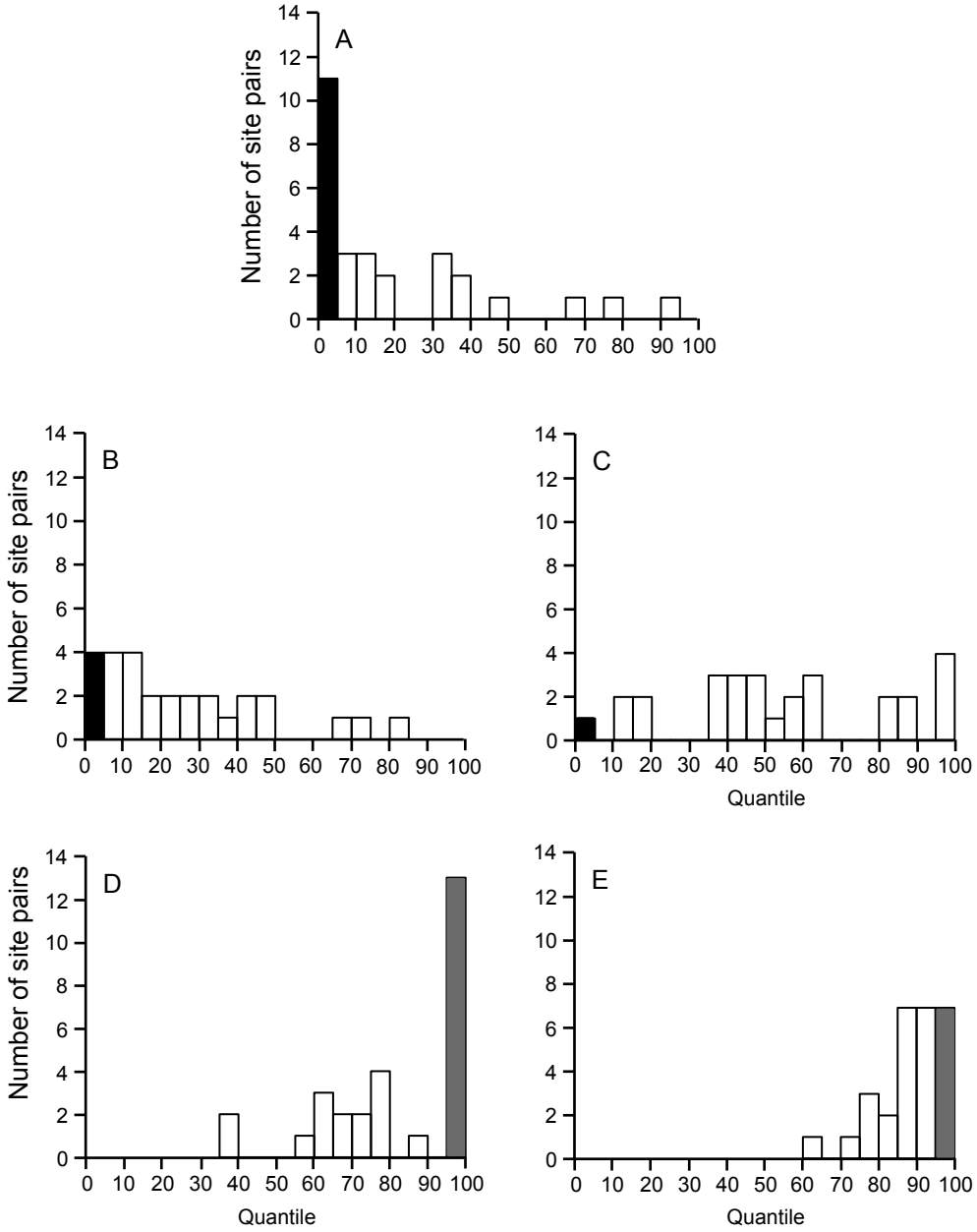

Figure 2.7. Null model results for A) taxonomic, B) phylogenetic nearest neighbour, C) functional trait nearest neighbour, D) phylogenetic pairwise, and E) functional trait pairwise beta diversity between pairs of sites in the Gulf of Maine. Histogram bars represent quantile scores for each pair of sites. Low quantiles (< 0.05) (black) indicate that beta diversity was less than expected by chance and high quantiles (> 0.95) (gray) indicate that beta diversity was greater than expected by chance.

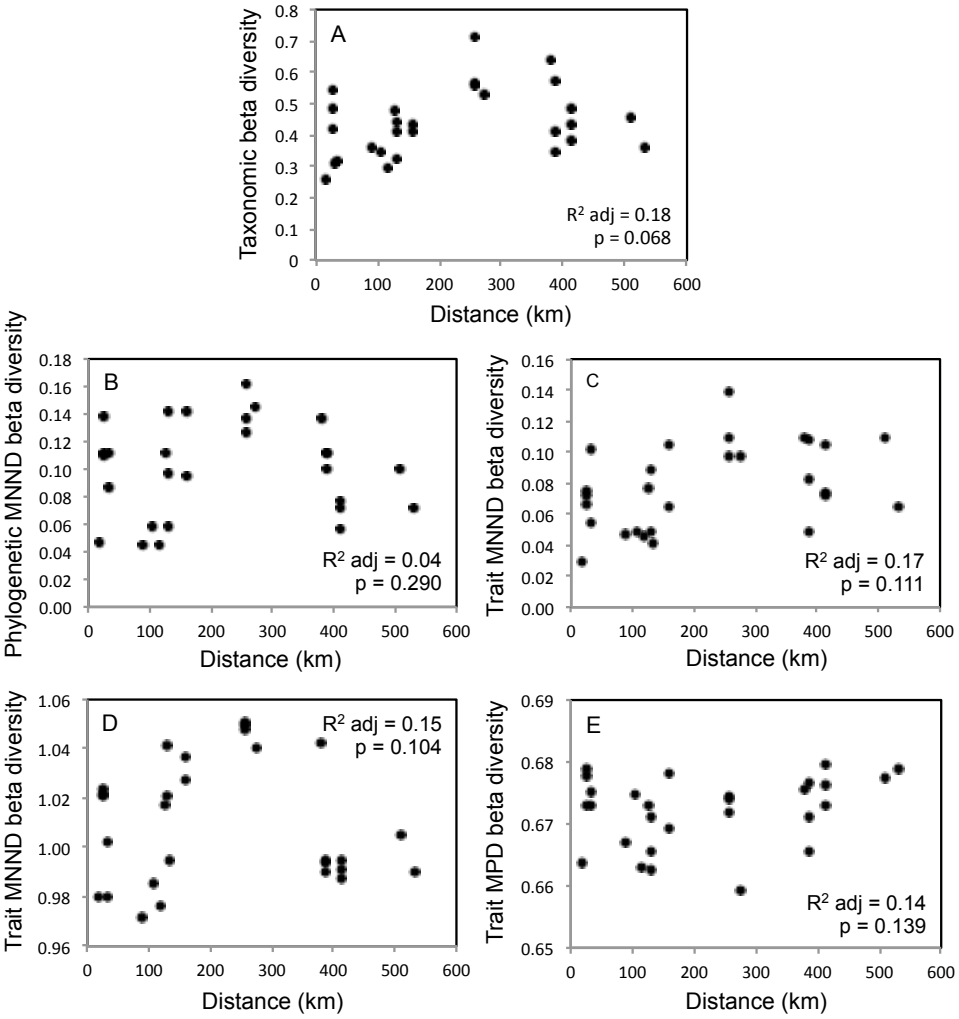

Figure 2.8. Relationship between geographical distance and A) taxonomic, B) phylogenetic nearest neighbor, C) functional trait nearest neighbor, D) phylogenetic pairwise, and E) functional trait pairwise beta diversity. Multiple regressions on distance matrices (MRM) were used to investigate curvilinear relationships. Adjusted R^2 and p-values are shown. No relationship was significant.

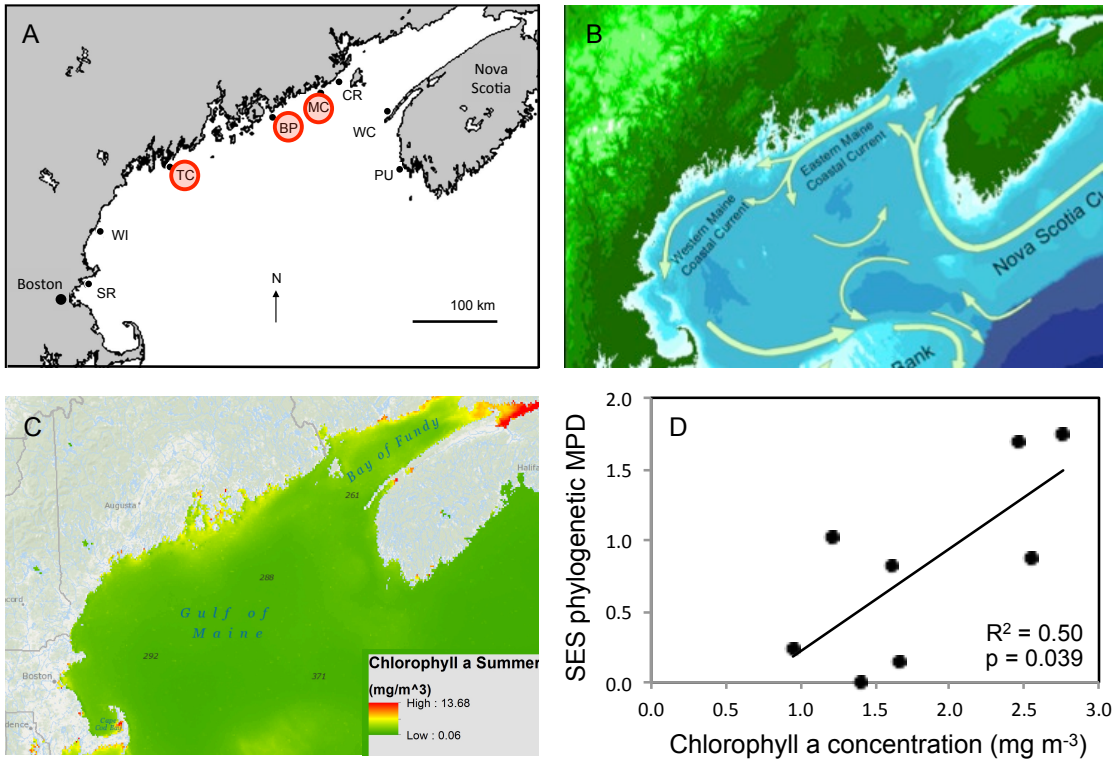

Figure 2.9. The relationship between chlorophyll a concentration and competitive exclusion. A) Circled sites had positive SES and high quantiles for phylogenetic MNND and MPD suggestive of community assembly by competitive exclusion. B) The Eastern Maine coastal current, a cold plume of nutrient dense water, coincides with these sites. C) Chlorophyll a concentration in the Gulf of Maine, showing that concentration is greater at the circled sites in A) than in the non-circled sites. D) Relationship between SES and chlorophyll a concentration for phylogenic MPD showing that the greater the chlorophyll a concentration the higher the SES (this relationship is not significant, however, with a Bonferroni corrected alpha set at 0.0125).

CHAPTER 3

INTERKINGDOM COMPETITION FOR SPACE OR FACILITATION? MACROALGAE VERSUS SESSILE INVERTEBRATES IN A RESOURCE LIMITED ENVIRONMENT

Abstract

Space is often the limiting resource in marine subtidal epibenthic communities, with organisms from many different phyla, including two different kingdoms, competing for this. Plant-animal interactions within these communities influence the relative abundance of heterotrophs and autotrophs and have important implications for ecosystem structure, function, and productivity. In rocky subtidal habitats, algae typically occupy shallow sunlit horizontal surfaces whereas invertebrates are more prevalent on deeper, darker, vertical surfaces. Recent experiments suggest that algae exclude sessile invertebrates on horizontal surfaces, but how that exclusion is mediated remains unclear. Here manipulative experiments, hung off the sides of floating docks, were used to test hypotheses about how macroalgae exclude sessile invertebrates. Algae did not negatively impact invertebrate assemblages and instead, had little effect on invertebrate abundance and diversity. Algae did, however, influence community composition and enhance invertebrate colonization in the early stages of community assembly. It appears that the factors that partition autotrophs and heterotrophs between horizontal and vertical surfaces

differ between floating docks and the rocky subtidal, and that man-made and natural subtidal habitats are fundamentally different.

Introduction

In subtidal marine epibenthic communities, sessile plants and animals often compete for the same limiting resource, space (Osman, 1977, Sutherland and Karlson 1977, Buss and Jackson 1979, Woodin and Jackson 1979, Sebens 1985). This direct competition between organisms from two kingdoms is a fundamental distinction between marine and terrestrial realms. In rocky subtidal systems, it has been hypothesized that benthic macroalgae can exclude sessile invertebrates from horizontal sunlit surfaces (Miller and Etter 2008) but it is unclear how competition is mediated. Identifying the mechanisms by which macroalgae exclude invertebrates will help to identify some of the ecological forces that control the balance between autotrophs and heterotrophs, which has important implications for ecosystem productivity, function, and services (Mineur et al. 2014, Strong et al. 2015), and is critical for understanding how subtidal communities might respond to anthropogenic impacts (Steneck et al. 2002, Brodie et al. 2014).

A striking pattern found in subtidal epibenthic ecosystems in the Gulf of Maine (Sebens 1985, Witman and Dayton 2001, Miller and Etter 2008, Miller and Etter 2011), and indeed worldwide (Withers and Thorpe 1977, Todd and Turner 1986, Baynes 1999, Irving and Connell 2002a), is that algae typically occupy horizontal, sunlit surfaces while animals dominate vertical walls and underhangs. Differences in abiotic factors between substrate angles, such as light (Gili and Coma 1998, Irving and Connell 2002a), flow

(Leichter and Witman 1997, Gili and Coma 1998, Genovese and Witman 1999), sedimentation, (Weinberg 1978, Airoldi and Cinelli 1997, Irving and Connell 2002a,b), and biological disturbance (Witman and Cooper 1983, Witman 1985, Sebens 1985, 1986), are thought to account for this pattern, although little experimental evidence exists to demonstrate the partitioning of taxa between different angles. Recently, in the Gulf of Maine, light was found to be an important factor controlling the distribution of autotrophs and heterotrophs between horizontal and vertical substrates respectively, and manipulative shading of horizontal surfaces prevented algal growth, causing a shift to invertebrate cover (Miller and Etter 2008). Furthermore, invertebrate larvae, previously assumed be negatively phototactic (Young and Chia 1984, Raimondi and Morse 2000), settled on shaded and unshaded horizontal plots in equal numbers. This suggests that larval phototaxis is not important in excluding invertebrates from horizontal surfaces with a role, instead, for invertebrate post-settlement mortality. These results suggest that, in sunlight, algae might exclude invertebrates on shallow surfaces (Miller and Etter 2008).

Algae can negatively impact invertebrates directly and indirectly. Indirectly, algae affect invertebrates by altering water flow, sedimentation, and light intensity, which can, in turn, negatively impact invertebrate recruitment, feeding, and growth (Duggins et al. 1990). For example, by altering flow, algae affect food delivery to, and capture by, sessile invertebrates (Morrow et al. 2008). Algae also provide habitat for micropredators that prey on invertebrate recruits (Osman and Whitlatch 2004, Stachowicz and Whitlatch 2005) and mesograzers that potentially do (Duffy and Hay 2000). Directly, algae can preempt space (Connell et al. 1997) and physically or

chemically interfere with invertebrates. Physically, algae fronds scour the substratum reducing invertebrate recruitment (Duggins et al. 1990, Connell 2003), and abrade (River and Edmunds 2001, Box and Mumby 2007, Titlyanov et al. 2007) and smother (Hughes 1989) adults, impacting invertebrate assemblages beneath the algal canopy (Kennely 1989). Algae can also overgrow (Young and Chia 1984, Lewis 1986, Coyer et al. 1993, Davis and White 1994, Jompa and McCook 2002) and dislodge invertebrates (Witman 1987). Chemically, algae can release allelopathic compounds that directly result in the mortality of invertebrate recruits and adults (Rasher and Hay 2010, Rasher et al. 2011) as well as release substances that alter the microbial activity on corals that, in turn, inhibits coral growth and survival (Smith et al. 2006, Vermeij et al. 2009 Thurber et al. 2012, Haas et al. 2013). Algae may also act as vectors for coral disease (Nugues et al. 2004, Sweet et al. 2013).

Directly, invertebrates can preempt space from algae (Bingham and Young 1991, Connell et al. 1997) and, when in close contact, some coral species can inhibit algal growth (van Steveninck et al. 1988, McCook 2001). The sweeper tentacles of gorgonian octocorals can abrade algae, possibly via anthozoan nematocysts or mucus (Sebens and Miles 1988). Invertebrates can overgrow encrusting algae (Breitburg 1984), and colonize arborescent forms (Dixon et al. 1981, Fraschetti et al. 2002) causing blade loss (Dixon et al. 1981) and a reduction in spore output (Saier and Chapman 2004). Like algae, invertebrates can produce allelopathic chemicals (Jackson and Buss 1975, Engel and Pawlik 2000, Pisut and Pawlik 2002) and inorganic acids (Dyrynda 1986) that can

prevent algal germination (Bak and Borsboom 1984). Finally, invertebrates can consume algal spores (Santelices and Martinez 1988).

Here we use manipulative experiments to investigate how macroalgae exclude sessile invertebrates in subtidal epifaunal communities in the Gulf of Maine. Specifically, two questions were asked: 1) Do macroalgae exclude sessile invertebrates, and 2) Does algal physical form increase mortality or decrease recruitment of invertebrates? Experiments were suspended from the sides of floating docks. In this system, algae did not negatively impact invertebrate assemblages and instead, had little effect on invertebrate abundance and diversity. It did, however, influence community composition.

Methods

Study site and experimental set-up

Experiments were conducted at a depth of 1 m on polycarbonate boards suspended vertically off floating docks at Dorchester Yacht Club, Boston, Massachusetts (42.305556°N, 71.046111°W). The fouling community consisted of sessile algae and invertebrates typical of this region including red and green algae, sponges, bryozoans, polychaetes, molluscs, barnacles, and colonial and solitary ascidians (see Table 3.1 for a full species list). These species are a subset of those commonly found in rocky subtidal habitats.

To address whether macroalgae exclude sessile invertebrates, two experiments were designed, 1) Assembly, which assessed if algae prevent the formation of

invertebrate assemblages in communities allowed to develop naturally, and 2) Displacement, which investigated if algae displace adult invertebrates. To test if algal physical form contributes to the prevention or reduction of invertebrate recruitment, two additional experiments were conducted, 3) Recruits, which tested if algae negatively impact of young, recently recruited invertebrates, and 4) Settlers, which tested if algae reduce invertebrate settlement. Here, settlers are defined as incoming larvae that might settle on plates, and recruits as newly settled individuals that have colonized the plates.

The Assembly experiment started with blank, standard 15 x 15 cm, 0.5 cm thick, polycarbonate settlement plates. The basic design of the other three experiments, Displacement, Recruits, and Settlers, was to create starting communities that varied in algal and invertebrate composition depending on the treatment and experiment. To create the initial community composition in the Displacement, Recruits, and Settlers experiments, pure stands of algae or invertebrates were grown on 3 x 3 cm, 0.5 cm thick, polycarbonate tiles on the side or underneath floating docks. Once seeded, 25 tiles were randomly pieced together to create a starting community of 15 x 15 cm depending on the experimental treatment. For example, in Treatment 3 of the Displacement experiment, inverts and algae, the starting community was comprised of 50% algae and 50% invertebrates. To create this starting community, 12 tiles with algae and 13 tiles with invertebrates were randomly assembled to create a larger 15 x 15 cm plate (Table 3.2; Fig. 3.1). This was achieved by securing tiles to a 17 x 17 cm, 0.5 cm thick, polycarbonate base plate. Threaded polycarbonate rods that were glued into blind holes on the underside of the tiles were passed through holes in the base plate and secured

using polycarbonate nuts (Fig. 3.1). 15 x 15 cm was selected as the size for settlement surfaces as this is the scale at which epifaunal species in the communities typically interact (Stachowicz et al. 2002). Starting communities for the other treatments in the Displacement, Recruits and Settlers experiments were formed in a similar way depending upon the required starting community composition and this is explained for each experiment and treatment below. The experiments were submerged and attached to a floating dock side facing north. Treatment replicates (n = 4 in all cases) were arranged in a Latin Square.

Experiments

Assembly. To test the hypothesis that algae prevent or hamper the formation of invertebrate assemblages, communities were allowed to form on bare substrate in the presence and absence of algae. Polycarbonate settlement plates (15 x 15 cm) were attached to polycarbonate boards. Treatments were: 1) natural community (control, without any manipulation), and 2) algae removal (with continual algae removal). Four replicates plates were used for each treatment. In the removal treatment, algae were carefully removed with a scraper, wire brush, sponge, or forceps without disturbing any invertebrate recruits. Algae removal was performed once a week in summer when growth was fast, bimonthly in spring and fall, and once a month in winter when growth was slower. The experiment was continued for 18 months, from 19th April 2012 to 20th October 2013. At the end of the experiment all algae and arborescent invertebrates were trimmed down to enable all primary space occupiers in the community to be

photographed using an Olympus Stylus Tough 8010 camera. Digital photographs were later enlarged on a screen with an overlay of 200 random dots. Species under the dots were identified and recorded to estimate percent cover and species richness of space occupying sessile invertebrates. Response variables were percent cover of primary space occupying sessile invertebrates and diversity of primary space occupying sessile invertebrates. All information was taken from the central 12 x 12 cm area of the settlement surface to avoid edge effects.

This experiment was designed to examine the effects of algae on the natural formation of epifaunal invertebrate communities in the presence and absence of algae and provide a contrast to the experiments described below. If algae increase invertebrate mortality or decrease recruitment invertebrates should be more prevalent in the algae removal treatment.

Displacement. To test the hypothesis that macroalgae displace sessile invertebrates, the percent cover of macroalgae and invertebrates were manipulated using pre-seeded tiles and community composition quantified at the end of the experiment. The four starting communities, or treatments, were: 1) algae (algae tiles and blank tiles); 2) inverts (invertebrate tiles and blank tiles); 3) inverts and algae (invertebrate tiles and algae tiles with no blank tiles); and 4) inverts and algae removal (invertebrate tiles and blank tiles with continual algal removal) (Table 3.2). This last treatment was a control to determine if invertebrate mortality was influenced by colonization of macroalgae in treatment 2. Communities were formed for the algae treatment above with 13 algae tiles (pre-seeded) and 12 blank tiles randomly assembled to create a larger, 15 x 15 cm plate.

Starting communities in the other treatments were assembled in a similar way (see Table 3.2 for details). Four replicates plates were used for each treatment. Algae were removed from the control treatment in the same way and frequency as described above for the Assembly experiment. The duration of the experiment, data collection, and response variables were also the same.

This experiment was designed to test the role of macroalgae in affecting the survivorship of adult invertebrates and invertebrate recruitment. It addresses a number of additional key questions including: is the shift to macroalgae influenced by the presence of invertebrates i.e. do macroalgae replace invertebrates regardless of initial community state; can invertebrates settle into macroalgae only communities; are invertebrates more likely to settle when adult invertebrates are present; do invertebrate mortality rates differ in the presence or absence of plant competitors; and do invertebrates persist when macroalgae are removed.

Recruits. The presence and absence of algae and plastic structural mimics were used to test if invertebrate recruits or juveniles were affected by algae or its structure. Tiles containing either normal algae, cut algae (distal thalli removed with scissors), or plastic algal mimics, were combined with tiles containing invertebrate recruits to create starting communities. Again, a 50:50 mix of algae and recruit tiles were combined to form 15 x 15 cm plates. Starting communities, or treatments, were 1) algae normal (intact algae tiles and invertebrate recruit tiles); 2) algae cut (modified algae tiles (distal thalli removed with scissors) and invertebrate recruit tiles); 3) algae mimic (plastic algae mimic tiles and invertebrate recruit tiles); and 4) no algae (blank tiles with algae absent

and continually removed and invertebrate recruit tiles) (control) (Table 3.2). Four replicate plates were used for each treatment. The distal thalli were cut from algae in the algae cut treatments to reduce frond length to about 1 cm. This was performed once a week and, at the same time, algae were removed from the no algae treatments. The algae present in the system at this time, *Ulva intestinalis,* was mimicked using green "Easter Grass" which was attached to tiles using Loctite Marine Epoxy. The experiment ran for five weeks, from 26th June until 31st July 2012. This experiment was shorter than the previous two, as the aim was to tease out the influence of algae on early ontogenetic stages of invertebrates. Data collection and response variables were as described for the previous experiments.

By removing the distal thalli in the algae cut treatment some of the algae's physical structure was eliminated. If the physical structure negatively impacted invertebrate recruits more recruits would be expected in this treatment than in the algae normal and algae mimic treatments where the structure was still present. The first centimeter of the frond was retained to investigate if factors other than structure, i.e. algae chemical composition, affected recruit survivorship. The use of plastic forms of algae in the algae mimic treatment removes the effect of any algal chemical or microbial exudates while retaining a mimic of its structure. The no algae treatment (control) allowed us to monitor recruitment without algae.

Settlers. This experiment was focused on whether the presence of algae, or its structure, influenced the settlement of invertebrate larvae. It was set up similar to the Recruits experiment above, but the algae tiles (normal, cut or mimicked) were combined

with blank tiles to investigate invertebrate settlement (Fig. 3.2). A 50:50 mix of algae and blank tiles were used to create a 15 x 15 cm plate. Starting communities, or treatments, were 1) algae normal (intact algae tiles and blank tiles); 2) algae cut (modified algae tiles (distal thalli removed with scissors) and blank tiles); 3) algae mimic (plastic algae mimic tiles and blank tiles); and 4) no algae (all blank tiles with algae absent and continually removed) (control). Four replicate plates were used for each treatment. The distal thalli were cut from the algae cut treatment, and algae removed from the no algae treatment as in the Recruits experiment. Distal thalli were again cut off to remove the physical structure but leave other characteristics of the algae present, e.g. chemical make-up (Fig. 3.2). In the algae mimic treatment the structure was present but any characteristic chemicals eliminated by using plastic forms. Green polyethylene bags were cut into the shape of *Ulva linza* fronds (the algae present in the system at the time of the experiment) and attached these to tiles using Loctite Marine Epoxy (Fig. 3.2). Data collection and response variables were as described in the Assembly experiment. The experiment ran for 5 weeks, from 8th August 2012 to 11th September 2012.

Statistical analyses

Experiments were analyzed as single factor MANOVAs with the number of fixed levels varying depending upon the experiment i.e. Assembly had two levels and Displacement, Recruits, and Settlers all had four levels (each level corresponding to a treatment). Response variables were percent cover of primary space occupying sessile invertebrates and diversity of space occupying sessile invertebrates. Percent cover data

was transformed to logits, ln[p/(1 - p)], to homogenize variances where p is the proportion of sessile inverts + 0.025 (0.025 was added to avoid proportions of 0 or 1). Diversity was calculated using Shannon's diversity index. MANOVAs were followed up with univariate ANOVAs to test for the contribution of each dependent variable using a Bonferroni-corrected α of 0.025. Post-hoc pairwise comparison tests, i.e. Tukey's HSD, were carried out when ANOVA results revealed significant differences among treatments. Normality was visualized with Q-Q plots and tested using the Shapiro-Wilk test. Homogeneity of variance was visualized with plots of residual versus fitted values and tested using Levene's median test. Compositional differences among treatments of primary space occupying sessile invertebrates were explored using PERMANOVA and non-metric multi-dimensional scaling (NMDS), both based on Bray-Curtis distances. Multivariate dispersion was tested using PERMDISP, which is a multivariate analog of Levene's test for homogeneity of variances. The contribution of individual species to the differences between groups was assessed using similarity percentage, or SIMPER. All analyses were carried out in R version 3.0.2 (R Core Team 2013) using the Vegan package (Oksanen et al. 2012).

Results

Assembly. Percent cover and diversity were not affected by the treatments (Tables 3.3 and 3.4; Fig. 3.3A). Community composition of space occupying sessile invertebrates did, however, differ among treatments (Pseudo-F = 2.47, p = 0.03; Table 3.5; Fig. 3.4A). In the algae removal treatment there were more colonial ascidians and in

the natural community treatment there were more *Mytilus edulis, Ostrea edulis, Crepidula plana,* and algae/mud/tube complex (Table 3.6). Across treatments barnacles and solitary ascidians did not vary (Table 3.6). These observations were supported by SIMPER.

Displacement. Percent cover and diversity were not affected by the treatments (Tables 3.3 and 3.4; Fig. 3.3B) but community composition was (Pseudo-F = 3.27, p = 0.001; Table 3.5; Fig. 3.4B), which is similar to the findings from the Assembly experiment. Composition in the algae removal treatment was different from the other treatments, which all overlapped in the NMDS plot (Fig. 3.4B). As in the Assembly experiment, there were more colonial ascidians in the algae removal treatment and more *M. edulis, O. edulis, C. plana,* and algae/mud/tube complex in the other three treatments (Table 3.6). Across treatments barnacles and solitary ascidians did not vary (Table 3.6).

