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An Urban Estuary in a Changing World: Diversity, Invasions, and Climate Change in San Francisco Bay

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An Urban Estuary in a Changing World:
Diversity, Invasions, and Climate Change in San Francisco Bay

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DISSERTATION

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To my family

for the inspiration to begin,
the love to continue,
and the courage to finish

Man marks the earth with ruin—his control stops with the shore;
—Byron, 1818

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ACKNOWLEDGEMENTS

This dissertation is composed of two manuscripts being prepared for publication in the scientific literature, a third (Chapter 3) that has been published in the journal Biological Invasions, and two Appendices. I am the primary researcher and have co-authored the manuscript for Chapter 1 with Christopher Brown, Jeffrey Crooks, and Gregory Ruiz. I am the primary researcher and sole author of Chapter 2. Chapter 3 (including Appendix I) was the product of a four year collaboration with a number of colleagues. I am the lead author and was responsible for final production of the manuscript for publication. I collaborated equally with three others (Judah Grossman, Teresa Sabol Spezio, and Heidi Weiskel) in designing the study, collecting and analyzing the data, and producing initial drafts of the manuscript. Julia Blum and Edwin Grosholz contributed significantly to the data analysis and the manuscript. Jennifer Burt, Adrianna Muir, Jonah Piovia-Scott, and Kari Veblen played an invaluable role in the initial conception of the study and in improving the final manuscript. I am primary researcher and sole author of Appendix II.

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It started with a science project in college. Jim Carlton gave me a dozen wooden panels. I tied them to a dish rack and hung them off the Mystic River Bridge to see what kind of estuarine life would grow on them. I didn't know what tunicates or bryozoans were, or hydroids or caprellids or nudibranchs. All I knew was that there was going to be some interesting stuff down there that I'd never seen before. Now, eleven years and over three thousand fouling panels later, I wonder if Jim knew what the future held when he gave me those first, few panels.

Some of the earliest and most enduring influences on my approach to science came from my undergraduate advisors: James Carlton, who introduced me to marine research, and to biogeography, ecology, taxonomy, and natural history, and Rónadh Cox, from whom I derived an interest in geology, oceanography, and climate change science. From them I learned to see the connections between the biological and physical worlds, and to embrace many different ways of looking at nature. Jim's continued support and advice in particular has been invaluable these many years.

Before entering graduate school, I spent three years working for an outpost of the Smithsonian Environmental Research Center based in the San Francisco Bay area. My experience there went a long way toward getting me to where I am now, as well as providing the starting material for this dissertation, including a portion of the data in Chapter 1. From Jeff Crooks I learned to think deeply about ecology, that all good scientists must spend at least some time taking apart televisions at the lab, that it's often worth going out of my way to do things the "easy" way (twice the thought equals half the labor), and especially how to work with and manage a wide variety of people. From Whitman Miller, I have absorbed the habits of endless tinkering and random, cheerful pranksterism along with careful, creative approaches to science. Cat deRivera's boundless energy and willingness to jump headfirst into new ventures inspired me to step beyond my scientific comfort zones. Greg Ruiz taught me to think big—bigger than I ever thought possible—and to reach far and wide for collaborators and funding while keeping a weather eye on how all the pieces fit together.

Ted Grosholz tried his best to undo a few of Greg's teachings (such as "take a lot of samples… you don't have to analyze all of them") so I could finish this dissertation in a finite amount of time. As my advisor, Ted's encouragement, support, and belief in me and in my ideas allowed me the freedom to make my own mistakes and learn from them, while ensuring that I didn't become too discouraged. I am also grateful to him for showing me how to fit my science into my life, rather than vice versa.

I thank my committee—Ted, Greg, Jay Stachowicz, and Eric Sanford, for their forbearance and guidance throughout this process, for allowing me to pursue several rather large projects simultaneously, and for thoughtful criticism and suggestions that helped me turn mountains of data into more coherent narratives.

Through the UC Davis IGERT program in Biological Invasions, I gained some lasting friendships and had some intense learning experiences with Heidi Weiskel, Judah Grossman, Teresa Sabol Spezio, Adrianna Muir, Jen Burt, Jonah Piovia-Scott, and Kari Veblen. As my co-authors for Chapter 3, along with Ted Grosholz and Julia Blum, they were very understanding every time I said, "I think I found another problem." I also thank Carole Hom for her constant enthusiasm and support.

Chris Brown, David Kimbro, and Gail Ashton have proven to be cheerful, dependable, and patient research partners these past few years; they somehow managed to put up with my ability to be just a few minutes late every time, and with the delays in our various projects incurred by my attempts to finish this dissertation.

Graduate school has been a much more enjoyable and stimulating experience than it would have been otherwise thanks to the kind attention, encouragement, and camaraderie of many current and former fellow Grosholz labmates, including Sarikka Attoe, Rachael Blake, Elizabeth Brusati, Christy Bowles, Brian Cheng, Nicole Christiansen, India Clarke, Caitlin Coleman-Hulbert, Anna Deck, Erika Gallo, Randall Hughes, David Kimbro, Becca Kordas, Ursula Mahl, Christy Tyler, Heidi Weiskel, Betsy Wells, and Eric Yoshizuka. I especially thank Betsy Wells for her friendship (after twelve years spread across three labs on two coasts), and hope that she will never again have to call my parents to say "Andy's okay now, but…" as she had to do twice, many years ago in Oregon, after I started a solo hike at the top of a cliff and arrived in very short order at the bottom.

The staff, faculty, and fellow students at Bodega Marine Lab have made my stay a pleasant one, even if I occasionally soured their airspace with buckets of organisms that had "gone off." In particular, Molly Engelbrecht's cheerful persistence has been invaluable in obtaining numerous otherwise impossible-to-find references. The physical plant staff graciously repaired the things that I broke, including two doors. Kitty Brown, Skyli McAfee, Jennifer Sauter, and Lisa Valentine have assisted me with many logistical and practical affairs. I thank Susan Williams for her early advice and continued encouragement.

At the Romberg Tiburon Center (RTC, San Francisco State University), Wim Kimmerer, Brita Larsson, Toby Garfield, and the Kimmerer Lab staff (especially Toni Ignoffo and Anne Slaughter) allowed me to work with many buckets of seawater and stinky fouling panels in the Kimmerer Lab. Current and former members of the Smithsonian lab at RTC, including Gail Ashton, Chris Brown, Basma Mohammad, and Chela Zabin gave me a home away from home (or more appropriately, a "lab away from lab") for my field and lab work in San Francisco Bay. Safra Altman and I spent many a late night at RTC as we looked through our respective fouling panels and mused about the ways of the world, foul and fair.

In the Graduate Group in Ecology and the Department of Environmental Science and Policy, Silvia Hillyer, Pat Conners, Shirley Holm, Stephani Shone, and Susan Trigilio helped me through both logistical and bureaucratic quagmires.

Many marinas and piers in the San Francisco Bay area have mysterious ropes hanging from inconspicuous places retaining fouling panels coated with estuarine life; some have been there as many as nine years now. Numerous harbormasters and boat owners put up with these panels and my constant presence, particularly Jim Walter (harbormaster at Richmond Marina Bay Yacht Harbor). Jim Nybakken (the harbormaster, not the marine biologist) at South Beach Harbor was similarly generous.

Almost every grad student relies on others to help get the field and lab work done; I was fortunate to work with two people who also greatly improved my experience in doing the work. Kristine Pierce had a wonderful ability to remain unruffled and to get the job done, no matter how many panels or tunicates I piled up in front of her. Tony Hoang was unfortunate enough to work with me for two summers. His unique sense of humor and sarcasm helped keep me grounded in real life as we picked tunicates and bryozoans off hundreds of panels, over and over ("leave them… they'll still be there tomorrow").

This dissertation was made possible by funding from the University of California, the National Science Foundation, Point Reyes National Seashore, and an internship with the NOAA Fisheries Southwest Restoration Center (for which I thank Natalie Cosentino-

Manning). Theresa Talley generously extended an invitation to teach with her as my funds began to dwindle near the end of graduate school.

A *sine qua non* of community ecology is the ability to identify the species in the community. Early on, Jim Carlton instilled in me an abiding appreciation for the value and power of accurate taxonomy. I have since benefited greatly from the expertise of a number of taxonomists, some of whom also graciously identified samples for this work: Rolando Bastida-Zavala, Chris Brown, Lea-Anne Henry, Natasha Hitchcock, Linda McCann, Gretchen Lambert, and Judy Winston.

I owe my greatest thanks to my family, especially my parents, Paul and Vivia Chang, and my brother Bryan and his wife Phung. Their love, constant encouragement, and interest in my work (no matter how incomprehensible I made it sound) has enabled me to stay the course, and to strive for greater things. My fiancée, Julia Blum, has been a tremendous source of comfort, peace, and happiness, without which I would be much poorer. She has consistently pushed me to think more critically and incisively about my work, and her insight and advice have improved this dissertation immeasurably.

Finally, I thank the many organisms that made the ultimate sacrifice for this dissertation. For them, one day, the tide went out… and it never came back.

ABSTRACT

Estuarine and coastal ecosystems face both large scale physical environmental stresses from climate change and local scale community changes, including non-native species introductions. In northern California, USA, climate change projections indicate increasingly severe droughts in the future punctuated by more frequent, intense winter storms, significantly affecting freshwater outflow to estuaries like San Francisco Bay. Using long-term physical and biological data sets coupled with experiments, I found drastic changes in epifaunal communities in the Bay between years with extreme high and low freshwater outflow (Chapter 1). In dry years (low outflow), competition determines community composition, but in wet years (high outflow), low salinity stress shifts the community to being recruitment limited, with community membership determined by the availability of larvae to recruit.

Shifts in outflow extremes drive changes in the dominant species, resulting in altered community performance (Chapter 2). I experimentally tested the effect of functional group diversity on community performance in different outflow regimes. Communities containing more functional groups generally occupied more space, but the strength of this effect varied from year to year. In the absence of normally dominant species during a wet year, resident community functional group diversity had no effect on subsequent invasion by non-resident species. These results suggest that outcomes of short-term, unreplicated studies of biodiversity-ecosystem function relationships may thus be contingent on prevailing conditions.

Finally, I assessed the risk posed by the aquarium fish trade for introducing non-native fish species to the San Francisco Bay–Delta using an inventory of fish species for sale, a model comparing fish physiological tolerances to environmental parameters, and a telephone survey of store representatives' knowledge and attitudes toward invasions (Chapter 3). This analysis showed that the local aquarium trade includes at least five and as many as twenty-seven species that could potentially survive in the Bay–Delta. Management efforts for this vector should focus on improving labeling and identification of fish species in stores, expanding available information on fish physiological tolerances, educating customers and store employees about the risks of pet release, and providing better options for responsible disposal of unwanted fish.

CHAPTER 1. Storms and Droughts: community consequences of altered estuarine hydrological cycles*

ABSTRACT

Climatic extremes are predicted to increase in frequency and severity due to global climate change. Extreme climatic events may often be more biologically relevant than mean climatic conditions, yet their biotic impacts are difficult to assess because they are rare and require long-term biological and physical data sets. Recent, mounting evidence indicates significant human-driven alterations to the hydrological cycle of the western United States over the last half of the twentieth century, including increasing variability and extreme fluctuations in flow. Previous work has documented these hydrological changes, and management policy has focused on ensuring water supply for human and agricultural uses and freshwater ecosystems. Little is known about the ecological impacts of human alterations to the hydrological cycle in downstream, estuarine systems, which are critical habitats that provide a range of ecosystem functions.

We combined long-term physical and biological data sets with experimental approaches to assess the effect of extreme high and low freshwater outflow levels on estuarine and marine epifaunal communities in San Francisco Bay, California, USA. We assessed the relative extremeness of outflow and salinity levels during our study by comparing them to the historical record, finding that approximately 20% of all years since 1600 were wetter and 20% were dryer than the extremes we observed during 2001–2008. Using time series of sessile invertebrate recruitment, we examined recruitment and

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* Co-authored with Christopher W. Brown, Jeffrey A. Crooks, and Gregory M. Ruiz

survival patterns in different outflow conditions. During high freshwater outflow (wet) years, low salinity levels caused mass mortality of resident adults and altered the composition of recruitment in epifaunal communities, significantly changing community composition throughout the estuary. In contrast, during dry years, epifaunal communities were dominated by non-native solitary tunicate species. Using removal experiments, we showed that these solitary tunicates depressed overall community diversity and greatly altered community composition. Our experiments mimicking the low salinity levels produced by a high freshwater outflow event demonstrated that these dominant solitary tunicate species were susceptible to low salinity levels, and the resulting communities were dominated by recruitment. The changes between the observed climatic wet and dry extremes thus appeared to shift these epifaunal communities from being space-limited in dry years to being recruitment-limited in wet years.

INTRODUCTION

A widely predicted consequence of global climate change is increasing frequency and intensity of climatic extremes (Easterling *et al.* 2000, Gutowski *et al.* 2008). These extremes include fluctuations in freshwater flow resulting from an augmentation of natural climate variability by significant human-driven alterations to the hydrological cycle in areas such as the western United States over the last half of the twentieth century (Knowles and Cayan 2002, Barnett *et al.* 2008). Previous work has documented these hydrological changes, and management policy has focused on ensuring water supply for human and agricultural uses and freshwater ecosystems (Kimmerer 2002, Knowles and Cayan 2002, Barnett *et al.* 2008). Relatively few studies have directly addressed the short and long-term ecological consequences of climatic precipitation extremes in general, and existing studies tend to focus either on drought or flood effects, but not both (*e.g.,* Marques *et al.* 2007, Thibault and Brown 2008). Especially little is known about the ecological impacts of climate regime changes and human alterations to the hydrological cycle in downstream, estuarine ecosystems.

Estuaries are at least partially enclosed bodies of water that serve as a transition zone between freshwater runoff and the ocean and supply critical habitat and nursing grounds for many terrestrial, aquatic, and avian species (Beck *et al.* 2001). They provide a range of ecosystem functions, from filtration and removal of nutrients from surface waters to buffering of shorelines from nearshore storms (Costanza *et al.* 1997, Levin *et al.* 2001). Despite decades of increasing awareness and efforts to reduce human impacts, estuaries remain among the most stressed ecosystems on Earth while population densities and demands on coastal zones continue to rise (Kennish 2002, Lotze *et al.* 2006).

The salinity gradient between the river and the ocean is one of the fundamental determinants of where different species can live in the estuary (Remane and Schlieper 1971). While some degree of seasonal change in this gradient is a near-universal feature of estuaries, the highly seasonal nature of precipitation in Mediterranean climates results in unusually large annual salinity fluctuations in Mediterranean estuaries (Emmett *et al.* 2000). Mediterranean climates, such as in the San Francisco Bay, California region are typified by two distinct precipitation seasons, with nearly all precipitation occurring during a wet season, and almost none during a dry season that lasts several months (Conomos *et al.* 1985).

Although global climate change is not expected to change the total amount of precipitation in California, USA, intense precipitation events are expected to increase both in frequency and magnitude (Knowles and Cayan 2002). As average winter temperatures have increased in the last sixty years, more precipitation has fallen as rain rather than as snow, a trend that is projected to continue with further warming (Kim *et al.* 2002, Kim 2005, Knowles *et al.* 2006). At the same time, drought frequency is also expected to increase (Cook *et al.* 2004, Hayhoe *et al.* 2004). The net effect is to have more intense storms punctuating longer intervals of dry weather (Gutowski *et al.* 2008). In estuaries, these climatic changes are amplified by large-scale watershed modification, notably in the San Francisco Bay–Delta system (Nichols *et al.* 1986). Extensive draining and filling of marshes, riprapping of banks and channelizing of rivers has essentially converted many estuaries from "sieves" that gradually filter and process precipitation into "funnels" that swiftly send precipitation downstream, enhancing the speed and severity of downstream water quality changes following storms (Nichols *et al.* 1986). While dams and water diversions for agriculture and drinking water use reduce the total amount of outflow, especially in the spring and summer, high outflow during winter storms can greatly exceed the influence of these water management activities (Knowles 2002).

Extreme climatic events may often be more biologically relevant than mean climatic conditions (Easterling *et al.* 2000, Stenseth *et al.* 2002), yet are difficult to measure because they are rare, and therefore, require long time series for effective comparison (*e.g.*, Thibault and Brown 2008, Cloern *et al.* 2008). Both physical and biological data sets are required to test for patterns of association between physical regime alterations and changes in biological conditions (Stenseth *et al.* 2002). We present evidence of large-scale mortality and changes to epifaunal communities throughout the San Francisco Estuary following a wet winter with high precipitation and freshwater outflow, as well as significant structural changes to these communities following dry winters with little precipitation and very low freshwater outflow. Using long- and short-term biological and physical data sets and both observational and experimental methods, we tested three hypotheses regarding estuarine community change in the face of altered hydrological regimes: (1) community diversity will be highest in moderate outflow conditions, and lower in extreme high or low outflow conditions; (2) in a more stable environment (*i.e.* lower seasonal and / or interannual variation in freshwater outflow), competitive exclusion by dominant species will result in lower community diversity; (3) in more variable environments (*i.e.* greater seasonal or interannual variation in freshwater outflow), community composition will be determined by recruitment rather than competition.

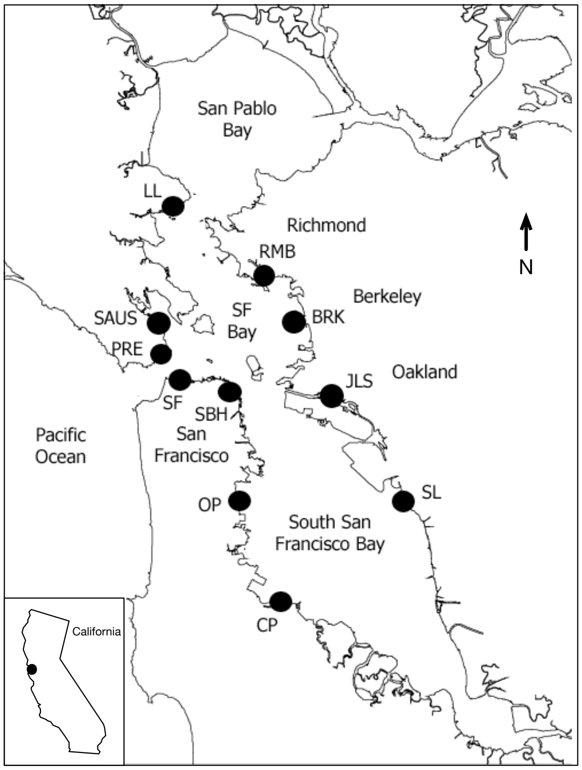
We first assessed the relative extremeness of precipitation, freshwater outflow, and salinity levels during the period of our study (2001–2008) by comparing them to historical patterns in the San Francisco Estuary. Second, we used time series of sessile invertebrate recruitment and community change along with temperature, salinity, and freshwater outflow conditions to assess patterns of recruitment and survival in different climatic conditions. We then experimentally tested the effects of salinity conditions associated with different climatic extremes on community development. Finally, as these epifaunal communities are often dominated by solitary tunicates that appear to be sensitive to salinity fluctuations, we used a series of manipulative experiments to assess community development in the presence and absence of solitary tunicates. We then discuss implications of our findings for predicting future responses of epifaunal communities to increased variance in freshwater outflow.

METHODS

Study System

Our study was conducted in the San Francisco Estuary, one of the largest estuaries on the Pacific Coast of North America (Figure 1.1). The San Francisco Estuary is a drowned river valley with a watershed of 163,000 km^2 (Nichols *et al.* 1986). Since the advent of major European settlement following the California Gold Rush in 1849, both the topography and hydrology of the estuary have been highly modified (Nichols *et al.* 1986). Major topographical changes include draining and filling of wetlands for development, damming and canalization of upstream waterways, and major flow diversions to provide fresh water for agricultural and drinking water use (Nichols *et al.* 1986). The local climate regime is Mediterranean in nature, with wet winters and dry summers. Like many large estuaries, the San Francisco Estuary has a persistent salinity gradient that is spread over a relatively large spatial area (tens of kilometers) and which fluctuates in response to changes in freshwater flow, typically as a result of precipitation and snowmelt (Conomos *et al.* 1985).

Figure 1.1: Study sites in the San Francisco Estuary



Site codes correspond to abbreviations listed in Table 1.1.

Table 1.1: Survey sites in the San Francisco Estuary, from north to south

Abbreviation	Site	Distance (km)	Survey Type	Survey Years
LL	Loch Lomond Marina	23	Quarterly	2006–2008
BRK	Berkeley Marina	15	Quarterly	2008
RMB	Richmond Marina	14	Monthly	2002, 2004–2008
			Quarterly	2001–2008
SAUS	Sausalito Marine Harbor	6	Quarterly	2006–2008
SF	San Francisco Marina	1	Quarterly	2006–2008
PRE	Presidio Yacht Harbor	0	Quarterly	2006–2008
SBH	South Beach Harbor	9	Monthly	2004–2006
JLS	Jack London Square Marina	18	Quarterly	2008
OP	Oyster Point Marina	25	Quarterly	2008
SL	San Leandro Marina	31	Quarterly	2006–2008
CP	Coyote Point Marina	33	Quarterly	2007–2008

Monthly panels were deployed serially for a duration of one month. Quarterly panels were deployed in the summer for a 3-month period from around June/July to September/October. Distance refers to distance along major channels from the Golden Gate (the mouth of the estuary).

Although numerous sites throughout the estuary were examined over the course of this study (2001–2008), much of the focal work was conducted at the Richmond Marina in the northeastern portion of the estuary (Figure 1.1; 37°54′41″N 122°21′05″W). This site was chosen because of its location in the mesohaline region of the estuary (average salinity 20–30 psu), allowing us to easily examine the influence of salinity changes on epifaunal communities. Richmond also is one of the deepest shoreline-accessible sites in the estuary, permitting easy investigation of the influences of salinity stratification with depth on epifaunal communities.

Sessile epifaunal invertebrates comprise a significant portion of the fauna in many estuaries and are relatively sensitive to changes in water quality parameters (McFarland and Peddicord 1980, Attrill and Power 2000). Changes to water quality can greatly impact these invertebrate communities (*e.g.,* MacGinitie 1939, Goodbody 1961), potentially with significant consequences for the ecosystem as a whole via altered water column filtration capacity and space availability. The sessile epifaunal invertebrates of the San Francisco Estuary include tunicates, bryozoans, mussels, barnacles, and numerous other groups, and are typical of those found in protected estuaries and sheltered rocky shores in temperate zones, with tunicates and bryozoans generally most abundant. Different tunicate species have distinct morphologies, with some species growing as larger solitary individuals and others as colonies composed of smaller individuals. Similarly, some bryozoan species can be calcified and grow as colonies in erect, arborescent forms, while other species have encrusting, sheet-like mophologies. Nearly all are filter feeders capable of removing very small particles from the water column, though bryozoans and tunicates generally prefer larger particles (> 15 μm) (Lesser *et al.* 1992), while mussels and barnacles can filter a wide range of particles from very small (< 5 μm) to large zooplankton (Anderson 1994). Most common species in San Francisco Estuary epifaunal communities are non-native; relatively few native estuarine epifaunal invertebrates exist in Pacific coast estuaries (Cohen and Carlton 1995). The vast majority of these species have approximately annual life cycles; most only live for several months, with only barnacles and mussels typically living longer than one year. Solitary tunicates such as *Ciona* spp., *Ascidia zara*, and *Styela clava* are often numerically dominant in epifaunal communities (*e.g.,* Sutherland 1974), and *C. intestinalis* has been demonstrated to depress community diversity and alter community composition when present in significant numbers (Blum *et al.* 2007).

How do temperature and salinity vary in wet, moderate, and dry years?

Temperature was recorded at hourly intervals at 1 m and 4 m depth in Richmond Marina using ibutton data loggers (Maxim IC Corp., model 1921G-F5) from June 2004 until August 2006, and Hobo Pendant loggers (Onset Computer, model UA-002-64) from August 2006 until March 2009. At South Beach Harbor, temperature was recorded at hourly intervals using ibuttons deployed at 1 m and 3.5 m depths from September 2004 until July 2006.

Salinity was recorded at 5 minute intervals at 1 m depth in Richmond Marina Bay using Star-Oddi DST-CT data loggers from January 2005 until March 2009, and at 4 m depth from January 2005 until April 2007. Instrument loss prevented further use of Star-Oddi data loggers at 4 m depth.

Data from loggers at Richmond Marina Bay were supplemented by and checked against temperature and salinity profiles taken at 1 m depth intervals at least twice weekly during the solitary tunicate removal experiments (June 2004-August 2006) using a YSI-85 multimeter and once or twice monthly from August 2006 until March 2009. Similarly, ibutton temperature data from South Beach Harbor was supplemented by and checked against temperature and salinity profiles taken at 1 m depth intervals (also including 3.5 m depth) at least monthly using the YSI-85 multimeter.

Monthly averages of temperature were calculated for each depth at each site from ibutton and Hobo Pendant logger data. Monthly averages of salinity were calculated for Richmond Marina from YSI profile data and Star-Oddi DST logger data. Since salinity logger data were not available for South Beach Harbor, we calculated monthly average salinity from a combination of YSI profile data as well as data from USGS water quality monitoring cruises taken at station 21 (Bay Bridge) (USGS 2009a).

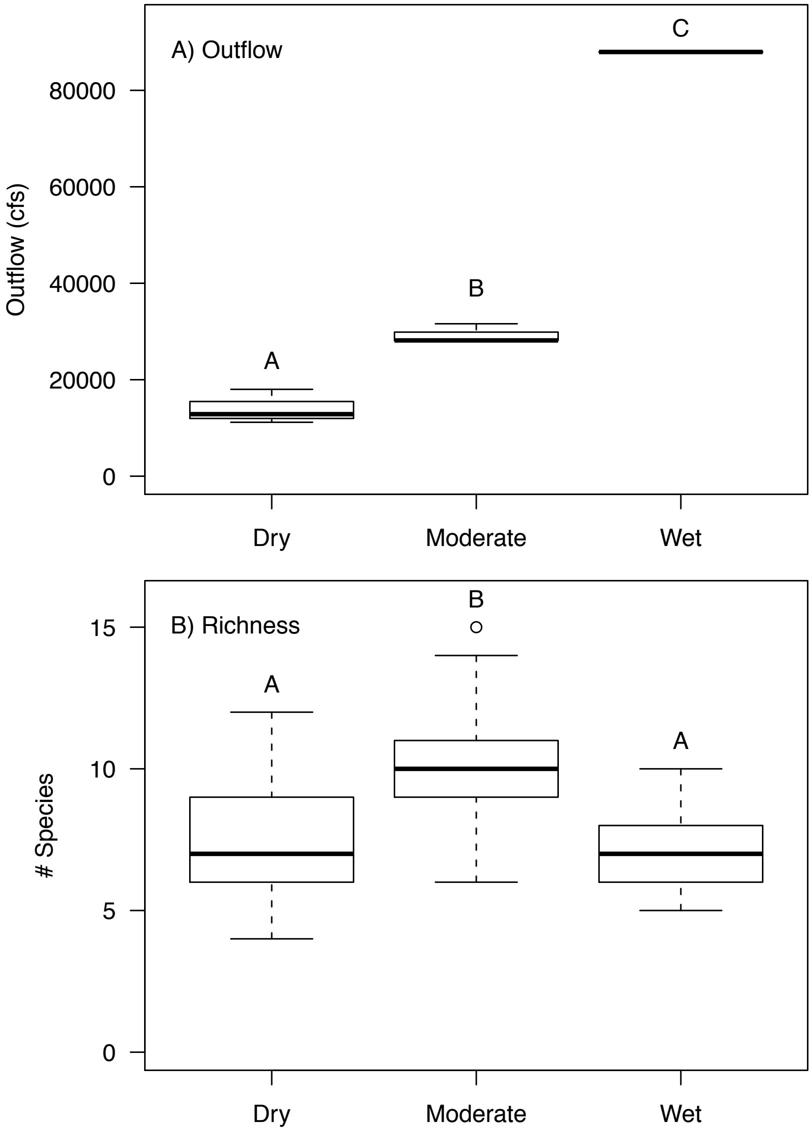
How do the wet and dry years in this study compare to historical patterns?

We examined historical precipitation, Net Delta Outflow, and salinity levels for the San Francisco Estuary to determine the historical frequency of droughts or floods matching or exceeding extremes observed during this study. Data describing daily Net Delta Outflow, a measure of the total volume of freshwater outflow from the Sacramento-San Joaquin Delta into San Francisco Bay from 1955 to 2009 was obtained from the California Data Exchange Center (USGS 2009b). Long-term (1604–1997) records of estimated salinity at Fort Point at the mouth of San Francisco Bay derived from blue oak tree ring data were obtained from Stahle *et al.* (2001). We used ordinary least squares regression (OLS) to assess the relationship between historical and current precipitation, Net Delta Outflow, and salinity records in San Francisco Bay. We used OLS with average monthly salinity levels at Richmond Marina regressed against USGS measurements of average monthly salinity at Point San Pablo from 2004–2006. This allowed us to determine the utility of the Point San Pablo record as a surrogate for probable hindcast salinity levels at Richmond. We also used OLS with salinity from Richmond Marina and Point San Pablo regressed against Net Delta Outflow (2004–2009 for Richmond Marina; 1990–2006 for Point San Pablo) to determine the utility of Net Delta Outflow as a predictor of salinity. Finally, we used OLS to determine the utility of tree ring-derived yearly average salinity records from Stahle *et al.* (2001) as an indicator of past salinity fluctuations in the Bay, regressing estimated salinity from the tree ring record against yearly average Net Delta Outflow (1955 to 1997) and salinity at Point San Pablo (1990–1997). Data were log-transformed before analysis if necessary to meet the assumption of normality. The coefficients of variation of 10-year moving averages of precipitation in San Francisco (1849–2009) and tree ring-derived salinity record were used to assess long-term trends in salinity variation. Precipitation data were obtained from the National Weather Service (National Weather Service 2009).

Based on the highly significant regression of Richmond Marina salinity levels on Net Delta Outflow levels (2004–2009), we used Net Delta Outflow as a proxy for Richmond salinity levels. We then used outflow levels in the extreme wet (2006) and dry (2007) years of this study to determine the historical frequency of wet and dry years matching these levels between 1955 and 2007. As the regression of tree ring-derived salinity on yearly average Net Delta Outflow was highly significant, we then used the much longer (393 years) tree ring record determine the historical frequency of wet and dry years matching the 2006 and 2007 extremes observed in this study.

Years from the current study (2001–2008) were classified as wet, dry, or moderate based on daily average Net Delta Outflow of the previous winter and spring (November to May) equivalent to the 25th percentile of the equivalent yearly tree ring derived salinity for wet years, the 75th percentile for dry years, and the 40th-60th percentile range for moderate years. Using this scheme, 2001, 2002, 2007, and 2008 were classified as dry (average Net Delta Outflow < 20,000 cfs); 2003, 2004, and 2005 were classified as moderate (average Net Delta Outflow 30,000 cfs), and 2006 was classified as wet (average Net Delta Outflow = 77,600 cfs) (Figure 1.2a).

Figure 1.2: Box plots of freshwater outflow and species richness in wet, moderate, and dry years



Wet ($n = 1$), moderate ($n = 3$), and dry years ($n = 4$). Rectangle represents interquartile range (25th to 75th percentile), horizontal line in rectangle represents the median, and whiskers extend to minimum and maximum values, up to 1.5 times the interquartile range, beyond which outliers are marked with open circles.

(**a**) Average monthly freshwater outflow in San Francisco Bay during November to May from 2000–2008. (**b**) Average species richness on panels deployed from the following June-to-October period at Richmond Marina. Error bars are ± 1 standard error.

Moderate years had significantly higher average richness, while both wet and dry year richness were lower on average (Kruskal-Wallis test, $p = 0.0014$). Letters indicate significantly different groups at $\alpha = 0.05$ (2-tailed, Steel-Dwass non-parametric multiple comparison tests).

How do community diversity and composition vary in wet, moderate, and dry years?

