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CLIMATE CHANGE AND INVASIVE SPECIES INTERACT TO IMPACT SUCCESSION AND DIVERSITY IN GULF OF MAINE MAIRNE FOULING COMMUNITIES

BY

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TABLE OF CONTENTS

ACKNOWLEGMENTS....................iii

LIST OF TABLES....................x

LIST OF FIGURES....................xi

ABSTRACT....................xiv

CHAPTER

GENERAL INTRODUCTION....................1

- Fouling communities....................5
- Natural history of invasive species....................7
    - *Botrylloides violaceus*....................7
    - *Diplosoma listerianum*....................8
    - *Didemnum* sp. 8
    - *Membranipora membranacea*....................9

I. DISTRIBUTION AND LONG-TERM TEMPORAL PATTERNS OF FOUR INVASIVE COLONIAL ASCIDIANS IN THE GULF OF MAINE....................11

- Abstract....................11
- Introduction....................13
    - Study Species....................14
- Materials and Methods....................15
    - Field Sites....................15

Predators....17

Temporal Patterns....17

Data Analysis....18

Results and Observations....19

Habitats....19

Predators....19

Temporal abundance patterns of invasive species....20

Annual patterns of abundance....20

Discussion....21

Timing and mechanism of the introductions of *Botryllus*, *Botrylloides*, *Diplosoma* and *Didemnum* in the Gulf of Maine....22

Temporal patterns of invasive ascidians....22

II. THE ROLE OF COLONIAL ASCIDIANS IN ALTERING BIODIVERSITY IN MARINE FOULING COMMUNITIES....34

Introduction....34

Materials and Methods....35

Results....36

Discussion....37

III. COMMUNITY LEVEL EFFECTS OF INVASIVE SPECIES....50

Abstract....41

Introduction....................42
Materials and Methods....................44
Results....................47
Discussion....................49
IV. RESPONSE OF TWO COLONIAL ASCIDIANS, BOTRYLLUS SCHLOSSERI AND BOTRYLLOIDES VIOLACEUS TO LOW SALINITIES....................63
Abstract....................63
Introduction....................65
Materials and Methods....................67
Species....................67
Laboratory experiments....................68
Results....................69
General condition of *Botryllus schlosseri* and *Botrylloides violaceus*.................... 69
Heart rates....................71
Discussion....................72
V. REDUCTION IN THE SEASONAL DEVELOPMENT OF A MARINE COMMUNITY: RESPONSE TO INCREASING SEA SURFACE TEMPERATURES 83

Abstract........83

Introduction........84

Materials and Methods........85

Results........87

Discussion........91

VI. REGIME SHIFTS IN MARINE COMMUNITIES: INVASIVE SPECIES ALTER SPECIES COMPOSITION, SUCCESSION AND THE MAINTENANCE OF SPECIES DIVERSITY........102

Abstract........102

Introduction........104

Materials and Methods........108

Study area........108

Data collection........109

Abiotic factors........109

Data analysis for community succession and diversity........110

Results........112

Community assemblage between 1979 to 1982 and 2003 to 2006........113

Species composition and succession........115

        Homogenization....................118

        Diversity....................119

        Abiotic factors....................119

    Discussion....................120

        Species composition and succession....................120

        Homogenization....................124

        Diversity....................125

        Abiotic factors....................127

    Conclusion....................129

GENERAL CONCLUSION....................149

LITERATURE CITED....................155

LIST OF TABLES

TABLE

1.1 Appearance and potential vectors for *Botryllus schlosseri*, *Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp. A 25

1.2 Distribution of the four dominant colonial ascidians in various habitats........26

2.1 Species assigned to functional groups in the 70% to 100% experiment.......38

3.1 Species assigned to functional groups in the 30% to 50% experiments......53

4.1 Average heart rates and standard deviations for *Botryllus schlosseri* and *Botrylloides violaceus*...76

5.1 Seasonal changes in species abundances...94

6.1 Temporal change in species abundances between 1979 to 1982 and 2003-2006...131

6.2 SIMPER (similarity percentage) results showing which species made the greatest contribution to the dissimilarity between 1979 to 1982 and 2003 to 2006 communities...132

6.3 SIMPER (similarity percentage) results showing which species made the greatest contribution to the similarity within communities............................133

LIST OF FIGURES

FIGURE

1.1: Distribution of *Botryllus schlosseri, Botrylloides violaceus, Didemnum* sp. A and *Diplosoma listerianum* in the coastal Gulf of Maine..............................27

1.2: Average annual percent cover of invasive colonial ascidians on panels between 1979 to 1980 and 2003 to 2005..28

1.3: Average annual percent cover of *Botrylloides violaceus*, *Didemnum* sp. A and *Botryllus schlosseri* on panels between 1979 to 1980 and 2003 to 2005...29

1.4: Average seasonal percent cover of invasive ascidians on panels observed in 1979 to 1980 and 2003 to 2005...30

1.5: Average seasonal (summer, fall, winter and spring) percent cover of individual species from 1979 to 1980 and 2003 to 2005..............................31

1.6A: Daily maximum and minimum water temperatures for Portsmouth Harbor at 5m below mean water from August 2003 to February 2005.........................32

1.6B: Percent cover of colonial ascidians in Portsmouth Harbor from August 2003 to February 2005..33

2.1: Mean number of individuals found on functional groups for the two experiments...39

2.2: Mean number of species found on functional groups for the two experiments...40

3.1: Map of study sites...54

3.2A: Box plots of individual recruits that settled on functional groups.............55

3.2B: Box plots of individual recruits onto bare space of panels with adult functional groups...56

3.3A: Box plots of species that recruited onto functional groups...................57

3.3B: Box plots of species that recruited onto bare space on panels with adult functional groups...58

3.4A: Cluster analysis of similarity values of recruits found on each of seven functional groups........59

3.4B: Non-metric multidimensional scaling of recruits counted on each of seven functional groups........60

3.5A: Dendrogram of recruits observed on bare space adjacent to each of seven functional groups........61

3.5B: Non-metric multidimensional scaling of recruits counted on bare space adjacent to each of seven functional groups........62

4.1A: Percent mortality of *Botryllus schlosseri* colonies exposed to salinities between 5 ppt. and 10 ppt........77

4.1B: Percent mortality of *Botrylloides violaceus* colonies exposed to salinities between 5 ppt. and 15 ppt........78

4.2A: Heart rates of *Botryllus schlosseri* exposed to 5 ppt., 10 ppt., 15 ppt., 20 ppt., 25 ppt. and 30 ppt........79

4.2B: The heart rates of *Botrylloides violaceus* exposed to 5 ppt., 10 ppt., 15 ppt., 20 ppt., 25 ppt. and 30 ppt........80

4.3A: Heart rates of *Botryllus schlosseri* as a function of increasing salinity......81

4.3B: Heart rates of *Botrylloides violaceus* as a function of increasing salinity...82

5.1A: Average temperature between 1973 to 1981 and 1996 to 2005 in Portsmouth Harbor........95

5.1B: Average temperature between 1979 to 1980 at the mouth of the Great Bay Estuary System........96

5.2A: Cluster analysis of data collected from July 1979 through August 1980 and July 2003 through August 2004........97

5.2B: Non-Metric Multi-dimentional Scaling ordination showing the seasonal development between July 1979 through August 1980 in Portsmouth Harbor, NH........98

5.3A: Cluster analysis of data collected from July 2003 through August 2004...99

5.3B: Non-Metric Multi-dimensional Scaling ordination showing the seasonal development between July 2003 and August 2004 in Portsmouth Harbor, NH.100

5.4: Cluster analysis of average monthly abundances of panels observed between July 1971 and July 1972 in Beauford, North Carolina......................101

6.1: Gulf of Maine Map with a subset of Portsmouth Harbor study site...........134

6.2: Percent cover patterns of invasive colonial ascidians, *Botrylloides violaceus* and *Didemnum* sp...135

6.3: Three dimensional non-metric multidimensional scaling plot representing species assemblages between 1979 to 1982 and 2003 to 2006..................136

6.4: Abundance of the blue mussel, *Mytilus edulis* between 1979 and 1982....137

6.5A and B: Succession observed in both communities (1979 to 1982 and 2003 to 2006)...138

Figure 6.6: Annual variance in similarity as a function of time for both studies (1979 to 1982 and 2003 to 2006)...139

6.7A and B: Average species richness (A) and abundance (B) for the two study periods (1979 to 1982 and 2003 to 2006)...140

6.7C and D: Average diversity (C) and evenness (B) for the two study periods (1979 to 1982 and 2003 to 2006)...141

6.8A and B: Box plots of successional patterns of abundance (A) and species richness (B) between 1979 and 1982..142

6.8C and D: Successional patterns of diversity (A) and evenness (B) between 1979 and 1982...143

6.9A and B: Successional patterns of abundance (A) and species richness (B) as a function of time since initial development between 2003 and 2006.............144

6.9A and B: Successional patterns of diversity (C) and evenness (D) as a function of time since initial development between 2003 and 2006...............145

6.10A and B: Measurements of dissolved inorganic nitrogen and phosphate between the studies..146

6.10C and D: Measurements of chlorophyll *a* and temperature taken during the two study periods...147

6.10E: Measurements of salinity taken during the two study periods...........148

ABSTRACT

CLIMATE CHANGE AND INVASIVE SPECIES INTERACT TO IMPACT SUCCESSION AND DIVERSITY IN GULF OF MAINE MAIRNE FOULING COMMUNITIES

By

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University of New Hampshire, September 2007

Gulf of Maine subtidal communities have changed in recent years resulting from anthropogenic pressures such as climate change and the introduction of non-indigenous species. Sea surface temperatures have risen while salinity is predicted to decline; concurrently invasive tunicates (ascidians) became conspicuous members of fouling communities. These abiotic and biotic changes may lead to shifts in species composition, diversity and succession. The goals of these studies were to examine the relationship between climate change and invasive species on diversity, succession, and community homogeneity.

Anthropogenic impacts on diversity, succession, species composition and homogeneity were assessed through field experiments and comparing community and environmental parameters between two studies (1979 to 1982 and 2003 to 2006). Since the

initial study between 1979 and 1982, three invasive colonial ascidians (*Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp.) and one encrusting bryozoan (*Membranipora membranacea*) have become members of the fouling community. Results from these studies revealed a 33% change in species composition since 1982, succession between 1979 and 1982 was directional, leading towards a stable climax community and diversity was maintained by secondary substrate provided by the hard shell of the blue mussel, *Mytilus edulis*. In contrast, succession between 2003 and 2006 was directional in the first two years only, becoming increasingly unpredictable in year 3 and year 4; diversity was not maintained by secondary substrate but by primary substrate due to the seasonal life-history characteristics of the invasive species. Interestingly a four season development was observed between 1979 and 1982 and a three season development was detected between 2003 and 2006. The smaller number of seasonal cycles was linked to a rise in temperature that facilitated the longevity and dominance of warmer water species.

GENERAL INTRODUCTION

Succession in terrestrial and marine ecosystems is the spatial and temporal change in species composition following natural or anthropogenic disturbances (Pickett 1976). It can be directional, seasonal and random and all have been observed for marine benthic communities (Weiss 1948, Sutherland & Karlson 1973, Osman 1977, Harris & Irons 1982, Connell et al. 1997). Directional succession is an orderly process of species replacement leading towards a stable climax. It is typically observed in environmentally stable regions such as old growth forests, coral reefs and communities inhabiting the deep sea and the polar regions (Sanders 1969, Connell 1978, Pearson 1981, Grassle & Morse-Porteous 1987, Gutt et al. 1996, Connell et al. 1997). Seasonal succession is observed in temperate environments, where species undergo cyclical cycles in abundances in response to predictable changes in abiotic factors (Weiss 1948, Sutherland & Karlson 1973, Underwood & Anderson 1994). Finally, random succession is unpredictable as frequent disturbances inhibit competitive exclusion by one or more species (Sutherland & Karlson 1977, Hughes & Connell 1999). In recent years there has been an increase in the rate of physical, biological and anthropogenic disturbances that changed ecosystem processes and have the potential to alter species composition and succession. A physical, biological or anthropogenic disturbance disrupts an ecosystem, population and substrate availability.

Physical disturbances e.g., ice scouring or wave action, clear swaths of substrate and “reset” the successional clock (Dayton et al. 1969, Gutt et al. 1996). In a seminal paper (Dayton 1971) demonstrated that physical disturbances resulted in a seascape of cleared spaces. Each space represents the potential for different levels of community development depending on interactions between competitively inferior species (typically pioneers), intermediate competitors and competitively superior species. Disturbance was found to mediate competitive interactions by forestalling competitive exclusion. This idea was further developed by (Connell 1978) and (Sousa 1979) who demonstrated that intermediate levels of disturbance maintains species coexistence and increases diversity by repeatedly restarting the successional clock, enabling competitively inferior species to coexist with competitive dominants.

Biological disturbance is similar to physical disturbance, though it affects a smaller scale. This form of disturbance disrupts succession by clearing small patches through processes such as predation or senescence of individual species (Connell 1961, Paine 1966, Dayton 1971, Osman 1977, Lauzon et al. 2002). Predation on competitively dominant species and senescence (process of deterioration after the development of an organism) create patches of open space that allow competitively inferior species to coexist in the community. These processes function at small scales and arrest competitive dominance by a single species, facilitating the coexistence of a greater number of species (Paine 1966).

Climate change or species introductions are relatively recent factors altering species composition, dominance and diversity patterns (Stachowicz et al. 2002, Parmesan & Yohe 2003, Parmesan et al. 2005). Recent trends for the North Atlantic show increased sea and air surface temperatures and reduced salinities (Curry et al. 2003, Clean-Air Cool Planet & Wake 2005). Temperature greatly influences the seasonal cycles of species composition through recruitment and growth of species (Sutherland & Karlson 1973, Osman 1977, Stachowicz et al. 2002, Stachowicz & Byrnes 2006). Average sea surface temperature in the Northwest Atlantic has increased by an average of 0.02 °C/yr. Sea surface temperatures off the coasts of Maine and New Hampshire have increased by 0.03 °C /yr since 1880 (Clean-Air Cool Planet & Wake 2005); the largest increase (0.027 °C/yr.) has occurred in the last 30 yrs In temperate environments, where abiotic factors change predictably, terrestrial and aquatic systems undergo cyclical fluctuations in species composition as a result of life cycles of the dominant species (Sutherland & Karlson 1973, Osman 1977, Harris & Irons 1982). Seasonal cycles are a reflection of the timing and magnitude of recruitment, growth, and mortality of species observed within a community. For example, a drop in temperature in the fall stimulates loss of polyps and tissue regression in hydroids while a rise in temperature in the spring stimulates the development and regeneration of both bryozoans and hydroids (Winston 1977, Calder 1990). Warmer winter sea surface temperatures have been linked to earlier spring recruitment of invasive species, while warmer summer temperatures may contribute to the dominance of invasive species during the summer months (Stachowicz et al. 2002). Thus, increasing sea surface temperatures may alter the seasonal development of communities by increasing the "growing season" of warm water species.

Unlike temperature, salinity has declined over the last 50 years in the North Atlantic resulting from an influx of fresh water from the Arctic (sea ice and glaciers), continental run-off from melting ice and increased precipitation (Curry et al. 2003). Increased precipitation has risen by an average of 7.5 cm/50 yrs. in the Northwest Atlantic. Increased rainfall coupled with lower salinity in the North Atlantic may lead to lower salinity regimes in the Gulf of Maine (Clean-Air Cool Planet & Wake 2005). Reduced salinity may delay or prevent settlement of species and alter local abundance patterns (Vázquez & Young 1996, Witman & Grange 1998, Vázquez & Young 2000), affecting successional development of communities.

Most investigations involving introduced species and climate in the Gulf of Maine have been concerned with documenting their abundance and distribution (Berman et al. 1992, Osman & Whitlatch 1995, 1999, Bullard et al. 2007), while only a handful of studies have quantified impacts of these invading species on Gulf of Maine resident communities (Lambert et al. 1992, Harris & Tyrrell 2001, Levin et al. 2002). Long-term studies coupled with field experiments are critical to evaluate the integrated response of communities e.g. species composition and diversity to disturbances such as exotic species and global climate change.

Fouling communities

Marine fouling communities are assemblages of sessile suspension feeders that typically inhabit vertical and shaded surfaces on natural and man-made substrates, e.g. floating docks in the coastal zone. Experiments on fouling communities are common because they are accessible and the organisms that inhabit them are easy to manipulate. Studies have shown the driving factor structuring species composition, succession and diversity is competition for space (Jackson 1977). Space is a limited resource and made available through mortality of individual species (e.g. senescence), predation or some type of physical disturbance. Succession in marine fouling communities has been random, where frequent disturbances inhibited dominance by superior competitors (Sutherland & Karlson 1977). In the southern and southwestern portion of the Gulf of Maine seasonal directional succession was observed, indicating competitive dominance by certain organisms takes place over time (Osman 1977, Harris & Irons 1982).

In recent years, invasive species (otherwise known as exotic or non-indigenous), particularly ascidians and bryozoans, have become conspicuous members of fouling and subtidal communities and have produced substantial changes in species composition (Levin et al. 2002, Lambert & Lambert 2003, Lambert 2005). Among the types of changes are a reduction in the abundance of native species and alterations from native to invasive dominated communities (Whitlatch et al. 1995, Lambert 2001). Ascidians are particularly effective invaders of communities as they have relatively high reproductive rates that allow for a rapid buildup in populations. They tend to grow quickly, particularly during the warm summer months (Yamaguchi 1975, Stachowicz et al. 2002), and are extremely efficient suspension feeders (Peterson, 2005). Their life histories, combined with their large size allow them to dominate a wide range of habitats including floating docks, pilings and rocks (Osman & Whitlatch 1999). In addition, they are found as epibionts on the kelp *Agarum* spp., the green non-native algae *Codium fragile* subsp. *tomentosoides*, a number of red algal species as well as hard-shelled bivalves such as *Mytilus edulis* and *Anomia simplex* (Dijkstra pers. obs). Their indeterminate growth (colonial ascidians) and their ability to hold space and inhibit recruitment of other species (solitary and colonial ascidians) make them effective spatial competitors (Sutherland 1978, Osman & Whitlatch 1995, Stachowicz et al. 2002, Dijkstra et al. 2007). For example, colonies of a colonial ascidian (*Didemnum* sp.) presently cover an area of 140.8 km2 at Georges Bank, where they are observed overgrowing a variety of sedentary invertebrates (Valentine et al. 2006).

In the last ~25 years six introduced species including three colonial ascidians (*Botrylloides violaceous*, *Diplosoma listerianum*, and *Didemnum* sp.) two solitary ascidians (*Ascidiella aspersa*, *Styela clava*), and an encrusting bryozoan (*Membranipora membranacea*) have become conspicuous space holders in Southwestern Gulf of Maine fouling communities, though their introduction has not resulted in local extinctions of native species. *B. violaceus* and *D. listerianum* have been observed on primary and secondary substrate overgrowing neighboring species including the blue mussel *Mytilus edulis*, the jingle shell *Anomia simplex*, barnacles, bryozoans as well as the hydroid *Ectopleura larynx* (Dijkstra, pers. obs.). While these species are common in the Gulf of Maine, few studies have examined their effect on species composition and no study has examined their impact on succession.

Natural history of invasive species

Botrylloides violaceous

Botrylloides violaceus, originally misidentified in the Gulf of Maine as *B. diegensis*, is a member of the sub-family Botrylloidea. It produces short-lived, lecithotrophic larvae that typically settle within 12 hours. Once settled, larvae metamorphose and colony growth is through asexual budding (Rabinowitz & Rinkevich 2004). Colonies of *B. violaceous* are generally orange-reddish but they can also be found in a wide variety of colors including yellow, purple and maroon.

Botrylloides violaceus is originally from the Pacific Ocean and has been documented in Aburatsubo-Moroisa Inlet, Guam (Yamaguchi 1975), off the coast of Alaska (Lambert & Sanamyan 2001) the Northeast Pacific from British Columbia to Ensanada, Baja California, the Northeast Atlantic (Cohen et al. 1998, Lambert & Lambert 1998, Cohen et al. 2005), Sydney Harbor, (Australia Australian Museum Business Services), and the Venetian Lagoon, Mediterranean (Zaniolo et al. 1998). In the Gulf of Maine, *B. violaceus* has a relatively wide distribution, occurring from Eastport, Maine (ME) to Cape Cod, Massachusetts (MA) (Dijkstra et al. 2007). This species was first observed at

Woods Hole, MA in 1980, at the mouth of the Great Bay Estuary, New Hampshire (NH) in 1981 and at the Darling Marine Center, ME in 1982 (Carlton 1989, Berman et al. 1992, Dijkstra et al. 2007). It colonizes a variety of habitats, including an epibiont on algae and kelp species, other tunicates and mussels (Dijkstra pers. obs.). Additionally, it is exposed to a wide variety of temperature (-2 °C to 25 °C) and salinity (5ppt. to 35 ppt.) regimes along the coast and in estuaries (Berman et al. 1992).

Diplosoma listerianum

Diplosoma listerianum belongs to the family Didemnidae and has asexual and sexual reproduction. It forms a thin (0.2 cm), flat, soft, gelatinous sheet that is translucent with scattered brown or grey pigmented cells.

Diplosoma listerianum is thought to originate form Europe. It first appeared at the Isles of Shoals in 1993 (Harris and Mathieson 2000), its current distribution is from Cape Cod to Casco Bay, preferring environmentally stable habitats (Dijkstra et al. 2007). Like many colonial ascidians, it commonly occurs as an epibiont on a variety of organisms and plants.

Didemnum sp.

Like all three colonial ascidians, once settled *Didemnum* sp. reproduces and grows by asexual budding. Colonies are tan or pink colored; in the spring and early summer *Didemnum* sp. can be unspiculated and mistaken for a sponge (Dijkstra, pers. obs.).

The origins of *Didemnum* sp. are currently unknown, though it is thought to originate from the Pacific (Lambert G., pers. comm.). It is an epibiont on many species and inhabits a wide variety of substrates (Coutts & Forrest 2007). Unlike *Botrylloides violaceus*, *Botryllus schlosseri* and *Diplosoma listerianum*, it occupies shallow to deep water (45-60 m; (Valentine et al. 2007). It occurs on both coasts of the United States from British Columbia to California on the west coast and from Maine to Long Island Sound on the east coast (Bullard et al. 2007). Its distribution in the Gulf of Maine is from Eastport, ME to Cape Cod, MA (Bullard et al. 2007, Dijkstra et al. 2007)

Membranipora membranacea

This is an encrusting bryozoan with rectangular shaped zooids. *Membranipora membranacea* produce plankton feeding larvae, cyphonautes, which can remain in the water column for a period of 2 months before settling upon suitable substrate and recruiting to the adult population (Williamson 1992). After larval settlement and metamorphosis, colonies grow by asexual multiplication.

M. membranacea is known to originate from the Northeast Atlantic (Schwaninger 1999). Its range extends from Maine to Connecticut (Pederson et al. 2005). It was first observed in 1987 on blades of the kelp *Saccharina latissima* at the Isles of Shoals (Berman et al. 1992). Since its arrival at the Isles of Shoals, *M. membranacea* has spread throughout the Gulf of Maine (Harris and Mathieson 1999; Whitlatch and Osman 1999). It has become the dominant epiphyte of not only kelp beds but also a variety of other algal species (Harris and Tyrrell 2001). Ecologically, this bryozoan has been shown to negatively impact kelp beds and facilitate the increase of exotic species (Lambert et al. 1992, Levin et al. 2002).

The purpose of the experiments described here is then to better understand the effect of climate change and invasive species on succession in marine fouling communities. To this end we: 1) Compared succession from 1979 to 1982 and 2003 to 2006. 2) Determined which abiotic factors have significantly changed over the last 35 years and examine their influence of species composition. 3) Examined the effect of established adults on diversity and species composition. 4) Determined mortality and physiological sensitivity of introduced species to low salinities.

CHAPTER I

DISTRIBUTION AND LONG-TERM TEMPORAL PATTERNS OF FOUR INVASIVE COLONIAL ASCIDIANS IN THE GULF OF MAINE

Abstract

Invasive ascidians are a growing concern for ecologists and natural resource managers, yet few studies have documented their long and short-term temporal patterns of abundance and distribution. This study focuses on the invasion of the Gulf of Maine by the colonial ascidians *Botryllus schlosseri*, *Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp. A. We examined the time of arrival and potential vectors of the ascidians using survey data (collected from 1969 onwards) and literature documentation. We also compared the dominance and seasonal abundance patterns of these species using data from two identical panel studies; one conducted from 1979 to 1980, the other from 2003 to 2005. For panel studies, eight vertical and eight horizontal 0.1m^2 Plexiglas panels were deployed (starting July 1979 and July 2003) and photographed monthly from 1979 to 1982 and 2003 to the present

. The first 17 months of panel photographs from each study were analyzed to determine the percent cover of ascidian species.

Didemnum and *Botrylloides* were most likely introduced into the Damariscotta River, Maine in the early 1970's through oyster aquaculture while *Botryllus* and *Diplosoma* were probably transported to the region by commercial and/or recreational vessels. The overall abundance of colonial ascidians has increased since 1979 and 1980. *Botryllus* was the only invasive colonial ascidian present during the 1979 to 1980 study and accounted for an average of 6.16% cover on panels. In 2003 to 2005, the more recently arrived invasive *Botrylloides* and *Didemnum* accounted for 7.38% and 2.08% cover respectively, while *Botryllus* covered only 1.16%. Our results revealed a shift in seasonal abundance between 1979 to 1980 and 2003 to 2004. In 1979 and 1980 colonial ascidians had the highest percent cover in fall and winter while in 2003 and 2005 they had highest percent cover in summer and fall. Seasonal patterns of space occupation by colonial ascidians corresponded to temperature. These results illustrate substantial changes in the total and seasonal abundance of colonial ascidians in the Gulf of Maine within the last 26 years.

Introduction

There is a growing awareness that invasive ascidians have caused changes in the structure and composition of benthic communities (Whitlatch et al. 1995; Lambert, 2001). Among the types of changes involved are alterations from native-dominated to invasive-dominated communities and a reduction in the abundance of native species (Osman and Whitlatch, 1995a; Lambert and Lambert, 1998). Invasive ascidians can be transported to new areas by a number of human related vectors (e.g. ship, aquaculture species) as juveniles or adults, and once they've become established in new locations, they can persist and become dominant members of the community (Lambert, 2001; Godwin, 2003; Lambert and Lambert, 2003).

Four species of invasive colonial ascidians are currently present in the Gulf of Maine, *Botryllus schlosseri*, *Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp. A (Carlton, 2003; Bullard et al.,2007). *Botryllus* is the oldest invader and has been present since at least 1870 (Gould, 1870). *Botrylloides*, *Diplosoma* and *Didemnum* are more recent invaders and have arrived within the last 24 years (Berman et al., 1992; Carlton 1989; Harris et al., 1998; Bullard et al., 2007). Since their introduction, these species have become well established in northern New England fouling and benthic communities and two species (*Botrylloides* and *Didemnum*) have become very abundant (i.e., Berman et al., 1992; Carman and Roscoe 2003). Despite the fact that the three most recent invaders have been well documented, little work has assessed temporal changes within fouling communities that have resulted from the invasions. Additionally, little is known regarding the susceptibility of these invasive ascidians to native predators. Juvenile *Botrylloides*, *Diplosoma* and *Botryllus* are consumed by the snails *Mitrella lunata* and *Anachis lafresnayi* (Osman and Whitlatch, 1995b), but it is unclear which predators consume adult colonies. The purpose of this study is to: 1) examine the probable vectors associated with the introduction of these four invasive ascidians. 2) compare long-term differences in dominance and seasonal abundance patterns of the ascidian species using data from two identical panel studies, one conducted from 1979 to 1980 the other from 2003 to 2005 and 3) identify two new predators of adult invasive ascidians.

Study species

Botryllus schlosseri has occurred in the Gulf of Maine since at least 1870 (Gould, 1870) and is presently well established from Cape Cod, MA to Eastport, ME (Figure 1). The remaining three species arrived much more recently. *Botrylloides violaceus* (originally misidentified as *Botrylloides diegensis*) was first observed in 1980 at Woods Hole, MA (Carlton, 1989) and in 1981 at Fox Point in the Great Bay Estuary, NH (Berman et al., 1992). It did not appear on artificial substrates deployed at the Coast Guard Station, Newcastle, NH until 1982 (Berman et al., 1992). Photographic records indicate the species was also present in high abundances in 1982 on oyster aquaculture lanterns deployed in the Damariscotta River, ME (R. Clime, pers. comm. 2005). Currently, *Botrylloides* is found from Cape Cod, MA to Eastport, ME. *Diplosoma listerianum* is a temporally patchy invasive ascidian in shallow waters in the Gulf of Maine. It has been observed from Cape Cod, MA to Casco Bay, ME and it first appeared at the Isles of Shoals in 1993 (Harris et al., 1998), and Portsmouth Harbor in 1999 (Harris, L., pers. obs.). *Didemnum* sp. A was found in Portsmouth Harbor, NH in summer 2001 and at the Isles of Shoals in fall, 2004 (Bullard et al., 2007). Of the three recent invaders, *Didemnum* and *Botrylloides* have the widest distribution within the Gulf of Maine. Although widespread populations of *Didemnum* were only noticed within the last five years, this species has been present in New England for some time. The earliest confirmed specimens were collected from the Damariscotta River, Maine in 1988 (Bullard et al., 2007) and high abundances of the species were observed in photographs taken in the Damariscotta River in 1982 (R. Clime, documented appearances). In addition, both *Botrylloides* and *Didemnum* became such pests on suspended oyster net grow-out cages in the Damariscotta Estuary by 1980 that oyster farmers changed from suspended culture cages to bottom culture (R. Clime, pers. comm.).

Materials and Methods

Field sites

Appearance, potential vectors and habitats for *Botryllus*, *Botrylloides*, *Didemnum* and *Diplosoma* are based upon photographic data that were taken at a variety of locations that include Eastport, Winter Harbor, Damariscotta and

Cape Neddick, ME, Newcastle, Isles of Shoals, NH as well as Salem and Cape Cod, MA over several years beginning in 1969.

Data on the temporal abundance patterns of the colonial ascidians and potential predators were obtained from two sites in the southern Gulf of Maine: Star Island at the Isles of Shoals, and Portsmouth Harbor, NH (Figure 1.1). Both sites have been studied since the mid 1970's (Hurlbert, 1980; Harris and Irons, 1982; Witman, 1985; Harris and Chester, 1996; Harris et al., 1998; Harris and Tyrrell, 2001). The Star Island site is located at a depth of 8m, and has recently undergone shifts in community composition (Harris and Tyrrell, 2001). The site was formerly dominated by kelp beds (*Laminaria* spp.) and urchin (*Strongelocentrotus drobaechiensis*) barrens. More recently the site has become dominated by the invasive green alga *Codium fragile* subsp. *tomentosoides* (Carlton and Scanlon, 1985; Harris and Tyrrell, 2001) and the invasive colonial ascidians *Botrylloides* and *Diplosoma*. The Portsmouth Harbor site is located at a depth of 5m at mean low tide under a cement pier. It is a fouling community that has also undergone dramatic changes in community composition during the last 26 years (Dijkstra and Harris, unpublished data). In the late 1970's this site was dominated by blue mussels (*Mytilus edulis*), the sea anemone *Metridium senile*, the sponge *Haliclona loosanoffi* and the colonial ascidian *Botryllus* (Harris and Irons 1982). Currently, it is dominated *Botrylloides* and *Didemnum*.

Predators

We identified predators of the invasive colonial ascidians using a combination of direct observations documented by photography of active invertebrate predation events, and stomach content analysis of winter flounder *Pleuronectes americanus*. For stomach content analysis, fifty flounder were captured at 8m depth at the Isles of Shoals (June 1979). The fish were brought to the laboratory, sacrificed and their stomachs dissected to determine the presence or absence of colonial ascidian remains.

Temporal patterns of ascidians

Temporal patterns of abundance of *Botrylloides*, *Diplosoma*, *Botryllus* and *Didemnum* were quantified using two panel studies from the Portsmouth Harbor site. The first study was initiated in 1979 to document epifauna community development between 1979 and 1982 (Harris and Irons, 1982). 8 horizontal and 8 vertical predator inaccessible 0.1m^2 Plexiglas were deployed in July 1979 at approximately 5m below mean low water under the cement pier at the Coast Guard Station, Newcastle, NH. All panels were photographed monthly from 1979 to 1982. The second panel study was established in July 2003 to document epifauna community development between 2003 and 2006. The second study replicated the experimental design of the first study and the panels were photographed monthly. For this study we analyzed the first ~17 months of data collection from both studies (July 1979 to December 1980; July 2003 to February 2005). Photographs (~550 images) were digitized and downloaded into Adobe

Photoshop 7.0 ©. To ensure each image was square, all images were skewed in Adobe Photoshop 7.0 © before analysis. A point count (100 grid points) analysis was used to assess percent cover of invasive ascidians on each panel.

Temperature data from the Portsmouth Harbor site were collected from a HOBO temperature data logger deployed at 5m below mean low water. Temperature was recorded every hour from the time the panels were deployed in July 2003 to present. Daily minimum and maximum temperatures were calculated for 2003, 2004 and 2005.

Data analysis

The percent cover data were arcsin transformed and analyzed using a one-way Analysis of Variance (ANOVA) test to detect long-term differences in abundance of colonial ascidians between years. A post-hoc Tukey's test was used to detect differences between seasons in the different years (1979 to 1980 and 2003 to 2004). Seasons were defined as: summer (June, July, August and September), fall (October and November), winter (December, January, February and March) and spring (April and May). Error bars in figures 1.2, 1.3, 1.4 and 1.5 were calculated using standard error.

Results and Observations

Habitats

Invasive ascidian species arrivals and habitat colonization were documented using historical aquaculture reports, dive reports and photographic data that Harris and his students have taken since 1969 (Table 1.1 and 1.2; Hidu, 1974; Richmond, 1975; Pratt and Hidu, 1976). These records indicated all four species of invasive colonial ascidians colonized multiple habitats including fouling and benthic communities (Table 1.2). In addition to colonizing primary free space, all species were found as epibionts on the kelps *Lamanaria* spp. and *Agarum* sp., the green invasive alga *Codium fragile* subsp. *tomatosoides,* and numerous species of red algae.

Predators

We witnessed the nudibranch *Flabellina verrucosa* feeding upon adult *Botryllus* and *Botrylloides* (Morse, 1969 and Harris, photographic documentation), the winter flounder *Pleuronectes americanus* and the starfish *Henricia sanguinolenta* feeding upon adult *Botryllus* and *Diplosoma*, respectively (Harris L., pers. obs.). Additionally, the starfish *Henricia sanguinolenta* were photographed between 1995 and 2005 feeding with extended stomachs on *Diplosoma* colonies. Winter flounder stomach content analyses revealed that two of the fifty individuals contained remains of *Botryllus* colonies.

Temporal abundance patterns of invasive colonial ascidians

Average percent cover of invasive colonial ascidians on the experimental substrate significantly increased ($P<0.0001$) between 1979 and 2005 (Figure 1.2). Figure 1.3 indicates that between 1979 to 1980 and 2003 to 2005, subsequent to the arrival of *Botrylloides* and *Didemnum*, the percent cover of *Botryllus* has decreased. In the early panel study *Botryllus* occupied, on average, 6.16 % of the panel, while in the later study, *Botryllus* averaged 1.16 % cover. *Botrylloides* and *Didemnum* averaged 7.38 % and 2.08 % cover, respectively.

Annual patterns of abundance

Annual patterns of colonial ascidian abundance have shifted within the last 26 years with *Botryllus* being the only invasive colonial ascidian present in the late 1970's and *Botryllus*, *Botrylloides* and *Didemnum* present in the early 2000's. In 1979 and 1980, *Botryllus* occupied the greatest amount of space in fall and winter (Figure 1.4). In contrast, summer and fall months were the periods of greatest space occupancy in 2003 and 2005 by invasive colonial ascidians. Percent cover of *Botrylloides* was greatest in summer and fall (Figure 1.5), while *Didemnum* had the highest percent cover in fall and winter. Furthermore, seasonal variability in percent cover of invasive colonial ascidians in 2003 and 2005 appeared to be correlated with seasonal variability in seawater temperature (Figs. 1.6A and 1.6B). Colonial ascidians were most abundant during the months of summer and fall when seawater temperatures were generally >15°C and became less abundant when temperatures were <5°C. Because panels were initially deployed in July 2003, colonies that recruited to these panels had a stunted growing season but over-wintered. Consequently, over-wintered colonies had an earlier and longer growing season in 2004 than 2003 and thus, occupied more space than summer 2003.

Discussion

Timing and mechanism of the introductions of *Botryllus*, *Botrylloides*, *Diplosoma* and *Didemnum* in the Gulf of Maine

At least three potential anthropogenic vectors are responsible for the introduction of non-native colonial ascidians to the Gulf of Maine: 1) ship hull fouling, 2) ship ballast water, and 3) introduction of non-native aquaculture shellfish species (e.g. oysters). Because colonial ascidians produce short-lived lecithotrophic larvae (Lambert, 1968; Olson, 1985; Svane and Young, 1989), the likelihood of larvae surviving in ballast water is very low (Carlton and Geller, 1993). Thus, the most likely transport vectors are hull fouling and/or non-native aquaculture species.