Recruits. Percent cover and diversity were not affected by the treatments (Tables 3.3 and 3.4; Fig. 3.3C) and neither was community composition (Table 3.5; Fig. 3.4C). Only an encrusting bryozoan and the colonial ascidians *Botryllus schlosseri* and *Botrylloides violaceus* occurred in these communities and their abundances did not vary across treatments (Table 3.6).

Settlers. The treatments altered percent cover (F = 726.60, p < 0.001; Tables 3.3 and 3.4; Fig. 3.3D), which was greatest in the algae normal and algae cut treatments, considerably less in the algae mimic treatment, and least in the no algae treatment (Fig. 3.3D). The treatments also altered diversity (F = 6.49, p = 0.007; Tables 3.3 and 3.4; Fig. 3.3D), which was similar in the algae normal, algae cut and algae mimic treatments, and

decreased slightly in the no algae treatment (Fig. 3.3D). The differences in percent cover and diversity among treatments in this experiment contrasts to the other three experiments were no differences were found. As in the Assembly and Displacement experiments, community composition differed among treatments (Pseudo-F = 46.76, p = 0.001; Table 3.5; Fig. 3.4D). Again, the treatment without algae was different from all of the treatments with algae, whether algae were normal, cut or mimicked (Fig. 4.3D). *Botrylloides violaceus* and *Diplosoma listerianum* were present in all communities but varied in abundance by treatment (Table 3.6). *Botryllus schlosseri* was present in all of the algae treatments (normal, cut and mimic) but not in the no algae treatment. *Crepidula plana* was present in treatments with natural algae (normal and cut).

Discussion

The ecological forces that control the balance between heterotrophs and autotrophs have the potential to profoundly influence community structure, function, and productivity. On shallow sunlit horizontal surfaces at 10 – 12 m depth in the rocky subtidal, light favors algae, which in turn appear to exclude invertebrates (Miller and Etter 2008). In contrast, algae had little effect on the percent cover and diversity of sessile invertebrates on vertical experimental communities at 1 m depth suspended from floating docks, although they facilitated invertebrates in the early stages of community assembly and altered invertebrate community composition. Communities with algae tended to have more native and less invasive species. Differences in biotic and abiotic factors between these two systems, such as algal composition, light, and substrate angle

(as well as flow, sedimentation, and disturbance that vary with the latter), may account for the contrasting results.

Algae had little impact on percent cover and diversity of invertebrates

In three out of four experiments —Assembly, Displacement, and Recruits — algal did not affect invertebrates, in comparison to the rocky subtidal where algae was hypothesized to exclude invertebrates. This may be due to differences between the two systems such algae composition and substrate angle. On horizontal surfaces in the rocky subtidal, the dominant species of algae were two brown species, *Laminaria longicruris* and *Desmarestia viridis*, and two red species, a crustose coralline and *Bonnemaisonia hamifera* (Miller and Etter 2008). In contrast, experimental floating dock communities were dominated by ephemeral green species, *U. intestinalis, U. linza,* and *Ulva lattuca* (see Table 3.1 for additional species), and the red algae *Ceramium virgatum.* Two species of red algae, *Chondrus crispus*, and *Polysiphonia* sp.were common to both systems. Thus the dominance of perennial brown algae in rocky subtidal versus ephemeral green algae in floating dock systems may have influenced the results.

The brown rocky subtidal algae, *Laminaria longicruris*, more commonly known as kelp, is functionally classified as a large leathery macrophyte (see Steneck and Dethier 1994). Algae from this group, such as other species of kelp (Kennelly 1989, Duggins et al. 1990, Coyer et al. 1993, Connell 2003), *Sargassum* (River and Edmunds 2001), *Ascophyllum* (Leonard 1999), and *Fucus* (Grant 1977), are known to negatively impact invertebrates by scouring (Duggins et al. 1990, Connell 2003) and dislodgement (Witman

1987). On coral reefs, brown algae physically abrade (River and Edmunds 2001, Box and Mumby 2007, Titlyanov et al. 2007) and overgrow (Lewis 1986, Coyer et al 1993, Jompa and McCook 2002) coral, reducing growth rates and causing direct mortality. The absence of similar species, i.e. brown algae including large leathery macrophytes, in floating dock systems may account for the lack of invertebrate exclusion in these systems.

With respect to alleleopathy, temperate species of algae generally do not produce as many secondary allopathic metabolites as their tropical counterparts (Norris and Fenical 1982, Bolser and Hay 1996, Hay 1996). Studies examining the alleopathic effects of algae on invertebrates, however, are weighted towards tropical reefs (Rasher and Hay 2010, Rasher et al. 2011), where coral damage by algae is of prime concern (Hughes 1989, 1994). In the Gulf of Maine there is some evidence that algal exudates negatively impact polychaete larvae (Warkus et al. 2011), but it is unclear whether this negative effect of algae on invertebrates is restricted to this taxon or this ontogenetic stage.

Substrate angle is also known to influence community composition in floating dock (Connell 1999, Glasby and Connell 2001) and rocky subtidal (Glasby and Connell 2001, Irving and Connell 2002a, Miller and Etter 2008, 2011, Dafforn et al. 2012) systems, most likely because abiotic factors differ between surface orientation (Glasby and Connell 2001, Witman and Dayton 2001). On shallow horizontal surfaces in the Gulf of Maine rocky subtidal, light is the most important factor partitioning autotrophs and heterotrophs between different substrate angles (Miller and Etter 2008). Light was

also found to be the most important factor controlling community structure on horizontal surfaces in Outer Harbor, South Australia (Irving and Connell 2002a), and in the southern Gulf of California (Baynes 1999). These shallow horizontal surfaces receive high levels of sunlight, which promote algae growth and limit invertebrates (Miller and Etter 2008).

Vertical substrates, conversely, experience increased flow (Leichter and Witman 1997, Gili and Coma 1998, Genovese and Witman 1999), decreased sedimentation (Weinberg 1978, Irving and Connell 2002a), and decreased biological disturbance (Witman and Cooper 1983, Witman 1985, Sebens 1985, 1986), all of which enhance invertebrate success. This perhaps gave invertebrates an advantage in vertical experimental communities, changing the nature of algae-invertebrate interactions. Invertebrates typically monopolize vertical subtidal surfaces (Miller and Etter 2011), in part because of reduced light (Miller and Etter 2008). The greater light intensity on these floating-dock experimental surfaces may have allowed the algae to persist. Thus, the vertical orientation favors the invertebrates while the greater sunlight favors the algae, resulting in vertical surfaces that support both invertebrates and algae, and little impact of the algae on invertebrate percent cover or diversity. Here we show that, in the Gulf of Maine, algae-invertebrate interactions at different depths appear to be mediated by different factors, which have profound effects on community structure. In the rocky subtidal, algae excluded invertebrates, whereas in floating dock communities algae had little effect. Different functional groups of algae occur with depth and appear to differentially exclude invertebrates, i.e. large leathery brown macrophytes, known to negatively impact invertebrates (Witman 1987, Duggins et al. 1990, Connell 2003), occur

in the rocky subtidal but are absent in shallower floating dock communities. Substrate angle (as well as factors such as light, flow, sedimentation, and disturbance that vary between horizontal and vertical surfaces) also likely influences assemblages. Light, in particular, is the dominant factor influencing algae-invertebrate interactions in the rocky subtidal (Baynes 1999, Irving and Connell 2002a, Miller and Etter 2008) and on horizontal surfaces light favors algae growth which in turn exclude invertebrates (Miller and Etter 2008). On shallow vertical surfaces light also enables algae growth but, because of increased flow, decreased sedimentation, and decreased disturbance relative to horizontal surfaces, invertebrates also persist, enabling organisms from both kingdoms to co-occur.

Algae facilitated invertebrates in the early stages of community assembly

In the Settlers experiment, algae enhanced invertebrate percent cover and diversity, which was opposite to expectations, i.e. instead of negatively impacting invertebrates, algae facilitated colonization. Natural algae (algae normal and algae cut) was more important than structure in mediating this process (Figs. 3.2 and 3.3), suggesting that properties unique to living algae play a role in enhancing invertebrate colonization. Invertebrate larvae may have been attracted to the natural algae's biofilm and chemical composition (Crisp 1974, Pawlik 1992, Dobretsov 1999, Hadfield and Paul 2001) and some coral species have been shown to settle preferentially into communities with natural rather than mimicked algae (Nugues and Szmant 2006, Diaz-Pulido et al. 2010). The importance of natural properties relative to structure is further suggested by

the natural treatments (algae normal and algae cut) being similar in percent cover. If, for example, structure was equally or more important than natural properties, percent cover would be lower in the algae cut treatment where algal frond length was reduced and some structure eliminated.

Structure was somewhat important, as percent cover of invertebrates was greater in the algae mimic treatment than in the no algae treatment. Structure, in general, may create eddies and increase deposition of recruits (Sebens 1983). It is interesting that the percent cover of invertebrates was lowest in the no algae communities, that started off as blank plates, as invasive ascidians, the main occupiers of space in the Setters experiment, are known to quickly enter and colonize open space (Stachowicz et al. 2002, Tyrrell and Byers 2007, Kremer et al. 2010, Janiak et al. 2013). Perhaps even these tenacious invaders require a natural cue, such as an accumulated biofilm, to settle on bare substrate (Crisp 1974, Pawlik 1992, Hadfield and Paul 2001) and/or structure. These species do not ultimately appear to distinguish between natural and plastic forms as diversity was the same in all treatments with algae, whether natural or mimicked (Fig. 3.3).

The different results between the long-term Assembly and Displacement experiments (18 months) and the short-term Settlers experiment (5 weeks) could be due to the different temporal scales of the experiments. Results suggest that algae facilitate invertebrates in the early stages of community assembly but that this effect is undetectable 18 months later. In Fig. 3.2, in the early stages of community formation, invertebrates are clearly associated with algae, and algae appear to facilitate invertebrates. This was also observed in the early stages of the Displacement experiment.

While facilitation is often common in the early stages of community assembly (Clements 1916, Odum 1969, Connell and Slatyer 1977, Dean 1981), communities in this study were manipulated to already contain algae, and thus the facilitation of invertebrates by algae in the early stages of community assembly may be an artifact of experimental design.

The results of the short-term Recruits experiment are similar to the results of the long-term Assembly and Displacement experiments, and not to the Settlers experiment of the same duration, i.e. there was no difference among treatments for percent cover or diversity of invertebrates in the Recruits experiment. This is likely because invertebrate recruits were already present in all of the starting communities. The percent cover of invertebrates is much lower in the Recruits experiment compared to all other experiments and this may be due to 1) the experiment running for less time than the Assembly and Displacement with invertebrates having less time to colonize, and 2) the absence of the rapidly colonizing *D. listerianum* (Vance et al. 2009) in the Recruits versus the Settlers experiment.

In the early stages of assembly in floating dock communities, algae appear to facilitate invertebrates. However, this early positive interaction is undetectable 18 months later and thus algal presence is unlikely to greatly influence invertebrate percent cover and diversity in the long term. Nonetheless, this apparent facilitation of invasive species by algae should be noted as these species continue to expand their distributions (Lambert and Lambert 2003, Karlson and Osman 2012).

Community composition

Algae influenced community composition in the Assembly, Displacement, and Settlers experiments, where treatments with algae were distinctly different from those without. In the Assembly and Displacement experiments, species diversity did not differ between treatments and neither did species identities (Table 3.6), suggesting species abundances alone account for differences in composition. Treatments with algae had greater abundances of *M. edulis, O. edulis,* and *C. plana,* which may be attracted to algal-associated natural cues and/or structure (Crisp 1974, Pawlik 1992, Hadfield and Paul 2001), and a greater abundance of algae/mud/tube complex, which appeared to form out of sediment trapped in algae. In the algae removal/no algae treatments, invasive colonial ascidians were the dominant taxa. These species are known to rapidly colonize open space in fouling communities (Stachowicz et al. 2002, Tyrrell and Byers 2007, Kremer et al. 2010, Janiak et al. 2013) (although findings from this study suggest that the presence of structure and natural cues facilitate this process). In the algae removal/no algae treatments, removing algae also created a disturbance, which is known to facilitate invasive colonial ascidian success (Altman and Whitlatch 2007).

In the Settlers experiments, invasive colonial ascidians were the dominant species in all treatments. Again, the only differences between the algae and non-algae treatments were species abundances. Other species — *C. plana*, *B. balanus*, encrusting bryozoans, *Ciona intestinalis,* and *Styela clava*—were found on plates in the treatments with natural algae (algae normal and algae cut) (see Appendix 3A) but were rare and did not fall under one of the 200 dots. These species may also be attracted to algal-associated natural cues.

If this experiment had been continued for a further 17 months, community composition in the treatments with algae may have been similar to those in the Assembly and Displacement experiments.

Overall, it appears that, in experimental floating dock communities, assemblages with algae also have a greater abundance of native species such as *M. edulis* and *C. plana*, whereas communities without algae are dominated by invasive species. This suggests that, in the longer term, algae play a role in invasion resistance (despite the initial facilitation of invasive ascidians by algae in the early stages of community assembly), with implications for algae conservation. Worldwide, algae are under threat from ocean warming, acidification, sedimentation loss of habitat, invasive species, pollution, sedimentation, and hypoxia (Walker and Kendrick 1998, Steneck et al. 2002, Schiel 2009, Brodie et al. 2014, Mineur et al. 2014) but have not yet been subject to serious conservation efforts (Walker and Kendrick 1998). Any factor that reduces algae cover thus has the potential to shift floating dock invertebrate communities towards those dominated by invasive species, which in turn increases the risk of invasion to nearby native habitats (Glasby and Connell 1999, Lambert and Lambert 2003, Ruiz et al. 2009, Simkanin et al. 2012).

Invasive species

Invasive colonial ascidians dominated communities without algae, possibly due to their ability to colonize open space (Stachowicz et al. 2002, Tyrrell and Byers 2007, Kremer et al. 2010, Janiak et al. 2013) and withstand physical disturbance (Altman and

Whitlatch 2007). They were also present in communities with algae, however, and are generally found to be more persistent in man-made floating dock versus natural rocky subtidal habitats (Glasby et al. 2007, Dafforn et al. 2012). Additional factors also structure communities in floating dock systems (Dafforn et al. 2012), which may have contributed to invasive invertebrate success. For example, substrates in floating dock systems are moving and positioned close to the water's surface. This contrasts to substrates in rocky subtidal habitats that are fixed and deeper in the water column. Indeed, invasive ascidians have been found in greater numbers on surfaces that are moving as opposed to fixed (Glasby 2007, Dafforn et al 2009, Simkanin et al. 2012), and on substrates that are close to the water's surface as opposed to deeper in the water column (Glasby et al. 2007, Dafforn et al. 2009). Furthermore, invasive ascidians do not appear to be regulated by the forces that regulate native taxa (Miller and Etter 2008). The dominance of floating dock communities by invasive species (Pederson et al. 2005, Dijkstra et al. 2007, Dijkstra and Harris 2009) complicates the comparison of algae-invertebrate interactions between these and rocky subtidal systems, especially given the different forces that regulate these taxa. However, marine invasions are on the rise (Ruiz et al. 2000, Bax et al. 2003) and thus identifying how these species enter and persist in communities is crucial.

Conclusions

The balance between heterotrophs and autotrophs in subtidal ecosystems has implications for marine productivity (Mineur et al. 2014, Strong et al. 2015): any

factor that disrupts the interactions between algae and invertebrates thus has the potential to impact this important ecosystem service. At 12 m depth in the rocky subtidal, light partitions algae and invertebrates between sunlit horizontal and darker vertical surfaces respectively but at shallower depths, the distribution of these taxa becomes more complex. On vertical surfaces at 1 m depth, for instance, high light levels promote algae growth but invertebrates also persist as vertical surfaces experience increased flow, decreased sedimentation, and decreased disturbance, all of which promote invertebrate growth. Organisms from both kingdoms thus co-occur. Although algae does not dominate over invertebrates at shallower depths, it does play an important role in invasion resistance, experimental communities with algae supporting greater abundances of native invertebrate species, whereas those without algae are characterized by greater abundances of invasive colonial ascidians.

Given 1) the importance of algae-invertebrate interactions for marine productivity and invasion resistance, 2) the complex nature of these interactions in terms of species identity, ontogenetic stage, and invasion status, and 3) the general interest to ecologists of organisms from two kingdoms competing for the same resource, factors that control the balance between autotrophs and heterotrophs in subtidal ecosystems warrant further research.

References

Airoldi, L., and F. Cinelli. 1997. Effects of sedimentation on subtidal macroalgal assemblages: an experimental study from a Mediterranean rocky shore. Journal of Experimental Marine Biology and Ecology 215:269–288.

Altman, S., and R. B. Whitlatch. 2007. Effects of small-scale disturbance on invasion success in marine communities. Journal of Experimental Marine Biology and Ecology 342:15–29.

Bak, R. P. M., and J. L. A Borsboom. 1984. Allelopathic interaction between a reef coelenterate and benthic algae. Oecologia 63:194–198.

Bax, N., A. Williamson, M. Aguero, E. Gonzalez, and W. Geeves. 2003. Marine invasive alien species: a threat to global biodiversity. Marine Policy 27:313–323.

Baynes, T. W. 1999. Factors structuring a subtidal encrusting community in the southern Gulf of California. Bulletin of Marine Science 64:419–450.

Bingham, B. L., and C. M. Young. 1991. Influence of sponges on invertebrate recruitment: a field test of allelopathy. Marine Biology 109:19–26.

Bolser, R. C. and M. E. Hay. 1996. Are tropical plants better defended? Palatability and defenses of temperate vs. tropical seaweeds. Ecology 77:2269–2286.

Box, S. J., and P. J. Mumby. 2007. Effect of macroalgal competition on growth and survival of juvenile Caribbean corals. Marine Ecology Progress Series 342:139–149.

Breitburg, D. L. 1984. Residual effects of grazing: inhibition of competitor recruitment by encrusting coralline algae. Ecology 65:136–1143.

Brodie J., C. J. Williamson, D. A. Smale, N. A. Kamenos, N. Mieszkowska, R. Santos, M. Cunliffe, M. Steinke, C, Yesson, K. M. Anderson, V. Asnaghi, C. Brownlee, H. L. Burdett, M. T. Burrows, S. Collins, P. J. Donohue, B. Harvey, A. Foggo, F. Noisette, J. Nunes, F. Ragazzola, J. A. Raven, D. N. Schmidt, D. Suggett, M. Teichberg, and J. M. Hall-Spencer. 2014. The future of the northeast Atlantic benthic flora in a high CO^2 world. Ecology and Evolution 4:2787–2798.

Buss, L. W., and J. B. C. Jackson. 1979. Competitive networks: nontransitive competitive relationships in cryptic coral reef environments. American Naturalist 113:223–234.

Clements, F. E. 1916. Plant succession: an analysis of the development of vegetation. Carnegie Institution of Washington, Washington D. C., USA.

Connell, J. H., T. P. Hughes, and C. C. Wallace. 1997. A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. Ecological Monographs 67:461–488.

Connell, J. H., and R. O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. American Naturalist 111:1119–1144.

Connell, S. D. 1999. Effects of surface orientation on the cover of epibiota. Biofouling 14:219–226.

Connell, S. D. 2003. Negative effects overpower the positive of kelp to exclude invertebrates from the understorey community. Oecologia 137:97–103.

Coyer, J. A., R. F. Ambrose, J. M. Engle, and J. C. Carroll. 1993). Interactions between corals and algae on a temperate zone rocky reef: mediation by sea urchins. Journal of Experimental Marine Biology and Ecology 167:21–37.

Crisp, D. J. 1974. Factors influencing the settlement of marine invertebrate larvae. Pages 177–265 in P. T. Grant, and A. M. Mackie, editors. Chemoreception in Marine Organisms. Academic Press, New York, New York, USA

Dafforn, K. A., T. M. Glasby, and E. L. Johnston. 2012. Comparing the invasibility of experimental 'reefs' with field observations of natural reefs and artificial structures. PLoS ONE 7:e38124.

Dafforn, K. A., E. L. Johnston, and T. M. Glasby. 2009. Shallow moving structures promote marine invader dominance. Biofouling 25:277–287

Davis, A. R., and G. A. White. 1994. Epibiosis in a guild of sessile subtidal invertebrates in south-eastern Australia: a quantitative survey. Journal of Experimental Marine Biology and Ecology 177:1–14.

Dean, T. A. 1981. Structural aspects of sessile invertebrates as organizing forces in an estuarine fouling community. Journal of Experimental Marine Biology and Ecology 53:163–180.

Diaz-Pulido, G., S. Harii, L. J. McCook, and O. Hoegh-Guldberg. 2010. The impact of benthic algae on the settlement of a reef-building coral. Coral Reefs 29:203–208.

Dijkstra, J. A., and L. G. Harris. 2009. Maintenance of diversity altered by a shift in dominant species: implications for species coexistence. Marine Ecology Progress Series 387:71–80.

Dijkstra J., L. G. Harris, and E. Westerman. 2007. Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. Journal of Experimental Marine Biology and Ecology 342:61–68.

Dixon, J., S. C. Schroeter, and J. Kastendiek. 1981. Effects of the encrusting bryozoan, *Membranipora membranacea*, on the loss of blades and fronds by the giant kelp, *Macrocystis pyrifera* (Laminariales). Journal of Phycology 17:341–345.

Dobretsov, S. V. 1999. Effects of macroalgae and biofilm on settlement of blue mussel (*Mytilus edulis* L.) larvae. Biofouling 14:153–165.

Duffy, J. E., and M. E. Hay. 2000. Strong impacts of grazing amphipods on the organization of a benthic community. Ecological monographs 70:237–263.

Duggins, D. O., J. E. Eckman, and T. A. Sewell. 1990. Ecology of understory kelp environments. II. Effects of kelps on recruitment of benthic invertebrates. Journal of Experimental Marine Biology and Ecology 143:27–45.

Dyrynda, P. E. J. 1986. Defensive strategies of modular organisms. Philosophical Transactions of the Royal Society of London B: Biological Sciences 313:227–243.

Engel, S., and J. R. Pawlik. 2000. Allelopathic activities of sponge extracts. Marine Ecology Progress Series 207:273–281.

Fraschetti, S., A. Giangrande, A. Terlizzi, M. Miglietta, L. Della Tommasa, and F. Boero. 2002. Spatio-temporal variation of hydroids and polychaetes associated with *Cystoseira amentacea* (Fucales: Phaeophyceae). Marine Biology 140:949–957.

Genovese, S. J., and J. D. Witman. 1999. Interactive effects of flow speed and particle concentration on growth rates of an active suspension feeder. Limnology and Oceanography 44:1120–1131.

Gili, J. M., and R. Coma. 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology and Evolution 13:316–321.

Glasby, T., and S. Connell. 2001. Orientation and position of substrata have large effects on epibiotic assemblages. Marine Ecology-Progress Series 214:127–135.

Glasby, T. M., and S. D. Connell. 1999. Urban Structures as Marine Habitats. Ambio 28:595–598.

Glasby, T.M., S. D. Connell, M. Holloway, and C. Hewitt. 2007. Nonindigenous biota on artificial structures: could habitat creation facilitate biological invasions? Marine Biology 151:887–895.

Grant, W. S. 1977. High intertidal community organization on a rocky headland in Maine, USA. Marine Biology 44:15–25.

Haas, A. F., C. E. Nelson, L. W. Kelly, C. A. Carlson, F. Rohwer, J. J. Leichter, A. Wyatt, and J. E. Smith. 2011. Effects of coral reef benthic primary producers on dissolved organic carbon and microbial activity. PLoS ONE 6:e27973.

Hadfield, M. G., and V. J. Paul. 2001. Natural chemical cues for settlement and metamorphosis of marine invertebrate larvae. Pages 431–461 in B. B. McClintock and B. J. Baker, editors. Marine Chemical Ecology. CRC Press, Boca Raton, Florida, USA.

Hay, M. E. 1996. Marine chemical ecology: what's known and what's next? Journal of Experimental Marine Biology and Ecology 200:103–134.

Hughes, T. P. 1989. Community structure and diversity of coral reefs: the role of history. Ecology 70:275–279.

Hughes, T. P. 1994. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science 265:1547–1551.

Irving, A., and S. Connell. 2002a. Sedimentation and light penetration interact to maintain heterogeneity of subtidal habitats: algal versus invertebrate dominated assemblages. Marine Ecology Progress Series 245:83–91.

Irving, A. D., and S. D. Connell. 2002b. Interactive effects of sedimentation and microtopography on the abundance of subtidal turf-forming algae. Phycologia 41:517–522.

Jackson, J. B. C., and L. Buss. 1975. Allelopathy and spatial competition among coral reef invertebrates. Proceedings of the National Academy of Sciences 72:5160–5163.

Janiak, D. S., R. W. Osman, and R. B. Whitlatch. 2013. The role of species richness and spatial resources in the invasion success of the colonial ascidian *Didemnum vexillum* Kott, 2002 in eastern Long Island Sound. Journal of Experimental Marine Biology and Ecology 443:12–20.

Jompa, J., and L. J. McCook. 2002. Effects of competition and herbivory on interactions between a hard coral and a brown alga. Journal of Experimental Marine Biology and Ecology 271:25–39.

Karlson, R. H., and R. W. Osman. 2012. Species composition and geographic distribution of invertebrates in fouling communities along the east coast of the USA: a regional perspective. Marine Ecology Progress Series 458:255–268.

Kennelly, S. J. 1989. Effects of kelp canopies on understory species due to shade and scour. Marine Ecology Progress Series 50:215–224.

Kremer, L. P., R. M. Rocha, and J. J. Roper. 2010. An experimental test of colonization ability in the potentially invasive *Didemnum perlucidum* (Tunicata, Ascidiacea). Biological invasions 12:1581–1590.

Lambert, C., and G. Lambert. 2003. Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Marine Ecology Progress Series 259:145–161.

Leichter, J. J., and J. D. Witman. 1997. Water flow over subtidal rock walls: relation to distributions and growth rates of sessile suspension feeders in the Gulf of Maine. Water flow and growth rates. Journal of Experimental Marine Biology and Ecology 209:293–307.

Leonard, G. H. 1999. Positive and negative effects of intertidal algal canopies on recruitment and survival of barnacles. Marine Ecology Progress Series 178:241–249.

Lewis, S. M. 1986. The role of herbivorous fishes in the organization at a coral reef community. Ecological Monographs 56:183–200.

McCook, L. 2001. Competition between corals and algal turfs along a gradient of terrestrial influence in the nearshore central Great Barrier Reef. Coral Reefs 19:419–425.

Miller, R. J., and R. J. Etter. 2008. Shading facilitates sessile invertebrate dominance in the rocky subtidal Gulf of Maine. Ecology 89:452–462.

Miller, R. J., and R. J. Etter. 2011. Rock walls: small-scale diversity hotspots in the subtidal Gulf of Maine. Marine Ecology Progress Series 425:153–165.

Mineur, F., J. Assis, A. J. Davies, A. H. Engelen, F. Fernandes, E. Malta, T. Thibaut, T. Van Nguyen, F. Vaz-Pinto, S. Vranken, E. A. Serrão, and O. De Clerck. 2014. European seaweeds under pressure: Consequences for communities and ecosystem functioning. Journal of Sea Research: http://dx.doi.org/10.1016/j.seares.2014.11.004

Morrow, K. M., and R. C. Carpenter. 2008. Macroalgal morphology mediates particle capture by the corallimorpharian *Corynactis californica*. Marine Biology 155:273–280.

Norris, J. N., and W. Fenical. 1982. Chemical defense in tropical marine algae. Pages 417–431 in K. Rutzler and I. G. McIntyre, editors. The Atlantic Barrier Reef ecosystem at Carrie Bow Cay, Belize, 1, structure and communities. Smithsonian Contributions to Marine Science 18:1–131.

Nugues, M. M., G. W. Smith, R. J. Hooidonk, M. I. Seabra, and R. P. Bak. 2004. Algal contact as a trigger for coral disease. Ecology letters 7:919–923.

Nuges, M. M., and A. M. Szmant. 2006. Coral settlement onto *Halimeda opuntia*: a fatal attraction to an ephemeral substrate? Coral Reefs 25:585–591.

Odum, E. P. 1969. The strategy of ecosystem development. Science 164:262–270.

Oksanen, J. F., G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner, H. 2012. Vegan: Community Ecology Package. R package version 2.0-4. http://CRAN.Rproject.org/package=vegan

Osman, R. W. 1977. Establishment and development of a marine epifaunal community. Ecological Monographs 47:37–63.

Osman R. W., and R. B. Whitlatch. 1995. Predation on early ontogenic life stage and its effect on recruitment into a marine epifaunal community. Marine Ecology Progress Series 117:111–126.

Pawlik, J. R. 1992. Chemical ecology of the settlement of benthic marine invertebrates. Oceanography and Marine Biology: an annual review 30:273–335.

Pederson, J. P., R. Bullock, J. Carlton, J. Dijkstra, N. Dobroski, P. Dyrynda, R. Fisher, L. Harris, N. Hobbs, G Lambert, E. Lazo-Wasem, A. Mathieson, M. Miglietta, J. Smith, J. Smith III, and M. Tyrrell. 2005. Marine Invaders in the Northeast: Rapid Assessment Survey of Non–native and native Marine Species of Floating Dock Communities. MIT Sea Grant College Program, Cambridge, Massachusetts, USA.