We surveyed epifaunal community composition in the San Francisco Estuary over an eight-year period (2001–2008), using monthly surveys to measure sessile invertebrate recruitment, 3-month summer surveys to examine community development during the summer, and longer-term (1–4 years) surveys to assess the effects of recruitment and survival on community development. We measured monthly recruitment of sessile epifaunal invertebrates at Richmond Marina in northeastern San Francisco Bay for a total of nearly five years (58 months), from 2002–2003 and 2004–2008. We supplemented this time series with monthly recruitment surveys at South Beach Harbor in the central portion of San Francisco Bay for 21 months, from 2004–2006 (Figure 1.1). 3-month studies of sessile invertebrate community development were conducted at Richmond Marina during the summer from June to September in each of eight years, from 2001 to 2008, and from 2006 to 2008 at six additional sites (Presidio Yacht Harbor, San Francisco Marina, Sausalito Marine Harbor, Loch Lomond Marina, Coyote Point Marina, and San Leandro Marina; Table 1.1).

We used 14 cm × 14 cm × 0.5 cm square grey PVC panels as passive recruitment collectors. We distributed panels throughout each marina using a stratified-random design in which the site was divided into five blocks. Each panel was lightly sanded, attached to a brick for weight, and suspended panel side-down from a rope tied to a floating dock. In 3-month summer surveys, one panel was placed at 1 m depth at a randomly chosen location in each block. In monthly recruitment surveys (Richmond Marina and South Beach Harbor), two panels were placed at randomly chosen locations in each block, one at 1 m depth and the other at either 3.5 m (South Beach Harbor) or 4 m (Richmond Marina). Only the 1 m depth was used at Richmond in 2002–2003. In general, two depths were used to measure recruitment differences that may occur due to light, temperature, salinity, and other differences between depths.

Recruitment panels were left in place for four to six weeks (monthly panels) or twelve to fourteen weeks (3-month summer panels) to record invertebrate settlement on the downward-facing side, then retrieved and replaced with new, blank panels at the same location. Recruitment as measured here thus includes both settlement and post-settlement processes, including mortality, that occurred during the entire deployment period. After retrieval, panels were analyzed for percent cover, biota volume (‘biovolume”), and species composition. Biovolume, a proxy for biomass, was measured using a two-step process. Each panel was first submerged in a full bucket of water equipped with a drainage spout, causing a volume of water equivalent to the volume of the panel and attached biota to overflow via the spout into a graduated cylinder. The volume of a blank panel was then subtracted from this value to obtain the volume of the biota itself. Differences in duration of panel deployment were corrected for before analysis by dividing biota volume by the number of days the panel was deployed. To estimate percent cover of dominant taxa, a grid of 100 points was placed over each panel and the taxon attached to the panel at each point (*i.e.* the “primary” cover organism) was identified to the lowest possible taxonomic level using a dissecting microscope at 40×. If other organisms were growing on top of the primary cover organism at a point, these “secondary cover” organisms were identified and recorded. Total percent cover was the sum of primary and secondary cover and could thus exceed 100%. A complete species inventory was then taken, removing organisms from the panel to ensure accurate identification if necessary. Identifications were made using criteria described in Appendix II.

Estimates of species richness at Richmond and South Beach Harbor based on monthly sampling were calculated using the software program EstimateS, version 8.0 (Colwell 2006). EstimateS uses Monte-Carlo resampling procedures to generate robust estimates of diversity in relation to sampling effort by randomizing sample order over a given number of replicates (in this case, 1000). The Chao2 nonparametric richness estimator was used, as it has proven to be a relatively robust estimator when compared to empirical estimates (Walther and Morand 1998, Foggo *et al.* 2003).

Changes in community composition through time were analyzed using multivariate ordinations, clustering analyses, and ANOSIM (Analysis of Similarities). ANOSIM is a permutational test that is a nonparametric analog to multivariate analysis of variance (MANOVA). It produces the R statistic, an absolute measure of separation between groups, with 0 indicating complete overlap and 1 indicating complete separation (Clarke and Gorley 2006). For ANOSIM, we used Bray-Curtis dissimilarity matrices of square root transformed percent cover data of each taxon on each panel at every timepoint. Square root transformation was used to reduce the effect of extremely abundant taxa while simultaneously emphasizing the effects of rare taxa. Hierarchical agglomerative clustering using the unweighted pair group method with arithmetic means was used to classify the dissimilarity matrices. The matrices were visualized using non-metric multidimensional scaling (NMDS). SIMPER, a similarity percentage procedure, was used to ascertain which species were most responsible for significant groupings identified in ANOSIM.

Univariate analyses were performed using the R Environment for Statistical Computing (R Development Core Team 2009). All multivariate analyses were carried out using the PRIMER software package (Plymouth Routines in Multivariate Ecological Research, version 6; Clarke and Gorley 2006).

How does solitary tunicate presence affect community development?

Based on preliminary results from the community surveys described above, we determined that solitary tunicates are a numerically dominant taxa in San Francisco Estuary epifaunal communities. Previous work indicated that one solitary tunicate species, *Ciona intestinalis*, can significantly depress community diversity when present in high abundance (Blum *et al.* 2007), but it was not known if other solitary tunicates have similar effects. Therefore, to determine the effects of solitary tunicate presence or absence on the rest of the epifaunal community, we carried out a series of experiments in which we compared panels from which we experimentally removed solitary tunicates to panels with naturally-occurring solitary tunicate densities. Experiments were carried out at Richmond Marina from June 2004 to August 2006, which included two years of high solitary tunicate abundance (2004 and 2005) and one year of lower solitary tunicate abundance (2006).

On 27 June 2004, 60 panels were deployed at Richmond Marina in a two-way factorial design arranged in randomized complete blocks. The marina was divided into five blocks and two replicate panels assigned to each of three treatments at each of two depths (1 m and 4 m). Treatments were solitary tunicate removal, unmanipulated control, and manipulated control, with ten replicate panels per treatment per depth. Solitary tunicate removal consisted of removing each panel from the water, submersing it in a tub of seawater on the dock and removing all visible solitary tunicates with forceps, then returning the panel to the water. Manipulated control panels were treated identically to solitary tunicate removal panels, except nothing was removed, accounting for any artifacts of the manipulation other than removing solitary tunicates. Unmanipulated control panels were not removed from the water except to take monthly photographs. Treatments were performed every two to four weeks until August 2006. Panels were analyzed for percent cover and species composition after 16 weeks (October 2004) and again after 2 years (July 2006).

An identical experiment with n = 8 replicates at 1 m depth only (arranged into 4 of the 5 blocks used in the 2004 experiment) was begun on 18 July 2005, lasting until 24 August 2006. Panels from this experiment were analyzed for percent cover and species composition after 16 weeks (November 2005). A third experiment with n = 8 replicates at both 1 m and 4 m depths was begun on 10 February 2006 and lasted until 24 August 2006. Panels were analyzed after 20 weeks (July 2006).

Community development was analyzed for solitary tunicate removal experiments using a one-way ANOSIM of treatment effects. Solitary tunicates were omitted from the data to examine the effect of solitary tunicate removal on the other components of the community. Pairwise comparisons of treatments were performed, but α was not adjusted for multiple comparisons. The p-value calculated by the ANOSIM procedure depends on the number of permutations examined, with p equal to the number of observed permutations with $R \geq R_{observed}$, divided by the total number of permutations examined. For complex data sets, PRIMER can only examine a limited number of permutations, so in these cases p-values for comparisons with very large values of R are likely quite inflated. Therefore common multiple comparison methods, such as the Bonferroni correction, are much too conservative. Here, we present the uncorrected p-values, but encourage the reader to focus attention on the R statistic. As a rule of thumb, pairwise $R > 0.75$ indicates well-separated treatments (Clarke and Gorley 2006).

A non-parametric Kruskal-Wallis test was used to compare richness data between treatments, as the data could not be transformed to meet the assumption of normality required for parametric tests.

How do low salinity pulses affect mortality of resident species and availability of space for new recruits?

Salinity varies more directly in proportion to freshwater outflow than numerous other factors, including turbidity, which is also often affected by wind shear (Conomos *et al.* 1985, Schoellhamer 1996). We used a combined field-lab experiment during the dry winter of 2006–2007 to determine whether salinity variation alone could be responsible for differences in sessile invertebrate survival and recruitment between wet and dry years. We examined epifaunal community responses to the simulated effect of increased freshwater runoff resulting from winter storms. A total of 24 panels was deployed at Richmond Marina in November 2006 and left in place for six weeks to allow sessile invertebrates to colonize. We used a randomized complete block design in which the marina was divided into six blocks and we randomly assigned panel locations within each block. After six weeks, 18 panels were retrieved and subjected to one of three salinity levels (5 psu, 20 psu, or 30 psu; 6 replicates per treatment) at the Bodega Marine Laboratory. The 5 psu and 20 psu salinity treatment levels were chosen based on average low salinities at the nearby Point San Pablo monitoring station (US Geological Survey) during the most severe storms (5 psu) or during a moderate storm (20 psu) of the period 1990–2007. The 30 psu treatment was the ambient salinity at Richmond Marina when panels were retrieved and, thus, served as a "no-storm" control, simulating the lack of salinity change observed during dry years such as 2007. Ambient salinity at Richmond Marina did not change more than 1–2 psu during the course of the experiment. Although no acclimation period was used when transferring panels from the field to treatment salinity levels in the lab, this is not unrealistic for estuarine organisms that may experience rapid changes in salinity due to heavy storms and runoff (*e.g.,* Vazquez and Young 2000). The remaining panels stayed in the field for the duration of the experiment, serving as controls to account for any overall effect of taking the treatment panels to the lab.

Each treatment panel was placed in a separate 2-gallon bucket outfitted with an air bubbler and filled with water of the appropriate salinity mixed from filtered seawater (35 microns) and distilled, deionized water. All buckets were placed in a temperature-controlled room on a 12-hour light/dark cycle at 12° C, matching the average temperature in the field during the laboratory portion of the experiment. Water was changed every 24 hours and invertebrates were fed MicroVert liquid invertebrate food (Kent Marine Inc., Acworth, GA, USA) just after water changes. Panels were subjected to salinity treatments for 2 days, simulating the effects of a brief storm. Following treatments, panels were then re-deployed in the same locations they had previously occupied at Richmond Marina. Recruitment following re-deployment was measured using an additional six blank panels that were deployed when the treatment panels were returned to the field.

All panels were photographed before salinity treatments were applied, immediately following salinity treatments, and at 8 weeks following re-deployment. Photographs were analyzed for percent cover, abundance of individuals, and species composition in the same manner as for the community surveys. In addition, we ground-truthed photographic analyses by comparing them with field notes as well as point counts and species compositions determined using live analyses under a dissecting microscope at 40× magnification immediately before salinity treatments.

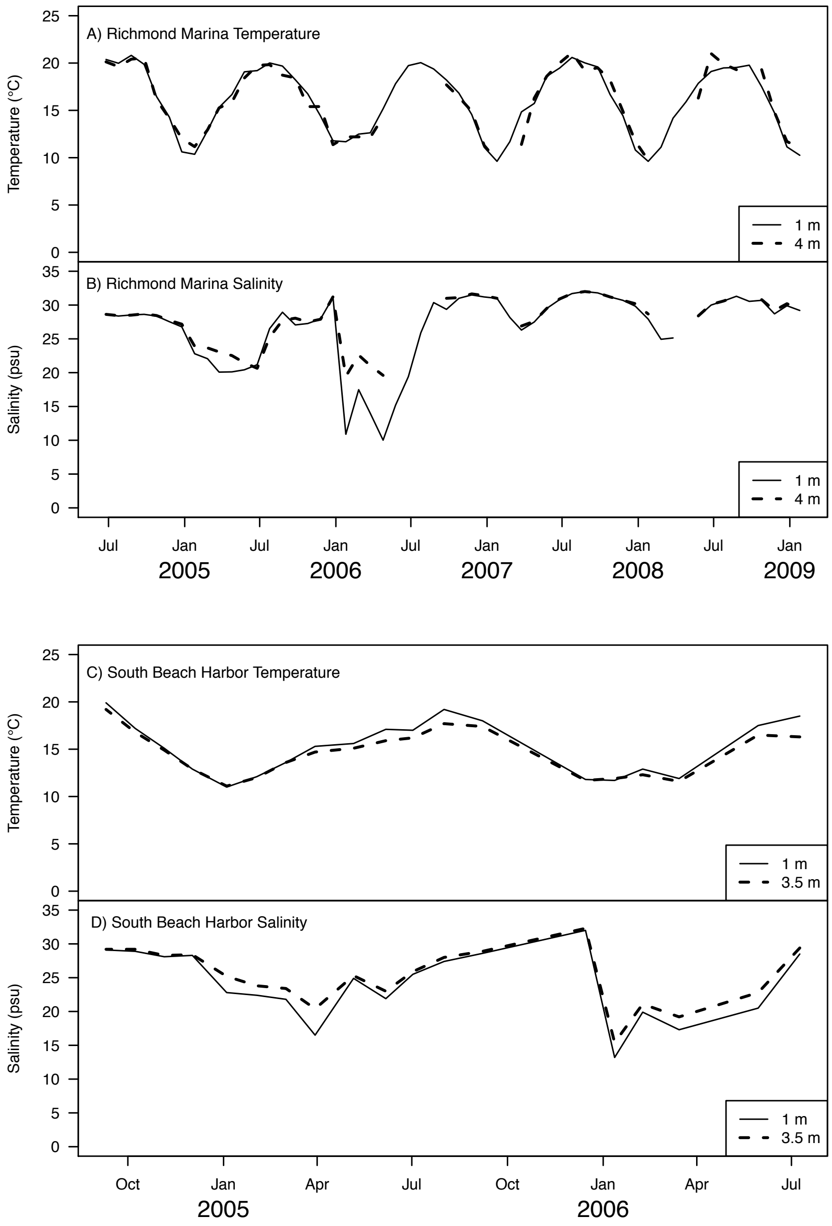
Community development on panels in the salinity tolerance experiment was analyzed in a similar manner to data from the solitary tunicate removal experiments. A one-way ANOSIM of salinity treatment effects was performed on a matrix of Bray-Curtis dissimilarities based on square root transformed percent cover data for each taxon. Pairwise comparisons of treatments were performed, but as before, α was not adjusted for multiple comparisons using a Bonferroni correction.

RESULTS

How do temperature and salinity vary in wet, moderate, and dry years?

At both Richmond Marina and South Beach Harbor, temperature showed an annual cycle of lows in the winter and highs in early fall (Figure 1.3). Winter minimum temperatures varied by several degrees between years, but summer/fall maximum temperatures varied little. Salinity levels also showed an approximately annual pattern, with lows in winter and highs in late summer and early fall. Winter minimum salinities varied greatly between years, from as low as 5 psu in January 2006 to as high as 26 psu in January 2007. Summer and fall salinities also varied, with slightly higher salinity in dry years (Figure 1.3).

Figure 1.3: Monthly average temperature and salinity at Richmond Marina (2004–2009) and South Beach Harbor (2004–2006)



Monthly average temperature and salinity at a shallow (1 m) and deep (3.5 m for South Beach Harbor and 4 m for Richmond Marina) depths.

How do the wet and dry years in this study compare to historical patterns?

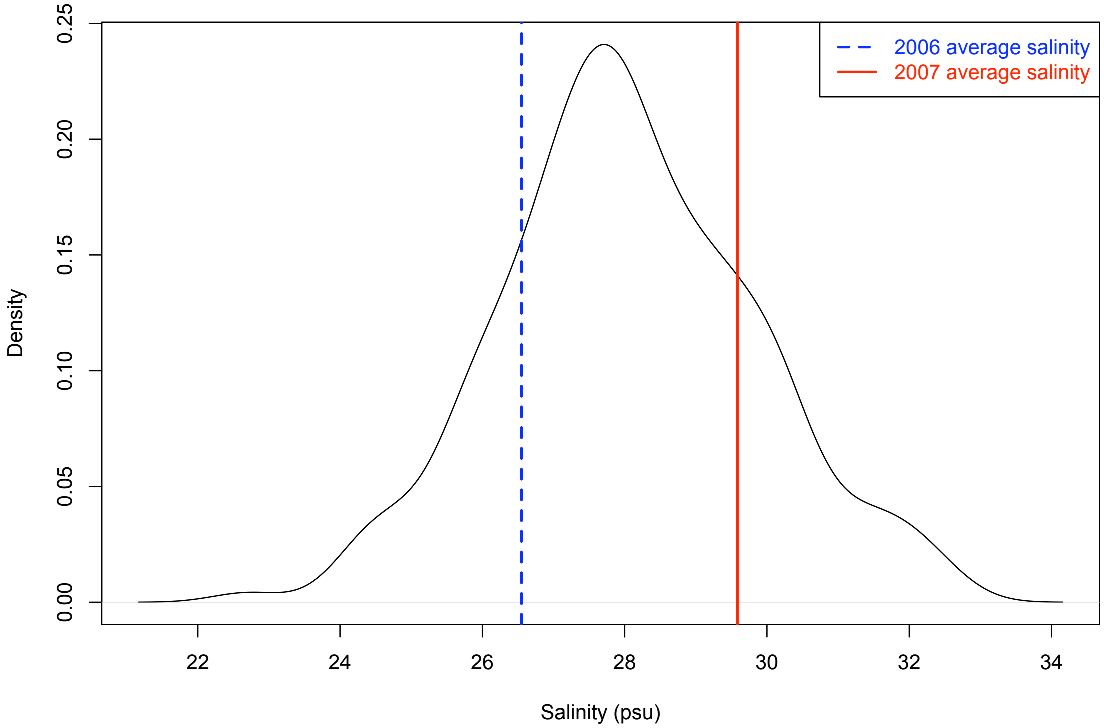
Analysis of historical patterns of salinity and outflow indicate that outflow can be used as a good proxy for salinity, and that over the past fifty years, outflow levels matching the high and low winter/spring extremes observed during this study have occurred approximately once or twice each decade (for each extreme). Using the maximum daily outflow of 10,432 m^3/s during the wet winter of 2005–2006 and the maximum daily outflow of 1331 m^3/s during the drought of 2007–2008, we determined that the 2005–2006 maximum had been equalled or exceeded in nine winters since 1955, while outflow levels at or below the 2007–2008 minimum occurred in eight winters. Extending the analysis back further using tree ring-derived salinity estimates from Stahle *et al.* (2001), we found that 19% of all years from 1604–1997 had lower average yearly salinity than in 2006, whereas 20% of all years from this period had higher average yearly salinity than in 2007 (Figure 1.4). Precipitation records for the city of San Francisco (NWS 2009) show that the wettest extreme observed during this study was unusual on a decadal scale, but not over longer time periods. Deviations from the annual average rainfall from 1849–2009 equal to or greater than the high recorded in 2006 (12.497 inches above the mean) have occurred eleven times since 1849.

A very significant relationship between monthly average salinities at Richmond Marina and the USGS Point San Pablo monitoring station during the period of overlap between these two records (2004–2006) indicates that we can use the Point San Pablo record as an accurate proxy for Richmond (OLS, adjusted $R^2 = 0.962$, $p < 0.0001$). As Richmond Marina salinity also shows a strong relationship with Net Delta Outflow for

2004–2009 (adjusted $R^2 = 0.814$, $p < 0.0001$), outflow levels can be used directly to predict Richmond Marina salinity. On longer time scales, a highly significant correlation between tree ring-derived salinity records for Fort Point at the mouth of San Francisco Bay and annual mean outflow for the period of overlap (1955–1997) indicates that the tree ring record can be used as an accurate approximation of the yearly average salinity in the Bay (adjusted $R^2 = 0.496$, $p < 0.0001$).

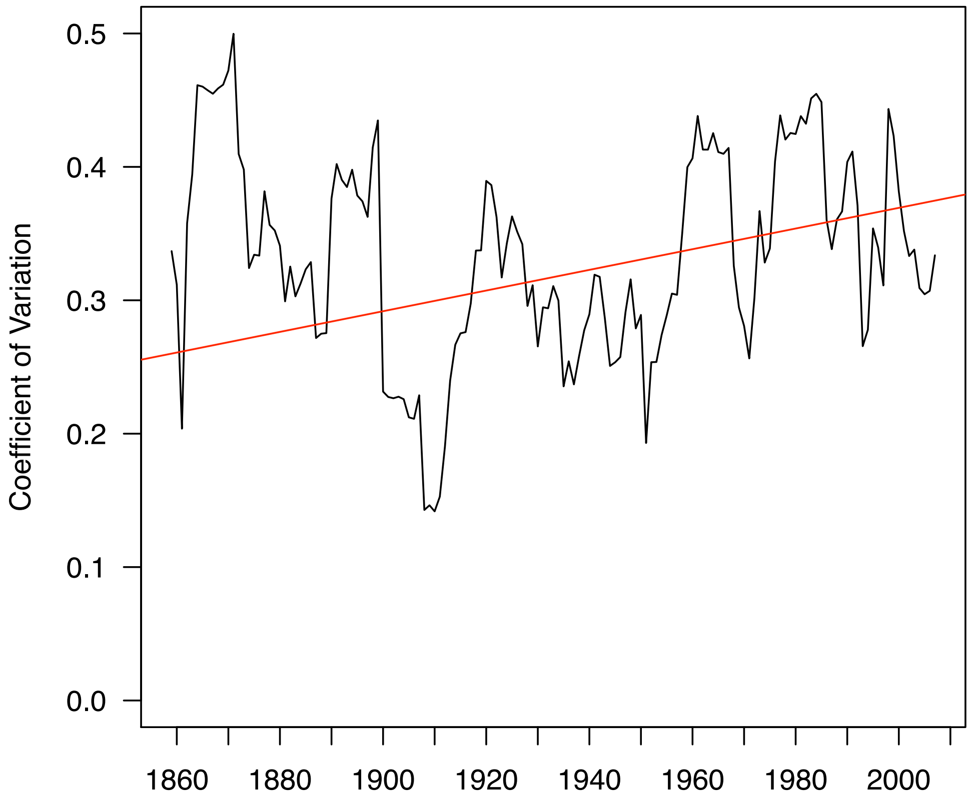
Both rainfall and tree ring-derived salinity records show increasing variability. Decadal average rainfall in San Francisco has increased in variation over the past 160 years (Figure 1.5, adjusted $R^2 = 0.104$, $p < 0.0001$). A significant increasing trend is also evident in the coefficient of variation of 10-year moving average reconstructed salinity at Fort Point over a 390-year timespan (Figure 1.6; adjusted $R^2 = 0.106$, $p < 0.0001$).

Figure 1.4: Probability density plot of reconstructed salinity at Fort Point from 1604 to 1997



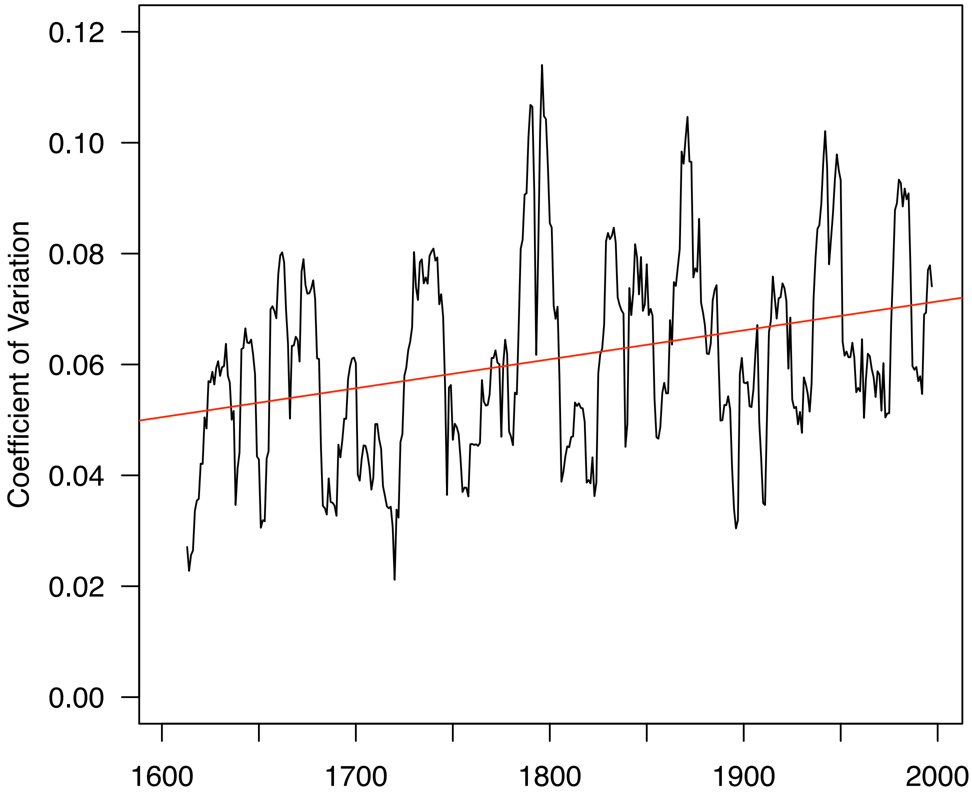
Relative distribution of the yearly average salinities at Fort Point observed during the 2006 wet year (dashed line) and the 2007 dry year (solid line). 19% of all years from 1604 to 1997 had lower average salinity than 2006, while 20% of all years during this period had higher average salinity than 2007. Data from Stahle *et al.* (2001).

Figure 1.5: Coefficient of variation of a 10-year moving average of total annual precipitation in San Francisco from 1849–2009



Data from NWS (2009).

Figure 1.6: Coefficient of variation of a 10-year moving average of a 390-year salinity record at Fort Point at the mouth of San Francisco Bay



Estimated yearly mean salinity as reconstructed from tree-ring chronologies (Stahle *et al.* 2001).

How do community diversity and composition vary in wet, moderate, and dry years?

Summer 3-month panels deployed at Richmond had significantly greater species richness following moderate outflow winters, while species richness on summer panels following both wet and dry winters was lower on average (Figure 1.2b, Kruskal-Wallis test $\chi^2 = 13.169$, $p = 0.0014$). Estimates of total species richness at Richmond based on monthly sampling during the same time period from 2004–2008 indicates similar trends, suggesting that the diversity trends shown on the 3-month panels were not simply a function of whichever species was recruiting at the time of panel deployment (Figure 1.7b). In moderate years, species richness was higher than in wet or dry years, with an estimated richness of 45.03 species in 2004 and 47.15 species in 2005. In the wet year, richness was somewhat lower, estimated at 35.83 species. In the dry year, richness was also low, estimated at 30.94 species (Figure 1.7b). While summer monthly recruitment data were only available for 2005 (a moderate year) at South Beach Harbor, estimated richness was 60.7 species (Figure 1.7d). Moderate year estimation curves did not reach asymptotes at either site, indicating incomplete sampling to capture the diversity of the site during this time period. These calculations thus appear to underestimate the actual differences between moderate years and wet and dry years. The major interannual physical differences were in winter salinity, with summer conditions largely similar from year to year, suggesting that these different estimates of richness in the summer are based on the previous winter's outflow levels.

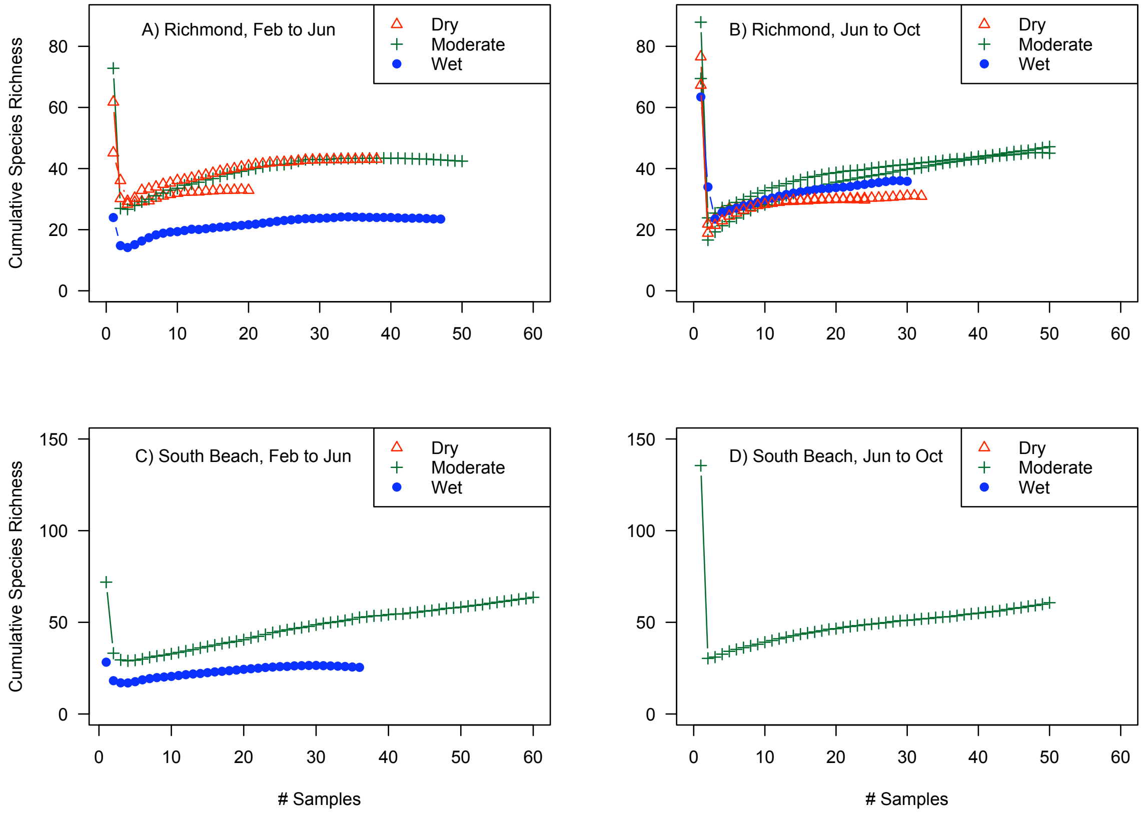
The diversity of sessile invertebrates recruiting at Richmond and South Beach Harbor during late winter and spring (February to June) also differed between years of wet, dry, and moderate outflow. As with the richness estimates based on monthly panels during the summer, the lowest estimated richness values in winter and spring occurred during the wet year, with 23.47 species at Richmond Marina and 25.43 species at South Beach Harbor. In contrast, dry and moderate year estimates of richness during winter and spring were greater than 50 species (Figures 1.7a and 1.7c). 2005 (moderate year) richness was estimated to be 63.63 species at South Beach Harbor and 42.43 species at Richmond Marina. Dry year richness was estimated at 32.9 species in 2002 and 43.09 species in 2007 at Richmond Marina. The lower estimate of richness in 2002 at Richmond is likely due to a smaller sample size (n = 5 panels per timepoint) that was taken at 1 m depth only, rather than at both 1 m and 4 m.

A general seasonal trend in species richness occurred on monthly recruitment panels at both Richmond Marina and South Beach Harbor, with high richness in summer and fall, and low richness in winter and early spring. Shannon diversity patterns were broadly similar to richness (Table 1.2).

Table 1.2: Species richness (*S*) and Shannon-Weiner diversity (*H*) per panel on monthly panels at Richmond Marina (RMB) and South Beach Harbor (SB)

Site	Depth (m)	Min *S*	Max *S*	Avg *S*	Min *H*	Max *H*	Avg *H*
RMB	1	2.6	22.75	11.95	0.15	2.88	1.68
RMB	4	2.4	17.60	11.55	0.31	3.05	1.90
SB	1	3.2	25.20	12.97	0	2.20	1.09
SB	3.5	1.7	22.60	11.88	0	2.29	0.99

Figure 1.7: Species accumulation curves for monthly panels at Richmond Marina and South Beach Harbor



Accumulation of species from monthly panels deployed at Richmond Marina during February to June (**a**) and June to October (**b**) in 2002, 2004–2007 and at South Beach Harbor during February to June in 2005 and 2006 (**c**) and June to October 2005 (**d**).