Though somewhat speculative, we suggest that *Botryllus* and *Diplosoma* may have been introduced to the Gulf of Maine by hull fouling. *Botryllus* has been resident in the area since before the 1870's (Gould 1870) and this was a period before the use of non-native shellfish species for aquaculture purposes. *Diplosoma* was first discovered south of Cape Cod, MA before it was found at the

Isles of Shoals, NH in 1993 (Harris and Tyrrell, 2001). The Isles of Shoals is a high traffic area for recreational vessels in the summer and we hypothesize vessels transiting from southern New England to the Gulf of Maine were the likely vector for introduction of *Diplosoma*.

In contrast we suggest *Botrylloides* and *Didemnum* may have been introduced by non-native oyster aquaculture (either the European flat oyster *Ostrea edulis* and/or the Pacific oyster *Crossastrea gigas*) in the Damariscotta River, ME. Large numbers of *Didemnum* and *Botrylloides* colonies were photographed in the Damariscotta River in 1982 (R. Clime, pers. comm., 2005), eleven years after oyster aquaculture began in the area (Hidu, 1974; Pratt and Hidu, 1976). Further support for this hypothesis comes from the fact that the Damariscotta River was the first site where *Didemnum* was observed in the U.S. coastal waters (Bullard et al., 2007).

Temporal patterns of invasive ascidians

Over the past 26 years, the percent cover of colonial ascidians in subtidal habitats in coastal portions of the Gulf of Maine has doubled. There has also been a change in composition; *Botryllus* was the dominant species between 1979 and 1980, while *Didemnum* and *Botrylloides* are now the major space occupants. The significant decline in *Botryllus* abundance is likely due to competitive interactions with *Botrylloides* and *Didemnum* (Dijkstra, J. unpubl. data).

Seasonal variations in seawater temperature appear to play a significant role in structuring community dynamics in this system. *Botrylloides* was the spatially dominant member during periods of high water temperatures while *Didemnum* was a major space occupant during periods of cooler water temperatures. These observations are consistent with results of recent laboratory studies that show *Botrylloides* has a faster growth rate than *Botryllus* and *Didemnum* in warmer water conditions (Stachowicz et al., 2002b; McCarthy et al., 2007) while *Didemnum* grows faster in cooler water temperatures (McCarthy et al., 2007). The marked decrease in percent cover by colonial ascidians in winter may be the result of extremely low water temperatures, characteristic of the Gulf of Maine, which inhibits ascidian proliferation.

Of particular ecological importance is the annual shift in colonial ascidian abundance that has occurred over the past 26 years in the coastal waters of the Gulf of Maine. Historically (i.e. late 1970's) colonial ascidians were most abundant during the late fall and winter. More recently, they are most abundant during the summer and early fall. This shift in abundance may have a profound impact on benthic communities. Many colonial ascidians are chemically defended from predators (Lindquist et al., 1992; Teo and Ryland, 1994; Joullie et al., 2003) and make settlement and growth difficult for other epifaunal organisms (Grosberg, 1981; Stachowicz et al., 2002a Bryan et al., 2003; Bullard et al., 2004; Dijkstra et al., 2007). Because many species reproduce, settle and experience high growth rates during the summer and fall (Fuller, 1946; Stachowicz et al., 2002b), the increased abundance of colonial ascidians could inhibit the recruitment of species and reduce the amount of space available for other species. This in turn could lead to dramatic changes in species composition.

Table 1.1: Appearance and potential vectors for *Botryllus schlosseri*, *Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp. A

Species	First documented	Distribution in the GOM	Potential Vectors
Botryllus schlosseri	1870 (Gould, 1870)	Cape Cod, MA to Penobscot Bay, ME	Shipping
Botrylloides violaceus	1980	Cape Cod, MA to Penobscot Bay, ME	Aquaculture
Diplosoma listerianum	1993	Cape Cod, MA to Casco Bay, ME	Shipping
Didemnum sp.	1982	Cape Cod, MA to Eastport, ME	Aquaculture

Table 1.2: Distribution of the four dominant colonial ascidians in various habitats. Fouling refers to man-made (artificial substrate) while benthic refers to natural subtidal substrate.

Species	Habitat					
	Fouling			Benthic		
	Vertical	Upper horizontal	Lower Horizontal	Vertical	Horizontal	Epibiont
Botryllus schlosseri	X		X	X	X	X
Botrylloides violaceus	X		X	X	X	X
Diplosoma listerianum	X	X	X	X	X	X
Didemnum sp. A	X	X	X	X	X	X

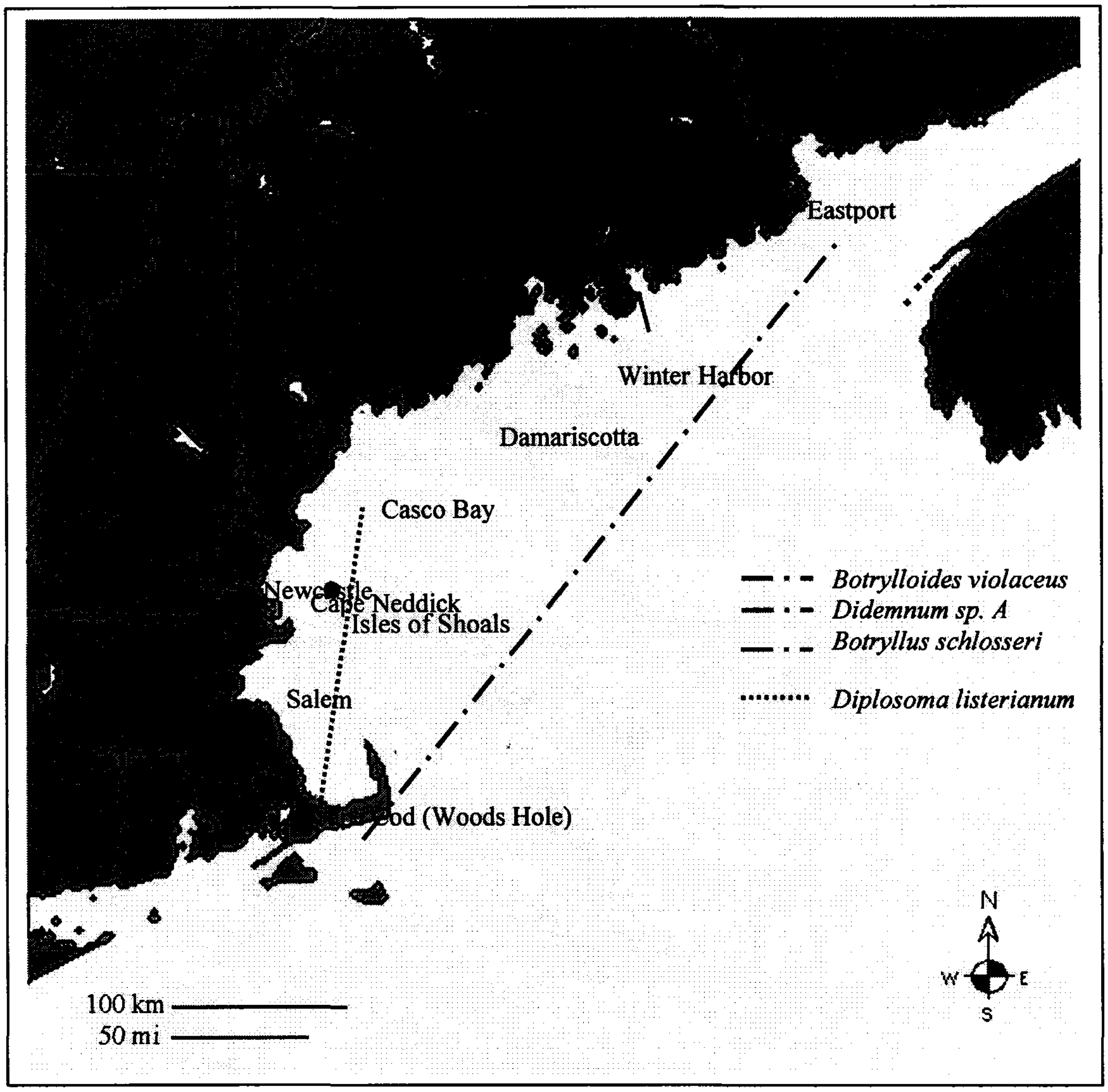


Figure 1.1: Distribution of *Botryllus schlosseri, Botrylloides violaceus, Didemnum* sp. A and *Diplosoma listerianum* in the coastal Gulf of Maine.

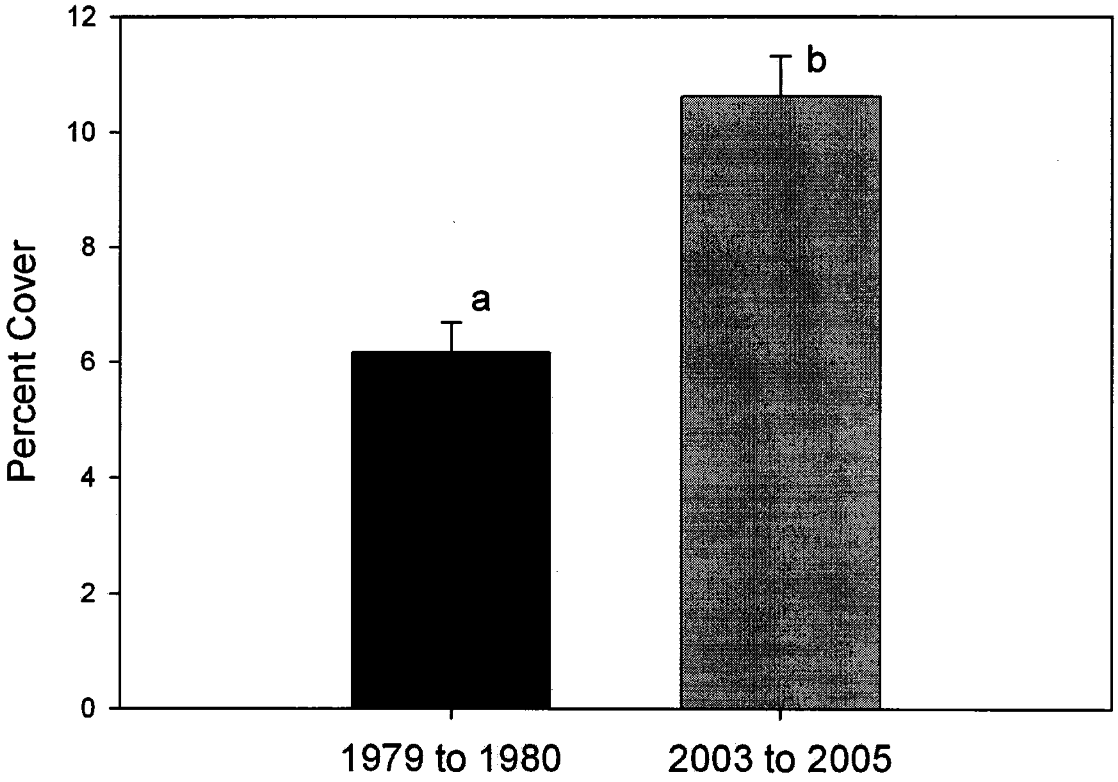


Figure 1.2: Average annual percent cover of invasive colonial ascidians on panels between 1979 to 1980 and 2003 to 2005 ($P<0.001$). In 1979 and 1980 *Botryllus schlosseri* was the only dominant invasive ascidian.

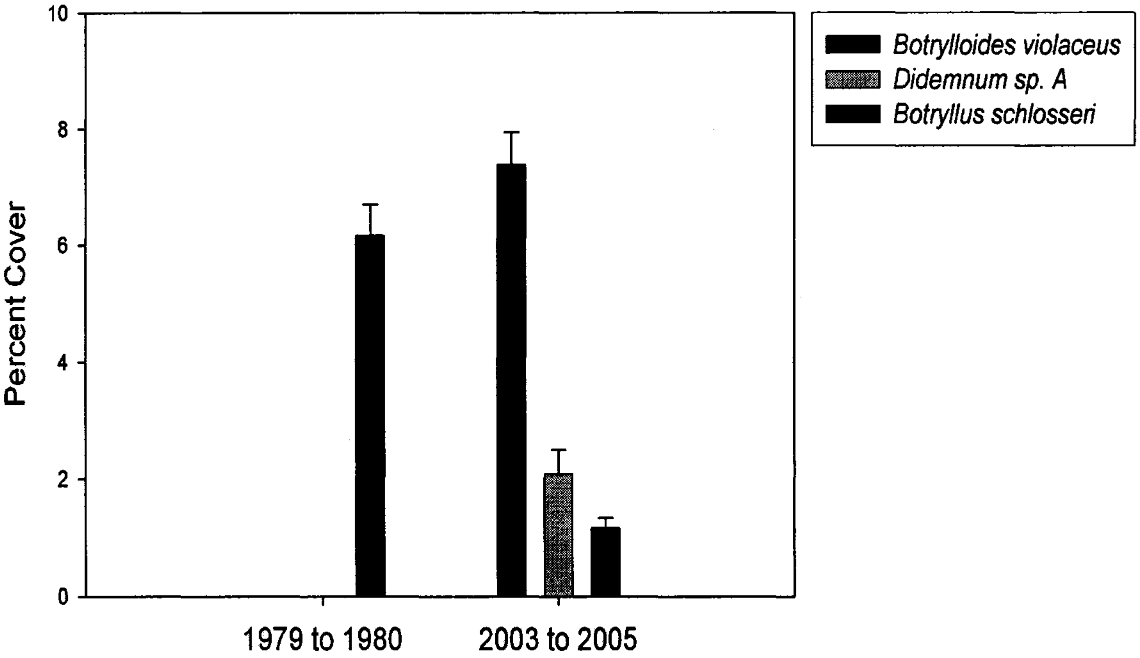


Figure 1.3: Average annual percent cover of *Botrylloides violaceus*, *Didemnum* sp. A and *Botryllus schlosseri* on panels between 1979 to 1980 and 2003 to 2005. In 1979 and 1980, *Botryllus schlosseri* was the only dominant invasive ascidian.

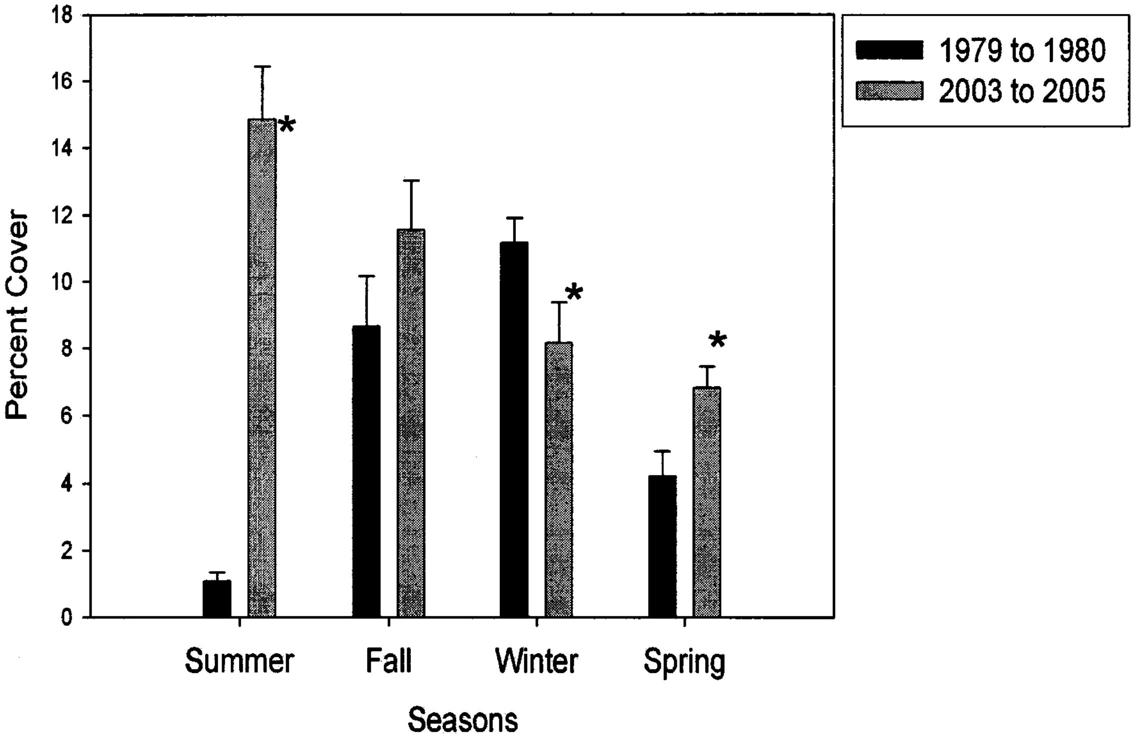


Figure 1.4: Average seasonal percent cover of invasive ascidians on panels observed in 1979 to 1980 and 2003 to 2005. Significant differences (*) were found in the abundance of colonial ascidians between the seasons of summer, winter and spring (*P*<0.05) 1979 to 1980 and 2003 to 2004.

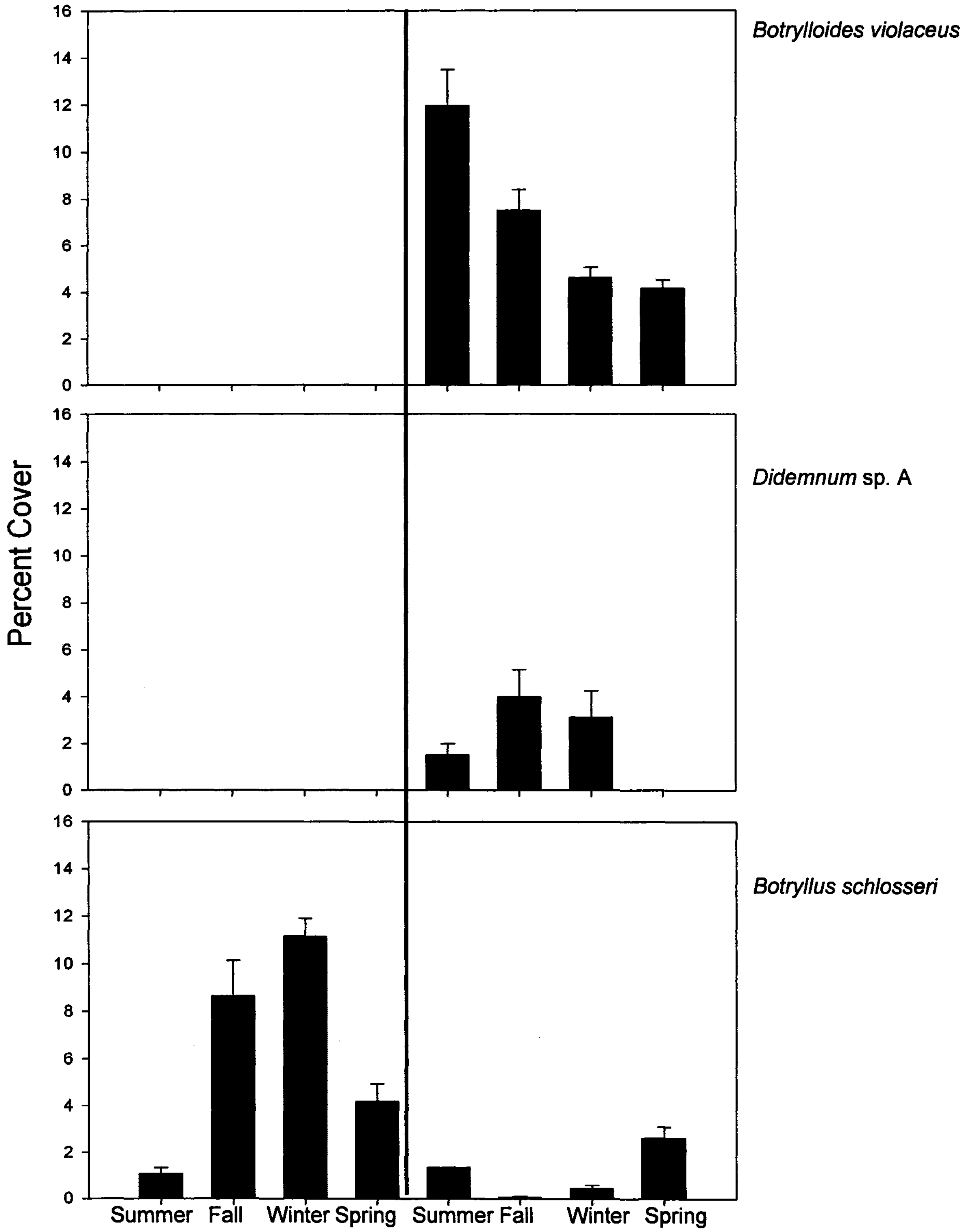


Figure 1.5: Average seasonal (summer, fall, winter and spring) percent cover of individual species from 1979 to 1980 and 2003 to 2005. 1979 to 1980 percent cover is to the left of the black line, while 2003 to 2005 percent cover is to the right of the black line.

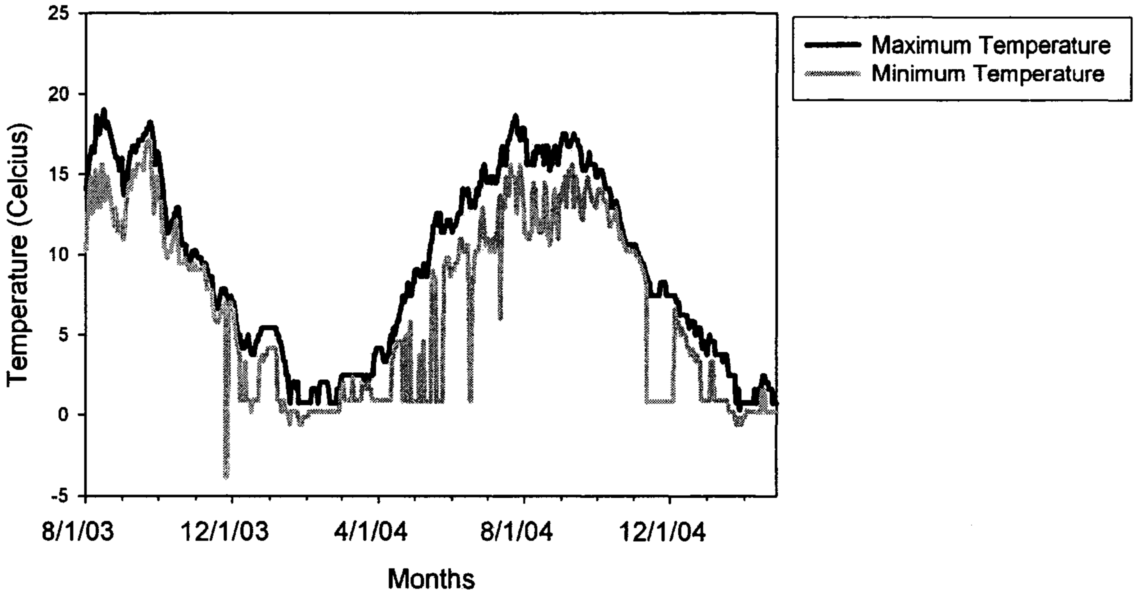


Figure 1.6A: Daily maximum and minimum water temperatures for Portsmouth Harbor at 5m below mean water from August 2003 to February 2005.

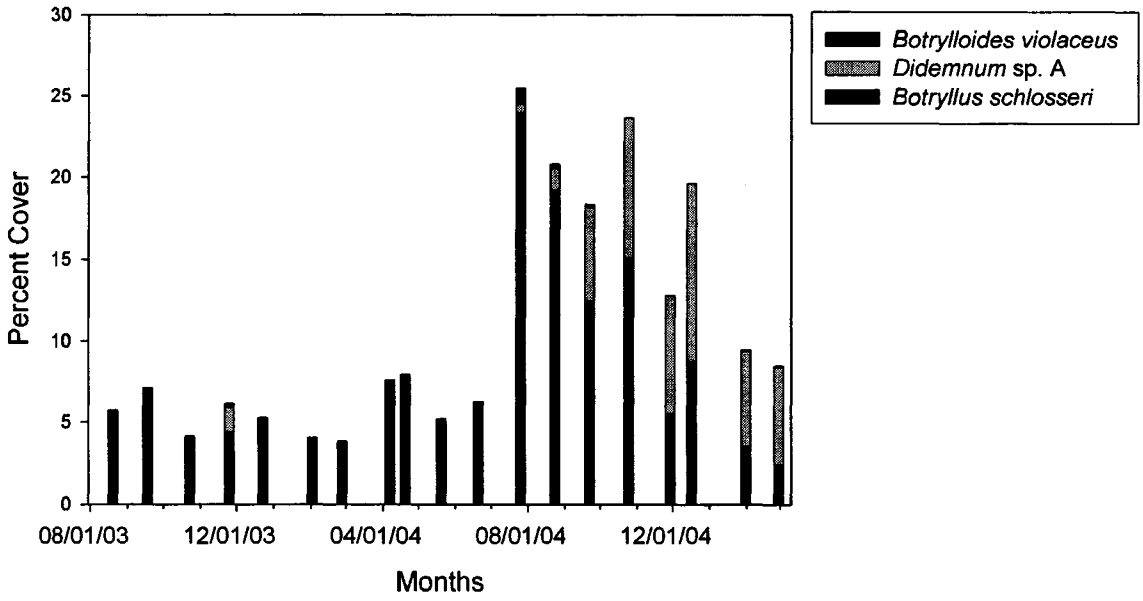


Figure 1.6B: Percent cover of colonial ascidians in Portsmouth Harbor from August 2003 to February 2005.

CHAPTER II

THE ROLE OF COLONIAL ASCIDIANS IN ALTERING BIODIVERSITY IN MARINE FOULING COMMUNITIES

Introduction

A suite of invasive species, each with their own unique morphologies, have recently become members of Gulf of Maine subtidal communities (Harris and Tyrrell 2001; Pederson et al. 2005). Three of these species, *Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp., are colonial ascidians and have become major space occupants in New England fouling communities (Berman et al., 1992; Bullard et al., in press; Osman and Whitlatch, 1995; Osman and Whitlatch, 1999; Whitlatch et al., 1995). They are warm water, 2-dimensional species that dominate during seasons of greatest recruitment (Stachowicz et al. 2002). Therefore, the presence of these species in our system will significantly influence the recruitment of species to the community. The goal of this study was to examine the role of colonial ascidians in the development of communities by comparing the recruitment of species onto panels containing organisms with different functional morphologies.

Materials and Methods

We conducted our study at two sites with similar species composition in Portsmouth Harbor, Newcastle, New Hampshire. The communities occupying the floating docks at these sites included the blue mussel *Mytilus edulis*, the sea anemone *Mitridium senile*, the encrusting sponges *Halichondria panicea*, *Haliclona loosanoffi* and *Leucosolenia* sp., the hydroids *Ectopleura larynx* and *Obelia* spp., the solitary ascidians *Molgula* spp. and *Ciona intestinalis* and the established colonial ascidian *Botryllus schlosseri*. Also, invasive colonial ascidians *Botrylloides violaceus* and *Didemnum* sp. A were abundant at both sites.

To examine the effects of adult colonial ascidians on recruitment of individuals and species, species observed in the fouling communities were divided into functional groups. A functional group of any species was based upon its morphology (encrusting or erect) and growth pattern (solitary or colonial; Jackson 1977). Two, two-week settlement panel experiments were conducted in July and August, 2004. Experiments were carried-out using 70%-100% cover treatments on 100cm^2 Plexiglas panels. To attain this percent cover, species from each functional group were collected from floating docks and glued, using plastic surgery glue, to panels. Each functional group consisted of two to four species (Table 2.1). Experiments consisted of three replicates of the following treatments: Encrusting bryozoans, bivalves, sponges, erect bryozoans, hydroids, solitary and colonial ascidians as well as two controls (glue and no glue). Two controls were used to determine if the plastic surgery glue would effect recruitment. Prior to the beginning of each experiment all epibionts were removed from the panels and from the treatment organisms on the panels. Panels were left in the field for two weeks, at which time they were collected and brought to the lab for analysis of recruits.

All individuals that settled upon adult individuals were counted and identified to species. Only panels where the treatment organism survived were analyzed and all analyses were performed on square root transformed data.

Results

We found differences in the number of individuals and species that recruited to functional groups ($P<0.05$, one-way ANOVA). A post-hoc Tukey pairwise comparisons test revealed significant differences in recruitment between colonial ascidians and sponges and all other functional groups ($P<0.05$) (Figures 1 and 2).

We found recruitment of individuals and species was significantly lower on colonial ascidians and sponges compared to the other 5 functional groups (Figures 2.1 and 2.2). Encrusting bryozoans (*Electra pilosa*, *Membranipora membranacea*) and solitary ascidians (*Styela clava*, *Ascidiella aspersa*, *Ciona intestinalis* and *Molgula* spp.) were found on adult hydroids, bivalves, solitary ascidians and erect bryozoans but not adult sponges or colonial ascidians. In addition, high numbers of *Mytilus edulis*, *Balanus* spp., *Molgula* spp., and *Ectopleura larynx* were found on adult hydroids, bivalves, solitary ascidians and erect bryozoans. In contrast, few individuals were observed on sponges and colonial ascidians.

Discussion

The disparity in recruitment among functional groups is most likely the result of two factors. First, erect and hard covered species are more structurally complex than the encrusting, 2-dimensional sponges and colonial ascidians (Dean and Hurd, 1980; Dean, 1981; Jackson, 1977). Hydroids and erect bryozoans, in particular, have been found to harbor large numbers of *Mytilus edulis* and *Molgula* spp. (Dean and Hurd, 1980; Dean, 1981). Bivalves and hard tunic solitary ascidians have surfaces that are also easily colonized by species (Dean and Hurd 1981; Jackson 1977). Second, many sponges and colonial ascidians synthesize allelopathic chemical compounds that inhibit the recruitment of individuals (Jackson and Buss, 1975; Bryan et al., 2003; Davis et al., 1998; Joullie et al., 2003). Consequently low abundance of individuals and species found on sponges and colonial ascidians is most likely a result of toxic chemicals produced by both sponges and colonial ascidians that inhibit the recruitment of individuals and a reduction in spatial dimensionality, from 3 to 2 dimensions.

Table 2.1: Species assigned to functional groups and used in both experiments

Functional group	Species
Erect bryozoans	*Bugula Turrita*
	Bugula simplex
Hydroids	*Obelia* sp.
	Ectopleura larynx
Bivalves	*Mytilus edulis*
Sponges	*Halichondria panicea*
	Haliclona sp.
	Leucosolenia sp.
Solitary ascidians	*Ciona intestinalis*
	Molgula sp.
	Styla clava
	Ascidiella aspersa
Colonial ascidians	*Botrylloides violaceus*
	Didemnum sp. A
	Botryllus schlosseri
Encrusting bryozoans	*Membranipora membranacea*
	Electra pilosa
	Cryptosula pallasiana

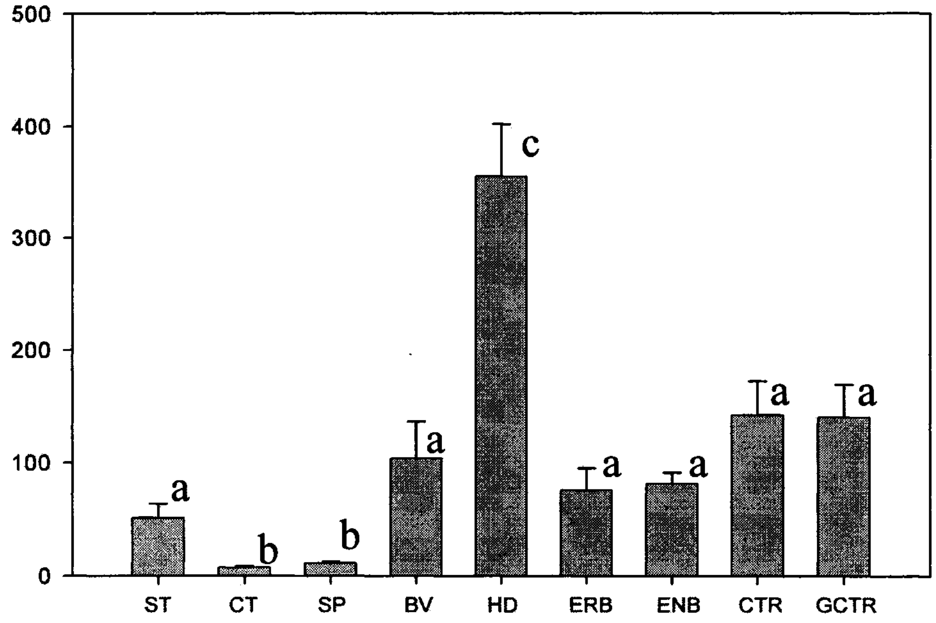


Figure 2.1: Mean number of individuals found on functional groups for the two experiments. ST: Solitary ascidians, CT: Colonial ascidians, SP: Sponge, BV: Bivalves, HD: Hydroids, ERB: Erect bryozoans, ENB: Encrusting bryozoans, CTR: Control, GCTR: Glue control

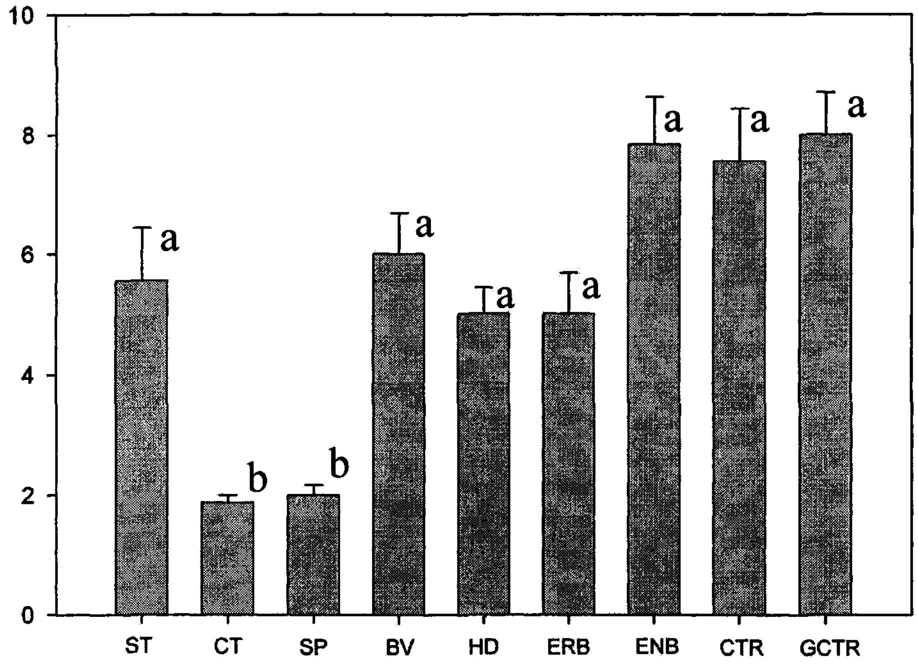


Figure 2.2: Mean number of species found on functional groups for the two experiments. ST: Solitary ascidians, CT: Colonial ascidians, SP: Sponge, BV: Bivalves, HD: Hydroids, ERB: Erect bryozoans, ENB: Encrusting bryozoans, CTR: Control, GCTR: Glue control

CHAPTER III

COMMUNITY LEVEL EFFECTS OF INVASIVE SPECIES

Abstract

Invasive species and their effect on secondary substrate of subtidal marine fouling communities have received little attention in recent years. In this study we examined the effects of invasive colonial ascidians on the recruitment of fouling species. To examine recruitment patterns onto panels with the non-native colonial ascidians, we divided adult species into functional groups based on morphology and growth patterns. Three, 2-week experiments were conducted using 30% to 50% cover of 7 different functional groups. Results indicate the non-native colonial ascidians reduce diversity and affect community composition in two ways: they reduce the recruitment of individuals and species and alter the composition of recruits. The presence of invasive species in subtidal fouling communities not only changes the composition of primary space occupants but also potentially alters the further development of these communities.

Introduction

Secondary space is often provided by established adults in hard substrate communities (Dayton 1971, Osman & Whitlatch 1995, 1995, Barnes 2001). Many species can create 3-dimensional structures that enable the coexistence of a greater number of individuals and species compared to the absence of these structures (Jackson 1977, Woodin & Jackson 1979, Barnes 2001). For example, the surfaces of corals and bivalves often provide substrates for the recruitment of species (Jackson 1977, Woodin & Jackson 1979, Osman & Whitlatch 1995, Barnes 2001). Arborescent species such as bryozoans and hydroids can provide vertical structure to fouling communities that can also act as a refuge against predators and facilitate succession (Standing 1976, Dean & Hurd 1980). However, not all species enhance recruitment or create 3-dimensional communities, particularly those that possess unique functional morphologies (encrusting or erect), growth patterns (solitary or colonial) or defenses that inhibit settlement of larvae (Buss 1976, Jackson 1977, Buss & Jackson 1979).