Pisut, D. P., and J. R. Pawlik. 2002. Anti-predatory chemical defenses of ascidians: secondary metabolites or inorganic acids? Journal of Experimental Marine Biology and Ecology 270:203–214.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Raimondi, P. T., and A. N. Morse. 2000. The consequences of complex larval behavior in a coral. Ecology 81:3193–3211.

Rasher, D. B., and M. E. Hay. 2010. Chemically rich seaweeds poison corals when not controlled by herbivores. Proceedings of the National Academy of Sciences 107:9683–9688.

Rasher, D. B., E. P. Stout, S. Engel, J. Kubanek, and M. E. Hay. 2011. Macroalgal terpenes function as allelopathic agents against reef corals. Proceedings of the National Academy of Sciences 108:17726–17731.

River, G. F., and P. J. Edmunds. 2001. Mechanisms of interaction between macroalgae and scleractinians on a coral reef in Jamaica. Journal of Experimental Marine Biology and Ecology 261:159–172.

Ruiz, G. M., P. W. Fofonoff, J. T. Carlton, M. J. Wonham, and A. H. Hines. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annual Review of Ecology and Systematics 31:481–531.

Ruiz, G. M., A. L. Freestone, P. W. Fofonoffand C. Simkanin. 2009. Habitat distribution and heterogeneity in marine invasion dynamics: the importance of hard substrate and artificial structure. Pages 321–332 in M. Wahl, editor. Marine Hard Bottom Communities. Springer-Verlag, Berlin Heidelberg, Germany.

Saier, B., and A. S. Chapman. 2004. Crusts of the alien bryozoan *Membranipora membranacea* can negatively impact spore output from native kelps (*Laminaria longicruris)*. Botanica Marina 47:265–271.

Santelices, B., and E. Martinez. 1988. Effects of filter-feeders and grazers on algal settlement and growth in mussel beds. Journal of Experimental Marine Biology and Ecology 118:281–306.

Schiel, D. R. 2009. Multiple Stressors and Disturbances. Pages 281–294 in M. Wahl, editor. Marine Hard Bottom Marine Hard Bottom Communities. Springer-Verlag, Berlin Heidelberg, Germany.

Sebens, K. P. 1983. Settlement and metamorphosis of a temperate soft-coral larva (*Alcyonium slderium* Verril): Induction by crustose algae. Biological Bulletin 165:286–304.

Sebens, K. P. 1985. The Ecology of the Rocky Subtidal Zone: The subtidal rock surfaces in New England support a diversity of encrusting species that compete for space and that recolonize patches cleared through predation. American Scientist 73:548–557.

Sebens, K. P. 1986. Community ecology of vertical rock walls in the Gulf of Maine, U.S.A.: small-scale processes and alternative community states. Pages 346–371 in P. G. Moore and R. Seed, editors. The ecology of rocky coasts. Hodder and Stoughton Educational Press, Kent, UK.

Sebens, K. P., and J. S. Miles. 1988. Sweeper tentacles in a gorgonian octocoral: morphological modifications for interference competition. The Biological Bulletin 175: 378–387.

Simkanin, C., I. C. Davidson, J. D. Dower, G. Jamieson, and T. W. Therriault. 2012. Anthropogenic structures and the infiltration of natural benthos by invasive ascidians. Marine Ecology 33:499–511.

Smith, J. E., M. Shaw, R. A. Edwards, D. Obura, O. Pantos, E. Sala, S. A. Sandin, S. Smriga, M. Hatay, and F. L. Rohwer. 2006. Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. Ecology Letters 9:835–845.

Stachowicz, J. J., and R. B. Whitlatch. 2005. Multiple mutualists provide complementary benefits to their seaweed host. Ecology 86:2418–2427.

Stachowicz, J. J., H. Fried, R. W. Osman, and R. B. Whitlatch. 2002. Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. Ecology 83:2575–2590.

Steneck, R. S., and M. N. Dethier. 1994. A functional group approach to the structure of algal-dominated communities. Oikos 69:476–498.

Steneck, R. S., M. H. Graham, B. J. Bourque, D. Corbett, J. M. Erlandson, J. A. Estes, and M. J. Tegner. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental conservation 29:436–459.

Sutherland, J. P., and R. H. Karlson. 1977. Development and stability of the fouling community at Beaufort, N.C. Ecological Monographs 47:425–446.

Sweet, M. J., J. C. Bythell, and M. M. Nugues. 2013. Algae as reservoirs for coral pathogens. PLoS ONE 8:e69717.

Thurber, R. V., D. E. Burkepile, A. M. Correa, A. R. Thurber, A. A. Shantz, R. Welsh, C. Pritchard, and S. Rosales. 2012. Macroalgae decrease growth and alter microbial community structure of the reef-building coral, *Porites astreoides*. PLoS ONE 7:e44246

Titlyanov, E. A., I. M. Yakovleva, and T. V. Titlyanova. 2007. Interaction between benthic algae (*Lyngbya bouillonii, Dictyota dichotoma*) and scleractinian coral *Porites lutea* in direct contact. Journal of Experimental Marine Biology and Ecology 342:282–291.

Todd, C. D., and S. J. Turner. 1986. Ecology of intertidal and sublittoral cryptic epifaunal assemblages. I. Experimental rationale and the analysis of larval settlement. Journal of Experimental Marine Biology and Ecology 99:199–231.

Tyrrell, M. C., and J. E. Byers. 2007. Do artificial substrates favor nonindigenous fouling species over native species? Journal of Experimental Marine Biology and Ecology 342:54–60.

van Steveninck, E. D. R., L. L. Van Mulekom, and A. M. Breeman. 1988. Growth inhibition of *Lobophora variegata* (Lamouroux) Womersley by scleractinian corals. Journal of Experimental Marine Biology and Ecology 115:169–178.

Vance, T., L. Lauterbach, M. Lenz, M. Wahl, R. A. Sanderson, and J. C. Thomason. 2009. Rapid invasion and ecological interactions of *Diplosoma listerianum* in the North Sea, UK. Marine Biodiversity Records 2:e59.

Vermeij, M. J. A., J. E. Smith, C. M. Smith, R. V. Thurber, and S. A. Sandin. 2009. Survival and settlement success of coral planulae: independent and synergistic effects of macroalgae and microbes. Oecologia 159:325–336.

Walker, D. I., and G. A. Kendrick. 1998. Threats to macroalgal diversity: marine habitat destruction and fragmentation, pollution and introduced species. Botanica Marina 41:105–112.

Warkus, E., M. Wagstaff, S. Morello, and R. Etter. 2011, February. Do macroalgae use allelochemicals to outcompete invertebrates for space in the Gulf of Maine? Poster session presented at the meeting of the American Association for the Advancement of Science, Washington, DC.

Weinberg, S. 1978. Mediterranean octocorallian communities and the abiotic environment. Marine Biology 49:41–57.

Withers, R. G., and C. H. Thorp. 1977. Studies on the shallow, sublittoral epibenthos of Langstone Harbour, Hampshire, using settlement panels. Pages 595–604 in B. F. Keegan, P. J. S. Boaden, and P. O. Ceidigh, editors. Biology of Benthic Organisms. Pergamon Press, London, UK.

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

Witman, J. D. 1987. Subtidal coexistence: storms, grazing, mutualism, and the zonation of kelps and mussels. Ecological Monographs 57:167–187.

Witman, J. D., and R. A. Cooper. 1983. Disturbance and contrasting patterns of population structure in the brachiopod *Terebratulina septentrionalis* (Couthouy) from two subtidal habitats. Journal of Experimental Marine Biology and Ecology 73:57-79.

Witman, J. D., and P. K. Dayton. 2001. Rocky subtidal communities. Pages 339–366 in M. Bertness, S. Gaines, and M. Hay, editors. Marine Community Ecology. Sinauer, Sunderland, Massachusetts, USA.

Woodin, S. A., and Jackson, J. B. C. 1979. Interphyletic competition among marine benthos. American Zoologist 19:1029–1043.

Young, C. M., and F. S. Chia. 1984. Microhabitat-associated variability in survival and growth of subtidal solitary ascidians during the first 21 days after settlement. Marine Biology 81:61–68.

Table 3.1. Species list for all algae and sessile invertebrates found on settlement plates at Dorchester Yacht Club.

Species		
Red algae	Porifera	Bryozoa
Aglaothamnion halliae	*Halichondria panicea*	*Bugula neritina*
Ceramium virgatum	*Halichondria permolis*	Encrusting bryozoan
Chondrus crispus		
Polysiphonia elongata	Cnidaria	Polychaeta
	Diadumene lineata	Tube complex
Green algae	Mollusca	Ascidiacea
Acrosiphonia arcta	*Crepidula plana*	*Ascidiella aspersa*
Cladophora sericea	*Mytilus edulis*	*Ciona intestinalis*
Ulva intestinalis	*Ostrea edulis*	*Molgula manhattensis*
Ulva linza	*Tellina agilis*	*Styela clava*
Ulva lattuca		*Botryllus schlosseri*
Spongomorpha aeruginosa	Arthropoda	*Botryllus violaceus*
	Balanus balanus	*Diplosoma listerianum*
		Didemnum vexillum

Table 3.2. Starting community manipulations for Displacement, Recruits, and Settlers experiments. 3 x 3 cm tiles were pre-seeded with algae or invertebrates, or kept blank. Tiles (12 or 13 of each type) were then randomly combined to make starting communities.

	Algae			Invertebrates			Blank space
	In starting community?	Life stage	Species	In starting community?	Life stage	Species	In starting community?
Displacement							
Algae	✔	Adult	All	✗			✔
Inverts	✗			✔	Adult	All	✔
Inverts and algae	✔	Adult	All	✔	Adult	All	✗
Inverts and algae removal	✗			✔	Adult	All	✔
Recruits							
Algae normal	✔	Adult	*U. intestinalis*	✔	Recruits	All	✗
Algae cut	✔	Adult	*U. intestinalis*	✔	Recruits	All	✗
Algae mimic	✔	Adult	Plastic mimic	✔	Recruits	All	✗
No algae	✗			✔	Recruits	All	✔
Settlers							
Algae normal	✔	Adult	*U. linza*	✗			✔
Algae cut	✔	Adult	*U. linza*	✗			✔
Algae mimic	✔	Adult	Plastic mimic	✗			✔
No algae	✗			✗			✔

Table 3.3. Results of MANOVAs for Assembly, Displacement, Recruits, and Settlers experiments. Percent cover data were logit transformed. Significant p-values are shown in bold.

Source	df	Wilks	Approx F	num df	den df	Pr (>F)
Assembly						
Treatment	1	0.96	0.10	2	5	0.904
Residuals	6					
Displacement						
Treatment	3	0.65	0.89	6	22	0.522
Residuals	12					
Recruits						
Treatment	3	0.90	0.20	6	22	0.974
Residuals	12					
Settlers						
Treatment	3	0.002	69.89	6	22	**< 0.001**
Residuals	12					

Table 3.4. Results of univariate ANOVAs for percent cover and diversity of primary space occupying sessile invertebrates for the Assembly, Displacement, Recruits, and Settlers experiments. Percent cover data was logit transformed. Diversity was calculated using Shannon's diversity index. Significant p-values with $\alpha = 0.025$ are shown in bold.

	Percent cover				Diversity			
Source	df	SS	F	p	df	SS	F	p
Assembly								
Treatment	1	0.33	0.20	0.668	1	0.005	0.06	0.809
Residuals	6	9.78			6	0.43		
Displacement								
Treatment	3	0.67	0.68	0.583	3	0.12	1.23	0.342
Residuals	12	3.95			12	0.38		
Recruits								
Treatment	3	0.31	0.26	0.85	3	0.004	0.14	0.934
Residuals	12	4.78			12	0.11		
Settlers								
Treatment	3	42.96	726.60	**< 0.001**	3	0.82	6.49	**0.007**
Residual	12	0.24			12	0.50		

Table 3.5. Results of PERMANOVA, using the Bray-Curtis distance metric, for community composition of primary space occupying sessile invertebrates for the Assembly, Displacement, Recruits, and Settlers experiments. Significant p-values are shown in bold.

Source	df	SS	Pseudo-F	p
Assembly				
Treatment	1	0.22	2.47	**0.03**
Residuals	6	0.54		
Displacement				
Treatment	3	0.822	3.27	**0.001**
Residuals	12	1.01		
Recruits				
Treatment	3	0.18	0.52	0.802
Residuals	12	1.40		
Settlers				
Treatment	3	2.84	46.76	**0.001**
Residual	12	0.24		

Table 3.6. Percent cover of primary space occupying sessile invertebrate taxa (mean ± SE) in Assembly, Displacement, Recruits, and Settlers experiments by treatment (continued overleaf).

	Halicondria panicea	*Mytilus edulis*	*Ostrea edulis*	*Crepidula plana*	*Balanus balanus*	Encrusting bryozoan	*Bugula neritina*	Algae, mud, tube complex
Assembly								
Natural community		9.25 ± 3.99	5.88 ± 5.88	19.00 ± 2.18	10.38 ± 0.88			16.88 ± 1.32
Algae removal		1.63 ± 0.56	0.88 ± 0.88	7.13 ± 2.71	13.13 ± 4.60	0.13 ± 0.13		9.75 ± 1.38
Displacement								
Algae	0.37 ± 0.38	4.12 ± 0.99	7.62 ± 1.25	11.50 ± 2.12	9.85 ± 3.95	1.37 ± 0.85		21.62 ± 2.67
Inverts	1.00 ± 0.71	3.87 ± 0.97		10.25 ± 2.71	10.00 ± 6.45	5.50 ± 2.59		23.75 ± 3.12
Inverts and algae	0.38 ± 0.24	4.88 ± 2.08	3.38 ± 2.61	6.63 ± 2.84	6.25 ± 2.66	1.5 ± 0.74		20.625 ± 1.85
Inverts and algae removal		0.50 ± 0.50	1.13 ± 0.66	6.00 ± 1.40	8.38 ± 4.49	0.63 ± 0.63	0.13 ± 0.13	7.25 ± 2.59
Recruits								
Algae normal						0.25 ± 0.25		
Algae cut						0.25 ± 0.25		
Algae mimic								
No algae								
Settlers								
Algae normal				0.13 ± 0.13				
Algae cut				0.13 ± 0.13				
Algae mimic								
No algae								

Table 3.6 continued.

	Ascidiella aspersa	*Ciona intestinalis*	*Styela clava*	*Botryllus schlosseri*	*Botrylloides violaceus*	*Diplosoma listerianum*	*Didemnum vexillum*
Assembly							
Natural community	1.75 ± 0.86	1.63 ± 0.56	1.50 ± 0.84	1.13 ± 0.97	3.75 ± 0.93		3.63 ± 1.07
Algae removal	1.13 ± 0.66		1.13 ± 0.43	2.88 ± 1.28	13.38 ± 7.85	4.38 ± 3.32	3.13 ± 1.66
Displacement							
Algae	2.75 ± 1.45	1.25 ± 0.72	5.12 ± 4.18	4.25 ± 1.64	2.75 ± 1.20		9.12 ± 3.41
Inverts	2.00 ± 0.98	4.75 ± 1.65	1.75 ± 1.09	1.75 ± 0.92	3.50 ± 1.70		14.12 ± 2.77
Inverts and algae	3.00 ± 1.47	1.75 ± 1.03	3.75 ± 1.96	1.13 ± 0/83	10.00 ± 1.58	3.13 ± 2.37	11.50 ± 0.96
inverts and algae removal	3.63 ± 3.46		1.50 ± 0.61	16.38 ± 5.36	16.00 ± 6.98	7.88 ± 2.01	3.25 ± 1.48
Recruits							
Algae normal				5.75 ± 1.09	5.63 ± 2.07		
Algae cut				7.25 ± 3.26	4.75 ± 1.93		
Algae mimic				4.00 ± 0.84	7.25 ± 2.15		
No algae				3.38 ± 1.21	4.75 ± 1.23		
Settlers							
Algae normal				9.75 ± 2.29	7.13 ± 0.83	50.75 ± 2.86	
Algae cut			0.13 ± 0.13	7.63 ± 1.96	5.88 ± 1.09	56.88 ± 3.78	
Algae mimic				5.25 ± 0.92	1.63 ± 0.24	9.88 ± 0.90	
No algae					0.38 ± 0.24	2.38 ± 0.43	

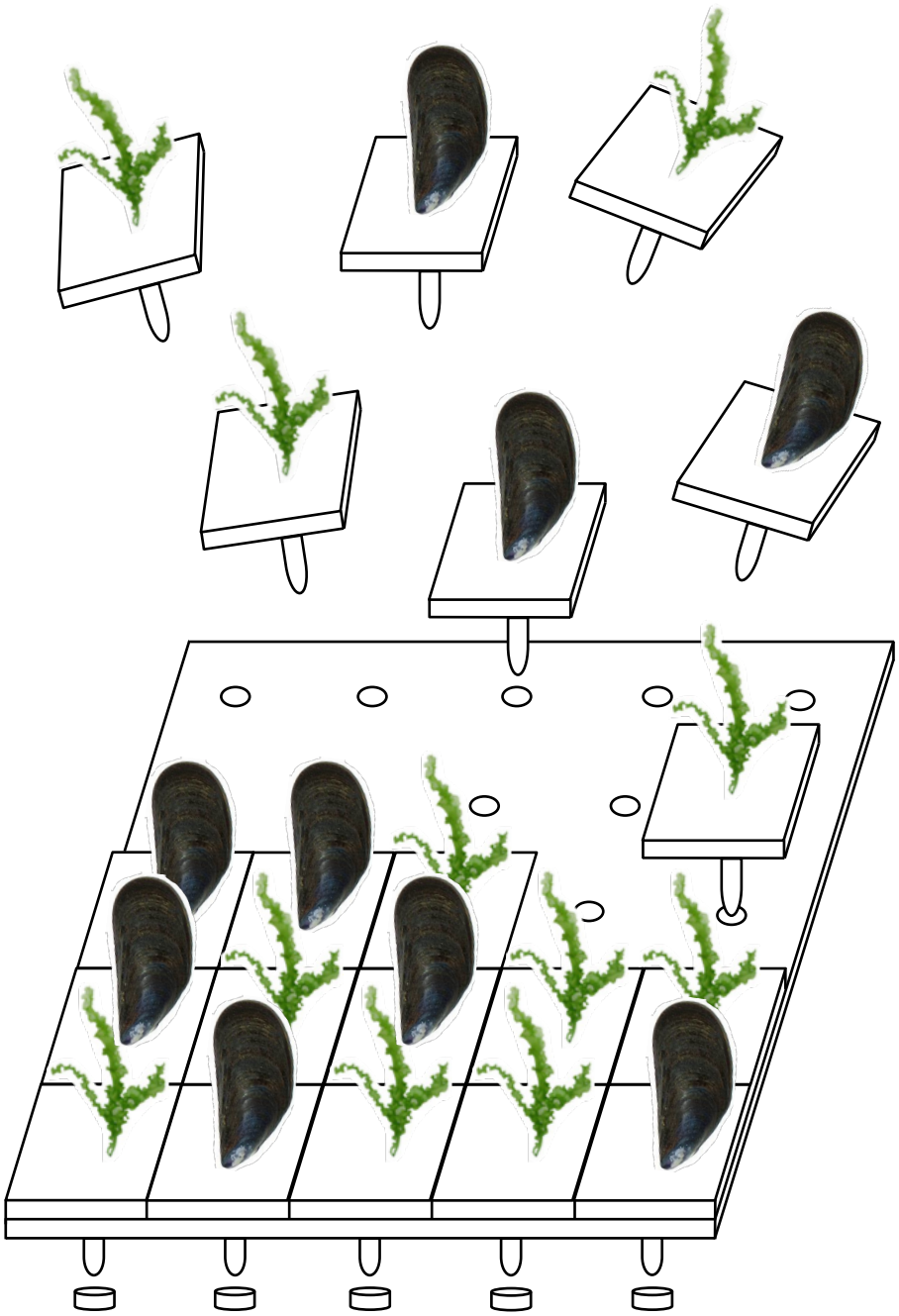

Figure 3.1. Starting community manipulations. Small 3 x 3 cm tiles pre-seeded with algae or invertebrates were assembled into a 15 x 15 starting community. This figure represents the algae and invertebrates treatment in the Displacement experiment. This diagram is a schematic only, and multiple species were present on the tiles.

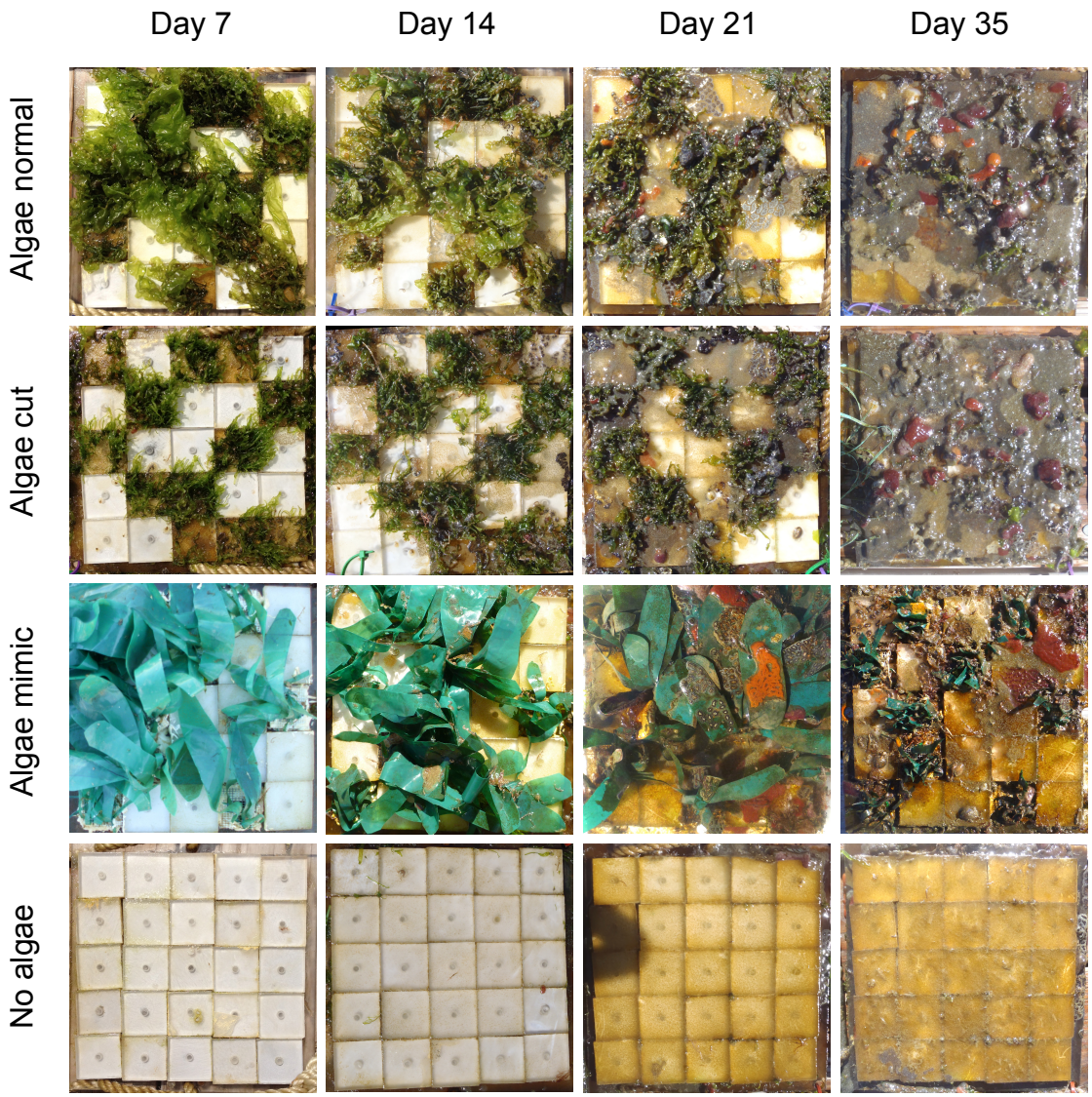

Figure 3.2. Treatments in the Settlers experiment through time. The figure shows the manipulations for the algae cut and the algae mimic treatment. It also shows that at the end of the experiment, day 35, the algae normal and algae cut treatments had high space coverage by colonial ascidians, the algae mimic treatment had intermediate coverage, and the no algae treatment had very little to no coverage.

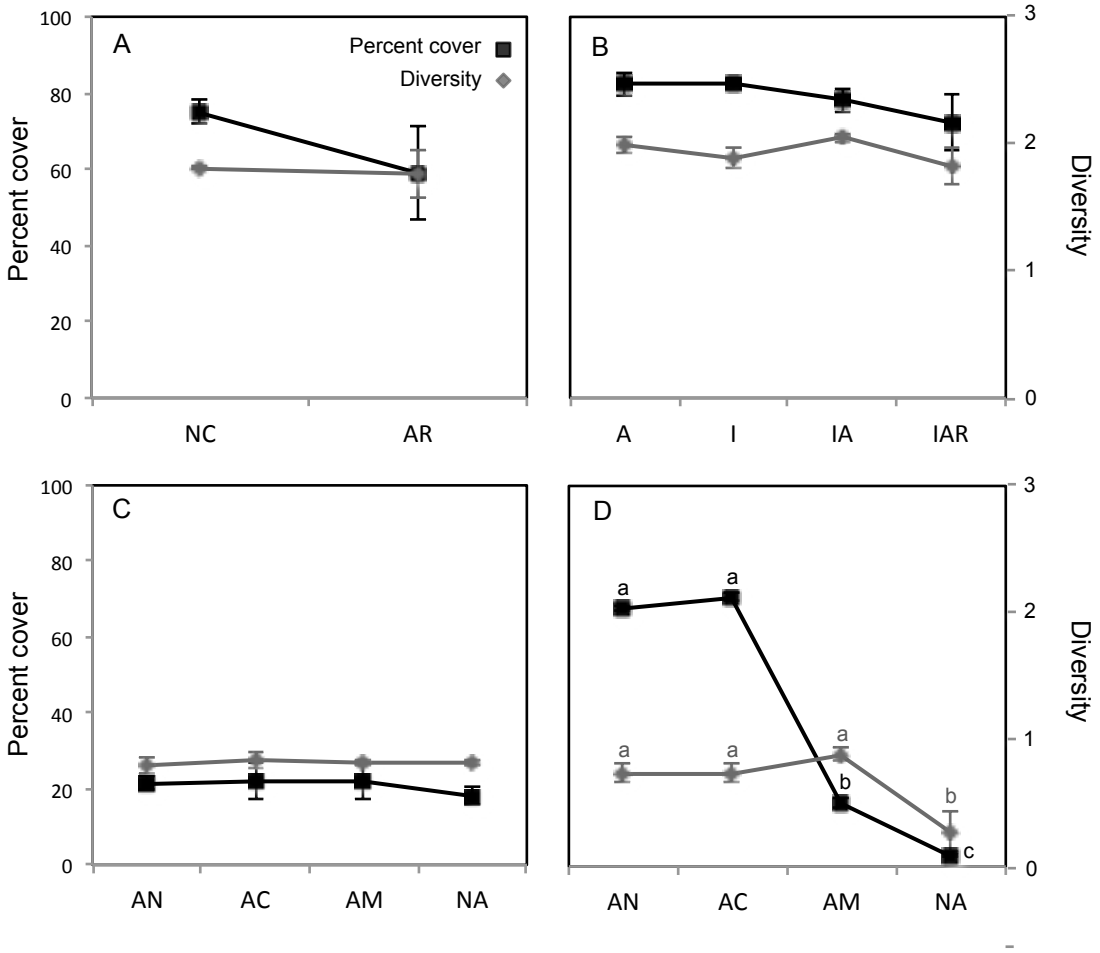

Figure 3.3. Centroid plots showing percent cover and diversity of primary space occupying sessile invertebrates, for all treatments in the A) Assembly, B) Displacement, C) Recruits, and D) Settlers experiments. Treatments are as follows: natural community (NC) and algae removal (AR) in the Assembly experiment; algae (A), inverts (I), inverts and algae (IA), inverts and algae removal (IAR) in the Displacement experiment; and algae normal (AN), algae cut (AC), algae mimic (AM), and no algae (NA) in the Recruits and Settlers experiments. Percent cover and diversity were not affected by the treatments in the Assembly, Displacement, and Recruits experiments, but were affected by the treatments in the Settlers experiment. Lowercase letters in plots D) denote the different groupings revealed by Tukey's HSD test. Error bars represent one standard error.