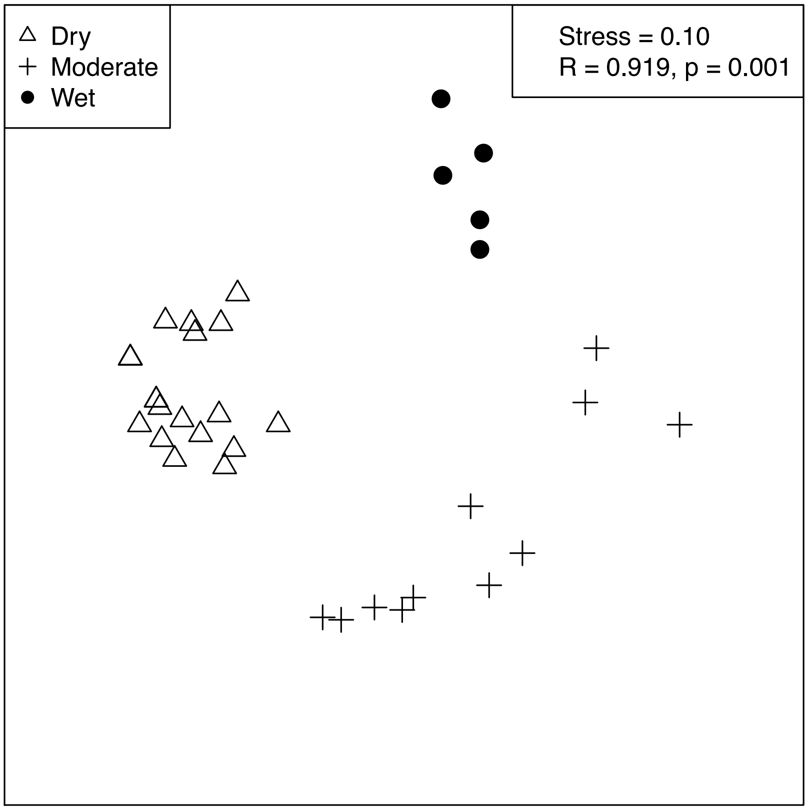
Each point of the species accumulation curve represents the average of 1000 estimates of richness per number of panels calculated using the Chao2 index. Open triangles are dry years (2002, 2007), crosses are a moderate (2005), and filled circles are the wet year (2006). Panels from both 1 m and 4 m (Richmond) or 1 m and 3.5 m (South Beach Harbor) are included.

Initial spikes come from variation in sample richness. Although many more samples were available for the February to June period during 2005 (a moderate year) at South Beach Harbor (c), the estimation curve shows that an asymptote has not yet been reached, whereas for the wet year (2006), an the curve levels off after 30 samples, indicating a sufficient sample size to capture the available diversity for this time period. Asymptotes also have not been reached for moderate year diversity in June to October at either Richmond (b) or South Beach Harbor (d).

Summer community composition as measured by 3-month panels at Richmond differed significantly between wet, moderate, and dry years (Figure 1.8). SIMPER analysis indicates that the major taxa responsible for these differences are the solitary tunicates *Ciona intestinalis* (Type A, *sensu* Nydam and Harrison 2007; only Type A was found in this study), *Ciona savignyi*, and the colonial tunicates *Botrylloides violaceus* and *Botryllus schlosseri*, which are most prevalent in dry, moderate, and wet years, respectively (Table 1.3).

Summer bay-wide surveys of community composition using 3-month panels showed that these composition patterns were not limited to Richmond. Two-way crossed SIMPER using Site and Year Type (wet or dry) as factors indicated that the major distinguishing taxa between wet and dry year communities again were *Ciona intestinalis* (most prevalent during dry years) and botryllid tunicates (highest abundance in the wet year). In addition, following the wet winter (2006), communities showed greater differences from each other than following dry winters (in 2007 and 2008). One-way ANOSIM on 2006 panels showed that most sites were distinct from each other. The main exception was San Francisco Marina, which was intermediate between several nearby sites, although those sites differed from each other (Table 1.4). Following the dry winters of 2007 and 2008, sites near the mouth of the Bay (Presidio Yacht Harbor, San Francisco Marina, Sausalito Marine Harbor) were indistinguishable from each other (Table 1.5). Similarly, two sites further from the mouth, but in different parts of the Bay (Richmond and Coyote Point) were statistically indistinguishable from each other, yet together were distinct from the three sites near the mouth of the Bay.

Figure 1.8: Summer community composition (on 3-month panels) differs between wet, dry, and moderate years



Non-metric multi-dimensional scaling plot based on a Bray-Curtis dissimilarity matrix of square root-transformed species percent cover data. Symbols indicate dry, moderate, or wet years. ANOSIM shows very significant differences in community composition between year types.

Table 1.3: SIMPER results indicating species most responsible for discriminating amongst dry, moderate, and wet years for summer 3-month panels at Richmond Marina

	Dry	**Moderate**	**% Contribution**
Ciona intestinalis	94 % *of points*	6 % *of points*	33.99
Ciona savignyi	5	7	15.83

	Dry	**Wet**	**% Contribution**
Ciona intestinalis	94 % *of points*	0 % *of points*	27.05
Botrylloides violaceus	5	63	17

	Moderate	**Wet**	**% Contribution**
Botrylloides violaceus	5 % *of points*	63 % *of points*	19.66
Botryllus schlosseri	1	42	13.77

Average abundance (percent cover on 14 cm x 14 cm panels) in each year type and percent contribution to overall differences between groups. All species shown are tunicates.

Table 1.4: 2006 (wet year) ANOSIM pairwise tests comparing community composition on summer 3-month panels from sites throughout San Francisco Estuary

Sites *(see Table 1.1)*	***R* Statistic**	***p*-value**	**Number ≥ Observed**
LL *vs* Pre	1	0.008	1
LL *vs* RMB	1	0.008	1
LL *vs* SF	0.769	0.016	2
LL *vs* SL	0.831	0.008	1
LL *vs* Saus	1	0.008	1
Pre *vs* RMB	0.66	0.016	2
Pre *vs* SF	0.18	0.103	13
Pre *vs* SL	0.98	0.008	1
Pre *vs* Saus	0.648	0.008	1
RMB *vs* SF	0.2	0.04	5
RMB *vs* SL	1	0.008	1
RMB *vs* Saus	0.828	0.008	1
SF *vs* SL	0.876	0.008	1
SF *vs* Saus	0.368	0.008	1
SL *vs* Saus	0.992	0.008	1

126 permutations, global $R = 0.759$, $p = 0.001$. Most sites are distinct from every other site, except SF, which is intermediate between Saus, Pre, and RMB (low pairwise R values). ‘Number ≥ Observed’ gives the number of random permutations whose R was greater than or equal to the observed data set’s R.

Table 1.5: 2007–2008 (dry years) ANOSIM pairwise test results comparing community composition on summer 3-month panels from sites throughout San Francisco Estuary

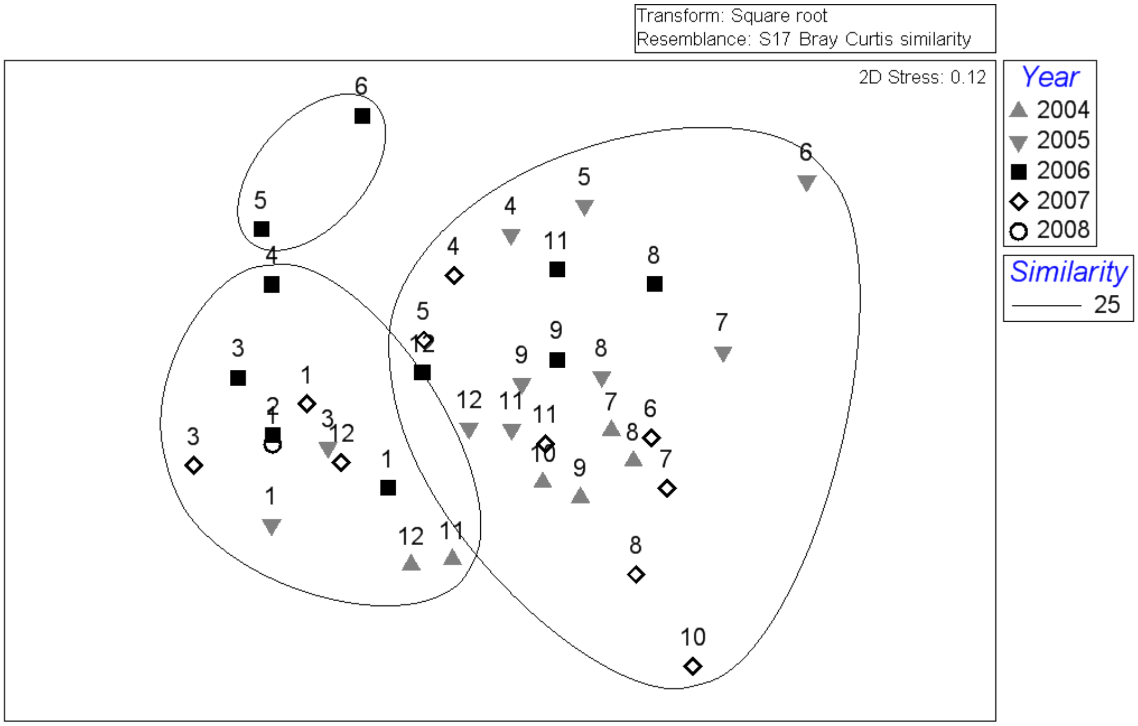
Sites *(see Table 1.1)*	***R* Statistic**	***p*-value**	**Number ≥ Observed**
CP *vs* LL	0.991	0.001	0
CP *vs* Pre	0.838	0.001	0
CP *vs* RMB	0.038	0.025	224
CP *vs* SF	0.537	0.001	0
CP *vs* SL	0.702	0.001	0
CP *vs* Saus	0.837	0.001	0
LL *vs* Pre	0.696	0.003	2
LL *vs* RMB	0.975	0.002	1
LL *vs* SF	0.355	1.004	13
LL *vs* SL	0.544	0.003	2
LL *vs* Saus	0.96	0.008	1
Pre *vs* RMB	0.756	0.001	0
Pre *vs* SF	0.055	0.214	213
Pre *vs* SL	0.497	0.001	0
Pre *vs* Saus	0.047	0.319	318
RMB *vs* SF	0.416	0.001	0
RMB *vs* SL	0.709	0.001	0
RMB *vs* Saus	0.833	0.001	0
SF *vs* SL	0.376	0.001	0
SF *vs* Saus	-0.095	0.704	703
SL *vs* Saus	0.572	0.005	4

999 permutations, global $R = 0.527$, $p = 0.0001$. ‘Number ≥ Observed’ gives the number of random permutations whose R was greater than or equal to the observed data set’s R.

Sites closer to the mouth of the bay (SF, Saus, Pre) are not different from each other; two sites further from the mouth (RMB, CP) are similar to each other but distinct from downstream sites.

Community composition differed dramatically both across the seasons as well as between 2006 and other years. Cluster analysis shows a split at the 25% similarity level between two groups of panels, one mostly from the winter months and the other including all other months. The only months were not included in these two clusters are May and June 2006, following the large rainstorms in the winter of 2006 (Figure 1.9). SIMPER analysis showed that these 2006 panels were united by the presence of diatoms, protozoans, the mussel *Mytilus* sp., the barnacle *Balanus improvisus*, and the encrusting bryozoan *Electra* sp. Recruitment to monthly panels during the summer and fall did not show a clear pattern of differences between years (Figure 1.9).

Figure 1.9: Average community composition on monthly recruitment panels from Richmond, 2004–2008



Non-metric multi-dimensional scaling plot based on a Bray-Curtis dissimilarity matrix of square root-transformed species percent cover data. Numbers indicate months of the year, while symbols indicate year.

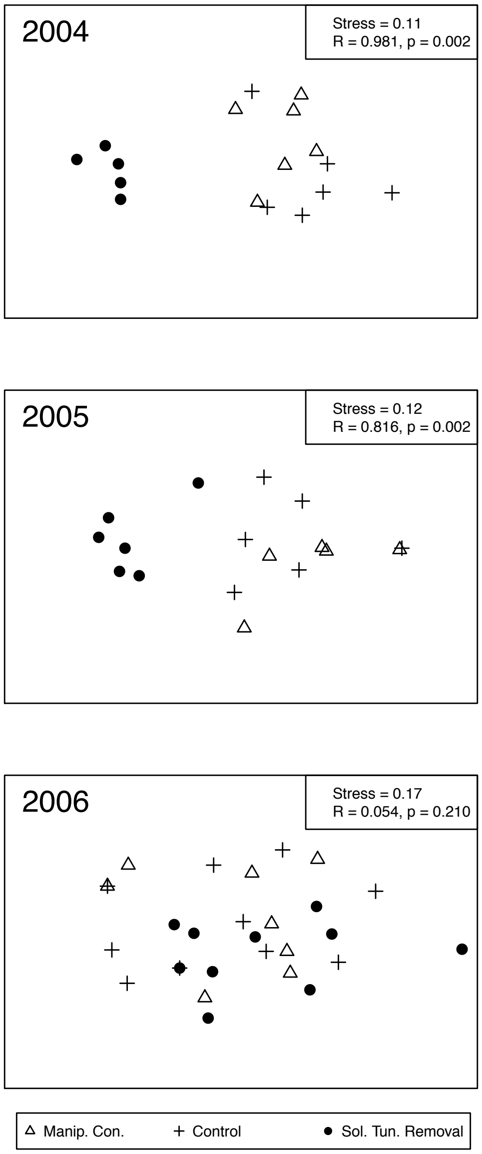
Cluster analysis shows a split at the 25% similarity level between panels that are mostly from the winter months and all others. A third group is formed by May and June 2006, following the large rainstorms in the winter of 2006.

How does solitary tunicate presence affect community development?

The dominance of solitary tunicates during dry and moderate years was underscored by significant correlations between solitary tunicate cover and biovolume of the community on monthly panels at Richmond (adjusted $R^2 = 0.642$, $p < 0.0001$). Seasonal trends in biovolume are clearly evident, with maximum biovolume generally occurring in late summer and fall and minimum biovolume occurring in the winter (January/February) of each year. Maximum annual biovolume increased greatly during the 2007–2008 drought, from an annual maximum of 7.32 mL per panel per day from 2004–2006, to a high of 10.16 mL per panel per day in 2007, and 25.29 mL per panel per day in 2008. These maxima in 2007 and 2008 coincided with large recruitment pulses and near-complete cover of *Ciona intestinalis*. There was a significant correlation between *C. intestinalis* percent cover and biovolume in 2007 and 2008 (adjusted $R^2 = 0.592$, $p = 0.0005$), but not in 2004–2006 (adjusted $R^2 = -0.055$, $p = 0.9443$).

We examined the consequences of solitary tunicate dominance for community diversity using three solitary tunicate removal experiments, each in a different year. Solitary tunicate removal treatments produced communities that differed dramatically in richness and composition (Figure 1.10). After four months in 2004 and 2005, panels from which solitary tunicates had been removed biweekly were significantly different. One-way SIMPER analysis indicated that aside from the solitary tunicates *Ciona savignyi* and *Ascidia zara*, the species most responsible for distinguishing between solitary tunicate and no-solitary tunicate panels in these two years were colonial botryllid tunicates. These results are very similar to the factors shown to distinguish between wet, dry, and moderate year community composition on 3-month summer panels at Richmond (Table 1.3, Figure 1.8). No solitary tunicates recruited to experimental panels during an attempted repetition of this experiment following the wet winter of 2006; thus, there were no differences between control panels and solitary tunicate "removal" panels (Figure 1.10).

Figure 1.10: Species composition of communities that developed in the presence or absence of solitary tunicates



Non-metric multi-dimensional scaling plots based on a Bray-Curtis dissimilarity matrix of square root-transformed species percent cover data from panels from which solitary tunicates were removed every two weeks (filled circles) and unmanipulated control panels (crosses). Manipulation control panels (open triangles) were treated identically to solitary tunicate removal panels, except that nothing was removed.

Pairwise ANOSIM *R* values and corresponding *p*-values are given for comparisons of solitary tunicate removal panels to unmanipulated control panels. Manipulated and unmanipulaed controls were not statistically different.

After four months in 2004 and 2005 (years of moderate outflow), biweekly removal of solitary tunicates created significantly different communities. Solitary tunicates failed to recruit during the five months immediately following the wet winter of 2006, and there was no difference between groups.

How do low salinity pulses affect mortality of resident species and availability of space for new recruits?

Experimental tests of the effect of lowered salinity on community development showed that a salinity level matching the extreme low observed during the wet winter of 2006 (5 psu) was sufficient to cause complete mortality of all taxa. Eight weeks following salinity treatments and re-deployment of panels to the field, community composition as measured by percent cover (point counts) differed significantly between two groups of panels. The low salinity (5 psu) and recruitment panels grouped separately from all other treatment panels (Table 1.6). In classification analyses, the recruitment panels again clustered with the 5 psu panels separately from all other treatments, indicating that the 5 psu treatment is functionally equivalent to deploying a blank panel. This cluster split at the 40% similarity level from all other treatments (Figure 1.11). No significant differences were evident between any other treatments, indicating no effect of the 20 psu treatment and no artifacts associated with placing panels in buckets during the application of treatments.

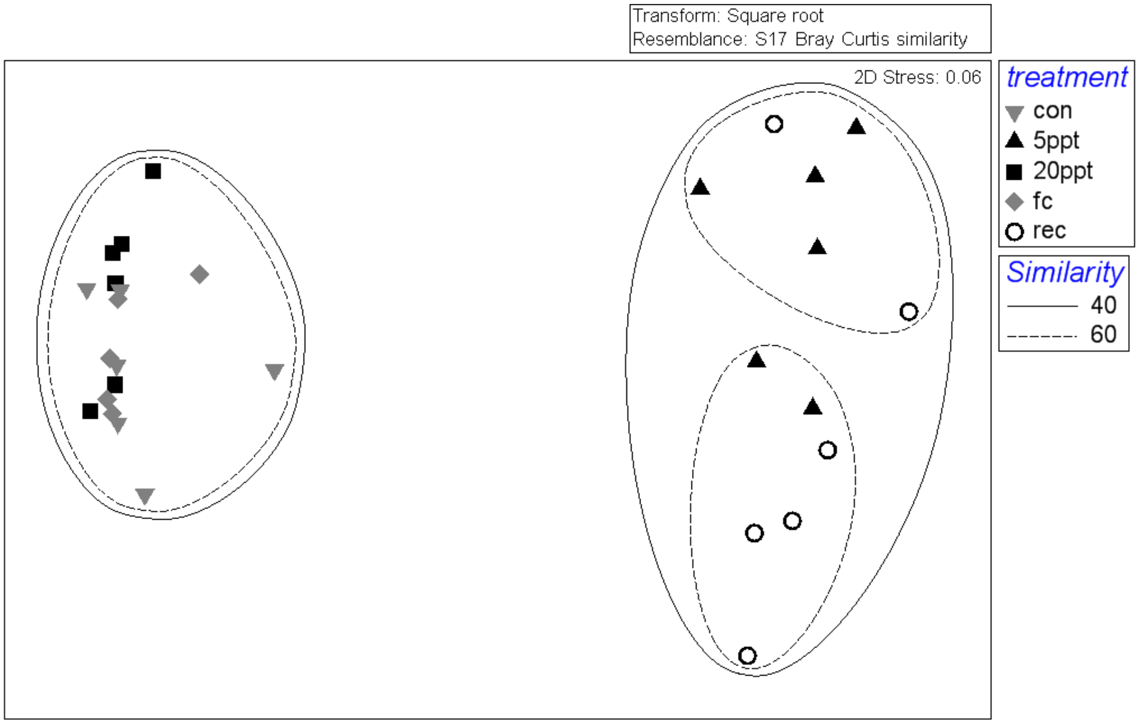
SIMPER indicates that the primary factors uniting the 5 psu and recruitment panels, respectively, were diatoms and bare space. The major factors separating both the 5 psu treatment and recruitment panels from other treatments were the high prevalence of diatoms and bare space and the absence of the solitary tunicates *Ascidia zara* and *Ciona savignyi*, both of which were the dominant taxa in all other treatments. These two solitary tunicates occupied dramatically less space on 5 psu and recruitment panels compared to all other treatments (Figure 1.12; Kruskal-Wallis test, $\chi^2 = 21.522$, $p < .001$).

Table 1.6: ANOSIM pairwise tests comparing treatments in salinity tolerance experiment

Treatments	*R* Statistic	*p*-value	Number ≥ Observed
5 PSU *vs* CON	1	0.002	1
5 PSU *vs* 20 PSU	1	0.002	1
5 PSU *vs* FC	1	0.002	1
5 PSU *vs* REC	0.365	0.03	14
CON *vs* 20 PSU	0.043	0.249	115
CON *vs* FC	−0.112	0.851	393
CON *vs* REC	0.991	0.002	1
20 PSU *vs* FC	−0.069	0.578	267
20 PSU *vs* REC	1	0.002	1
FC *vs* REC	0.997	0.002	1

462 permutations, global $R = 0.602$, $p = 0.0001$. Panels with 6-week old communities were exposed to 5 psu, 20 psu, or 30 psu ("CON") salinity levels for 2 days in the lab, then re-deployed in the field. Field control ("FC") panels remained in the field for the duration of the experiment; recruitment ("REC") panels were deployed when experimental panels were re-deployed in the field. Community composition was assessed after 8 weeks showing two groups: 5 psu and recruitment panels grouped together, separately from all other treatments.

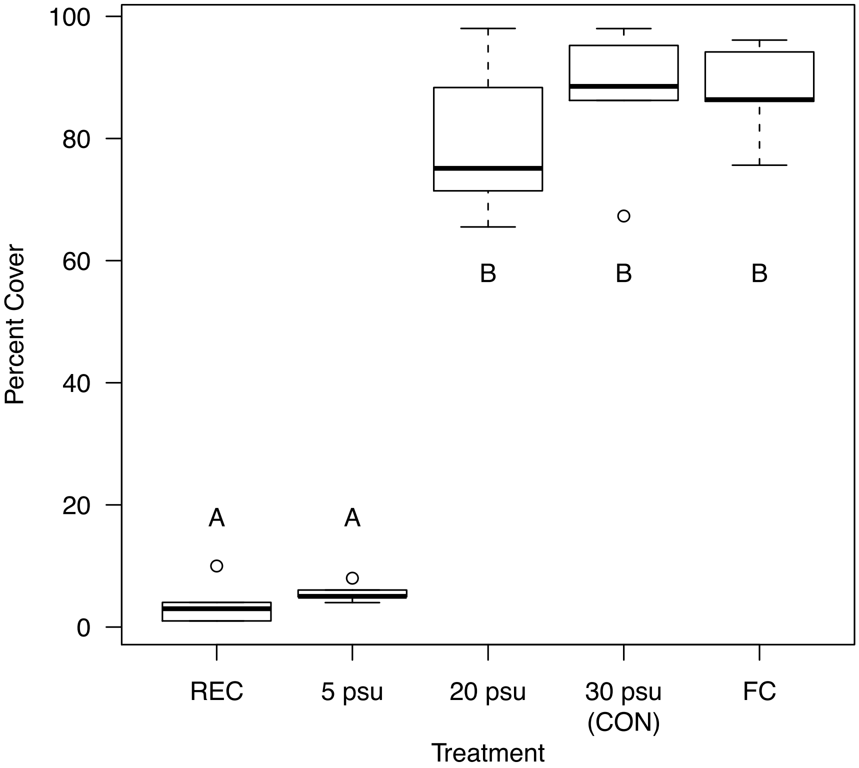
Figure 1.11: Community composition is significantly different on panels exposed to low salinity (5 psu) mimicking wet winter conditions



Non-metric multi-dimensional scaling plot based on a Bray-Curtis dissimilarity matrix of square-root transformed species percent cover data from panels exposed to low salinity (5 psu), moderately low salinity (20 psu), and controls (30 psu) for 2 days in the lab, then re-deployed in the field for 8 weeks.

Field control ("FC") panels remained in the field for the duration of the experiment and served to control for any effect of placing panels in buckets in the lab during the treatments. Recruitment panels ("REC") were blank panels used to measure new recruitment following re-deployment of treatment panels in the field. Community composition was assessed after 8 weeks showing two groups: 5 psu and recruitment panels grouped together, separately from all other treatments.

Figure 1.12: Space occupied by the solitary tunicates *Ascidia zara* and *Ciona savignyi* on panels in low salinity experiment



Solitary tunicate cover is significantly lesser on panels exposed to 5 psu for two days in the lab, and on recruitment panels ("REC") deployed when all experimental panels were re-deployed following salinity treatments. There were no statistically significant differences in solitary tunicate cover between panels exposed to 20 psu for two days, control panels exposed to 30 psu ("CON") for two days, and field control panels ("FC") that remained in the field for the duration of the experiment.

Letters indicate treatments found to be significantly different using Steele-Dwass type non-parametric multiple comparison tests.

DISCUSSION

Community Assembly Consequences of Changing Hydrological Regimes

Salinity levels measured during the wettest and dryest years of this study (2001–2008) are relatively modest in the long run, falling at the 20th and 80th percentile of all estimated salinity levels in the last four centuries (Figure 1.4). Yet even these extremes drove strong shifts in epifaunal community composition in San Francisco Bay. Dry years appear to promote sustained dominance by non-native solitary tunicates in large parts of San Francisco Bay, resulting in lower average community diversity and greater epifaunal biovolume. In contrast, following the wet winter of 2005–2006, decreased salinity throughout San Francisco Bay prevented solitary tunicate dominance while altering community diversity by two mechanisms. First, the apparent low tolerance of several common solitary tunicates (*Ciona intestinalis*, *C. savignyi*, and *Ascidia zara*) to decreased salinity conditions appears to prevent their dominance in wet years. Second, lower salinity conditions are highly correlated with greatly increased recruitment by several non-tunicate species, notably barnacles and mussels.

Epifaunal community diversity is highest on average during years of moderate freshwater flow, which accords with predictions of the Intermediate Disturbance Hypothesis (Grime 1973, Connell 1978). According to the Intermediate Disturbance Hypothesis, low and high levels of disturbance are expected to be less effective than intermediate levels at maintaining community diversity. Competitive exclusion by dominant species reduces diversity in low disturbance conditions, whereas high disturbance levels directly eliminate many species. Intermediate disturbance levels allow coexistence of competitive dominants and species tolerant of some disturbance (Grime 1973, Connell 1978). Here, high freshwater outflow levels are analogous to high disturbance, causing significant changes to salinity levels in the more saline portions of San Francisco Bay. Low freshwater outflow levels result in less seasonal salinity change, and less disturbance.

Species richness is depressed in both wet and dry years, but for different reasons, as predicted by the Intermediate Disturbance Hypothesis. During dry years, communities are dominated by the solitary tunicate *Ciona intestinalis*, which has previously been shown to reduce community diversity (Blum *et al.* 2007). Removals of solitary tunicates produced communities with higher diversity, indicating that space occupation by *C. intestinalis*, rather than recruitment limitation, is the major factor driving apparent low community diversity when *C. intestinalis* is abundant (Blum *et al.* 2007). Although our removal experiments were performed mainly during moderate years (2004–2006) when *C. intestinalis* was absent, similar (albeit less numerically dominant) solitary tunicate species were present, and their removal subsequently increased community diversity (Chang, unpublished data)

During the 2006 wet year, low salinity caused mass mortality among most adult organisms, simultaneously opening bare space and altering recruitment patterns. In contrast to communities typical of high salinity conditions, the communities that developed following the wetter winter of 2005–2006 were more morphologically, structurally, and phyletically diverse, but with lower biovolume. Due to mass mortality of adult organisms of most species at most sites in the Bay, it is likely that many (but not all) species of lacked sufficient numbers to produce significant amounts of new larvae even as water conditions returned to higher salinity levels. A lesser number of species recruited during the months immediately following large storms in January 2006 (Figure 1.7), and those that did were different from the species that normally recruit at this time of year (Figure 1.9). These altered patterns of recruitment persisted for months after salinity had returned to more typical levels (Chang, unpublished data).

The changes between the observed climatic extremes of dry and wet years thus appear to shift these epifaunal communities from being space-limited in dry years to being recruitment-limited in wet years. The similarity between the 5 psu treatment panels and recruitment panels deployed in the salinity tolerance experiment indicates that exposure to salinity levels that low essentially "resets" the community assembly process, starting it anew (Figure 1.11). Sites defaunated by the low salinity conditions during the wet winter of 2006 were eventually recolonized by most, but not all, of their former inhabitants. In the absence of the formerly dominant competitors, new recruits appear to have come from (1) survivors of the wet winter, (2) long-distance recruitment from elsewhere in the Bay, or (3) adult organisms newly transported to the region on boat hulls or other anthropogenic vectors. Exposure to wet conditions may have triggered some species to reproduce and recruit in greater numbers than usual. Some species also recruited in places where they normally would not (Chang, unpublished data), possibly due to transport by oceanographic processes.

Community recovery patterns following mass mortality events appeared to depend on the severity and duration of salinity decreases as well as the dispersal ability of larvae and proximity of surviving adults. Sudden, severe rainfall of relatively short duration tends to produce a layer of freshwater that floats on top of more saline waters, creating a refuge below the freshwater lens for many organisms that would otherwise be killed (Smith and Witman 1999, Rutger and Wing 2006, McLeod and Wing 2008). In the absence of further disturbance or predation, such short events may be followed by relatively swift recolonization of the denuded areas (*e.g.,* MacGinitie 1939, Goodbody 1961). However, with more sustained or greater outflow volume, the freshwater lens can occupy the entire water column, especially in shallower areas, effectively pushing saline waters out of significant portions of an estuary and removing these depth refuges. While our observations indicate that the resident adult community at many mid-Bay sites was essentially eradicated by low salinity conditions following the wet winter of 2005–2006, mortality appeared greater at shallower depths and in shallower marina basins (Chang, unpublished data). Over time, species that were formerly present may return to defaunated areas via stepping-stone recruitment from deeper refuges and locations closer to the mouth of the Bay where salinity levels did not decrease as much during low salinity events.

Our results agree with those of Ritter *et al.* (2005), who used a series of short-term defaunation experiments of varying durations to simulate the effects of salinity stress-induced mortality on infaunal community development. Ritter *et al.* (2005) found that the duration of defaunated sediment sample deployment (between 2 and 8 weeks) and environmental conditions at the time of deployment were less important in explaining observed community patterns than environmental conditions at the time of sample retrieval. This pattern resulted from dominance of samples in all treatments by recent recruitment to the samples from surrounding communities, similar to recruitment dominance of the 5 psu treatment panels in our salinity tolerance experiment. Here, we extend these results by showing that environmental conditions during the preceding winter can significantly affect the following summer's community development. Generally similar conditions occurred during the summer months of each year, but community development differed due to differential recruitment linked to the previous winter's outflow levels, which determined whether adults in the community survived or died.

Although a number of water column variables change in response to increased outflow levels during storms, including turbidity and contaminant levels, salinity is the most directly affected and easily measured of these. Salinity has long been known to be a physiological determinant of an organism's ability to inhabit a given area (Remane and Schlieper 1972). Our salinity tolerance experiment showed that decreased salinity levels alone are sufficient to cause widespread mortality in the epifaunal community. Aside from salinity, increased suspended sediment levels during periods of high outflow may also significantly affect epifaunal communities. However, in the San Francisco Estuary, high outflow is not always correlated with high suspended sediment concentrations, nor do high suspended sediment concentrations always occur during periods of high outflow (Powell *et al.* 1989). In addition, previous work examining the tolerance of estuarine organisms to high suspended sediment concentrations has shown that two exotic estuarine solitary tunicates taken from San Francisco Bay, *Molgula manhattensis* and *Styela clava*, were able to tolerate continuous exposure to 100,000 mg/L concentrations of clay for 12 days with just 10% mortality occurring (McFarland and Peddicord 1980). The most sensitive species in the study by McFarland and Peddicord (1980), the California coastal native solitary tunicate *Ascidia ceratodes*, suffered just 10% mortality after 4 days' continuous exposure to a lower suspended sediment concentration of 7000 mg/L. To place these values in context, the highest recorded suspended sediment concentrations at the USGS Point San Pablo monitoring station in San Francisco Bay from 1990–2006 was 1582 mg/L following a major storm in January 1997, and this concentration showed high daily variation due to tidal influences (USGS 2009a). Nevertheless, any negative effects of high suspended sediment concentrations on the epifaunal community are likely to add to deleterious effects of decreased salinity. Our salinity tolerance experiment could thus be considered a conservative test of epifaunal community tolerances for outflow changes in resulting from storms.

While higher salinity conditions (*i.e.* 30+ psu) are prevalent near the mouth of the San Francisco Estuary, sites in this region are generally not dominated by solitary tunicates, suggesting that the high abundances of solitary tunicates in the mid-Bay during dry years are related to additional factors other than salinity levels. One likely factor in mid-Bay solitary tunicate dominance is the higher maximum temperatures reached in the mid-Bay compared to the mouth of the Bay (USGS 2009a), as solitary tunicate reproduction and recruitment are constrained in part by temperature and salinity conditions (*e.g.,* Dybern 1965, Thiyagarajan and Qian 2003, Clarke and Therriault 2007). While high salinity conditions are most prevalent near the mouth of the Bay, solitary tunicates are somewhat uncommon. This may be due to lower recruitment, as this region has a lower maximum temperature (compared to the mid-Bay) as a result of greater ocean influence. Alternatively, it is possible that solitary tunicates do recruit in high abundance near the mouth of the Bay, but are susceptible to greater predation pressure by marine species that cannot tolerate the more estuarine conditions upstream.