Additionally, the presence of established superior competitive adults may also inhibit or facilitate recruitment to bare space (Grosberg 1981, Young & Chia 1985). Some species may act as settlement cues for conspecific larvae while others may release allelopathic chemical compounds that harm competitors or settling larvae (Raimondi 1990, Thacker et al. 1998, Engel & Pawlik 2000, Koh & Sweatman 2000). Consequently, significant alterations in species composition as a result of invasive species have the potential to change the composition of recruits.

In recent years, invasive species have caused significant changes in the structure and composition of sessile communities and have often become dominant primary space occupants (Carlton 1996, Ruiz et al. 1997, Cohen & Carlton 1998, Cohen et al. 2005, Pederson et al. 2005, Reise et al. 2006). For example, the blue mussel, *Mytilus edulis*, the hydroid *Ectopleura larynx*, the sponge *Haliclona* sp. and the colonial ascidian *Botryllus schlosseri* were dominant members of fouling communities from 1979 through 1982 (Harris & Irons 1982). *Mytilus edulis*, in particular covered 40% to 100% of panels for approximately 2 years beginning in April, 1980 (Dijkstra and Harris, unpubl. data). Unlike the historical community, invasive ascidians and an invasive encrusting bryozoan now dominate Gulf of Maine fouling communities (Dijkstra et al. 2007). Though their morphologies, basic body plans, structure and growth patterns are functionally similar to other resident species, their dominance of primary space can potentially affect the availability of secondary space.

Three invasive species observed in fouling communities in the Gulf of Maine, *Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp., are colonial ascidians, two species are solitary ascidians *Ascidiella aspersa* and *Styela clava* and one is an encrusting bryozoan *Membranipora membranacea* (Berman et al. 1992, Whitlatch et al. 1995, Whitlatch & Osman 1999). Many of these species have altered the dominance and composition of primary space occupants (Lambert et al. 1992, Whitlatch et al. 1995, Osman & Whitlatch 1999, Lambert 2001, Bullard et al. 2007, Dijkstra et al. 2007). Additionally, they tend to dominate during seasons of greatest species recruitment and therefore can potentially change the availability of secondary space (Stachowicz et al. 2002, Pederson et al. 2005, Dijkstra et al. 2007). In a previous study using 70% to 100% cover treatments of seven functional groups (sponges, hydroids, erect bryozoans, encrusting bryozoans, solitary ascidians, colonial ascidians, and bivalves), we found colonial ascidians and sponges significantly reduced species diversity (Dijkstra et al. 2007). However, it was unclear if we would obtain similar results if the substrate was only partially covered (50%) by each functional group and if each functional group similarly affected the species composition of recruits.

The purpose of our investigation was twofold: 1) to compare the recruitment of species onto functional groups and bare space on panels containing 30% to 50% cover of organisms with different functional morphologies and 2) to compare these results to those obtained for the 70% to 100% cover (Dijkstra et al. 2007). Examining partial coverage of a substrate would allow us to compare the impact of functional groups on recruits and species composition.

Materials and methods

We conducted our study at 3 sites with similar fouling species composition in Portsmouth Harbor, Newcastle, New Hampshire (Figure 3.1). The communities occupying the floating docks at these sites included the blue mussel

Mytilus edulis, the sea anemone *Metridium senile*, the sponges *Halichondria panicea*, *Haliclona loosanoffi* and *Leucosolenia* sp., the hydroids *Ectopleura larynx* and *Obelia* spp., the solitary ascidians *Molgula* spp. and *Ciona intestinalis* and the established colonial ascidian *Botryllus schlosseri.* More recently, the invasive colonial ascidians *Botrylloides violaceus* and *Didemnum* sp. and the bryozoan *Membranipora membranacea* have become dominant members of these communities (Dijkstra et al. 2007).

To examine the effect of functional groups on recruitment and species composition, species in the fouling communities were divided into 7 functional groups based on their size, form, growth pattern, and reproductive strategies (Jackson 1977). Three, 2-week experiments were conducted in July and August, 2004 and July 2005 on 10 X 10 cm Plexiglas® panels. Experiments were carried out using 30% to 50% cover of individual functional groups (colonial ascidians, solitary ascidians, sponges, hydroids, erect, encrusting bryozoans and bivalves) and two controls (panels with glue and no glue). 50% of the surface of the control glue panels was covered in glue. Each experiment had 3 replicates of each of the 7 functional groups and controls. Species from each functional group were collected from floating docks and glued, using Superhold Plastic Surgery glue®, to panels. Each panel consisted of two to four species of each functional group and was left in the field for ~ 1-week before the start of an experiment (Table 3.1). Prior to the beginning of each experiment all epibionts were removed from panels and also from treatment organisms glued to panels. Individual panels were attached to bricks and hung, with a nylon rope, off floating docks. They were left in the field for two weeks, at which time they were collected and brought to the laboratory for analysis of recruits.

All individuals that settled upon adult individuals and bare panel were counted and identified to species. Only panels where the treatment organism survived were analyzed and all analyses were performed on square root transformed data. A one-way Analysis of Variance (ANOVA) was used to test for differences in recruitment of individuals and number of species onto the various functional groups and bare panel surface (Zar 1999). Data were grouped by treatment and surface type (bare space or functional group), resulting in two groups of surfaces that were analyzed using one-way ANOVA. A post-hoc Tukey pairwise comparisons test was used to detect differences in recruitment of species and individuals among functional groups. All ANOVA statistical analyses were generated using JMP software© (JMP 6, SAS Institute, Cary, NC).

To examine divergence in faunal composition of the recruits among functional groups, a Bray-Curtis similarity matrix was constructed using abundances that were first square root transformed (Clarke & Warwick 2001). A multi-dimensional scaling plot (MDS) and cluster analysis were performed using average linkage on the Bray-Curtis similarity matrix. In addition an analysis of similarities (ANOSIM) was calculated from the Bray-Curtis similarity matrix. To assess these differences in species composition we used the PRIMER 6.0 software package (Clarke & Warwick 2001).

Results

An average of 67 individuals and 4 species per panel settled on 7 functional groups (Figs. 2a and 3a), whereas 105 individuals and 10 species were counted on bare space (Figs. 3.2b and 3.3b). The number of recruits were significantly ($p \leq 0.01$) higher on control panels (both glue and no glue) than individual functional group panels, with an average of 170 individuals (control = 164 individuals; glue control = 177 individuals) and 10 species (control = 9.6 species; glue control = 10.6 species) per panel.

Overall, significantly fewer individuals settled on sponges, colonials ascidians, encrusting bryozoans and solitary ascidians ($p \leq 0.05$, Figs. 3.2b). Significantly fewer species settled on colonial ascidians and sponges than other functional groups and controls ($p \leq 0.05$). Encrusting (*Membranipora membranacea*, *Electra pilosa*, *Cryptosula pallasiana*), erect (*Bugula simplex* and *Bugula turrita*) bryozoans and solitary ascidians (*Molgula* sp. and *Ciona intestinalis*) were not found on the tunics of colonial ascidians. Fewer individuals of *Mytilus edulis*, *Hiatella arctica*, sponges, *Ectopleura larynx* and the colonial ascidians *Botrylloides violaceus* and *Botryllus schlosseri* recruited on colonial ascidians and sponges. In contrast, several species recruited to arborescent hydroids and bryozoans than other functional groups. Most of the individuals were found nestled amongst the stems and branches of *E. larynx* and *Bugula* species. Individuals were mainly two taxa, *M. edulis* and *Molgula* sp. Unlike *M. edulis* and *Molgula* spp., recruits of the colonial ascidians *B. violaceus* and *B.*

schlosseri and sponges were often observed wrapped around the hydrocauli of both *Obelia* sp. and *E. larynx* and in between and behind the zooecia of *Bugula* spp. While many species recruited to one of the functional groups, species from the group “encrusting Bryozoa” recruited to open primary space only.

No significant differences were detected for number of species settling on bare space on panels with functional groups or control panels (Figure 3.3b, $p \leq 0.05$). Significantly fewer individuals settled on bare space with adult colonial ascidians, solitary ascidians, hydroids, bivalves and sponges than encrusting or erect bryozoans (Figure 3.2b; $p \leq 0.05$).

The dendrogram derived from recruitment densities onto the different functional groups revealed a 78% dissimilarity between colonial ascidians and sponges compared to other functional groups and controls (Figs. 3.4a,b). Similar groupings were found when the data were plotted in a non-metric multidimensional scaling plot. The results of an ANOSIM test corroborated the groupings and found significant differences between the assemblage of individuals and species that recruited to sponges and colonial ascidians versus other functional groups and controls (Figure 3.4a,b; $R = 0.793$, $p \leq 0.001$). No significant differences were detected between assemblages of recruits onto open space on panels with functional groups (Figure 3.5a,b; $R = 0.095$, $p = 0.114$).

Discussion

The invasive colonial ascidians *Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp. have recently become dominant primary and secondary space occupants of subtidal hard substrate communities in the Gulf of Maine (Berman et al. 1992, Bullard et al. 2007, Dijkstra et al. 2007). Previous studies have shown colonial ascidians are often superior spatial competitors by overgrowing other sessile invertebrates (Grosberg 1981, Young & Chia 1985, Stachowicz et al. 2002, Bullard et al. 2004). In contrast, our results showed little difference in the density of recruits or species to bare space on panels with competitively superior species e.g. colonial ascidians or sponges (Figure 3.2b and 3.3b). One hypothesis for describing this result may be the relatively high number of invasive ascidian species that have recently become seasonally dominant members (summer and fall) of Gulf of Maine fouling communities (Harris & Tyrrell 2001, Dijkstra et al. 2007). Non-native ascidians had the highest overall recruitment, making up 28.5% of recruits onto functional groups and 27.6% on bare space. Ascidian larvae tend to be indiscriminate settlers, recruiting to a variety of primary (wood, rock and cement pilings) and secondary (algae, bivalves, other ascidians, hydroids and sponges) substrates (Berrill 1950, Standing 1976, Dean & Hurd 1980). Elevated abundances of invasive ascidian species may have resulted in a higher level of homogeneity among recruits to bare space on panels with functional groups. In addition, chemical defenses possessed by the tadpole larvae of colonial ascidians may facilitate ubiquitous settlement on substrates regardless of the presence of competitively superior adults (Young & Bingham 1987).

Number of species recruiting onto functional groups was similar to the results of our 70% to 100% cover experiments (Dijkstra et al. 2007). While significantly fewer individuals settled on colonial ascidians and sponges than other functional groups in the 70% to 100% cover experiments, density of recruits on sponges, colonial ascidians, encrusting bryozoans and solitary ascidians was similar in the 30% to 50% cover experiment (Dijkstra et al. 2007). Though the difference in recruitment of individuals between experiments is unclear, it may be that open space on panels in the 30% to 50% cover experiments may attract larvae that would avoid a panel with 100% cover of colonial ascidians and sponges.

Hydroids and erect bryozoans enhanced species diversity, acting as refuges for the solitary ascidians *Molgula* spp. and the blue mussel *Mytilus edulis*; their stems were colonized by sponges and introduced colonial ascidians. In Bodega Bay, CA and Delaware River, NJ, hydroids enhanced the settlement of solitary ascidians (Standing 1976, Dean & Hurd 1980). In both systems hydroids facilitated different species composition relative to controls and other functional groups, resulting in disparate combinations of species. *Ectopleura crocea* enhanced settlement of *Molgula* spp. and *Obelia dichotoma* enhanced recruitment of the solitary ascidian *Ascidia ceratodes* thereby increasing diversity by harboring numerous polychaetes and amphipod species (Standing 1976, Dean 1981). Our results were consistent with these results and showed arborescent organisms (e.g., hydroids) facilitated recruitment. Solitary ascidians and bivalves provided additional substrate for larval settlement, though less than arborescent groups and controls. The outer surfaces supplied by these groups increased recruitment in areas as diverse the sub-Antarctic, Caribbean, Connecticut and North Carolina (Jackson 1977, Sutherland & Karlson 1977, Sutherland 1978, Dean & Hurd 1980, Osman & Whitlatch 1995, Barnes 2001).

The species composition of recruits settling onto sponges and colonial ascidians was dissimilar to the other functional groups and controls. The disparity in species composition is most likely the result of two factors. First, erect and hard shelled species are structurally complex and facilitate recruitment (Jackson 1977, Sutherland & Karlson 1977, Sutherland 1978, Dean & Hurd 1980, Dean 1981, Barnes 2001). Colonial ascidians are structurally 2-dimensional organisms with a smooth surface that often inhibit the attachment of species (Seed, 1979). Dean (1981) compared the structural complexity of mimic species and found arborescent individuals to be important in facilitating succession and maintaining species composition. Bivalves and hard tunic solitary ascidians have surfaces that are also easily colonized by species (Jackson 1977, Osman 1977, Dean 1981). Second, many sponges and colonial ascidians harbor antifouling chemical compounds that deter predation and hinder recruitment of individuals (McClintock 1987, Davis et al. 1998, Tarjuelo et al. 2002, Bryan et al. 2003, Joullie et al. 2003, Marti et al. 2003). Our results indicate that the impact of invasive species on subtidal communities may be dependent upon the functional group and dominance patterns of exotic species.

While short-term dominance of introduced colonial ascidians decrease diversity (Dijkstra et al. 2007), it is unclear whether diversity would be depressed at longer time scales. In the Gulf of Maine, population densities of adult colonial ascidians vary seasonally and among sites within regions (Yund & Stires 2002, Dijkstra et al. 2007). Unlike some dominant space occupants (e.g. *Mytilus edulis*) that occupy space for months and potentially years, adult colonial ascidians have a relatively short life-span and undergo senescence after a number of reproductive cycles (asexual and sexual) (Chadwick-Furman & Weissman 1995). Senescing colonies slough off substrate, leaving open space for the recruitment of resident species.

Colonial ascidians and sponges create distinct assemblages of recruits that are dissimilar to assemblages of recruits observed on other functional groups. Over the last 30 years there has been a change in dominant space occupants, from mussels and hydroids to invasive colonial ascidians during the summer and early fall months (Harris and Irons 1982, Dijkstra and Harris, unpubl. data). This shift in dominance patterns of primary space occupants during periods of peak recruitment as a result of invasive species may, therefore, may delay and alter the trajectory of succession.

Table 3.1: Species assigned to functional groups in the 30% to 50% experiments

Functional group	Species
Erect bryozoans	*Bugula turrita*
	Bugula simplex
	Bugula stolonifera
Hydroids	*Obelia* sp.
	Ectopleura larynx
Bivalves	*Mytilus edulis*
	Anomia simplex
Sponges	*Halichondria panicea*
	Haliclona sp.
	Leucosolenia sp.
Solitary ascidians	*Ciona intestinalis*
	Molgula sp.
	Styla clava
	Ascidiella aspersa
Colonial ascidians	*Botrylloides violaceus*
	Didemnum sp. A
	Botryllus schlosseri
Encrusting bryozoans	*Membranipora membranacea*
	Electra pilosa
	Cryptosula pallasiana

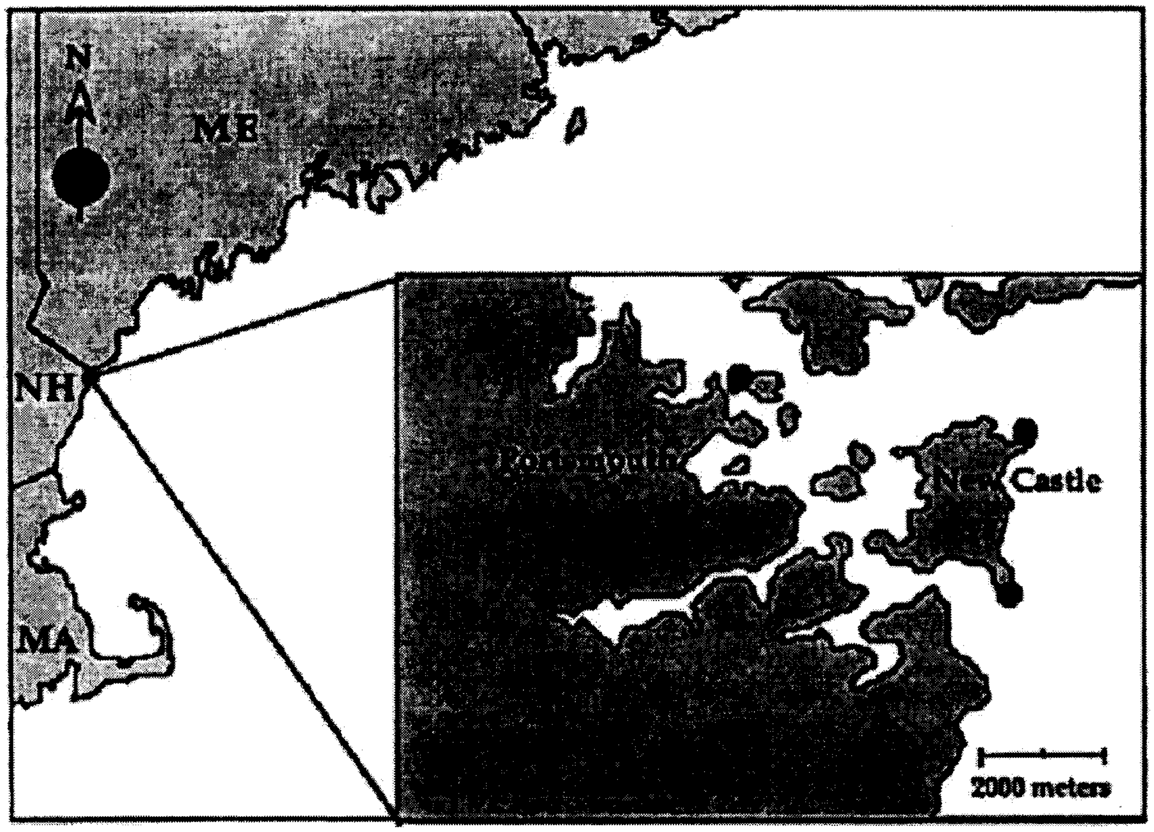


Figure 3.1: Map of Gulf of Maine. Black dots represent study sites.

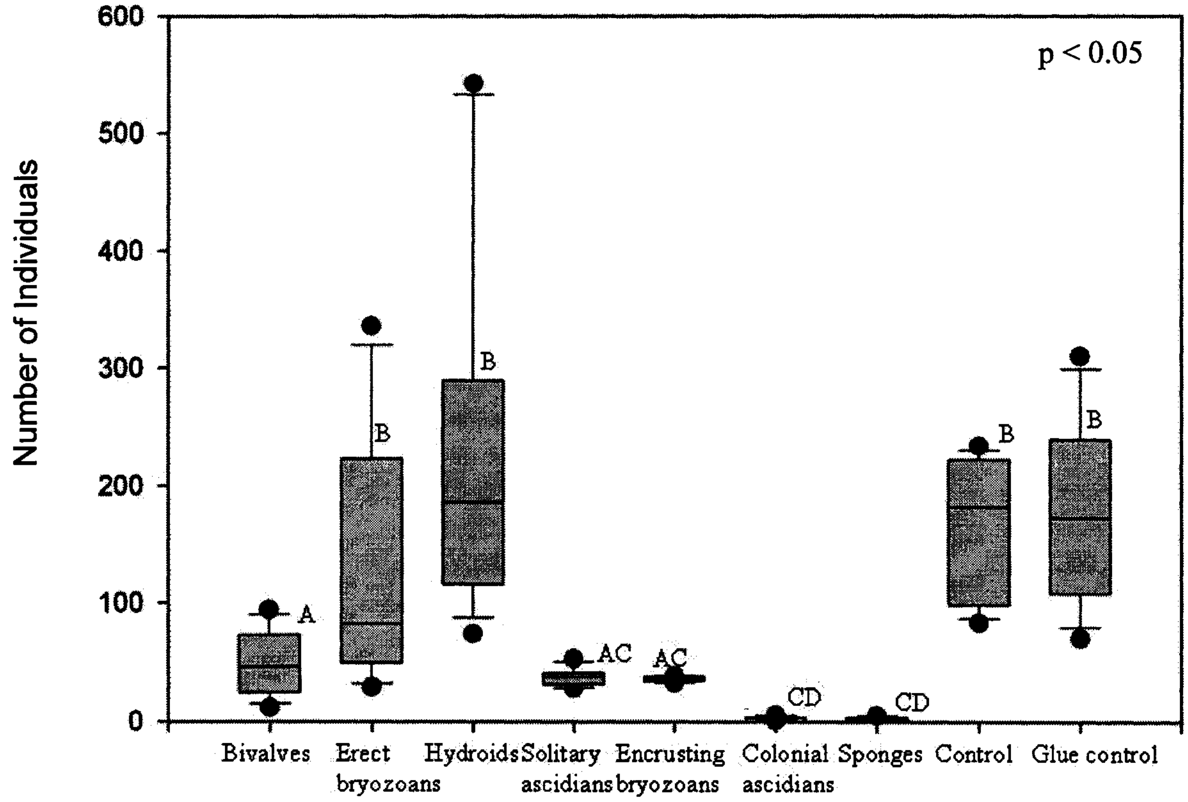


Figure 3.2A: Box plots of individual recruits that settled on functional groups. Tukey test revealed no significant differences in density of recruits between sponges, colonial ascidians, solitary ascidians and encrusting bryozoans. Density of individuals was similar among bivalves, erect bryozoans, encrusting bryozoans and solitary ascidians.

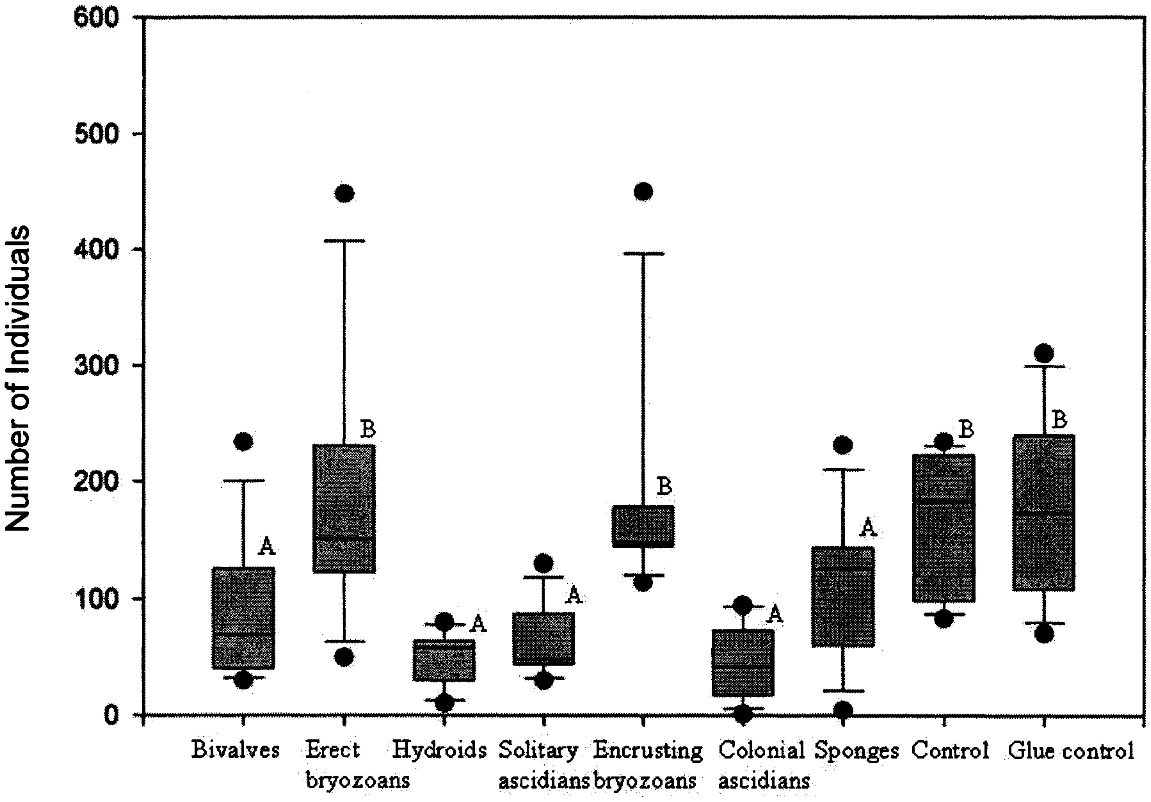


Figure 3.2B: Box plots of individual recruits onto bare space of panels with adult functional groups. Tukey-test revealed significant differences in density of recruits between bryozoans (erect and encrusting) and all other functional groups and controls to bare space adjacent to functional groups.

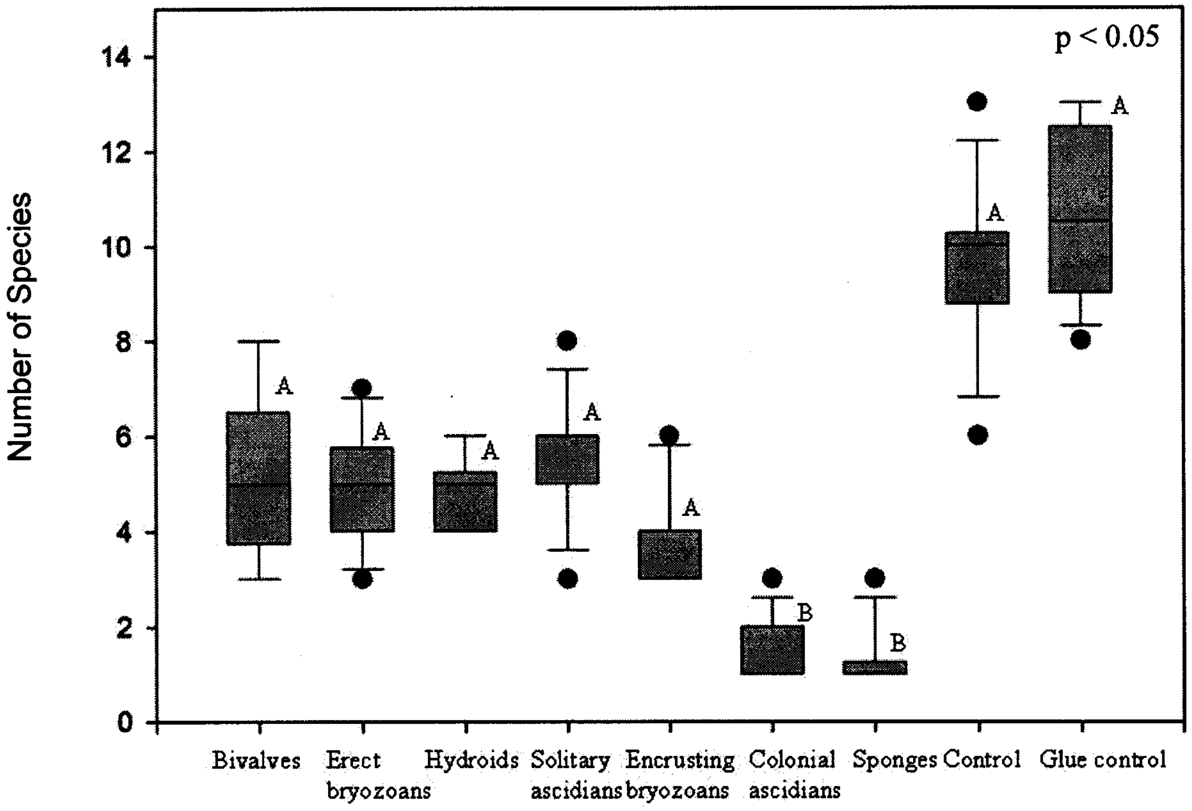


Figure 3.3A: Box plots of species that recruited onto functional groups. Tukey test revealed significant differences ($p \leq 0.05$) between number of species settling on colonial ascidians and sponges and other functional groups and controls. Controls were also found to be significantly different ($p \leq 0.05$) from functional groups.

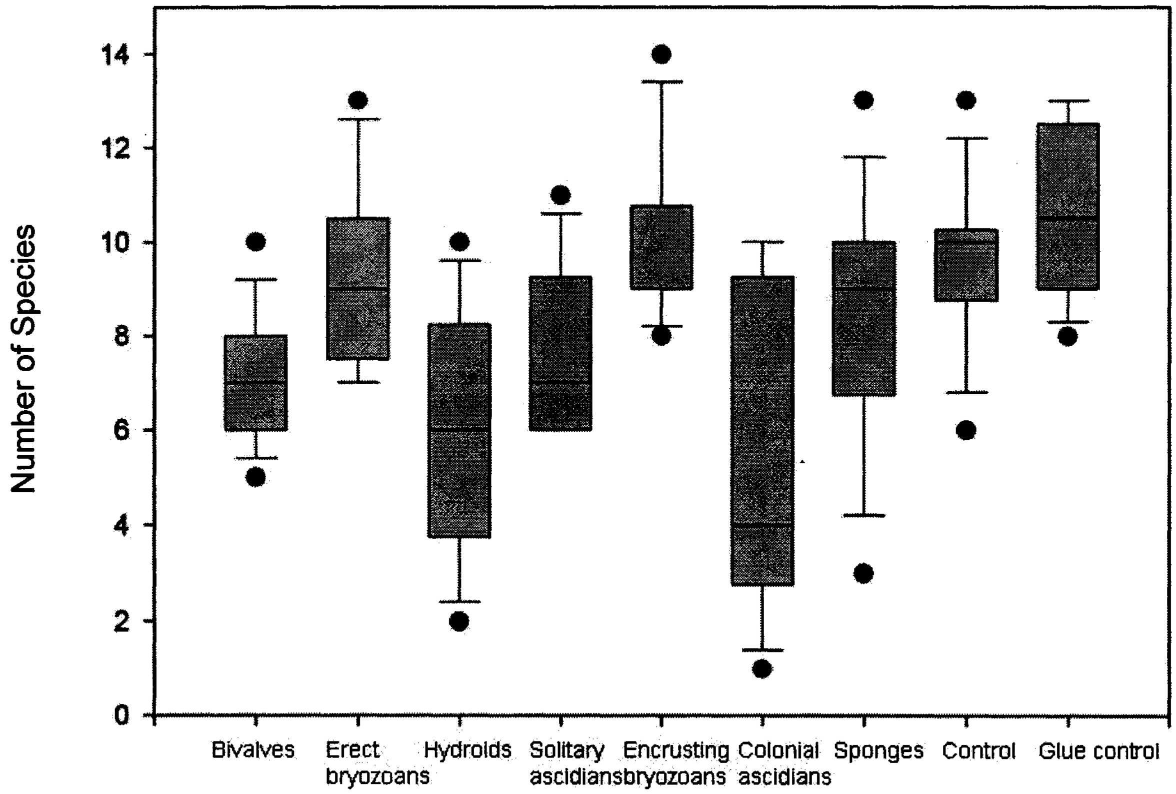


Figure 3.3B: Box plots of species that recruited onto bare space on panels with adult functional groups. Tukey test revealed no significant differences in recruitment of species on bare space.

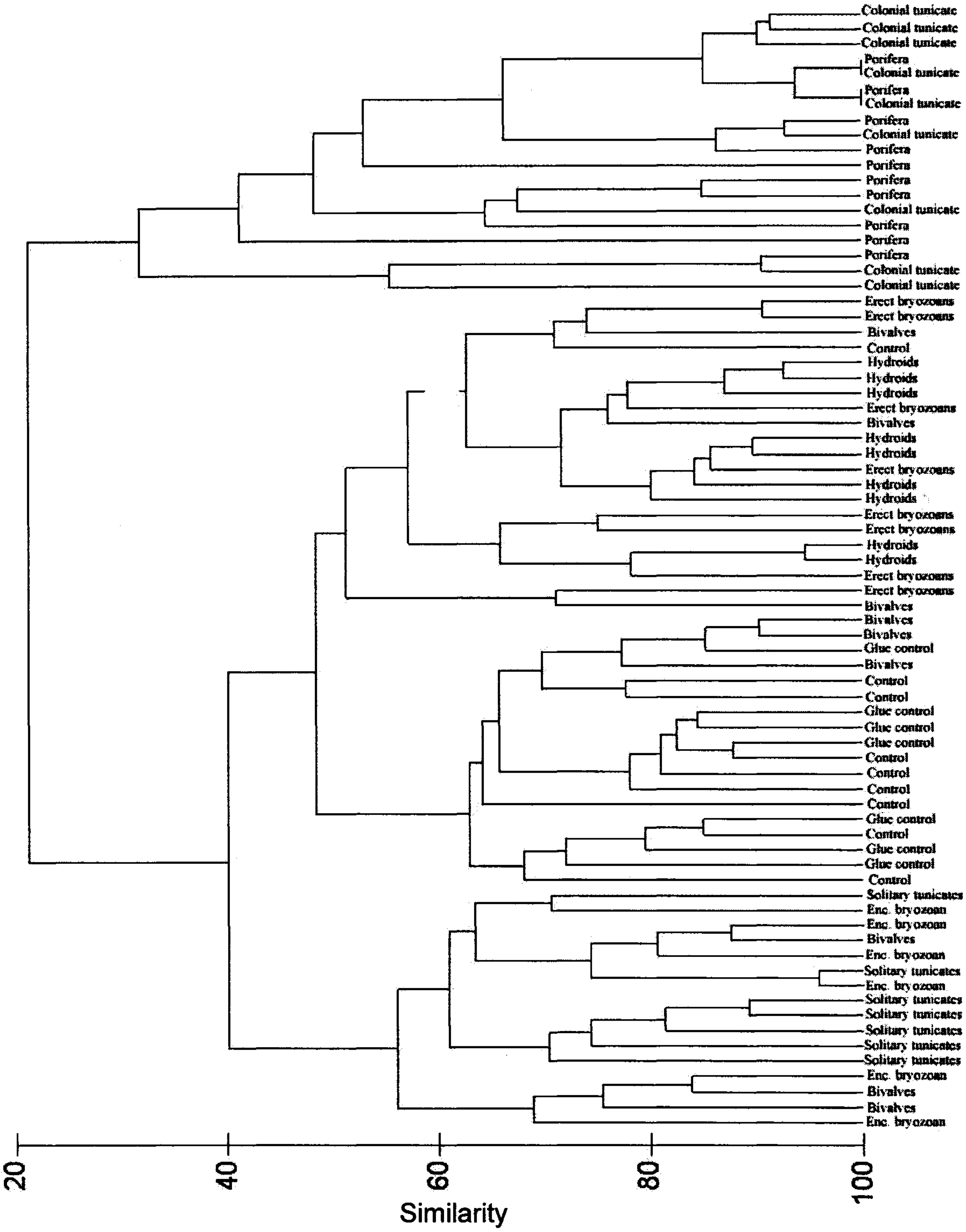


Figure 3.4A: Cluster analysis of similarity values of recruits found on each of seven functional groups. Dendrogram revealed 78% dissimilarity between assemblages of recruits found on colonial ascidians and sponges than other functional groups. Circle identifies colonial ascidians and sponges.

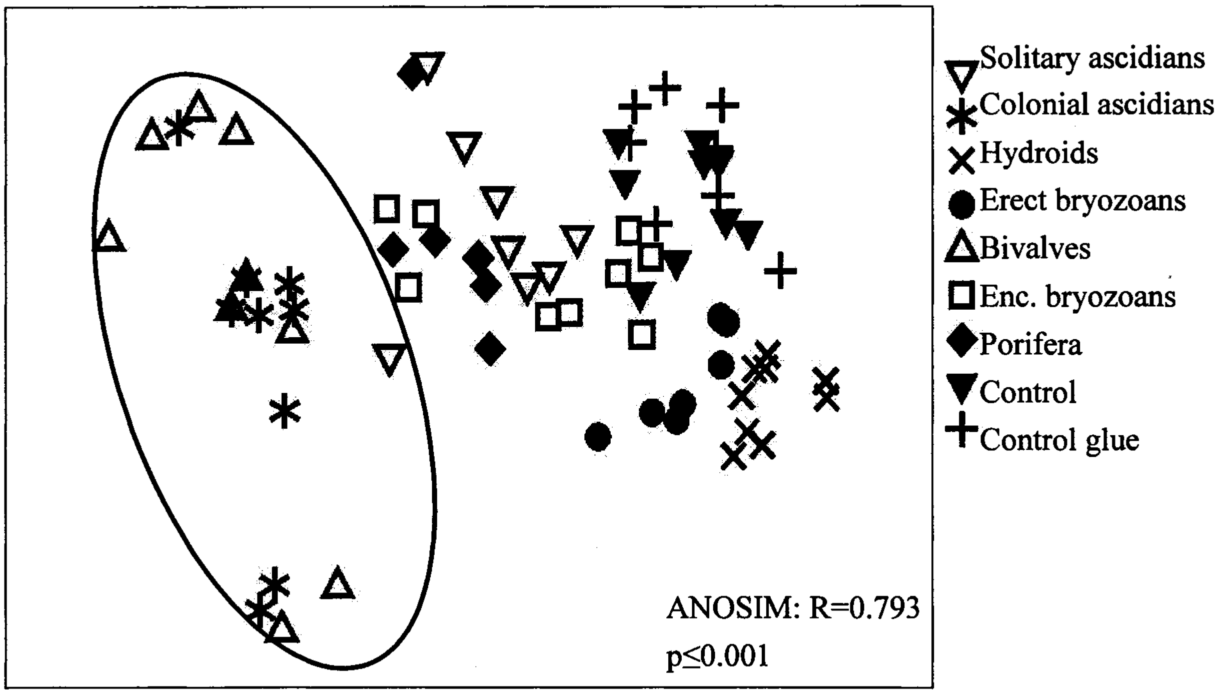


Figure 3.4B: Non-metric multidimensional scaling of recruits counted on each of seven functional groups. Colonial ascidians and sponges created distinct assemblages that were separate from other functional groups. Hydroids and erect bryozoans are also separated form other functional groups.