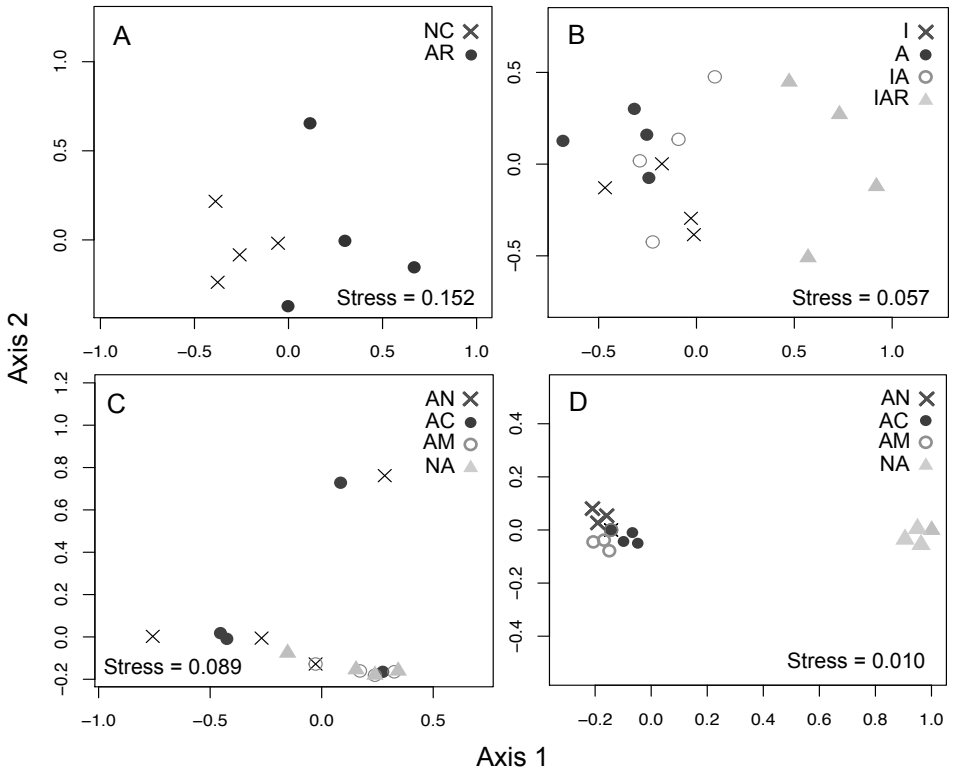

Figure 3.4. NMDS ordination plots of community replicates separated by treatments using Bray-Curtis dissimilarities for communities of space occupying sessile invertebrates for the A) Assembly, B) Displacement, C) Recruits, and D) Barrier experiments. See Fig. 3.3 for treatment abbreviations. Treatments affected community composition in the Assembly, Displacement, and Settlers experiments but not in the Recruits experiment.

CHAPTER 4

THE RESPONSE OF AN INVASIVE SPECIES, *BOTRYLLOIDES VIOLACEUS*, TO NOVEL HABITATS IN THE GULF OF MAINE

Abstract

Species invasions are characterized by range expansions during which invasive species respond to new environmental conditions. Any resulting changes in life history traits may be due to phenotypic plasticity, local adaptation, or both. Identifying differences in life history traits is the first step in identifying an evolutionary basis to invasions. It can also help determine how invaders integrate themselves into local ecosystems, which is a poorly understood aspect of marine ecology and management. In the Gulf of Maine, the invasive ascidian *Botrylloides violaceus* is found on man-made substrates and has also entered natural subtidal habitats. To investigate if *B. violaceus* is changing in new habitats, colonies were grown on polycarbonate plates in floating dock, eelgrass bed, and rocky subtidal habitats, and life history traits were monitored from settlement until death from June 2012 to July 2013. This was replicated at three sites along the Massachusetts coast. Settlement density differed among habitats and was highest in floating dock, lower in eelgrass bed, and least in rocky subtidal habitats. Terminal age was not different among habitats. Terminal size and maximum growth rates were higher in floating dock and eelgrass bed habitats than in the rocky subtidal. The

duration of colony regression did not differ among habitats, but the pattern of regression varied considerably within and among habitats. In floating dock habitats, distinct, seasonal cohorts were observed. These results suggest that *B. violaceus* is most successful in man-made versus natural habitats. Its integration into natural habitats may be in an early phase, however, with *B. violaceus* still posing a threat to native species and ecosystems.

Introduction

Organisms have been expanding their ranges for millennia (Vermeij 1991, Webb 1991), but the rate at which humans are transporting species today far exceeds their natural spread (Drake 1989). Second only to land use changes, species invasions are thought to be one of the greatest causes of modern species extinctions (D'Antonio and Vitousek 1992, Vitousek et al. 1997). Additional impacts of invasive species include transport of pathogens and disease, rapid and extensive hybridization between invaders and native species, and unknown effects on native species and ecosystems (Grosholz 2002). While invasions of animals and plants on land are well characterized (Elton 2000), less is known about them in marine systems (Steneck and Carlton 2001). How invaders integrate themselves into marine communities and the scale of their impact, for example, is an important but poorly understood aspect of marine ecology and resource management (Whitlatch et al. 1995).

In the Gulf of Maine, a highly degraded ocean region (Halpern et al. 2008), invasive species are an increasing problem (Pederson et al. 2005, Dijkstra et al. 2007). In

benthic habitats, the invasive colonial ascidian, *Botrylloides violaceus,* is a major occupier of space (Dijkstra et al. 2007) and poses problems for native diversity (Dijkstra and Harris 2009) and the aquaculture industry (Arenas et al. 2011). Invasive ascidians are transported around the world on boat hulls, from which they can colonize floating docks and subsequently expand into natural habitats (Glasby and Connell 1999, Lambert and Lambert 2003, Ruiz et al. 2009, Simkanin et al. 2012). Indeed, in the Gulf of Maine, *B. violaceus* can be found in floating dock (Pederson et al. 2005, Dijkstra et al. 2007, Dijkstra and Harris 2009), rocky subtidal (Sebens et al. 1997, Miller and Etter 2008, 2011), and eelgrass bed (Berman et al. 1992) habitats. Once in a new range or habitat, invasive species may then exhibit variation in life history traits in response to new environmental conditions (Hanfling and Kollmann 2002). Such variation can be a result of phenotypic plasticity, local adaptation, or both (Grosholz 2001).

Within habitats in the Gulf of Maine, the major life history traits of ascidians — growth, reproduction, and survival — are highly correlated with environmental variables between sites (Grosholz 2001, Yund and Stires 2002). Given the strong abiotic and biotic differences that exist among habitats, it is likely that life history traits will also vary (see Table 4.1). Worldwide, invasive marine invertebrates in general (Glasby et al. 2007, Ruiz et al. 2009, Dafforn et al. 2012), and invasive ascidians in specific (Lambert 2002, Marins 2010, Simkanin et al. 2012), occur in greater numbers in man-made versus natural habitats. Differences in settlement, growth, and survival among habitats may account for this pattern.

To test if life history traits of invasive ascidians differ among habitats, polycarbonate plates were deployed in floating dock, rocky subtidal, and eelgrass bed habitats. Life history traits of *B. violaceus* were monitored from settlement until senescence with the expectation of finding pronounced differences among habitats. Identifying any differences in life histories will elucidate how this species changes as it invades new habitats. This can, in turn, provide insights into the selective regimes acting on individual invaders as well as evolutionary processes that may be key in determining their success in novel ecosystems and regions. By quantifying these processes we can better predict the impacts of invasive species on native species diversity and ecosystems and seek to mitigate their ecological and economic costs.

Methods

Study species

B. violaceus is a colonial tunicate that forms sheet-like colonies composed of zooids embedded in a gelatinous, transparent tunic. Zooids are 2 to 4 mm in size, distributed in long irregular rows around a common exhalent siphon, and are connected by a common vascular network that extends throughout the colony (Carver et al. 2006). Different color morphs exist including white, yellow, orange, red, purple and black. *B. violaceus* is invasive to the Gulf of Maine and may have been introduced with oyster aquaculture in the Damariscotta River, Maine, in the early 1970s (Dijkstra et al. 2007). Believed to have originated in Japan (Saito et al. 1981), *B. violaceus* can now be found along the east coast of North America from the Canadian Maritimes to Virginia, on the

west coast from Alaska to Ensenada, Mexico, and in Europe (Lambert and Lambert 2003, Karlson and Osman 2012).

B. violaceus is very similar in colony development to *Botryllus schlosseri*, a well-studied confamilial taxon (Berrill 1947, Oka and Watanabe 1959). Botryllid ascidians are hermaphrodites and reproduce both asexually and sexually (Milkman 1967). A new colony forms when a sexually produced larva settles and metamorphoses into an oozoid (Grave and Woodbridge 1924). The colony then grows exponentially through several asexual cycles in which adult zooids give rise to, and are replaced by, more adult zooids (Milkman 1967). After about five to ten cycles, sexual reproduction begins (Grosberg 1988), which often coincides with terminal size (Harvell and Grosberg 1988). The sexual cycle is also synchronized and is locked in phase with the asexual cycle (Harvell and Grosberg 1988). The asexual cycles maintain the size of the colony and the sexual cycles result in the synchronized release of brooded larvae (Milkman 1967). After about ten of these cycles (Grosberg 1988), colonies senesce and regress (Chadwick-Furman and Weissman 1995). Little is known about colony regression in *B. violaceus* but in *B. schlosseri,* colony regression proceeds through four distinct phases and results in the death of all zooids (Chadwick-Furman and Weissman 1995). The phases are: 1) blood vessels narrow slowing blood flow, 2) zooids shrink and became densely pigmented, 3) groups of zooids become disconnected, and 4) the tunic softens and disintegrates, with a film of tunic material persisting for at least 1week after death marking the former extent of the colony.

Experimental design

Settlement density. To record settlement density of *B. violaceus* in different habitats, polycarbonate plates (20 x 20 cm, 0.5 cm thick) were deployed in floating dock, rocky subtidal, and eelgrass bed habitats. This was replicated at three sites along the Massachusetts coast, in Gloucester, Beverly and Sandwich (Figure 4.1). In total, 36 plates were deployed (three habitats x three sites x four replicate plates in each habitat-site combination). This is two-way experimental design with habitat type as a fixed factor with three levels (floating docks, rocky subtidal, and eelgrass beds), and replicate sites as a random factor (Gloucester, Beverly, and Sandwich).

Plates were attached to the undersides of floating docks using ropes tied to cleats on either side of the dock. Sufficient rope was left coiled at one end to allow a pulley system to operate (Figure 4.2A). In rocky subtidal habitats, patches of rock were scrubbed bare with a scraper and wire brush. A small mound of underwater epoxy putty (A-788 Splash Zone Compound; Kop-Coat, Rockaway, New Jersey, USA) was then attached with a dry wall anchor embedded. The putty was allowed to dry for three days, after which plates were attached by passing a stainless steel screw with a polycarbonate cap through the plate and by securing into the anchor (Figure 4.2B). In eelgrass bed habitats, plates were suspended in the water column using cinder blocks as anchors and empty laundry detergent bottles as floats (Figure 4.2C). In both rocky subtidal and eelgrass bed habitats plates were fixed and suspended, respectively, approximately 4 m below the mean low water level. Polycarbonate plates were used instead of natural substrate to standardize settlement area, as substrate size is known to affect colony size in

B. schlosseri (Harvell and Grosberg 1988). This does, however, remove the influence of substrate type.

Settlement density was recorded from June 2012 after first settlement was observed (see Appendix 4A) and continued through December 2012. At each sampling date, plates were photographed with an Olympus Stylus Tough 8010 camera. In floating dock habitats, plates were photographed by pulling them up onto docks. In eelgrass bed and rocky subtidal habitats, plates were photographed underwater with SCUBA. At each sampling date, plates were also scraped clean to allow room for additional settlement. From photographs, the number of recruits on each plate (400 cm^2) was counted at each sampling date and, at the end of the season, the cumulative number of recruits was calculated per replicate plate. Plates were sampled once a week. All habitats in each site were sampled on the same day. Gloucester and Beverly were sampled at the same time but Sandwich, being further away, was sampled on a different day. When weather did not permit SCUBA, underwater habitats were sampled at the next opportunity.

Life history traits. To record terminal age, terminal size, maximum growth rate and the duration of colony regression, colonies of *B. violaceus* were followed on a different set of polycarbonate plates (20 x 20 cm, 0.5 cm thick). These plates were also deployed in floating dock, rocky subtidal, and eelgrass bed habitats and replicated at three sites along the Massachusetts coast (Figure 4.1). For this study 72 plates were used (three habitats x three sites x eight replicate plates in each habitat-site combination). This is also a two-way experimental design with habitat type as a fixed factor with three levels

(floating docks, rocky subtidal, and eelgrass beds), and replicate sites as a random factor (Gloucester, Beverly, and Sandwich). Plates were secured in each habitat as above.

Life history traits were recorded from June 2012 after first settlement was observed (see Appendix 4A) and continued through October 2012 when the last *B. violaceus* colony had disappeared. After settlement, a *B. violaceus* recruit close to the center of each plate was selected for monitoring and, at this time, all other recruits were removed to allow for colony growth in the absence of competition. At each sampling date, colonies on plates were photographed with an Olympus Stylus Tough 8010 camera. Photographs were taken as above, i.e. in floating dock habitats, plates were photographed by pulling them up onto docks and, in eelgrass and rocky subtidal habitats, plates were photographed underwater with SCUBA. Again, at each sampling date all new recruits were removed to allow for continued colony growth in the absence of competition. Colonies were monitored by photography until no tissue remained. The sampling routine was as described above.

Terminal age, terminal size, maximum growth rate, and the duration of colony regression were calculated from photographs (Figure 4.3). Terminal age was recorded as the number of days between the date of settlement (estimated using a key of recruit stages (Bullard and Whitlatch 2004)) and the date at which the colony was last observed to be intact i.e. before the onset of senescence. Terminal size was estimated, using Image J (Rasband and US Image J 1997–2014), as the area attained on the date at which the colony was last observed to be intact before the onset of senescence. Maximum growth rate was calculated as the change in colony area (area calculated using Image J (Rasband

and US Image J 1997–2014)) between sampling dates during the exponential phase of growth. The duration of colony regression was measured as the number of days taken for the colony to disappear after the onset of senescence. Patterns of colony regression were observed.

Cohorts. In floating dock habitats, distinct seasonal cohorts were observed whereby the original cohort of eight recruits produced a new generation of colonies before senescence, and so on. These cohorts were further monitored by photography until July 2013, i.e. for a full year, to further identify differences between habitats. During this time eelgrass bed and rocky subtidal habitats were also monitored. During winter months when growth was slow, the sampling interval was reduced to a bimonthly schedules.

Algae removal experiment in the rocky subtidal. After observing differences in settlement density among habitats, a post-hoc experiment was performed to investigate low settlement on rocky subtidal plates. This experiment tested the hypothesis that algae prevent settlement of *B. violaceus* in subtidal rocky habitats, as algae are known to physically (River and Edmunds 2001, Box and Mumby 2007, Titlyanov et al. 2007) and chemically (Rasher and Hay 2010, Rasher et al. 2011) inhibit invertebrates and might prevent settlement or cause post-settlement mortality. Algae were removed continually from a 1 m^2 area surrounding eight polycarbonate plates and left intact around a further eight (control). Settlement was monitored throughout the recruitment and growth period, from June 2013 to December 2013.

Statistical analyses

To test for differences in settlement density among habitats, the cumulative number of recruits was analyzed using a two-way, balanced ANOVA with habitat as a fixed factor with three levels (floating docks, rocky subtidal, eelgrass beds) and site as a random factor with three levels (Gloucester, Beverly, Sandwich). This analysis was conducted using the aov function in R version 3.0.2 (R Core Team 2013). A post-hoc Tukey's HSD test was used to test for differences among habitat means.

To test for differences in terminal age, terminal size, maximum growth rate, and duration of colony regression among habitats, two-way, univariate, unbalanced ANOVAs were employed, with habitat as a fixed factor with three levels (floating docks, rocky subtidal, eelgrass beds) and site as a random factor with three levels (Gloucester, Beverly, Sandwich). Models were fit using the lmer function in the lme4 package (Bates et al. 2014) and the difference between group means assessed using the Anova function, with the default type II sums of squares (Lansgrud 2003), in the car package (Fox and Weisberg 2011) in R version 3.0.2 (R Core Team 2013). Model parameters were estimated using residual (or restricted) maximum likelihood (REML) and, as such, the output of the ANOVAs are Analysis of Deviance tables and Wald χ^2 test-statistics. In maximum likelihood, deviance is a measure of lack of fit between a model and the data, and can be used to compare models in an Analysis of Deviance, where deviance has a similar role to residual variance in ANOVA. The difference in deviances for the compared models follows an approximate chi-squared distribution with *k*-degrees of freedom. The Wald χ^2 test statistic is a result of comparing the maximum likelihood

estimate of the parameters of interest to the proposed value. In all ANOVAs, a Bonferroni-corrected significance level of 0.0125 was used, followed by Tukey's HSD to test for the differences among habitat means. Settlement density, terminal size and maximum growth rate were log transformed. Terminal age and duration of colony regression were not transformed. Normality and homogeneity of variances were visualized at the replicate and group level using Q-Q plots and plots of residual versus fitted values respectively.

Patterns of colony regression are described, as are patterns of cohort dynamics. The algae removal experiment in the rocky subtidal was not statistically analyzed due to low settlement.

Results

Settlement density

Settlement density was statistically different among habitats ($F = 70.04$, $p < 0.001$, Table 4.2, Fig. 4.4) and all habitats were different from each other. Settlement was highest in floating dock, lower in eelgrass bed, and least in rocky subtidal habitats (Fig. 4.4).

Life history traits

Terminal age did not differ among habitats (Table 4.3, Fig. 4.5A) but terminal size did ($\chi^2 = 14.26$, $p < 0.001$, Table 4.5, Fig. 4.7B). Colonies in floating dock and eelgrass bed habitats were larger than colonies in rocky subtidal habitats. Maximum growth rate was different among habitats ($\chi^2 = 14.20$, $p < 0.001$, Table 4.3, Fig. 4.5C) and

again, colonies in floating dock and eelgrass bed habitats grew faster than those in rocky subtidal ones. The duration of colony regression was not different among habitats (Table 4.3, Fig. 4.5D).

Patterns of colony regression

Colony regression did not progress through four distinct stages as noted by Brunetti (1974), Rinkevich et al. (1992), and Chadwick-Furman and Weissman (1995) for *B. Schlosseri*. Instead, four different patterns of regression were found with no distinct phases observed in any of these. The four types of regression were: 1) regression to a small amount of intact or disintegrated tissue with or without film, 2) colony middle regresses leaving no deteriorated tissue or film, zooids on the outside remain intact for some time, 3) colony maintains its shape but zooids shrink and become densely pigmented, or 4) rapid with no observations (Fig. 4.6, Appendix 4A). Patterns of regression varied within and among habitats (see Appendix 4A).

Cohorts

Distinct seasonal cohorts were only identified in dock habitats (Figs. 4.7A-C). In Gloucester and Beverly, three cohorts were observed throughout the year, in early summer, late summer, and overwinter (Figs. 4.7A and B). In Sandwich there was only one summer cohort followed by an overwintering cohort (Fig. 4.7C). In rocky subtidal and eelgrass bed habitats only a few colonies were observed and these were at the start of summer (Figs. 4.7D-I).

In floating dock habitats, settlement rate corresponded to cohort dynamics (Fig. 4.8). For example, in Gloucester dock habitats, settlement peaked as colonies in the early summer cohort peaked in size. Settlement then declined as the early summer cohort senesced, but increased as colonies in the late summer cohort increased in size. Settlement decreased as colonies in the late summer cohort declined, reaching a plateau in October as colonies senesced slowly. This sustained settlement in October likely ensured the settlement and establishment of the overwintering cohort. The overwintering cohort grew steadily until December at which point growth was halted until the following April. During these winter months no settlement was detected. In late April, growth of the overwintering cohort resumed until early June, after which the colony senesced.

Algae removal experiment in rocky subtidal habitats

In rocky subtidal habitats, despite removing $1m^2$ of algae from around plates, only one settler of *B. violaceus* was observed on a control plate and none on the experimental plates.

Discussion

B. violaceus is a tenacious invader that is expanding its range (Karlson and Osman 2012), and increasing in dominance (Dijkstra et al. 2007). In this study, differences in settlement density and some life history traits of *B. violaceus* were found among habitats. In general, *B. violaceus* was most successful in floating dock habitats, where distinct seasonal cohorts were also observed, and least successful in rocky subtidal habitats.

Settlement density

Settlement density differed among habitats and was highest in floating dock, lower in eelgrass bed, and least in rocky subtidal habitats. Differences in settlement may be due to propagule pressure, differential settlement, and/or post-settlement mortality. In general, floating dock habitats receive a large number of propagules via boat traffic (Carlton and Geller 1993, Ruiz et al. 2000), and invasive ascidians can be transported to these habitats on boat hulls (Lambert 2001, Lambert and Lambert 2003) and in ballast water (Svane and Young 1989, Carlton and Geller 1993). This may account for the high settlement density observed in these habitats. If floating docks are hubs for invasive species (Lambert and Lambert 2003, Arenas et al. 2006, Ruiz et al. 2009), acting as a stepping-stone for invasions into natural habitats (Glasby and Connell 1999, Ruiz et al. 2009, Simkanin et al. 2012), then propagules must be able to disperse between these habitats. Lower settlement density in eelgrass bed and rocky subtidal habitats, however, suggests that the larvae of *B. violaceus* do not, in this system, disperse in large numbers from man-made to natural habitats. This is consistent with *B. violaceus* having a short larval duration time of minutes to hours (Saito et al. 1981) and in British Columbia, larvae did not disperse among habitats separated by 15 m (Simkanin 2013). Eelgrass beds were slightly closer than the rocky subtidal habitats to floating docks at each site, and this might explain the intermediate settlement density in eelgrass beds.

Differences in settlement density may also be explained by larval choice, and ascidian larvae are know to select for different light levels and substrate angle (Young and Chia 1984, Ruis et al. 2010). Recently, invasive ascidians have been shown to settle

in greater numbers on surfaces close to the water's surface (Glasby et al. 2007, Dafforn et al. 2009) as in floating dock habitats, and on floating versus fixed substrates (Glasby 2007, Dafforn et al. 2009, Simkanin et al. 2012) as in floating dock and eelgrass bed habitats versus the rocky subtidal. These observations are consistent with greater settlement in floating dock habitats, intermediate settlement in eelgrass bed habitats, and lower settlement in rocky subtidal habitats.

Differences in observed recruitment could also be due to differential post-settlement mortality of recruits among habitats. Floating docks may provide a refuge from predation and, in Chile, recruits of the invasive ascidian, *Ciona intestinalis,* were preyed upon in rocky subtidal habitats but not in floating dock habitats (Dumont et al. 2011). In this study, a small gastropod, *Mitrella lunata*, which is known to prey on ascidian recruits (Osman and Whitlatch 1995, 2004), was observed in eelgrass bed habitats. It was found in greater densities on eelgrass plates in Beverly and Sandwich than in Gloucester, which could explain why more colonies of *B. violaceus* were observed in Gloucester (Fig. 4.7). In Sandwich, low numbers of the snail were also found on rocky subtidal plates, which could explain why only one colony was observed at this site.

Life history traits

Colonies of *B. violaceus* grew faster and attained larger sizes in floating dock and eelgrass bed habitats than in the rocky subtidal. Interestingly, despite differences in settlement between floating dock and eelgrass bed habitats, size and growth rates of *B.*

violaceus colonies were comparable between these habitats. Differences in colony growth between floating dock and eelgrass bed habitats versus rocky subtidal habitats could be due to biotic factors such as substrate movement and temperature. Invasive species are successful on floating substrates such as those in floating dock and eelgrass bed habitats, and least successful on fixed rocky subtidal habitats (Glasby et al. 2007, Dafforn et al. 2009, Simkanin et al. 2012). This may be due to the interaction of the differing hydrodynamic regimes with abiotic and biotic factors (Glasby 2001, Perkol-Finkel et al. 2008). Temperature may have influenced life history traits as temperature is known to influence growth and survival of *B. violaceus* in the Gulf of Maine (Grosholz 2001), as well as reproductive output in *B. schlosseri* (Yund and Stires 2002). Again, given the proximity of eelgrass bed and rocky subtidal habitats to each other as opposed to floating dock habitats, it would be expected that colonies in these habitats would experience similar temperatures and thus be more similar to each other. However, this was not the case.

Biotic factors that could influence life history traits in different habitats include predation and diversity of native species. Predation may vary between floating versus fixed habitats as fixed habitats, due to their contiguity with the benthos, may be more accessible to benthic predators (Dumont et al. 2011). In Chile, adults of the invasive solitary ascidian *C. intestinalis* were preyed upon by echinoderms, fish and crustaceans in rocky subtidal habitats, but were not preyed upon in floating dock habitats (Dumont et al. 2011). No predation on adult colonies, however, was directly observed in this study. Greater species diversity in rocky subtidal habitats (Glasby et al. 2007) could also hinder

the success of *B. violaceus* in these habitats via competitive interactions. Colonies were grown in the absence of direct competition for space but species interactions occurring on larger scales could have influenced growth.

To test if differences in terminal size and maximum growth rates of *B. violaceus* are a result of phenotypic plasticity, local adaptation, or both, reciprocal transplants of *B. violaceus* colonies among habitats would be necessary. Within rocky subtidal habitats in the Gulf of Maine, Grosholz (2001) found a genetic basis for differences in growth rates of *B. violaceus* in response to temperature, suggesting evolutionary change and population differentiation among sites separated by less that 60 km. Invasive species that exhibit phenotypic plasticity in size and growth and can adapt to local environmental conditions are able to compensate for changing environments (Langerhans and DeWitt 2004) and expand their niche and geographic range (Price et al. 2003, Schlichting 2004, Pigliucci et al. 2006). Indeed, invasive species tend to exhibit greater phenotypic plasticity than native species (Davidson et al. 2011), as well as greater growth and reproduction rates which can threaten and feedback to existing species (Ghalambor 2007).

Terminal age (life span) and colony regression (duration of senescence) did not vary among habitats and these traits could be heritable. A heritable aspect to lifespan was found in lab-raised populations of *B. schlosseri* (Rinkevich et al. 1992) and the duration of colony regression appeared to be genetically programmed in both lab (Rinkevich et al. 1992) and field populations (Brunetti 1974, Chadwick-Furman and Weissman 1995). Whether lifespan, ageing, and senescence are programmed is contentious (Kirkwood and

Melov 2011, Goldsmith 2012) but this does appear to be the case with colonial ascidians. A predetermined lifespan may increase fitness by facilitating ongoing evolutionary adaptation by promoting a succession of generations and genetic variants (Goldsmith 2008). Such evolvability (Goldsmith 2008) would certainly contribute to range expansions and may also explain the paradigm that, despite being a clonal organism, new colonies of *B. violaceus* are generally formed by sexually produced larvae, and rarely by clonal propagation (Grosberg 1988)

Colony regression

Differences in duration and patterns of colony regression within and among habitats were unexpected as regression is remarkably consistent across *B. schlosseri* colonies raised in different field and laboratory settings (Chadwick-Furman et al. 1995, Brunetti 1974, Rinkevich et al. 1992). Perhaps the two species cannot be compared for this trait. Investigating regression further in a controlled laboratory setting might prove insightful.

Cohorts

Distinct season cohorts were observed only in floating dock habitats but not in eelgrass beds or the rocky subtidal. The absence of seasonal cohorts in these natural habitats is not surprising given low settlement density in these natural habitats and the general lack of success of *B. violaceus* in the rocky subtidal. In eelgrass habitats, the absence of year-round cohorts might also be explained by the loss of actual substrate, and hence reduction in the number of propagules, on a seasonal basis. In winter, for instance,

eelgrass fronds die back to the rhizome and then reemerge the following spring resetting available space (Osman et al. 2010). The establishment of distinct, seasonal cohorts in floating dock habitats might contribute to the invasion success of *B. violaceus* as it ensures the presence of reproducing colonies or overwintering colonies that are ready to reproduce when environmental conditions are favorable. Furthermore, it ensures that space, a limiting resource in benthic habitats, is always occupied by this species.

Algae removal experiment in the rocky subtidal

The removal of algae from the area surrounding polycarbonate plates did not enhance invertebrate success. It is possible that settlement is too low in these habitats to test the hypothesis that algae physically and/or chemically *inhibit B. violaceus* in these habitats. The consistent lack of success of invasive species in rocky subtidal habitats (Lambert 2002, Glasby et al. 2007, Ruiz et al. 2009, Marins 2010, Dafforn et al. 2012, Simkanin et al. 2012) is interesting, and warrants further investigation.

Conclusions

In general, *B. violaceus* was most successful in floating dock habitats where settlement density and growth rates were high, colony sizes large, and distinct seasonal cohorts observed. Factors unique to man-made floating docks might promote invader success (Glasby et al. 2007, Dafforn et al. 2009, 2012, Simkanin et al. 2012) such as greater propagule pressure, floating substrates close to the water's surface, and refuge from predation. Although growth rates and colony size were also high and large in eelgrass beds, possibly due to the floating nature of the substrate, this habitat may be

protected from invasion due to its distance from a propagule source, predation on recruits, and substrate loss over winter. Invasion resistance in the rocky subtidal might be also be conferred by distance from a propagule source, as well as the fixed nature of the substrate, predation on recruits and adults, and greater species diversity. Identifying these differences in life history traits, as well as the factors that might influence them, represents the first step in elucidating how *B. violaceus* responds to, and integrates into, new habitats and how this species might continue to spread.

As floating docks serve as entry points for invasions (Glasby and Connell 1999, Lambert and Lambert 2003, Arenas et al. 2006, Ruiz et al. 2009, Simkanin et al. 2012), invasive species may also have had longer to establish in these systems. It should not, therefore, be assumed that natural habitats are somewhat protected from invasive species and instead, these habitats should be continually monitored. The mechanisms that contribute to invader success in different habitats should be investigated further, as well as the factors that confer invasion resistance in the rocky subtidal. This could provide us with the information necessary to protect natural subtidal habitats and to appropriately manage man-made ones towards this aim.