Recent anthropogenically-linked increases in drought frequency and severity, coupled with increasing frequency and intensity of storms (Gutowski *et al.* 2008), create an estuarine environment that is at once more constant on annual time scales (Knowles 2002), yet increasingly variable in the long run (Figure 1.6; Knowles and Cayan 2002). Water diversions for agriculture and drinking water use in the Central Valley and southern California have also significantly lowered and smoothed the Bay's hydrograph, such that salinity levels remain higher and less variable for longer periods of time each year during the spring and early summer (Knowles 2002). Alterations to freshwater flow entering San Francisco Bay with projected warming are forecasted to further increase spring and summer salinity levels (Knowles and Cayan 2002). On shorter time scales of several months, the salinity conditions in dry and wet years favor vastly different estuarine communities; the epifaunal species studied here thus provide a sensitive indicator of biological responses to changing water column conditions.

Consequences of Changing Hydrological Regimes for Ecosystem Function

Longer and more severe droughts will likely promote sustained dominance by solitary tunicates in significant portions of San Francisco Bay, which may have community-level diversity effects as well as consequences for ecosystem function (see Chapter 2). In the summer of 2008, a dry year, 7 out of 10 sites surveyed in San Francisco and San Pablo

Bays were dominated by solitary tunicates; *Ciona intestinalis* was the dominant taxa at 6 of these 7 sites. While previous research has demonstrated the ability of *C. intestinalis* to depress community diversity levels (Blum *et al.* 2007), *C. intestinalis* is also a capable filter feeder (Lesser 1992). Other benthic primary consumers such as the introduced clam *Corbula amurensis* have been shown to have a tremendous impact on phytoplankton levels in the estuary (Kimmerer *et al.* 1994). While the impact of *C. intestinalis* on planktonic communities likely does not approach that of *Corbula*, high abundances of this and similar species are likely to have at least a significant local impact on water column particulate and plankton concentrations. In addition, transitions from dry to wet years would likely alter community filtration abilities significantly as high densities of barnacles and mussels replace solitary tunicate populations, such as was observed following the wet winter of 2006.

Byrnes and Stachowicz (2009) suggested that community dominance by a single invader would likely result in unpredictable changes in any given ecosystem function. Community dominance by *Ciona intestinalis*, such as happens during dry years, is correlated with tremendous seasonal fluctuations in both biomass and community diversity. In San Francisco Bay, *C. intestinalis* has a propensity for recruiting in high numbers (upwards of 400+ recruits per 14 × 14cm panel), rapidly reaching prodigious sizes (adults up to 9 inches long have been measured after 3 months in the summer), and then suddenly synchronously sloughing off the substrate (Chang, unpublished data). Sloughing events appear most related to the accumulated weight of the animal outstripping the strength of its attachment to the substrate (Chang, pers. obs.).

The mass occupation and sudden release of space caused by *Ciona intestinalis* blooms significantly influences the cold weather availability of space. As *C. intestinalis* often recruits in high numbers concurrent with sloughing events, rapid recolonization by juvenile *C. intestinalis* occurs within two weeks at warmer temperatures. In cooler temperatures, juvenile *C. intestinalis* grow more slowly, allowing taxa that preferentially recruit at cooler temperatures to gain a temporary foothold on primary space. Unlike mussels and barnacles, *C. intestinalis* and *C. savignyi* (which is more abundant in moderate outflow years) are generally not settled on by other organisms, so fluctuations in *Ciona* spp. abundance as well as shifts between dominant species also may significantly affect the persistence of other species in the community (see Chapter 2). Examinations of communities over longer time scales would help reveal any effects of *C. intestinalis* on the stability of community assemblages at time scales longer than the annual life span of most epifaunal organisms.

Although relatively few quantitative descriptions exist of storm or drought-related effects on estuarine communities, a number of qualitatively similar accounts of significant alterations to community composition have been observed in other estuarine and marine systems in times of drought and flood. Mass mortality resulting from extraordinarily high freshwater flow events has been documented in both epifaunal and infaunal marine and estuarine communities on several different coasts in both temperate and tropical climates. Broadly similar sessile epifaunal communities in California, Texas, Chesapeake Bay, and Jamaica all exhibited mass mortality in response to severe rainfall (MacGinitie 1939, Gunter 1955, Hoese 1960, Goodbody 1961, Andrews 1973, Nydam and Stachowicz 2007).

In each case shell-less and tube-less epifaunal invertebrates were found to be most susceptible to low salinity pulses, a group that includes tunicates and bryozoans, two of the numerically dominant groups in all cases. Bivalves and gastropods that could withdraw into a shell, and tubicolous polychaete worms that could seek refuge in a tube were generally less affected, but did suffer extensive mortality with protracted low salinity events (MacGinitie 1939, Andrews 1973).

Mortality and altered recruitment effects of low salinity events are not limited to epifaunal communities. Working in the much larger Chesapeake Bay, Andrews (1973), Boesch *et al.* (1976), and Orth (1976) described high mortality and altered recruitment patterns in both epifaunal and infaunal communities following Tropical Storm Agnes in 1972, much as was observed following the 2006 wet winter in San Francisco Bay. Interestingly, many of the same species found in Andrews' 1973 study were also found in San Francisco Bay, and although a number of these are native to the Chesapeake Bay but introduced to San Francisco Bay, similar responses to low salinity were observed in each case. Hoese (1960) documented analogous patterns in both infaunal and epifaunal molluscs in a central Texas estuary following heavy rains that marked the end of a nine-year drought. Likewise, Chollett and Bone (2007) showed similar patterns for seagrass meadows and their associated infauna in Venezuela, while Montagna and Kalke (1992) documented changes in meiofaunal and macrofaunal infauna in two Texas estuaries in response to freshwater flow variation.

Dominance of exotic taxa and paucity of native estuarine taxa

Significantly, every species of solitary tunicate and 98% of all animal taxa recorded here are not native to San Francisco Bay. An exception was the barnacle *Balanus crenatus*, one of the few definitively native species, which was more abundant immediately following the wet winter of 2006 than in other years. While the dominance of introduced species may be partly a function of habitat (floating docks and pier pilings being relatively unusual habitat, *e.g.*, Glasby 1999), North American Pacific coast estuaries have long been noted for a paucity of native species, with the highest native species diversity occurring near the mouths of estuaries (Hedgpeth 1968, Carlton 1979, Cohen and Carlton 1995). Native taxa near the mouth of the Bay or entering the Bay from the outer coast may be better adapted to the colder, more saline conditions there and may be less able to tolerate the warmer, more variable salinities found upstream. For example, Tomales Bay and Bodega Harbor are two nearby embayments with lesser salinity fluctuations than San Francisco Bay, and both have a number of native species absent from San Francisco Bay (Chang and Kimbro, unpublished data; J. Stachowicz pers. comm.). Salinity levels near the mouth of the Bay do remain high for extended periods of time during multi-year droughts, but our results indicate that the occasional occurrence of low salinity pulses would likely eliminate any native outer coast taxa that manage to disperse into the Bay (in addition to affecting non-native species within the Bay). Overall, the high level of invasion and the paucity of native estuarine fauna in the Bay mean that community and ecosystem dynamics are governed by fluctuations in non-native species abundances (Cohen and Carlton 1995, Nichols and Thompson 1985).

Very large floods may set the stage for subsequent invasions by non-native species by extirpating any resident species that might resist incipient invasions, a notion proposed by Hedgpeth (1979, 1993). He specifically cites the Great Flood of 1862, which dwarfs any other outflow event in San Francisco Bay since the advent of Western colonization in 1849. Retrospective analyses of the 1862 flood show that outflow during this flood rivaled that of the Amazon River (120,000 m^3/s in 1862, Peterson *et al.* 1985) and is over six times greater than any value measured in the last fifty years (17,825 m^3/s in 1986, based on daily Net Delta Outflow (USGS 2009b)). Nichols *et al.* (1990) suggested that this high outflow level in 1986 may have set the stage for the invasion of *Corbula amurensis* to San Francisco Bay by removing many potential benthic competitors. Here, we have shown that even the relatively modest wet year of 2006 (maximum flow 10,432 m^3/s) resulted in significant community mortality, increased space availability, and altered recruitment patterns long after water column conditions had returned to more normal conditions, suggesting that an inoculation of non-native species during this time period might enhance the likelihood of successful invasion.

Historical and Future Conditions

Studies examining the ecological effects of extreme climate events generally have a small number of observations of extreme events (often just one), look at one climatic extreme rather than opposite extremes, and often fail to place their results in context by comparing their observed climatic extremes to the historical record. Here, we documented the ecological effects of both wet (2006) and dry (2007–2008) conditions over an eight-year period from 2001–2008, which were are among the more extreme wet and dry conditions experienced by the San Francisco Bay region during the past several decades. While historical fluctuations of much greater magnitude have occurred in the past millenium, such as the Great Flood of 1862 (Hedgpeth 1993) and a long drought during medieval times (Stine 1994), our results show that even the moderately severe extremes observed during the relatively short time span of this study were enough to cause significant changes to communities throughout the Bay.

Although watershed modification and management have significantly affected freshwater outflow regimes, the dominant influence on outflow is still climatic forcing (Stahle *et al.* 2001, Knowles 2002). The trends of increasing variation evident in long-term time series of precipitation and reconstructed salinity suggest that we will see increasing variation in outflow in the future (Figures 1.5, 1.6). We speculate that greater outflow variation will in turn cause communities to toggle rapidly from year to year between the extreme states of dominance by non-native solitary tunicates in dry years and mussel-barnacle communities present in wet years. The shorter period between extreme low salinity events will reduce recovery times for resident species, particularly affecting those that require longer recovery times. Rare and less tolerant species are likely to be lost. As communities were most diverse in moderate years this study, a reduction in the occurrence of moderate years in favor of more extreme years seems likely to result in the loss of species that do best in those years. The reduction in resident diversity also may present greater opportunities for invasions, particularly by euryhaline non-native species. Further investigation is of critical importance, as the fluctuations documented here are likely to become increasingly frequent and extreme in the coming years.

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CHAPTER 2. Environmental conditions mediate the effect of functional group diversity on community functioning

ABSTRACT

The degree to which local diversity versus functional identity of resident species dictates the productivity and invasibility of communities is a critical issue for ecologists. Most tests of the effects of diversity on ecosystem function are relatively short, on the order of weeks, and are usually not replicated through time, hindering our ability to determine whether the results are generally applicable. In addition, while most experimental work testing biodiversity effects on ecosystem function has examined species diversity effects, the importance of other types of diversity is increasingly recognized.

I present the results of several manipulative experiments assessing the roles of functional group diversity and identity in the assembly of epifaunal communities in Richmond, San Francisco Bay, USA. These experiments were conducted across three separate years that varied in environmental conditions and community composition. I used removal experiments incorporating both settlement and post-settlement processes in the life cycle of resident species to test whether communities composed of varying levels of functional diversity and composition differed in space occupation. I then assessed whether these different levels of resource use among communities translated into changes in a related emergent function, the resistance of communities to subsequent invasion by species not already present. These invasion resistance tests integrated both settlement and survival of the invading species.

Communities containing more functional groups generally occupied more space, although the strength of this effect varied from year to year. Resident community functional group diversity had no effect on subsequent invasion by non-resident species, apparently due to the absence of high-performing residents that had previously suffered high mortality due to a large environmental disturbance. Results of short-term, "snapshot" studies of biodiversity-ecosystem function relationships are thus likely to be contingent on prevailing conditions, suggesting that caution must be used in interpreting the results of studies of short duration or which are not replicated among years.

INTRODUCTION

Ecologists have long been interested in the causes and consequences of diversity for community structure and functioning, including the relationship between a community's pre-existing diversity levels and its susceptibility to subsequent invasion by non-resident species (Elton 1958, Tilman 1997, Loreau *et al.* 2001, Stachowicz *et al.* 2002, Cardinale *et al.* 2006). Most work has focused on species diversity, and while much research has been spurred by recent recognition that other sorts of diversity such as functional group diversity may play key roles in community assembly and functioning, most of these studies were short-term and were not replicated through time (*e.g.,* Hooper and Vitousek 1997, Fargione *et al.* 2003, Von Holle and Simberloff 2005). Relatively little is known about how biodiversity-ecosystem function relationships vary over time or in response to environmental changes.

One specific community function that has generated much interest is invasion resistance, or the ability of a resident community to repel invasion by non-resident species

(Elton 1958). Elton (1958) proposed that more speciose communities would be better able to resist invasion by virtue of more complete resource use. A contrarian view posed by Stohlgren *et al.* (1999, 2003), among others, is that more speciose communities would be more invasible because these communities possess a wider range of resources that could potentially benefit invading species as well as existing residents. Many recent studies have attempted to test these and similar hypotheses using both experimental and observational approaches in a variety of conditions (*e.g.,* Levine 2000, Von Holle and Simberloff 2005, Stachowicz *et al.* 2008). At small spatial scales, experimental work showed reduced invasion success in the face of increased diversity, while observational evidence supported the opposite trend of increased invader diversity with higher diversity levels at large spatial scales (Stohlgren *et al.* 2003). Several attempts have been made to reconcile these apparently conflicting observations, suggesting that the explanation may lie in spatial heterogeneity and the operation of different processes at small versus large spatial scales (Shea and Chesson 2002, Davies *et al.* 2005).

Many biodiversity-ecosystem function experiments involve the construction of communities of regularly varying levels of diversity whose performance is then tested in some function, such as productivity (or biomass), nutrient cycling, or invasion resistance (Tilman *et al.* 1996, Emmerson and Raffaelli 2000, Hector *et al.* 2002, Cardinale *et al.* 2006, Stachowicz *et al.* 2008). The various levels of community diversity used in such experiments are generally created either through synthetic assemblage or via removal treatments (Diaz *et al.* 2003). Terrestrial studies have chiefly used grasslands or similar systems in which communities can both be easily constructed and "invaded" using the seeds of the desired plants (*e.g.,* Tilman 1997, Symstad 2000). One difficulty with such experiments is that only the very largest experiments can test most of the species in the community, and for logistical reasons, higher diversity treatments must choose only selected random combinations of species (*e.g.,* Tilman *et al.* 1996, Tilman 1997). Most experiments are only able to test a relatively small number of species or a limited proportion of the species pool. For example, Stachowicz *et al.* (2002) constructed marine invertebrate communities on fouling panels, creating mosaics of specific species richnesses and compositions with a maximum of four species per panel.

Grouping species according to their functional properties and then manipulating the resulting groups may be a way of more completely assessing performance of the community and its constituent components. This approach collapses individual species properties into a much smaller number of general functional types, the manipulation of which can allow the experimenter to work with a greater proportion of the community while keeping the size of the experiment manageable. Given that the prevailing mechanistic hypothesis for invasion resistance is that more speciose communities reduce invader success by using resources more completely, it stands to reason that the diversity of functional types in the community may matter more than the number of species per se (Arenas *et al.* 2006). Existing evidence indicates that both the number and identity of functional groups in a community can be important to overall performance (*e.g.,* Tilman *et al.* 1997, Symstad 2000, Dukes 2001, Arenas *et al.* 2006, Cardinale *et al.* 2006).

Most existing diversity–function experiments also suffer two shortcomings having to do with time. First, most experiments are short-term, lasting only a few weeks (but see

Tilman *et al.* 1996, 1997; Stachowicz *et al.* 2008). Short-term experiments may not account for temporal complementarity between species or groups (Stachowicz *et al.* 2007). Second, few such experiments are replicated through time and thus cannot account for year-to-year changes in community composition or environmental conditions (but see Bruno *et al.* 2005). While the same species are not always present in otherwise similar communities in a region at different times or places, those using resources in a similar manner would presumably have similar effects on community structure and functioning.

Here, I present the results of several manipulative experiments assessing the roles of functional group diversity and identity in the assembly of San Francisco Bay fouling (marine and estuarine hard substrate-colonizing) communities. These experiments were conducted across three separate years that varied in environmental conditions and community composition. I used removal experiments incorporating both settlement and post-settlement processes in the life cycle of resident species to test whether communities composed of varying levels of functional diversity and composition differed in space occupation. I then assessed whether these varying levels of resource use among communities translated into differences in a related emergent function, the resistance of communities to subsequent invasion by species not already present. These invasion resistance tests integrated both settlement and survival of the invading species.

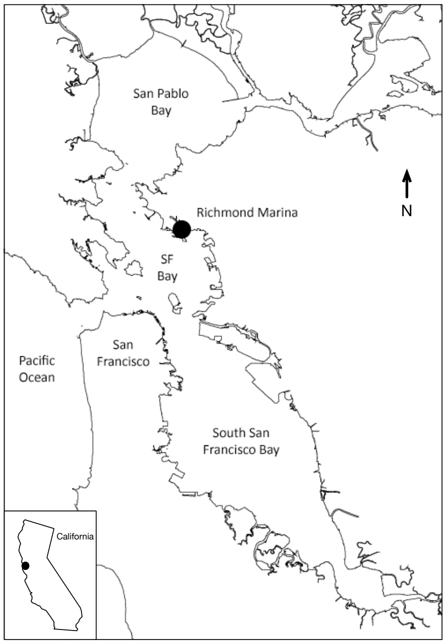
I hypothesized that communities containing more functional groups would perform more consistently, both in use of the space resource and in resisting invasion by non-resident species. Conversely, communities containing fewer functional groups would show greater variation in performance. Second, I hypothesized that the identity of the particular functional groups present would play a larger role in the community's performance than functional group or species diversity per se.

METHODS

Study System

All work described here was conducted at the Richmond Marina in the northeastern part of San Francisco Bay, one of the largest estuaries on the Pacific Coast of North America (Figure 2.1; 37°54′41″N 122°21′05″W). This site was chosen because of its location in the mesohaline region of the estuary (average salinity 20–30 psu), allowing me to easily examine the influence of salinity changes on epifaunal communities. Previous work at this site showed significant interannual fluctuations in community composition, suggesting a high likelihood of encountering different community compositions if an experiment was replicated in several different years.

Figure 2.1: Map of San Francisco Bay showing location of the Richmond Marina study site



Sessile epifaunal invertebrate ("fouling") communities are an excellent system for experimental manipulation and have been used in numerous investigations of diversity effects (*e.g.,* Sutherland 1974, Stachowicz *et al.* 2002, Johnston and Clark 2007). The organisms in these communities have short generation times, relatively short lifespans, have high diversity on small spatial scales, and are relatively small and easily manipulated. The sessile epifaunal invertebrates of the San Francisco Estuary include tunicates, bryozoans, mussels, barnacles, and numerous other groups, and are typical of those found in protected estuaries and sheltered rocky shores in temperate zones, with tunicates and bryozoans generally most abundant. Tunicates generally grow as either larger solitary individuals or colonies composed of smaller individuals. Solitary tunicates such as *Ciona* spp. and *Ascidia zara* are often numerically dominant. Bryozoans can be calcified and grow as colonies in either erect, arborescent forms or encrusting, sheet-like forms. Nearly all are filter feeders capable of removing very small particles from the water column, though bryozoans and tunicates generally prefer larger particles (> 15μm) (Lesser *et al.* 1992), while mussels and barnacles can filter a wide range of particles from very small (< 5μm) to large zooplankton (Anderson 1994). Most common species in San Francisco Estuary epifaunal communities are non-native; relatively few native estuarine epifaunal invertebrates exist in Pacific coast estuaries (Cohen and Carlton 1995). The vast majority of these species have approximately annual life cycles; most live for several months, with only barnacles and mussels typically living longer than one year.

As space has long been recognized as one of the main limiting resources in fouling and other epifaunal communities (*e.g.,* Dayton 1971, Buss and Jackson 1979, Connell and

Keough 1985, Altman and Whitlatch 2007), I classified San Francisco Bay fouling species into functional groups according their mode of space occupation and growth form. I defined three general functional groups: (1) "spot-type" space holders that generally have a single point of attachment that changes relatively little as the organism grows, (2) "arborescent organisms" that generally attach to the substrate with rootlets or runners, and whose primary space occupancy increases with growth, and (3) "laterally-spreading" species where growth generally occurs by spreading over the primary substrate (hereafter referred to as SPOT, ARB, and LAT, respectively). Spot-type organisms include solitary tunicates, mussels, and barnacles. Arborescent organisms include typical branching taxa like bryozoans, hydroids, and most algae. Laterally-spreading species include colonial tunicates, encrusting bryozoans, and encrusting sponges.

I monitored temperature and salinity at Richmond Marina for the duration of this study, using iButton data loggers (Maxim IC Corp., model 1921G-F5) to record temperature at hourly intervals at 1 m and 4 m depth from June 2004 until August 2006, and Hobo Pendant loggers (Onset Computer, model UA-002-64) from August 2006 until January 2008. I used Star-Oddi DST-CT data loggers to record salinity at 5 minute intervals at 1 m depth from January 2005 until January 2008. Data from loggers were supplemented by and checked against temperature and salinity profiles taken at 1 m depth intervals at least twice weekly using a YSI-85 multimeter from June 2004 until August 2006, and once or twice monthly from August 2006 until January 2008. Monthly averages of temperature were calculated from ibutton and Hobo Pendant logger data. Monthly averages of salinity were calculated from YSI profile data and Star-Oddi DST logger data.

The local climate regime is Mediterranean in nature, with wet winters and dry summers. Like many large estuaries, the San Francisco Estuary has a persistent salinity gradient that is spread over a relatively large spatial area (tens of kilometers) and which fluctuates in response to changes in freshwater flow, typically as a result of precipitation and snowmelt (Conomos *et al.* 1985). I classified years from the current study (2001–2008) as Wet, Dry, or Moderate based on daily average Net Delta Outflow of the previous winter and spring (November to May). Net Delta Outflow is a measure of the total volume of freshwater outflow from the Sacramento–San Joaquin Delta into San Francisco Bay. Net Delta Outflow data were obtained from the California Data Exchange Center (USGS 2009). Using this scheme, 2001, 2002, 2007, and 2008 were classified as Dry (average Net Delta Outflow < 20,000 cfs); 2003, 2004, and 2005 were classified as Moderate (average Net Delta Outflow 30,000 cfs); and 2006 was classified as Wet (average Net Delta Outflow = 77,600 cfs).

Recruitment

Sessile invertebrate recruitment varies both seasonally and interannually, and is affected by environmental perturbations such as winter storms (see Chapter 1). I assessed these variations by measuring monthly recruitment of sessile epifaunal invertebrates at Richmond Marina from June 2004 to January 2008 using 14 cm × 14 cm × 0.5 cm square grey PVC panels. One face of each panel was lightly sanded and acted as the collecting surface. Two panels were randomly distributed within each of five blocks throughout the marina, with one panel deployed at 1 m depth and the other at 4 m depth. Each panel was attached to a brick for weight and suspended from a rope tied to a floating dock, with the collecting surface facing down.

Panels were left in place for four to six weeks to allow invertebrate settlement on the collecting surface, then retrieved and replaced with new, blank panels at the same location. Recruitment as measured here thus includes both settlement and post-settlement processes, including mortality, that occurred during the entire four to six week deployment period. After retrieval, panels were analyzed for percent cover and species composition. To estimate percent cover of dominant taxa, a grid of 100 points was placed over each panel and the taxon attached to the panel at each point (*i.e.* the "primary" cover organism) was identified to the lowest possible taxonomic level using a dissecting microscope at 40×. If other organisms were growing on top of the primary cover organism at a point, these "secondary cover" organisms were identified and recorded. Total percent cover was the sum of primary and secondary cover and could thus exceed 100%, though this rarely occurred. A complete species inventory was then taken, removing organisms from the panel to ensure accurate identification if necessary. Identifications were made using criteria described in Appendix II and Carlton (2007).

In addition, I measured recruitment and growth of invertebrates on panels that were deployed for three-month durations during the summer months (generally June to September) of 2001–2008. Similar procedures to those described above were used for analysis.

All statistical analyses were performed using the R Environment for Statistical Computing (R Development Core Team 2009).

How do functional group richness and composition affect resource use?

Previous work indicated that one solitary tunicate species, *Ciona intestinalis*, can significantly depress community diversity when present in high abundance (Blum *et al.* 2007), but it was not known if other SPOT group organisms have similar effects, or how these effects compared to other structural components of the community. I therefore compared panels from which I experimentally removed various functional groups to panels with naturally-occurring densities of these groups. Three separate experiments were carried out at Richmond Marina from June 2004 to August 2006 (hereafter referred to as 2004, 2005, and 2006 experiments, according to the year in which they began; Table 2.1).

On 27 June 2004, I deployed 30 panels at 1 m depth in a two-way factorial design arranged in randomized complete blocks. The marina was divided into six blocks, each of which contained five randomly distributed panels. Each panel in a block was assigned to one of five treatments, which comprised three target group removal treatments, an unmanipulated control, and a manipulated control. Target group removals consisted of removing each panel from the water, submersing it in a tub of seawater on the dock and removing all visible target group members with forceps, then returning the panel to the water. Target group removals generally took about 20 minutes per panel. I made no attempt to control the number of species within each functional group, and therefore could not separate the effect of species richness from functional group richness. Assemblages of higher functional group diversity generally also had higher species diversity, although the exact species number and identity differed from panel to panel. In this experiment, only SPOT, ARB, and SPOT+ARB group removal treatments were conducted, creating ARB+LAT polycultures, SPOT+LAT polycultures, and LAT monocultures, respectively. Manipulated control panels (hereafter referred to as 3-group polycultures) were treated identically to target group removal panels, except nothing was removed, accounting for any artifacts of the manipulation other than removing solitary tunicates. Unmanipulated control panels (hereafter referred to as control panels) were not removed from the water except to take monthly photographs (Table 2.1). Treatments were performed every two to four weeks until October 2004. Communities in each treatment were thus allowed to develop essentially in the absence of the functional group(s) targeted for removal. Panels were analyzed for percent cover and species composition after 16 weeks (October 2004).

A second experiment (“2005 experiment”) with *n* = 8 replicates per treatment (arranged into the same six blocks used in the 2004 experiment, plus two more) was begun on 18 July 2005. In this experiment, I created a full factorial set of monocultures of each functional group as well as their respective combination polycultures, for a total of 8 treatments. 3-group polycultures and control panels were used as in the 2004 experiment (Table 2.1). Panels from the 2005 experiment were analyzed for percent cover and species composition after 16 weeks (November 2005).

A third experiment (“2006 experiment”) with *n* = 8 replicates was begun on 10 February 2006 and lasted until 24 August 2006. This experiment was identical to the 2005 experiment in design, possessing a full factorial set of monocultures and polycultures, with the addition of a biomass removal control treatment, for a total of nine treatments

(Table 2.1). One concern with removal experiments is that the removal of material will create a local disturbance that is confounded with the diversity treatment, so I tested for this using a treatment in which I removed a random sample of organisms whose total mass equaled the greatest amount of mass removed in any removal treatment. This biomass removal treatment mimicked the effect of removing randomly selected organisms equal in biomass to the greatest amount of biomass removed from any single treatment, allowing me to assess the effect of disturbances associated with performing removal treatments. Panels were analyzed after 20 weeks (July 2006).

I used regressions to evaluate the effect of experimentally-manipulated functional group richness levels on total cover of organisms per panel. In addition, I assessed the relationship between species richness and total cover. While I did not directly manipulate species richness, this analysis helps indicate the degree to which observed total cover might simply be a function of species richness. Because cover at a given richness level could vary for reasons unrelated to richness, I focused on the relationship between richness and minimum cover using quantile regression, a method of estimating the functional relationships between variables for all portions of a probability distribution (Koenker and Bassett 1978). Here, I used regression quantiles based on a weighted absolute deviance model, which is robust to outliers and non-normality in the data (Cade *et al.* 1999). I used the "boot" option of the `rq` procedure in the R package `quantreg` to create bootstrapped estimates of regression slope and intercept at the 10th quantile using 10,000 bootstrapped replicates (R Development Core Team 2009). I selected the minimum percentile that could be used to approximate the lower limit of total cover following the conservative procedure outlined in Scharf *et al.* (1998), whereby the maximum quantile (q) to be examined is selected based on $n > 10 / (1 - q)$ for n samples.

I then used one-factor ANOVAS to examine the contributions of functional group identity and diversity to the overall treatment effect on total cover. Following Bruno et al. (2005) and Duffy et al. (2005), I partitioned the effect of functional group treatment into variation due species identity (*i.e.* variation among monocultures) and variation between monoculture and the 3-group polyculture (*i.e.* diversity effects).

I calculated the average value of the D_{max} statistic for each treatment to test for transgressive overyielding, a condition in which a mixture outperforms the best performing monoculture of any component of the mixture (Loreau 1998). Transgressive overyielding indicates the operation of some form of complementarity between species or groups in the mixture, such that the dominant species or group alone cannot account for the performance of the mixture (Loreau 1998, Schmid *et al.* 2008). D_{max} is essentially the proportional deviation of the total mixture yield from the highest performing component functional group's yield in monoculture:

$$D_{max} = \frac{O_T - Max(M_i)}{Max(M_i)}$$

where O_T is the observed total cover of the polyculture, and $\max(M_i)$ is the maximum total cover in monoculture of any component of the polyculture (Loreau 1998). D_{max} was calculated for total cover for all polycultures in the 2005 and 2006 experiments, but not for the 2004 experiment, which lacked the full complement of treatments (*i.e.* some monocultures) required to calculate D_{max}.

Table 2.1: Treatments, duration, and response variables of functional group experiments

	Duration (weeks)	S	A	L	SA	SL	AL	SAL	CON	BIO	COVER	INVASION
2004	16			×		×	×	×	×		×	
2005	16	×	×	×	×	×	×	×	×		×	
2006	20	×	×	×	×	×	×	×	×	×	×	×

The invasion portion of the 2006 experiment occurred after the rest of the experiment. S = SPOT group present, A = ARB group present, L = LAT group present; SA, SL, AL, SAL are combinations of these groups, CON is unmanipulated control, BIO is biomass removal control, and COVER is the total cover response variable.

How do functional group richness and composition affect invasion resistance?

A severe winter storm in January 2006 caused large declines in salinity throughout San Francisco Bay, resulting in significant die-offs of many species, including several that normally are very abundant (see Chapter 1). I took advantage of this situation to see if communities that developed in the absence of those normally dominant species could repel subsequent settlement of those species. I ceased maintenance of diversity treatments in the 2006 experiment, allowing all panels to experience natural levels of settlement and mortality. Experimental treatments were not maintained after 24 August 2006, and communities were then assessed 4–5 months later. Here, I use “invader” to refer to subsequent recruitment of species that either were not previously present in the community or were only present at very low levels (< 5% cover). In this sense, the term is not related to the evolutionary origin of the species in question (*i.e.* whether it is native or non-native to the San Francisco Bay region). The same pool of species served as invaders for all treatments. Naturally-occurring recruitment during the period following cessation of removal treatments was assessed by deploying blank panels ($n = 5$) on 24 August 2006, then analyzing for percent cover and species composition when the experimental panels were analyzed.

I used quantile regressions to assess the relative success of species invading the experimental communities, focusing on the maximum level of invader success for each diversity level using 90th regression quantiles. In this case, maximum invader success is hypothesized to be constrained by resource availability, which in turn depends on resident community diversity. When resident diversity is high, few resources are available, and invader success will be low. Invaders could potentially be much more successful at low levels of resident diversity when many more resources are available. However, under those conditions, factors other than resident diversity (and resource pre-emption), such as predation or environmental factors, may prevent invaders from reaching their maximum potential success (Cade *et al.* 1999, Brown and Peet 2003).

The performance of the resident community in rejecting invaders can be due to the strong effects of a single group (*i.e.* sampling effect) or due to species complementarity, in which the combined effect of resident groups is greater than that of any one component group. I assessed these effects by calculating D_{min} for all polycultures in the 2006 experiment using percent cover of invaders as a response variable. Greater performance by the resident community here results in lower invader cover; the relevant question is thus how much the resident community can depress invader success. In this adaptation of Loreau (1998)'s formula, the quantity of interest is the minimum of invader cover in a polyculture, so the observed invader cover in a polyculture is compared to the minimum invader cover achieved by any component of the polyculture when in monoculture (*e.g.*, Stachowicz *et al.* 2002). Values less than zero would indicate transgressive overyielding.