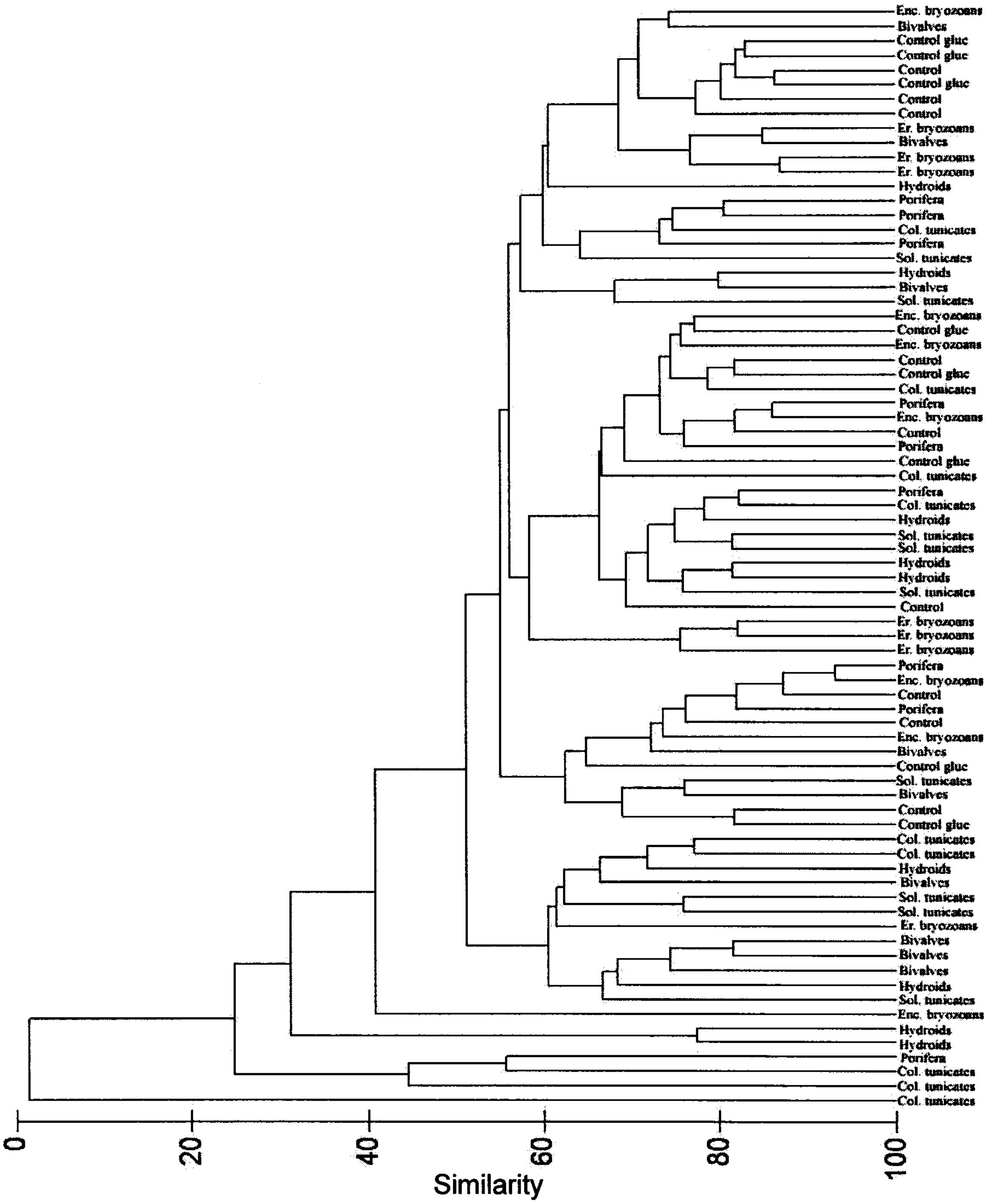


Figure 3.5A: Dendrogram of recruits observed on bare space adjacent to each of seven functional groups. No clear cluster or pattern was found for recruits settling near functional groups.

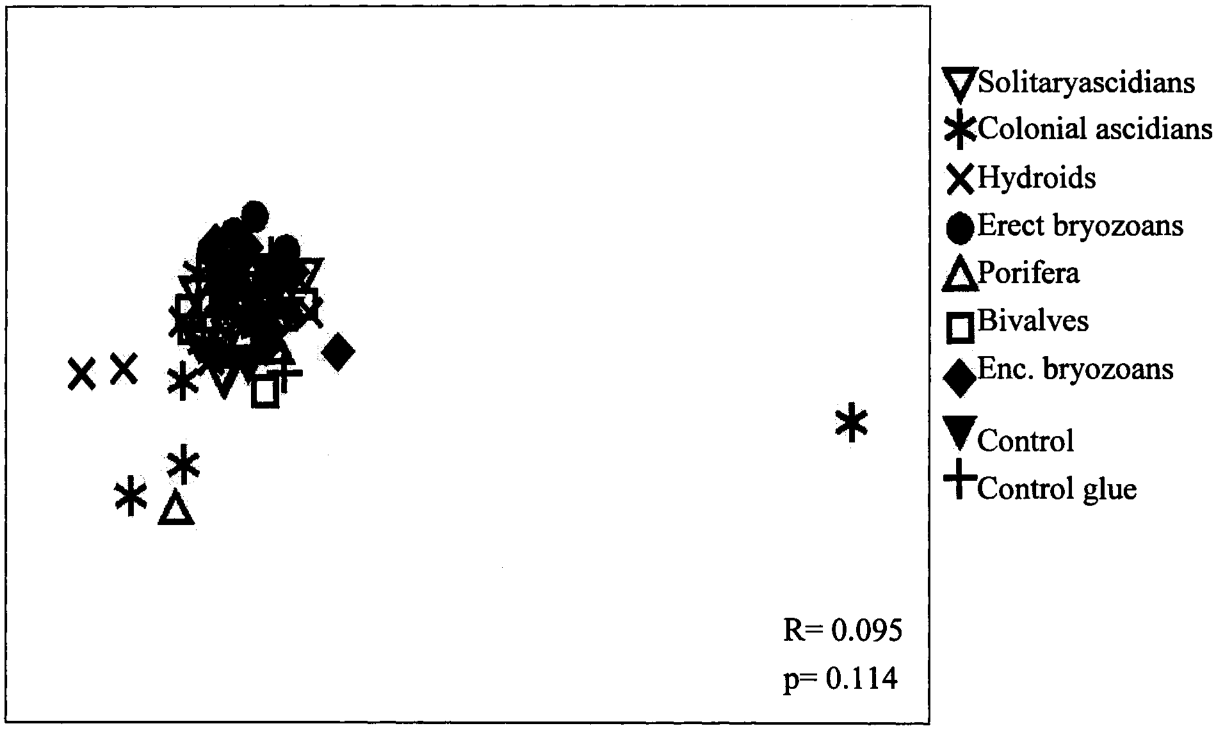


Figure 3.5B: Non-metric multidimensional scaling of recruits counted on bare space adjacent to each of seven functional groups. Results corroborated those of the cluster analysis. Assemblages of recruits on bare space on panels with functional groups did not reveal distinct clusters.

CHAPTER IV

RESPONSE OF TWO INTRODUCED COLONIAL ASCIDIANS BOTRYLLUS SCHLOSSERI AND BOTRYLLOIDES VIOLACEUS TO LOW SALINITIES

Abstract

How abiotic factors influence the establishment and success of invasive species is often difficult to determine for most marine ecosystems. However, examining this relationship is critical for predicting the spread of invasive species and which habitats will be most vulnerable to invasion. Here we examine the mortality and physiological sensitivity to salinity of adult colonies of the colonial ascidians *Botryllus schlosseri* and *Botrylloides violaceus*. Adult colonies of each species were exposed to abrupt changes in salinity (5, 10, 15, 20, 25, 30 ppt) in the laboratory. Salinity ranges used in the laboratory corresponded with those of the field distributions of *B. violaceus* and *B. schlosseri* in the Great Bay Estuary, NH. Heart rate was used as a proxy for health to assess the condition of individual colonies. Heart rates were monitored daily for ~2 week. Results revealed that both species experienced 100% mortality after 1 day at 5 ppt and

that their heart rates declined with decreasing salinity. Heart rates of *B. schlosseri* remained consistent between 15 ppt and 30 ppt and slowed at 10 ppt. Heart rates of *B. violaceus* remained constant between 20 ppt and 30 ppt but slowed at 15 ppt. These laboratory results corresponded to the distribution of these species in estuaries indicating salinity is a key factor in the distribution and dominance of *B. schlosseri* and *B. violaceus* among coastal and estuarine sites.

Introduction

Rates of establishment of non indigenous tunicates in shallow-water subtidal and fouling habitats has risen in recent years (Cohen & Carlton 1998, Osman & Whitlatch 1999, Lambert & Lambert 2003, Bullard et al. 2007, Dijkstra et al. 2007). Their distribution patterns are dependent upon many factors including predation (Osman & Whitlatch 1995, 1998), larval behavior (Keough & Downes 1982, Raimondi & Keough 1990, Hurlbut 1993), water flow (Eckman & Duggins 1993, Guichard & Bourget 1998), light (Hurlbut 1993), temperature (Brunetti et al. 1980, Stachowicz et al. 2002), and salinity (Sims 1984, Vázquez & Young 1996, 2000). However, the success of non indigenous tunicates at local and regional scales is often determined by environmental extremes rather than average conditions (Dybern 1967, Stachowicz et al. 2002). Average sea surface temperature in the Northwest Atlantic has increased by an average of 0.61°C while salinity over the last 100 years, on the other hand has declined over the last 40 years (Curry et al. 2003, Clean-Air Cool Planet & Wake 2005). Precipitation in the Northwest Atlantic has risen by an average of 8.34 cm over the last century (Clean-Air Cool Planet & Wake 2005). Increased precipitation combined with lower salinity regimes in the North Atlantic may affect the abundance and distribution patterns of tunicate species, particularly in near shore coastal zones, estuaries and lagoons that frequently exhibit high variability in environmental parameters.

Temperature and salinity are often two of the most variable factors in near-shore habitats and two of the most important factors limiting the distribution of introduced tunicates (Grosholz 2001). While temperature generally determines seasonally programmed reproduction and growth (Sabbadin 1955, Brunetti et al. 1980, Stachowicz et al. 2002) mortality caused by extreme temperatures are rarely reported (but see Sabbadin 1958). Salinity, in contrast, can result in mass mortalities (Lambert & Lambert 1998, Lambert 2005). Most tunicates lack tubular excretory organs that assist in ionic and osmotic regulation (Berrill, 1930), and consequently abrupt salinity changes characteristic of many near-shore and estuarine habitats may restrict their establishment and population abundance (Vázquez & Young 1996, 2000, Thiyagarajan & Qian 2003). Vásquez and Young (1996, 2000) reported dilute seawater acted as a barrier for settlement to tadpoles of four colonial tunicates (*Eudistoma hepaticum*, *Eudistoma olivaceum*, *Ecteinascidia turbinate* and *Polyandrocarpa zorritensis*) and was responsible for delayed settlement and 100% mortality of juvenile *E. hepaticum*, *E. olivaceum* and *E. turbinate*.

While most tunicates are frequently observed in areas experiencing salinities ≥ 25 ppt. (Lambert 2005), some can tolerate widely fluctuating salinity regimes commonly observed in shallow water estuaries and lagoons (Millar 1971, Shumway 1978, Sims 1984, Vázquez & Young 1996, 2000). Both of the introduced colonial ascidians, *Botryllus schlosseri* and *Botrylloides violaceus* are observed in areas experiencing fluctuating salinity regimes, particularly those observed in the Great Bay Estuary (Short 1992).

While their distribution in the Gulf of Maine and the Great Bay Estuary has been documented, relatively little is known of their tolerance to low salinities (Berman 1992, Dijkstra et al. 2007). The purpose of this study was: 1) examine the response of two introduced colonial tunicates (*Botryllus schlosseri* and *Botrylloides violaceus*) to low salinities (< 30 ppt.) in the laboratory. Examining tolerances of these species to low salinities would allow us to predict the success of these species in bays and estuaries.

Materials and Methods

Species

Botryllus schlosseri is a compound ascidian belonging to the sub-family Botryllidea. It is commonly found in shallow subtidal habitats on both the east and west coasts of the United States, and populations are often exposed to widely fluctuating salinities (16 ppt. to 44 ppt; (Osman & Whitlatch 1999, Cohen et al. 2005, Pederson et al. 2005). Within the Gulf of Maine it is the older of the two introduced species and is commonly found in habitats exposed to salinities above 15 ppt. However exposure to episodic salinities between 7 ppt. and 10 ppt. can occur in harbors and estuaries within the region (Gould 1870, Berman 1992).

Like *Botryllus schlosseri*, *Botrylloides violaceus* is a member of the sub-family Botrylloidea and is also found on both coasts of the U.S. It is commonly found in shallow subtidal communities, however occurrences <25 ppt. are rare (but see Berman 1992). In the Gulf of Maine, *B. violaceus* has a relatively wide distribution, occurring from Eastport, ME to Cape Cod, MA (Dijkstra et al. in press). This species was first observed at Woods Hole, MA in 1980, at the mouth of the Great Bay Estuary, NH in 1981 and at the Darling Marine Center, ME in 1982 (Carlton 1989, Berman et al. 1992, Dijkstra et al. in press). Although commonly found in habitats exposed to salinities >20 ppt., limited exposure to salinities of 10 ppt. to 15 ppt. can occur in some harbors and estuaries (Berman 1992, Lambert & Sanamyan 2001, Cohen et al. 2005, Pederson et al. 2005).

Colonies of *B. schlosseri* and *B. violaceus* were collected from 100cm^2 Plexiglas ® panels. Panels were deployed horizontally ~0.5m below the surface for two weeks off floating docks. Panels were brought to the laboratory and the colonies were removed and placed on microscope glass slides in a closed container. Once the colonies reattached, the slides were placed in open microscope slide boxes in aerated aquaria. Colonies were maintained at 15 C° and 30 ppt. for one 1 week prior to use, and fed 2 drops of Invertebrate Smorgasborg© twice daily throughout the experiments.

Laboratory experiments

Seven colonies of *B. schlosseri* and six colonies of *B. violaceus* were kept at each of 6 salinities (5 ppt., 10 ppt., 15 ppt., 20 ppt., 25 ppt., and 30 ppt.) for a period of 10 days. Prior to the beginning of the experiments, general condition of individual colonies of both species were noted. Heart rates were recorded under a dissecting microscope once a day for 10 days. Heart beats were recorded at 15 seconds intervals and a single peristaltic wave (undulation) was considered one heart beat. Heart rates from individual colonies were randomly recorded from 3 zooids lining the edge of the colony. The pumping action of the tunicate heart is produced by a peristaltic wave and the direction of peristalsis reverses periodically (Hideo et al. 1982) . Heart beats of both species had a higher rate of reversal at lower (e.g. 10 ppt. to 20 ppt.) than higher (e.g. 25 ppt. to 30 ppt.) salinities.

Statistical analyses were generated using JMP software© (JMP 6, SAS Institute, Cary, NC). To detect differences in heart rates among salinities between and within species, average heart rates per colony were square root transformed and analyzed using repeated measures one-way Analysis of Variance (ANOVA) using the colony as a replicate. A non-linear cubic regression was used to detect correlations between salinity and heart rates within species.

Results

General condition of *Botryllus schlosseri* and *Botrylloides violaceus*

Tunicate colonies were in good condition at the beginning of each experiment and heart rates of both species were between 84 to 120 heart beats/min and zooids responded by closing their oral siphon when gently poked with a probe. During the salinity acclimation period all colonies of both species held at five ppt. suffered 100% mortality after 24 hr. Colonies of *B. schlosseri* placed in the ten ppt. treatment experienced 100% mortality by day six. Blood flow in colonies ceased towards ampullae by day two but continued to flow in the core colony. By the third day ampullae had shrunk. Between the fifth and sixth days colonies were surrounded by parasites; zooids in 78% of the colonies began to separate and some zooids within colonies experienced mortality. In addition, mortality of colonies held at 10 ppt. increased with number of days; colonies had 0% mortality on day 1, 11% by day 2 and 3, 22% by day 4, 33% by day 5, 78% by day 6 and 100% mortality by day 7. At 15 ppt. blood flow within colonies was less stable than colonies held between 20 ppt. and 30 ppt., however colonies experienced 0% mortality (Figure 4.1a). Colonies held between 20 ppt. and 30 ppt. showed no difference in blood flow and mortality was 0%.

Botrylloides violaceus colonies exposed to 10 ppt. experienced 17% mortality rate on day one, 67% mortality by day two and 100% mortality by day three (Figure 4.1b). Colonies that survived the first 2 days of the experiment showed reduced blood flow after day one. Hearts of some zooids within a colony stopped beating and zooids began to separate. At 15 ppt. 33.33% of colonies died on day 4 of the experiment; zooids in the remaining colonies began to separate and shrink into balls; siphons were no longer visible. Blood kept flowing amongst zooids, though it ceased flow to ampullae and eventually they were reabsorbed in the tunic. Colonies held between 20 ppt. and 30 ppt. experienced 0 % mortality and during the experiment blood flowed consistently and continuously throughout the colony. Zooids of colonies exposed to 20 ppt. were arranged in systems forming common cloacal openings for approximately eight days, at which time zooids connecting excurrent siphons began to separate.

Heart rates

Botryllus schlosseri and *Botrylloides violaceus* colonies exposed to salinities between 25 ppt. and 30 ppt. experienced few reversals of heart beats. However, reversals became increasingly sporadic and heart beats intermittent with decreasing salinities (Dijkstra, pers. obs.)

Botryllus schlosseri colonies exposed to 10 ppt. initially had only slightly lower heart rates than those of colonies exposed to higher (15 ppt. to 30 ppt.) salinities (Figure 4.2a). However during the experiment heart rates dramatically declined, ending in the death of all colonies by day seven. Although heart rates were similar for *B. schlosseri* colonies held between 15 ppt. and 30 ppt., heart rates in the 15 ppt. treatment were more variable than those exposed to salinities between 20 ppt. and 30 ppt. (Figure 4.2a).

Botrylloides violaceus colonies exposed to 10 ppt. showed slower heart rates than colonies exposed to higher salinities (Figure 4.2b), and 67 % of colonies died by day 2 and 100% mortality occurred by day 3. At 15 ppt. colonies had significantly ($p \leq 0.01$) lower heart rates than colonies held between 20 ppt. and 30 ppt, and 17 % of colonies exposed to 15 ppt. died on day two and

33 % of colonies died by day four. Heart rates of colonies exposed to salinities above 20 ppt. were similar and mortality was not observed.

Non-linear regression revealed a strong positive correlation between heart rate and salinity in both tunicates (adj.$R^2 = 0.818$ and 0.936; $p \leq 0.001$; Figs. 4.3a and 4.3b). Overall, heart rates of *B. schlosseri* were about 1.5 times higher than those of *B. violaceus* (Table 4.1). At 10 ppt. heart rates of *B. schlosseri* were approximately six times higher than *B. violaceus.* Similarly, heart rates of *B. schlosseri* colonies kept at 15 ppt. were approximately twice as fast as those of *B. violaceus.*

Discussion

Botryllus schlosseri is a ubiquitous species and is frequently exposed to a wide range of salinities (Brunetti 1974, Lambert & Lambert 1998, Cohen et al. 2005, Pederson et al. 2005). Optimum salinity for colonies of *B. schlosseri* from the Venetian Lagoon were between 25 ppt. and 40 ppt., although colonies survived at 16 ppt. (Brunetti 1974, Brunetti et al. 1980). On the west coast of Norway, Northeast Atlantic and Pacific Northeast *B. schlosseri* colonies were found in habitats exposed to salinities between 28 ppt. and 33 ppt. (Dybern 1969, Cohen et al. 1998, Cohen et al. 2005, Pederson et al. 2005).

Our results revealed that while *B. schlosseri* colonies exposed to 10 ppt. experienced 100% mortality after six days of exposure, 67% of the colonies survived through five days of exposure. Between 15 ppt. and 30 ppt. colonies had 0% mortality and heart rates were similar and relatively high. In contrast (Brunetti et al. 1980) found lower heart rates in *B. schlosseri* colonies exposed to 16 ppt. and 24 ppt. than in colonies exposed to salinities above 30 ppt from the Venice Lagoon. The discrepancy between our results and those of Brunetti et al. (1980) may indicate local adaptation to environmental parameters. The Venice Lagoon was becoming less estuarine and more marine; therefore *B. schlosseri* populations in the Venice Lagoon no longer needed to survive in brackish waters. Consequently selection for survival in low saline waters was no longer required. In contrast, floating docks in harbors and estuaries along the Gulf of Maine are frequently subjected to changes in salinity due to river run-off or heavy rainfall. Thus, colonies of *B. schlosseri* are constantly exposed to fluctuating salinities that may have lead to higher tolerance of a wide range of salinities. Our laboratory results corresponded to the distribution of *B. schlosseri* in the Great Bay Estuary (Berman 1992).

Botrylloides violaceus is originally from the Pacific Ocean and has been documented in Aburatsubo-Moroisa Inlet, Guam (30.9 ppt. to 34.2 ppt.(Yamaguchi 1975), off the coast of Alaska (27 ppt.; Lambert and Sanamyan 2001), in the Northeast Pacific from British Columbia to Ensanada, Baja California (26 ppt. to 36.5 ppt., the Northeast Atlantic (26 ppt. to 36 ppt.; (Cohen et al. 1998, Lambert & Lambert 1998, Cohen et al. 2005), Sydney Harbor, Australia (36 ppt.; Australian Museum Business Services), and the Venice Lagoon, Mediterranean (Zaniolo et al. 1998). Our results showed *B. violaceus* colonies can survive at much lower salinities than previously recorded (15 ppt. to 30 ppt.) and for 2 days colonies can persist at 10 ppt. However, heart rates of colonies exposed to 10 ppt. were much lower than heart rates of colonies exposed to salinities between 15 ppt. and 30 ppt. Lower heart rates in colonies exposed to 15 ppt. suggest they were under stress but most colonies survived and heart rates of those remaining colonies were consistent over the 10 day experimental trials.

Overall, heart rates of *B. schlosseri* were higher than *B. violaceus.* Several explanations may elucidate this difference. Firstly, *B. schlosseri* tends to grow faster than *B. violaceus* at temperatures between 14.5 °C and 16.5 °C (Stachowicz et al. 2002). Our experiments were conduced at 15 °C, potentially a sub-optimal temperature for growth of *B. violaceus.* Higher growth rate in colonies of *B. schlosseri* at lower temperatures may indicate increased heart rates that facilitate blood flow and consequently growth. Secondly, zooids are larger in *B. violaceus* colonies (Dijkstra, pers. obs.). Larger zooids may indicate a slower metabolic rate and consequently slower cardiac contractions.

Non-linear regression revealed salinity explained 82% of the variance in heart rates of *B. schlosseri* and 94% of the variance in *B. violaceus,* suggesting *B. schlosseri* is better adapted to live in more euryhaline habitats than *B. violaceus.* The long-term exposure of *B. schlosseri* to extreme fluctuating salinities observed in Gulf of Maine coastal zones and notably estuaries subjected to spring-run off, may have facilitated an increased level of adaptation to low salinities. *B. violaceus* has not had as much time as *B. schlosseri* to adapt to low salinities and is commonly observed in areas with salinities above 20 ppt. (Grosberg 1988, Grosholz 2001, Yund & Stires 2002, Newlon et al. 2003).

Though temperature is one of the foremost abiotic factors thought to control large-scale geographic distribution and abundance patterns of colonial tunicates (Brunetti et al. 1980, Stachowicz et al. 2002), mortality caused by extreme high's and low's has rarely been observed (but see Sabbadin 1958). Salinity, on the other hand, causes mass mortality of tunicates and is a key factor controlling their dominance (Millar 1971, Sims 1984, Toop & Wheatly 1993, Vázquez & Young 2000, Lambert & Lambert 2003). Predicted lower salinity regimes coupled with increased flooding events for the Northwestern Atlantic may counteract the effects of increasing sea surface temperatures and therefore, limit the spread and dominance of *Botrylloides violaceus* in New England near-shore coastal and estuarine environments (Stachowicz et al. 2002).

Table 4.1: Average heart rates and standard deviations for *Botryllus schlosseri* and *Botrylloides violaceus*

Salinity (ppt.)	***Botryllus schlosseri*** (mean ± SE)	***Botrylloides violaceus*** (mean ± SE)
5	0 ± 0	0 ± 0
10	6.93 ± 0.931	1.039 ± 0.383
15	22.85 ± 0.423	13.19 ± 0.308
20	25.26 ± 0.319	19.76 ± 0.192
25	24.81 ± 0.228	21.24 ± 0.209
30	24.24 ± 0.219	21.17 ± 0.210

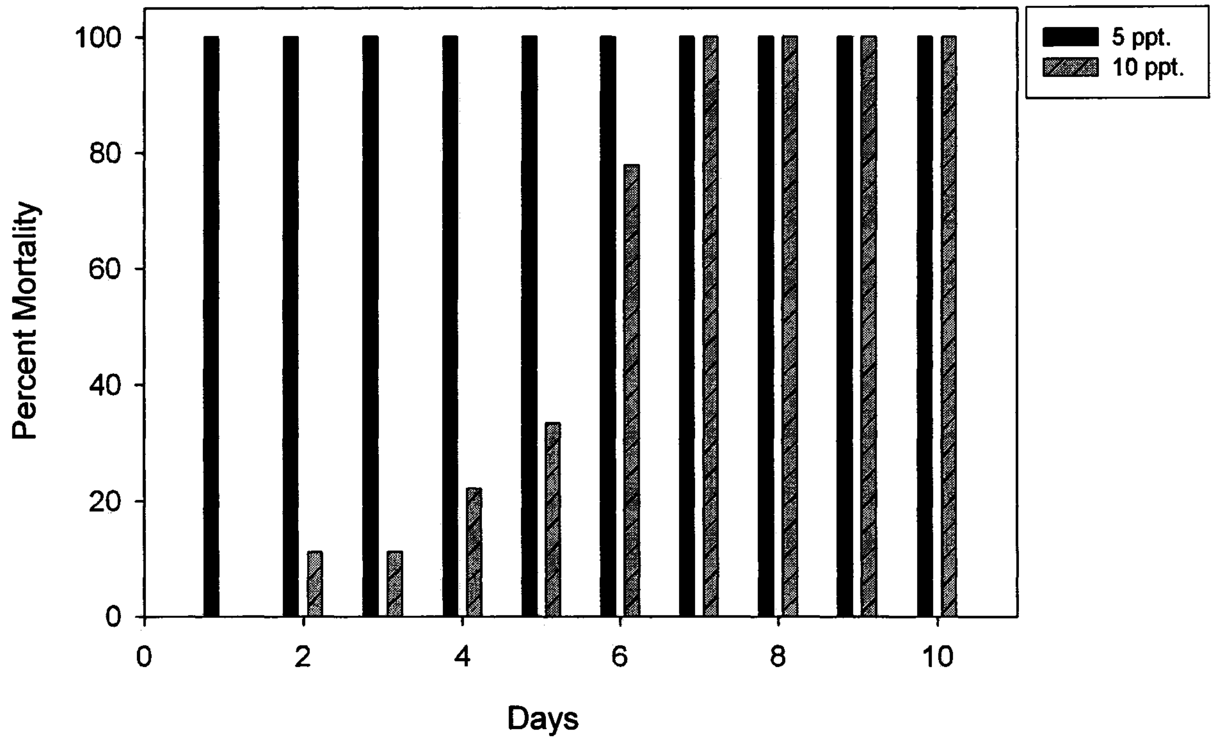


Figure 4.1A: Percent mortality of *Botryllus schlosseri* colonies exposed to salinities between 5 ppt. and 10 ppt. Mortality of colonies held between 15 ppt. and 30 ppt. was 0% and are not shown on the graph.

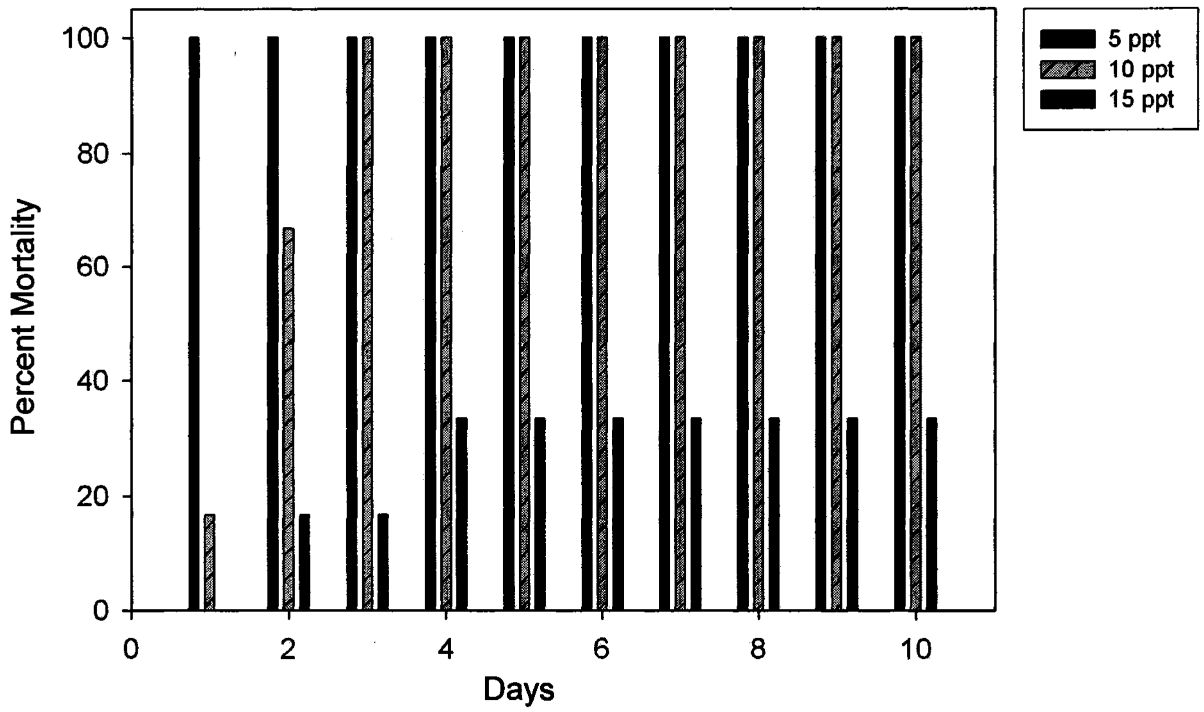


Figure 4.1B: Percent mortality of *Botrylloides violaceus* colonies exposed to salinities between 5 ppt. and 15 ppt. Mortality of colonies held between 20 ppt. and 30 ppt. was 0% and are not shown on the graph.

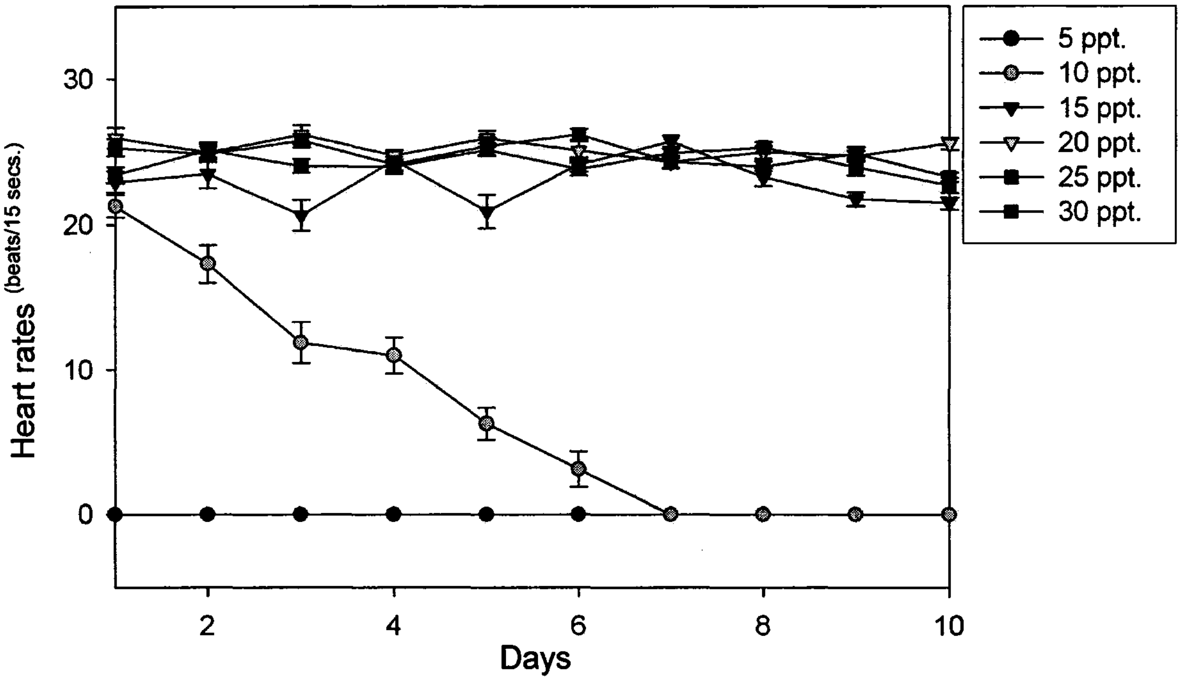


Figure 4.2A: Heart rates of *Botryllus schlosseri* exposed to 5 ppt., 10 ppt., 15 ppt., 20 ppt., 25 ppt. and 30 ppt. Repeated measures revealed a significant difference ($p \leq 0.0001$) amongst heart rates.

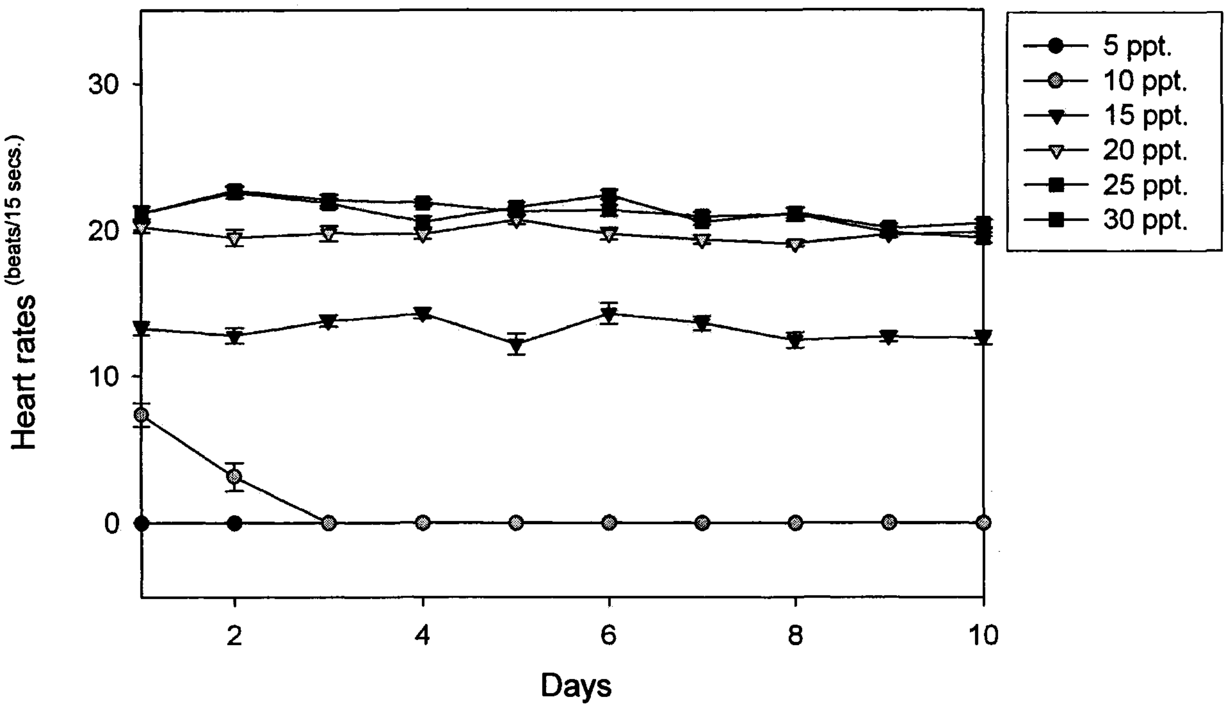


Figure 4.2B: The heart rates of *Botrylloides violaceus* exposed to 5 ppt., 10 ppt., 15 ppt., 20 ppt., 25 ppt. and 30 ppt. Repeated measures revealed a significant difference ($p \leq 0.0001$) amongst heart rates. Heart rates of colonies exposed to salinities between 10 ppt. and 15 ppt. had significantly ($p<0.01$) lower heart rates than colonies exposed to salinities between 20 ppt. and 30 ppt.