Supplemental material

Appendix 4A. Summary of life history trait data.

Appendix 4B. Chapter 4 raw data.

References

Altman, S., and R. B. Whitlatch. 2007. Effects of small-scale disturbance on invasion success in marine communities. Journal of Experimental Marine Biology and Ecology 342:15–29.

Arenas, F., J. D. D. Bishop, J. T. Carlton, P. J. Dyrynda, W. F. Farnham, D. J. Gonzalez, M. W. Jacobs, C. Lambert, G. Lambert, S. E. Nielsen, J. A. Pederson, J. S. Porter, S. Ward, and C. A. Wood. 2006. Alien species and other notable records from a rapid assessment survey of marinas on the south coast of England. Journal of the Marine Biological Association of the United Kingdom 86:1329–1337.

Arenas, C. J., S. C. Paetzold, A. Ramsay, and J. Davidson. 2011. Pressurized seawater as an antifouling treatment against the colonial tunicates *Botrylloides violaceus* and *Botryllus schlosseri* in mussel aquaculture. Aquatic Invasions 6:465–476.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014a. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-6. http://CRAN.R-project.org/package=lme4

Bates, D., M. Maechler, B. Bolker, and S. G. Walker. 2014b. Fitting linear mixed-effects models using lme4. Journal of Statistical Software. http://arxiv.org/abs/1406.5823

Berman, J., L. Harris, W. Lambert, M. Buttrick, and M. Dufresne. 1992. Recent invasions of the Gulf of Maine: three contrasting ecological histories. Conservation Biology 6:435–441.

Berrill, N. J. 1947. The developmental cycle of *Botrylloides*. Quarterly Journal of Microscopical Science 88:393–407.

Biebl, R., and C. P. McRoy. 1971. Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. Marine Biology 8:48–56.

Box, S. J., and P. J. Mumby. 2007. Effect of macroalgal competition on growth and survival of juvenile Caribbean corals. Marine Ecology Progress Series 342:139–149.

Brunetti, R. 1974. Observations on the life cycle of *Botryllus schlosseri* (Pallas)(Ascidiacea) in the Venetian lagoon. Italian Journal of Zoology 41:225–251.

Bullard, S. G., and R. B. Whitlatch. 2004. A guide to the larval and juvenile stages of common Long Island Sound ascidians and bryozoans. Connecticut Department of Environmental Protection, Connecticut, USA.

Bulthuis, D. A. 1987. Effects of temperature on photosynthesis and growth of seagrasses. Aquatic Botany 27:27–40.

Carlton J. T., and J. B. Geller. 1993 Ecological Roulette: the global transport of nonindigenous marine organisms. Science 261:78–82.

Carver, C. E., A. L. Mallet, and B. Vercaemer. 2006. Biological synopsis of the colonial tunicates (*Botryllus schlosseri* and *Botrylloides violaceus*). Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada.

Chadwick-Furman, N. E., and L. L. Weissman. 2005. Life Histories and Senescence of *Botryllus schlosseri* (Chordata, Ascidiacea) in Monterey Bay. Biological Bulletin 189:36–41.

Connell, S. D. 1999. Effects of surface orientation on the cover of epibiota. Biofouling 14:219–226.

Connell, S. D. 2001. Urban structures as marine habitats: an experimental comparison of the composition and abundance of subtidal epibiota among pilings, pontoons and rocky reefs. Marine Environmental Research 52:115–125.

D'Antonio, C. M., and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics 23:63–87.

Dafforn, K. A., T. M. Glasby, and E. L. Johnston. 2012. Comparing the invasibility of experimental 'reefs' with field observations of natural reefs and artificial structures. PLoS ONE 7:e38124.

Dafforn, K. A., E. L. Johnston, and T. M. Glasby. 2009. Shallow moving structures promote marine invader dominance. Biofouling 25:277–287.

Davidson, A. M., M. Jennions, and A. B. Nicotra. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecology letters 14:419–431.

Dayton, P. K. 1971. Competition, Disturbance, and Community Organization: The Provision and Subsequent Utilization of Space in a Rocky Intertidal Community. Ecological Monographs 41:351–389.

Dennison, W. C. 1987. Effects of light on seagrass photosynthesis, growth and depth distribution. Aquatic Botany 27:15–26.

Dijkstra, J. A., and L. G. Harris. 2009. Maintenance of diversity altered by a shift in dominant species: implications for species coexistence. Marine Ecology Progress Series 387:71–80.

Dijkstra J., L. G. Harris, and E. Westerman. 2007. Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. Journal of Experimental Marine Biology and Ecology 342:61–68.

Drake, J., H. A. Mooney, F. Di Castri, R. Groves, F. J. Kruger, M. Rejmánek, and M. Williamson, editors. 1989. Biological Invasions: a Global Perspective. Wiley, Chichester, UK.

Dumont, C. P., C. F. Gaymer, and M. Thiel. 2011. Predation contributes to invasion resistance of benthic communities against the non–indigenous tunicate *Ciona intestinalis*. Biological Invasions 13:2023–2034.

Elton C. S. 2000. The ecology of invasions by animals and plants. University of Chicago Press, Chicago, Illinois, USA.

Fonseca, M. S., and S. S. Bell. 1998. Influence of physical setting on seagrass landscapes near Beaufort, North Carolina, USA. Marine Ecology Progress Series 171:109–121

Fonseca, M. S., J. C. Zieman, G. W. Thayer, and J. S. Fisher. 1983. The role of current velocity in structuring eelgrass *Zostera marina* meadows. Estuarine, Coastal, and Shelf Science 17:367–380.

Fox, J., and S. Weisberg. 2011. An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Fredette, T. J., R. Diaz, J. J. van Montfrans, and R. J. Orth. 1990. Secondary production within a seagrass bed (*Zostera marina* and *Ruppia maritima*) in lower Chesapeake Bay. Estuaries 13:431–440.

Gaines, S. J., and J. Roughgarden. 1985. Larval settlement rate: a leading determinant of structure in an ecological community of the marine intertidal zone. Proceedings of the National Academy of Sciences 82:3707–3711.

Genovese, S. J., and J. D. Witman. 1999. Interactive effects of flow speed and particle concentration on growth rates of an active suspension feeder. Limnology and Oceanography 44:1120–1131.

Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology 21:394–407.

Glasby, T. M. 2001. Development of sessile marine assemblages on fixed versus moving substrata. Marine Ecology Progress Series 215:37–47.

Glasby, T. M., and S. D. Connell. 1999. Urban Structures as Marine Habitats. Ambio 28:595–598.

Glasby, T., and S. Connell. 2001. Orientation and position of substrata have large effects on epibiotic assemblages. Marine Ecology-Progress Series 214:127–135.

Glasby, T.M., S. D. Connell, M. Holloway, and C. Hewitt. 2007. Nonindigenous biota on artificial structures: could habitat creation facilitate biological invasions? Marine Biology 151:887–895.

Goldsmith, T. C. 2008. Aging, evolvability, and the individual benefit requirement, medical implications of aging theory controversies. Journal of Theoretical Biology 252:764–768.

Goldsmith, T. C. 2012. On the programmed/non-programmed aging controversy. Biochemistry (Moscow) 77:729–732.

Grave, C., and H. Woodbridge. 1924. *Botryllus schlosseri* (Pallas): The behavior and morphology of the free-swimming larva. Journal of Morphology 39:207–247.

Grosberg R. K. 1988. Life-history variation within a population of the colonial ascidian *Botryllus schlosseri*. I. The genetic and environmental control of seasonal variation. Evolution 42:900–920.

Grosholz, E. 2001. Small spatial-scale differentiation among populations of an introduced colonial invertebrate. Oecologia 129:58–64.

Grosholz, E. 2002. Ecological and evolutionary consequences of coastal invasions. Trends in Ecology and Evolution 17:22–27.

Halpern, B. S., S. Walbridge, K. A. Selkoe, C. V. Kappel, F. Micheli, C. D'Agrosa J. F. Bruno, K. S. Casey, C. Ebert, H. E. Fox, R. Fujita, D. Heinemann. H. S. Lenihan, E. M.P. Madin, M. T. Perry, E. R. Selig, M. S. Spalding, R. Steneck, and R. Watson. 2008. A Global Map of Human Impact on Marine Ecosystems. Science 319:948–952.

Hanfling, B., and K. Kollman. 2002. An evolutionary perspective of biological invasions. Trends in Ecology and Evolution 17:545–546.

Harris, L. G., and K. P. Irons. 1982. Substrate angle and predation as determinants in fouling community succession. Pages 131–174 in J. Cairns, editor. Artificial substrates, Ann Arbor Science, Ann Arbor, Michigan, USA.

Harvell, C. D., and R. K. Grosberg. 1988. The Timing of Sexual Maturity in Clonal Animals. Ecology 69:1855–1864.

Holloway, M., and S. Connell. 2002. Why do floating structures create novel habitats for subtidal epibiota? Marine Ecology Progress Series 235:43–52.

Irving, A., and S. Connell. 2002a. Sedimentation and light penetration interact to maintain heterogeneity of subtidal habitats: algal versus invertebrate dominated assemblages. Marine Ecology Progress Series 245:83–91.

Jackson, J. B. C. 1977. Competition in marine hard substrata: the adaptive significance of solitary and colonial strategies. American Naturalist 111:743–767.

Kamermans, P., M. A. Hemminga, and D. J. de Jong. 1999. Significance of salinity and silicon levels for growth of a formerly estuarine eelgrass (*Zostera marina*) population (Lake Grevelingen, The Netherlands). Marine Biology 133:527–539.

Karlson, R. H., and R. W. Osman. 2012. Species composition and geographic distribution of invertebrates in fouling communities along the east coast of the USA: a regional perspective. Marine Ecology Progress Series 458:255–268.

Kenworthy, W. J., and M. Fonseca. 1977. Reciprocal transplant of the seagrass *Zostera marina* L. Effect of substrate on growth. Aquaculture 12:197–213.

Kirkwood, T. B., and S. Melov. 2011. On the programmed/non–programmed nature of ageing within the life history. Current Biology 21:R701–R707.

Lambert, G. 2002. Nonindigenous ascidians in tropical waters. Pacific Science 56:291–298.

Lambert, G. 2001. A global overview of ascidian introductions and their possible impact on the endemic fauna. Pages 267–269 in H. Sawada, H. Yokosawa, and C. Lambert, editors. The Biology of Ascidians. Springer-Verlag, Tokyo, Japan.

Lambert, C., and G. Lambert. 2003. Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Marine Ecology Progress Series 259:145–161.

Langerhans, R. B., and T. J. DeWitt. 2004. Shared and unique features of evolutionary diversification. American Naturalist 164:335–349.

Langsrud, O. 2003. ANOVA for unbalanced data: Use Type II instead of Type III sums of squares. Statistics and Computing 13:163–167

Lee, K. S., S. R. Park, and Y. K. Kim. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. Journal of Experimental Marine Biology and Ecology 350:144–175.

Leichter, J. J., and J. D. Witman. 1997. Water flow over subtidal rock walls: relation to distributions and growth rates of sessile suspension feeders in the Gulf of Maine. Water flow and growth rates. Journal of Experimental Marine Biology and Ecology 209:293–307.

Marins, F. O., R. L. M. Novaes, R. M. Rocha, and A. O. R. Junqueira. 2010. Non-indigenous ascidians in port and natural environments in a tropical Brazilian bay. Zoologia 27:213–221.

Milkman, R. 1967. Genetic and developmental studies on *Botryllus schlosseri*. Biological Bulletin 132:229–243.

Miller, R. J., and R. J. Etter. 2008. Shading facilitates sessile invertebrate dominance in the rocky subtidal Gulf of Maine. Ecology 89:452–462.

Miller, R. J., and R. J. Etter. 2011. Rock walls: small-scale diversity hotspots in the subtidal Gulf of Maine. Marine Ecology Progress Series 425:153–165.

Moore, K. A., and F. T. Short. 2006. *Zostera*: Biology, Ecology and Management. Pages 361–386 in A. W. D. Larkum, R. J. Orth and C. M. Duarte, editors. Seagrasses: Biology, Ecology and Conservation. Springer, Netherlands.

Nandakumar, N., M. Tanaka, and T. Kikuchi. 1993. Interspecific competition among fouling organisms in Tomioka Bay, Japan. Marine Ecology Progress Series 94:43–50.

Nejrup, L. B., and M. F. Pedersen. 2008. Effects of salinity and water temperature on the ecological performance of *Zostera marina*. Aquatic Botany 88:239–246.

Oka, H., and H. Watanabe. 1959. Vascular budding in *Botrylloides*. The Biological Bulletin 117:340–346.

Osman, R. W. 1977. Establishment and development of a marine epifaunal community. Ecological Monographs 47:37–63.

Osman, R. W., P. Munguia Matute, R. Whitlatch, R. Zajac, and J. Hamilton. 2010. Thresholds and multiple community states in marine fouling communities: integrating natural history with management strategies. Marine Ecology Progress Series 413:277–289.

Osman R. W., and R. B. Whitlatch. 1995. Predation on early ontogenic life stage and its effect on recruitment into a marine epifaunal community. Marine Ecology Progress Series 117:111–126.

Osman, R. W., and R. B. Whitlatch. 2004. The control of the development of a marine benthic community by predation on recruits. Journal of Experimental Marine Biology and Ecology 311:117–145.

Pederson, J. P., R. Bullock, J. Carlton, J. Dijkstra, N. Dobroski, P. Dyrynda, R. Fisher, L. Harris, N. Hobbs, G Lambert, E. Lazo-Wasem, A. Mathieson, M. Miglietta, J. Smith, J. Smith III, and M. Tyrrell. 2005. Marine Invaders in the Northeast: Rapid Assessment Survey of Non–native and native Marine Species of Floating Dock Communities. MIT Sea Grant College Program, Cambridge, Massachusetts, USA.

Peralta, G., J. L. Pérez-Lloréns, I. Hernández, and J. J. Vergara. 2002. Effects of light availability on growth, architecture and nutrient content of the seagrass *Zostera noltii* Hornem. Journal of Experimental Marine Biology and Ecology 269:9–26.

Perkol-Finkel, S., G. Zilman, I. Sella, T. Miloh, and Y. Benayahu. 2008. Floating and fixed artificial habitats: Spatial and temporal patterns of benthic communities in a coral reef environment. Estuarine, Coastal and Shelf Science *77*:491–500.

Pigliucci, M. 2001. Phenotypic plasticity: beyond nature and nurture. JHU Press, Baltimore, Maryland, USA.

Piola, R., and E. Johnston. 2008. Pollution reduces native diversity and increases invader dominance in marine hard–substrate communities. Diversity and Distributions 14:329–342.

Price, T. D., A. Qvarnström, and D. E. Irwin. 2003. The role of phenotypic plasticity in driving genetic evolution. Proceedings of the Royal Society of London. Series B: Biological Sciences 270:1433–1440.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R–project.org/.

Randall, J. E. 1965. Grazing effect on sea grasses by herbivorous reef fishes in the West Indies. Ecology 46:255–260.

Rasband, W. S., and U. S. ImageJ. 1997-2014. National Institutes of Health, Bethesda, Maryland, USA.

Rasher, D. B., and M. E. Hay. 2010. Chemically rich seaweeds poison corals when not controlled by herbivores. Proceedings of the National Academy of Sciences 107:9683–9688.

Rasher, D. B., E. P. Stout, S. Engel, J. Kubanek, and M. E. Hay. 2011. Macroalgal terpenes function as allelopathic agents against reef corals. Proceedings of the National Academy of Sciences 108:17726–17731.

Renn, C. E. 1936. The Wasting Disease of *Zostera marina*. I. A Phytological investigation of the diseased plant. Biological Bulletin 70:148–158.

Rinkevich, B., R. J. Lauzon, B. W. Brown, and I. L. Weissman. 1992. Evidence for a programmed life span in a colonial protochordate. Proceedings of the National Academy of Sciences 89:3546–3550.

Rius, M., G. M. Branch, C. L. Griffiths, and X. Turon. 2010. Larval settlement behaviour in six gregarious ascidians in relation to adult distribution. Marine Ecology Progress Series 418:151–163.

Ruiz, G. M., P. W. Fofonoff, J. T. Carlton, M. J. Wonham, and A. H. Hines. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annual Review of Ecology and Systematics 31:481–531.

Ruiz, G. M., A. L. Freestone, P. W. Fofonoffand C. Simkanin. 2009. Habitat distribution and heterogeneity in marine invasion dynamics: the importance of hard substrate and artificial structure. Pages 321–332 in M. Wahl, editor. Marine Hard Bottom Communities. Springer-Verlag, Berlin Heidelberg, Germany.

Saito, Y., H. Mukai, and H. Watanabe. 1981. Studies on Japanese compound styelid ascidians: 2. A new species of the genus *Botrylloides* and redescription of *B. violaceus* Oka. Publications of the Seto Marine Biological Laboratory 26:357–368.

Schlichting, C. D. 2004. The role of phenotypic plasticity in diversification. Pages 191–200 in T. J. deWitt, and S. M. Scheiner, editors. Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press, Oxford, UK.

Sebens, K. P. 1985. The Ecology of the Rocky Subtidal Zone: The subtidal rock surfaces in New England support a diversity of encrusting species that compete for space and that recolonize patches cleared through predation. American Scientist 73:548–557.

Sebens, K. P. 1986. Spatial relationships among encrusting marine organisms in the New England subtidal zone. Ecological Monographs 56:73–96.

Sebens, K. P., E. J. Maney, and A. Gordon. 1997. Long term research in the rocky subtidal zone (Massachusetts 1977–1997). Pages 141–159 in E. J. Maney and C. H. Ellis, editors. Diving for Science – 1997. American Academy of Underwater Science, Nahant, Massachusetts, USA.

Short, F. T. 1983. The seagrass, *Zostera marina*: Plant morphology and bed structure in relation to sediment ammonium in Izembek, Lagoon, Alaska. Aquatic Botany 16:149–161.

Short, F. T. 1987. Effects of sediment nutrients on seagrasses: Literature review and mesocosm experiments. Aquatic Botany 27:41–57.

Short, F. T., B. W. Ibelings, and C. Den Hartog. 1988. Comparison of a current eelgrass disease to the wasting disease in the 1930s. Aquatic Botany 30:295–304.

Simkanin, C. 2013. Marine Bioinvasions in Anthropogenic and Natural Habitats: an Investigation of Nonindigenous Ascidians in British Columbia. Thesis. University of Victoria, British Columbia, Canada.

Simkanin, C., I. C. Davidson, J. D. Dower, G. Jamieson, and T. W. Therriault. 2012. Anthropogenic structures and the infiltration of natural benthos by invasive ascidians. Marine Ecology 33:499–511.

Stachowicz, J. J., H. Fried, R. W. Osman, and R. B. Whitlatch. 2002. Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. Ecology 83:2575–2590.

Steneck, R. S., and J. T. Carlton. 2001. Human Alterations of Marine Communities: Students Beware! Pages 445–468 in M. D. Bertness, S. Gaines, and M. E. Hay, editors. Marine Community Ecology. Sinauer Associates, Sunderland, Massachusetts, USA.

Sutherland, J. P. 1974. Multiple stable points in natural communities. American Naturalist 108:849–873.

Sutherland, J. P. 1978. Functional roles of *Schizoporella* and *Styela* in the fouling community at Beaufort, North Carolina. Ecology 59:257–264.

Sutherland J. P., and R. H. Karlson. 1977. Development and stability of the fouling community at Beaufort, North Carolina. Ecological Monographs 47:425–446.

Svane, I., and C. M. Young. 1989. The ecology and behaviour of ascidian larvae. Oceanography of Marine Biology Annual Review 26:45–90.

Titlyanov, E. A., I. M. Yakovleva, and T. V. Titlyanova. 2007. Interaction between benthic algae (*Lyngbya bouillonii, Dictyota dichotoma*) and scleractinian coral *Porites lutea* in direct contact. Journal of Experimental Marine Biology and Ecology 342:282–291.

Valentine, J. F., and J. E. Duffy, J. E. 2006. The central role of grazing in seagrass ecology. Pages 463–501 in A. W. D. Larkum, R. J. Orth, C. M. Duarte. Seagrasses: Biology, Ecology and Conservation. Springer, Netherlands.

Valentine, J. F., and K. L. Heck Jr. 1999. Seagrass herbivory: evidence for the continued grazing of marine grasses. Marine Ecology Progress Series 176:291–302.

Vermeij, G. J. 1991. Anatomy of an Invasion: the Trans-Arctic Interchange. Paleobiology 17:281–307.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science 277:494–499.

Webb, D. S. 1991. Ecogeography and the Great American Interchange. Paleobiology 17:266–280.

Whitlatch, R. B., R. W. Osman, A. Frese, R. Malatesta, P. Mitchell, and L. Sedgewick. 1995. The ecology of two introduced marine ascidians and their effects of epifaunal organisms in Long Island Sound. Pages 29–48 in N. Balcom, editor. Proceedings of the Northeast Conference on Non–Indgenous Aquatic Nuisance Species. Connecticut Sea Grant College Program, Cromwell, Connecticut, USA.

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

Witman, J. D. 1998. Natural disturbance and colonization on subtidal hard substrates in the Gulf of Maine. Pages 30–37 in E. M. Dorsey and J. Pederson, editors. Effects of fishing gear on the sea floor of New England. MIT Sea Grant Publication 98–4, Cambridge, Massachusetts, USA.

Witman, J. D., and P. K. Dayton. 2001. Rocky subtidal communities. Pages 339–366 in M. D. Bertness, S. Gaines, and M. E. Hay, editors. Marine Community Ecology. Sinauer Associates, Sunderland, Massachusetts, USA.

Witman, J. D., J. C. Ellis, and W. B. Anderson WB. 2004. The influence of physical processes, organisms, and permeability on cross- ecosystem fluxes. Pages 225–358 in G. A. Polis, M. E. Power, and G. R. Huxel, editors. Food webs at the landscape level. University of Chicago Press, Chicago, USA.

Young, C. M., and F. S. Chia. 1984. Microhabitat-associated variability in survival and growth of subtidal solitary ascidians during the first 21 days after settlement. Marine Biology 81:61–68.

Yund, P. O., and A. Stires. 2002. Spatial variation in population dynamics in a colonial ascidian (*Botryllus schlosseri*). Marine Biology 141:955–963.

Table 4.1. Abiotic and biotic characteristics of floating dock, rocky subtidal, and eelgrass bed habitats.

Abiotic	Biotic
FLOATING DOCKS	
Sedimentation (Harris and Irons 1982)	Competition (Harris and Irons 1982, Nandakumar et al.1993)
Temperature (Lambert and Lambert 2003)	Recruitment (Sutherland 1974, 1978)
Salinity (Lambert and Lambert 2003)	Senescence (Sutherland and Karlson 1977)
Angle of substrata (Connell 1999, Glasby and Connell 2001)	Priority effects (Sutherland 1974, 1978)
Depth of structure (Holloway and Connell 2002, Dafforn et al. 2009)	Temporal niches (Stachowicz et al. 2002)
Flow (Glasby and Connell 1999)	Predation (Harris and Irons 1982)
Disturbance (Altman and Whitlatch 2007)	
Pollution (Piola and Johnston 2008)	
Floating versus fixed structure (Connell 2001, Holloway and Connell 2002, Glasby et al. 2007, Dafforn et al. 2009)	
Swash (Glasby and Connell 1999, Holloway and Connell 2002)	
Substrate type (Glasby and Connell 1999, Glasby et al. 2007)	
ROCKY SUBTIDAL	
Sedimentation (Witman and Dayton 2001, Irving and Connell 2002, Dafforn et al. 2012)	Competition (Jackson 1977, Sebens 1985, 1986)
Temperature (Witman and Dayton 2001)	Recruitment (Gaines and Roughgarden 1985)
Angle of substrata (Glasby and Connell 2001, Irving and Connell 2002, Miller and Etter 2008, 2011 Dafforn et al. 2012)	Predation (Witman and Cooper 1983, Witman 1985, Sebens 1986)
Depth (Witman and Dayton 2001)	
Flow (Leichter and Witman 1999, Genovese and Witman 1999)	
Disturbance (Dayton 1971, Witman 1998)	
Light/irradiance (Irving and Connell 2002, Miller and Etter 2008)	
Internal waves (Witman et al. 2004)	
EELGRASS BEDS	
Sedimentation (Short 1983, Short 1987)	Disease (Renn 1936, Short et al. 1988)
Temperature (Bulthuis 1987, Nejru and Pedersen 2008)	Grazing (Randall 1965, Valentine and Heck 1999, Valentine and Duffy 2006)
Salinity (Biebl and McRoy 1971, Kamermans et al. 1999, Nejru and Pedersen 2008)	
Flow (Fonseca et al.1983, Fonseca and Bell 1978)	
Day length (Dennison 1987, Moore and Short 2006)	
Light/irradiance (Peralta et al. 2002, Lee et al. 2007)	
Substrate type (Kenworthy and Fonseca 1977)	
Nutrients (Short 1987, Lee et al. 2007)	

Table 4.2. Results of ANOVA, testing for a difference in cumulative settlement density among habitats, with site as a random factor. Settlement data was log transformed. Significant p-values are shown in bold.

Source	df	SS	F	p
Habitat	2	55.05	70.04	**< 0.001**
Site	2	0.67	2.16	0.135
Habitat:Site	4	1.57	2.51	0.065
Residuals	27	4.22		

Table 4.3. Results of univariate, habitat X site ANOVAs for terminal age, terminal size, maximum growth rate, and duration of colony regression. As models had a random effect (site) and were unbalanced, parameters were estimated with REML. The outputs are Analysis of Deviance tables and Wald χ^2 test statistics. Terminal size and maximum growth rate were log transformed. Significant p-values, with a Bonferroni-corrected alpha of 0.0125, are shown in bold.

Source	df	χ^2	p
Terminal age			
Habitat	2	2.01	0.366
Site	2	3.15	0.207
Habitat:Site	4	7.87	0.096
Terminal size			
Habitat	2	14.26	**< 0.001**
Site	2	1.83	0.401
Habitat:Site	4	7.01	0.135
Maximum growth rate			
Habitat	2	14.20	**< 0.001**
Site	2	3.89	0.143
Habitat:Site	4	11.18	0.025
Colony regression			
Habitat	2	2.35	0.309
Site	2	0.85	0.653
Habitat:Site	4	3.60	0.464

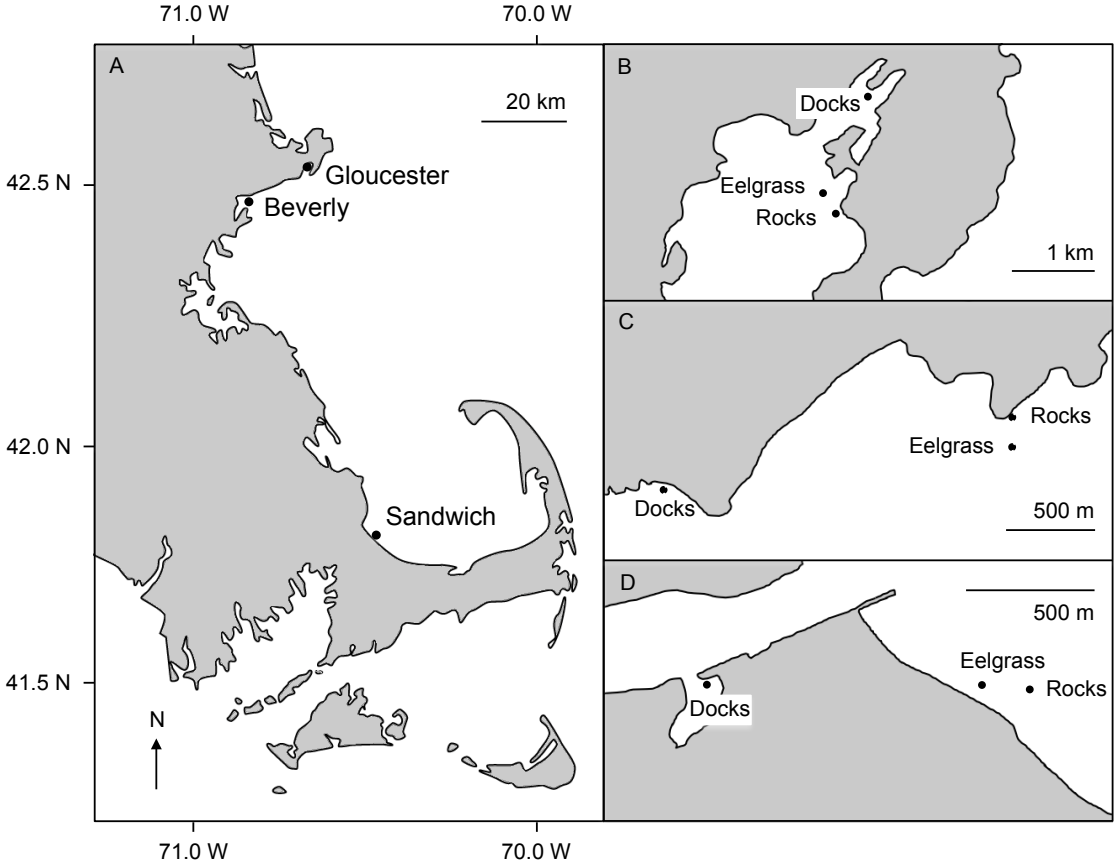

Figure 4.1. A) Map of Massachusetts Bay showing location of three replicate sites. B-D) Maps of replicates sites — B) Gloucester, C) Beverly, and D) Sandwich — showing locations of floating docks, rocky subtidal, and eelgrass bed habitats. In Gloucester, floating dock habitats (42.612715°N, 70.652762°W) were located at the State Fish Pier, and rocky subtidal (42.598323°N, 70.656900°N) and eelgrass bed (42.598394°N, 70.657418°N) habitats were located off Niles Beach. In Beverly, floating dock habitats (42.544391°N, 70.860191°W) were located at Beverly Port Marina, and rocky subtidal (42.544138°N, 70.860374°N) and eelgrass bed (42.543403°N, 70.860277°N) habitats were located off Lynch Park. In Sandwich, floating dock habitats (41.770187°N, 70.503685°W) were located at Sandwich Marina, and rocky subtidal (41.771951°N, 70.485725°N) and eelgrass bed (41.772147°N, 70.486852°N) habitats were located off Town Neck Beach.