RESULTS

Monthly Recruitment Surveys

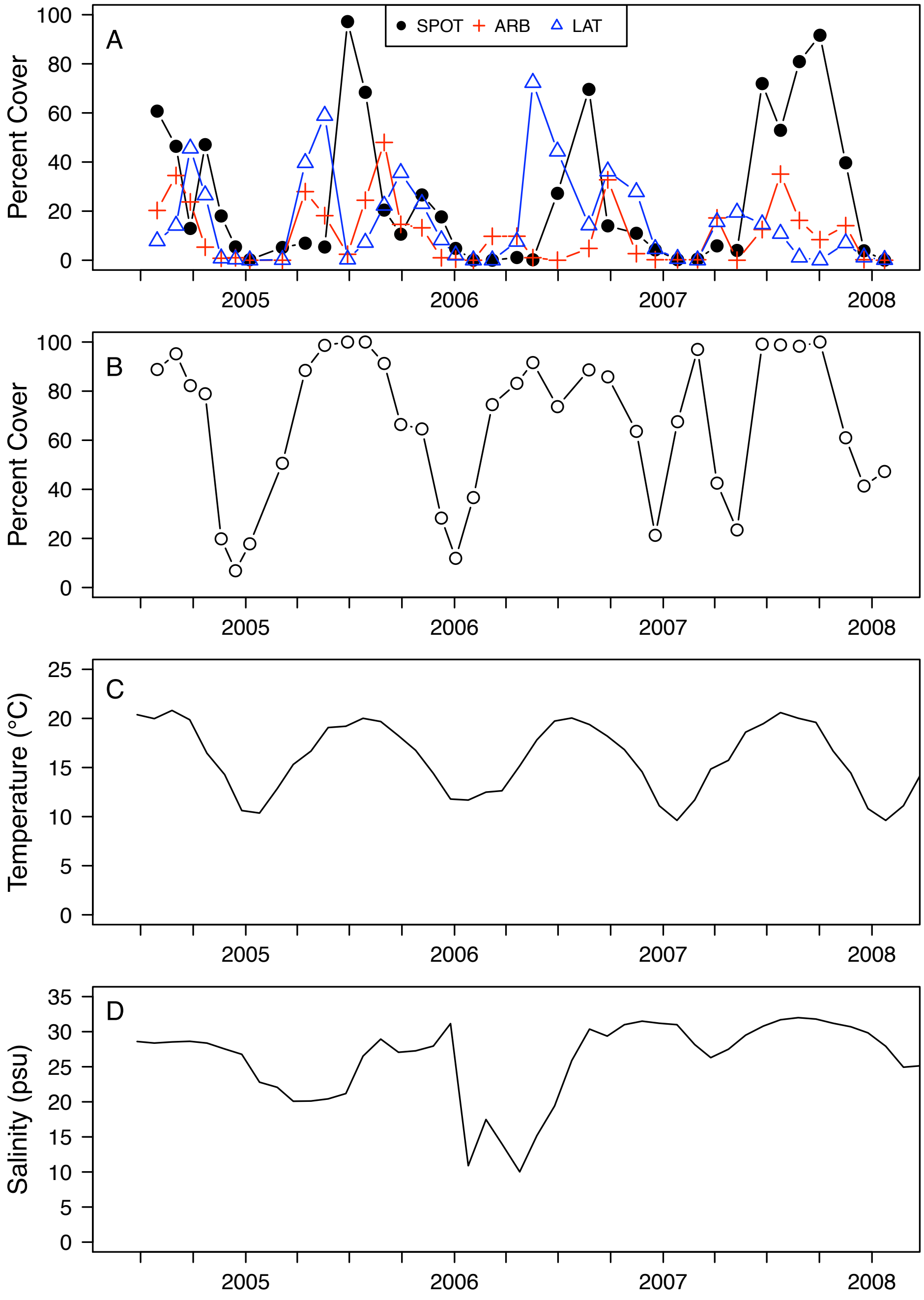
Space occupation approached 100% on monthly recruitment panels in late summer and early fall each year, indicating both dense settlement as well as rapid growth coinciding with annual maximum temperatures (Figure 2.2a). Complementary peaks of settlement were evident for the duration of the monthly recruitment survey (Figure 2.2a). The SPOT group showed maximal percent cover during periods of high salinity and high temperature, which occurred during late summer and early fall (Figure 2.3a). LAT group cover was highest on monthly recruitment panels during somewhat cooler temperatures and lower salinity levels in late spring. ARB group cover showed bimodal peaks, one in late spring and one in late fall. However, when considering all monthly survey panels together, regression analyses showed no relationship between total cover and species richness (OLS regression: adjusted $R^2 = -0.017$, $p = 0.5386$; 90th quantile regression: $t = 0.43$, $p = 0.6696$). Within each functional group, different species were dominant at different times and during different years (Figure 2.3). Total cover of each group was dominated by one or two species each year, though not necessarily the same species every year (Figure 2.3).

A large decrease in salinity in the first half of 2006, caused by severe winter storms in January 2006, produced the lowest salinity readings in San Francisco Bay during the past ten years (1999–2009; approximately 5 psu at Richmond Marina; Figure 2.2d). These storms resulted in drastically lowered salinity levels that devastated fouling communities throughout the Bay, including the Richmond Marina study site used in these experiments (see Chapter 1). Populations of many taxa were decimated, including those of most solitary tunicates, previously the dominant space users in the SPOT group, and arborescent bryozoans, which had been the ARB group taxa occupying the greatest space (Figures 2.2, 2.3). Most of these taxa either did not recruit at all in the spring and summer of 2006 or began to recruit several months later than normal (see Chapter 1). The monthly survey panels showed a temporary shift from SPOT-dominated recruitment to LAT-dominated recruitment (Figure 2.2a). Summer communities resulting from post-storm (2006) recruitment were similar in some ways to pre-storm (2005) experimental LAT monocultures, as the dominant SPOT and ARB group taxa were no longer present after the storms. The LAT group, normally scarce, became much more abundant (Figure 2.4).

Summer Surveys

The 4-month summer panel surveys, performed over a longer time span (2001–2008) than the monthly survey (2004–2008), showed very large changes in functional group composition and abundance of communities that corresponded with the previous winter's outflow levels (Figure 2.4). The SPOT group dominates the community in Dry years, and to a lesser degree during Moderate years. The lone Wet year during this study (2006) showed a high abundance of the LAT group, with low cover of the other two groups (Figure 2.4).

Figure 2.2: Functional group cover on monthly recruitment panels and temperature and salinity conditions



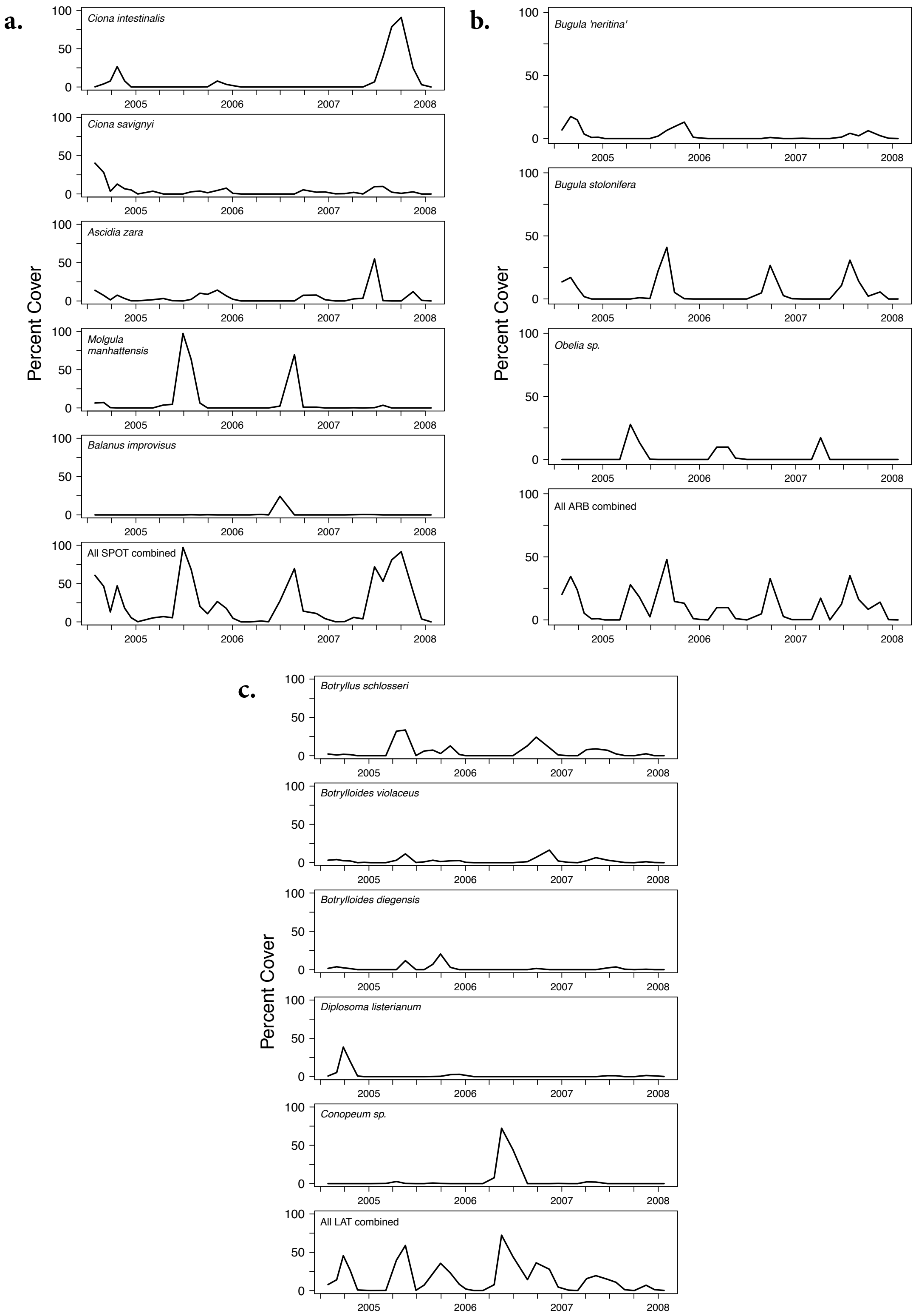
(a) Percent cover of each functional group on Richmond Marina monthly recruitment panels at 1 m depth. Filled circles are SPOT, open squares are LAT, and crosses are ARB functional groups.

(b) Mean total cover of community on Richmond Marina monthly recruitment panels at 1 m depth.

(c) Monthly mean temperature at 1 m depth at Richmond Marina.

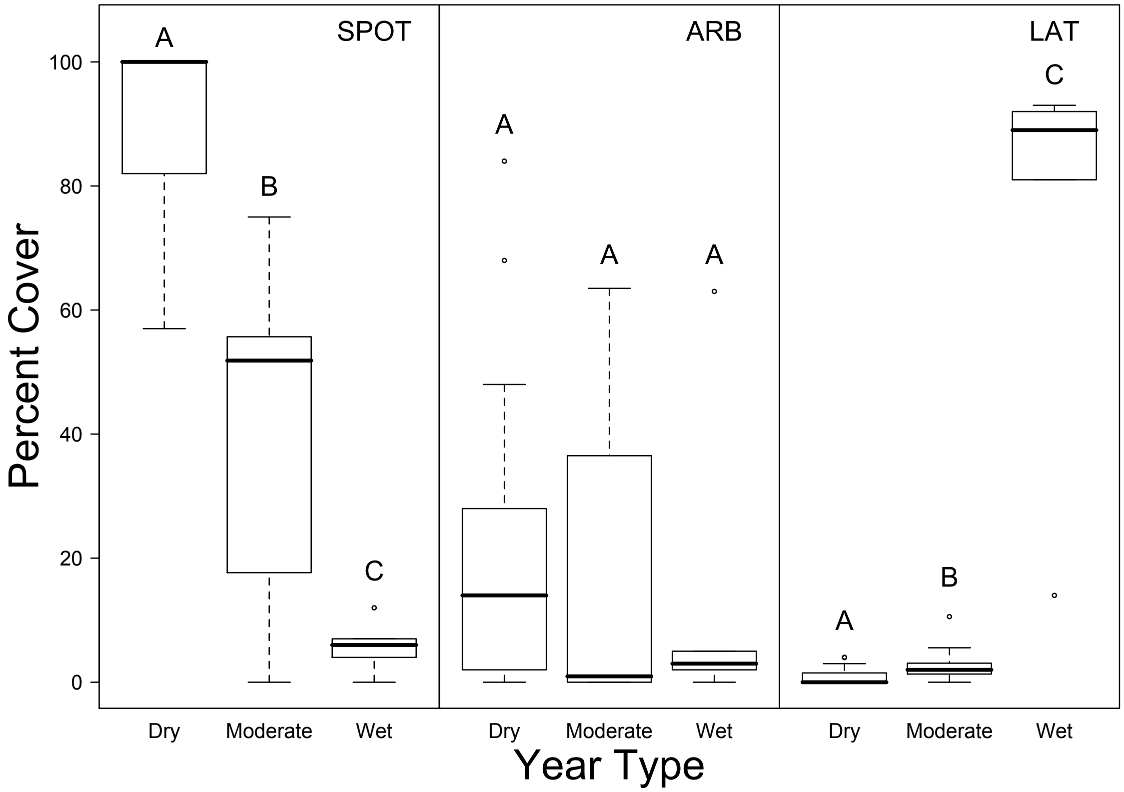
(d) Monthly mean salinity at 1 m depth at Richmond Marina.

Figure 2.3: Percent cover of key species in each functional group on monthly recruitment panels



(**a**) SPOT group species, (**b**) ARB group species, and (**c**) LAT group species.

Figure 2.4: Box plots of percent cover of each functional group on 3-month panels deployed during the summer at Richmond Marina, 2001-2008



Rectangle represents interquartile range (25th to 75th percentile), horizontal line in rectangle represents the median, and whiskers extend to minimum and maximum values, up to 1.5 times the interquartile range, beyond which outliers are marked with open circles.

Letters indicate significant differences ($p < 0.05$) between Dry, Moderate, and Wet years for each functional group (Steel-Dwass non-parametric multiple comparison tests). See text for definition of Dry, Moderate, and Wet years.

How do functional group richness and composition affect resource use?

Total cover showed no relationship with the number of functional groups present in 2004, but was positively correlated with functional group richness in 2005 and 2006 (Table 2.2, Table 2.3). However, analysis of total cover as a function of the species richness of each community showed positive relationships in all three years (Table 2.2, Figure 2.5). Inspection of the data and panel photographs shows that the differing trends in 2004 result from sloughing events on some panels. Sloughing occurs when the weight of an organism (and whatever is attached to it) exceeds the strength of the organism's attachment to the substrate (Chang, pers. obs.); large numbers of organisms can be lost this way, reducing species richness. Unfortunately, the panels where the most sloughing occurred were part of the 3-group polyculture, so while these panels were still considered as having a functional group richness level of 3, they had fewer species than other panels in the treatment.

Within each level of functional group richness, resource use (as measured by total cover) depended on functional group identity in 2004 and 2006, but not 2005 (Table 2.3, Figure 2.6). In 2004, the ARB+LAT polycultures had signficantly greater cover than the other treatments, which were statistically indistinguishable (Figure 2.6). This result is perhaps misleading, since numerically dominant, large-bodied organisms sloughed off numerous panels in the 3-group polyculture and control treatments. Based on photographs taken two weeks prior to panel retrieval, before sloughing occurred, peak cover was actually highest in the 3-group polyculture and control treatments. In 2005, functional group richness, but not identity, had a significant effect on total cover. All monocultures had significantly lower cover than all polycultures, but variation among monocultures was insignificant (Figure 2.6). In 2006, within-treatment variation was generally much greater, and the only clear trend was that the ARB monocultures had much lower cover than any other treatment (Figure 2.6). This was in part due to the general absence of ARB species recruitment during the 2006 experiment, even during months when ARB recruitment is typically high (June, July, August; Figure 2.2a).

Transgressive overyielding, as indicated by positive D_{max} values, was found in all 2005 polyculture treatments. The SPOT+LAT polycultures had the highest average D_{max} (0.2). In contrast, none of the 2006 polyculture treatments showed significant transgressive overyielding (Figure 2.7).

Table 2.2: Bootstrapped regression quantiles of total cover on functional group and species richness

Experiment	Regression Quantile	Variable	Estimate	Std. Error	t-value	Pr < t
2004	33	# Species	2.25364	0.82320	2.73766	**0.01082**
	33	# Functional Groups	−6.45105	4.82478	−1.33707	0.19236
2005	20	# Species	1.78571	0.54292	3.28906	**0.00187**
	20	# Functional Groups	13.45238	2.39975	5.60575	< **0.00001**
2006	20	# Species	8.00000	2.59041	3.08832	**0.00352**
	20	# Functional Groups	30.50000	10.66589	2.85958	**0.00652**

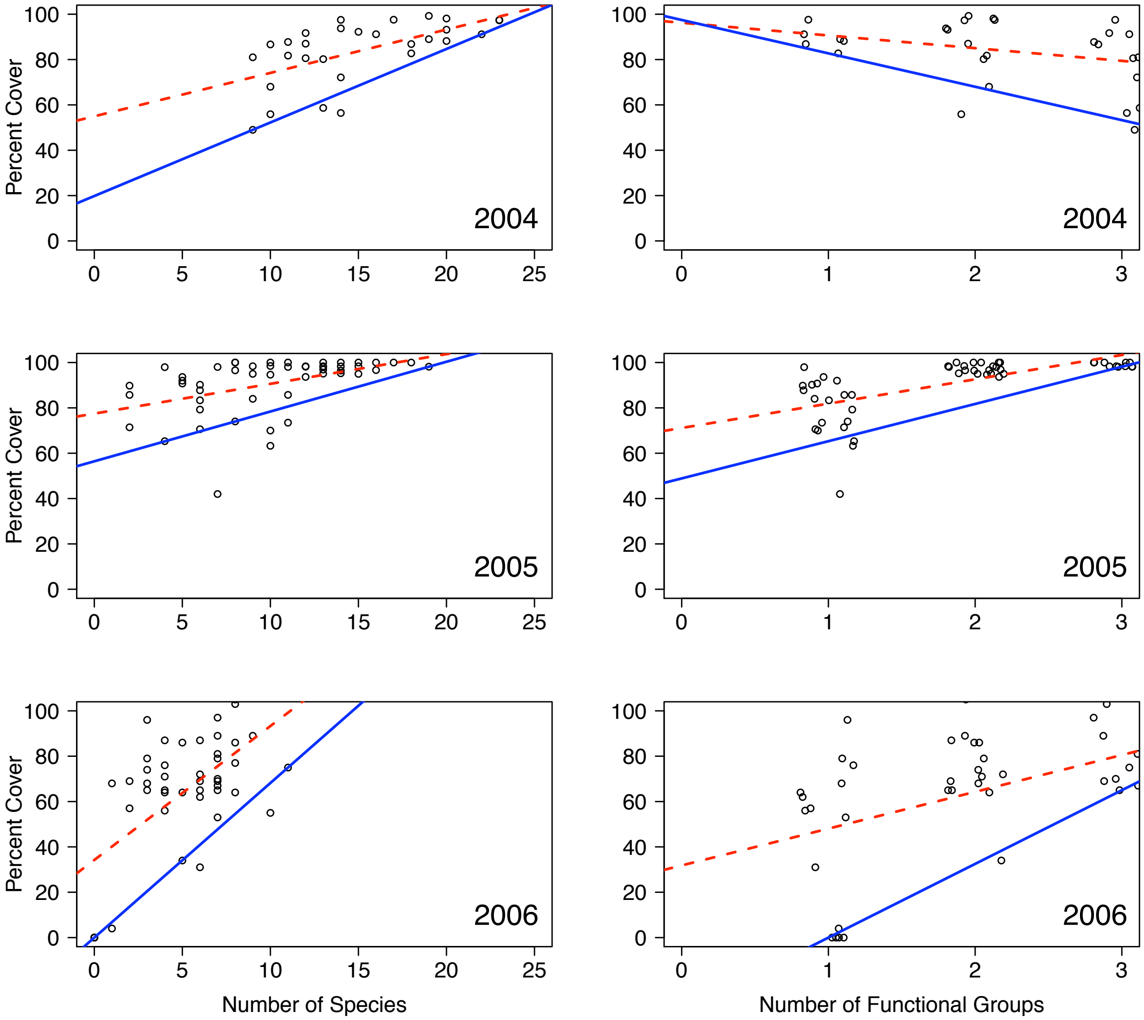
The regression quantile that was used was calculated based on Scharf *et al.* (1998): $n > 10 / (1 - q)$, in which n is the number of samples available and q is the regression quanitle to be used.

Table 2.3: Tests of significance and estimated magnitudes of effect of functional group diversity on total community cover

Year	Effect	SS	d.f.	F	P	ω^2
2005	Whole model	2057.2695	3	4.4471	**0.0150**	
	Richness	1812.2762	1	11.7525	**0.0027**	0.55
	Identity	244.9933	2	0.7944	0.4656	0.00
2006	Whole model	19868.5500	3	30.587	**<0.0001**	
	Richness	5980.0167	1	27.6181	**<0.0001**	0.41
	Identity	13888.5333	2	32.0722	**<0.0001**	0.48

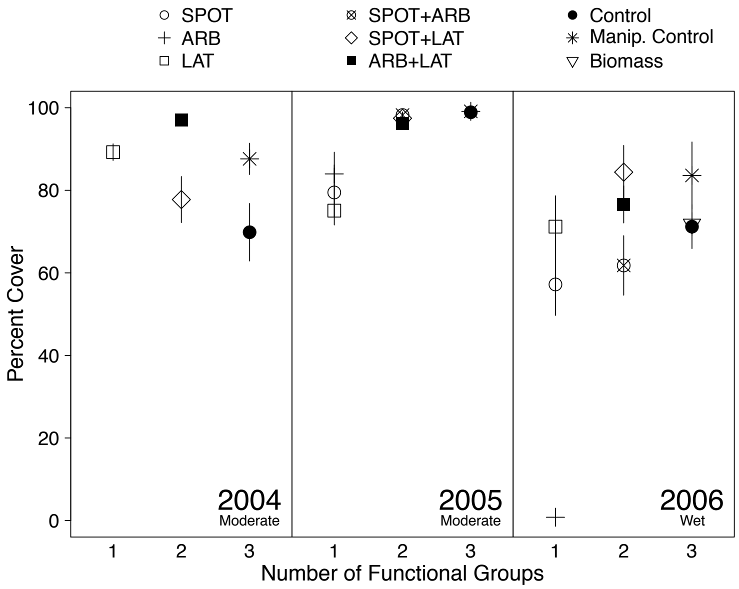
Functional group richness and identity effects were tested using orthogonal planned contrasts as in Duffy et al. (2005). Effect sizes were estimated as ω^2, with negative estimates reported as zero.

Figure 2.5: Total cover of communities as a function of species and functional group richness in the 2004, 2005, and 2006 experiments



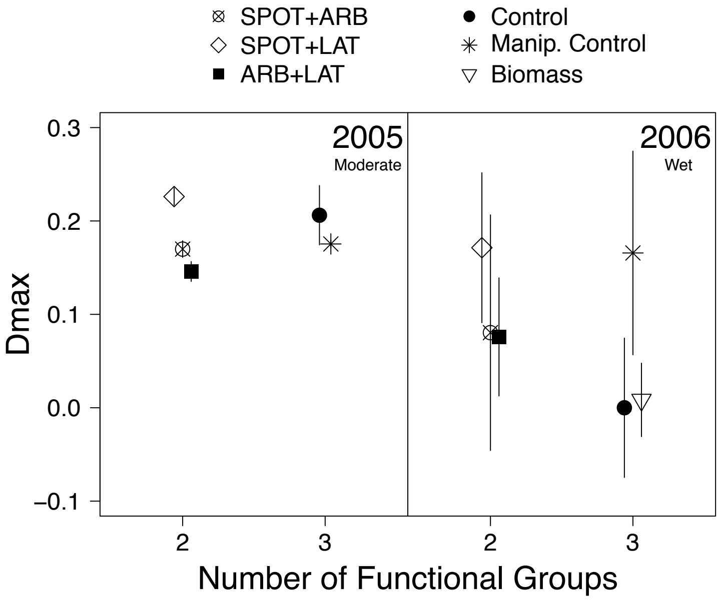
Total cover of communities (jittered) as a function of species richness **(left)** and functional group richness **(right)** in the 2004, 2005, and 2006 experiments. Each point represents one panel. Dashed lines indicate OLS (Ordinary Least Squares) regression lines. Solid lines indicate 10th quantile regression lines, representing the minimum limit of cover in each year as a function of richness.

Figure 2.6: Total cover of communities in each treatment in the 2004, 2005, and 2006 experiments



Total cover (mean ± SE) on panels from which functional groups were removed to create monocultures of each functional group (SPOT, ARB, or LAT) or two-group communities. Unmanipulated control panels contained all three groups and thus served as a three-group polyculture treatment. Manipulated control panels were treated identically to removal treatments except nothing was removed, and the biomass removal panels ("BIOMASS") controlled for any effect of removing biomass.

Figure 2.7: D_{max} for each polyculture treatment in the 2005 and 2006 experiments



D_{max} (mean ± SE) was calculated for two-group communities, polycultures, manipulated control panels, and biomass removal control panels. See text for explanation of D_{max}.

How do functional group richness and composition affect invasion resistance?

While ANOVA showed a non-significant negative trend in mean total cover of all invaders relative to pre-existing functional group richness, quantile regressions reveal a significant negative relationship between maximum invader cover and both functional group and species richness levels (Table 2.3, Table 2.4). Mean invader cover was significantly higher in the ARB monoculture than in other treatments. The solitary tunicate *Ascidia zara* settled more abundantly on recruitment panels than on experimental panels, but did not show differential cover with increasing functional group and species richness, indicating that the presence of any competition seemed to reduce recruitment, but more diverse competition did not have an additional effect (Table 2.4). The arborescent bryozoan *Bugula "neritina"* showed no relationship with functional group richness, but a negative relationship with species richness (Table 2.4). In contrast, the solitary tunicate *Ciona savignyi*, encrusting sponge *Halichondria* sp., colonial tunicates *Botrylloides violaceus* and *B. diegensis*, and the arborescent bryozoan *Bugula stolonifera* showed no relationship with increasing functional and species richness (Table 2.4). D_{min} was greater than zero in all cases, indicating no transgressive overyielding.

The availability of bare space on panels at the beginning of the invasion experiment in August 2006 did not predict invader success as measured by total invader cover in December 2006 (Table 2.5). The sponge *Halichondria* sp., however, showed a significant positive relationship with increasing bare space availability, and the colonial tunicate *Botrylloides diegensis* similarly showed a nearly significant positive relationship (Table 2.5).

The local community was dominated by very different taxa in 2005 (solitary tunicates) compared to 2006 (barnacles and mussels), so I measured how many organisms settled on top of these taxa to determine how secondary settlement contributed to overall invasion resistance. Examining only 3-group polyculture and control panels, I found that on a per-panel basis, the proportion of points with any secondary cover on top of barnacles and mussels was significantly greater than the proportion of points with any secondary cover on top of solitary tunicates (Mann-Whitney U test, $W = 49.5$, $p < 0.0001$; Figure 2.8).

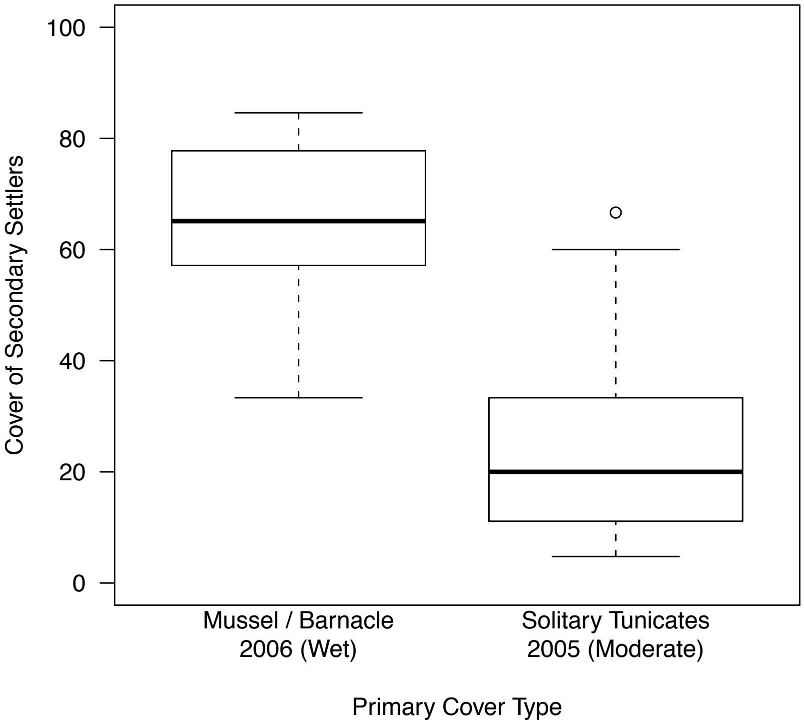
Table 2.4: Bootstrapped 80th regression quantiles of percent cover of individual invading species and total invader cover on functional group and species richness

Species	**Variable**	**Slope Estimate**	**Std. Error**	***t*-value**	**Pr < *t***
Ascidia zara	# Species	−1.14286	0.77670	−1.47143	0.15065
	# Functional Groups	−3.00000	2.36631	−1.26780	0.21185
Ciona savignyi	# Species	0.25000	0.61185	0.40860	0.68547
	# Functional Groups	0	1.57653	0	1
Halichondria sp.	# Species	−0.88889	0.72065	−1.23345	0.22612
	# Functional Groups	1	1.32589	0.75421	0.45493
Botrylloides violaceus	# Species	0.75000	1.31149	0.57187	0.57129
	# Functional Groups	0	2.39673	0	1
Botrylloides diegensis	# Species	−1.16667	0.85153	−1.37008	0.17991
	# Functional Groups	−1.50000	2.32540	−0.64505	0.52240
Bugula "neritina"	# Species	-0.75000	0.35745	−2.09822	**0.04362**
	# Functional Groups	0.50000	1.18857	0.42067	0.67614
Bugula stolonifera	# Species	0	0.16196	0	1
	# Functional Groups	−1.00000	0.58233	−1.71723	0.09330
Total Cover of Invaders	# Species	−2.80000	0.91510	−3.05977	**0.00438**
	# Functional Groups	−6.00000	2.32929	−2.57589	**0.01361**

Table 2.5: Bootstrapped 80th regression quantiles of percent cover of individual invading species and total invader cover on bare space

Species	**Slope Estimate**	**Std. Error**	**t-value**	**Pr < t**
Ascidia zara	0	0.30556	0	1
Ciona savignyi	0.06667	0.17179	0.38807	0.70013
Halichondria sp.	0.42857	0.17252	2.48416	**0.01751**
Botrylloides violaceus	−0.07143	0.52991	−0.13479	0.89349
Botrylloides diegensis	0.36735	0.18348	2.00212	0.05245
Bugula "neritina"	0.10870	0.08765	1.24017	0.22251
Bugula stolonifera	0.02174	0.04234	0.51341	0.61063
Total Cover of Invaders	0.34043	0.31977	1.06458	0.29378

Figure 2.8: Secondary settlement on mussels and barnacles in the 2006 experiment compared to settlement on solitary tunicates in the 2005 experiment



Greater secondary settlement occurred on the mussels and barnacles that dominated primary cover in the 2006 experiment than on the solitary tunicates that dominated primary cover on 2005 experiment panels (Mann-Whitney U test, $W = 49.5$, $p < 0.00001$).

DISCUSSION

These results suggest that the richness, identity, and species composition of functional groups present are all important determinants of the community's overall functioning. Environmental conditions play a large role in determining both the species and functional composition of the resident community (Figures 2.2, 2.4; Chapter 1). Species and functional group composition in turn are likely to affect the community's ability to use limiting resources and resist invading species via resource pre-emption. The apparent dependence of dominant species in the SPOT functional group on freshwater outflow levels suggests that the community's functional properties will change according to the prevailing environmental conditions (Figures 2.3a and 2.4).