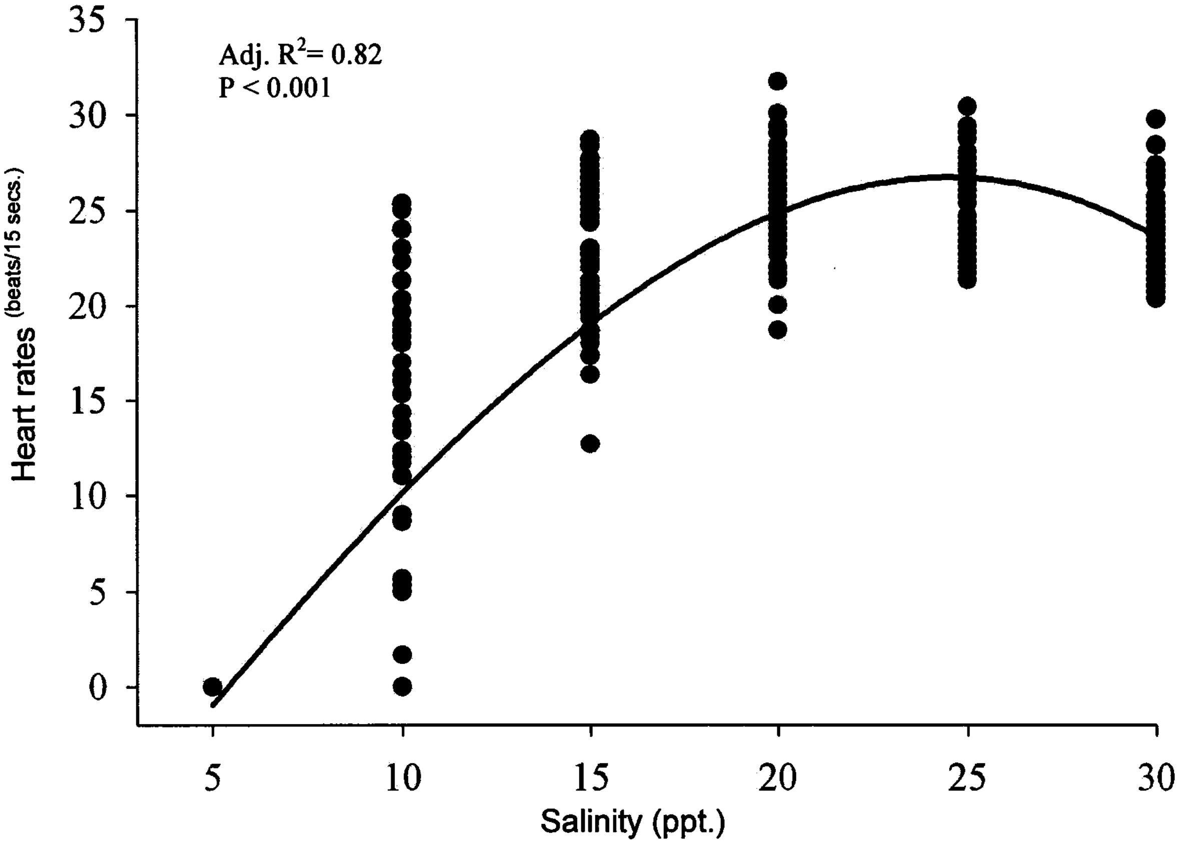


Figure 4.3A: Heart rates of *Botryllus schlosseri* as a function of increasing salinity. Polynomial cubic regression revealed a significant correlation between heart rate and increasing salinity.

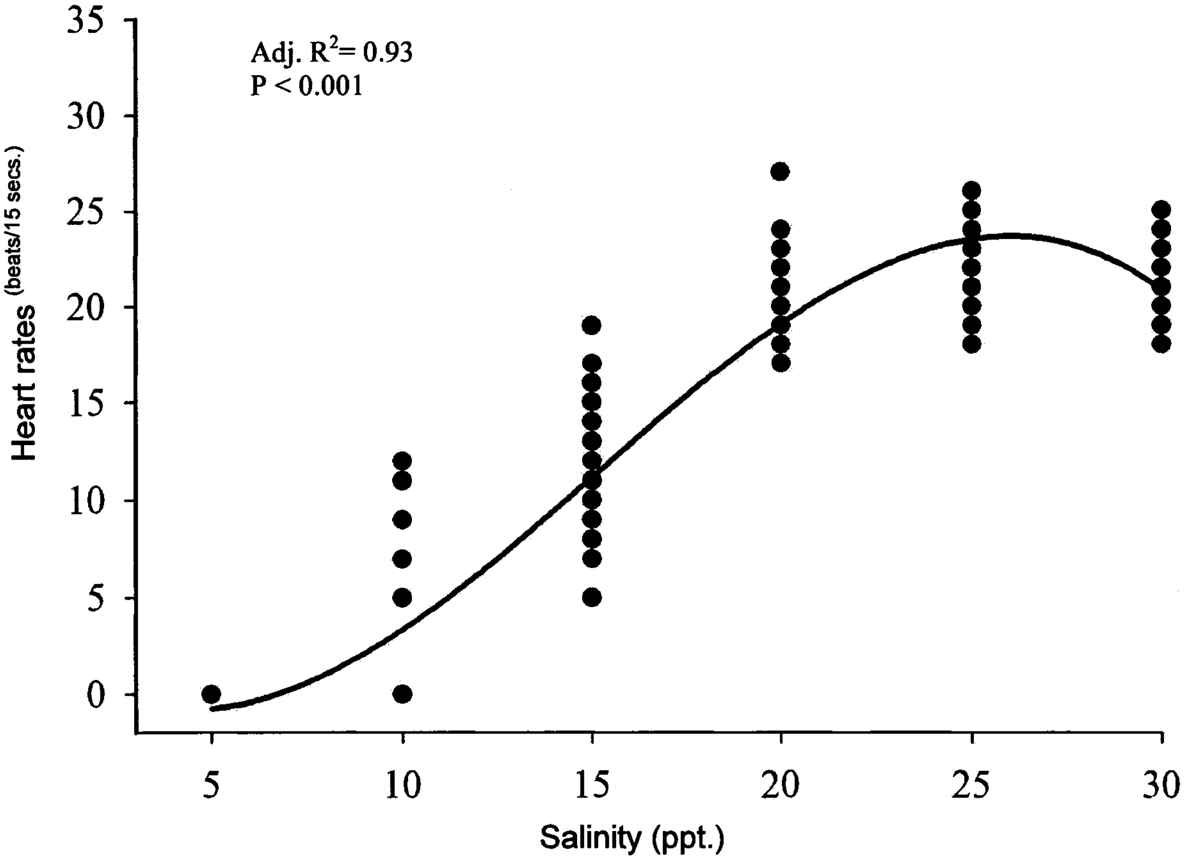


Figure 4.3B: Heart rates of *Botrylloides violaceus* as a function of increasing salinity. Polynomial cubic regression revealed a significant correlation between heart rate and increasing salinity.

CHAPTER V

REDUCTION IN THE SEASONAL DEVELOPMENT OF A MARINE COMMUNITY: REPONSE TO INCREASING TEMPERATURES

Abstract

The seasonal development of a New England temperate marine fouling community has shifted over the last 25 years. Historically, four stages of community development were present that corresponded to the seasons (spring, summer, autumn and winter). Now three stages are present, a condition similar to communities found in warmer, southern waters. The smaller number of stages is linked to a rise in temperature that facilitated the longevity and dominance of warmer water species. This link is established by simultaneous monthly records of temperature, nitrogen, phosphate, total suspended solids and abundance data of species, for the periods between 1979 to 1980 and 2003 to 2004.

Introduction

Annual cycles of species composition in terrestrial and marine communities have long been known to reflect seasonal phenologies and abundances of species (Weiss 1948, Osman 1977, Minchin 1992, Morse & Bazzaz 1994, Stachowicz & Byrnes 2006). In both terrestrial and aquatic systems annual fluctuations in dominant species drive seasonal cycles of species composition, particularly in temperate environments that undergo cyclical changes in environmental parameters (Osman 1977, Sutherland & Karlson 1977, Minchin 1992, Manning & Bucklin 2005). Seasonal cycles of species composition reflect the timing and magnitude of recruitment, growth, and mortality of species and are primarily driven by abiotic factors (Osman 1977, Sutherland & Karlson 1977, Harris & Irons 1982, Stachowicz et al. 2002, Stachowicz & Byrnes 2006).

Temperature has been widely shown to affect the timing of recruitment and growth of marine and terrestrial species (Winston 1977, Calder 1990, Minchin 1992, Crick et al. 1997, Menzel & Fabian 1999, Planque & Fredon 1999, Stachowicz & Byrnes 2006). Recent trends in rising temperatures have led to a number of changes in the timing of reproduction and growth of species (Minchin 1992, Crick et al. 1997, Brown et al. 1999, Dunn & Winkler 1999), and in the annual growing season of plants (Menzel & Fabian 1999). Higher abundances of warm-water species, and lower abundances of cold-water species were linked to warmer waters in an intertidal zone in central California (Barry et al. 1995, Sagarin et al. 1999). Rising temperatures have led to earlier spawning in the clam *Macoma balthica* and the polycheate, *Nireis virens* (Edwards & Richardson 2004, Lawrence & Soame 2004) as well as earlier egg laying in birds (Crick et al. 1997, Brown et al. 1999). Invasive species have also benefited from rising temperatures, increasing their success and longevity in temperate environments by facilitating an earlier spring appearance and contributing to increased growth rates (Stachowicz et al. 2002). While it is evident an interaction exists between temperature and short-term seasonal appearance and growth of species, little is known of long-term shifts occurring in the seasonal development of marine communities. Here, we report shifts in the seasonal development of a shallow water marine community between the period 1979 to 1980 and 2003 to 2004 and associate these changes with concurrent changes in temperature and biota.

Materials and Methods

We replicated an earlier study conducted by Harris and Irons (1982) that documented the development of a marine fouling (assemblages of species occupying man-made structures) community on 0.1m^2 Plexiglas® panels from 1979 to 1982 beneath a cement pier at the mouth of the Great Bay Estuary in Portsmouth Harbor, Newcastle, New Hampshire. Photographs and observations of the 1979 to 1982 community on the pier indicated the fouling community was dominated by sponges, hydroids, mussels and large barnacles (Harris and Irons

1982). Soft corals, tunicates, encrusting and erect bryozoans were interspersed throughout these assemblages.

Eight vertical and eight horizontal 0.1m^2 Plexiglas® panels were deployed in July 1979 and photographed monthly for 3 years to document succession. A follow-up study beginning in July 2003 used identical materials, methods and deployment site to compare community development (1979 to 1982; 2003 to 2006). Though seasonal cycling of species composition was observed throughout the entire study period (1979 to 1982; 2003 to 2006; Dijkstra and Harris, in prep.), cycles were less apparent with each successive year due to the later establishment of adults that inhibit the settlement of larvae (Karlson & Jackson 1981, Grigg 1984, Breitburg 1985, White & Pickett 1985, Drake 1991, Roberts et al. 1991, Osman & Whitlatch 1998). Slides of the 1979 to 1980 panels and photographs of the 2003 to 2004 panels (~ 400 images) were digitized and downloaded into Adobe Photoshop 7.0©. All individuals were counted and identified to the species level while hydroids were assessed as percent cover using point count analysis. The group *Molgula* sp./*Obelia* sp. is the result of *Obelia* sp. settling on *Molgula* sp. with *Obelia* sp. as secondary space occupants. We compared the seasonal development of the 1979 to 1980 and 2003 to 2004 fouling community in Newcastle, New Hampshire to a fouling community in Beaufort, North Carolina using monthly data reported from panels deployed horizontally between July 1971 and June 1972 (Sutherland & Karlson 1973).

In addition to biotic data, temperature records have been collected within 0.5 km of our study site from 1973 to 1981 and 1996 to 2005 while dissolved oxygen has been taken since 2001 (Trowbridge 2006). Non-parametric analysis of variance by ranks, Kruskal-Wallis test, was used to examine monthly differences in temperature between 1973 to 1981 and 1996 to 2005. Chlorophyll *a*, ammonium, nitrite, nitrate, phosphate and total suspended solids have been reported from 1974 to 1981 and 1997 to 2006 at a site approximately 10 km inland from our study site (Trowbridge 2006).

A Mann-Whitney U test was used to detect differences in monthly abundances of single species between communities (1979 to 1980 and 2003 to 2004). Non metric-Multi-Dimensional Scaling plot (nMDS) and cluster analysis were used to detect within year seasonal development between 1979 to 1980 and 2003 to 2004 in Portsmouth Harbor and 1971 to 1972 in Beaufort, North Carolina. Non-Metric Multidimensional Scale (nMDS) and cluster analysis are methods which preserves species-specific formation and are sensitive in detecting changes in assemblage pattern (Clarke & Ainsworth 1993). Bray-Curtis similarities were the basis for these statistics and were calculated on fourth root transformed data. Community related statistics were performed using the Primer 6.0 and JMP 6.0 software packages (Clarke & Warwick 2001).

Results

Since the 1979 study, 6 invasive species have been observed in the fouling community in Portsmouth Harbor. These included the encrusting bryozoan, *Membranipora membranacea*, the solitary ascidians *Ascidiella aspersa* and *Styela clava* and the colonial ascidians, *Didemnum* sp., *Botrylloides violaceus* and *Diplosoma listerianum*. All three colonial ascidians and the encrusting bryozoan were documented on the panels beneath the pier at our study site in Newcastle, N.H. by 2004, but the solitary ascidians have not yet been observed at this site.

In addition to the arrival of invasive species, abiotic factors have also changed at this location. Annual mean temperatures in Newcastle have increased by 1.29 °C since 1973 (Figure 5.1a). During the 1979 to 1980 study water temperatures ranged from 1.30°C in January to 14.54°C in August (Figure 5.1b). In contrast, temperatures between 2003 and 2004 ranged from 2.14°C in January to 16.24°C in August. Average monthly temperature comparisons between 1973 to 1981 and 1996 to 2005 show that November and December temperatures have increased by 0.47 °C, and January through April temperatures have increased by 0.74°C. The largest increase in temperature occurred during the months of May through October with an average increase of 2.03 °C (Figure 5.1a and b). This overall trend, maximum warming during the spring and summer, corresponds to regional trends, though values observed at the Portsmouth Harbor site were higher than those calculated for the Gulf of Maine (Clean-Air Cool Planet & Wake 2005). The higher temperatures observed in Portsmouth Harbor are most likely a result of the shallow depth of much of the Great Bay Estuary.

Hypoxia is not occurring at our study site in Newcastle as dissolved oxygen levels are equal to or above 75% saturation (Trowbridge 2006). Total suspended solids increased by an average of 7.1mg/L (81% increase), mean dissolved inorganic nitrogen (DIN) has increased from 0.107 mg N/L to 0.169 mg N/L. Although DIN levels have risen over the last 32 years, they are still within an acceptable range that does not suggest a high level of eutrophication (Jaworski 1971, Jaworski & Villa 1981). Finally average PO_4 concentrations fell from 0.027 to 0.023 mg P/L and are well within the limits that sustain a healthy system (Pritchard 1969). Chlorophyll *a* has not significantly changed between 1974 to 1981 and 1997 to 2006 (Trowbridge 2006).

Comparison of monthly invertebrate abundances from the 1979 to 1980 and 2003 to 2004 surveys documented two prominent patterns. First, annual abundances of many species found in the 1979 to 1980 community have significantly changed over the last twenty five years (Table 5.1). Second, there has been a shift in the seasonal stages of community development between July 1979 to August 1980 and July 2003 to August 2004 (Figures 5.2 a and b and 5.3 a and b). Of the 28 fauna analyzed, 18 revealed significant statistical differences ($p \leq 0.05$) in abundances over 3 consecutive months (Table 1), indicating a substantial shift in the annual abundances of species between 1979 to 1980 and 2003 to 2004.

Four species exhibited strong changes in their seasonal cycles in Portsmouth Harbor over the last twenty five years. The seasonally dominant hydroid *Ectopleura larynx* increased in abundance, arrived earlier and remained later in the 2003 to 2004 community compared to the 1979 to 1980 community. The bryozoan, *Bugula simplex*, shifted its seasonal cycle, with peak abundances moving from August through November (1979 to 1980) to May through October (2003 to 2004). Abundances of *Cryptosula pallasiana* (an encrusting bryozoan) were highest from October through January in 1979 and 1980 while abundances were highest from March through June in 2003 to 2004. Lastly, periods of peak abundances for *Schizoporella unicornis* (another encrusting bryozoan) shifted from May through August in 1979 and 1980 to January through April in 2003 and 2004. *Bortylloides violaceus*, an invasive species not found in the 1979 to 1980 community, exhibited strong seasonal patterning, dominating the community during the warm summer months of July, August, September and October.

We observed 4 season development of species composition between 1979 and 1980 that corresponded to the seasons of summer, autumn, winter and spring (Figure 5.2a and 5.2b). In contrast, we observed only 3 season development in species composition between 2003 and 2004 that do not correspond to the seasons (Figures 5.3a and 5.3b). This new seasonal pattern of community development is very similar to that found in North Carolina in the early 1970's (Figure 5.4), a shallow water fouling community with a three season development in species composition that is associated with a warm-water habitat.

Discussion

Temperature is a key factor in the seasonal distribution and abundance of many sessile marine organisms (Winston 1977, Calder 1990, Minchin 1992, Stachowicz et al. 2002). A drop in temperature in autumn stimulates loss of polyps and tissue regression in seasonally dominant hydroids while a rise in temperature in the spring stimulates the development and regeneration of both bryozoans and hydroids (Winston 1977, Calder 1990). Recent studies have linked warmer winters to earlier spring recruitment of the seasonally dominant invasive colonial ascidian, *Botrylloides violaceus* (Stachowicz et al. 2002). Futhermore, a 2 °C increase in temperature enabled a 2-fold increase in the growth rate of *B. violaceus* (Stachowicz et al. 2002). The two degree increase in temperature from 1979 (May through October) to 2005 may have resulted in the dominance of *B. violaceus* in the summer and the increased longevity of both *B. violaceus* and the hydroid *Ectopleura larynx* within the community. The greater longevity of dominant warmer water species (e.g. *E. larynx*) observed in this community mirrors that found in terrestrial habitats for birds and plants (Crick et al. 1997, Brown et al. 1999, Dunn & Winkler 1999, Menzel & Fabian 1999). Statistically significant trends correlated rising temperatures to earlier bird nesting and egg-laying for sites in Europe (Brown et al. 1999, Dunn & Winkler 1999) and the southern United States (Brown et al. 1999) while plants in Europe have experienced a 10.8 day increase in their annual growing season (Menzel & Fabian 1999).

A dramatic shift in temperate marine community structure has occurred in New England over the last 25 years. Historically, four season community development was present that corresponded to the seasons (spring, summer, autumn and winter). Now three season community development is present, a condition similar to communities found in warmer southern waters of North Carolina. Given the relatively small changes in nutrients and large changes in sea surface temperature that facilitate the seasonal dominance and longevity of warm water species (native and invasive) over the past 25 years, we suggest increasing temperature is the main factor influencing the seasonal development of the fouling community.

These findings may reflect larger scale temporal trends on the effects of climate related factors on shallow water marine ecosystems. The similarity between the historic North Carolina fouling community and the present day New Hampshire fouling community suggests that rising temperatures increased the longevity of warmer water species and have made it possible for a northern community to take on characteristics of seasonal patterns of a southern community. Because reproduction, appearance and growth rates of species are critically tied to seasonal changes in temperature (Winston 1977, Calder 1990, Minchin 1992, Planque & Fredon 1999, Edwards & Richardson 2004), a reduction (e.g. from 4 to 3) in the seasonal development of communities towards a longer warm period provides a competitive advantage to warm water species, while at the same time impedes cold water species. Consequently the shift in the seasonal development of the fouling community can have dramatic consequences in ecosystem function and stability. Longer "summer" period may result in a temporal mismatch in predator-prey populations as well as change the relative competitive abilities amongst members of the community (Planque & Fredon 1999, Anderson 2001, Stachowicz et al. 2002, Edwards & Richardson 2004).

Table 5.1: Seasonal changes in species abundances. *** Significant differences in species abundances on panels between months in different years (e.g. August 1979 to August 2003, September 1979 to September 2003). * Analyses of abundances were determined using eight, 0.1m2 horizontal (downward facing) Plexiglass® panels.

Species	**Aug.**	**Sept.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**
Porifera													
Haliclona loosanofi	NS	NS	NS	NS	NS	NS	***	NS	***	***	***	***	NS
Halichondria panicea	NS	NS	***	***	NS	NS	NS	NS	NS	***	***	***	***
Leucosolenia sp.	NS	NS	***	***	NS	NS	NS	***	***	***	NS	NS	NS
Halisarca sp.	NS	NS	NS	NS	NS	NS	NS	NS	***	***	***	NS	NS
Cnidaria													
Ectopleura larynx	NS	NS	***	***	NS	NS	NS	NS	NS	***	***	NS	***
Obelia spp.	NS	NS	***	NS	***	NS	NS	***	***	NS	NS	NS	NS
Metridium senelis	NS	NS	NS	NS	NS	NS	NS	NS	***	***	NS	***	NS
Mollusca													
Anomia simplex	NS	NS	***	NS	NS	NS	NS	NS	NS	***	NS	NS	NS
Mytilus edulis	NS	***	NS	NS	NS	***	***	***	***	***	***	***	***
Hiatella arctica	NS	NS	***	NS	***	NS	NS	***	NS	NS	***	NS	NS
Bryozoa													
Membranipora membranacea	***	***	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Bugula simplex	NS	NS	NS	***	NS	NS	NS	NS	NS	***	***	***	NS
Bugula turrita	NS	NS	NS	***	NS	NS	NS	NS	NS	NS	NS	***	NS
Schizoperella unicornis	NS	NS	NS	NS	NS	***	NS	NS	NS	***	***	***	NS
Electra pilosa	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cryptosula pallasina	NS	NS	NS	NS	***	***	NS	***	NS	***	***	***	NS
Encrusting bryozoans	***	***	NS	NS	NS	***	NS	***	***	***	NS	***	NS
Tunicata													
Botrylloides violaceus	***	***	***	***	***	***	***	***	***	***	***	***	***
Botryllus schlosseri	***	***	***	***	***	NS	***	***	***	***	NS	NS	NS
Didemnum sp.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	***
Ciona intestinalis	NS	NS	***	***	***	***	***	NS	NS	NS	NS	***	NS
Molgula spp.	NS	NS	***	***	***	NS	NS	***	***	NS	NS	NS	NS
Aplidium constellatum	NS	NS	NS	NS	NS	***	***	***	NS	***	NS	NS	NS
Didemnum albidum	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Crustacea													
Balanus spp.	NS	***	NS	NS	NS	***	***	***	***	***	***	NS	NS
Molgula spp./*Obelia* spp.	NS	NS	NS	***	NS	NS	***	***	***	***	***	***	***

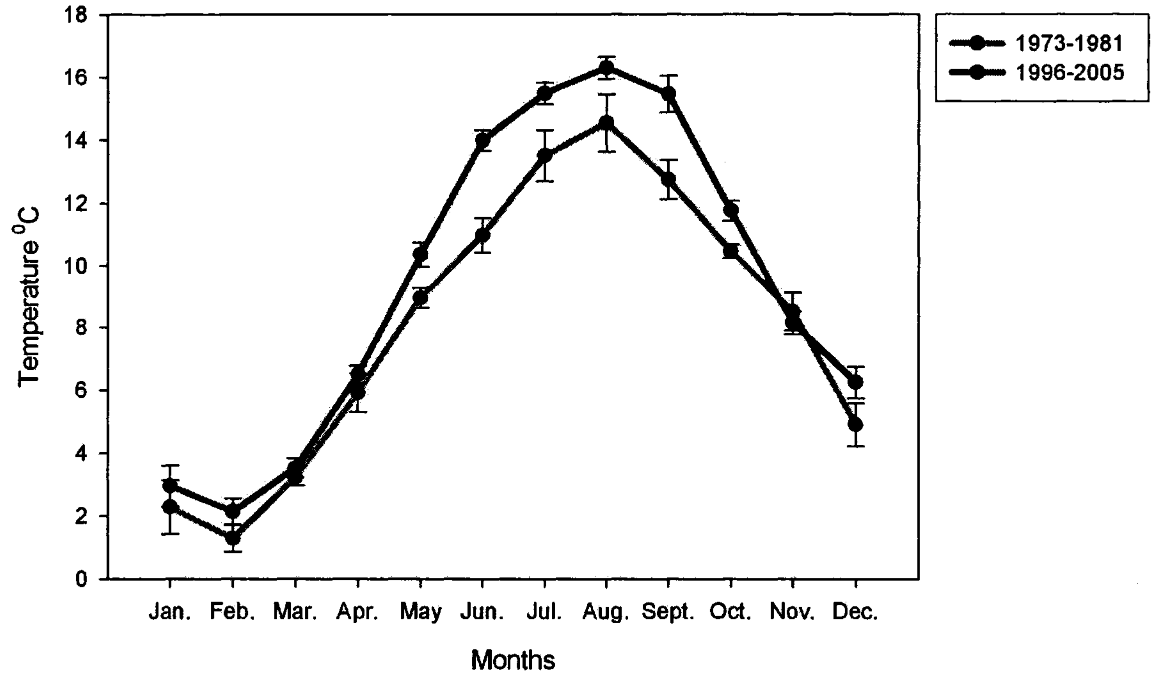


Figure 5.1A: Average temperature between 1973 to 1981 and 1996 to 2005 in Portsmouth Harbor. Error bars are standard error. Kruskal-Wallis test revealed significant ($p \leq 0.05$) differences in monthly temperatures between 1973 to 1981 and 1996 to 2005 were found for the months of May, June, July, August, September and October.

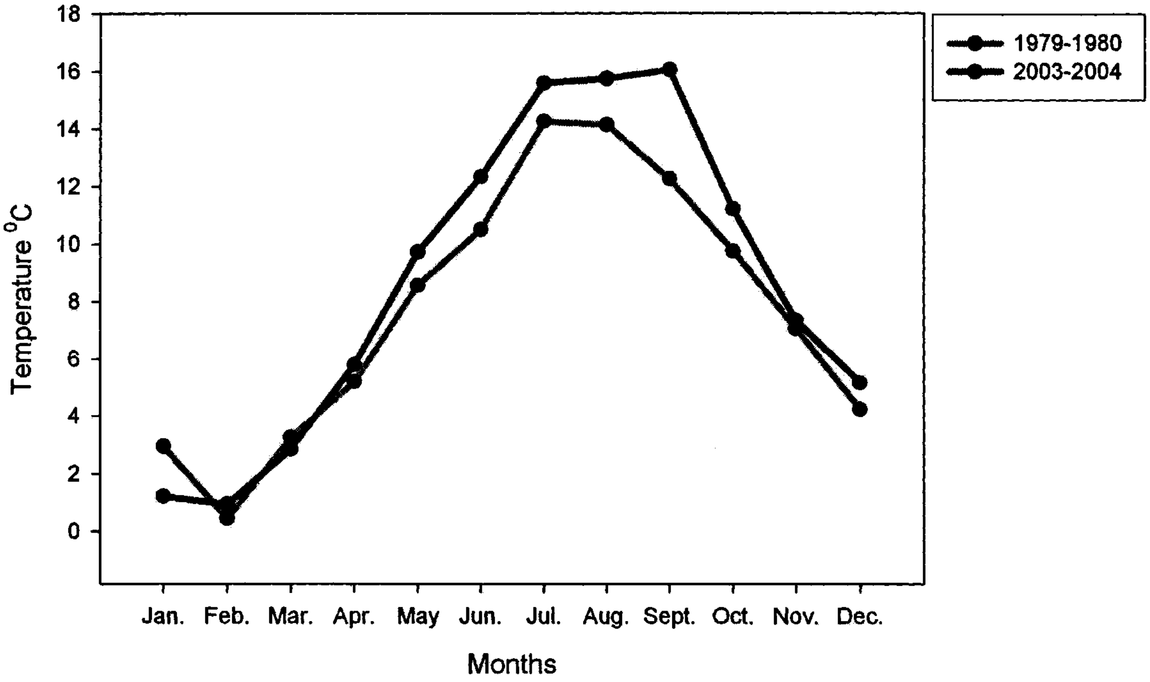


Figure 5.1B: Average temperature between 1979 to 1980 and 2003 to 2004 at the mouth of the Great Bay Estuary System.

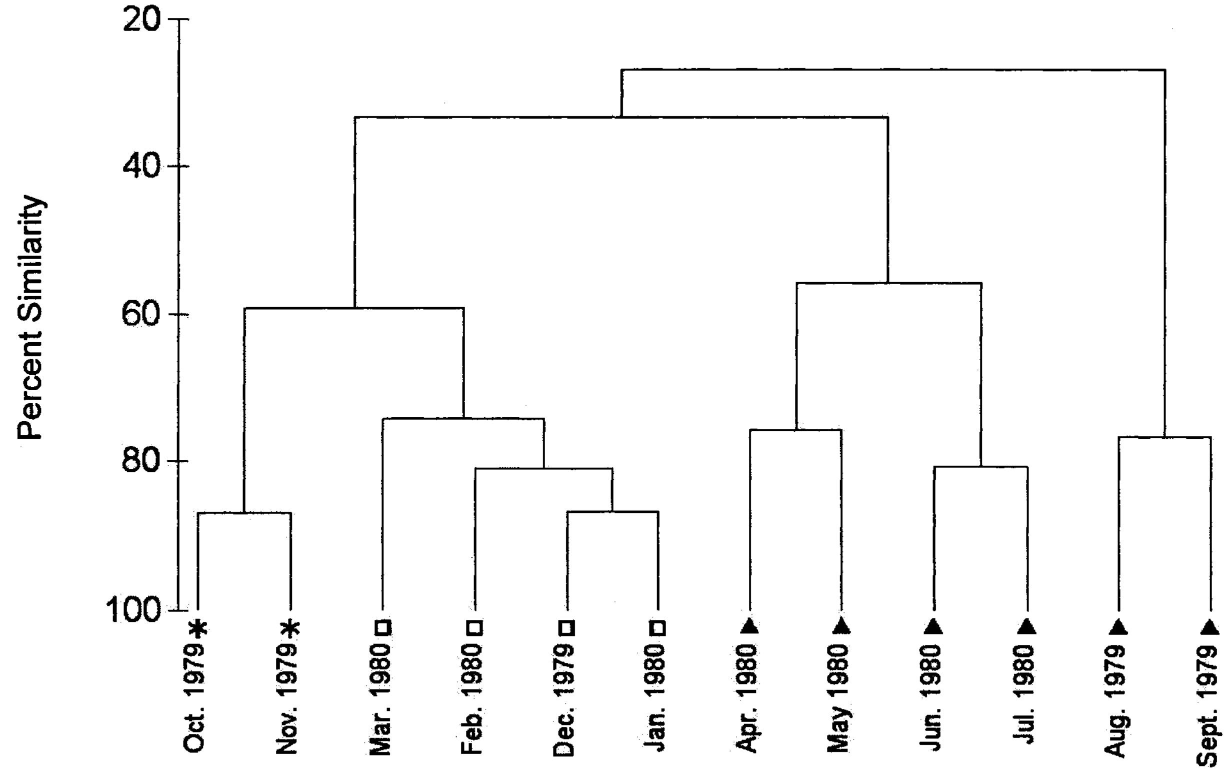


Figure 5.2A: Cluster analysis separating data collected from July 1979 through August 1980 into stages of community development that correspond to the four seasons of Summer (June through September), autumn (October and November), winter (December through March) and spring (April through May). Points represent monthly average abundances of species found on panels.

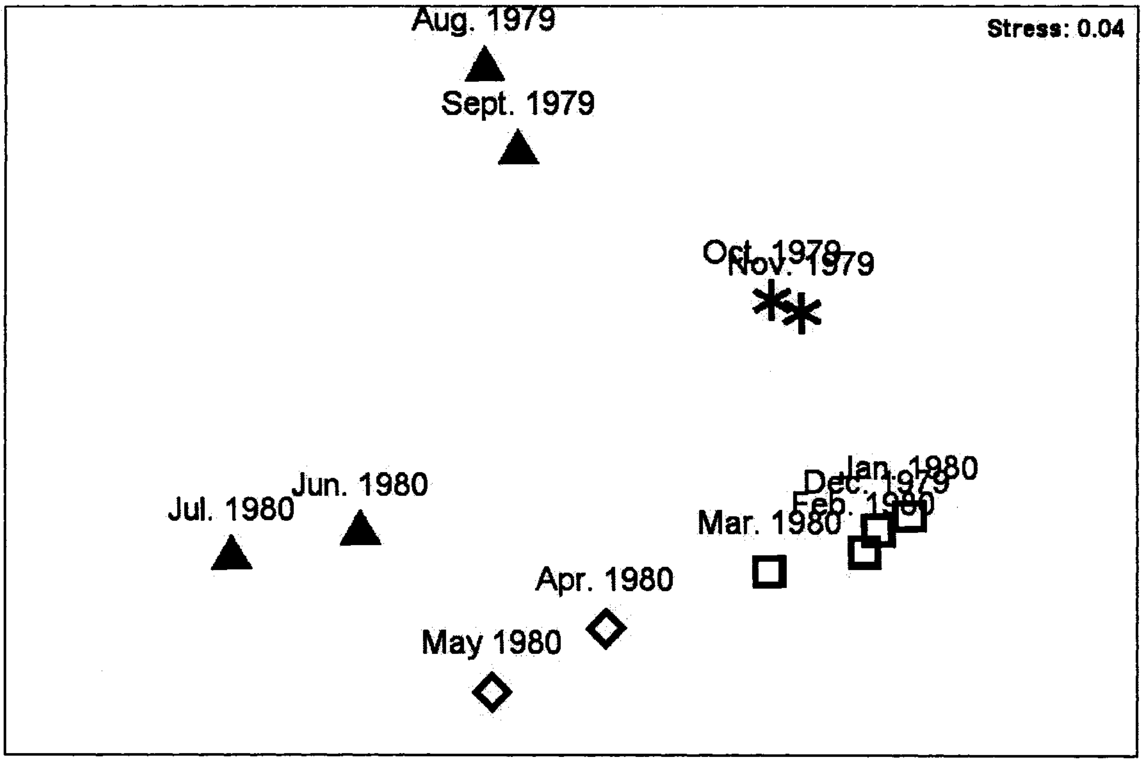


Figure 5.2B: Non-Metric Multi-dimentional Scaling ordination showing the seasonal development between July 1979 through August 1980 in Portsmouth Harbor, NH. Points represent monthly average abundances of species found on panels.