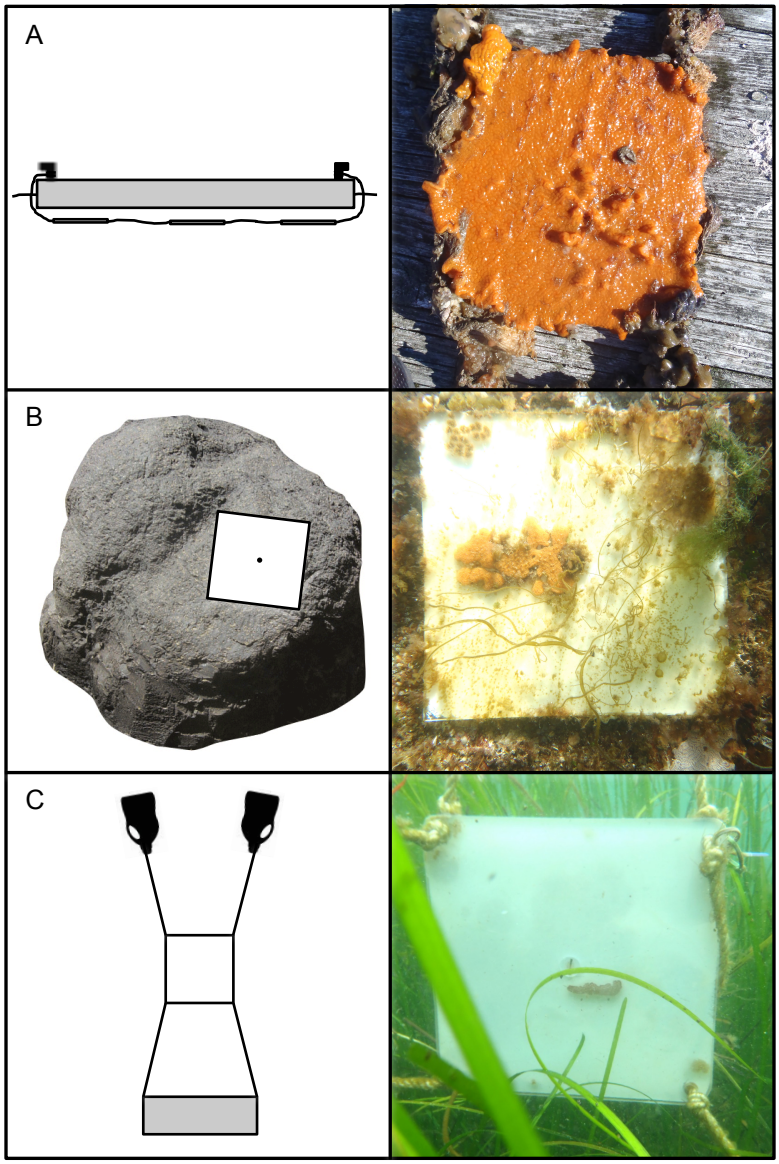

Figure 4.2. Experimental set-up and example plates. A) Floating dock, B) rocky subtidal, and C) eelgrass bed habitats.

Figure 4.3. Colony growth from settlement until terminal size (Colony ID is G_D_G_ES, see Appendix 4A).

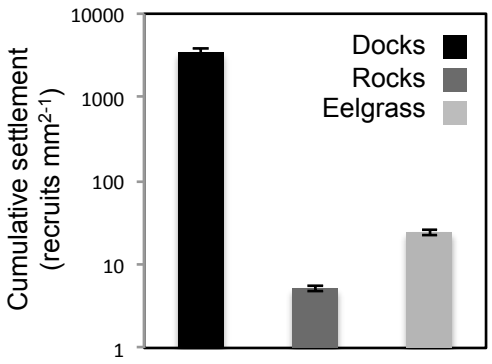

Figure 4.4. Cumulative settlement of *B. violaceus* in the 2012 season. Settlement was different among habitats and lower case letters in the plot denote the different groupings revealed by Tukey's HSD test.

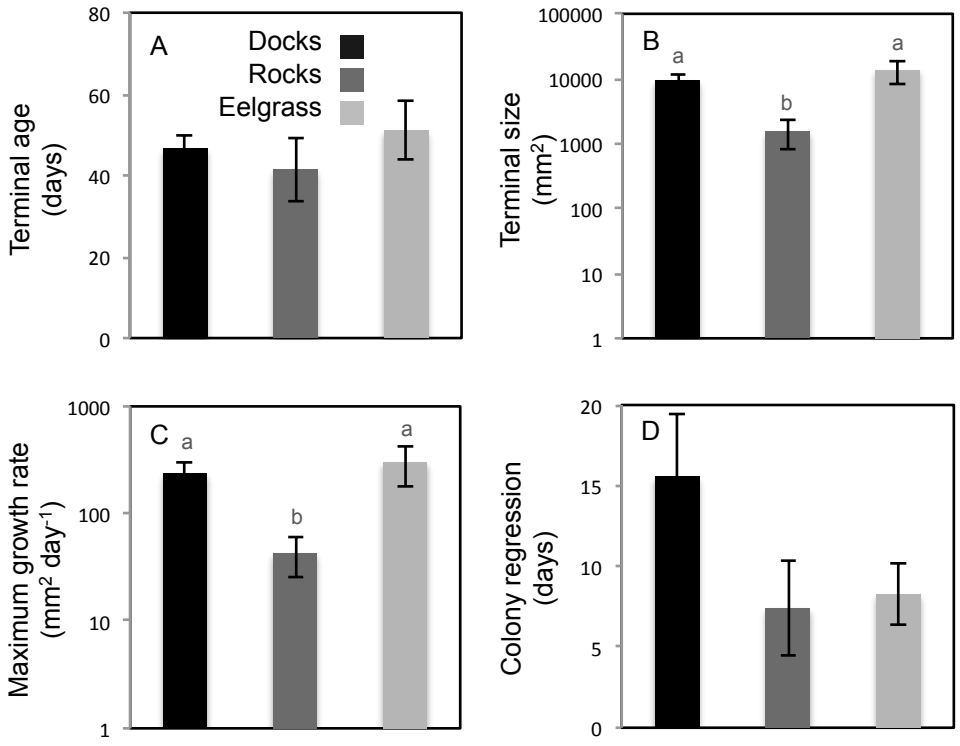

Figure 4.5. Life history traits of *B. violaceus* colonies in floating dock, rocky subtidal, and eelgrass bed habitats for A) terminal age, B) terminal size, C) maximum growth rate, and D) duration of colony regression. Terminal age and the duration of colony regression did not differ among habitats but terminal size and maximum growth rate were different among habitats. Lower case letters in plots B) and C) denote the different groupings revealed by Tukey's HSD test.

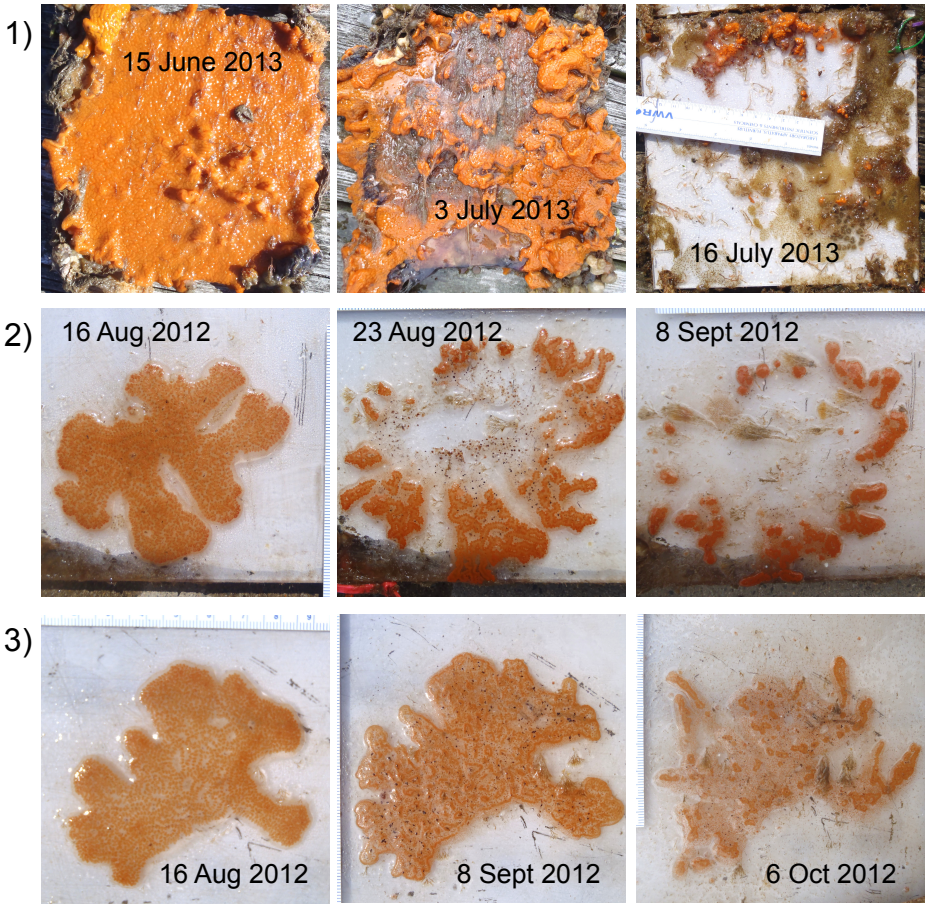

Figure 4.6. Patterns of *B. violaceus* colony regression in floating dock colonies. 1) Regression to varying amounts of intact or disintegrated tissue with or without film (colony ID G_D_Y_OW), 2) colony middle regresses leaving no deteriorated tissue or film, zooids on the outside remain intact for some time (colony ID S_D_P_LS), 3) colony maintains its shape but zooids shrink and become densely pigmented (colony ID S_D_Y_LS, see Appendix 4A for colony IDs).

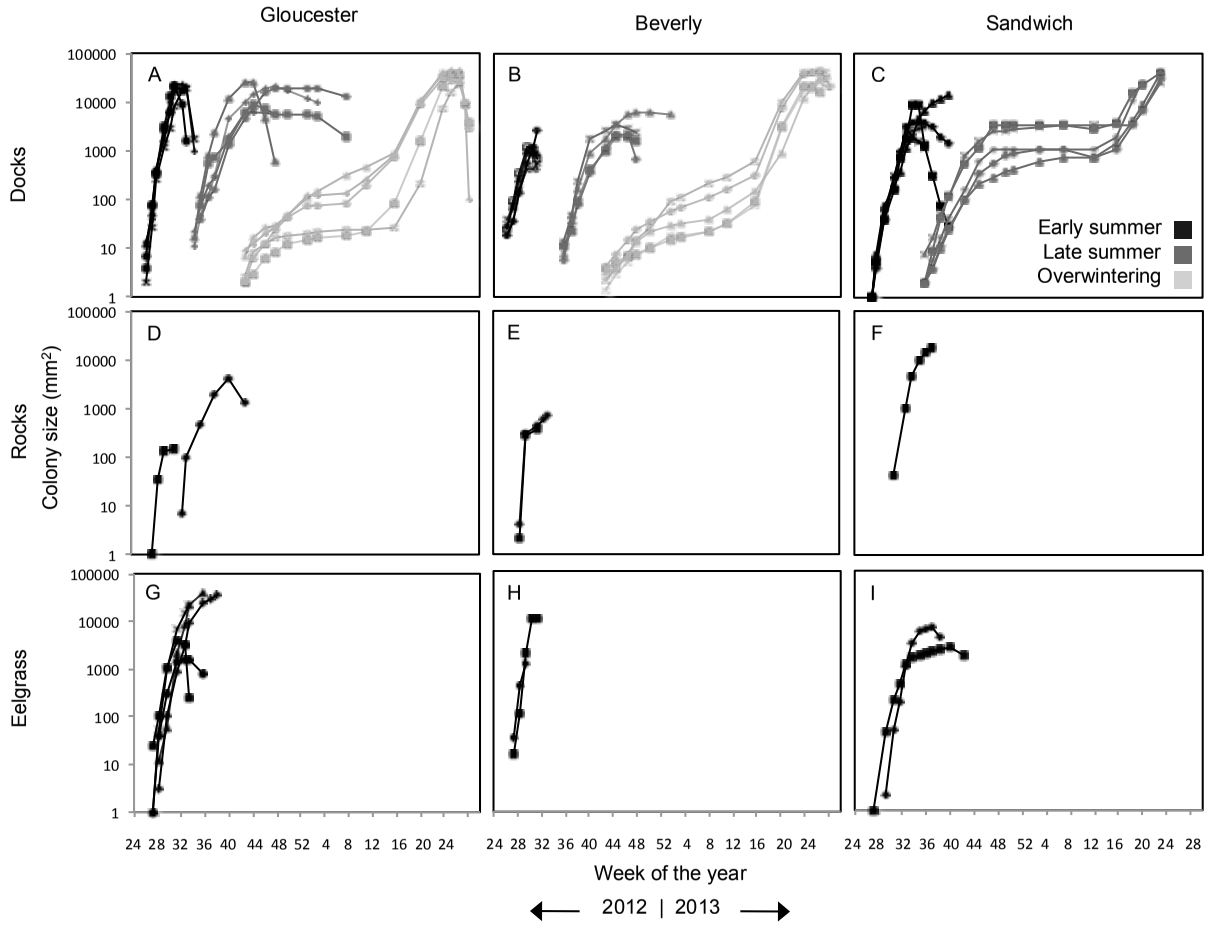

Figure 4.7. Growth curves for *B. violaceus* colonies in all habitats at all sites. Each individual curve represents a colony. Note the presence of three distinct seasonal cohorts in dock habitats. With the exception of Gloucester eelgrass no other cohorts were identified in rocky subtidal and eelgrass bed habitats. The few colonies observed in these habitats were found at the start of summer only with no observations in late summer or overwinter.

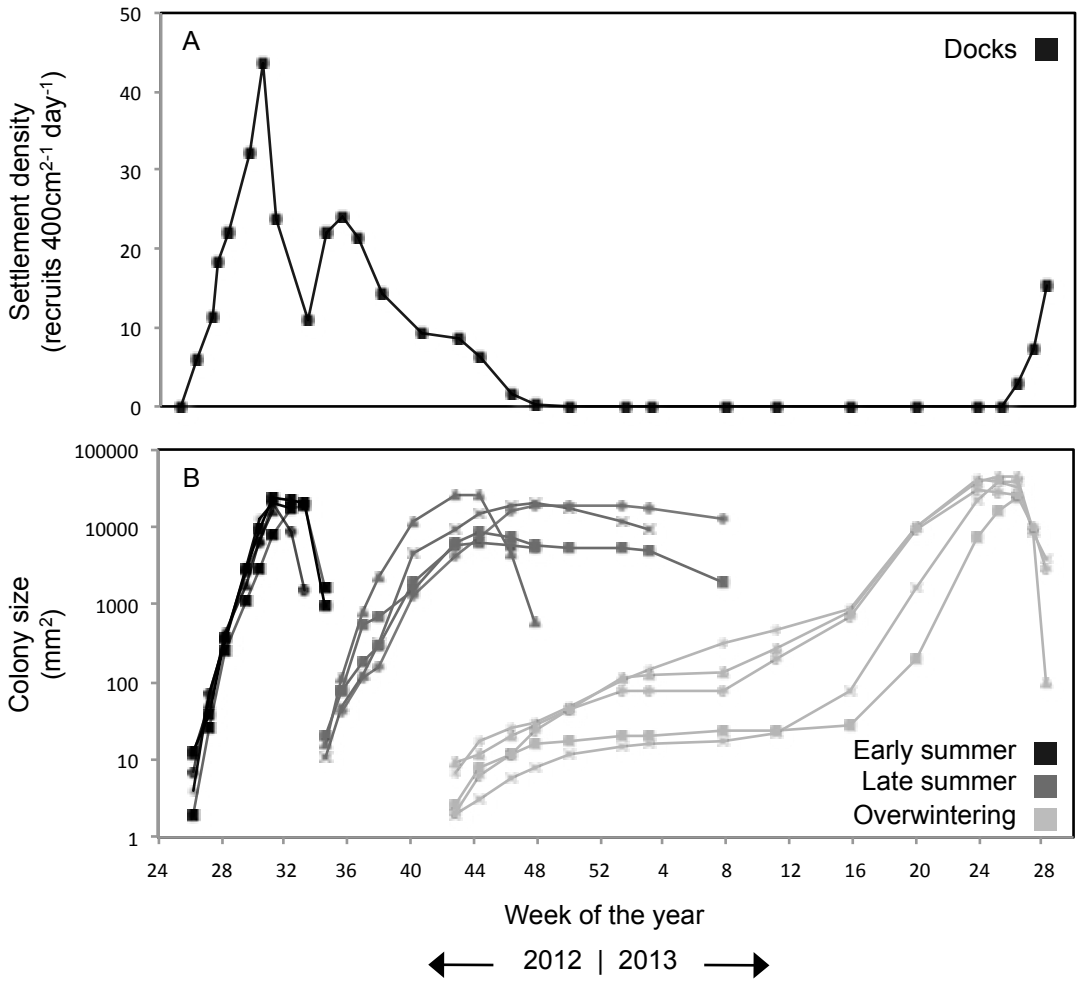

Figure 4.8. A) Daily settlement of *B. violaceus*, and B) growth curves for individual *B. violaceus* recruits colonies in floating dock habitats in Gloucester showing relationship between settlement rate and cohort dynamics.

CHAPTER 5

FINAL CONCLUSIONS

Given the increasing number of threats to the marine environment (Worm et al. 2006, Halpern et al. 2008, Crain et al. 2009), and the lack of knowledge in this realm compared to terrestrial systems (Ray and Grassle 1991, Jackson 2001, Hendriks and Duarte 2008), I set out to investigate some of the basic ecological and evolutionary forces that structure subtidal epibenthic habitats in the Gulf of Maine. Such studies are important in this region, as it is one of the most highly impacted in the world (Halpern et al. 2008).

A central goal of ecology is to understand how species assemble to form communities (Weiher and Keddy 1999, Chase and Leibold 2003), and results suggest that local subtidal epifaunal communities in the Gulf of Maine assemble via deterministic forces. Based on phylogenetic community analyses, the assembly of local communities appeared to be influenced by competition, but among sites, at a regional scale, environmental filtering was also important. This supports existing knowledge, that local communities in the Gulf of Maine are structured by competitive exclusion (Sebens et al. 1995, 1996), and also increases our understanding of the processes that operate at a regional scale. Thus the application of community phylogenetic and functional trait analyses, developed in terrestrial systems, to the marine environment revealed novel

insights into ecological processes, with implications for further work in a marine setting. By borrowing from the more developed terrestrial literature (Hendriks and Duarte 2008), marine scientists can accelerate studies in a marine setting and produce work that enables cross-ecosystem comparisons, which are necessary for identifying common ecological processes and predicting the response of communities to environmental change (Webb 2012).

In subtidal communities on artificial substrates suspended vertically below floating docks, algae did not outcompete invertebrates as expected, but did influence invertebrate community composition. Algae also appeared to facilitate invertebrates in the early stages of community formation. These results contrast to those found in rocky subtidal communities where algae excluded invertebrates on sunlit shallow horizontal surfaces (Miller and Etter 2008). In rocky subtidal communities, light appears to be the most important factor influencing the distribution of autotrophs and heterotrophs between horizontal and vertical surfaces respectively (Miller and Etter 2008) whereas biotic factors unique to floating docks, such as the floating nature the substrate and its position at the water's surface and in a swash zone (Holloway and Connell 2002), appear to structure communities in this habitat. The differences in macroalgal composition may also have contributed to the findings. Investigating the factors that influence the distribution between autotrophs and heterotrophs, i.e. those that ultimately affect productivity, can help us understand how this important ecosystem service might respond to anthropogenic impacts.

When comparing the life history characteristics of an invasive colonial ascidian between floating dock, rocky subtidal, and eelgrass bed habitats, *B. violaceus* was more successful in man-made versus natural habitats. *B. violaceus* in floating dock habitats settled in greater densities, attained larger colony sizes, experienced higher growth rates, and produced seasonal cohorts. The success of *B. violaceus* in these habitats may be due to greater propagule pressure in floating dock habitats (Carlton and Geller 1993, Ruiz et al. 2000), differential settlement of *B. violaceus* recruits (Simkanin et al. 2012) and of invasive ascidians in general (Glasby et al. 2007, Dafforn et al. 2009) on floating substrates close to the waters surface, and refuge from predation (Dumont et al. 2011). Low settlement in eelgrass bed habitats but high growth rates, may be explained by the inability of *B. violaceus* larvae to disperse between floating dock invasion hubs and natural habitats (Simkanin 2013) but, by success on floating substrates (Glasby 2007, Dafforn et al. 2009, Simkanin et al. 2012) for those propagules that do infiltrate this habitat. Ultimately, eelgrass beds appear to be protected against invasion by seasonal dieback of fronds and elimination of substrate. Rocky subtidal habitats may be protected against invasion due to fixed substrate Glasby 2007, Dafforn et al. 2009, Simkanin et al. 2012) and biotic resistance (Glasby 2007, Dumont et al. 2011, Dafforn et al. 2012). Identifying differences in life history traits among habitats is the first step in investigating if *B. violaceus* is changing as it expands its range, and provides a potential mechanism for the integration of this species into new habitats. The identification of potential forces that influence this integration can help us predict and mitigate further spread.

In conclusion, the results presented here indicate that subtidal communities in the Gulf of Maine were assembled by deterministic forces, with different processes operating at different spatial scales. At a regional scale, environmental differences likely influenced community structure. At local sites competitive exclusion was important and, on artificial substrates suspended below floating docks, algae appeared to facilitate invertebrates in the early stages of community formation and alter invertebrate community composition. Within the region, different subtidal habitats appear to be structured by different abiotic forces, such as light, substrate angle, and substrate movement, and by biotic factors such as algae composition, predation, species diversity, and propagule pressure. These can strongly influence invertebrate community composition, as well as the success of invasive species. These results contribute to our understanding of how communities assemble in the Gulf of Maine and how, once assembled, various abiotic and biotic factors unique to different habitats can influence community structure and invasive species success, providing insight into the critical forces that structure subtidal assemblages, and how invasive species integrate into them. With knowledge of these forces, we can better predict the response of ecological communities to global change.

References

Carlton J. T., and J. B. Geller. 1993 Ecological Roulette: the global transport of nonindigenous marine organisms. Science 261:78–82.

Chase, J. M., and M. A. Leibold. 2003. Ecological niches: linking classical and contemporary approaches. University of Chicago Press, Chicago, Illinois, USA.

Crain, C. M., B. S. Halpern, M. W. Beck, and C. V. Kappel. 2009. Understanding and Managing Human Threats to the Coastal Marine Environment. Annals of the New York Academy of Sciences 1162:39–62.

Dafforn, K. A., T. M. Glasby, and E. L. Johnston. 2012. Comparing the invasibility of experimental 'reefs' with field observations of natural reefs and artificial structures. PLoS ONE 7:e38124.

Dafforn, K. A., E. L. Johnston, and T. M. Glasby. 2009. Shallow moving structures promote marine invader dominance. Biofouling 25:277–287

Dumont, C. P., C. F. Gaymer, and M. Thiel. 2011. Predation contributes to invasion resistance of benthic communities against the non–indigenous tunicate *Ciona intestinalis*. Biological Invasions 13:2023–2034.

Glasby, T.M., S. D. Connell, M. Holloway, and C. Hewitt. 2007. Nonindigenous biota on artificial structures: could habitat creation facilitate biological invasions? Marine Biology 151:887–895.

Halpern, B. S., S. Walbridge, K. A. Selkoe, C. V. Kappel, F. Micheli, C. D'Agrosa, J. F. Bruno, K. S. Casey, C. Ebert, H. E. Fox, R. Fujita, D. Heinemann, H. S. Lenihan, E. M. P. Madin, M. T. Perry, E. R. Selig, M. S. Spalding, R. Steneck, and R. Watson. 2008. A Global Map of Human Impact on Marine Ecosystems. Science 319:948–952.

Hendriks, I. E., and C. M. Duarte. 2008. Allocation of effort and imbalances in biodiversity research. Journal of Experimental Marine Biology and Ecology. 360:15–20.

Holloway, M. and S. Connell. 2002. Why do floating structures create novel habitats for subtidal epibiota? Marine Ecology Progress Series 235:43–52.

Jackson, J. B. C. 2001. What was natural in the coastal oceans? Proceedings of the National Academy of Sciences USA 98:5411–5418.

Miller, R. J., and R. J. Etter. 2008. Shading facilitates sessile invertebrate dominance in the rocky subtidal Gulf of Maine. Ecology 89:452–462.

Ray, G. C. and J. F. Grassle. 1991. Marine biological diversity: a scientific program to help conserve marine biological diversity is urgently required. BioScience 41:453–458.

Ruiz, G. M., P. W. Fofonoff, J. T. Carlton, M. J. Wonham, and A. H. Hines. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annual Review of Ecology and Systematics 31:481–531.

Sebens, K. P. 1984. Water flow and coral colony size: interhabitat comparisons of the octocoral *Alcyonium siderium*. Proceedings of the National Academy of Sciences, 81:5473–5477.

Sebens, K. P. 1985. The Ecology of the Rocky Subtidal Zone: The subtidal rock surfaces in New England support a diversity of encrusting species that compete for space and that recolonize patches cleared through predation. American Scientist 73:548–557.

Sebens, K. P. 1986 . Spatial relationships among encrusting marine organisms in the New England subtidal zone. Ecological Monographs 56:73–96.

Simkanin, C. 2013. Marine Bioinvasions in Anthropogenic and Natural Habitats: an Investigation of Nonindigenous Ascidians in British Columbia. Thesis. University of Victoria, British Columbia, Canada.

Simkanin, C., I. C. Davidson, J. D. Dower, G. Jamieson, and T. W. Therriault. 2012. Anthropogenic structures and the infiltration of natural benthos by invasive ascidians. Marine Ecology 33:499–511.

Webb, T. J. 2012. Marine and terrestrial ecology: unifying concepts, revealing differences. Trends in ecology and evolution 27:535-541.

Weiher, E., and P. A. Keddy. 1995. The assembly of experimental wetland plant communities. Oikos 73:323–335.

Worm, B., E. B. Barbier, N. Beaumont, J. E. Duffy, C. Folke, B. S. Halpern, J. B. C. Jackson, H. K. Lotze, F. Micheli, S. R. Palumbi, E. Sala, K. A. Selkoe, J. J. Stachowicz, and R. Watson. 2006. Impacts of biodiversity loss on ocean ecosystem services. Science 314:787–790.

APPENDIX 2A

SUMMARY OF TRAIT DATA

Table 2A.1. Table of trait data for subtidal epibenthic invertebrates in the Gulf of Maine.