Transgressive overyielding of polycultures in 2005 suggests that polycultures were more consistently able to use the space resource, since the component functional groups performed better together than any single group did in monoculture. The complementary growth forms and diversity of attachment mechanisms of the different functional groups appeared to allow the polyculture to achieve greater overall cover. Individual organisms were held in place by neighboring organisms in addition to their own attachments to the substrate. The varying strengths and modes of attachment of organisms in the polyculture meant that any one organism was likely to be reliant on several neighbors with different attachment strategies in order to remain in place. In contrast, monocultures lacked such a diversity of reinforcements to individual organisms' attachment to the panel.

However, no such complementarity was evident in post-storm communities in early 2006 (Figures 2.6 and 2.7), when barnacles and mussels replaced solitary tunicates as the dominant cover organisms in the SPOT group. This lack of complementarity is curious given that mussels create tight matrices and have byssal threads that should help hold clusters of organisms together, leading to greater overall use of space. It thus appears that this result is caused by the paucity of species remaining in the ARB and LAT groups following the winter storms in early 2006.

When shifts occur in species and functional group composition, such as from solitary tunicates to barnacles and mussels, community performance is unlikely to be affected in the same way for different functions. While both groups of taxa proved adept at occupying bare space, solitary tunicates appear to be more effective at preventing secondary settlement by other species (Figure 2.8), so the barnacle-mussel dominated community may not have provided equivalent functioning in terms of invasion resistance. Tunicate species can repel secondary settlers via either chemical antifoulants or via physical defenses such as surface cell sloughing, mucus secretions, or simply the flexible nature of their tunics (Davis 1991, Davis 1998, Hirose *et al.* 2001). In contrast, the rigid, calcareous shells of barnacles and mussels are easily colonized by a diverse array of species (*e.g.,* Witman 1985, Thiel and Ullrich 2002, Dijkstra and Harris in press). Stachowicz and Byrnes (2006) suggested that facilitators could counteract biotic resistance by lessening space limitation. Here, barnacles and mussels likely act as facilitators, providing secondary substrate for other species (including invaders) that are excluded from primary substrate, enhancing the persistence in the community of these secondary recruits (Dijkstra and Harris, in press). Thus, while most experimental investigations of invasion resistance in marine systems have demonstrated a negative relationship between diversity and invader abundance or survival (Stachowicz *et al.* 2007), facilitative effects of mussels and barnacles could explain the lack of such a negative relationship between resident community diversity and invader cover observed in my experiment.

Previous work in the same system during a Dry year (2002) provides further evidence that solitary tunicates can strongly impact space availability, and perhaps also the invasion resistance properties of the community. Blum *et al.* (2007) showed that the when the solitary tunicate *Ciona intestinalis* was highly abundant, it depressed species richness and altered community composition, apparently by both being a superior competitor for primary space and a poor substrate for secondary settlement by other organisms. I observed similar inhibitory effects of the SPOT group on non-SPOT species richness and composition in 2005 (pre-storm), a year in which *C. intestinalis* was rare and the SPOT group was composed primarily of the congeneric species *C. savignyi* as well as the solitary tunicate *Ascidia zara.* However, in post-storm communities (2006) without solitary tunicates, the presence of the SPOT group had little effect on non-SPOT species richness and composition (Chang, unpublished data). Although I did not conduct an invasion resistance experiment in 2005, these data suggest that the outcome of such an experiment might be different if the resident community resembled the tunicate-dominated communities common in 2005, rather than the barnacle-mussel community of 2006. Similarly, Cardinale and Palmer (2002) found that disturbance moderated biodiversity-ecosystem function relationships in stream mesocosms by controlling the abundance of a dominant species of caddisfly larvae.

While disturbance caused by high freshwater outflow conditions limits solitary tunicate abundance in San Francisco Bay on a large scale in Wet years, disturbance via sloughing can cause local disruptions to solitary tunicate-dominated communities during Dry and Moderate years. Sloughing is not restricted to dead or dying organisms. Massive growth of mussels or of solitary tunicates, for example, can result in large masses of organisms held to the substrate with a relatively small attachment point that often abruptly gives way, such as occurred in several treatments in the 2004 experiment.

In general, disturbances that open up bare substrate can occur year-round in dock fouling and other epifaunal communities via bulldozing by grazing organisms, predation, human-derived disturbances such as abrasion from boats, and large-scale physical disturbances (see Chapter 1; Sousa 2001, Altman and Whitlatch 2007). Bare patches can thus appear in dock and piling communities on both small (< 1 m^2) and relatively large (> 10 m^2) spatial scales at any time of year. Rapid colonization of these newly bare surfaces creates monocultures or low diversity polycultures of the dominant settlers on small spatial scales similar to those that I created on panels in the present study (Chang, pers. obs.). On larger spatial scales, these processes create patchwork mosaics of species and functional groups; the greater resulting spatial heterogeneity may increase the available opportunities for invasion by non-resident species (Davies *et al.* 2005, Stachowicz and Byrnes 2006, Altman and Whitlatch 2007).

In cases where an invader's success appears to be affected by some quality of the resident community, invader characteristics may be just as important as resident community characteristics such as richness or composition (Stachowicz and Tilman 2005, Strauss *et al.* 2006). Such species-specific differences in successful settlement and persistence can arise from myriad factors, including different settlement cues, varying responses to species already present in the community, hydrodynamics, different post-settlement survival, and so on. This experiment integrated across all of these processes, perhaps leading to a more realistic outcome than approaches that isolate one of these potential causative factors, but additional work will be required to distinguish the effects of these processes on the overall success of invading species.

For example, Von Holle and Simberloff (2005) have shown that propagule pressure can overwhelm ecological resistance factors such as physical environmental regimes and resident species diversity. This may explain the observed patterns in the present study, as I observed high recruitment of invading species on my recruitment panels (*i.e.* high propagule pressure) and did not explicitly control propagule pressure in the invasion experiment, instead allowing a natural settlement regime. Future work should therefore also attempt to control propagule pressure to ascertain its role relative to the richness and identity of resident species in determining invader success.

I began this chapter with a proposal that examining the effects of functional group diversity might prove more fruitful than looking at species diversity effects. Had I stopped after performing this experiment in 2004 or 2005, I would have reached quite different conclusions—that the functional group approach effectively encompasses the major properties of the species in the community, and that communities with greater functional group diversity more consistently and effectively use space. The addition of a third year of experimentation immediately following a major disturbance highlighted the importance of the specific identities of species comprising these functional groups, as well as the environmental context in which different groups of species might play an important role.

In conclusion, specific functional groups had a greater effect on invasion success than either functional group or species diversity, but this effect was controlled by environmental conditions that varied from year to year. Thus, the results of short-term, "snapshot" views of ecosystem function are contingent upon prevailing conditions, suggesting that a great deal of caution be exercised when interpreting the results of short-term biodiversity–ecosystem function studies not replicated among years. Longer-term investigations will also generate a more complete picture of diversity–function relationships by integrating over these short-term variations.

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CHAPTER 3. Tackling aquatic invasions: risks and opportunities for the aquarium fish industry*

ABSTRACT

The aquarium trade is an important and rapidly growing vector for introduced species in the United States. We examined this vector by surveying pet stores in the San Francisco Bay–Delta region to compile a list of aquarium fish species commonly stocked. We identified which of these species might be able to survive in the Bay–Delta, and investigated store representatives' knowledge and attitudes about biological invasions. A restrictive analysis using conservative estimates of fish temperature tolerances and environmental conditions found that the local aquarium trade includes 5 fish species that can survive in a temperate system such as the Bay–Delta. Under more inclusive parameters, up to 27 fish species met the criteria for survival in the Bay–Delta. We further explored these results by comparing potential invader incidence between different types of stores. In the more restrictive analysis, three national retail chains stocked significantly more potentially invasive species than independent aquarium stores, but there was no difference in the more inclusive analysis. A significantly higher percentage of fish taxa were easily identifiable and well-labeled in chain stores than in independent stores. Most aquarium store representatives indicated willingness to take action to reduce the threat of

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trade-related introductions, although chain store employees were more willing to assign responsibility for reducing this threat to the aquarium industry than were independent store employees. Management efforts for this vector should focus on (a) improving labeling and identification of fish species in stores, (b) expanding the often spotty data on fish physiological tolerances, especially for saltwater species, (c) educating customers and store employees about the risks posed by pet release, and (d) providing better options for responsible disposal of unwanted fish.

INTRODUCTION

Invasive species are a growing source of ecological and economic harm worldwide (Chapin *et al.* 2000; Sala *et al.* 2000; Worm *et al.* 2006; Lotze *et al.* 2006). Non-indigenous organisms are transported throughout the United States via international and domestic trade at an ever-increasing rate, making the introduction of new species inevitable (Jenkins 1996; Levine and D'Antonio 2003). The aquarium trade represents one of five major avenues for introduction of non-indigenous aquatic species (Ruiz *et al.* 1997) and has been linked to over 150 species invading natural ecosystems around the world (Fuller 2003; Siguan 2003; Padilla and Williams 2004). We examined the retail trade in ornamental fishes, which are the centerpiece of the rapidly growing aquarium industry and consist largely of Indo-West Pacific and South American tropical species marketed and sold in other regions of the world (Chapman *et al.* 1997).

Our study considers both biological and sociological aspects of the aquarium trade to assess its potential as a vector for human-mediated introductions of non-native fish species. The risk posed by an invasion vector has both a biological component (the ability of species to establish self-sustaining populations) and a human component (the delivery of species to the new habitat), so an assessment of invasion risk must consider both elements. Since local environmental conditions, trade practices, and consumer preferences for particular fish species are subject to regional variation, the aquarium trade-related invasion risk profile likely exhibits substantial geographic variation. Therefore, we focused our research on our local area, the San Francisco Bay–Delta, and we offer our work here both as an assessment of one particular location and as a model for future studies investigating the role of the commercial pet industry in biological invasions.

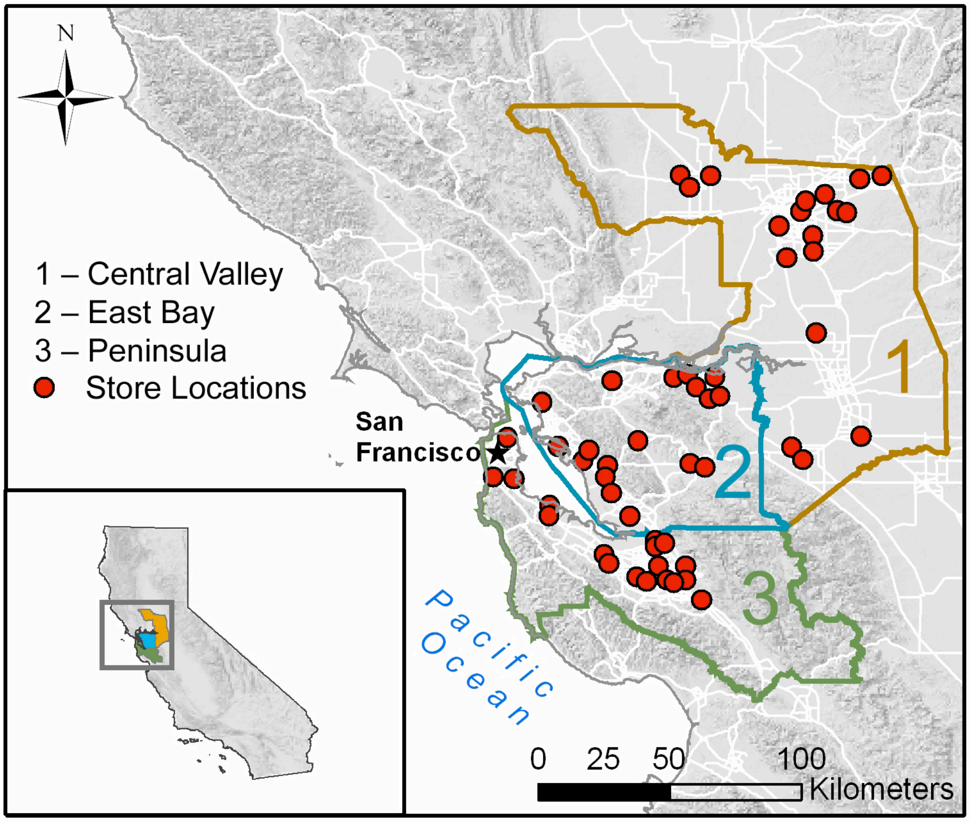
The San Francisco Bay–Delta region (henceforth, Bay–Delta) in California, U.S.A., is a major Pacific coast trade hub and one of the largest estuarine and freshwater ecosystems in North America. The Bay–Delta includes over 1500 km^2 of aquatic habitat of varying water temperatures and salinities and serves as a critical juncture between the Pacific Ocean and a watershed comprising 40 percent of the state of California (Conomos 1979). A heavily urbanized area, the Bay–Delta is subject to ever-increasing rates of invasion by non-native species (Cohen and Carlton 1995). Over 7 months (December 2004–June 2005), we determined which locally-sold aquarium species might be potential invaders in the Bay–Delta by first conducting an extensive inventory of aquarium fish stocked in Bay–Delta stores, then identifying which species might be able to survive in the Bay–Delta by comparing fish physiological tolerances to regional environmental parameters. We examined the risk posed by different segments of the aquarium industry by analyzing where potential invaders were sold and by investigating in-store practices that might mitigate risk such as product labeling and employee awareness of invasive species. We specifically considered both independent and chain stores, two major types of retail outlet in the aquarium trade. While our study did not consider sales volume or the frequency of releases into waterways, we did describe which species are for sale, determine which might survive in local waterways, and explore the vector's social dynamics, which are key first steps in determining the importance of this invasion pathway.

METHODS

Inventory of aquarium fish for sale in the Bay–Delta

We identified 168 stores that sold aquarium fish in the Bay–Delta using a keyword search of online business directory listings (Verizon 2004; Yahoo! 2004). We searched for stores selling items related to "fish" and/or "aquarium" in the greater San Francisco Bay–Delta area and called each store to confirm that they sold aquarium fish. Based on proximity to freshwater or saltwater parts of the Bay–Delta system, we separated the geographical area under study into three regions (Figure 3.1). We classified each store as either an independent or a chain store and then randomly selected and surveyed nine independent and nine chain stores per region for a total of 54 stores. Individually owned and operated stores were classified as independent, while retailers that were part of a chain with multiple retail outlets and centralized management were classified as chain stores. The nine chain stores per region were equally divided among the three possible chain stores: a large international general discount retailer with a freshwater aquarium pet section (hereafter referred to as General Discount Chain), Pet Chain A (a large international specialty retailer of pet supplies and services with a mostly freshwater aquarium pet section), and Pet Chain B (a large national specialty retailer of pet supplies and services with an aquarium pet section of both salt and freshwater species). We conducted an exhaustive inventory of fish stocked in all 54 stores, generating an extensive, representative listing of aquarium fish offered for sale throughout the Bay–Delta. For each store, we obtained verbal permission to conduct our survey from a manager or supervisor before starting (none of the stores we visited declined to give permission for the study). We then recorded the store's last stocking date, and for each fish tank recorded: (1) species listed on the tank, (2) species present in the tank, and (3) additional labeling information where available. When possible, we identified each organism to species level using Axelrod *et al.* (1995) and Burgess (2000) as references. The same team of three observers (A. L. Chang, J. D. Grossman, H. W. Weiskel) visited each store to reduce observer bias, with frequent cross-checking of identifications in the store both with other observers and with store representatives to ensure accurate species identification.

Figure 3.1: Regional divisions and locations of stores visited during the store survey in the San Francisco Bay–Delta



Analysis of invasion potential of aquarium fish for sale in the Bay–Delta

We developed a model to assess the invasion potential of aquarium fish by comparing temperature and salinity requirements of aquarium fish found in our inventory to environmental data for the Bay–Delta region. We used FishBase, a publicly-available database (Froese and Pauly 2005), to determine temperature and salinity requirements for each aquarium fish we could identify to species level during our inventory. The minimum temperature tolerance limit is of particular interest because the aquarium trade generally focuses on tropical species, so cold winter temperatures would likely be the limiting factor for survival of most aquarium fish in the temperate Bay–Delta system (P. Moyle pers.

comm.). Precise temperature tolerances are not well known for most saltwater species, so we used numerical temperature data for freshwater species and FishBase's somewhat broader climatic classifications for saltwater species. Climatic categories are temperate, subtropical, and tropical, corresponding to minimum temperature tolerances of 0–10, 10–20, and over 20°C, respectively (Froese and Pauly 2005).

We then characterized two regions in the Bay–Delta that consistently differed in salinity based on U.S. Geological Survey (USGS) records: marine (average salinity greater than 30 psu) and freshwater (average salinity less than 2 psu) (USGS 2006). We excluded the brackish region of the Bay–Delta since few fish sold in the aquarium trade are categorized as brackish or live predominantly in brackish regions. Exact salinity tolerance limits for aquarium fish species are seldom known, but many species classified as either "freshwater" or "saltwater" have reported salinity tolerance ranges that would appear to let them live in brackish waters. Therefore, our exclusion of brackish habitat from consideration in this study means that our conclusions are a conservative estimate of the overall invasion potential of fish species in the aquarium trade.

We defined a "potentially invasive" fish as one that could survive in the Bay–Delta according to available physiological and environmental data. Although a non-native species must successfully pass through numerous steps to establish a population in a new region (in addition to surviving, it must first be transported to the region and then also reproduce), we focused on survival because the greatest amount of data was available to evaluate this portion of the invasion process for aquarium fish. As low temperature tolerance is assumed to be a major limiting factor in fish survival in the Bay–Delta, we inferred that introduced fish might move to more favorable locations during cooler seasons. We therefore chose the warmest temperature in a salinity region during winter (i.e. the warmest minimum temperature) as our environmental criterion for determining survival potential. We examined winter temperatures throughout the Bay–Delta using USGS water quality data collected over 10 years (1996–2005) at 1 m depth in mid-channel throughout the Bay–Delta system along a transect from the Sacramento River to the Golden Gate Bridge (USGS 2006). The warmest minimum temperature for the freshwater zone (<2 psu) was 8.8°C and the warmest minimum temperature for the saltwater zone (>30 psu) was 10.2°C.

Preliminary examination of model results suggested that we were underestimating the invasion potential for aquarium fish because these criteria failed to include three non-native species known to be established in the Bay–Delta (Table 3.2). Because the USGS data represent mid-channel (i.e. not shoreline) temperatures throughout the Bay–Delta system, they do not capture the presence of temperature refugia such as power plant effluents or lagoons that may be the warmest locations in the Bay–Delta during the coldest times of year. Likewise, reported values for fish physiological tolerances are unlikely to be fully representative of a species' tolerances, given intraspecific variation and our incomplete knowledge of temperature limits for many species. We therefore modified our original, restrictive model (Colder Scenario) for both freshwater and saltwater fish to generate a more inclusive Warmer Scenario. Under the Colder Scenario for freshwater fish, we compared the lowest recorded temperature tolerance for each fish species (Froese and Pauly 2005) to the warmest minimum temperature recorded in the freshwater region

(8.8°C; USGS 2006). In the Warmer Scenario, we adjusted the warmest minimum temperature upward 3°C, while fish temperature tolerances were adjusted downward 3°C. Although this scenario may be more inclusive in terms of identifying potential fish invaders, it is more likely to realistically reflect the confluence of actual fish temperature tolerances and environmental conditions (P. Moyle pers. comm.).

Similarly, we constructed Colder and Warmer Scenarios for saltwater fish based on the warmest minimum temperature found in the saltwater zone of the Bay–Delta (10.2°C). Because winter water temperatures in the Bay–Delta often fall below 10°C, we used FishBase's temperate classification (lower temperature limit of 0–10°C) as the criterion for determining survivorship of saltwater fish in the Colder Scenario and the subtropical classification (lower temperature limit of 10–20°C) as the relevant criterion for the Warmer Scenario.

Statistical analysis

We used our store inventory data to perform two analyses. First, we tested for differences in the number of potentially invasive fish sold in each store among geographical regions and store types. Second, we tested for differences among regions and store types with regard to: (1) the percentage of correctly labeled fish taxa in each store and (2) the percentage of fish taxa in each store that we were able to identify to species level. For each analysis, we performed ANOVA as well as ANCOVA using the total number of taxa identified to species level in each store as a covariate. This approach allowed us to account for the effect of more diverse store inventories, as one might expect a greater number of potential invaders to be found in stores with a greater total number of species (i.e. a sampling effect).

To meet the assumptions of normality and homogeneity of variances in the first analysis, we performed a log (x + 1) transformation on the data where necessary, and in some cases also weighted the ANOVA or ANCOVA using the reciprocal of the variance. In addition, four extreme values in the Colder Scenario data were winsorized to the 5% and 95% levels to meet the assumption of normality before running the ANOVA and ANCOVA (Tukey 1962). For all analyses, differences between levels within a factor were detected using least-squares means comparisons. All analyses were performed using SAS 9.1.2 (SAS Institute Inc. 2005).

Telephone survey of store representatives in the Bay–Delta

We developed a telephone survey to investigate the level of knowledge regarding invasive species among Bay–Delta aquarium store representatives and to explore their willingness to address potential threats posed by invasive species. The survey was conducted in July 2005. Using the same list of stores that we identified as selling aquarium fish for our store inventory, we randomly selected and telephoned 114 stores that were not visited during the store inventory. A total of 30 stores (12 chain stores and 18 independent stores) participated in the telephone survey (26.3% response rate).

Our survey consisted of 17 multi-part, closed-ended questions with opportunity for further comment afterwards (see Appendix I). We asked to speak with managers or employees who dealt specifically with aquarium fish at the highest responsibility level possible. The survey was designed to minimize response bias, with survey topics progressing from general to specific. We measured respondents' awareness of invasive species using several different questions that addressed knowledge of the term "invasive species" and perception of invasive species as an environmental problem. To measure respondents' sense of responsibility for preventing introductions of potentially invasive fish species, we asked about the aquarium trade's role in the spread, prevention, and introduction of invasive species. We also questioned respondents about their own involvement in the aquarium trade and their assessment of consumer behavior regarding aquarium fish. Finally, to measure willingness to alter behavior, we asked respondents to indicate whether they would be willing to sell alternative species that would not be potentially invasive.

Analysis of store representatives' knowledge and attitudes

We tested for differences in respondents' knowledge of invasions (awareness), sense of the industry's responsibility regarding invasions, and willingness to alter behavior based on the respondents' store type (independent or chain) and level of involvement in the aquarium trade. We first examined answers to groups of questions addressing awareness and responsibility, respectively, using principal component analyses. We evaluated the principal components using ANCOVA to determine whether store type and expertise predicted awareness of invasive species, and whether store type, expertise, and awareness of invasive species predicted the respondents' assignment of responsibility and their willingness to take action regarding invasive species. Our covariate was an index of the respondents' expertise in the aquarium industry that we created by combining answers to questions that asked whether the respondent owned an aquarium at home and whether s/

he bred or traded fish as a hobby. We also tested for differences between independent and chain store respondents' willingness to assign responsibility to different aquarium trade stakeholders. We supplemented these analyses with more specific examinations of responses to individual survey questions using contingency tables evaluated with Fisher's exact test. All analyses were performed in SAS 9.1.2 (SAS Institute Inc. 2005).

RESULTS

Inventory and analysis of aquarium fish for sale

We observed 1009 unique fish taxa in the 54 stores surveyed and were able to identify 867 of these to species level, comprising 432 freshwater, 23 brackish and 412 marine species. Independent stores as a group had the highest average number of species, with an average of 100.2 species per store, while the General Discount Chain had the fewest, at 34.1 species per store (Table 3.1). The General Discount Chain sold exclusively freshwater fish whereas Pet Chain A sold saltwater fish in addition to freshwater fish in 5 out of 9 stores, but any one of those 5 stores had at most 2 saltwater taxa. One independent store sold only saltwater fish. All other stores sold both freshwater and saltwater fish. Of these species, we found that under the Colder Scenario, 3 of the freshwater and 2 of the saltwater fish species could potentially survive if released into the Bay–Delta (Table 3.2). In the Warmer Scenario, these numbers increased to 9 freshwater and 18 saltwater species, respectively (Table 3.2). All 3 of the freshwater species identified by the Colder Scenario and 3 additional freshwater species identified by the Warmer Scenario have already been introduced to the Bay–Delta (Figure 3.2), although only one of these introductions (the goldfish *Carassius auratus*) was the result of the aquarium industry; the others were introduced for angling or mosquito control purposes (Moyle 2002, Moyle pers. comm.). None of the saltwater fish identified as potentially invasive by the Colder and Warmer Scenarios are currently established in the Bay–Delta.

Table 3.1: Average store sizes (in total gallons per store) and average number of species per store identified during store inventory survey.

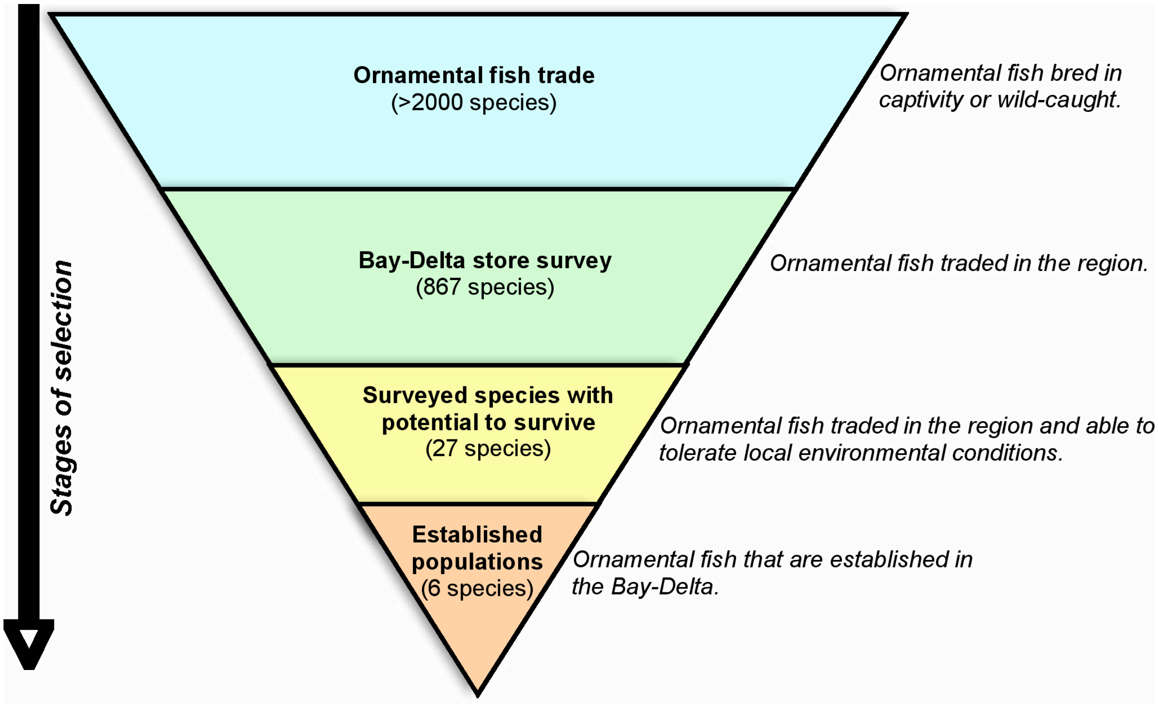
Store type	Average number of gallons	Median number of gallons	Range of gallons	Average number of freshwater species	Average number of saltwater species	Average total number of species
Independent	3639.4	2663	485–11446	62.9	34.8	100.2
Chain (all)	1092.7	1101	250–2240	57.6	4.6	67.7
General Discount Chain	265.6	270	250–260	32.2	0	34.1
Pet Chain A	1679.3	1750	1018–2225	81.4	0.6	83.6
Pet Chain B	1333.2	1250	660–2240	59	13.2	74.1

Table 3.2: Non-native ornamental fish species present in stores during this survey and identified under the Colder and Warmer Scenarios as having the potential to survive in the San Francisco Bay–Delta

	Colder Scenario	**Warmer Scenario**
Freshwater species	**Goldfish (*Carassius auratus*)*** **Koi (*Cyprinus carpio*)*** **Rosy red minnow (*Pimephales promelas*)***	**Goldfish (*Carassius auratus*)*** **Koi (*Cyprinus carpio*)*** **Rosy red minnow (*Pimephales promelas*)*** **Western mosquitofish (*Gambusia affinis*)*** **Channel catfish (*Ictalurus punctatus*)*** **Blue catfish (*Ictalurus furcatus*)*** Dojo loach (*Misgurnus anguillicaudatus*) Bull rout (*Myoxocephalus scorpius*) Garra pingi (*Garra pingi pingi*)
Saltwater species	Red Scorpionfish (*Rhinopias argolipa*) Yasha Hase goby (*Stonogobiops yasha*)	Red Scorpionfish (*Rhinopias argolipa*) Yasha Hase goby (*Stonogobiops yasha*) Flying Gurnard (*Dactylopterus volitans*) Sergeant Major (*Abudefduf saxatilis*) Scrawled Cowfish (*Acanthostracion quadricornis*) Orange Filefish (*Aluterus schoepfii*) Clown goby (*Microgobius gulosus*) Porcupine pufferfish (*Diodon holocanthus*) Red grouper (*Epinephelus morio*) Banded Cat Shark (*Halaelurus lineatus*) Sargassumfish (*Histrio histrio*) Western Jumping blenny (*Lepidoblennius marmoratus*) Bigeye squirrelfish (*Myripristis jacobus*) Green Chromis Damsel (*Chromis viridis*) White angelfish (*Chaetodipterus faber*) Pygmy angelfish (*Centropyge argi*) Blueface angelfish (*Chaetodontoplus personifer*) Harlequin tuskfish (*Choerodon fasciatus*)

* Non-native species already known to have established in the Bay–Delta

Figure 3.2: Magnitude of the aquarium trade in the San Francisco Bay–Delta region.



The number of non-native aquarium species currently found in the San Francisco Bay–Delta region is affected by the size of the ornamental fish trade, the number of species sold regionally, the physiological and environmental parameters of the system, number of releases (incidental or intentional) and the species that have been correctly identified in the system.

Our results comparing store types (chain versus independent) indicate that potentially invasive fish are sold in nearly all chain and independent stores. In the Colder Scenario, the chain store inventories had a significantly greater number of potential invaders compared to independent store inventories ($F_{1,\,42} = 9.78$, $p = 0.0032$), with Pet Chain A offering the greatest number of potentially invasive fish for sale (Table 3.3). Results were similar when adjusting for inventory size (Table 3.4, Figure 3.3).

Table 3.3: Weighted ANOVA results from store survey under the Colder and Warmer Scenarios.