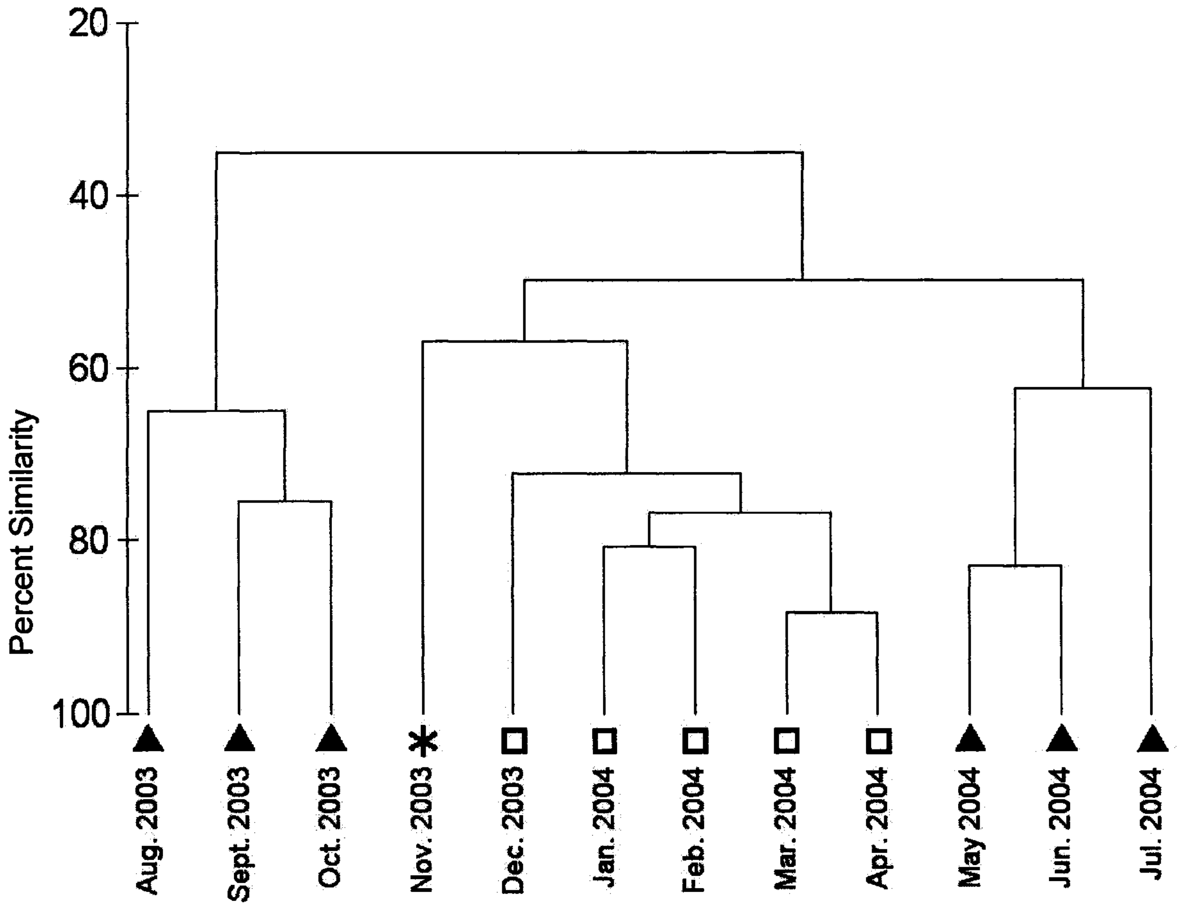


Figure 5.3A: Cluster analysis separating data collected from July 2003 through August 2004 into stages of community development that do not correspond to the four seasons: 1. May through October, 2. November, and 3. December through April. Points represent monthly average abundances of species found on panels.

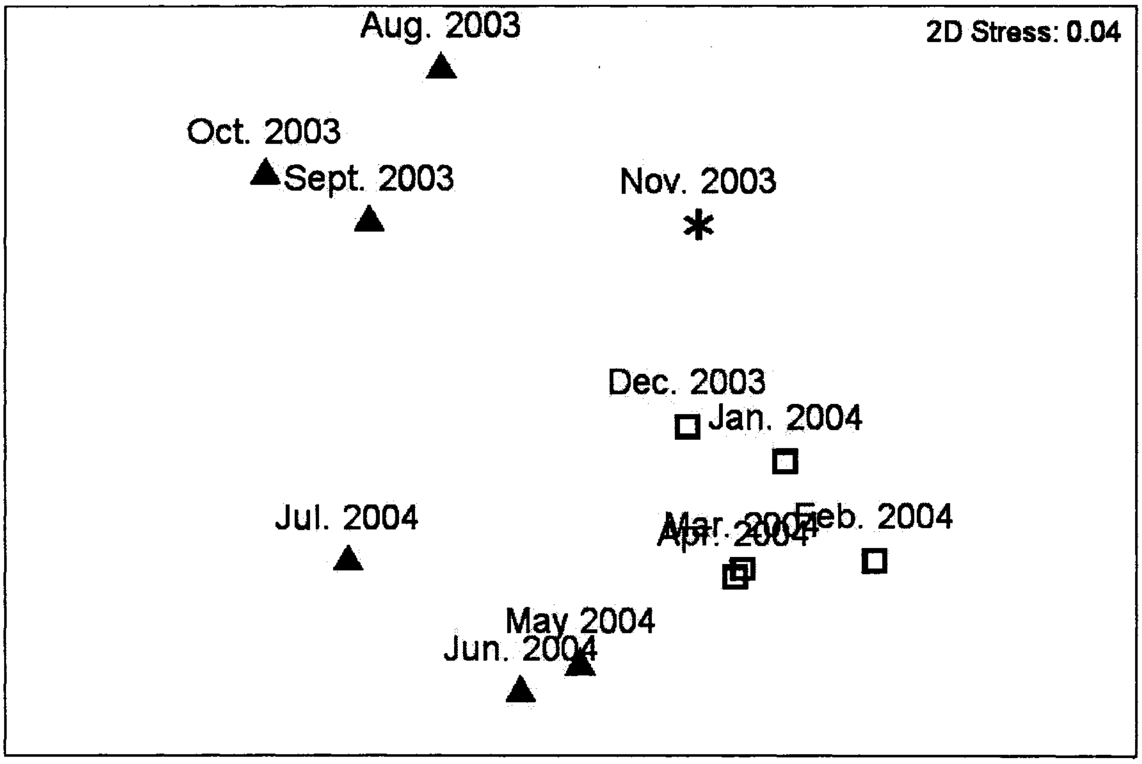


Figure 5.3B: Non-Metric Multi-dimensional Scaling ordination showing the seasonal development between July 2003 and August 2004 in Portsmouth Harbor, NH. Points represent monthly averages of panels. Points represent monthly average abundances of species found on panels.

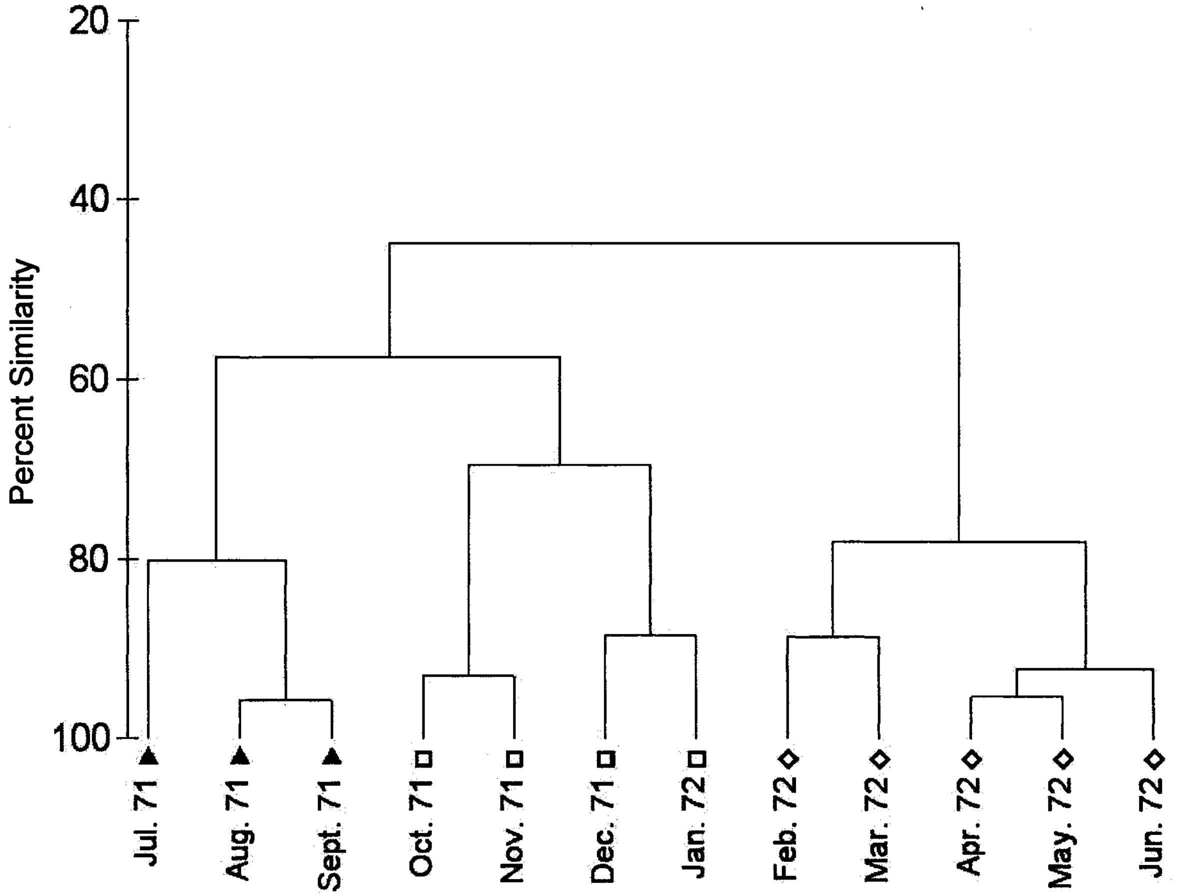


Figure 5.4: Cluster analysis of average monthly abundances of panels observed between July 1971 and July 1972 in Beauford, North Carolina (Sutherland and Karlson, 1973). Like the 2003 to 2004 community in New Hampshire, community development is separated into three stages that don't correspond to seasons.

CHAPTER VI

REGIME SHIFTS IN MARINE COMMUNITIES: INVASIVE SPECIES ALTER SPECIES COMPOSITION, SUCCESSION AND THE MAINTENANCE OF SPECIES DIVERSITY

Abstract

We examined the combined effects of invading species and increasing sea surface temperatures on community development by comparing temporal changes in succession at the mouth of the Great Bay Estuary in Portsmouth Harbor, Newcastle, New Hampshire. Two experiments that took place between 1979 to 1982 and 2003 to 2006 recording community development revealed a dramatic shift in species composition, succession and the maintenance of species diversity. The 2003 to 2006 study was a follow-up of an earlier study conducted by Harris and Irons (1982) which documented the development of a marine fouling community on Plexiglas® panels beneath a cement pier in Portsmouth Harbor. Photographs and observations of the 1979 to 1982

community on the pier indicates that the fouling community was dominated by encrusting and erect bryozoans, and mussels were interspersed throughout these assemblages. Succession was seasonal, leading towards increased homogeneity, and diversity was maintained as secondary substrate provided by the dominant bivalve *Mytilus edulis*.

Since the 1979 to 1982 study, summer and autumn temperatures have risen, facilitating the dominance of warmer water native and invasive species. Invasive species observed on panels in the 2003 to 2006 experiment were the encrusting bryozoan *Membranipora membranacea* and the colonial ascidians *Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp. Unlike the 1979 to 1982 community, succession in the 2003 to 2006 community was directional in the first two years only, leading towards increased heterogeneity. Diversity was not maintained by secondary substrate but by primary substrate due to the seasonal life-history characteristics of the invasive species. The combined effect of rising temperature and invading species has created a novel community driven by invasive colonial ascidians.

Introduction

Invasive species (otherwise known as exotics or non-indigenous species) have become ubiquitous members of terrestrial and marine communities. Once established they can disrupt ecosystems by altering species composition, trophic structure, habitats and species distribution patterns (Lambert & Lambert 1998, Byers 2000, Grosholz et al. 2000, Noonburg & Byers 2005, Blum et al. 2007, Dijkstra et al. 2007), characteristics of communities that can influence the trajectory of succession and lead to disparate patterns of diversity and homogeneity. Long-term intertidal studies of invasive green crabs found they impacted multiple trophic levels, reducing the abundance of native crab species as well as competing with fish and bird species for amphipods and polycheates (Grosholz et al. 2000). Byers (2000) showed invasive snails displaced native snails by altering local habitats while Harris and Tyrrell (2001) witnessed a shift in the development of a subtidal system from a community that was once dominated by kelp beds to a community comprised of invasive species, including a green alga (*Codium fragile* spp. *tomotosoides),* a bryozoan (*Membranipora membranacea*), and colonial ascidians (*Diplosoma listerianum* and *Botrylloides violaceus*). In Long Island Sound, Osman and Whitlatch (1995) also observed a substantial shift in species composition from native to invasive dominated fouling communities that include the solitary ascidians, *Ascidiella aspersa* and *Styela clava* and the colonial ascidians, *Diplosoma listerianum* and *Botrylloides violaceus.*

Invasive species can also promote biotic homogenization. Biotic homogenization generally refers to an increase in the compositional similarity amongst species at many locations due to the establishment of invasive species (McKinney & Lockwood 2005). Most studies investigating homogenization by exotics have been examined using fish (Radomski and Goeman 1995, Rahel 2002), plants (Vitousek et al. 1987, McKinney 2004), land snails (Cowie 2001) and birds (Harrison 1993). For example, plant inventories at 20 locations in the United States revealed a link between increased exotic species and compositional homogenization (McKinney 2004). Fewer studies have focused on marine invertebrates (Carlton 1996), and less have examined changes in biotic homogenization along a successional gradient.

Succession in both terrestrial and marine ecosystems is the directional change in species over time where development rests upon rates of disturbance and biological interactions such as competition and predation (Dayton 1971, Greene & Schoener 1982, Breitburg 1985, Osman & Whitlatch 1995, Osman & Whitlatch 1998, Wootton 2002, Keeton & Franklin 2005). In subtidal marine communities, succession can be directional, seasonal or random (Weiss 1948, Sutherland & Karlson 1973, Osman 1977, Harris & Irons 1982, Connell et al. 1997). Directional succession involves an orderly process of species replacement leading towards a stable climax. It has been reported for communities in environmentally stable regions e.g. coral reefs and communities inhabiting the deep sea and the poles (Sanders 1969, Pearson 1981, Grassle & Morse-Porteous 1987, Gutt et al. 1996, Connell et al. 1997). Seasonal succession is typical of temperate environments, where abiotic factors change predictably, and aquatic systems undergo cyclical fluctuations in species composition that are the result of life-history characteristics of species within communities (Weiss 1948, Sutherland & Karlson 1973, Underwood & Anderson 1994). Finally, random succession is a disorderly process where the direction and pattern of community development is unpredictable. It often results from recurrent physical or biological disturbances, that generate open space and prevent competitive exclusion by one or more species (Sutherland & Karlson 1977, Hughes & Connell 1999). In subtidal benthic and fouling (species inhabiting man made structures) communities, space is a limiting resource and competition amongst individuals and species can be intense (Sebens 1986). Primary space can be freed by the death of an organism, e.g. senescence, predation or by physical disturbance e.g. flooding, heavy wave action. Release of primary space "resets" succession by enabling the settlement of a wide variety of species that comprise both inferior and superior competitors. While all three types of succession have been observed, few studies have examined the interactive effects of climate change and invasive species on succession in marine communities.

In recent years, increasing sea surface temperature has been found to alter species composition, dominance and diversity patterns of subtidal communities (Osman & Whitlatch 1995, Connell et al. 1997, Harris & Tyrrell 2001, Stachowicz et al. 2002, Schiel et al. 2004). Global sea surface temperatures have risen by 0.4°C to 0.8°C in the past century and temperatures are expected to accelerate in coming years (IPCC 2001). In the Northwest Atlantic, average sea surface temperature has increased by an average of 0.61°C over the last century (Curry et al. 2003, Clean-Air Cool Planet & Wake 2005). Temperature generally determines seasonally programmed reproduction and growth of marine species (Goodbody 1961, Millar 1971, Winston 1977, Minchin 1992, Witman & Grange 1998, Lambert 2005). Seasonally fluctuating temperature is often one of the most important factors that cause species replacement in near-shore temperate habitats (Goodbody 1961, Stachowicz & Byrnes 2006). Species observed within communities have disparate tolerances to temperature, thus an increase in temperature can favor growth or earlier reproduction one species over another (Sagarin et al. 1999, Vázquez & Young 2000, Parmesan & Yohe 2003). In central California, rising sea surface temperatures in the intertidal zone have led to higher abundances of warmer water species and lower abundances of colder water species (Barry et al. 1995, Sagarin et al. 1999, Schiel et al. 2004). Additionally, increasing water temperatures in Long Island Sound, Connecticut, facilitated the dominance of warmer water invasive species (Stachowicz et al. 2002).

Here we link changes in species composition, succession and the maintenance of species diversity spanning 27 years (1979 to 1982 and 2003 to 2006) to increasing temperatures and invasive species. We compared succession between 1979 and 1982 to 2003 and 2006 in a marine community, determined which abiotic and biotic factors have significantly changed over the last 27 years and examined the influence of invasive species on species composition, diversity and successional homogeneity.

Materials and Methods

Study area

The study site was located underneath a cement pier in Newcastle, NH at the University of New Hampshire's Coastal Marine Laboratory (Figure 6.1). The pier extends out (~0.5 km) into the channel of Portsmouth Harbor. The area between the pilings under the pier is mostly fine sand/mud mixed with large stones. Photographs and observations of the 1979 to 1982 community on the pier indicate the fouling community was dominated by sponges, mussels, anemones and large barnacles (Harris and Irons 1982). Soft corals, ascidians, encrusting and erect bryozoans, and hydroids were interspersed throughout these assemblages. Since the 1979 study, 6 species have invaded artificial substrate communities in Portsmouth Harbor. These include the encrusting bryozoan, *Membranipora membranacea*, the solitary ascidians *Ascidiella aspersa* and *Styela clava* and the colonial ascidians, *Didemnum* sp., *Botrylloides violaceus* and *Diplosoma listerianum*. All three colonial ascidians and the encrusting bryozoan *M. membranacea* had been documented on the panels beneath the pier at our study site in Newcastle, NH by 2003, but the solitary ascidians have not yet been observed at this site.

Data collection

Comparison of succession and diversity were quantified using two panel studies. The first study was initiated in 1979 with the goal of documenting epifaunal community development between 1979 and 1982 (Harris and Irons, 1982). 8 horizontal and 8 vertical predator inaccessible 0.1m^2 Plexiglas ® panels were deployed in July 1979 at approximately 5m below mean low water under the cement pier at the Coast Guard Station, Newcastle, NH and photographed monthly from 1979 to 1982. Original slides were still available for analysis.

The second panel study was established in 2003 to document epifauna succession from 2003 to 2006 and to compare it to the results of the 1979 to 1982 study. The 2003 to 2006 study replicated the experimental design of the first study and the panels were photographed monthly. Slides and photographs of panels taken from 1979 to 1982 and 2003 to 2006 (~ 1200 images) were digitized and downloaded into Adobe Photoshop 7.0©. All individuals were counted and identified to the species level and percent cover of colonial hydroids and dominant structural members of each community were recorded (1979 to 1982, *Mytilus edulis*; 2003 to 2006, *Botrylloides violaceus*). *Obelia* sp. was observed primarily as secondary space occupants on the solitary tunicate, *Molgula* sp. It was impossible to enumerate colonies of *Obelia* sp. on *Molgula* sp. resulting in the group *Obelia* sp./*Molgula* sp.

Abiotic factors

Monthly water samples were collected within 0.5 km of our study site from 1978 to 1981 (Norrel et al. 1982). The data recorded include water temperature, salinity, dissolved oxygen, dissolved inorganic nitrogen (DIN), phosphorus (PO_4), and chlorophyll *a* (see Norell et al. 1982 for full description of methodology). Monthly water samples of DIN, PO_4, and chlorophyll *a* were collected April to December 2003 through 2006 at the Newcastle study site. Temperature, salinity, and dissolved oxygen were collected every half hour April 2003 through December 2006 using a YSI environmental data logger for this period. One way ANOVA's were used to detect differences in nutrients, temperature, and salinity between 1979 to 1982 and 2003 to 2006.

Data analysis for community succession and diversity

A number of uni- and multivariate analyses were used to detect differences in community assemblage and structure on panels between 1979 to 1982 and 2003 to 2006. These include analyses between eras (1979 to 1982 and 2003 to 2006) and between corresponding years of the experiment e.g., 1979 and 2003. The Mann-Whitney U test was used to detect differences in abundances of single species between era's (1979 to 1982 and 2003 to 2006). Monthly average abundance between 1979 to 1982 and 2003 to 2006 were used for the analyses [n= 33 (1979 to 1982); n = 33 (2003 to 2006)].

Bray-Curtis similarities were the basis for the community-related statistics between eras and were calculated using fourth root transformed raw data; fourth root transformations were used because the results were not strongly affected by seasonal effects. Within eras, Bray-Curtis similarities were also calculated, but on data that were square root transformed; square root transforms allow for the persistence of seasonal effects in the results. Non metric-Multi-Dimensional Scaling plot (nMDS) and analysis of similarities (ANOSIM) were used to examine within era succession (1979 to 1982 and 2003 to 2006) and to detect differences in faunal composition between 1979 to 1982 and 2003 to 2006. NMDS is a method which preserves species-specific information and is sensitive in detecting changes in assemblage pattern (Clarke & Ainsworth 1993). Analysis of Similarities was used to detect differences between groups; it is a non-parametric permutation test that applies the same (rank) similarity matrix used for the nMDS ordination (after Clarke and Warwick 2001).

To examine changes in temporal heterogeneity, within community variance (e.g. 1979 to 1982 and 2003 to 2006) and within year variance (e.g. 1979 and 2003) were calculated using Bray-Curtis similarities. Discriminator species were calculated between 1979 to 1982 and 2003 to 2006 using the SIMPER statistic (SIMilarities PERcentages). The discriminator species were calculated using a pairwise test for each pair of assemblages (Clarke 1993). It computes the average dissimilarity between all pairs of inter-assemblage areas and then breaks this average into the separate contributions within each species (Clarke & Warwick 2001). A discriminator species has a relatively high and evenly distributed abundance in most areas of one assemblage but is rare or not at all present in areas of another assemblage. A good discriminator species is characterized by having a high average dissimilarity (δ_i) between assemblages and a high value for the quotient $\delta_i/SD(\delta_i)$ (Clarke and Warwick, 2001). The ratio is the average dissimilarity of a species divided by the standard deviation. If the average dissimilarity of a species is large and the standard deviation small, that species contributes much to the average dissimilarity and does so consistently in inter-comparisons of all samples in both assemblages. Community analyses and graphs were generated using Primer 6.0 (Primer-E Ltd. Plymouth, UK).

In addition to multivariate tests, univariate tests were used to examine differences in structural characteristics within community succession (1979 to 1982; 2003 to 2006) and between 1979 to 1982 and 2003 to 2006. Diversity is expressed as the Shannon-Wiener index and combines the number of species with distribution of individuals among the taxa (Shannon & Weaver 1949). Evenness was calculated using Pielou's eveness index and describes the relative distribution of individuals among the different taxa (Pielou 1969). Abundance data were log transformed, though species richness, evenness and diversity were not as these data were relatively evenly distributed. A one-way ANOVA was used to test for differences in abiotic conditions between 1979 to 1982 and 2003 to 2006. ANOVAs were calculated using the statistical software package JMP (6.0).

Results

Four invasive species not present in the 1979 to 1982 community have become key members of the 2003 to 2006 community. They include three invasive colonial ascidians, *Botrylloides violaceus*, *Diplosoma listerianum* and

Didemnum sp. along with an invasive encrusting bryozoan, *Membranipora membranacea*. The encrusting bryozoan *M. membranacea* was a pioneer species with greatest abundance during the first three months of community development (July to September). *D. listerianum* was rarely observed in the 2003 to 2006 community. *B. violaceus* and *Didemnum* sp., on the other hand, were seasonally dominant species (Figure 6.2). Highest spatial coverage occurred in summer (June through August 2004 and July through September 2005) for *B. violaceus* and fall and winter for *Didemnum* sp. (October through March, 2004-2005 and November through April, 2005-2006). Colonies of *Didemnum* sp. regressed in February and almost dropped out of the system by May in each year.

Community assemblage between 1979 to 1982 and 2003 to 2006

Long-term fouling panels (1979 to 1982 and 2003 to 2006) were analyzed to examine the influence of invasive species and climate change on succession. A total of 37 species and > 600,000 individuals were recorded between the two studies.

The 1979 to 1982 community was dominated by Bivalvia and Crustacea, 44% and 31% respectively (Appendix I). Ascidiacea, Cnidaria, Porifera and Bryozoa comprised 18%, 3%, 2% and 1% respectively. Dominant member of the Crustacea and Bivalvia were *Balanus* spp. (99%) and the blue mussel, *Mytilus edulis* (82%). *Molgula* sp., the species complex *Molgula* spp./*Obelia* spp. and *Botryllus schlosseri* were dominant members of the Ascidiacea comprising 47%, 43% and 5%, respectively. The native hydroid *Ectopleura larynx* comprised 63% of the Cnidaria while the group encrusting bryozoan and *Bugula simplex* accounted for 37% and 18% of the bryozoans. The native sponge *Haliclona loosanofi* comprised 43% of the Porifera.

In contrast, the 2003 to 2006 community was dominated by ascidiacea (75.28%) and to a lesser extant Bivalvia (13%) and Crustacea (7%). Furthermore, there has been a shift in the species that dominated individual groups between 1979 and 2003. The assemblage of *Molgula* spp. and *Obelia* spp. were the dominant members of the group Ascidiacea between 2003 and 2006 comprising 85% while the invasive colonial ascidians *Botrylloides violaceus* and *Didemnum* sp. composed 5%. The *Molgula* spp. and *Obelia* spp. assemblage is abundant during the winter months while *B. violaceus* and *Didemnum* sp. are abundant in the summer and fall (Dijkstra et al. 2007). Two species (instead of one for the 1979 to 1982 community) were dominant members of Bivalvia (*Mytilus edulis*, 50%, and *Anomia simplex*, 47.57%) and three species for the group Porifera (*Halichondria panicea*, 37%, *Haliclona loosanofi*, 23% and *Leucosolenia* sp., 21%) The erect bryozoan *Bugula simplex* increased in abundance and contributed 39% to the group Bryozoa. The resident encrusting bryozoan *Schizoperalla unicornis* and the invasive encrusting bryozoan *Membranipora membranacea* composed 10% and 9%, respectively. Like the 1979 to 1982 community *Balanus* spp. dominated the Crustacea while the hydroid *Ectopleura larynx* governed the cnidaria, though less than in 1979 to 1982 (Harris and Irons, 1982).

Overall, the abundance of individuals in Ascidiacea and Bryozoa significantly increased from 1979 (Table 6.1). The ascidiacea expanded to include three invasive colonial ascidians *Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp. while the encrusting bryozoan *Membranipora membranacea* is included as a member of the group Bryozoa. The assemblage *Molgula* spp. and *Obelia* spp. have significantly increased since 1979 while *Botryllus schlosseri, Ciona intestinalis* and *Molgula* spp. have declined. Members of the group Bryozoa and cnidaria have either shown no significant difference in abundance or have significantly increased. Interestingly most hard shelled organisms including the historically dominant species *Mytilus edulis* and *Balanus* spp. and sponges have significantly declined since 1979 (Table 6.1).

Species composition and succession

Faunal composition between 1979 and 2006 was significantly different with apparent shifts in species composition, dominance of groups and species (Figure 6.3; ANOSIM $R = 0.326$ $p < 0.01$; Table 6.2). According to a one-way SIMPER analysis the invasive colonial ascidians *Botrylloides violaceus* and *Didemnum* sp., the mussel *Mytilus edulis*, the sponges *Haliclona loosanofi* and *Halichondria panicea* were the most important and consistent determinants between the 1979 to 1982 and 2003 to 2006 communities (Table 6.2). *Botrylloides violaceus* and to a lesser extent *Didemnum* sp., invasive colonial ascidians, characterized the 2003 to 2006 and have displaced the historically prevalent bivalve, *Mytilus edulis* (Table 6.3). *M. edulis* was a persistent special occupant throughout succession between 1979 and 1982 (Figure 6.4). *B. violaceus* and *Didemnum* sp., on the other hand, are ephemeral between 2003 and 2006 (Figure 6.2). Other notable species contributing to the difference in faunal composition were the mussel *Mytilus edulis*, the sponges *Halichondria panicea* and *Haliclona loosanofi* (Table 6.2). Furthermore, we detected dissimilarities in the pattern of succession between 1979 to 1982 and 2003 to 2006; succession was directional, marked by patterns of seasonality in the 1979 to 1982 community (Figure 6.5a). Succession in the 2003 to 2006 community was directional for the first two years of succession, but becoming less predictable in years 3 and 4 (Figure 6.5b).

Seasonal directional succession culminating in a community dominated by long-lived species e.g. sponges was observed between 1979 and 1982 (Figure 6.5a). The cycles in the plot reflect seasonal life cycles of individual species, though the seasonal cycle in the first year was more apparent than in subsequent years. Winter months (December through March) were clearly separated from all other months. The hydroid *Ectopleura larynx* quickly colonized recently deployed panels. After the regression of *E. larynx* in October, the jingle shell *Anomia simplex* along with the *Molgula* sp./*Obelia* sp. species complex were abundant on panels until the recruitment of the blue mussel, *Mytilus edulis* in April, 1980. Other common species observed before the recruitment of *M. edulis* were the sponges, *Leucosolenia* sp., *Haliclona loosanofi* and *Halichondria panicea* and the colonial ascidian *Botryllus schlosseri*. Once established, *M. edulis* remained the dominant member of the community for ~ 2 years (Figure 6.4). During this time, ephemeral species used its hard shell as a substrate. *E. larynx* was the dominant "summer species" observed from June through September. Other common species observed during this time were the erect bryozoan *Bugula simplex* and the group encrusting Bryozoa, the colonial ascidians *Botryllus schlosseri* and the barnacles *Balanus* sp. In September and October, the solitary ascidian *Molgula* sp. and the species complex *Molgula* sp./*Obelia* sp. recruited onto the shells of *M. edulis*, remaining until May.

In contrast, seasonal succession was less apparent between 2003 and 2006 than 1979 and 1982. Initial development between years 1 and 2 was directional. However unlike the 1979 to 1982 community, the directional pattern did not continue to years 3 and 4, years of peak abundance of colonial ascidians (Figure 6.5b). Additionally, succession did not lead towards long-lived species as it had in the 1979 to 1982 community but was dominated by ephemeral invasive species. Like the 1979 to 1982 community seasonal cycles were clear and distinct during the first year of community development (Figure 6.5a and 6.5b). However, winter months (December through March) in 2004, 2005 and 2006 were not separated from the spring or autumn months. Succession began with an abundance of the invasive encrusting bryzoan, *Membranipora membranacea* and the hydroid *Ectopleura larynx* (August through October, 2003). During this time the colonial ascidians (*Botrylloides violaceus* and *Botryllus schlosseri*) and bryozoans (*Bugula simplex* and species from the group encrusting Bryozoa) were also observed on panels. After regression of *M. membranacea* and *E. larynx* in October and November, 2003, the jingle shell *Anomia simplex* and small colonies of *B. violaceus* and *Didemnum* sp. recruited to the panels. Most small colonies of *Didemnum* sp. did not survive the 2003 winter while only 1/3 of the overwintering *B. violaceus* colonies survived. In May sea surface temperatures increased enough to facilitate growth of overwintering *B. violaceus*. During the winter and early spring months, the species complex *Molgula* sp./*Obelia* sp. was the most abundant species on panels. This complex survived and grew during the winter months, but individuals were consumed by the wrasse *Tautogolabrus adspersus* in late spring leaving primary space open for colonization by competent larvae (Harris and Dijkstra, unpub.data). *B. violaceus*, *M. edulis* and *B. simplex* occupied space left by the consumed *Molgula* sp./*Obelia* sp. complex and characterized the "summer" community. Though relative abundance of *B. violaceus* is substantially lower than *M. edulis*, individual colonies were much larger than individuals of *M. edulis* and consequently *B. violaceus* was the dominant spatial occupant from June through September (Figure 6.4).

Homogenization

Overall variance between the two communities showed no significant difference, but communities showed remarkably opposing trends in variance of species similarity as a function of succession (Figure 6.6). In the 1979 to 1982, annual assemblages of species became increasingly homogeneous during succession (adj. $R^2 = 0.928$, $p < 0.02$). In contrast species assemblages became less homogeneous with time in the 2003 to 2006 community, though the trend was not significant (adj. $R^2 = 0.136$, $p < 0.35$).

Diversity

Overall, we found no significant difference in abundance, evenness or diversity, though there was a significant increase in species richness (Figures 6.7a to 6.7d). Subsequent examination of these measurements as a function of succession revealed an increasing pattern of abundance and species richness between 1979 and 1982 (Figures 6.8a and 6.8b). Diversity was highest during initial development, stabilizing over the following years; no apparent pattern was observed for evenness (Figures 6.8 c and 6.8 d). In the 2003 to 2006 community, species richness and abundance slightly increased from 2003 to 2006 (Figures 6.9 a and 6.9b). In contrast, evenness and diversity declined over time, reaching their lowest point in 2006 (Figures 6.9 c and 6.9d).

Abiotic factors

Water quality measurements taken between 1979 to 1982 and 2003 to 2006 included DIN, PO_4, dissolved oxygen and Chlorophyll *a*, temperature, and salinity. Mean DIN increased from 0.0698 mgN/L (standard error (SE) 0.00647) to 0.1178 mgN/L (SE: 0.00940), though not significantly (Figure 10a). Average PO_4 concentrations have risen ($p < 0.0269$) from 0.0208 mgP/L (SE: 0.00125) to 0.0234 mgP/L (SE: 00523; Figure 10b). Chlorophyll *a* concentrations have marginally ($p < 0.0469$) increased from 1.1979 µg/L (SE: 0.1025) to 1.5889 µg/L (SE: 0.1397; Figure 10c). Furthermore, temperature and salinity have also significantly changed between the study periods (Figures 10d to 10e). Average temperatures rose from 7.446 °C to 9.269 °C with maximum increases occurring from May through October (previous chapter, $p < 0.001$; SE: 0.353 and 0.024). In contrast, salinity decreased from an average of 31.35 ppt. to 29.17 ppt. ($p < 0.01$; SE: 0.100 and 0.013). Lowered salinity values, particularly during the months between March and June, may have resulted from increased rain and flooding events. Finally hypoxia is not occurring at our study site as dissolved oxygen levels were equal to or above 75% saturation.

Discussion

Species composition and succession

Since 1982 we have observed a shift in species composition and a replacement in dominant species from the native blue mussel *Mytilus edulis* to the invasive colonial ascidian *Botrylloides violaceus* and to a lesser extent *Didemnum* sp. *B. violaceus* and *Didemnum* sp. are seasonally abundant, most likely the result of their complex life history coupled with extreme fluctuations in temperature (Yamaguchi 1975). The colonial ascidian *Botryllus schlosseri*, the sponges *Halichondria panicea* and *Haliclona loosanofi* and the blue mussel *M. edulis* have decreased in abundance. *B. schlosseri* and sponges recruited during the summer months beginning in June while recruitment of *M. edulis* began in May, one month later than the 1979 to 1982 community (Harris & Irons 1982, Berman 1992). May and June are times of substantial growth in overwintering colonies of *B. violaceus* (Figure 2). Many colonial ascidians can overgrow and smother neighboring species or new recruits (Valentine et al. 2007); they also possess toxic chemicals that inhibit the settlement of other species (Bryan et al. 2003). The decline in abundances of *M. edulis*, *B. schlosseri* and sponges may result from rapid growth of *B. violaceus* coupled with temporal recruitment patterns of historically dominant species. Support for this hypothesis comes from increased recruitment and abundance of *M. edulis* at a number of sites in the Gulf of Maine, including the Isles of Shoals. However, recruitment of marine invertebrates is variable in space and time and may have been low during the study period at this site.

In contrast to *Mytilus edulis*, *Botryllus schlosseri* and sponges, members of the group Bryozoa have significantly increased in abundance. Recruitment of *Bugula simplex* and *Bugula turrita* began in mid-July with highest recruitment occurring in August and September (Dijkstra and Westerman, unpub. data), during a time when some parent colonies of *Botrylloides violaceus* are undergoing regression, senescence and sloughing off panels. While the ephemeral life-cycle of *B. violaceus* may have contributed to reduced abundances of competitively superior species, it may have favored competitively inferior species that recruit during times of open space

One of the most striking differences between the 1979 to 1982 and 2003 to 2006 communities was the process of succession. Seasonal cycling of species composition was observed throughout succession in both communities (1979 to 1982; 2003 to 2006), though cycles were less apparent with each successive year as succession depended in part on the degree to which larvae could invade existing adult assemblages and the composition of the surrounding fauna (Karlson & Jackson 1981, Grigg 1984, Breitburg 1985, White & Pickett 1985, Drake 1991, Roberts et al. 1991, Osman & Whitlatch 1998).

Succession in the 1979 to 1982 community was clearly directional, marked by patterns of seasonality. Before the arrival of *Mytilus edulis* in April 1980, panels were dominated by species with seasonal life-cycles. After their recruitment little space was made available and *M. edulis* dominated panels for ~ 2 years, precluding take over of primary space. However, seasonally abundant species occupied secondary space provided by the hard shell of *M. edulis* and contributed to the clear seasonal structure observed in each year between 1979 and 1982.