Species	Trait		
	Coloniality	Reproduction	
	Type	Type	References
Porifera			
Leucosolenia botryoides	Colonial	Asexual and sexual, vivipary	
Sycon ciliatum	Colonial	Asexual and sexual, vivipary	
Halisarca dujardini	Colonial	Asexual and sexual, vivipary	Ereskovsky 2000
Haliclona oculata	Colonial	Asexual and sexual, vivipary	Maldonado 2006
Haliclona cinerea	Colonial	Asexual and sexual, vivipary	Maldonado 2006
Halichondria panicea	Colonial	Asexual and sexual, vivipary	Maldonado 2006
Cliona celata	Colonial	Asexual and sexual, ovipary	Marainai et al. 2000
Isodictya palmata	Colonial	Asexual and sexual, vivipary	Maldonado 2006
Iophon nigricans	Colonial	Asexual and sexual, vivipary	Ereskovsky 2000
Hymedesmia similis	Colonial	Asexual and sexual, vivipary	Maldonado 2006
Myxilla fimbriata	Colonial	Asexual and sexual, vivipary	Ereskovsky 2000
Plocamionida ambigua	Colonial	Asexual and sexual, vivipary	Maldonado 2006
Cnidaria			
Ectopleura crocea	Colonial	Asexual and sexual, vivipary	Miller 1976
Obelia geniculata	Colonial	Asexual and sexual, ovipary	Brusca and Brusca 2003
Halecium sessile	Colonial	Asexual and sexual, ovipary	Calder 2003
Alcyonium siderium	Colonial	Asexual and sexual, vivipary	Sebens 1983
Metridium senile	Individual	Asexual and sexual, ovipary	Hoffman 1986
Edwardsiella lineata	Individual	Asexual and sexual, ovipary	Reitzel et al. 2006
Urticina crassicornis	Individual	Asexual and sexual, ovipary	Wedi and Dunn 1983
Ascidiacea			
Aplidium glabrum	Colonial	Asexual and sexual, vivipary	Durante and Sebens 1994
Didemnum vexillum	Colonial	Asexual and sexual, vivipary	Valentine et al. 2007
Didemnum albidum	Colonial	Asexual and sexual, vivipary	Marks 1994
Trididemnum tenerum	Colonial	Asexual and sexual, vivipary	Berrill 1947
Diplosoma listerianum	Colonial	Asexual and sexual, vivipary	Burighel and Martinucci 1994
Molgula manhattensis	Individual	Sexual, ovipary	Pollock 1998
Molgula citrina	Individual	Sexual, vivipary	Durante and Sebens 1994
Halocynthia pyriformis	Individual	Sexual, ovipary	Mercier and Hamel 2010
Boltenia ovifera	Individual	Sexual, ovipary	Lacalli 1981
Boltenia echinata	Individual	Sexual, ovipary	
Dendrodoa carnea	Individual	Sexual, ovipary	Millar 1954
Botryllus schlosseri	Colonial	Asexual and sexual, vivipary	Milkman 1967
Botrylloides violaceus	Colonial	Asexual and sexual, vivipary	Mukai et al. 1987
Annelida			
Myxicola infundibulum	Individual	Sexual, ovipary	Dean et al. 1987
Spirorbis sp.	Individual	Sexual, vivipary	Hess 1993
Filograna implexa	Individual	Asexual and sexual, vivipary	Nishi 1993, Glasby 2000
Brachiopoda			
Terebratulina septentrionalis	Individual	Sexual, vivipary	Webb et al. 1976
Mollusca			
Heteranomia squamula	Individual	Sexual, ovipary	
Modiolus modiolus	Individual	Sexual, ovipary	
Mytilus edulis	Individual	Sexual, ovipary	
Arthropoda			
Semibalanus balanoides	Individual	Sexual, vivipary	
Balanus balanus	Individual	Sexual, vivipary	
Bryozoans			
Tubulipora liliacea	Colonial	Asexual and sexual, vivipary	Hayward 1985
Crisia eburnea	Colonial	Asexual and sexual, vivipary	Ostrovsky et al. 2009
Caberea ellisii	Colonial	Asexual and sexual, vivipary	Ostrovsky et al. 2009
Parasmittina jeffreysi	Colonial	Asexual and sexual, vivipary	Ostrovsky et al. 2009
Membranipora membranacea	Colonial	Asexual and sexual, ovipary	Temkin 1994
Schizomavella auriculata	Colonial	Asexual and sexual, vivipary	Ostrovsky et al. 2009
Bugula turrita	Colonial	Asexual and sexual, vivipary	Ostrovsky et al. 2009
Dendrobeania murrayana	Colonial	Asexual and sexual, vivipary	Hancock 1943

Table 2A.1 continued.

Species	Trait		
	Body size	Growth form	Food capture
	Type	Type	Type
Porifera			
Leucosolenia botryoides	Medium	Erect	Collar sieving
Sycon ciliatum	Large	Erect	Collar sieving
Halisarca dujardini	Very large	Encrusting	Collar sieving
Haliclona oculata	Very large	Erect	Collar sieving
Haliclona cinerea	Very large	Encrusting	Collar sieving
Halichondria panicea	Medium	Domal	Collar sieving
Cliona celata	Medium	Domal	Collar sieving
Isodictya palmata	Medium	Erect	Collar sieving
Iophon nigricans	Small	Erect	Collar sieving
Hymedesmia similis	Small	Encrusting	Collar sieving
Myxilla fimbriata	Large	Domal	Collar sieving
Plocamionida ambigua	Large	Encrusting	Collar sieving
Cnidaria			
Ectopleura crocea	Very large	Erect	Prey capture
Obelia geniculata	Very large	Erect	Prey capture
Halecium sessile	Very small	Erect	Prey capture
Alcyonium siderium	Very small	Erect	Prey capture
Metridium senile	Very large	Erect	Prey capture
Edwardsiella lineata	Very large	Erect	Prey capture
Urticina crassicornis	Very large	Erect	Prey capture
Ascidiacea			
Aplidium glabrum	Large	Encrusting	Mucus nets
Didemnum vexillum	Large	Encrusting	Mucus nets
Didemnum albidum	Very large	Encrusting	Mucus nets
Trididemnum tenerum	Very large	Encrusting	Mucus nets
Diplosoma listerianum	Small	Encrusting	Mucus nets
Molgula manhattensis	Small	Erect	Mucus nets
Molgula citrina	Very large	Erect	Mucus nets
Halocynthia pyriformis	Large	Erect	Mucus nets
Boltenia ovifera	Large	Erect	Mucus nets
Boltenia echinata	Very large	Erect	Mucus nets
Dendrodoa carnea	Large	Erect	Mucus nets
Botryllus schlosseri	Very large	Encrusting	Mucus nets
Botrylloides violaceus	Medium	Encrusting	Mucus nets
Annelida			
Myxicola infundibulum	Large	Erect	Ciliary downstream collecting
Spirorbis sp.	Medium	Encrusting	Ciliary downstream collecting
Filograna implexa	Large	Erect	Ciliary downstream collecting
Brachiopoda			
Terebratulina septentrionalis	Very large	Erect	Ciliary sieving
Mollusca			
Heteranomia squamula	Large	Encrusting	Cirri trapping
Modiolus modiolus	Very large	Erect	Cirri trapping
Mytilus edulis	Medium	Erect	Cirri trapping
Arthropoda			
Semibalanus balanoides	Very large	Erect	Filter setae
Balanus balanus	Very large	Erect	Filter setae
Bryozoans			
Tubulipora liliacea	Very large	Encrusting	Ciliary sieving
Crisia eburnea	Very large	Erect	Ciliary sieving
Caberea ellisii	Very large	Erect	Ciliary sieving
Parasmittina jeffreysi	Very large	Encrusting	Ciliary sieving
Membranipora membranacea	Very large	Encrusting	Ciliary sieving
Schizomavella auriculata	Large	Encrusting	Ciliary sieving
Bugula turrita	Medium	Erect	Ciliary sieving
Dendrobeania murrayana	Small	Erect	Ciliary sieving

Table 2A.1 continued.

Species	Trait	
	Defense	
	Type	References
Porifera		
Leucosolenia botryoides	Spicules	Jackson 1977, Dyrynda 1986
Sycon ciliatum	Spicules	Jackson 1977, Dyrynda 1986
Halisarca dujardini	Spicules	Jackson 1977, Dyrynda 1986
Haliclona oculata	Spicules	Jackson 1977, Dyrynda 1986
Haliclona cinerea	Spicules	Jackson 1977, Dyrynda 1986
Halichondria panicea	Spicules	Jackson 1977, Dyrynda 1986
Cliona celata	Spicules	Jackson 1977, Dyrynda 1986
Isodictya palmata	Spicules	Jackson 1977, Dyrynda 1986
Iophon nigricans	Spicules	Jackson 1977, Dyrynda 1986
Hymedesmia similis	Spicules	Jackson 1977, Dyrynda 1986
Myxilla fimbriata	Spicules	Jackson 1977, Dyrynda 1986
Plocamionida ambigua	Spicules	Jackson 1977, Dyrynda 1986
Cnidaria		
Ectopleura crocea	Nematocysts	Jackson 1977, Dyrynda 1986
Obelia geniculata	Cnidocytes	Jackson 1977, Dyrynda 1986
Halecium sessile	Cnidocytes	Jackson 1977, Dyrynda 1986
Alcyonium siderium	Cnidocytes	Jackson 1977, Dyrynda 1986
Metridium senile	Cnidocytes	Jackson 1977, Dyrynda 1986
Edwardsiella lineata	Cnidocytes	Jackson 1977, Dyrynda 1986
Urticina crassicornis	Cnidocytes	Jackson 1977, Dyrynda 1986
Ascidiacea		
Aplidium glabrum	Chemicals	Pisut and Pawlik 2002
Didemnum vexillum	Spicules	Jackson 1977, Dyrynda 1986
Didemnum albidum	Chemicals	Pisut and Pawlik 2002
Trididemnum tenerum	Chemicals	Pisut and Pawlik 2002
Diplosoma listerianum	Chemicals	Pisut and Pawlik 2002
Molgula manhattensis	Cellulose tunic	Jackson 1977, Dyrynda 1986
Molgula citrina	Cellulose tunic	Jackson 1977, Dyrynda 1986
Halocynthia pyriformis	Cellulose tunic	Jackson 1977, Dyrynda 1986
Boltenia ovifera	Cellulose tunic	Jackson 1977, Dyrynda 1986
Boltenia echinata	Cellulose tunic	Jackson 1977, Dyrynda 1986
Dendrodoa carnea	Cellulose tunic	Jackson 1977, Dyrynda 1986
Botryllus schlosseri	Chemicals	
Botrylloides violaceus	Chemicals	Simoncini and Miller 2007
Annelida		
Myxicola infundibulum	Refuge	Giangrande et al. 2014
Spirorbis sp.	Hard shell	Jackson 1977, Dyrynda 1986
Filograna implexa	Hard shell	Jackson 1977, Dyrynda 1986
Brachiopoda		
Terebratulina septentrionalis	Hard shell	Jackson 1977, Dyrynda 1986
Mollusca		
Heteranomia squamula	Hard shell	Jackson 1977, Dyrynda 1986
Modiolus modiolus	Hard shell	Jackson 1977, Dyrynda 1986
Mytilus edulis	Hard shell	Jackson 1977, Dyrynda 1986
Arthropoda		
Semibalanus balanoides	Hard shell	Jackson 1977, Dyrynda 1986
Balanus balanus	Hard shell	Jackson 1977, Dyrynda 1986
Bryozoans		
Tubulipora liliacea	Avicularia	Jackson 1977, Dyrynda 1986
Crisia eburnea	Avicularia	Jackson 1977, Dyrynda 1986
Caberea ellisii	Avicularia	Jackson 1977, Dyrynda 1986
Parasmittina jeffreysi	Avicularia	Jackson 1977, Dyrynda 1986
Membranipora membranacea	Avicularia	Jackson 1977, Dyrynda 1986
Schizomavella auriculata	Avicularia	Jackson 1977, Dyrynda 1986
Bugula turrita	Avicularia	Jackson 1977, Dyrynda 1986
Dendrobeania murrayana	Avicularia	Jackson 1977, Dyrynda 1986

References

Berrill, N. J. 1947. The ascidians *Trididemnum alleni* and *Distaplia garstangi*, new species from the Plymouth area. Journal of the Marine Biological Association of the United Kingdom 26:609–615.

Burighel, P., and G. B. Martinucci. 1994. Sexual reproduction in the compound ascidian *Diplosoma listerianum* (Tunicata). I. Metamorphosis, storage and phagocytosis of sperm in female duct. Marine Biology 118:489–498.

Calder, D. R. 2003. Subtidal hydroids Cnidaria of Northumberland Strait, Atlantic Canada, with observations on their life cycles and distributions. The Canadian Field-Naturalist 117:555–564.

Dean, D., S. R. Chapman, and C. S. Chapman. 1987. Reproduction and development of the sabellid polychaete *Myxicola infundibulum*. Journal of the Marine Biological Association of the United Kingdom 67:431–439.

Durante, K. M., and K. P. Sebens. 1994. Reproductive ecology of the ascidians *Molgula Citrina* Alder & Hancock 1848 and *Aplidium Glabrum* (Verrill 1871) from the Gulf of Maine, USA. Ophelia 39:1–21.

Dyrynda, P. E. J. 1986. Defensive strategies of modular organisms. Philosophical Transactions of the Royal Society of London B: Biological Sciences 313:227–243.

Ereskovsky, A. V. 2000. Reproduction cycles and strategies of the cold-water sponges *Halisarca dujardini* (Demospongiae, Halisarcida), *Myxilla incrustans* and *Iophon piceus* (Demospongiae, Poecilosclerida) from the White Sea. The Biological Bulletin 198:77–87.

Glasby, C. J. 2000. Family serpulidae. Pages 184–189 in P. L. Beeskey, G. J. B. Ross, and C. J. Glasby, editors. Polychaetaes and their allies: the southern synthesis. CSIRO Publishing, Melbourne, Australia.

Hancock Pacific expeditions. 1943. Allan Hancock Pacific Expeditions. Los Angeles, Calif: University of Southern California Press, California, USA.

Hayward, P. J. 1985. Ctenostome Bryozoans: keys and notes for the identification of the species. Synopsis of the British Fauna (New Series) 33:1–169.

Hess, H. C. 1993. The evolution of parental care in brooding spirorbid polychaetes: the effect of scaling constraints. American Naturalist 141:577–596.

Hoffmann, R. J. 1986. Variation in contributions of asexual reproduction to the genetic structure of populations of the sea anemone *Metridium senile*. Evolution 40:357–365.

Jackson, J. B. C. 1977. Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. American Naturalist 111:743–767.

Lacalli, T. 1981. Annual spawning cycles and planktonic larvae of benthic invertebrates from Passamaquoddy Bay, New Brunswick. Canadian Journal of Zoology 593:433–440.

Maldonado, M. 2004. Choanoflagellates, choanocytes, and animal multicellularity. Invertebrate Biology 1231:1–22.

Mariani, S., M. J. Uriz, and X. Turon. 2000. Larval bloom of the oviparous sponge Cliona viridis: coupling of larval abundance and adult distribution. Marine Biology 1375:783–790.

Marks, J. A. 1996. Three sibling species of didemnid ascidians from northern Norway: *Didemnum albidum* (Verrill, 1871), *Didemnum polare* (Hartmeyer, 1903), and *Didemnum romssae* sp. nov. Canadian Journal of Zoology 742:357–379.

Mercier, A., and J. F. Hamel. 2010. Synchronized breeding events in sympatric marine invertebrates: role of behavior and fine temporal windows in maintaining reproductive isolation. Behavioral Ecology and Sociobiology 64:1749–1765.

Milkman, R. 1967. Genetic and developmental studies on *Botryllus schlosseri*. Biological Bulletin 132:229–243.

Millar, R. H. 1954. The annual growth and reproductive cycle of the ascidian *Dendrodoa grossularia* (van Beneden). Journal of the Marine Biological Association of the United Kingdom 33:33–48.

Miller, R. L. 1976. Some observations on sexual reproduction in Tubularia. Pages 299–308 in G. O. Mackie, editors. Coelenterate ecology and behavior. Plenum Press, New York, New York, USA.

Mukai, H., Y. Saito, and H. Watanab. 1987. Viviparous development in *Botrylloides* compound ascidians. Journal of Morphology 193:263–276.

Nishi, E. 1993. Notes on Reproductive Biology of Some Serpulid Polychaetes at Sesoko Island, Okinawa, with Brief Accounts of Setal Morphology of Three Species of *Salmacina* and *Filograna implexa*. Marine Fouling 101:11–16.

Ostrovsky, A. N., D. P. Gordon, and S. Lidgard. 2009. Independent evolution of matrotrophy in the major classes of Bryozoa: transitions among reproductive patterns and their ecological background. Marine Ecology Progress Series 378:113–124.

Pisut, D. P., and J. R. Pawlik. 2002. Anti-predatory chemical defenses of ascidians: secondary metabolites or inorganic acids? Journal of Experimental Marine Biology and Ecology 270:203–214.

Pollock, L. W. 1998. A practical guide to the marine animals of northeastern North America. Rutgers University Press, New Brunswick, New Jersey, USA.

Reitzel, A. M., J. C. Sullivan, and J. R. Finnerty. 2006. Qualitative shift to indirect development in the parasitic sea anemone *Edwardsiella lineata*. Integrative and Comparative Biology 466:827–837.

Sebens, K. P. 1983. The larval and juvenile ecology of the temperate octocoral *Alcyonium siderium* Verrill. II. Fecundity, survival, and juvenile growth. Journal of Experimental Marine Biology and Ecology 723:263–285.

Simoncini, M., and R. J. Miller. 2007. Feeding preference of *Strongylocentrotus droebachiensis* (Echinoidea) for a dominant native ascidian, *Aplidium glabrum*, relative to the invasive ascidian *Botrylloides violaceus*. Journal of Experimental Marine Biology and Ecology 342:93–98.

Temkin, M. H. 1994. Gamete spawning and fertilization in the gymnolaemate bryozoan *Membranipora membranacea*. The Biological Bulletin 187:143–155.

Valentine, P. C., M. R. Carman, D. S. Blackwood, and E. J. Heffron. 2007. Ecological observations on the colonial ascidian *Didemnum* sp. in a New England tide pool habitat. Journal of Experimental Marine Biology and Ecology 342:109–121.

Webb, G. R., A. Logan, and J. P. A. Noble. 1976. Occurrence and significance of brooded larva in a Recent brachiopod, Bay of Fundy, Canada. Journal of Paleontology 50:869–871.

Wedi, S. E., and D. F. Dunn. 1983. Gametogenesis and reproductive periodicity of the subtidal sea anemone *Urticina lofotensis* (Coelenterata: Actiniaria) in California. The Biological Bulletin 165:458–472.

Feeding traits categorized according to:
Riisgård, H. U., and P. S. Larsen. 2010. Particle capture mechanisms in suspension-feeding invertebrates. Marine Ecology Progress Series 418:255–293.

APPENDIX 2B

R CODE FOR BETA DIVERSITY NULL MODELS

```
#### Read in files and organize data ################################

library(picante)
library(FD)
library(abind)
library(vegan)
phy <- read.tree (file = "bayesian_newick.txt")
comm <- read.csv("community_data.csv", header = TRUE, row.names = 1)
traits <- read.csv("traits_nominal.csv", header = TRUE, row.names = 1)
# check for mismatches/missing species, and order information
combined1 <- match.phylo.comm(phy, comm)
phy <- combined1$phy
comm <- combined1$comm
combined2 <- match.phylo.data(phy, traits)
traits <- combined2$data
# Get a dissimilarity matrices for phylogenetic and trait distances
phydist <- cophenetic (phy)
trait.dist <- as.matrix(gowdis(traits))


#### Taxonomic beta diversity ######################################

# function that calculates bray-curtis dissimilarity after shuffling presence
 and absence data within the matrix while maintaining species diversity or row
 totals
tax.shuff <- function (x) {
  as.matrix (vegdist (randomizeMatrix(x,null.model = "richness",iterations =
  1), method ="bray"))
}
# create nulls
nulls.tax <- replicate(999, tax.shuff(comm))
# calculate nulls mean and sd
mean.nulls.tax <- apply(nulls.tax, c(1:2), mean, na.rm = T)
sd.nulls.tax <- apply(nulls.tax, c(1:2), sd, na.rm = T)
# generate obs
obs.tax <- as.matrix (vegdist(comm, method = "bray"))
# calculate SES
ses.tax <- (obs.tax - mean.nulls.tax) / sd.nulls.tax
# generate p-values
obs.nulls.tax <- abind(obs.tax,nulls.tax)
# rank the values
temp.rank.tax <- array (dim = dim (obs.nulls.tax),
  t(apply (apply (obs.nulls.tax, c(1,2), rank), 3, t)))
temp.rank.tax [ , ,1]
write.csv(temp.rank.tax [ , ,1], "p_tax.csv")
```

```
#### Phylogenetic pairwise beta diversity #########################

# function that calculates comdist after shuffling species on the phylogeny
comdist.shuff <- function (x) {
  as.matrix (comdist(comm, cophenetic(tipShuffle(x))))
}
# generate nulls
nulls.phylo.pw <- replicate(999, comdist.shuff(phy))
# calculate nulls mean and sd
mean.nulls.phylo.pw <- apply(nulls.phylo.pw, c(1:2), mean, na.rm = T)
sd.nulls.phylo.pw <- apply(nulls.phylo.pw, c(1:2), sd, na.rm = T)
# generate obs
obs.phylo.pw <- as.matrix (comdist (comm, cophenetic(phy)))
# calculate SES
ses.phylo.pw <- (obs.phylo.pw - mean.nulls.phylo.pw) / sd.nulls.phylo.pw
# generate p-values
obs.nulls.phylo.pw <- abind(obs.phylo.pw,nulls.phylo.pw)
# rank the values
temp.rank.phylo.pw <- array (dim = dim (obs.nulls.phylo.pw),
  t(apply (apply (obs.nulls.phylo.pw, c(1,2), rank), 3, t)))
temp.rank.phylo.pw [ , ,1]
write.csv(temp.rank.phylo.pw [ , ,1], "p_phylo_pw.csv")


#### Phylogenetic nearest neighbour beta diversity #################

# function that calculates comdistnt after shuffling species on the phylogeny
comdistnt.shuff <- function (x) {
   as.matrix (comdistnt (comm, cophenetic(tipShuffle(x)), exclude.conspecifics =
   F))
}
# generate nulls
nulls.phylo.nn <- replicate(999, comdistnt.shuff(phy))
# calculate nulls mean and sd
mean.nulls.phylo.nn <- apply(nulls.phylo.nn, c(1:2), mean, na.rm = T)
sd.nulls.phylo.nn <- apply(nulls.phylo.nn, c(1:2), sd, na.rm = T)
# generate obs
obs.phylo.nn <- as.matrix (comdistnt (comm, cophenetic(phy)))
# calculate SES
ses.phylo.nn <- (obs.phylo.nn - mean.nulls.phylo.nn) / sd.nulls.phylo.nn
# generate p-values
obs.nulls.phylo.nn <- abind(obs.phylo.nn, nulls.phylo.nn)
# rank the values
temp.rank.phylo.nn <- array (dim = dim (obs.nulls.phylo.nn),
  t(apply (apply (obs.nulls.phylo.nn, c(1,2), rank), 3, t)))
temp.rank.phylo.nn [ , ,1]
write.csv(temp.rank.phylo.nn [ , ,1], "p_phylo_nn.csv")
```

```
#### Trait pairwise beta diversity ################################

# function that calculates comdist after shuffling species names in the trait
 matrix
comdist.shuff.traits <- function (x) {
  row.names (x) <- sample (rownames (x))
  as.matrix (comdist (comm, as.matrix(gowdis(x))))
}
# generate nulls
nulls.trait.pw <- replicate(999, comdist.shuff.traits(traits))
# calculate nulls mean and sd
mean.nulls.trait.pw <- apply(nulls.trait.pw, c(1:2), mean, na.rm = T)
sd.nulls.trait.pw <- apply(nulls.trait.pw, c(1:2), sd, na.rm = T)
# generate obs
obs.trait.pw <- as.matrix (comdist (comm, as.matrix(gowdis(traits))))
# calculate SES
ses.trait.pw <- (obs.trait.pw - mean.nulls.trait.pw) / sd.nulls.trait.pw
# generate p-values
obs.nulls.trait.pw <- abind(obs.trait.pw, nulls.trait.pw)
# rank the values
temp.rank.trait.pw <- array (dim = dim (obs.nulls.trait.pw),
  t(apply (apply (obs.nulls.trait.pw, c(1,2), rank), 3, t)))
temp.rank.trait.pw [ , ,1]
write.csv(temp.rank.trait.pw [ , ,1], "p_trait_pw.csv")


#### Trait nearest neighbour beta diversity ########################

# function that calculates comdistnt after shuffling species names in the trait
 matrix
comdistnt.shuff.traits <- function (x) {
  row.names (x) <- sample (rownames (x))
  as.matrix (comdistnt (comm, as.matrix(gowdis(x)), exclude.conspecifics = F))
}
# generate nulls
nulls.trait.nn <- replicate(999, comdistnt.shuff.traits(traits))
# calculate nulls mean and sd
mean.nulls.trait.nn <- apply(nulls.trait.nn, c(1:2), mean, na.rm = T)
sd.nulls.trait.nn <- apply(nulls.trait.nn, c(1:2), sd, na.rm = T)
# generate obs
obs.trait.nn <- as.matrix (comdistnt (comm, as.matrix(gowdis(traits))))
# calculate SES
ses.trait.nn <- (obs.trait.nn - mean.nulls.trait.nn) / sd.nulls.trait.nn
# generate p-values
obs.nulls.trait.nn <- abind(obs.trait.nn, nulls.trait.nn)
# rank the values
temp.rank.trait.nn <- array (dim = dim (obs.nulls.trait.nn),
  t(apply (apply (obs.nulls.trait.nn, c(1,2), rank), 3, t)))
temp.rank.trait.nn [ , ,1]
write.csv(temp.rank.trait.nn [ , ,1], "p_trait_nn.csv")
```

APPENDIX 2C

RELATIONSHIP BETWEEN CHLOROPHYLL A CONCENTRATION AND COMPETITIVE EXCLUSION

Methods

To investigate if chlorophyll a concentration could predict standard effect size (SES) of phylogenetic and functional trait mean nearest neighbour (MNND) and mean pairwise distances (MPD) for local sites (see Figs. 2.2 and 2.9 and Tables 2.3 and 2.5), and thus mechanisms of community assembly, regression analyses were performed. A composite value of chlorophyll a concentration for each local site was obtained from Northeast Ocean Data Portal (2015), which uses remotely sensed SeaWiFS data. The data set comprises seasonally averaged data from January 1998 to December 2006. Each GIS cell has a resolution of 1.1 km which was considered sufficient to estimate chlorophyll a for a given site as photquadrat transects, that were used to generate local site species pools, spanned 50 m but are likely influenced by food resources integrated over a much larger area due to factors such as ocean currents. As sites were separated by 10s or 100s of kms no two sites fell within the same GIS cell. We selected the summer data set, July to September (Fig 2.9C), as this was when photoquadrats were taken. The data is also conservative as the chlorophyll a concentration is lower in the summer than in the Spring and Fall and thus the differences in concentrations between sites is also less.

Results

Although clear directional trends can be seen, i.e. the greater chlorophyll a concentration the higher the SES indicative of community assembly by competitive exclusion, these relationships were not significant with alpha set by the Bonferroni correction at 0.0125 (Fig 2C.1).

References

Northeast Ocean Data Portal. http://www.northeastoceandata.org/files/metadata/Biology/ChlorophyllAsummer.pdf (Accessed September 11, 2015).

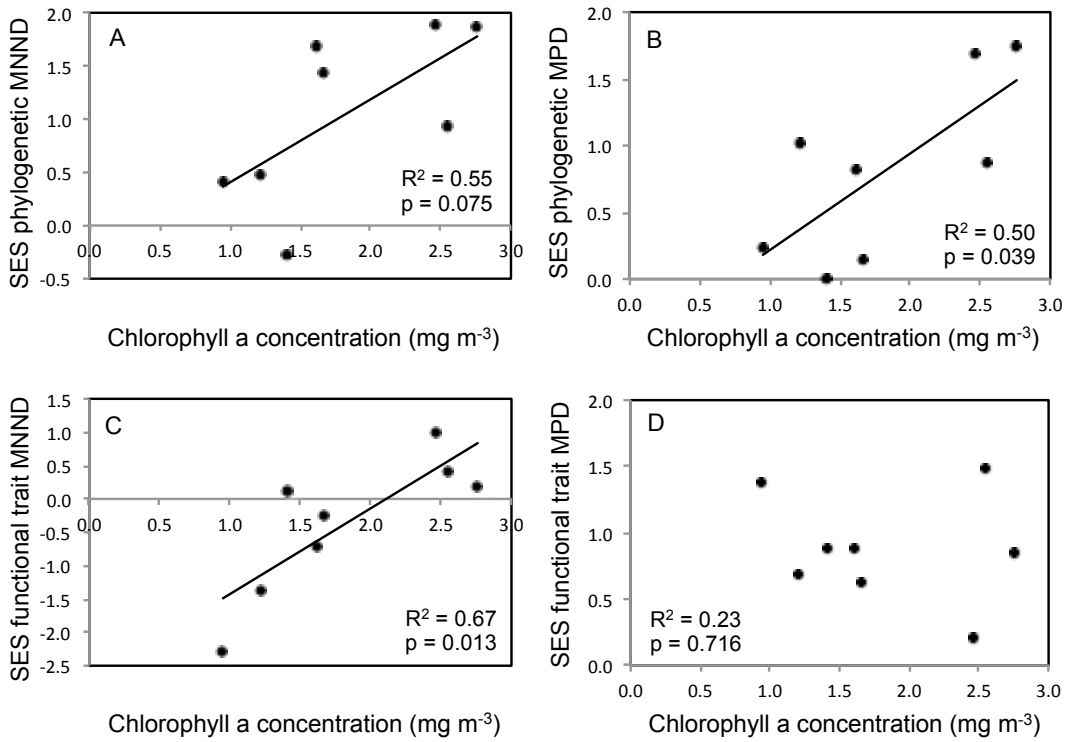

Figure 2C.1. Relationship between chlorophyll a and SES for A) phylogenetic MNND, B) phylogenetic MPD, C) functional trait MNND, and D) functional trait MPD. No relationship was significant with alpha set at 0.0125.