Source	**DF**	**MS**	***F***	***p*-value**
Colder Scenario				
Region	2	0.991	0.87	0.4277
Store type	1	11.172	9.78	**0.0032**
Region × store type	2	0.755	0.66	0.5220
Store name (store type)	2	10.167	8.90	**0.0006**
Region × store name (store type)	4	0.392	0.34	0.8473
Warmer Scenario				
Region	2	1.305	1.05	0.3576
Store type	1	1.283	1.04	0.3146
Region × store type	2	1.195	0.97	0.3892
Store name (store type)	2	53.259	43.02	<**0.0001**
Region × store name (store type)	4	3.635	2.94	**0.0315**

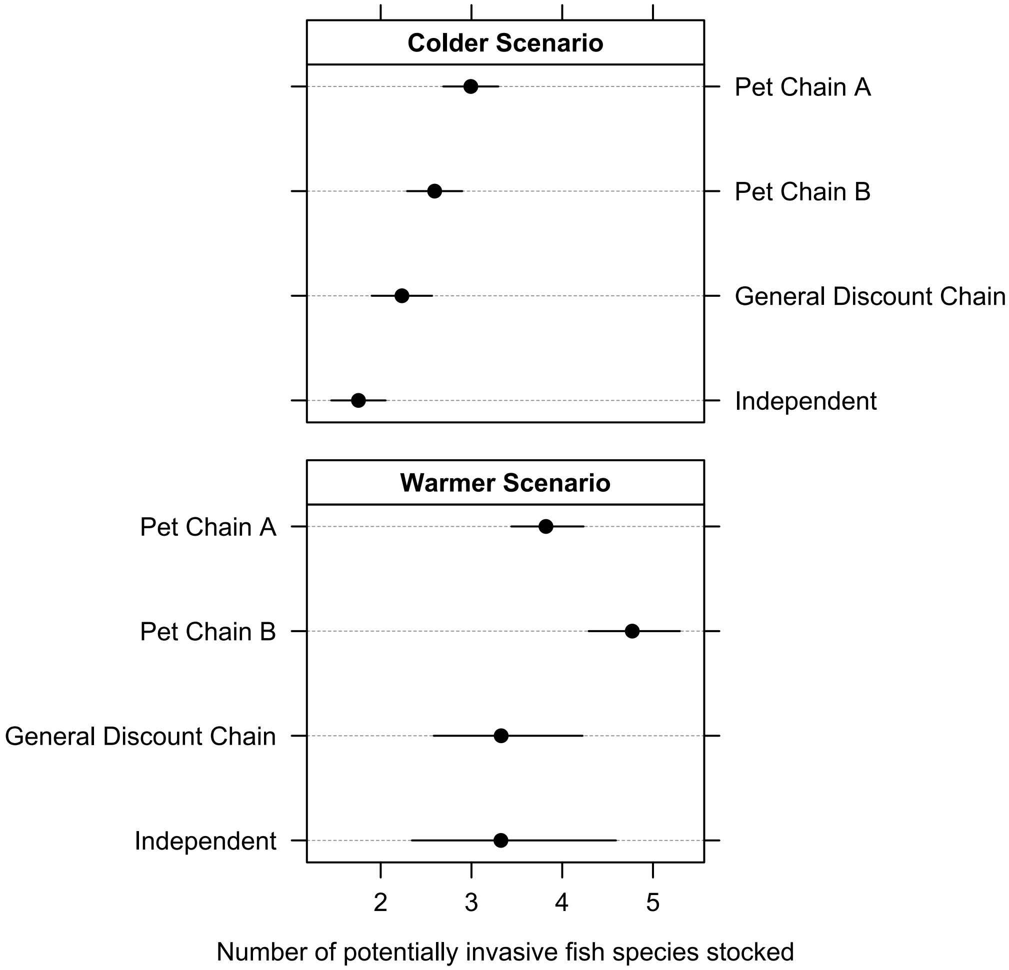
The dependent variable is the number of potentially invasive non-native species for sale at each store. 'Region' is Delta, East Bay, or Peninsula; 'Store Type' is Chain or Independent; 'Store Name' refers to Independents or individual chain stores (General Discount Chain, Pet Chain A, or Pet Chain B), and is nested within Store Type

Table 3.4: Weighted ANCOVA results from store survey under the Colder and Warmer Scenarios.

Source	**DF**	**MS**	***F***	***p*-value**
Colder Scenario				
Region	2	1.012	1.09	0.3447
Store type	1	16.735	18.09	**0.0001**
Region × store type	2	0.448	0.48	0.6198
Store name (store type)	2	5.113	5.53	**0.0075**
Region × store name (store type)	4	0.453	0.49	0.7430
Store diversity	1	10.064	10.88	0.0020
Warmer Scenario				
Region	2	1.069	1.26	0.2935
Store type	1	.753	0.89	0.3511
Region × store type	2	0.236	0.28	0.7585
Store name (store type)	2	7.416	8.76	**0.0007**
Region × store name (store type)	4	2.209	2.61	**0.0493**
Store diversity	1	17.297	20.44	**<0.0001**

The dependent variable is the percentage of potentially invasive non-native species for sale at each store. ‘Region’ is Delta, East Bay, or Peninsula; ‘Store Type’ is Chain or Independent; ‘Store Name’ refers to Independents or individual chain stores (General Discount Chain, Pet Chain A, or Pet Chain B), and is nested within Store Type; ‘Store Diversity” (total number of taxa identified to species level) is treated as a covariate.

Figure 3.3: Average number of potential invaders in Colder Scenario (top) and Warmer Scenario (bottom).



Dots represent least squares means for each store type; whiskers indicate 95% confidence intervals.

In the Warmer Scenario, there was no difference between chain and independent stores ($F_{1, 42} = 1.04$, $p = 0.3146$) (Table 3.3), although when considering individual chain stores, the General Discount Chain carried significantly fewer potential invaders than independent stores and Pet Chains A and B (Figure 3.3). When adjusting for store inventory size, chain and independent stores again were not significantly different (Table 3.4), but when considering the individual chain stores (along with independent stores as a group), Pet Chain B carried significantly more potential invaders than the General

Discount Chain and Pet Chain A, while independent stores were not significantly different from any other group. Significant interactions between region and store name in both the ANOVA and ANCOVA (using store inventory size as a covariate) for the Warmer Scenario suggest that there were differences among local regions in terms of the number of potential invaders found in a particular kind of store, and that these differences were not due to store inventory size (Tables 3.3 and 3.4).

A greater proportion of fish taxa sold in chain stores were identifiable to species level compared to independent stores ($F_{1,\,42} = 49.29$, $p < 0.0001$). In addition, a greater percentage of fish species were correctly labeled in chain stores versus independent stores ($F_{1,\,21} = 5.35$, $p = 0.0310$) (Table 3.5).

Table 3.5: ANOVA results from analyses of aquarium store labeling practices.

Source	**DF**	**MS**	***F***	***p*-value**
Taxonomic resolution: percentage of fish taxa in each store that could be identified to species level				
Region	2	0.816	0.66	0.5226
Store type	1	73.406	59.29	**<0.0001**
Region × store type	2	2.296	1.85	0.1691
Store name (store type)	2	10.162	8.21	**0.0010**
Region × store name (store type)	4	2.562	2.07	0.1020
Labeling accuracy: percentage of species present in store that were correctly labeled				
Region	1	0.000	0.04	0.8534
Store type	1	0.041	5.35	**0.0310**
Region × store type	1	0.000	0.00	0.9685
Store name (store type)	2	0.013	1.71	0.2053
Region × store name (store type)	2	0.000	0.06	0.9375

Survey of store representatives

(1) Awareness

A majority of respondents (18/30 = 60%) had previously heard the term "invasive species", representing 66.7% (12/18) of independent store respondents and 50% (6/12) of chain store respondents. When given a definition of invasive species—"a species that establishes populations in an area where it is not native"—90% (27/30) of respondents reported that invasive species were an important environmental concern. Although it was not signficant at the $\alpha = 0.05$ level, there was a trend that respondents who had more experience in the aquarium industry as a hobby (i.e. those who owned an aquarium or bred/traded fish) more frequently reported having heard of invasive species or having heard of aquarium pets or plants invading natural areas (owned an aquarium: Fisher's exact test, $p = 0.0524$; bred/traded fish: Fisher's exact test, $p = 0.0680$). A majority (21/30 = 70%) of store representatives in this survey reported that they thought aquarium stores sold potentially invasive fish or plants, but there was no significant difference between responses from chain store vs. independent store representatives (Fisher's exact test, $p = 1.0$). We found no significant difference between chain store and independent store respondents in knowledge of invasive species (ANCOVA, $p = 0.6978$).

(2) Responsibility

Most respondents (26/30 = 86.7%) agreed that scientists should identify which plants and animals sold in the aquarium trade are potentially invasive. Many respondents (19/30 = 63.3%) also reported believing that the aquarium trade plays a role in the introduction of aquatic invasive species. A slightly higher percentage (23/30 = 76.7%) reported believing that the aquarium trade has a role in the prevention of aquatic introductions. When asked to assign responsibility to specific stakeholder groups in the aquarium trade, including the aquarium industry, consumers, government, and scientists, chain store respondents were significantly more likely than independent store respondents to assign responsibility for preventing invasions to industry stakeholders (ANOVA, $p = 0.0280$). Examination of answers to individual survey questions indicates that this result is driven by chain store representatives' assignment of more responsibility to retailers (Fisher's exact test, $p = 0.0378$), while the amount of responsibility assigned to other aquarium industry sectors did not differ between store types. Interestingly, a greater percentage of chain store respondents held sales associate or equivalent positions without managerial responsibility, as compared to independent store respondents (Fisher's exact test, $p < 0.001$).

(3) Willingness to take preventative action

Nearly all respondents (29/30 = 96.7%) reported that customers have asked them what to do with unwanted fish, and a majority (24/30 = 80%) of store representatives reported that they would be willing to sell different fish species in place of fish known to pose an invasion risk. Willingness to sell alternative species did not differ significantly among store types (Fisher's exact test, $p = 0.6372$).

DISCUSSION

Our store survey results indicate that aquarium stores in the San Francisco Bay–Delta region sell as many as 27 fish species that could potentially survive if released into Bay–Delta waters, underscoring the significance of the aquarium trade as a potential vector for non-native species introductions. While this is a small number compared to the total number of fish species identified during the survey (867 taxa identified to species level), we caution that this does not mean the risk is low. First, many of the species listed as potential invaders are among the most commonly-stocked species in the industry, and are likely sold in high volumes. Second, given global warming trends, the occurrence of warm water refugia in the region, and imperfect knowledge of fish species' lower temperature tolerances, we suggest that our Colder Scenario analysis likely underestimates the number of potentially invasive fish species available for sale in the Bay–Delta region. For example, the Dojo loach (*Misgurnus anguillicaudatus*) was found to be a potential invader in the Bay–Delta region only in our Warmer Scenario (minimum temperature 12°C) due to its listed 10°C minimum temperature in FishBase (Froese and Pauly 2005). In contrast, Rixon *et al.* (2005) used a more restrictive minimum temperature criterion of 5.5°C for the Great Lakes, yet listed the Dojo loach as a likely invader there. In fact, the Dojo loach is already established near the Great Lakes in Michigan's Shiawassee River system, and has been documented to survive water temperatures as low as 2°C, which meets our Colder Scenario criteria (Schultz 1960; Logan *et al.* 1996). Finally, our analysis examines a snapshot of the species stocked in Bay–Delta aquarium stores during one 6-month time period and does not consider all species that may be part of the aquarium trade in this area, or in the future. Querying Fishbase (Froese and Pauly 2005) for species associated with the aquarium industry returns an additional 187 saltwater and 100 freshwater fish species that were not found in our inventory but which could survive Warmer Scenario conditions.

In contrast, our Warmer Scenario may overpredict the number of potential invaders, especially for saltwater fish, as the available temperature tolerance data for these fish are much less precise than for freshwater fish. This analysis may therefore include some species that are unlikely to be able to invade the Bay–Delta in today's climate. From a risk-assessment standpoint aimed at reducing the number of successful invasions, however, use of the Warmer Scenario results as a guide for management would more effectively lower invasion risk than using the Colder Scenario results. Improved estimations of invasion risk will require more accurate data describing both environmental characteristics of recipient areas, including potential refugia, as well as physiological requirements of aquarium species, especially saltwater species. Meanwhile, the Bay–Delta is likely to experience warming over the next 50–100 years due to global climate change (Hayhoe *et al.* 2004), potentially increasing the hospitability of this system to non-native aquarium species, many of which are from more tropical climes.

Significantly, the risk posed by potentially invasive aquarium fish species in the Bay–Delta is not evenly distributed across the industry. In our Colder Scenario, chain store inventories contained a significantly greater number of potentially invasive species compared to independent store inventories. This pattern is driven entirely by freshwater fish inventories, due in part to the fact that compared to independent stores, chain stores generally carried a much smaller percentage of saltwater fish. This pattern in store inventory diversity may exist because chain stores likely cater to a more general clientele than the more hobbyist-focused independent stores, and freshwater fish are likely to appeal to a broader clientele because they tend to be less expensive and easier to keep than saltwater fish (Chang *et al.* pers. obs.). In addition, since the chain store business model revolves around lower prices and high volume (Spector 2004), these stores' inventories might emphasize species that best survive the rigors of capture, transport, and stocking, traits also conducive to survival if released into a local waterway. However, these risks might be mitigated by clear and accurate labeling of fish species for sale and a high level of employee knowledge regarding invasive species.

In fact, while chain store inventories consistently contained a greater number of species identified by the Colder Scenario that could potentially invade the Bay–Delta, current practices that might mitigate these risks were also more widespread in chain stores than in independent stores. Specifically, labeling practices in chain stores were consistently better than in independent stores, and chain store inventories were more limited and generally contained easily identifiable fish. Independent stores, in contrast, had wide-ranging and variable inventories as well as relatively less well-labeled fish.

Our results also suggest that chain store representatives may be better positioned to mitigate invasion risk through customer assistance and interaction. Effective mitigation by these means requires not only that an employee be aware of invasive species issues but also that he or she recognize his or her own ability to help counteract any risk posed by a particular fish being sold. Our phone survey results indicate that 60% of all respondents had heard of the term "invasive species." While 90% of all respondents considered invasive species to be a concern, independent store respondents were significantly less likely than chain store respondents to consider it the retailer's responsibility to take action regarding invasive species. However, differences between respondents from independent and chain stores may well be confounded with differences in the respondent's level of authority in the store; on average, independent store respondents had a significantly greater level of authority compared to chain store respondents. The majority of independent store respondents had managerial-level duties, and many were store owners, whereas most of the chain store respondents worked at sales associates or equivalent lower-level positions.

Finally, and most hopefully, a majority of both independent and chain store respondents indicated that they would be willing to sell a different species in place of one that might pose an invasion risk. This positive result suggests that some biological invasions may be avoided if aquarium industry representatives are consulted to identify and make available for sale alternative species that pose a lesser threat. The horticulture industry, which plays a similar role to the aquarium trade in terrestrial invasions, has successfully collaborated with managers to create voluntary codes of conduct to reduce risks posed by non-native species (Baskin 2002). Although awareness of these codes remains the major hurdle to their adoption (Burt *et al.* 2007), our results indicate that similar programs in the aquarium trade may have a reasonable likelihood of success.

Suggested research and management actions

We suggest two key management actions to reduce the overall risk of invasion via the aquarium trade. The first is to implement programs to enhance invasive species awareness and education among store representatives, focusing especially on higher-level management at both chain and independent stores. These education initiatives would increase the ability of store employees to advise customers on the risks of their purchases and would complement general consumer education programs regarding invasive species.

Education has been a key factor in encouraging the adoption of risk-lowering behavior in other industries (Burt *et al.* 2007), but changes to store inventories and policies often require action at the managerial level. Our results also indicate that education might have the greatest impact at this level, since employees in managerial positions were less likely to report that they believed retailers bear responsibility for preventing aquatic invasions.

Second, we recommend improving fish labeling practices, particularly in independent stores; compared to chain stores, a relatively greater proportion of fish in independent stores were mislabeled or unlabeled. In part, this reflects chain stores' greater focus on freshwater species, which are generally better described (Wabnitz *et al.* 2003). In independent stores, employee expertise might sometimes substitute for less thorough labeling practices, but clear and accurate identification of fish for sale is still key to informing consumers about the potential risks of their purchases. We further suggest that readily available information on key life history traits (maximum size, growth rate, aggressiveness, etc.) would help consumers avoid buying fish that wind up as unwanted pets and which may be at higher risk for inappropriate disposal into local waterways (e.g., Crossman and Cudmore 1999). This information is often available on labels used in chain stores (Chang *et al.*, pers. obs.) Such information would ideally be complemented by warnings to customers about the potential hazards of releasing pets, which we observed at just 1 out of the 54 stores we visited during the course of this study. At that particular store, the information provided was from a commercial pet industry campaign, HabitattitudeTM.

Further research is needed to determine which of the potentially invasive species identified by our store inventory are most likely to be released by consumers and what, if any, significant ecological impacts they might have. The most threatening of these species could then be targets for aquarium store representative and consumer education initiatives. Effort also could be directed toward finding alternative, less potentially-invasive species, since our results indicate that people working in the aquarium trade may be willing to substitute less risky species for those demonstrated to be potentially invasive. While the relatively low response rate (26.3%) of our telephone survey could be interpreted as a lack of willingness to engage with the issue of invasive species, we argue that this more likely reflects a general lack of willingness to participate in telephone surveys, especially since 100% of the stores we visited in person allowed us to conduct inventories. Future studies might consider interviewing store representatives in person or distributing printed survey forms to increase response rates.

In assessing the invasion risk posed by the aquarium fish trade in the Bay–Delta region, our approach considered the interaction of specific local factors: environmental parameters, species availability patterns, and awareness and attitudes of trade workers. As such, our specific conclusions should not be extrapolated beyond the region we studied, but instead studies similar to our own should be repeated in as many regions as possible. Work along these lines has been attempted in Florida (Semmens *et al.* 2004) and the Great Lakes (Rixon *et al.* 2005), but other regions remain less well studied. Perhaps the most fruitful approach would be to combine the techniques used in this study with the complementary methods used by Cohen *et al.* (2007) to examine aquarium plant releases in the St. Lawrence Seaway. Our study determined which species were available for sale in our local region, assessed each species' likelihood of survival under local environmental conditions, determined which stores or types of stores carried more of the species identified as potential invaders, and examined industry attitudes toward invasive species. Cohen *et al.* (2007) more directly measured propagule pressure of aquarium plant releases by combining sales volume data for individual species with consumer surveys assessing the likelihood of various methods of release. In combination, these approaches allow a comprehensive analysis of the risk posed by an invasion vector at each major phase of the introduction process, from propagule delivery to survival and establishment, while the integration of sociological information points the way to the most productive targets for management action.

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APPENDIX I: Text of telephone survey used to assess aquarium store representatives' knowledge about and attitudes toward invasive species

For our eyes only: *Name of Aquarium Store* ____________________

Name of Contact ____________________

Date ____________________

TELEPHONE SURVEY

[Please be informed that the responses provided in this survey are anonymous. Neither your name nor that of your business will be presented with these data at any time.]

PART I

[I am going to start with some general background questions about your aquarium store]

1. What is your job title and what are your primary duties? (*Circle duties for coding)*

purchasing
inventory
management
customer service
owner
sales
other: _______________

2. Does your store sell salt or fresh water fish or both?

Salt Fresh Both

3. Have you heard the term "invasive species"?

Yes No Don't Know

[*Thanks, I'm interested in finding out your opinions on invasive species. To make sure that everyone is on the same page, we will use the following definition of invasive species: "**A species that establishes populations in an area where it is not native.**"]*

4. Have you previously heard of aquarium pets or plants becoming invasive in natural areas?

Yes No Don't know

5. Do you think aquarium hobbyists ever think of their pets or plants as being potentially invasive?

Yes No Don't know

6a. Do you think the aquarium trade has a role to play in the spread of invasive species?

Yes No Don't know

6b. Do you think the aquarium trade has a role to play in the prevention of invasions?

Yes No Don't know

7. What do you think people do with their aquarium pets when they can't keep them anymore (i.e., moving).

Suggestions to lead them if necessary (not to be read to the survey respondent):

Return it to the store where they bought it
Give it away to a friend
Release it in their backyard
Release it in a nearby park or waterway
Humanely dispose
Other?

8. Has a customer ever asked you what to do with their unwanted pets?

Yes No Don't know

9. Do you think aquarium store customers ever release their pets into a local stream or sewer system? (Optional—this could be duplicative if they answered "release it" in Question 7…but if they didn't, it would be important to ask here.)

Yes No Don't know

10. Do you think aquarium stores sell fish or plants that may become invasive?

Yes No Don't know

11. I'm going to read a short list of statements, for each statement, please rate yourself on a scale ranging from agreeing to disagreeing with that statement.

Statement	**agree**	**neither**	**disagree**
Aquatic invasive species are an important environmental concern			
The aquarium trade plays a role in the introduction of aquatic invasive plants and animals			
The aquarium trade should evaluate which aquatic plants and animals could become invasive			
Scientists and researchers should evaluate which aquatic plants and animals could become invasive			
It is okay for aquarium stores to sell plants and animals that are known to be invasive			

12. I will mention seven groups that I would like you to rate in terms of responsibility for preventing aquatic invasions. Please rank each group on a scale from 1 to 5, with 1 being who you think should be most responsible and 5 being the least responsible for preventing invasions via the aquarium trade.

Consumers	1	2	3	4	5
Retailers	1	2	3	4	5
Wholesalers	1	2	3	4	5
Breeders/Collectors	1	2	3	4	5
Policy Makers	1	2	3	4	5
Scientists	1	2	3	4	5
Government Agencies	1	2	3	4	5

13. Would you be willing to sell a different species in place of one that could become invasive in the area?

Yes No Don't know

14. How many different fish types do you have in your store now?

less than 75 75-300 more than 300 Actual number________

15. Do you own an aquarium of your own?

Yes No

16. Have you ever bred fish yourself or traded fish with friends?

Yes No

17. If it was determined that some fish could be invasive, would you prefer that the aquarium trade address the issue with….

Voluntary Code of Conduct regulations Other____________________

***Do you have any questions, or comments you'd like to add?*

Well, that's it. I really appreciate you taking the time to talk to me. Just to reiterate, this survey was designed to help us figure out how environmental issues affect the aquarium trade, and we care about your opinions and those of other professionals. All of your answers for you and your business are anonymous; we're just looking for general patterns and are extremely grateful for your willingness to participate. Thanks again for your time. [If you have any further questions, or comments you'd like to add at a future date, feel free to email/call me at ____].

Thank you very much.

APPENDIX II: Taxonomy and natural history of common sessile epifaunal invertebrates in the San Francisco Estuary

> "If you do not know the names of things, the knowledge of them is lost, too."
> —Linnaeus, 1751

INTRODUCTION

Determining the provenance of any given marine or estuarine sessile invertebrate species in northern California can pose several challenges. Alice Robertson, who originally described many of the bryozoan species on the North American Pacific Coast, worked primarily around the late 1800s and early 1900s, a time when "the bryozoa of the west coast of North America constitute[d] a fauna practically unknown to science" (Robertson 1905). Yet there was enormous human activity, including ship traffic and oyster shipments, that likely caused massive translocations of species to this region prior to Robertson's investigations, particularly beginning with the California Gold Rush in 1849 (Carlton 1979). A further blow to modern attempts to distinguish native from non-native species in this region was the loss of the California Academy of Sciences' collections in the 1906 San Francisco earthquake and fire (Williams 2007). As the Academy was the premier site for collections from the region prior to 1906, this loss immeasurably increases the challenge of knowing what species once were present in San Francisco Bay, and when, and where. The primary sources of modern evidence regarding a species' provenance are therefore genetic and biogeographic comparisons, of which an increasing number are being made (L. McCann, C. Zabin, pers. comm.). While such comparisons will greatly increase our knowledge of the provenance of existing flora and fauna, we cannot reconstruct records of species that are no longer present unless they have left some trace in fossils or sediments.

In this Appendix, I include several dichotomous keys and a section describing many of the species or morphospecies from major groups identified in this work in the hopes that it may be easier for others to determine what species I encountered, and to match up species that I found with species found in their work. I have tried to minimize jargon, but some terminology is unavoidable. Terms are used as defined in *The Light & Smith Manual*, 4th ed. (Carlton 2007). Specimens of most species reported here may be available from me upon request and will be deposited in museum collections for future reference. Generally speaking, the geographic scope of this work is the San Francisco Estuary from Antioch to the Golden Gate, including southern San Francisco Bay. I focus on species most often found at the shallow subtidal sites that I visited regularly from 2000–2009, although some taxa covered here are also intertidal. This is most of the marinas and harbors in the estuary, but not the Napa and Petaluma River region, or Lake Merritt in Oakland.

Studies that center around diversity are of limited use if suspect taxonomy has been applied to identify the species in question. Community ecology, particularly biodiversity–ecosystem function studies, and works on biological invasions require consistently accurate identification of species. The general state of knowledge of taxonomy is not equal among the many and varied groups of organisms, being much sparser among smaller organisms (*e.g.* Wasson *et al.* 2000) and those encounted less frequently by humans. While some groups are so small or so rare as to be studied mainly by specialists, sessile, epifaunal marine invertebrates appear to inhabit a zone in between: unknown to most people, yet familiar and common enough to most marine biologists to often be used in invertebrate classes, and easily encountered by anyone with access to a dock, boat, or pier piling. As such, their familiarity, diversity, and apparently accessible (at first glance) taxonomy lead many to be blithely casual about the precise details required for exact identification, eschewing caution and generating a flurry of inaccurate and ultimately useless identifications; as Alexander Pope (1711) wrote, "A little Learning is a dang'rous Thing."

Few published ecological works include descriptions of the species that were studied, and sadly, taxonomic work is not of equal quality among all published ecological studies. Quality taxonomy requires both competence on the part of the taxonomist as well as good guides to the taxa in question. Many ecological studies address the issue of taxonomy with a claim that species were identified according to the relevant general guide for that region (if one exists), but this statement still forces the reader to assume some level of taxonomic competence on the part of the author, or whoever performed the identifications. Specialists are sometimes, but often not consulted. It therefore seems that the clearest way for me to say how I identified things is to attempt to describe the criteria myself. I make no claim either to great expertise nor to superior taxonomic ability. I have been ably assisted along the way by several recognized experts, but any errors here are entirely my own. I document the criteria I found most useful and efficient for identifying major taxa in my studies so that future investigators may more accurately know to what species, morphospecies, or semi-species-like thing I refer in my ecological work.

I have focused on external morphology and readily apparent features that may be used to distinguish specimens in the field and in the lab without extensive dissection. The descriptions given here are intended to guide the worker with some knowledge of systematics in the correct matching of a freshly-found specimen to a particular species or morphospecies referred to in this study. For truly accurate taxonomy, however, some species simply require dissection and a full working-out using good dichotomous keys. Even these dissections, even if expertly performed, will often fail to provide a definitive identification, particularly if the organism in question is a juvenile, is generally small, is from a poorly-known group, or (most insidiously) is a member of a cryptic species group. The latter case is likely far more common than most of us realize, a fact borne out by increasing reports of molecular analyses that identify two or more species where previously (based on morphology), we had only recognized one (*e.g.*, Geller *et al.* 1994, Folino-Rorem *et al.* 2008).

In any investigation requiring morphological taxonomy that one wishes to compare to previous work, it is useful to know whether those who identified organisms in the previous work (as well as the authors of the relevant taxonomic keys used) are "lumpers" or "splitters." That is, were they more likely to group together or to separate organisms of similar appearance? In the first case two or more species may be lumped together, reducing the number of taxa reported, while the second instance risks a spurious report of more species than are actually present. In investigations requiring identification of species from multiple phyla, workers usually are not equally skilled at identifying organisms from all phyla, with natural inclinations toward lumping and splitting in general being magnified according to how familiar they are (or think they are) with particular groups.

While some have accused me of being a splitter, others no doubt find me a lumper. I leave that determination to others, and especially to molecular analyses.

An additional difficulty often encountered is that various life stages often bear little resemblance to each other. Taxonomic keys are usually written for adult organisms, and thus can be useless for identifying planktonic larvae, juveniles or even small adults. In my case, I am familiar with all sizes of settled sessile organisms, and less familiar with their planktonic larvae. I am generally best at identifying bryozoans, barnacles, and tunicates. I am less comfortable with sponges and hydroids, partly because these groups are difficult to identify morphologically and partly because I am not very practiced at identifying these groups. I also am less familiar with algae. Please keep this information in mind if using the keys and species descriptions given here.

Warning!

Unless noted otherwise, I have only included some of the more common species that I have observed and identified to the lowest taxonomic level identified here. This Appendix should **NOT** be treated as an exhaustive listing of all species known from this region for any of the groups described here!

KEY TO THE COMMON BRYOZOANS OF THE SAN FRANCISCO ESTUARY

1. Erect, branching, muddy, uncalcified ....*Anguinella palmata*
– Stolonate (single zooids connected at bases by stolons; soft, uncalcified)....................*2*
– Erect (branching, arborescent, calcified) *3*
– Encrusting (gelatinous or calcified)...*13*

Stolonate Bryozoa

2. Low, slender tubes 3 mm in height or less arising from mats of interconnected stolons; each zooid has 8 tentacles; estuarine....................................*Bowerbankia* sp. A
– As above, but with 10 tentacles per zooid; usually more marine......*Bowerbankia* sp. B
– As above, but with 20 or more tentacles per zooid; zooids taller and slenderer than *Bowerbankia*, occasionally branching, and found in brackish to fresh water ....*Victorella pavida*

Erect Bryozoa

3. Zooids heavily calcified tubes fused together; sometimes only the apertures may be visible; frontal wall may appear to have small pores; no avicularia or spines, colonies whitish, stiff, jointed *4*
– Zooids lightly calcified, colony flexible, often with avicularia and spines *8*

4. Zooids in alternating pairs...*5*
– Zooids randomly arranged, tubes partially protruding from main branch, colony stiff, whitish, usually with many zooids between yellow joints ....*Crisulipora occidentalis*

5. Joints white, yellowish, or light brown..*6*
– Joints black or dark brownish-black, colony fragile, zooids long and slender, in alternating series, 1 to 3 zooids between branches.........................*Filicrisia franciscana*

6. Joints white, tubes indistinct and almost completely immersed in branches..............*7*
– Tubes distinct, joined in pairs, short point at distal ("top") end of aperture, 12-20 zooids between joints, older joints dark brown.......................................*Crisia maxima*

7. Short point at outer distal end of aperture, giving outer edge of colony a serrated .... appearance...*Crisia serrulata*
– As above, but with ridge or keel running down center of each branch between apertures...*Crisia occidentalis*

8. Erect, not purple *9*
– Erect, purple, no avicularia ....*Bugula "neritina"*

9. Erect, tan, zooids in biserial rows, has avicularia but not vibracula or scuta, spines generally shorter than ½ length of zooid (if present) *10*
– Has vibracula and/or scuta, colony tufted, stiff *11*
– Erect, generally whitish or tan, branching, with long hair-like spines (at least as long as zooids are tall) projecting up from tops of zooids; no vibracula or scuta, few avicularia .... *Caulibugula ciliata*

10. Tan, avicularia near bottom of zooid, whorl branching pattern.......... *Bugula pacifica*
– Avicularia near top of zooid, random branching pattern .... *Bugula stolonifera*

11. Erect, whitish or tan, branching, has both vibracula and scuta *12*
– Erect, whitish or tan, but has scuta only (no vibracula) .... *Tricellaria* sp.

12. Vibracula approximately 3 zooids long, thick, robust, rectangular zooids .... *Scrupocellaria* cf. *diegensis*
– Vibracula slightly longer than 1 zooid .... *Scrupocellaria* cf. *californica*
– Vibracula shorter than the length of 1 zooid .... *Scrupocellaria varians*

Encrusting Bryozoa

13. Gelatinous, uncalcified, often muddy *14*
– Frontal membranous, uncalcified (sides of zooid calcified) 15
– Frontal at least partly calcified *17*

14. Smooth frontal, hexagonal or octagonal zooids .... *Alcyonidium* sp. A
– Papillated frontal, often muddy, with tubular protrusions in center, often resembles a dense mat of *Bowerbankia* sp .... *Alcyonidium* sp. B

15. Does not have lateral spines interlocking to form a shield over frontal 16
– Has central proximal spine set at up to 45° angle to frontal, "mask" of wide, fused lateral spines over frontal, two stout distal spines, zooids small, translucent, and colorless; small colonies crescent-shaped .... *Aspidelectra melolontha*

16. Encrusting but often upright branching growth form, lacks spines, single ancestrula.......... *Membranipora chesapeakensis*
– Lacks central spine proximal to membranous frontal, may have lateral spines.......... *Conopeum* sp.
– Has central spine (proximal to membranous frontal) that appears distinct from any lateral spines present; often stouter than proximal spine .... *Electra* sp.

17. Frontal porous all over *18*
– Frontal smooth in center with pores around edges of zooid 20

18. Operculum with sinus (notch at proximal end) *19*
– Operculum squarish and flared out at one end and lacking a sinus, frontal with large pores, colony often whitish or orange colored .... *Cryptosula pallasiana*

19. Operculum with sinus (notch flaring out at proximal end), frontal porous with small pores, colony is a brick red color with black opercula..........*Watersipora "subtorquata"*
– Frontal porous, operculum with sinus, zooids generally with 3:1 length-to-width ratio, colony is shiny orange or orangish-white in color, avicularia occasionally present and located proximal to operculum and off to one side ......*Schizoporella* sp. A
– Frontal porous, operculum with sinus, zooids hexagonal or generally squat rectangles, colony is a dull whitish or orange color, avicularia occasionally present and located proximal to the operculum and off to one side ....*Schizoporella* sp. B

20. Zooids opaque and whitish, small, tapered proximally, with a small ledge just below the proximal rim of the aperture. Ovicells, if present, are porous, and egg masses often pinkish (sometimes orange)..*Smittoidea prolifica*
– Zooids translucent, glassy, colorless (sometimes looks white), pores may be visible around edges of zooids, indentations visible on both sides of proximal rim of aperture, ovicells, if present, are globular and porous, with yellowish egg masses.........
....*Celleporella hyalina*
– Zooids squared off proximally, with one line of large pores around the edge of each zooid, frontal shiny, median denticle..*Parasmittina* sp.