In contrast to the 1979 to 1982 community, seasonal cycling of species composition was less apparent between the 2003 and 2006 community; directional succession was observed for years 1 and 2 but not for years 3 and 4. While many factors may account for these differences the most obvious factor is the large numbers of colonial organisms with distinct seasonal life history cycles. The life-cycle of *Botrylloides violaceus* begins with asexual growth followed by sexual and larval reproduction that lasts for approximately 3 weeks, ending with regression and senescence of the parent colony (Yamaguchi 1975). If sea water temperatures are above 10 °C throughout the year, this cycle can persist annually, otherwise small sized colonies over winter until temperatures are warm enough to initiate growth and reproduction (Mukai 1977). In Portsmouth Harbor, *B. violaceus* undergoes asexual reproduction in summer reaching peak abundances in August, followed by a rapid decline (Figure 2). Unlike *B. violaceus*, *Didemnum* sp. grew during the winter months, November through February, reaching peak abundances in January and February. In late January to early February temperatures fall below 4 °C, a potentially critical temperature that initiates regression in *Didemnum* sp. (Valentine et al. 2007). Senescence and regression of both species "reset" succession by creating open primary space for colonization by other species.

Succession between 2003 and 2006 resembled that of Sutherland and Karlson (1977). Both communities (2003 to 2006 and Sutherland and Karlson, 1977) experienced regular changes in species composition following initial development and were the result of the life cycles of the seasonally dominant species. Succession in Sutherland and Karlson (1977) was unpredictable owing to the continued generation of primary space as a result of life-history cycles of colonial and solitary ascidians and bryozoans. Between 2003 and 2006 succession was directional in years 1 and 2 becoming less so in years 3 and 4, years of higher abundances of seasonally dominant primary space occupants. Seasonal cycling of primary space occupants generated bare space for the recruitment of competent settling larvae. Unlike (Sutherland 1974, 1981) and Osman (1977), panels in this study were not monopolized by one species. This was most likely due to the size of the panels used in each of the studies. Sutherland (1974, 1981) used $0.0232 m^2$ panels while Osman (1977) used

$0.01m^2$ and $0.0014m^2$. In contrast panels used in this study were larger ($0.1m^2$) and inhibited exclusion by other species (Harris & Irons 1982).

Homogenization

Biotic homogenization is an increase in spatial and temporal similarity of taxonomic abundances. In the absence of mortality, the process of succession replacement leads to homogeneity since initial development by a few competitively superior or long-lived species monopolize space. This pattern has been observed for coral reefs (Connell 1973, 1978, 1999), rocky intertidal algal and invertebrate communities (Paine, 1966, Dayton 1971, Lubchenco and Menge 1978, Sousa 1979) and marine fouling communities (Osman 1977, Harris and Irons 1982). Mortality of species either by predation, senescence or physical disturbance frees primary space and can lead to a greater number of species and therefore increased heterogeneity by enabling the coexistence of pioneer and long-lived species (Connell 1978, Sousa 1979).

Between 1979 and 1982, homogeneity increased as a function of time from initial development. Greater heterogeneity during year 1 and part of year 2 was attributed to the seasonal life cycles of primary space occupants. After mussel settlement in April 1980, heterogeneity decreased owing to their continued dominance of primary space. In the summer and fall of 1981 mussels fell off panels, leaving open primary space. However, sponges that had settled prior to the dominance of mussels had apparently survived and grew, leaving little space for colonization by other species. Sponges together with the remaining mussels became the dominant primary space occupants and remained the dominant species over the next 24 years (Harris and Dijkstra, unpubl. data). The culmination of succession by species such as *M. edulis* and slow growing sponges indicated the 1979 to 1982 community was becoming increasing homogeneous and developing towards a stable climax (Barthel & Gutt 1992, Petraitis 1995).

In contrast to the 1979 to 1982 community, heterogeneity increased as a function of time between 2003 and 2006. Fewer mussels and sponges, coupled with greater numbers of ephemeral invasive species contributed to increasing heterogeneity as irregular temporal patterns of mortality enabled greater coexistence of species (Osman 1977, Sutherland & Karlson 1977, Sousa 1979).

Diversity

Short and long-term temporal patterns of species richness and diversity have been described and multiple explanations have been used to clarify patterns observed along a successional gradient (Paine 1966, Dayton 1971, Connell 1978, Sousa 1979). Competition amongst species has been shown to adversely impact diversity by facilitating the exclusion of one species by another (Sousa 1979). This is particularly true of habitats with limited resources or those that are relatively undisturbed (Sanders 1968). In these areas species richness, abundance and evenness will increase after initial disturbance, and then subsequently decreased. Diversity in highly competitive ecosystems is often maintained by mortality of individuals, inhibiting dominance of competitively superior species (Paine 1966, Dayton 1971, Connell 1978, Sousa 1984). For instance, predation and the natural death of organisms create space by removing species that might otherwise displace competitively inferior species.

Between the two studies (1979 to 1982 and 2003 to 2006) species richness increased while abundance, evenness and diversity were equivalent (Figures 6.7a through 6.7d). An increase in species richness most likely resulted from the larger number of species in the regional species pool (Karlson et al. 2004, Witman et al. 2004, Freetone & Harrison 2006). Karlson et al. (2004) found a direct link between a five fold increase in regional species richness and local species richness while Witman et al. (2004) found 75% of the local species variation was explained by the number of species found in the regional species pool.

Though abundance and diversity patterns were indistinguishable between 1979 to 1982 and 2003 to 2006, the maintenance of these patterns has changed as a result of a shift in dominant species (from the resident blue mussel *Mytilus edulis* to the invasive colonial ascidian *Botrylloides violaceus*). The hard shell of the long-term space occupant, *M. edulis*, provides a surface that is readily colonized by species and as such diversity was maintained by secondary substrate (Jackson 1977, Osman 1977, Dean 1981, Barnes 2001, Dijkstra et al. 2007). The invasive colonial ascidian, *B. violaceus* replaced *M. edulis* as the dominant species in the 2003 to 2006 community. Unlike *M. edulis*, *B. violaceus* inhibits the settlement of species and thus does not provide secondary substrate (Bryan et al. 2003, Dijkstra et al. 2007). *M. edulis* was the dominant primary space occupant covering 40% to 80% of panels ~ 2 years while *B. violaceus* and *Didemnum* sp. are seasonally dominant, covering only 40% to 50% of panels during the summer months and 5% to 20% during winter months (Figure 2). Therefore, a higher percentage of primary space was available for colonization in 2003 and 2006 than in 1979 and 1982. Increased availability of primary space in 2003 to 2006 may have counteracted the inhibitory characteristics of colonial ascidians as higher numbers of individuals and species settle on primary vs. secondary substrate (Osman & Whitlatch 1995, Dijkstra et al. 2007). While the hard shell of mussels facilitated species coexistence between 1979 and 1982, the ephemeral life-cycle of invasive species provided more primary space that supported the similar numbers of species.

Abiotic factors

Environmental parameters that have changed between 1979 to 1982 and 2003 to 2006 include temperature, salinity, PO_4, and chlorophyll *a*. While salinity has significantly decreased, data from 1973 to 1981 were sampled monthly and low saline events resulting from flooding or high rainfall may have been missed. Though average PO_4 and DIN concentrations have risen over the last 27 years, they are still within an acceptable range that sustains a healthy system and that does not suggest a high level of eutrophication (Pritchard 1969, Jaworski 1971, Jaworski & Villa 1981).

Temperature has significantly increased since 1982, particularly during summer and fall months. Increasing temperatures have been linked to range expansions and contractions of many marine organisms (Barry et al. 1995, Sagarin et al. 1999, Parmesan & Yohe 2003), though correlations between temperature and species abundances are not always clear (Sagarin & Gaines 2002, Schiel et al. 2004). This is particularly true of long-term studies where replication over a wide spatial scale is difficult (Parmesan et al. 2005). In these cases long-term changes in species abundances may not be driven by climate related changes but by local processes such as species introductions. For instance community composition and the abundances of species in the fouling community in Portsmouth Harbor have clearly changed over the last 27 years. However, the direct correlation between changes in species composition and temperature is interrupted by the introduction of invasive colonial ascidians. Their rapid growth and ability to inhibit recruitment of other species may enable greater recruitment of some species while at the same time inhibit recruitment of others. However, temperature does affect the timing of biological parameters of marine organisms e.g. reproduction and growth (Winston 1977, Calder 1990, Minchin 1992, Stachowicz et al. 2002), that in turn impacts the seasonal development of a community (see chapter 5).

While it is difficult to predict the effect of temperature on range expansions or contractions, its affect on seasonal dominance of invasive species may be easier to elucidate. *Botrylloides violaceus* first appeared in Portsmouth Harbor in 1981, however it was in the Gulf of Maine since the 1970's (Berman et al. 1992, Dijkstra et al. 2007). 1978 to 1981 were cold years relative to 1982 to present (Clean-Air Cool Planet and Wake 2005), winter temperatures in the estuary were regularly -2 °C during the winter months (Harris, pers. obs.). In 1982 temperatures began to rise and have continued to rise with record temperatures occurring in 2006 for the northern hemisphere and New England (Clean-Air Cool Planet and Wake 2005). While winter temperatures have increased, the most striking increases have occurred in late spring through autumn (Clean-Air Cool Planet and Wake 2005, Dijkstra et al. preceding chapters). Recent studies have linked warmer winters to earlier spring recruitment of *B. violaceus* (Stachowicz et al. 2002). Furthermore, a 2 °C increase in temperature enabled a 2-fold increase in the growth rate of *B. violaceus* (Stachowicz et al. 2002). The two degree increase in temperature from 1979 to 2006 (May through October) may have contributed to the seasonal dominance of *B. violaceus* in the fouling community in Portsmouth Harbor.

Conclusion

Long-term studies correlating species composition with climate change and invasive species are useful but due to infrequent sampling are often uncertain of the underlying mechanisms of species turnover and community change. For example, Schiel et al. (2004) found significant changes in species composition following a 3.5 °C rise in temperature but did not find direct correlations between increasing temperature and the rise and fall of individual species in subtidal benthic communities. Here, we documented a substantial shift in species composition from a long-term primary space occupant, *Mytilus edulis* to seasonally dominant species *Botrylloides violaceus* and *Didemnum* sp. Furthermore, succession has changed over the last 27 years; 1979 to 1982 succession was seasonally directional leading towards a stable climax community while 2003 to 2006 was less seasonal and did not lead towards a stable climax. Though overall diversity patterns have not changed since 1979, the maintenance of these patterns has dramatically altered. The observed changes in species composition, succession and maintenance of diversity have resulted from the introduction of invasive species with ephemeral life-cycles. More frequent sampling may identify the timing of species introductions and increasing temperatures that together will elucidate the mechanisms behind the response of communities to climate change and invasive species.

Table 6.1: Temporal change in species abundances between 1979 to 1982 and 2003-2006. *** Significant difference at the 0.01 level in species abundances between months in different years (e.g. August 1979 to August 2003, September 1979 to September 2003) while ** indicates significant differences at the 0.05 level.

Porifera	**Significance level**	**Level of change in abundance**
Haliclona loosanofi	***	**decrease**
Halichondria panicea	NS	
Leucosolenia sp.	***	**decrease**
Halisarca sp.	***	**decrease**
Yellow encrusting sponge	**	**increase**
Cnidaria		
Ectopleura larynx	NS	
Obelia spp.	**	**increase**
Metridium senelis	***	**increase**
Ectopleura indivisa	**	**decrease**
Hydractinia	NS	
Mollusca		
Anomia simplex	NS	
Mytilus edulis	***	**decrease**
Hiatella arctica	NS	
Crepidula spp.	NS	
Bryozoa		
Membranipora membranacea		**Not present in the 1979-1982 community**
Bugula simplex	***	**increase**
Bugula turrita	**	**increase**
Schizoperella unicornis	***	**increase**
Electra pilosa	NS	
Cryptosula pallasina	NS	
Bowerbankia	NS	
Hypothoa hyalina	NS	
Encrusting bryozoans	NS	
Tunicata		
Botrylloides violaceus		**Not present in the 1979-1982 community**
Diplosoma listerianum		**Not present in the 1979-1982 community**
Botryllus schlosseri	***	**decrease**
Didemnum sp.		**Not present in the 1979-1982 community**
Ciona intestinalis	***	**decrease**
Molgula spp.	**	**decrease**
Aplidium constellatum	NS	
Didemnum albidum	NS	
Molgula spp./ *Obelia* spp.	***	**increase**
Crustacea		
Balanus spp.	NS	
Amphipod tubes	**	**increase**

Table 6.2: SIMPER (similarity percentage) results showing which species made the greatest contribution to the dissimilarity between 1979 to 1982 and 2003 to 2006 communities.

	Average abundance		**Dissimilarity/SD**	**Percent contribution**
	1979-1982	**2003-2006**		
Botrylloides violaceus	0.00	2.04	4.47	8.62
Mytilus edulis	2.69	1.99	1.63	7.9
Ciona intestinallis	0.99	0.47	1.43	3.23
Didemnum sp.	0.00	0.84	1.37	3.53
Schizoperella unicornis	0.27	0.7	1.36	2.64
Haliclona loosanofi	1.13	0.77	1.31	2.89
Halichondria panicea	0.86	0.73	1.31	2.78

Table 6.3: SIMPER (similarity percentage) results showing which species made the greatest contribution to the similarity within communities.

1982 to 1979	**Average abundance**	**Average similarity**	**Similarity/SD**	**Percent contribution**
Mytilus edulis	2.69	7.67	0.97	13.5
Anomia simplex	1.95	6.57	3.1	11.57
Molgula sp.	1.96	6.29	1.74	11.08
Mogula sp./*Obelia* sp.	1.88	4.84	0.92	8.52
Botryllus schlosseri	1.33	4.78	2.28	8.42
Balanus spp.	1.94	4.08	0.82	7.18
Haliclona loosanofi	1.13	3.53	1.27	6.22
Leucosolenia sp.	1.02	3.38	1.68	5.94
Ciona intestinalis	0.99	2.98	1.48	5.25
Halichondria panicea	0.86	2.08	0.95	3.66
2003 to 2006				
Botrylloides violaceus	2.04	7.76	4.37	13.75
Mogula sp./*Obelia* sp.	2.96	6.84	0.89	12.12
Mytilus edulis	1.99	6.1	1.73	10.8
Anomia simplex	1.69	4.25	1.29	7.54
Molgula sp.	1.56	3.58	0.98	6.34
Botryllus schlosseri	1.05	3.36	1.65	5.95
Balanus spp.	1.5	3.09	0.92	5.48
Haliclona loosanofi	0.77	2.29	1.19	4.05
Didemnum sp.	0.84	2.12	0.94	3.76
Schizoperella unicornis	0.7	1.81	1.04	3.21
Bugula simplex	0.84	1.79	0.72	3.17
Leucosolenia sp.	0.72	1.76	0.97	3.12
Halichondria panicea	0.73	1.7	0.94	3.02

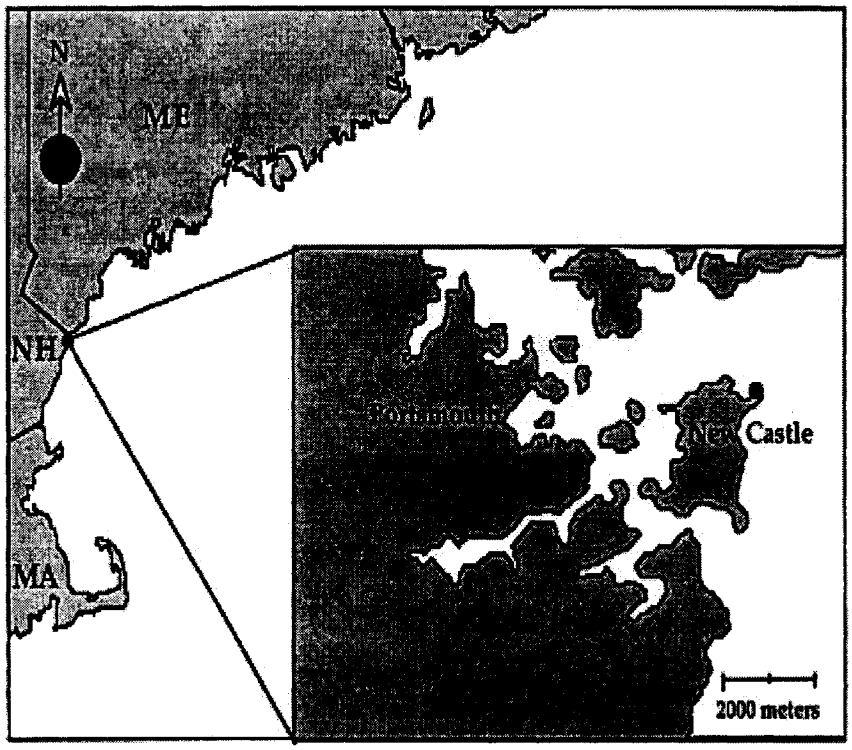


Figure 6.1: Gulf of Maine Map with a subset of Portsmouth Harbor. Study site is black round dot.

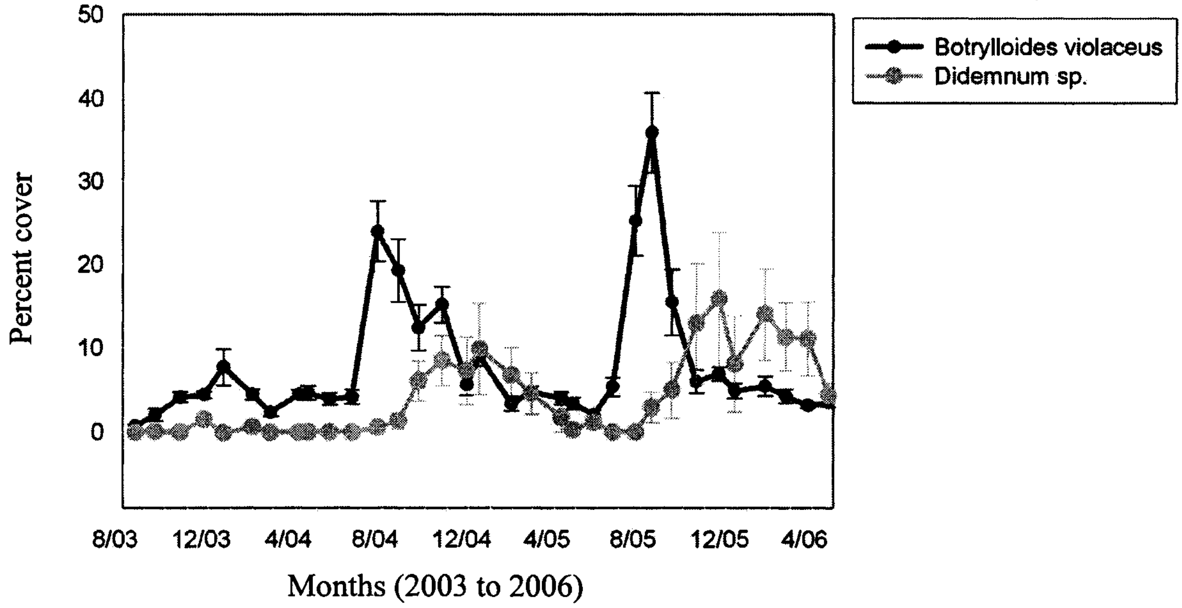


Figure 6.2: Annual percent cover patterns of invasive colonial ascidians, *Botrylloides violaceus* and *Didemnum* sp. Peak abundance of the two species is offset. Spatial cover is greatest between June through September for *B. violaceus* while highest percent cover occurred in November through February for *Didemnum* sp.

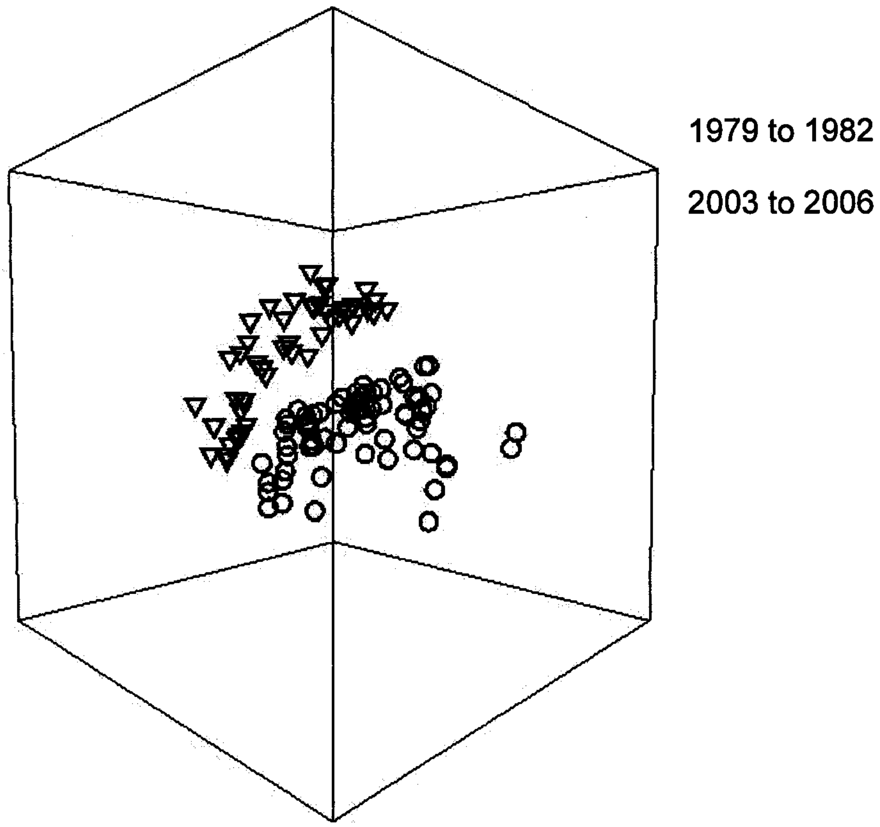


Figure 6.3: Three dimensional non-metric multidimensional scaling plot representing species assemblages between 1979 to 1982 (△) and 2003 to 2006 (○ / stress 0.13). Significant differences in species composition were detected between the two communities (ANOSIM: R = 0.326, $p <$ 0.01).

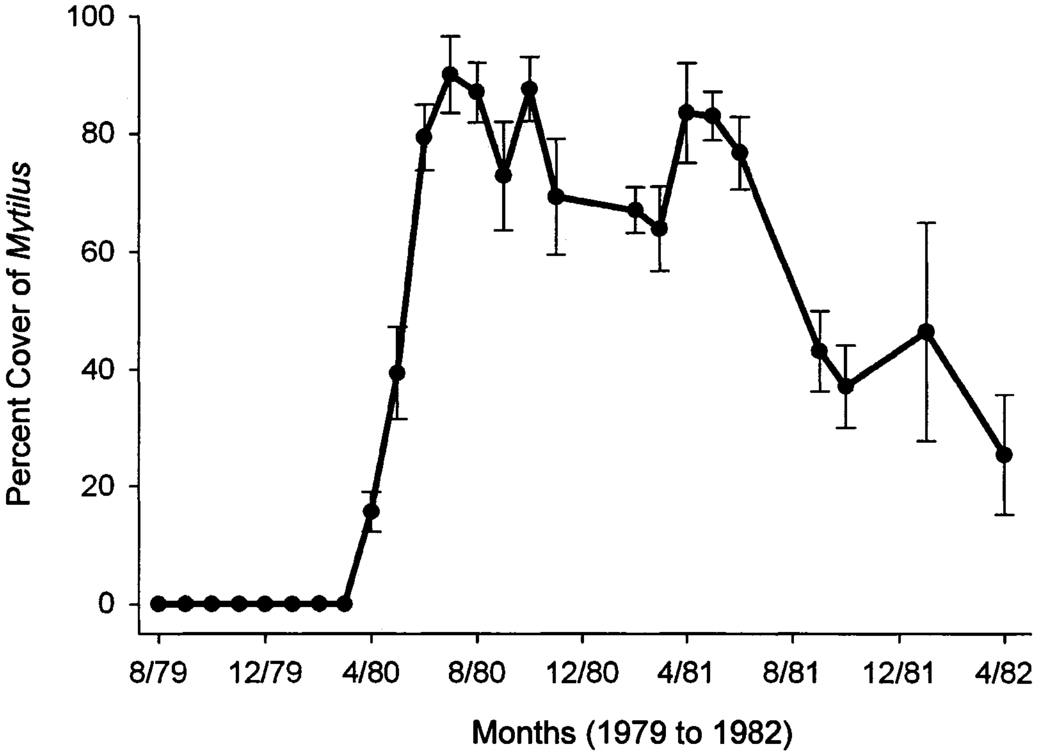


Figure 6.4: Abundance of the blue mussel, *Mytilus edulis* between 1979 and 1982. *M. edulis* dominated the panels for ~ 21 months beginning in April, 1980.

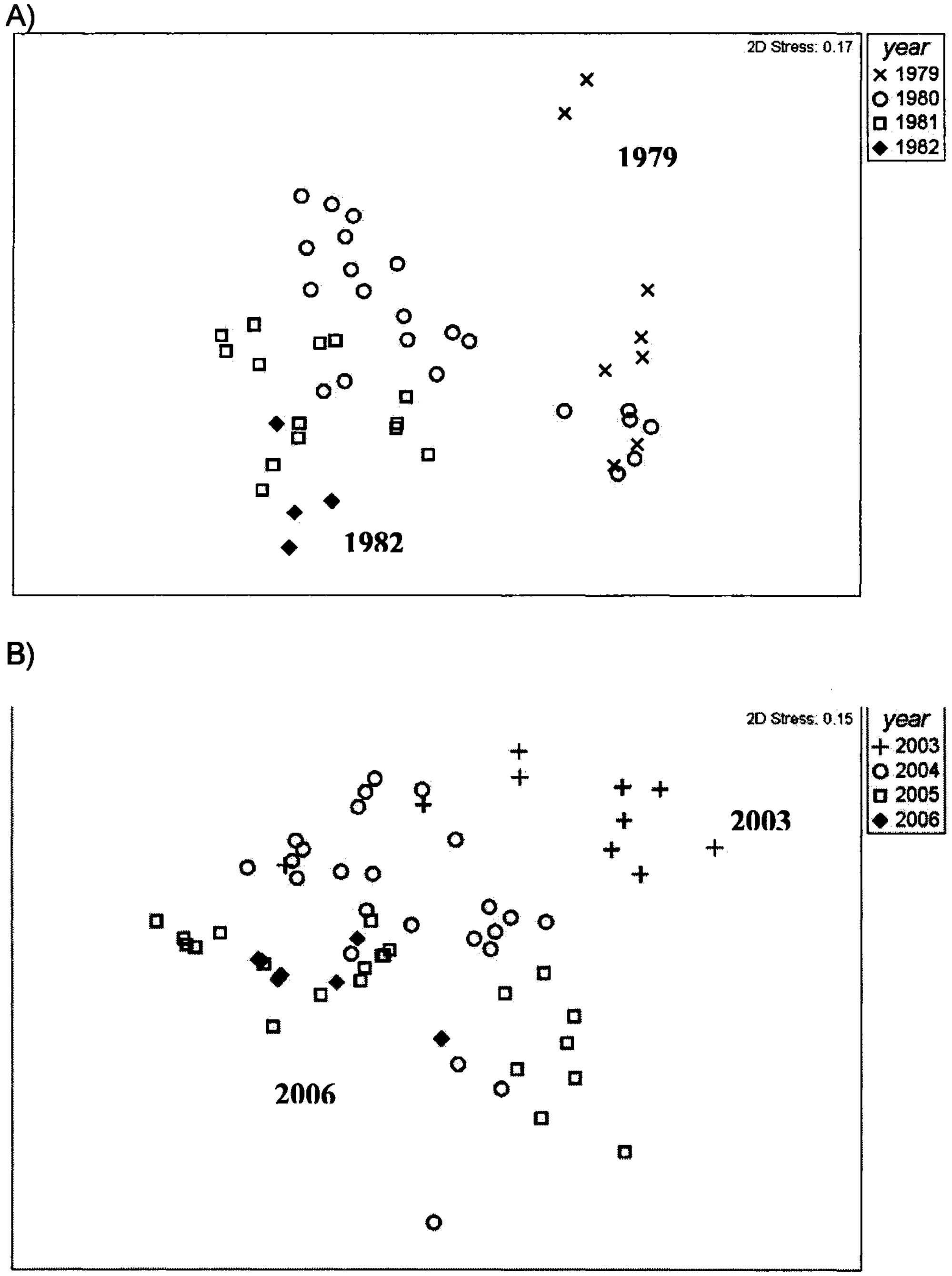


Figure 6.5: Succession observed in both communities (1979 to 1982 and 2003 to 2006). A) 1979 to 1982, clear seasonal patterns were observed during succession, though patterns were less apparent with each successive year. Succession was seasonal leading towards a stable climax dominated by mussels and bivalves. B) Succession between 2003 and 2006. Seasonal patterns are clear during initial development becoming less clear in subsequent years.

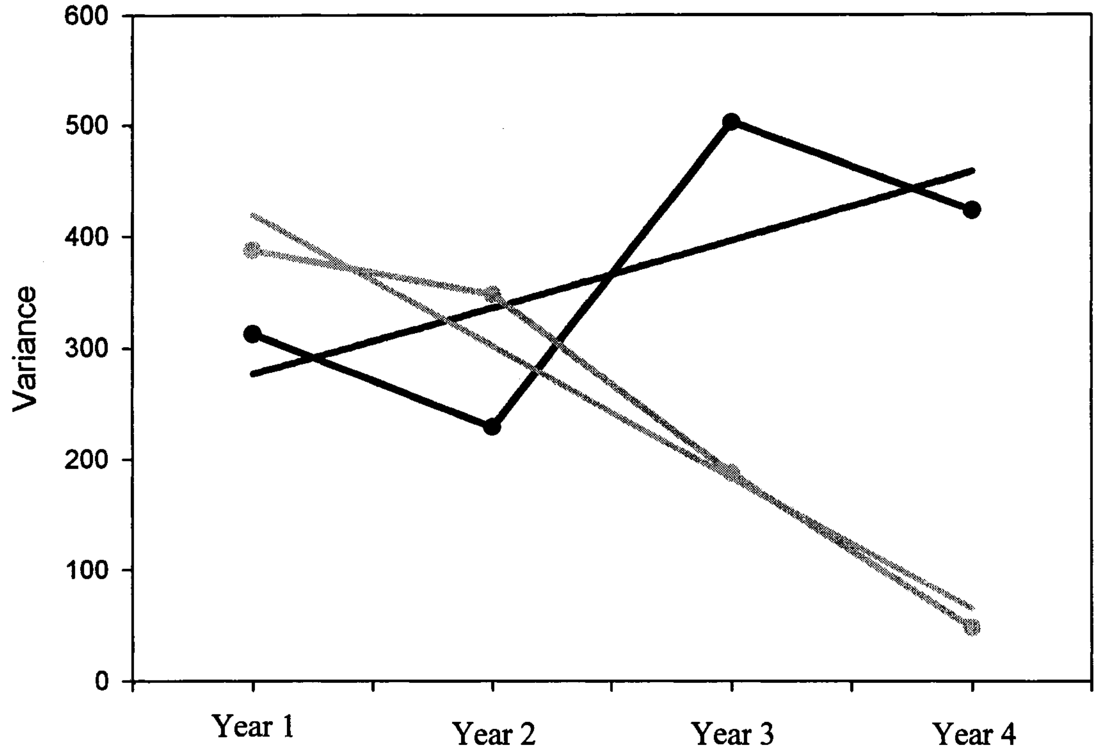
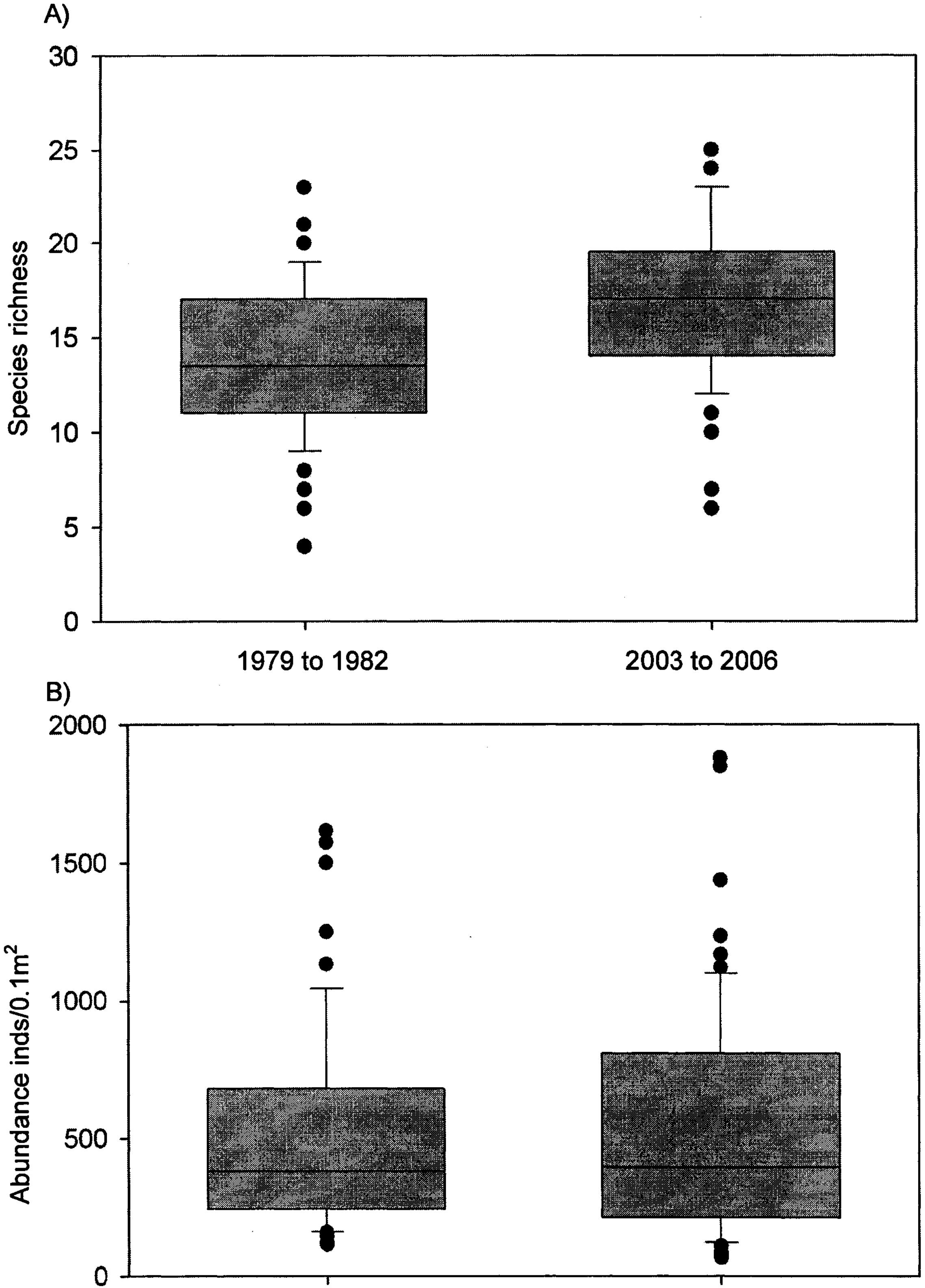
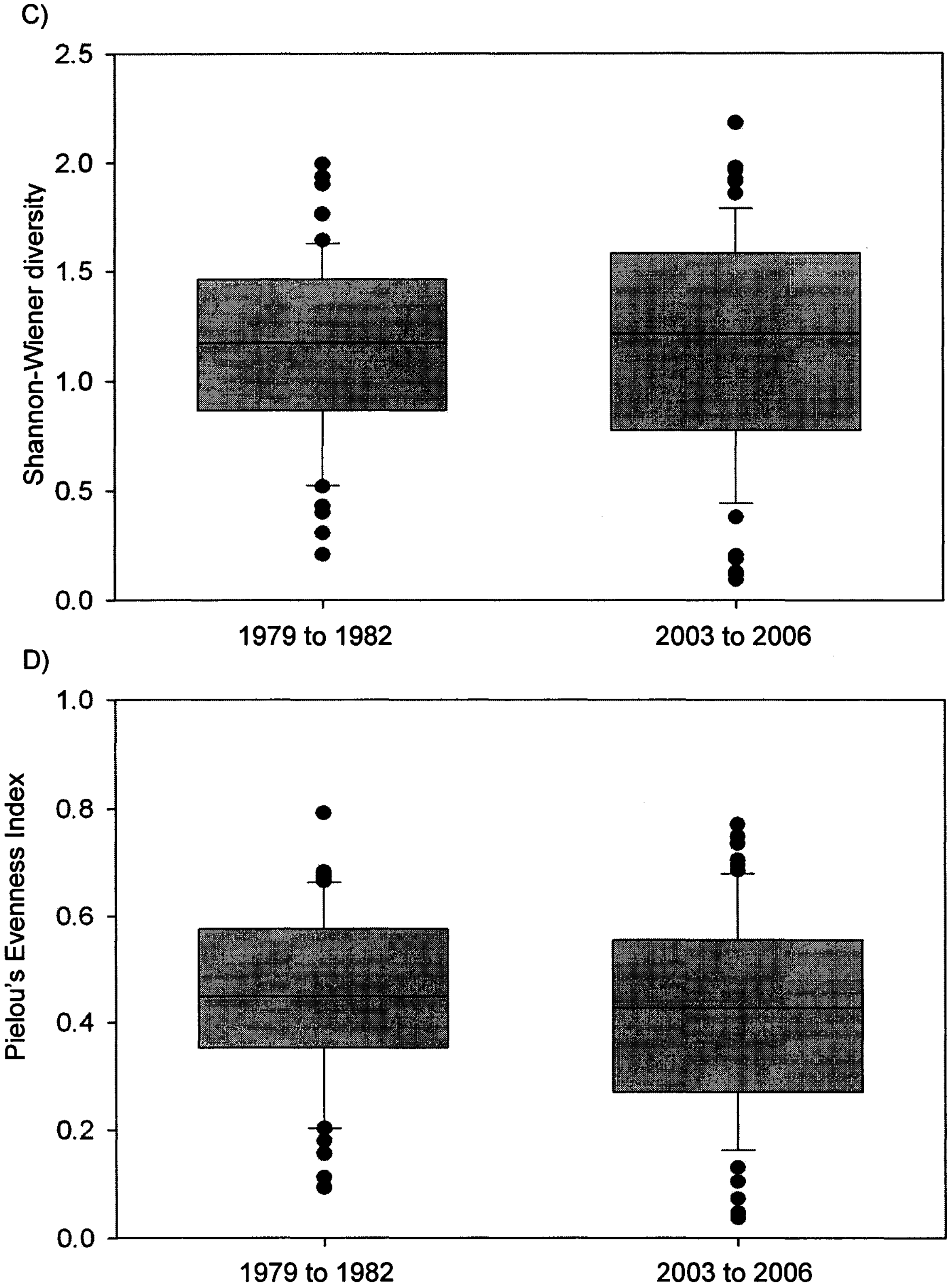