APPENDIX 3A

CHAPTER 3 RAW DATA

Table 3A.1. Species by treatment replicates for Assembly, Displacement, Recruits, and Settlers experiments. Numbers indicate the frequency of species occurrences, i.e. the number of times that species was found under one of 200 dots. x indicates that a species was found in the community either on the plates but not falling under one of the two hundred dots (i.e. was rare) or was found on an algae frond.

				Species																	
Experiment	Treatment	Replicate	Replicate ID	*Halichondria panicea*	*Diadumene lineata*	*Mytilus edulis*	*Tellina agilis*	*Ostrea edulis*	*Crepidula plana*	*Balanus balanus*	Encrusting bryozoan	*Bugula neritina*	Mud, algae tube complex	*Ascidiella aspersa*	*Ciona intestinalis*	*Molgula Manhattensis*	*Styela clava*	*Botryllus schlosseri*	*Botryllus violaceus*	*Diplosoma listerianum*	*Didenmmum vexillum*
Assembly	NC	Y	A_NC_Y			26	x	47	25	19			37	4	x		1	x	4		2
		G	A_NC_G				x		43	22			37	8	4		2	8	9	x	6
		Gr	A_NC_Gr	x		36	x		41	25			26	x	4		8	a	5	x	9
		lB	A_NC_lB			12	x	x	43	17			35	2	5		1	1	12	x	12
	AR	Y	A_AR_Y			6	x		9	19		x	25	2			2	13	19	7	4
		G	A_AR_G			4	x	7	17	9			22		x		4	4	10		16
		Gr	A_AR_Gr	x		1			28	25	1		19	6			3	1	73	28	4
		lB	A_AR_lB			2			3	52			12	1	x		p	5	5		1

Table 3A.1 continued

Experiment	Treatment	Replicate	Replicate ID	*Halichondria panicea*	*Diadumene lineata*	*Mytilus edulis*	*Tellina agilis*	*Ostrea edulis*	*Crepidula plana*	*Balanus balanus*	Encrusting bryozoan	*Bugula neritina*	Mud, algae tube complex	*Ascidiella aspersa*	*Ciona intestinalis*	*Molgula Manhattensis*	*Styela clava*	*Botryllus schlosseri*	*Botryllus violaceus*	*Diplosoma listerianum*	*Didemnum vexillum*
				Species																	
Displacement	AN	Br	D_A_Br			7	x	22	15	24	x		52	x				12	9	x	8
		B	D_A_B	x		14		14	27	x		x	28	13	5		35	3	3	x	31
		Y	D_A_Y			7	x	10	33	17	7		44	2	5		6	3	10	x	29
		R	D_A_R	3		5		15	17	38	4		49	7			x	16	x	x	5
	I	Br	D_I_Br	2		4	x		25	18	x	x	59	2	2		x	5	10		34
		B	D_I_B			10	x		22		10		45	7	14		9	8	15	x	18
		Y	D_I_Y	6		12	x	x	5	57	9		31	7	6		x		x		41
		R	D_I_R	x		5			30	5	25	x	55	x	16		5	1	3		20
	IA	Br	D_IA_Br			4	x	x	x	4	6		48	14	x		19	x	28	20	26
		B	D_IA_B	1		11		5	20	10	1		37	4	8		3	x	14		26
		Y	D_IA_Y	x		3			25	28	5		33	6	6		2	2	16	5	22
		R	D_IA_R	2		21		22	8	8			47	x	x		6	7	22		18
	IAR	Br	D_IAR_Br			4		4	7	42			9	28	x		5	17	9	19	12
		B	D_IAR_B				x		20	15		1	10	1	x		2	13	25	24	11
		Y	D_IAR_Y					5	10	10			30				5	43	72	5	
		R	D_IAR_R						11		5		9					58	22	15	3

Table 3A.1 continued

Experiment	Treatment	Replicate	Replicate ID	Species																	
				Halichondria panicea	*Diadumene lineata*	*Mytilus edulis*	*Tellina agilis*	*Ostrea edulis*	*Crepidula plana*	*Balanus balanus*	Encrusting bryozoan	*Bugula neritina*	Mud, algae tube complex	*Ascidiella aspersa*	*Ciona intestinalis*	*Molgula Manhattensis*	*Styela clava*	*Botryllus schlosseri*	*Botryllus violaceus*	*Diplosoma listerianum*	*Didemnum vexillum*
Recruits	AN	Gr	R_AI_Gr				x	x	x		x							15	3		
		GP	R_AI_GP				x	x	x									15	15		
		B	R_AI_P				x	x	x		x							10	6		
		P	R_AI_B				x	x			2		x					6	21		
	AC	Gr	R_AC_Gr				x		x									10	4		
		GP	R_AC_GP				x		x		x							1	2		
		B	R_AC_P				x		x		2							15	18		
		P	R_AC_B					x	x									32	14		
	AM	Gr	R_AM_Gr								x							7	11		
		GP	R_AM_GP															9	21		
		B	R_AM_P						x									12	22		
		P	R_AM_B					x										4	4		
	NA	Gr	R_NA_Gr															2	3		
		GP	R_NA_GP					x										8	15		
		B	R_NA_P						x									13	10		
		P	R_NA_B					x										4	10		

Table 3A.1 continued

Experiment	Treatment	Replicate	Replicate ID	Species																	
				Halichondria panicea	*Diadumene lineata*	*Mytilus edulis*	*Tellina agilis*	*Ostrea edulis*	*Crepidula plana*	*Balanus balanus*	Encrusting bryozoan	*Bugula neritina*	Mud, algae tube complex	*Ascidiella aspersa*	*Ciona intestinalis*	*Molgula Manhattensis*	*Styela clava*	*Botryllus schlosseri*	*Botryllus violaceus*	*Diplosoma listerianum*	*Didemnum vexillum*
Settlers	AN	P	S_AI_P		x		x		x									25	18	96	
		Gr	S_AI_Gr						x							1	x	9	14	111	
		Y	S_AI_Y						1	x							x	29	15	88	
		B	S_AI_B						x							x		15	10	111	
	AC	P	S_AC_P						x	x							1	24	18	94	
		Gr	S_AC_Gr						1	x							x	5	10	130	
		Y	S_AC_Y						x		x							15	11	112	
		B	S_AC_B						x		x						x	17	8	119	
	AM	P	S_AM_P															15	3	19	
		Gr	S_AM_Gr															7	2	23	
		Y	S_AM_Y															8	4	15	
		B	S_AM_B															12	4	22	
	NA	P	S_NA_P																	7	
		Gr	S_NA_Gr																2	4	
		Y	S_NA_Y																x	3	
		B	S_NA_B																1	5	

APPENDIX 4A

SUMMARY OF LIFE HISTORY TRAIT DATA

Table 4A.1. Summary of life history data for all *Botrylloides violaceus* colonies (all cohorts in all habitats at all sites)

Habitat	Site	Cohort	Colony ID	Settlement date	Date death	Age (days)	Size (mm^2)	Maximum growth rate (mm^2 day^{-1})	Regression duration (days)	Regression pattern
Docks	Gloucester	Early summer	G_D_R_ES	23-Jun-12	07-Aug-12	45	23483	671	23	4
			G_D_Y_ES	30-Jun-12	07-Aug-12	38	11383	405	< 8	4
			G_D_O_ES	30-Jun-12	07-Aug-12	38	16803	598	< 8	4
			G_D_lB_ES	30-Jun-12	07-Aug-12	38	20541	731	< 8	4
			G_D_Gr_ES	01-Jul-12	21-Aug-12	51	18404	292	9	1
			G_D_G_ES	23-Jun-12	07-Aug-12	45	20678	597	14	1
		Late summer	G_D_P_LS	17-Aug-12	06-Nov-12	81	6218	147	25	3
			G_D_R_LS	17-Aug-12	01-Dec-12	106	20334	339	60*	3
			G_D_Y_LS	19-Aug-12	06-Nov-12	79	26480	733	25	1
			G_D_B_LS	31-Aug-12	06-Nov-12	67	8743	249	109*	3
			G_D_G_LS	22-Aug-12	01-Dec-12	101	19195	212	84*	2
		Overwinter	G_D_P_OW	23-Oct-12	03-Jul-13	253	23508	518	< 7	4
			G_D_Y_OW	20-Oct-12	15-Jun-13	238	40000	434	8	1
			G_D_O_OW	20-Oct-12	03-Jul-13	256	38499	767	13	1
			G_D_lB_OW	20-Oct-12	03-Jul-13	256	44812	602	13	1
			G_D_Gr_OW	25-Oct-12	15-Jun-13	233	30217	602	13	1
	Beverly	Early summer	B_D_R_ES	22-Jun-12	26-Jul-12	34	953	39	12	2
			B_D_Y_ES	22-Jun-12	26-Jul-12	34	1162	47	12	2
			B_D_O_ES	22-Jun-12	26-Jul-12	34	1004	41	12	2
			B_D_Gr_ES	22-Jun-12	07-Aug-12	46	2512	35	< 7	4
			B_D_G_ES	22-Jun-12	07-Aug-12	46	757	26	< 7	4
		Late summer	B_D_P_LS	27-Aug-12	06-Nov-12	71	3347	68	11	2
			B_D_Y_LS	03-Sep-12	01-Dec-12	89	6024	27	39*	2
			B_D_O_LS	30-Aug-12	06-Nov-12	68	1937	22	25	2
			B_D_Gr_LS	02-Sep-12	06-Nov-12	65	2159	26	25	2

Table 4A.1 continued.

Habitat	Site	Cohort	Colony ID	Settlement date	Date death	Age (days)	Size (mm^2)	Maximum growth rate (mm^2 day^{-1})	Regression duration (days)	Regression pattern
Docks	Beverly	Overwinter	B_D_P_OW	26-Oct-12	25-Jun-13	242	28542	104	7	1
			B_D_Y_OW	25-Oct-12	25-Jun-13	243	39130	166	13	1
			B_D_lB_OW	26-Oct-12	25-Jun-13	242	27035	91	13	1
			B_D_B_OW	25-Oct-12	25-Jun-13	243	45094	180	13	1
			B_D_Gr_OW	25-Oct-12	03-Jul-13	251	31656	127	< 7	4
	Sandwich	Summer	S_D_P_S	07-Jul-12	19-Aug-12	43	3684	101	23	2
			S_D_Y_S	07-Jul-12	23-Aug-12	47	3726	102	54	3
			S_D_lB_S	07-Jul-12	06-Oct-12	91	13601	38	8	4
			S_D_B_S	04-Jul-12	23-Aug-12	50	9067	57	54	3
			S_D_G_S	04-Jul-12	08-Sep-12	66	3041	79	8	4
		Overwinter	S_D_P_OW	08-Sep-12	18-May-13	252	14149	219	< 7	4
			S_D_YR_OW	07-Sep-12	18-May-13	253	24591	427	< 7	4
			S_D_YYR_OW	07-Sep-12	09-Jun-13	275	40231	353	< 7	4
			S_D_O_OW	08-Sep-12	09-Jun-13	274	38702	152	< 7	4
			S_D_B_OW	04-Sep-12	09-Jun-13	278	24835	62	< 7	4
			S_D_G_OW	08-Sep-12	09-Jun-13	274	31026	112	< 7	4
Rocks	Gloucester	Summer	G_R_lBY	10-Jul-12	07-Aug-12	28	147	11	< 7	4
			G_R_lBG	09-Aug-12	08-Oct-12	60	3973	110	19	2
	Beverly	Summer	B_R_RP	16-Jul-12	07-Aug-12	23	704	27	< 9	4
			B_R_RY	15-Jul-12	21-Aug-12	36	378	26	< 8	4
	Sandwich	Summer	S_R_NL	27-Jul-12	25-Sep-12	60	2501	40	< 7	4
Eelgrass	Gloucester	Summer	G_EG_PGr	10-Jul-12	21-Aug-12	42	1613	70	16	1
			G_EG_BP	18-Jul-12	06-Sep-12	50	38325	856	< 7	4
			G_EG_Po	07-Jul-12	07-Aug-12	34	3788	134	6	1
			G_EG_BlB	17-Jul-12	23-Sep-12	68	35080	355	< 7	4
			G_EG_lB	10-Jul-12	21-Aug-12	42	22478	826	< 9	4
	Beverly	Summer	B_EG_WR	04-Jul-12	07-Aug-12	34	10116	90	8	2
			B_EG_Br	05-Jul-12	26-Jul-12	21	1218	58	< 6	4
	Sandwich	Summer	S_EG_OY	22-Jul-12	06-Oct-12	90	3851	28	17	2
			S_EG_OR	09-Jul-12	25-Sep-12	79	6697	118	9	2

APPENDIX 4B

CHAPTER 4 RAW DATA

Table 4B.1. Number of *Botrylloides violaceus* settlers per 400 cm^2 in floating dock, rocky subtidal, and eelgrass bed habitats in Gloucester, Beverly, and Sandwich in 2012.

	Floating docks settlement density (settlers 400 cm^{2-1})											
	Gloucester				Beverly				Sandwich			
	Replicate plate number				Replicate plate number				Replicate plate number			
Date	1	2	3	4	1	2	3	4	1	2	3	4
26-Jun-12	0	0	0	0	0	0	0	0	–	–	–	–
01-Jul-12	–	–	–	–	–	–	–	–	0	0	0	0
03-Jul-12	11	9	8	14	5	9	6	7	–	–	–	–
08-Jul-12	–	–	–	–	–	–	–	–	0	0	0	0
10-Jul-12	19	15	23	22	12	9	16	13	–	–	–	–
12-Jul-12	–	–	–	–	–	–	–	–	0	0	0	0
17-Jul-12	12	13	14	13	5	3	8	5	–	–	–	–
22-Jul-12	–	–	–	–	–	–	–	–	7	9	6	6
26-Jul-12	53	34	42	43	27	32	28	15	–	–	–	–
01-Aug-12	68	71	58	65	31	28	43	36	–	–	–	–
02-Aug-12	–	–	–	–	–	–	–	–	11	7	8	7
07-Aug-12	39	33	35	36	25	27	31	30	–	–	–	–
09-Aug-12	–	–	–	–	–	–	–	–	6	4	3	7
16-Aug-12	–	–	–	–	–	–	–	–	6	5	6	5
21-Aug-12	41	31	42	38	33	25	22	31	–	–	–	–
23-Aug-12	–	–	–	–	–	–	–	–	3	4	6	6
30-Aug-12	42	55	63	40	35	26	33	31	–	–	–	–
06-Sep-12	17	15	20	18	27	26	30	37	–	–	–	–
08-Sep-12	–	–	–	–	–	–	–	–	9	13	10	8
13-Sep-12					23	30	25	33	–	–	–	–
23-Sep-12	33	42	40	29	26	22	19	28	–	–	–	–
25-Sep-12	–	–	–	–	–	–	–	–	11	11	8	14
06-Oct-12	–	–	–	–	–	–	–	–	9	6	6	7
08-Oct-12	30	43	30	37	–	–	–	–	–	–	–	–
11-Oct-12	–	–	–	–	22	26	32	22	–	–	–	–
23-Oct-12	–	–	–	–	–	–	–	–	6	8	8	5
27-Oct-12					18	19	16	21	–	–	–	–
06-Nov-12	16	11	20	17	8	11	10	7	–	–	–	–
10-Nov-12	–	–	–	–	–	–	–	–	14	6	8	7
20-Nov-12	2	7	6	5	2	3	1	2	–	–	–	–
27-Nov-12	–	–	–	–	–	–	–	–	6	12	9	8
01-Dec-12	2	0	1	1	0	1	1	0	–	–	–	–
10-Dec-12	–	–	–	–	–	–	–	–	0	0	0	0
18-Dec-12	0	0	0	0	0	0	0	0	–	–	–	–

Table 4B.1 continued.

	Eelgrass bed settlement density (settlers 400 cm^{2-1})											
	Gloucester				Beverly				Sandwich			
	Replicate plate number				Replicate plate number				Replicate plate number			
Date	1	2	3	4	1	2	3	4	1	2	3	4
26-Jun-12	0	0	0	0	0	0	0	0	–	–	–	–
01-Jul-12	–	–	–	–	–	–	–	–	0	0	0	0
03-Jul-12	0	0	0	0	0	0	0	0	–	–	–	–
08-Jul-12	–	–	–	–	–	–	–	–	0	1	1	0
10-Jul-12	0	0	2	0	0	0	1	0	–	–	–	–
12-Jul-12	–	–	–	–	–	–	–	–	1	0	0	0
17-Jul-12	0	1	0	0	0	0	0	0	–	–	–	–
22-Jul-12	–	–	–	–	–	–	–	–	0	0	0	0
26-Jul-12	1	0	0	0	0	0	0	0	–	–	–	–
01-Aug-12	0	0	0	0	0	0	1	0	–	–	–	–
02-Aug-12	–	–	–	–	–	–	–	–	0	1	0	0
07-Aug-12	1	0	0	1	0	0	0	0	–	–	–	–
09-Aug-12	–	–	–	–	–	–	–	–	0	0	0	0
16-Aug-12	–	–	–	–	–	–	–	–	0	1	0	0
21-Aug-12	0	1	0	0	0	0	0	0	–	–	–	–
23-Aug-12	–	–	–	–	–	–	–	–	1	1	0	0
30-Aug-12	0	0	0	0	0	0	0	0	–	–	–	–
06-Sep-12	0	0	0	1	0	0	0	0	–	–	–	–
08-Sep-12	–	–	–	–	–	–	–	–	0	1	0	2
13-Sep-12	0	0	0	0	1	0	0	1	–	–	–	–
23-Sep-12	1	1	0	0	0	0	0	0	–	–	–	–
25-Sep-12	–	–	–	–	–	–	–	–	0	0	0	0
06-Oct-12	–	–	–	–	–	–	–	–	0	0	0	0
08-Oct-12	0	0	0	0	–	–	–	–	–	–	–	–
11-Oct-12	–	–	–	–	0	0	0	0	–	–	–	–
23-Oct-12	–	–	–	–	–	–	–	–	0	0	0	0
27-Oct-12	0	0	0	0	0	0	0	0	–	–	–	–
06-Nov-12	0	0	0	0	0	0	0	0	–	–	–	–
10-Nov-12	–	–	–	–	–	–	–	–	0	0	0	0
20-Nov-12	0	0	0	0	0	0	0	0	–	–	–	–
27-Nov-12	–	–	–	–	–	–	–	–	0	0	0	0
01-Dec-12	0	0	0	0	0	0	0	0	–	–	–	–
10-Dec-12	–	–	–	–	–	–	–	–	0	0	0	0
18-Dec-12	0	0	0	0	0	0	0	0	–	–	–	–

Table 4B.1 continued.

	Eelgrass bed settlement density (settlers 400 cm^{2-1})											
	Gloucester				Beverly				Sandwich			
	Replicate plate number				Replicate plate number				Replicate plate number			
Date	1	2	3	4	1	2	3	4	1	2	3	4
26-Jun-12	0	0	0	0	0	0	0	0	–	–	–	–
01-Jul-12	–	–	–	–	–	–	–	–	0	0	0	0
03-Jul-12	0	0	0	0	0	0	0	0	–	–	–	–
08-Jul-12	–	–	–	–	–	–	–	–	0	0	0	0
10-Jul-12	0	0	0	0	0	0	0	0	–	–	–	–
12-Jul-12	–	–	–	–	–	–	–	–	0	0	0	0
17-Jul-12	0	0	0	0	0	0	0	0	–	–	–	–
22-Jul-12	–	–	–	–	–	–	–	–	0	0	0	0
26-Jul-12	0	0	0	0	0	0	0	0	–	–	–	–
01-Aug-12	0	0	0	0	0	0	0	0	–	–	–	–
02-Aug-12	–	–	–	–	–	–	–	–	0	1	0	0
07-Aug-12	0	1	0	0	0	0	0	0	–	–	–	–
09-Aug-12	–	–	–	–	–	–	–	–	0	0	0	0
16-Aug-12	–	–	–	–	–	–	–	–	0	0	0	0
21-Aug-12	0	0	0	1	0	0	0	0	–	–	–	–
23-Aug-12	–	–	–	–	–	–	–	–	0	0	0	0
30-Aug-12	0	0	0	0	0	0	0	0	–	–	–	–
06-Sep-12	0	0	0	0	0	0	0	0	–	–	–	–
08-Sep-12	–	–	–	–	–	–	–	–	0	0	0	0
13-Sep-12	0	0	0	0	1	0	0	0	–	–	–	–
23-Sep-12	0	0	0	0	0	0	0	0	–	–	–	–
25-Sep-12	–	–	–	–	–	–	–	–	0	0	0	0
06-Oct-12	–	–	–	–	–	–	–	–	1	0	0	0
08-Oct-12	0	0	0	0	–	–	–	–	–	–	–	–
11-Oct-12	–	–	–	–	0	0	0	0	–	–	–	–
23-Oct-12	–	–	–	–	–	–	–	–	0	0	0	0
27-Oct-12	0	0	0	0	0	0	0	0	–	–	–	–
06-Nov-12	0	0	0	0	0	0	0	0	–	–	–	–
10-Nov-12	–	–	–	–	–	–	–	–	0	0	0	0
20-Nov-12	0	0	0	0	0	0	0	0	–	–	–	–
27-Nov-12	–	–	–	–	–	–	–	–	0	0	0	0
01-Dec-12	0	0	0	0	0	0	0	0	–	–	–	–
10-Dec-12	–	–	–	–	–	–	–	–	0	0	0	0
18-Dec-12	0	0	0	0	0	0	0	0	–	–	–	–

Tables 4B.2. Raw size data (area mm^2) for all *Botrylloides violaceus* colonies in all cohorts in all habitats at all sites. (G_D_R_ES = colony ID: G is Gloucester (site), D is docks (habitat), R is red (label color, equivalent to number system) and ES is early summer (cohort).

Gloucester docks early summer cohort

Date	G_D_R_ES	G_D_Y_ES	G_D_O_ES	G_D_lB_ES	G_D_Gr_ES
3-Jul-12	13	17	12	4	2
10-Jul-12	38	34	54	72	26
17-Jul-12	377	128	434	355	259
26-Jul-12	2982	1078	1742	3206	1156
1-Aug-12	9514	3261	6867	12764	2867
7-Aug-12	23483	11383	16803	20541	8191
15-Aug-12	22347			16839	16832
21-Aug-12	21001				18404
30-Aug-12	991				1683

Gloucester docks late summer cohort

Date	G_D_P_LS	G_D_R_LS	G_D_Y_LS	G_D_B_LS	G_D_G_LS
30-Aug-12	20	25	21	11	16
6-Sep-12	78	47	117	78	40
13-Sep-12	189	122	849	563	112
23-Sep-12	298	315	2316	723	156
8-Oct-12	1951	4663	11797	1517	1297
27-Oct-12	5843	9471	25729	6092	4334
6-Nov-12	6218	14484	26480	8743	7455
20-Nov-12	5929	18972	4716	7220	16439
1-Dec-12	5247	20334	615	5635	19195
16-Dec-12		18001		5512	18635
9-Jan-13		12193		5291	18399
21-Jan-13		9645		5006	18145
23-Feb-13				1911	13077

Gloucester docks overwinter cohort

Date	G_D_R_OW	G_D_Y_OW	G_D_O_OW	G_D_lB_OW	G_D_Gr_OW
27-Oct-12	3	7	2	9	2
6-Nov-12	8	17	3	12	6
20-Nov-12	12	26	6	20	12
1-Dec-12	16	29	8	27	24
16-Dec-12	18	47	12	45	44
9-Jan-13	20	105	15	116	77
21-Jan-13	21	141	16	122	78
23-Feb-13	23	307	18	135	80
18-Mar-13	24	471	22	262	192
20-Apr-13	27	891	80	834	712
19-May-13	205	9963	1652	10258	8986
15-Jun-13	7356	40000	21351	38434	30217
25-Jun-13	16359	38129	37671	44801	28349
3-Jul-13	23508	32371	38499	44812	25761
10-Jul-13			8931	9285	9860
16-Jul-13			3926	100	2806

Beverly docks early summer cohort

Date	B_D_R_ES	B_D_Y_ES	B_D_O_ES	B_D_Gr_ES	B_D_G_ES
3-Jul-12	18	25	28	19	38
10-Jul-12	37	89	68	35	77
17-Jul-12	157	354	261	138	166
26-Jul-12	953	1162	1004	591	429
1-Aug-12	941	1089	950	1209	631
7-Aug-12	418	669	899	2512	757

Beverly docks late summer cohort

Date	B_D_P_LS	B_D_Y_LS	B_D_O_LS	B_D_Gr_LS
6-Sep-12	14	8	11	6
13-Sep-12	46	45	22	32
23-Sep-12	251	156	88	74
11-Oct-12	1763	944	412	355
27-Oct-12	2578	1730	985	1185
6-Nov-12	3347	3634	1937	2159
20-Nov-12	2989	5436	1873	2091
1-Dec-12	2368	6024	1592	683
16-Dec-12		5923		
9-Jan-13		5673		

Beverly docks overwinter cohort

Date	B_D_P_OW	B_D_Y_OW	B_D_lB_OW	B_D_B_OW	B_D_Gr_OW
27-Oct-12	1	4	4	2	3
6-Nov-12	3	8	5	4	4
20-Nov-12	5	15	7	8	7
1-Dec-12	7	23	8	15	13
16-Dec-12	10	35	10	27	21
9-Jan-13	18	56	15	88	30
21-Jan-13	19	67	17	108	32
23-Feb-13	21	107	21	210	37
18-Mar-13	32	163	31	287	63
20-Apr-13	79	309	91	595	152
19-May-13	2774	7250	3217	10402	899
15-Jun-13	17965	39130	21035	40098	12097
25-Jun-13	22317	40844	20983	43671	19878
3-Jul-13	25849	41346	15914	44762	31656
10-Jul-13	22181	30772		40314	
16-Jul-13		21468			

Sandwich docks summer cohort

Date	S_D_P_S	S_D_Y_S	S_D_lB_S	S_D_B_S	S_D_G_S
8-Jul-12	1	1	1	1	1
12-Jul-12	6	4	8	5	6
22-Jul-12	52	38	78	40	64
2-Aug-12	301	291	194	159	278
9-Aug-12	896	986	387	742	809
16-Aug-12	2975	3001	1190	1689	2360
23-Aug-12	1833	3726	1853	9067	2659
31-Aug-12	1625	3871	4573	8815	2919
8-Sep-12	1203	3583	6924	1260	3041
13-Sep-12		2984	9826	322	
25-Sep-12		1963	11983	78	
6-Oct-12		1444	13601	28	

Sandwich docks overwinter cohort

Date	S_D_YR_OW	S_D_YYR_OW	S_D_O_OW	S_D_B_OW	S_D_G_OW
8-Sep-12	2	2	2	8	2
16-Sep-12	6	9	4	16	4
25-Sep-12	16	43	10	51	11
6-Oct-12	41	123	25	111	26
23-Oct-12	160	532	99	820	103
10-Nov-12	592	1203	222	1662	348
27-Nov-12	1071	3505	288	2499	575
10-Dec-12	1127	3512	366	2628	782
20-Dec-12	1123	3492	429	2786	855
20-Jan-13	1108	3414	627	3214	1124
16-Feb-13	1068	3345	718	3501	1131
24-Mar-13	1090	2899	723	3421	737
19-Apr-13	2009	3577	1383	3488	1038
7-May-13	10182	14966	3976	3516	3380
18-May-13	24591	22304	9101	6844	6902
9-Jun-13		40231	38702	24835	31026

Gloucester rock

Date	G_R_lBY	G_R_lBG
10-Jul-12	1	
17-Jul-12	36	
26-Jul-12	135.64	
7-Aug-12	147.24	
15-Aug-12		7
21-Aug-12		102
6-Sep-12		464
23-Sep-12		1991
8-Oct-12		3973
27-Oct-12		1342

Beverly rocks

Date	R_R_RP	B_R_RY
17-Jul-12	4	2
26-Jul-12	296.81	265.83
7-Aug-12	426.3	378.387
15-Aug-12	561	
21-Aug-12	704.13	

Sandwich rocks

Date	S_R_N
2-Aug-12	20
16-Aug-12	253.453
23-Aug-12	851.723
31-Aug-12	1562
8-Sep-12	2116
25-Sep-12	2501

Gloucester eelgrass

Date	G_EG_PGr	G_EG_BP	G_EG_PO	G_EG_BlB	G_EG_BR
10-Jul-12	1		24		1
17-Jul-12	38	12	101	3	54
26-Jul-12	303.228	58	1060.62	100	1000
7-Aug-12	1499.01	2215	3788	911.46	7069
15-Aug-12	1532	8092	3091	3257	14560
21-Aug-12	1613	22314	259	9318	22478
6-Sep-12	765	38325		23647	
13-Sep-14				30193	
23-Sep-12				35080	

Beverly eelgrass

Date	B_EG_WR	B_EG_Br
10-Jul-12	15	35
17-Jul-12	112	407
26-Jul-12	1981.71	1218.67
7-Aug-12	10116.27	
15-Aug-12	10067	

Sandwich eelgrass

Date	G_EG_OY	G_EG_OR
8-Jul-12	1	
22-Jul-12	42.31	2
2-Aug-12	196.59	45
9-Aug-12	434.87	188
16-Aug-12	1102.931	1002
23-Aug-12	1613.85	3171
31-Aug-12	1763	5231
8-Sep-12	1874	6213
16-Sep-12	2018	6607
25-Sep-12	2239	4203
6-Oct-12	2551	
23-Oct-12	1778	