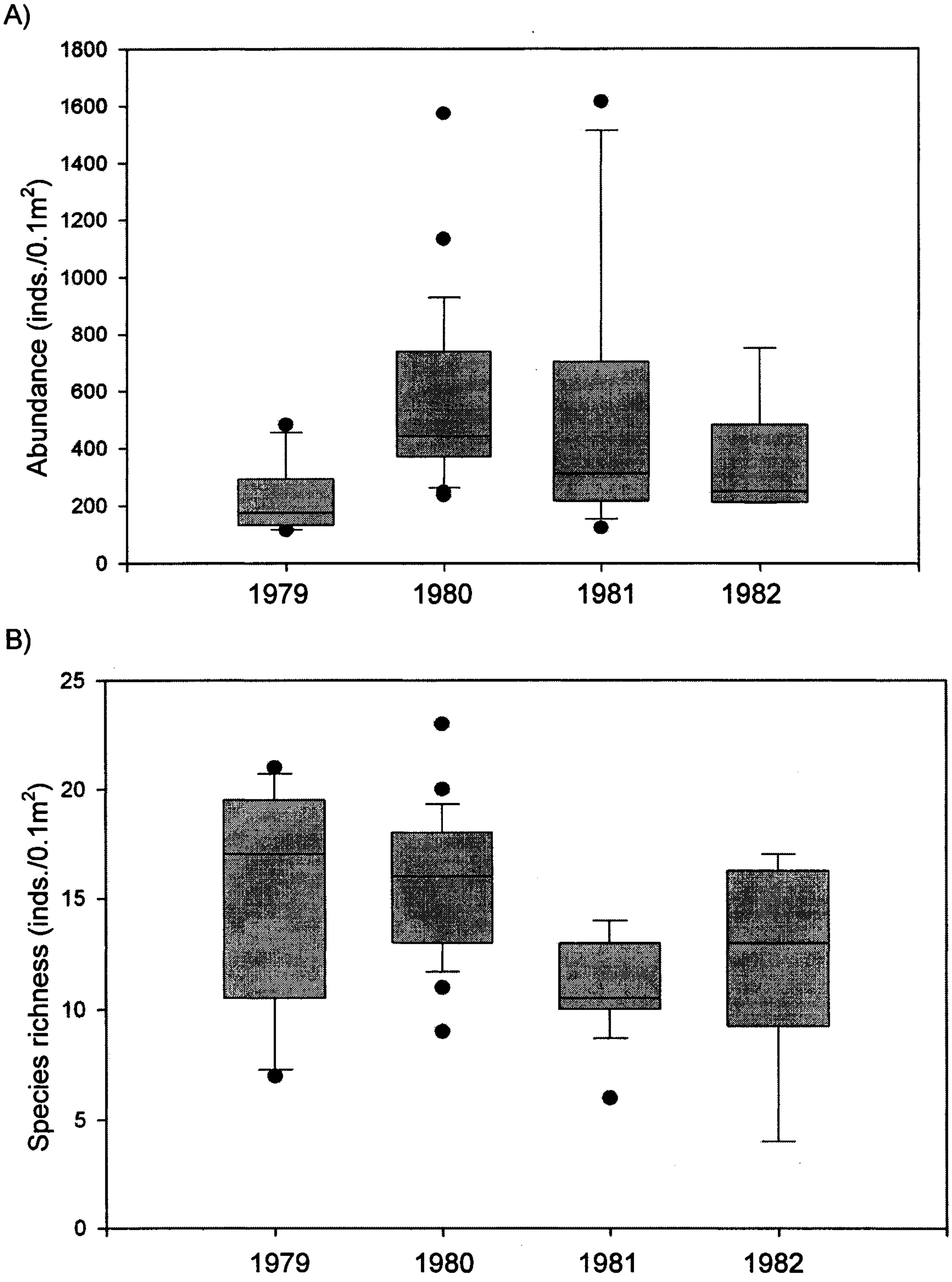
Figure 6.6: Annual variance in similarity as a function of time with year 1 as first year of development (e.g. 1979 and 2003) and year 4 as final year of development (e.g. 1982 and 2006). Between 1979 and 1982 similarity among members of the community was lowest (high variance) in year 1, increasing (declining variance) with time since panel deployment (adj. R^2 = 0.983, $p < 0.024$). In contrast, variance between 2003 and 2006 increased since initial development, though not significantly (adj. R^2 = 0.136, $p <$ 0.339).



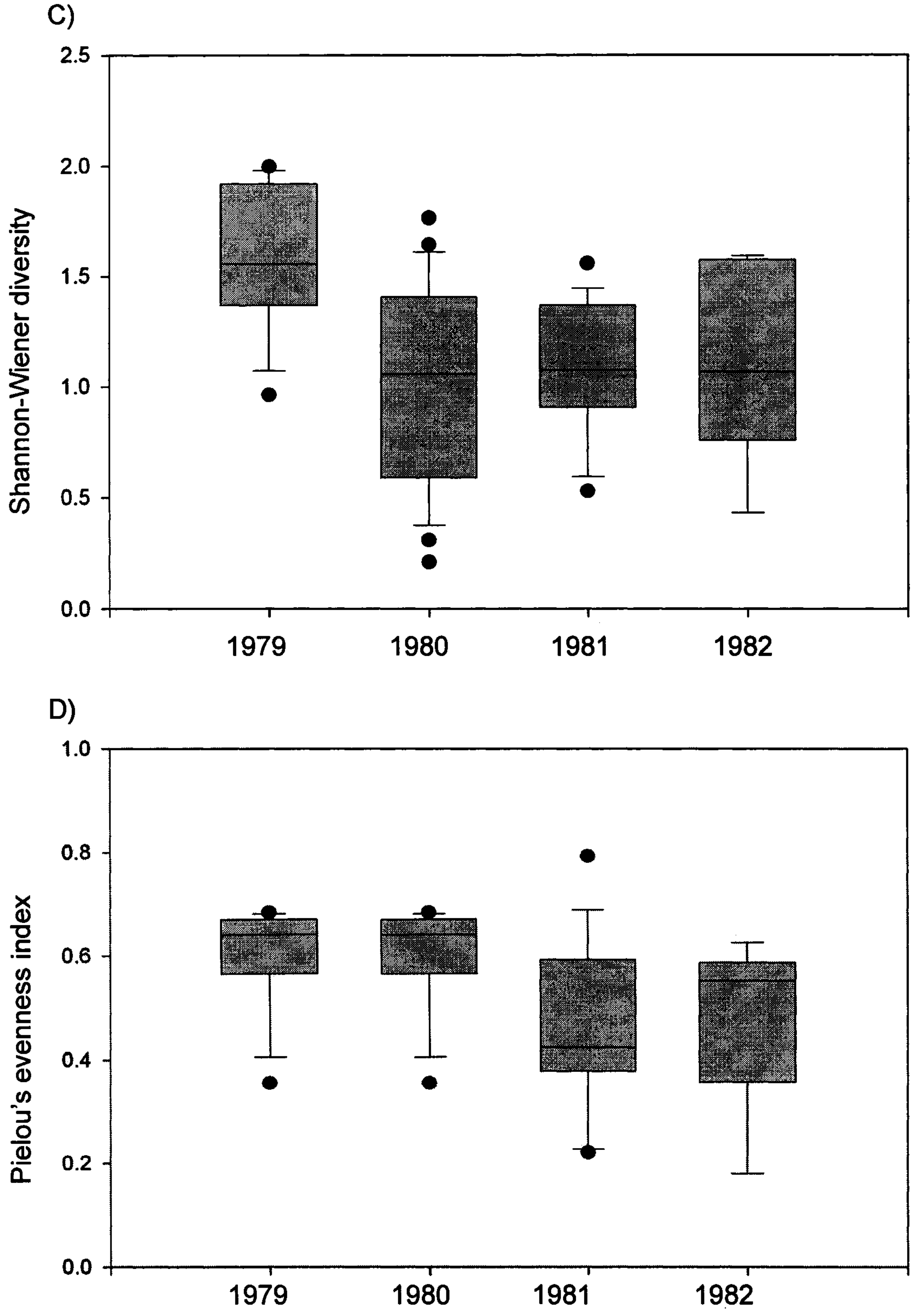
Figures 6.7 A and B: Average species richness (A) and abundance (B) for the two study periods (1979 to 1982 and 2003 to 2006).



Figures 6.7 C and D: Average diversity (C) and evenness (B) for the two study periods (1979 to 1982 and 2003 to 2006).



Figures 6.8 A and B: Box plots of successional patterns of abundance (A) and species richness (B) between 1979 and 1982. Abundance initially increased and then decreased while species richness slightly decreased with each successive year.



Figures 6.8 C and D: Successional patterns of diversity (A) and evenness (B) between 1979 and 1982. Diversity decreased after year 1, remaining stable for successive years. No apparent pattern was observed for evenness.

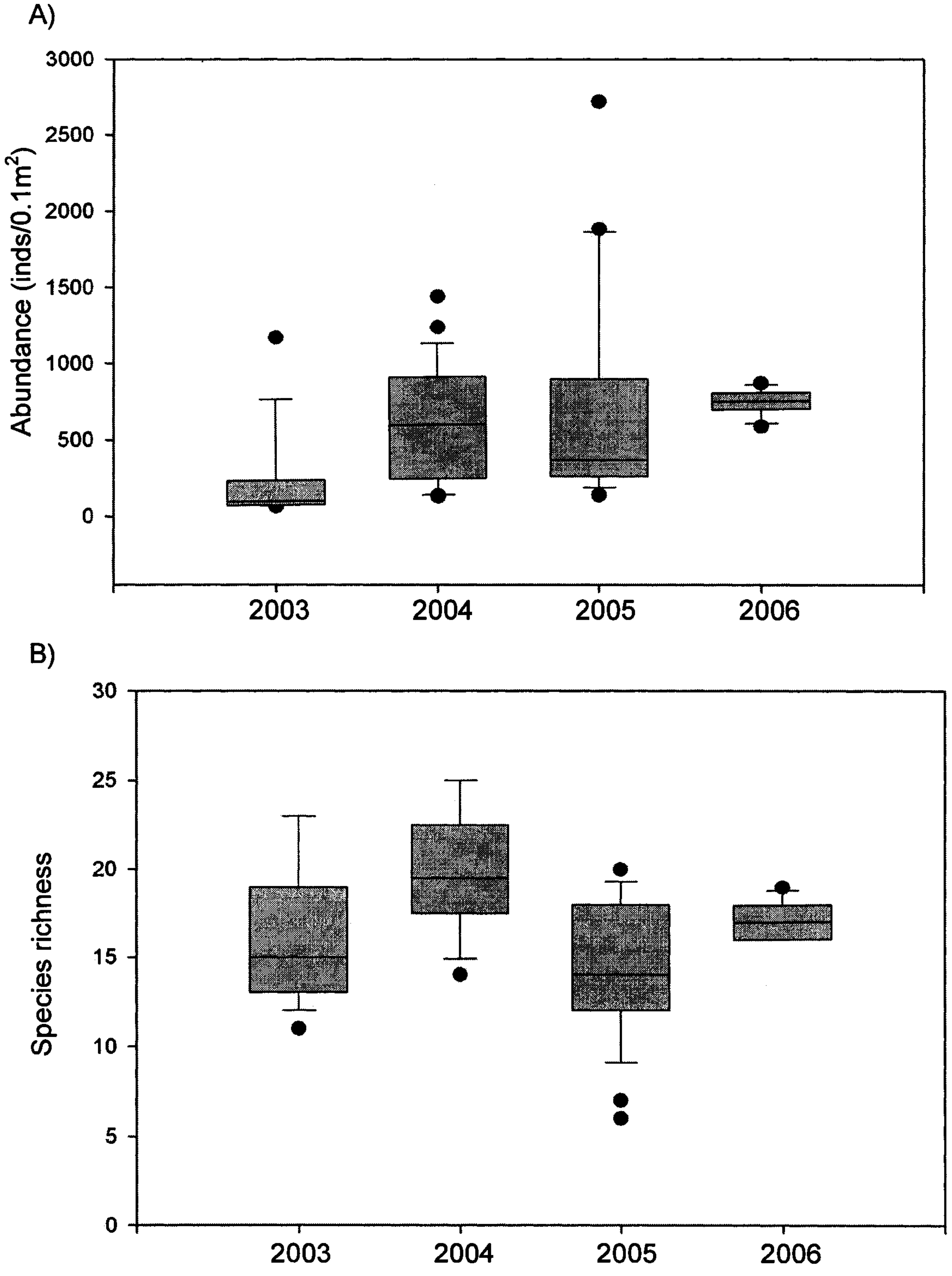
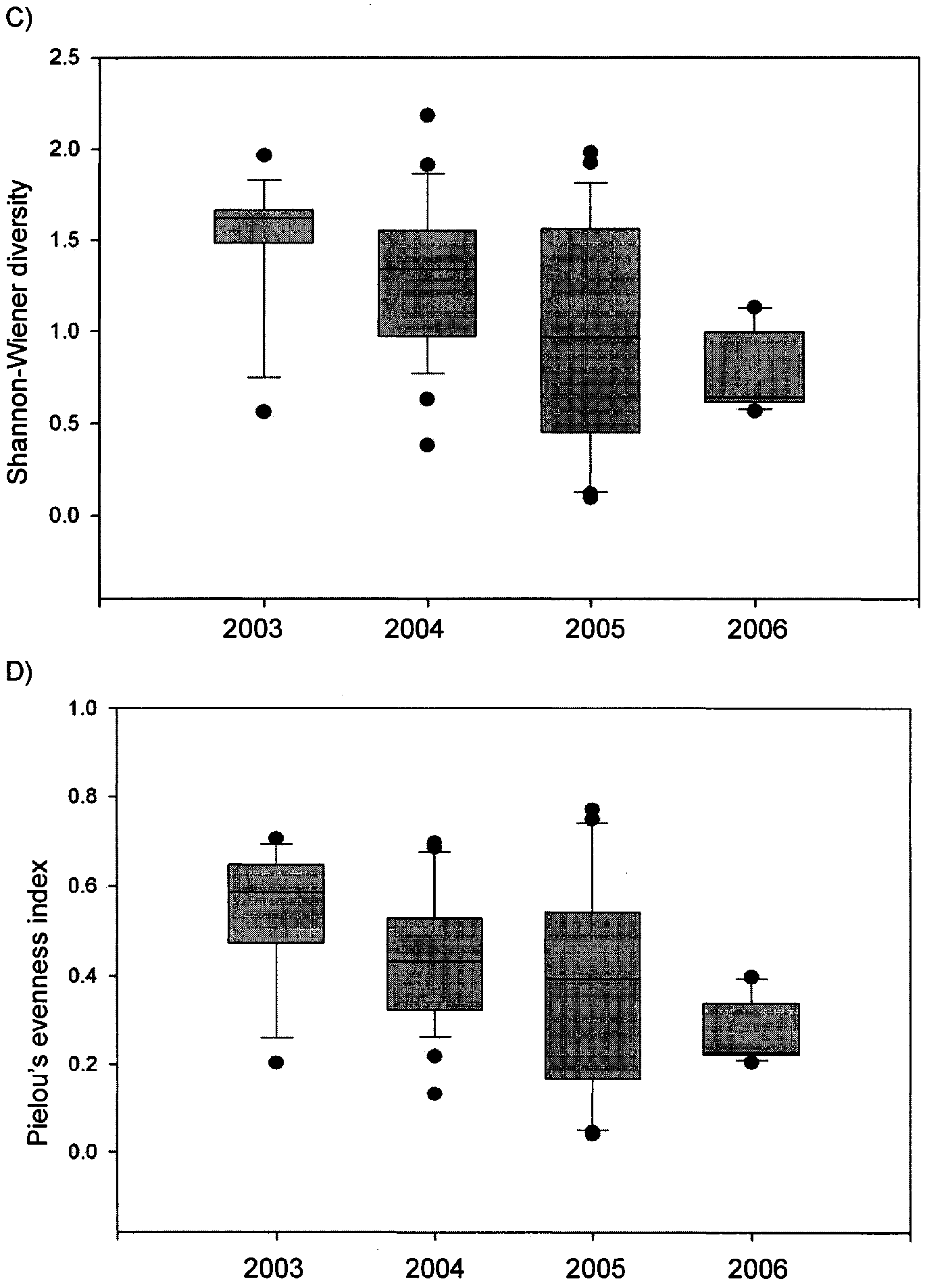
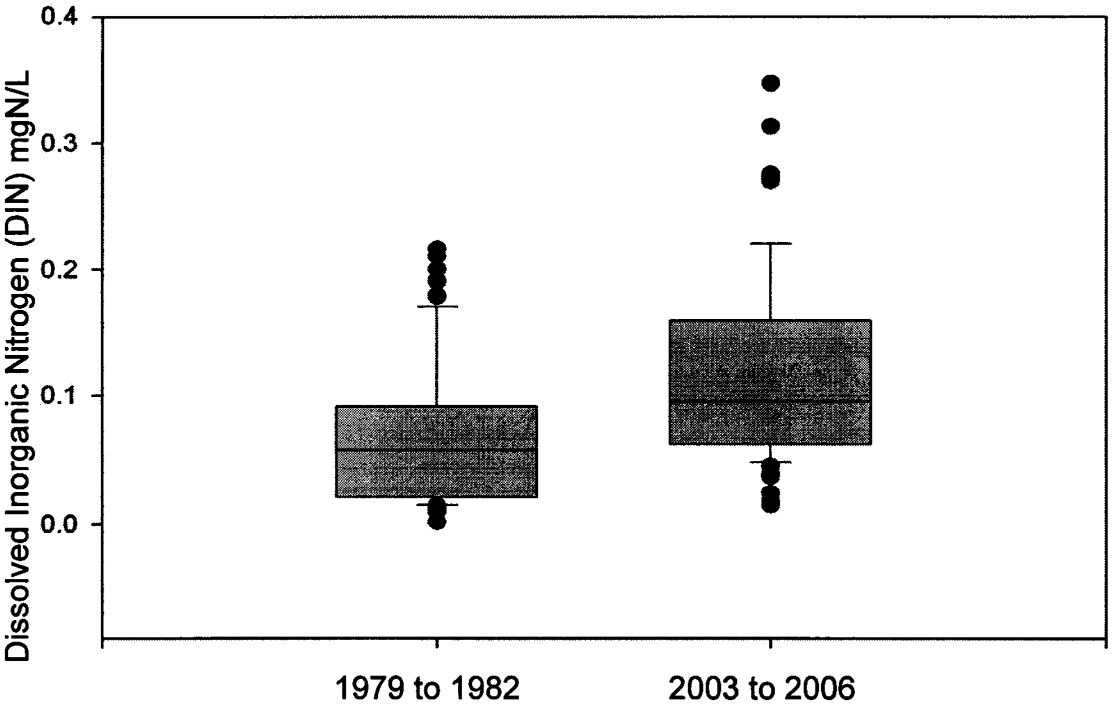


Figure 6.9 A and B: Successional patterns of abundance (A) and species richness (B) as a function of time since initial development between 2003 and 2006. Abundance slightly increased throughout succession no pattern was observed for species richness.



Figures 6.9 A and B: Successional patterns of diversity (C) and evenness (D) as a function of time since initial development between 2003 and 2006. Both diversity and evenness decreased as a function of time since initial development.

Figure 6.10A)



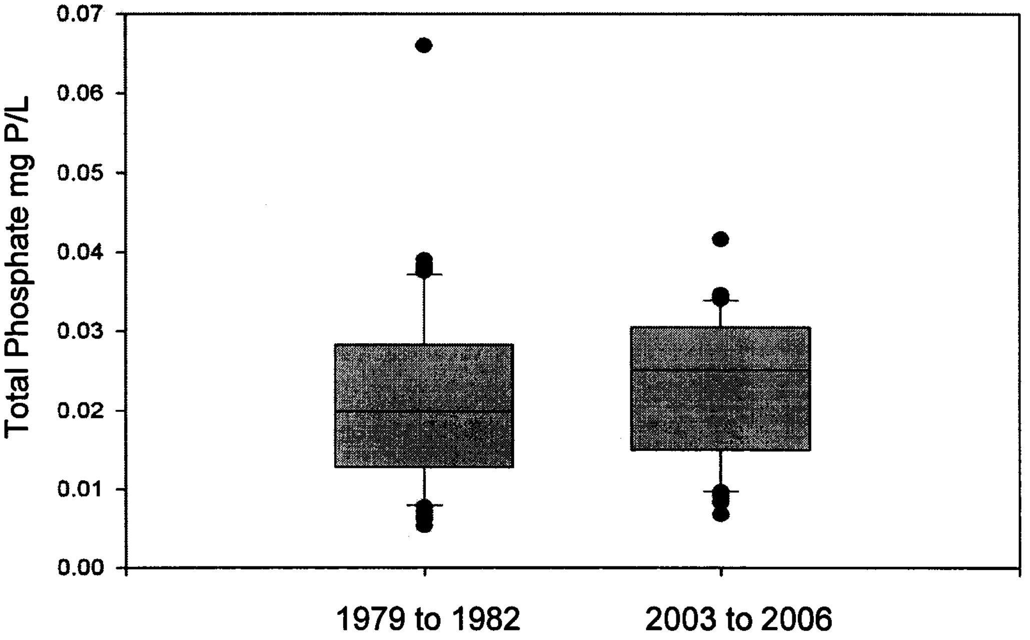


Figure 6.10A and B: Comparison of dissolved inorganic nitrogen and phosphate between the studies revealed that DIN had significantly increased while phosphate had not.

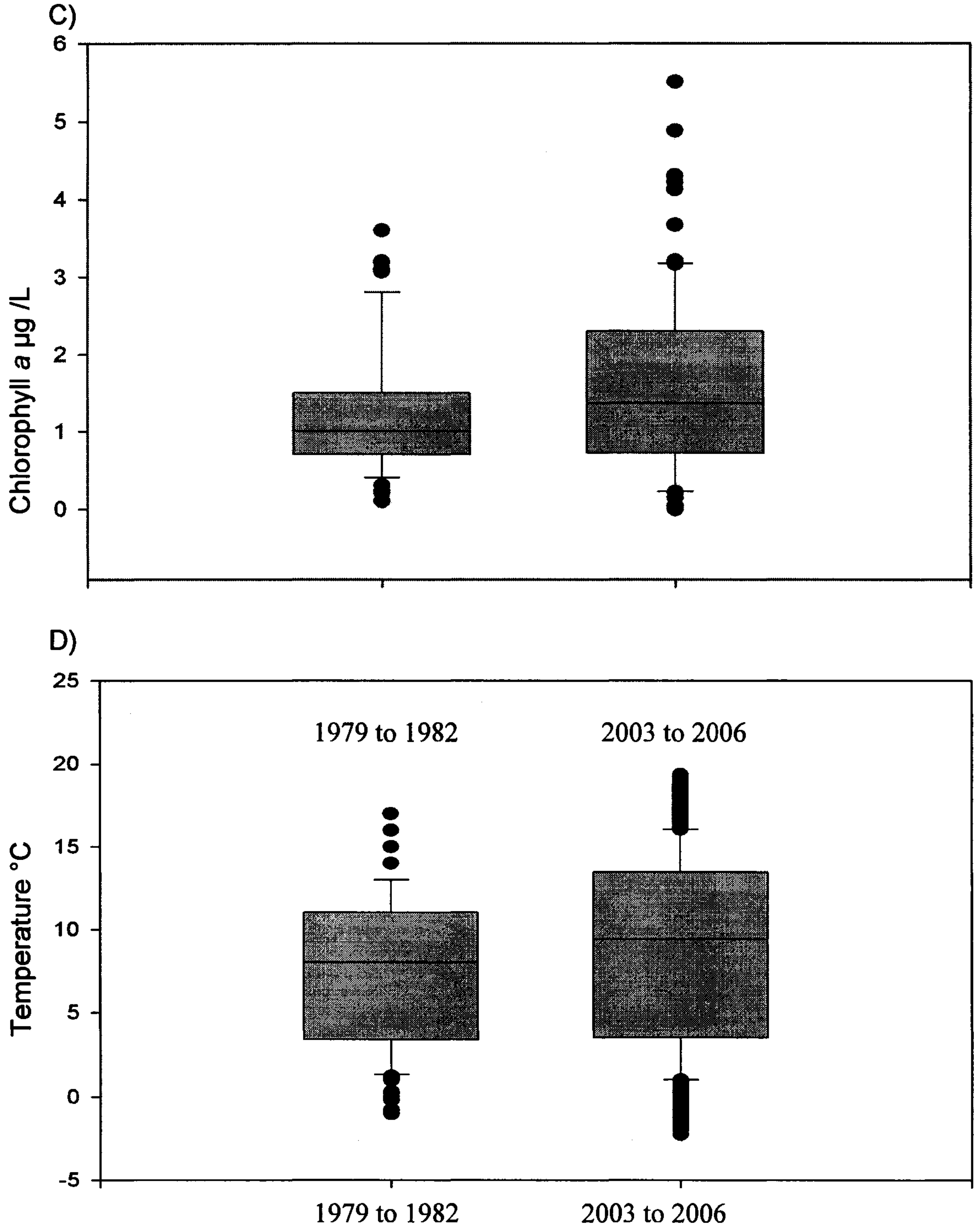


Figure 6.10 C and D: Measurements of chlorophyll a and temperature taken during the two study periods. Temperature significantly increased ($p < 0.001$) between 1979 to 1982 and 2003 to 2006 while chlorophyll *a* marginally increased ($p < 0.047$).

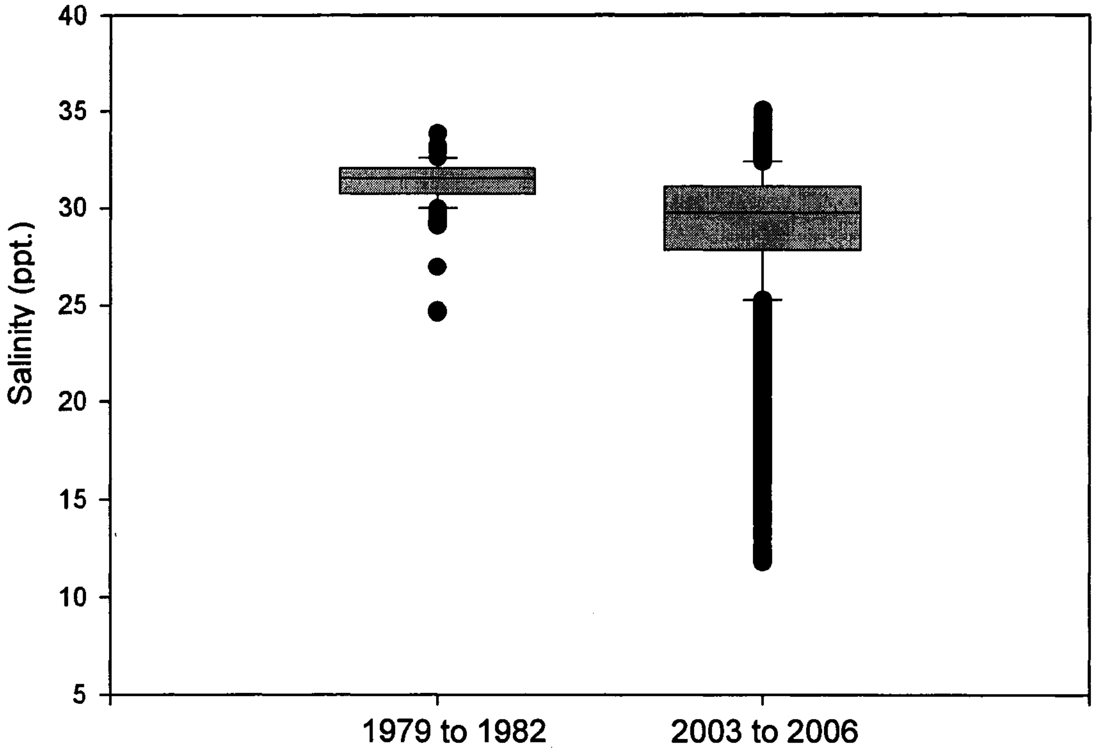


Figure 6.10E: Between the two study periods salinity significantly ($p < 0.001$) decreased (for further description see text).

GENERAL CONCLUSIONS

The main objectives of this research were to compare species composition and succession in a fouling community between 1979 and 1982 to 2003 and 2006 and identify key factors that contribute to the observed changes. Since the original panel study in 1982, several invasive species have been observed on panels underneath the cement pier. These include three colonial ascidians (*Botrylloides violaceus*, *Diplosoma listerianum* and *Didemnum* sp.) and an encrusting bryozoan (*Membranipora membranacea*). Additionally, temperatures have risen by 2 °C from late spring through autumn, facilitating the dominance of warm water invasive species.

The purpose of chapter 1 was to examine potential invasion pathways to the Gulf of Maine of the four most common colonial ascidians (*Botryllus schlosseri*, *Botrylloides violaceus*, *Diplosoma listerianum*, and *Didemnum* sp.) and to examine their seasonal distribution patterns. Results of surveys and observations suggested *B. schlosseri* and *D. listerianum* may have been introduced by hull fouling. *B. schlosseri* is the oldest of the colonial ascidians, having been present since the late 1800's (Gould 1870); a time before the advent of aquaculture. *Diplosoma listerianum*, on the other hand, was first discovered at the Isles of Shoals, NH in 1993 (Harris & Tyrrell 2001).

The Isles of Shoals is a relatively high traffic area for recreational boaters in the summer months. Consequently, the likely method of introduction is hull fouling on recreational vessels. In contrast, *B. violaceus* and *Didemnum* sp. were most likely introduced through aquaculture in the Damariscotta river estuary, ME. Large numbers of colonies of both species were observed on aquaculture nets in 1982, 11 years after initiation of oyster aquaculture (R. Clime, pers.comm.). This suggests both species were well established by this time.

Since 1982 the abundance of *Botryllus schlosseri* has declined, most likely the result of competition with *Botrylloides violaceus* and *Didemnum* sp. Temporal patterns of ascidian abundance have also shifted from late fall and winter to summer and fall, a time of high recruitment for other species. Because colonial ascidians possess toxic chemicals that inhibit the recruitment of species (Teo & Ryland 1994, Bryan et al. 2003, Joullie et al. 2003), a shift in dominance from a period of low (fall and winter) to high (summer and fall) recruitment may reduce overall biodiversity.

Chapter 2 and 3 build upon the long-term shift in seasonal abundance patterns of colonial ascidians. The purpose of chapter 2 was to examine the effect of colonial ascidians on recruitment with plates using 70% to 100% cover. This chapter illustrated that dominance of colonial ascidians will eventually lead to low diversity. Chapter 3 was designed to test whether individual functional groups influenced the identity of species recruiting to secondary substrate and bare space adjacent to competitively superior species. Results indicate that only certain species will settle on the tunics of colonial ascidians and sponges vs.

other groups and there was little difference in the density of recruits or species to bare space on panels with competitively superior species e.g. colonial ascidians or sponges.

Climate change in the North Atlantic not only involves rising temperatures but reduced salinities resulting from increased precipitation, flooding events and melting ice caps (Milly et al. 2002, Curry et al. 2003). While warmer temperatures enhance the dominance of some species, lowered salinity values may limit their abundance and distribution, particularly in estuaries. The purpose of chapter 4 was to examine the response of an older (*Botryllus schlosseri*) and a newer (*Botrylloides violaceus*) invader to low salinity values. Laboratory results indicated that *B. schlosseri* has a higher tolerance than *B. violaceus* for lower salinities. Results for *B. violaceus* showed colonies can survive at much lower salinities than previously recorded. Depressed salinities in near-shore coastal zones may therefore, moderate the effects of warmer sea surface temperatures. Because *B. violaceus* structures the community, changes in its abundance is then reflected in species composition. Questions involving the impacts of climate change on community composition should address multiple factors and not only increasing temperatures.

Temperate environments undergo annual changes in environmental parameters that influence the timing and magnitude of recruitment, growth, and mortality of species observed within a community (Osman 1977, Sutherland & Karlson 1977, Harris & Irons 1982). Species composition is therefore a reflection of the seasonal cycles of dominant members of the community. Chapter 5 was designed to compare the seasonal development between 1979 to 1982 and 2003 to 2006. Results indicate that four stages of community development were present between 1979 and 1980 that corresponded to the seasons (spring, summer, autumn and winter). Between 2003 and 2004 three stages are present, a condition similar to communities found in warmer, southern waters (Sutherland & Karlson 1973). The smaller number of stages is linked to a 2 °C rise in temperature from May through October that facilitated the longevity and dominance of warmer water species.

Chapter 6 was designed to compare community composition and succession between 1979 and 1982 to 2003 to 2006. Overall results indicate a change in species composition from a community structured by the blue mussel (*Mytilus edulis*) to one characterized by the invasive colonial ascidian (*Botrylloides violaceus*). Succession between 1979 and 1982 was seasonal leading towards increased homogeneity, a stable climax and diversity was maintained through the utilization of as a secondary substrate provided by the dominant bivalve *M. edulis*. Succession between 2003 and 2006 was unpredictable, leading to increased heterogeneity. Diversity is not maintained as secondary substrate but as primary substrate due to the ephemeral nature of the invasive species. The combined effect of rising temperature and invading species has created a novel community driven by invasive colonial ascidians.

Frequent sampling over longer time scales is necessary to understand the underlying causes of species composition and/or abundance patterns. Annual or quarterly observations can detect changes in species composition but they often fail to incorporate seasonal changes or detect the mechanisms behind the observed change (Schiel et al. 2004, Parmesan et al. 2005). For example, quarterly observations of a subtidal community in central California found increased abundances in both cold and warm water species, though temperatures had risen by 3.5 °C. Parmesan et al. (2005) also noted that long-term local studies are inadequate for correlating abundances with temperature as local factors other than temperature my be driving community change. The studies presented here showed a correlation between increasing temperature and changes in species composition. However during this period an ephemeral invasive species, *Botrylloides violaceus*, replaced the native mussel (*Mytilus edulis*) as the structuring member of the 2003 to 2006 community. *M. edulis* dominated the historical panels for ~2 years, leaving little open primary space. In contrast, *B. violaceus* dominated 40% to 50% of the panels during the summer months, after which time it sloughed off the panels leaving open space for re-colonization by other species. The timing and greater availability of open space enabled the recruitment of multiple species as a higher number of species settle on bare space vs. secondary space (Osman & Whitlatch 1995). While temperatures have risen over the last 27 years, changes in species composition most likely resulted from a biotic disturbance that facilitated increased availability of open primary space.

One overall impression resulting from studies presented here are short-term studies examining the impacts of invasive species on diversity, species composition or succession may not reflect long-term community dynamics. This is particularly true of temperate regions where environmental parameters govern the seasonal life-cycles of dominant species. For example, short-term studies examining the effect of functional groups, particularly bivalves and colonial ascidians (structural members of the 1979 to 1982 and 2003 to 2006 communities) on recruitment revealed colonial ascidians inhibited recruitment and lowered diversity (Chapters 2 and 3). However, comparing overall diversity patterns between 1979 to 1982 and 2003 to 2006 revealed no significant differences in abundance, evenness and diversity. In fact species richness significantly increased. While the results of chapters 2 and 3 are correct and suggest that increasing dominance of colonial ascidians will eventually lead to lower diversity patterns, the life-history of dominant species may be more important in explaining community-wide diversity patterns. Clearly caution is required using short-term experiments to explain long-term patterns in community structure and composition.

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