KEY TO THE COMMON TUNICATES OF THE SAN FRANCISCO ESTUARY

1. Solitary..........*2*
– Colonial..........*9*

2. Tunic both translucent and either soft or thin (easily torn)..........*3*
– Tunic opaque, firm, often yellowish or orangish..........*6*

3. Tunic flexible, tough, elastic; can be punctured with forceps, but not easily torn..........*4*
– Tunic translucent, colorless, thin and papillated, easily torn with forceps..........*5*

4. Yellowish, whitish, and/or pale orange coloration around siphon rims (yellowish patches visible in juveniles), body wall may contain flecks of white pigment, tip of vas deferens white..........*Ciona savignyi*
– Siphon rims with small red dots (ocelli) embedded in tunic and no other coloration (in juveniles, red patches instead of discrete dots are visible), small finger-like projections around each siphon in large specimens, no pigment flecks in body wall, tip of vas deferens red..........*Ciona intestinalis* Type A

5. Mushroom-shaped body with narrow attachment to substrate and widening near siphons, inflated brood chamber visible around anal siphon in adult specimens with siphonal rim below surrounding tissue..........*Corella inflata*
– Flattened body with two siphons widely spaced; oral siphon sometimes elongated (up to ½ length of body), particularly in crowded conditions; papillated, innards sometimes orangish..........*Ascidia zara*

6. Stalked..........*7*
– Not stalked..........*8*

7. Body long and thin, at least 5 times longer than wide, tunic with longitudinal wrinkles, one siphon turned laterally..........*Styela montereyensis*
– Body club-shaped, about 3 times longer than wide, tunic bumpy, especially near siphons, siphons with four lengthwise dark stripes that form a cross shape when closed siphons are viewed from top)..........*Styela clava*

8. Broadly attached, adults with thick, tough tunic, siphons far apart..........*Ascidia ceratodes*
– Body round, firm, often mud-covered, semi-opaque tunic..........*Molgula manhattensis*

9. Zooids connected by stolons..........*10*
– Zooids connected by thin or thick sheets or lobes of tunic..........*11*

10. Greenish color, stolons connecting small, round zooids, star-shaped terminal buds at ends of stolons..........*Perophora japonica*
– Stolons without star-shaped terminal buds..........*Perophora annectens*

11. Zooids distinct, connected by or embedded in thin bands or sheets of tissue..........*12*
– Zooids not easily distinguishable, embedded in thick sheets or lobes of tunic..........*14*

12. Zoooids form long chains (may be roughly circular)..*13*
– Zooids form distinct circular or star-shaped clusters (as many as 10 per cluster)..*Botryllus* sp.

13. Entire colony is of a single color (commonly orange, yellow, red; purple less common) or uniform coloration pattern (red or orange speckled with white); oral siphons are not distinctly colored compared to rest of zooid; large tadpoles with 20-30 ampullae..*Botrylloides violaceus*
– Colony is two distinct colors, with oral siphon and sometimes entire top surface of zooid usually bearing a lighter color (orange or yellow / white) and the edges and sides of the zooids having a darker color (often purplish); small tadpoles with 8 ampullae..*Botrylloides diegensis*

14. Tunic tough...*15*
– Tunic thin, easily torn, colonies fragile, lobate, often greyish, black, white, or yellow-gold; common excurrent siphons sometimes rimmed with white...*Diplosoma listerianum*

15. Tunic tough, whitish or yellowish, sometimes with shiny, pearlescent streaks on surface; larger colonies form mushroom shapes with a distinct stalk........*Distaplia* sp.
– Tunic tough, whitish or yellowish; with tiny calcareous spicules embedded in tunic; larger colonies often have long, dark lines where spicules are absent and form lobes that hang off the substrate...*Didemnum vexillum*

KEY TO THE COMMON PORIFERA OF THE SAN FRANCISCO ESTUARY

1. Tubular..*2*
– Encrusting...*3*

Tubular sponges

2. Bright, whitish, long, thin tube with short oscular fringe, sometimes grows in clusters..*Leucosolenia* sp.
– Bright whitish to dirty grayish, sometimes silt-covered, usually a short, fat tube with widest point at midsection, long oscular fringe (spicules in fringe are as long as oscule is wide)...*Scypha* sp.

Encrusting or blob-like sponges

3. Dense, darker color (anywhere from medium yellow to orange to reddish), but can be light yellow to whitish in freshwater...*4*
– Soft, not dense, lighter color (light yellow to tan to pinkish)......................................*5*

4. Medium to dark yellowish (or whitish in freshwater), dense, large colonies wispy, long diaxine spicules (pointed at both ends)......................................*"Halichondria"* sp.
– Orangish to reddish color, dense..*Clathria prolifera*

5. Light to medium yellow, soft, large colonies have inflated, rounded shapes with prominent oscules and may be tan to pink in color, spicules short and diaxine (pointed on both ends)...*"Haliclona"* sp.
– Light to medium yellow, soft to medium density, prominent oscules, has both short diaxine spicules (pointed on both ends) and anisochelate spicules (short rods with mushroom-shaped caps on both ends featuring anchor-like projections) ...*Mycale* sp.

KEY TO THE COMMON HYDROZOANS OF THE SAN FRANCISCO ESTUARY

1. Athecate (may have theca-like covering around hydranth, but hydranth cannot withdraw completely) *2*

– Hydranth with clearly defined rigid theca into which it can retract; stalks smooth except where annulated *8*

2. Tentacles in two or more whorls; gonophores (if present) among tentacles *3*

– Tentacles in one whorl or otherwise concentrated around the mouth; gonophores, if present, are below the tentacles *4*

3. Hydranths colonial, arising from interconnected stolons, perisarc (chitinous outer covering) covering stolons and stems up to base of hydranth, colonies large (up to 15 cm height), branched, forming tangled tufts, hydranths pink with reddish or purple gonophores resembling grapes among tentacles .... *Pinauay crocea*

– Hydranths solitary, though sometimes occurring in groups, perisarc as above, colonies smaller, up to 5 cm height, gonophores resembling strings of grapes among tentacles .... *Pinauay marina*

4. Perisarc (chitinous outer covering) covering stolons and stalks, but not hydranths *5*

– No perisarc, hydranth solitary, with tentacles at oral end only, relatively fat stalk not tapering toward mouth, often pink or pinkish-orange, fresh to brackish water .... *Hydractinia* spp.

5. Tentacles concentrated in a whorl *6*

– Hydranths pink or white and diamond-shaped in profile, covered with scattered filiform tentacles (not in a whorl), stalks connected by stolons; larger colonies have branched stalks .... *Cordylophora* spp.

6. Hydranth partially covered by pseudohydrotheca (bell-shaped, dirty, brownish extension of perisarc; hydranth cannot completely withdraw) *7*

– Hydranth naked, filiform tentacles, trumpet or urn-shaped hypostome, short annulated stem attaches hydranth to stalk .... *Eudendrium* sp.

7. Bright orange, stems polysiphonic (composed of several tubes joined together), annulated throughout .... *Garveia annulata*

– Hypostome dome-shaped, brownish with orange hydranths, stems monosiphonic (one tube), few annulations, estuarine .... *Garveia franciscana*

8. Does not have operculum 9

– Has operculum, stolonate (short stalks connected at bases via stolons) 10

9. Each stalk supports one hydranth; stalks short and connected by stolons; stalks annulated at junction with stolons and often just below hydranths; hydranths bell- or cup-shaped; hydranth rims generally crenulate .... *Clytia* spp.

– Colony upright with each stalk supporting multiple hydranths; stalks connected at bases via stolons but growing up off the substrate; hydranths on both sides of stalks, often (but not always) alternating, forming a "zig-zag" pattern when examined from the side; stems narrow and often annulated at branching points and just below hydranths; hydranths bell- or cup-shaped on a short pedicel branching off the main stalk. Rim of hydranth may be smooth or scalloped. Colony whitish (when small) to light brown. ....*Obelia* spp.

10. Hydranths are short, fat cylinders attached to stolons via short pedicels; operculum cone-shaped; polyps have webbing between tentacles....................*Blackfordia virginica*

– Hydranths are cylindrical, perched atop a short, annulated pedicel connected to the stolon; flap-shaped opercula composed of numerous lateral, pointed flaps that come together to form a cone..*Phialella* spp.

SPECIES DESCRIPTIONS

Bryozoa

The Bryozoa are one of the most diverse and challenging groups in the northern-central California region. Unfortunately, the extensively revised 4th edition of *The Light and Smith Manual* (2007), the premier taxonomic manual for this region, includes dichotomous keys to genus level only—not to species level (Soule *et al.* 2007). More specialized taxonomic literature must be consulted for species-level keys (e.g. Soule *et al.* 1995). The taxa included in the key and descriptions here are distinguishable using light microscopy, and most occur primarily in shallower estuarine waters, such as would be sampled by panels suspended from rafts, docks, or pilings. I have based the key on extensive collections from San Francisco and Tomales Bays, examinations of material in the California Academy of Sciences, and keys and descriptions from several sources, especially Robertson (1905, 1908, 1910), Osburn (1950), and Soule *et al.* (1995, 2007).

***Alcyonidium* sp. A and B**
KEY: p. 143, couplet 14

Muddy, gelatinous, and encrusting, *Alcyonidium* spp. are often overlooked or mistaken for patches of mud. *Alcyonidium* appear to favor very shallow and/or intertidal waters, appearing in mid-to-high salinity waters. *Alcyonidium* sp. A may correspond to *A. gelatinosum* (Linnaeus, 1767) or *A. polyoum* (Hassall, 1841), while *Alcyonidium* sp. B may be *A. mammilatum* (Alder, 1857). *Alcyonidium* sp. A settles in more protected areas, and I have taken it on monthly panels at Richmond Marina. *Alcyonidium* sp. B seems more common in the low intertidal and shallow subtidal on piers in more open waters.

***Anguinella palmata* (Van Beneden, 1845)**
KEY: p. 142, couplet 1

This muddy, branching bryozoan is an Atlantic species that occurs infrequently in marina basins and most often in more exposed areas such as pier pilings and seawalls in the Bay.

***Aspidelectra melolontha* (Busk, 1852)**
KEY: p. 143, couplet 15

This encrusting translucent and white bryozoan was described from Europe. It has distinctive small zooids with a thick mask of fused spines arched over the frontal. Membraniporids (*Conopeum*, *Membranipora*) have larger zooids and do not have the mask of spines over the frontal. This species is rare in San Francisco Bay and settles in low numbers in late winter / early spring in high salinity (over 30 psu) regions of the bay.

***Bowerbankia* sp. A and B**
KEY: p. 142, couplet 2

***Victorella pavida* (Saville Kent, 1870)**
KEY: p. 142, couplet 2

Bowerbankia creates furry mat-like colonies on ropes, rocks, docks, and pilings. I have referred to two morphospecies in the key: Species A has 8 tentacles, while Species B has 10. These may in fact represent different species. *Bowerbankia* sp. A, with 8 tentacles, appears to be a more estuarine variety, occurring throughout the mesohaline regions of the estuary. *Bowerbankia* sp. B (10 tentacles) appears to prefer more marine waters and is generally only present near the mouth of the bay (San Francisco Marina, Presidio Yacht Harbor, Sausalito). I have found both forms in the back regions of Tomales Bay.

In this region, *Bowerbankia* in general are distinguished from the similarly stolonate, tubular, mat-forming bryozoan *Victorella pavida* by the much smaller number of tentacles (generally 10 or less) and absence of branching zooids. *Victorella* zooids have at least 20 tentacles, and occasionally have branching zooids. The origin of *Victorella* is uncertain, but it appears to be introduced.

***Bugula "neritina"* (Linnaeus, 1758)**
KEY: p. 142, couplet 8

The purple erect bryozoan *Bugula "neritina"* is a cryptogenic species in San Francisco Bay. Molecular analyses based on limited sampling along the California coast demonstrated the presence of at least two crypic species fitting the general description of *B. "neritina."* Although San Francisco Bay was not sampled, both species were found in Humboldt Bay (Davidson and Haygood 1999).

***Bugula pacifica* (Robertson, 1905)**
KEY: p. 143, couplet 10

The tan erect bryozoan *Bugula pacifica* is a native species that occurs in mesohaline to saline areas of San Francisco Bay. It begins to recruit in cooler temperatures (spring and early summer), but continues to recruit during summer.

***Bugula stolonifera* (Ryland, 1960)**
KEY: p. 143, couplet 10

The tan erect bryozoan *Bugula stolonifera* is apparently an introduced species in San Francisco Bay that recruits in the fall. Overall colony morphology, spines (if present), and location of avicularia attachment help to distinguish *B. stolonifera* from the native *B. pacifica*. Compared to *B. pacifica*, *B. stolonifera* has a "bushier" appearance with branches appearing as a tangled mass, especially in larger colonies. *B. pacifica*'s branching pattern is much more orderly and whorl-like, such that larger colonies will have multiple levels of whorls of increasing size toward the base of the colony, contributing to an overall Christmas tree-like appearance.

A third species of tan or whitish *Bugula* often reported inside San Francisco Bay is *B. californica* (Robertson, 1905). *B. californica* is a putatively native species that has been reported from numerous localities along the coast of California, including its type location just inside the Golden Gate (Robertson 1905). Much confusion surrounds the distinction of *Bugula stolonifera* from *B. californica*, with some workers humorously referring to any tan, erect, branching bryozoan with avicularia attached near the top of the zooids as "*Bugula stolifornica*." Having examined tens of thousands of specimens taken from San Francisco and Tomales Bays over the past nine years, and having compared these to vouchers (including lectotypes) of *B. californica* deposited in the California Academy of Sciences' collections, I am convinced that *B. stolonifera* and *B. californica* cannot accurately be differentiated without genetic analyses — if, in fact, they are separate species. Several workers have remarked that a series of specimens can be constructed that perfectly grades from a morphology thought to be *B. stolonifera* to something that one might more readily call *B. californica* (N. Hitchcock and L. McCann, pers. comm.).

Caulibugula ciliata (Robertson, 1905)
KEY: p. 143, couplet 9

The whitish, erect bryozoan *Caulibugula ciliata* is apparently a native species that occurs in deeper, cooler waters and less protected, more marine regions of San Francisco Bay. It is a distinctive, delicate looking species with long curved spines projecting off the distal end of each zooid.

Celleporella hyalina (Linnaeus, 1767)
KEY: p. 144, couplet 20

This distinctive translucent, colorless bryozoan has small, perforated zooids. Larger colonies often grow in several layers jumbled on top of each other. Colonies larger than a silver dollar inevitably begin reproducing, with igloo-like ovicells containing pinkish or pinkish-orange egg masses.

***Crisia maxima* (Robertson, 1910)**
KEY: p. 142, couplet 6

***Crisia occidentalis* (Trask, 1857)**
KEY: p. 142, couplet 7

***Crisia serrulata* (Osburn, 1953)**
KEY: p. 142, couplet 7

***Crisulipora occidentalis* (Robertson, 1910)**
KEY: p. 142, couplet 4

***Filicrisia franciscana* (Robertson, 1910)**
KEY: p. 142, couplet 5

These cyclostomatous bryozoans are relatively rarely encountered in shallow marina basins in San Francisco Bay. I have more commonly found them in deeper, less protected Bay waters, such as off seawalls or fishing piers.

***Cryptosula pallasiana* (Moll, 1803)**
KEY: p. 143, couplet 18

This Atlantic orange encrusting bryozoan is one of the most frequently encountered species in the estuary. It frequently grows in dock fouling communities, and I have found it throughout the Bay from the Golden Gate to San Rafael and Richmond, but not upstream of there. It appears able to settle nearly year-round, though it settles the least in the winter months.

***Membranipora*, *Conopeum*, and *Electra* spp.**
KEY: p. 143, couplet 16

Lacy, white, encrusting bryozoans have been confusing Pacific coast bryozoan taxonomists for over a century (J.T. Carlton, pers. comm.). Recent combined genetic and morphological analyses show several different species present in the San Francisco Bay region, including the introduced species *Membranipora chesapeakensis* (L. McCann, pers. comm.). This encrusting bryozoan recruits heavily in brackish waters in the Carquinez Strait during the spring and summer and can grow up off the substrate in a distinctive, bushy, spiky, branching morphology that superficially resembles more marine species such as *Zoobotryon* and *Thalamoporella*.

Several other membraniporids also appear recruit in the Carquinez Strait in the summer months. In more saline Bay waters, several membraniporids recruit during late winter and early spring, and less so in the summer.

***Parasmittina* sp.**
KEY: p. 144, couplet 20

This whitish encrusting bryozoan seems to be relatively rare in the shallow waters of the Bay. I have only found it off a seawall in Tiburon.

***Schizoporella* sp. A**
KEY: p. 144, couplet 19

***Schizoporella* sp. B**
KEY: p. 144, couplet 19

The *Schizoporella* species in the Bay are ill-described. Here, I describe the forms of *Schizoporella* that I encountered in the Bay as two morphospecies. I have frequently found *Schizoporella* sp. A in dock fouling communities near the mouth of the Bay, and less frequently in mesohaline waters. I also have found this species throughout Tomales Bay. *Schizoporella* sp. B appears to be a more intertidal form. I have infrequently found it in dock fouling communities near the mouth of the Bay, and more often in less protected open waters in mesohaline to saline waters in the Bay. Recent taxonomic work has ascribed various identities to these morphospecies, including *S. pseudoerrata* (Soule, Soule, and Chaney 1995), *S. errata* (Waters, 1878), and *S. unicornis* (Johnston, 1874), among others. Recent genetic analyses may reveal the identities of these two morphospecies (C. Zabin, pers. comm.).

***Scrupocellaria* cf. *diegensis* (Robertson, 1905)**
KEY: p. 143, couplet 12

***Scrupocellaria varians* (Hincks, 1882)**
KEY: p. 143, couplet 12

***Scrupocellaria* cf. *californica* (Robertson, 1905)**
KEY: p. 143, couplet 12

These spiky-looking colonies are distinguished from other upright bryozoans by having vibracula, whip-like structures that perform a variety of functions including cleaning, defense, and feeding. I have infrequently found each species in the Bay, and they all seem to occur most commonly in less protected waters.

Smittoidea prolifica
KEY: p. 144, couplet 20

Smittoidea prolifica is a fairly common native species generally found in more exposed, mesohaline to marine areas of the estuary. It is common among pilings along the open bay shoreline as far upstream as Point Richmond, and rarely as far as Vallejo, and is less often found in marina basins. It is distinguished by an imperforate frontal with pores around edges of small, triangular zooids with a denticle just below proximal rim of aperture; early zooids often have two distal spines; ovicells are perforate and arise from distal end of zooid with pink or pinkish-orange egg masses. *S. prolifica* appears to favor cooler temperatures and begins settling in early spring.

***Tricellaria* sp.**
KEY: p. 143, couplet 11

This erect bryozoan grows in spiky-looking colonies in the saline and mesohaline portions of the Bay. It is abundant at Richmond Marina in dry years. I have been unable to determine whether this species is *Tricellaria occidentalis* (Trask, 1857), *T. circumternata* (Soule, Soule and Chaney 1995), *T. inopinata* (d'Hondt and Occhipinti Ambrogi 1985), or a similar species.

Watersipora "subtorquata"

KEY: p. 144, couplet 19

This introduced reddish bryozoan with black opercula is frequently found in the more saline portions of the Bay. In some places, such as South Beach Harbor, and in Sausalito, it grows into large reef-like colonies that can support a variety of associated mobile invertebrates and fish (e.g. Nydam and Stachowicz 2007).

Cirripedia

The common subtidal barnacles of San Francisco Bay are well described. Newman's chapter on barnacles in Light and Smith (2007) is an excellent taxonomic reference for barnacles from the San Francisco Estuary. Here, I only add a few notes on distribution, abundance, and taxonomy for those three species that most commonly occur in the shallow subtidal.

***Amphibalanus amphitrite* (Darwin, 1854)**

KEY: *No key provided*

Amphibalanus amphitrite appears to be introduced from the southern hemisphere (Newman 2007). San Francisco Bay specimens are easily distinguished from other subtidal barnacles by reddish vertical stripes on their outer walls. *A. amphitrite* is generally rare in the San Francisco Estuary, occurring in low numbers subtidally in the central and brackish portions of the bay (give distribution). It is most reliably found on rocks, pilings, and other hard substrates in the high intertidal, at or above the highest levels at which *Balanus glandula* settles. In the back waters of San Leandro Bay near Doolittle Drive, *A. amphitrite* can thickly encrust *Spartina* stalks; otherwise, it is rarely found in densities above three or four individuals per square meter.

***Amphibalanus improvisus* (Darwin, 1854)**

KEY: *No key provided*

Amphibalanus improvisus, introduced from the Atlantic, is the commonest subtidal estuarine barnacle in the more brackish portions of the San Francisco Estuary. In the Carquinez Strait, it occurs in the low intertidal as well, a fact remarked on by Newman (1967); I have observed on low intertidal rocks near Benicia in most years. It recruits heavily in a distinct band in the Carquinez Strait in dryer years, with heavy settlement as far upstream as Pittsburg in a drought, right at about X2 (the 2 psu line used by fish and zooplankton researchers in the Bay as a measure of habitat quality). Downstream of 2 psu, settlement tapers off as salinity levels rise above 15–20 psu. Some settlement still occurs, but the highest densities are far upstream (Chang, unpublished data).

The shell of *A. improvisus* is relatively thin and less white compared to that of *B. crenatus*. The base of the shell appears round when viewed from the top. Dark vertical purple or reddish-purple stripes mark the tissue around the opening of the opercular plates; this is one easy way of identifying live *A. improvisus* that are at least 3–4 mm in basal diameter. In individuals at least 4–5 mm in basal diameter, the terga develops a distinctive long spike that is much reduced in *B. crenatus.*

***Balanus crenatus* (Brugiere, 1789)**

KEY: *No key provided*

The most common native barnacle occurring subtidally in the Bay, *Balanus crenatus* favors more marine to mesohaline waters than *A. improvisus*, though it can recruit heavily as far upstream as Richmond and San Rafael and beyond in wetter years. I have not found *B. crenatus* in the Carquinez Strait, however. Its shell is often brilliant white, with crenulate indentations in the carinolateral plates preventing it from having a perfectly round basal shape when viewed from the top. A continuous reddish or pinkish band of tissue encircles the opening of the opercular plates.

Hydrozoa

The Hydrozoa of the San Francisco Estuary are poorly known. Molecular analyses will likely be needed to distinguish between some species, particularly for *Obelia*-like species and *Cordylophora*. Several studies in progress promise to elucidate the taxonomy and ecology of hydrozoans in the San Francisco Estuary, particularly in the brackish regions (San Pablo Bay, Napa and Petaluma Rivers, and Carquinez Strait; *e.g.*, Folino-Rorem *et al.* 2008, and by M. Meek and A. Wintzer [UC Davis], A. Wintzer, pers. comm.).

I have focused on the sessile (polypoid) stages of hydrozoans, since these are most commonly encountered by researchers examining epifaunal communities; I recognize that complete identification often also requires examination of medusae. The key is based largely on Marques *et al.* (2007), supplemented by my observations. I have only included species that I have actually observed and identified. Closer examination of the specimens I collected will undoubtedly reveal additional species I have missed.

***Blackfordia virginica* (Mayer, 1910)**
KEY: p. 149, couplet 10

Blackfordia virginica is found in brackish regions of the San Francisco Estuary, usually as a low, stolonate hydroid with short, nubby polyps. Mills and Sommer (1995) and Mills and Rees (2000) describe its invasion and occurrence in San Francisco Bay. I have found it in brackish regions, from San Rafael to the outer portions of the Carquinez Strait around Benicia.

Clytia spp.
KEY: p. 148, couplet 9

This small, often inconspicuous hydroid can be mistaken for juvenile *Obelia* if one does not examine the hydranths. It recruits in cooler, more marine waters of the Bay and is generally not found in mesohaline waters.

Cordylophora spp.
KEY: p. 148, couplet 5

Cordylophora spp. is a species complex that previously was described as both *C. lacustris* and *C. caspia* (Folino-Rorem *et al.* 2008). Folino-Rorem *et al.* (2008) have recently shown that samples taken from the San Francisco Estuary, including my samples from the Antioch and Pittsburg Marinas, encompass two morphologically identical, yet genetically distinct species. One species was found at both Antioch and Pittsburg, while the other was found only at Pittsburg. I have found that *Cordylophora* spp. begins recruiting in late spring and early summer, and continues to recruit until fall.

Garveia franciscana (Torrey 1902)
KEY: p. 148, couplet 7

This species was first described from San Francisco Bay as *Bimeria franciscana*, but was subsequently discovered to be present in many locations around the world (Marques *et al.* 2007). This species may be native to the northern Indian Ocean (Cohen and Carlton 1995), but a detailed biogeographic study has not been conducted.

Garveia franciscana tends to inhabit mesohaline-to-marine, less protected regions in the San Francisco Estuary. I have infrequently collected it in marina basins, finding it most often near fixed pilings in the open waters of San Francisco Bay, as well as in open waters in Tomales Bay. It occasionally will colonize floating dock habitats in the Richmond to San Pablo Bay region in dryer years.

Obelia spp.
KEY: p. 148, couplet 9

The hydroid *Obelia* spp. as described here most likely encompasses at least five distinct species as described by Marques *et al.* (2007), including *Laomedea calceolifera*, *O. bidentata*, *O. dichotoma*, *O. longissima*, and *Gonothyraea loveni*. I have observed forms matching all of these species in the San Francisco Estuary. The revision of local *Obelia* taxonomy produced by Marques *et al.* (2007) has been of great use, but I have not used it sufficiently to feel comfortable making species-level identifications and thus do not include them here.

Although Cohen and Carlton (1995) describe *Obelia* spp. as occurring in relatively low numbers throughout the Bay's fouling communities, I have found that *Obelia* spp. can be very abundant seasonally in late winter and spring. In the summer months, *Obelia* recruitment decreases, and other fouling species tend to settle on top of older *Obelia* colonies, eventually causing the entire assemblage to slough off the substrate. Following wet winters, *Obelia* may persist in settling at relatively higher densities into the summer months.

Pinauay crocea (Agassiz, 1862)
KEY: p. 148, couplet 3

Previously known as *Ectopleura crocea* and *Tubularia crocea*, this hydroid is introduced from the Atlantic and occurs in cooler mesohaline waters of the Bay with at least some flow. I have found it to be relatively rare in marina basins, and commoner on piers and pilings in the open Bay.

Pinauay marina (Torrey, 1902)
KEY: p. 148, couplet 3

I have included *P. marina* (= *Tubularia marina*) in the key for comparison with *P. crocea*, though I have only observed *P. marina* in Tomales Bay (not San Francisco Bay).

Phialella spp.
KEY: p. 149, couplet 10

Boero (1987) has described several *Phialella* species from Bodega Harbor. I have collected many specimens matching descriptions of *Phialella* spp. from the outer and middle portions of Tomales Bay, and rarely from the mouth of San Francisco Bay.

Porifera

The Porifera of the San Francisco Estuary are poorly known and difficult to distinguish. I have grouped taxa into five commonly-occurring morphospecies based on morphological analyses. More specific identification of these morphospecies awaits genetic analysis. The key is based on my observations, consultations with Christopher Brown, and the key by Lee *et al.* (2007).

Clathria prolifera
KEY: p. 147, couplet 4

This Atlantic sponge grows slowly, but can eventually attain rather large sizes. It has a distinctive reddish-orange coloration and is relatively dense. Settles in spring and summer.

***"Halichondria"* sp.**
KEY: p. 147, couplet 4

Specimens with generally similar gross appearance to more mesohaline specimens have been found as far upstream as Antioch. This far upstream, however, specimens generally have a somewhat looser matrix, are whitish in color, and have a low, encrusting morphology, never growing into the dense, wispy masses found further downstream. It is possible that the forms found in fresh and brackish water are not the same as those downstream, but genetic analyses are probably required to determine if this is so.

***"Haliclona"* sp.**
KEY: p. 147, couplet 5

This group of cryptic, soft, tan and light yellow sponges includes a number of different species (Lee et al. 2007). Large light tan and yellow colonies tinged with pink developed in many places in the Bay during the summer following the wet winter of 2006.

***Leucosolenia* sp.**
KEY: p. 147, couplet 2

This long, smooth, brilliant white tube sponge has a short oscular fringe and is not constricted, unlike *Scypha* sp. This species seems to be most common during moderate and dry years at Richmond Marina and elsewhere in the Bay, and is generally found in mesohaline to saline areas.

***Mycale* sp.**
KEY: p. 147, couplet 5

Mycale sp. is a cryptic yellow sponge that I have found difficult to distinguish from "*Haliclona*" sp. without looking at spicules; at least some specimens appear to be *M. macginitiei*. *Mycale* sp. appears to inhabit mesohaline and saline areas.

***Scypha* sp.**
KEY: p. 147, couplet 2

These fat, tubular (vase-shaped) sponges have a thin, dense attachment to the substrate and a long fringe around the osculum, or opening, and generally appear to be a dirty white color. Frequently found in mesohaline regions of the Bay.

Tunicata

The Tunicata, especially solitary forms, are generally the dominant group in the more saline waters (mean salinity > 18 psu) of San Francisco Bay. No native forms have recently been found in the Bay itself, although one native species (*Ascidia ceratodes*) is found in more consistently saline bays to the north and south, including Bodega Harbor, Tomales Bay, and Monterey Bay. The key is generally based on my own observations from extensive collections in the Bay combined with keys and descriptions such as Van Name (1945), Abbott *et al.* (2007) and communications with Gretchen Lambert on various points.

Ciona intestinalis (Linnaeus, 1767)
KEY: p. 145, couplet 4

Ciona intestinalis is a solitary tunicate of unknown introduction status in this region. It previously was thought to be introduced from the Atlantic coast of the United States (*e.g.*, Carlton 1979). Recent genetic work indicates that this species is in fact at least two distinct species (Nydam and Harrison 2007). On the Pacific coast, most records of "*Ciona intestinalis*" refer to the morphologically and genetically distinct form known as Type A, rather than the Type B form that is found on the Atlantic Coast of the United States and in Europe (Nydam and Harrison 2007). Previous records of "*Ciona intestinalis*" in the Pacific Northwest have been re-examined and found to be *C. savignyi* (G. Lambert pers. comm.).

Ciona intestinalis Type A appear to be reliably characterized in this region by the presence of a red dot at tip of vas deferens and red ocelli in the folds of the siphonal rims. *Ciona savignyi* is an introduced Japanese congener also present in San Francisco Bay and Bodega Harbor, but not Tomales Bay (Chang and Kimbro, unpublished data). Juvenile (< 1 cm height) *Ciona* spp. cannot be distinguished to species level until pigmentation has developed at the tip of the vas deferens, or around the siphonal rim. Once coloration develops, *C. intestinalis* have a red patch in the location where the red dot develops at the end of the vas deferens in sexually mature individuals; slightly larger juveniles will also have visible red ocelli at the tips of the siphons, in the folds of the siphonal rims. Juvenile *C. savignyi* have either white or yellowish patches in the location that becomes the tip of the vas deferens.

In this dissertation, all *Ciona intestinalis* recorded were of Type A (Nydam and Harrison 2007). To my knowledge, *C. intestinalis* Type B has never been recorded on the Pacific Coast of North America. However, this species is apparently physically quite similar to *C. savignyi*, and sampling efforts examining both morphology and genetics on this coast have been relatively low, decreasing the chances of detecting *C. intestinalis* Type B.

In dry years, *Ciona intestinalis* is common and indeed dominant throughout the more saline portions of the San Francisco Estuary, from about the Richmond-San Rafael bridge on south (see Chapter 1). This species appears to be dominant in the warmer, more sheltered portions of the estuary and has been found in great abundance in Loch Lomond, Richmond, and Berkeley marinas, as well as Jack London Square, San Leandro, Coyote Point, and Oyster Point marinas. High abundances also have been documented at the Port of Oakland.

When present in high abundance, *Ciona intestinalis* has been shown to depress species richness and to significantly alter epifaunal communities (Blum *et al.* 2007). In this study, I found *C. intestinalis* at most sites in the lower estuary at one time or another. While *C.*

intestinalis was very abundant in the estuary in 2002 (the period during which the Blum *et al.* 2007 study was done), it became much less common in the following years (2003–2005), and almost completely disappeared following the low salinity conditions brought on by winter storms in January 2006. Subsequently, during the drought years of 2007–2008, *C. intestinalis* recruited in very high numbers at many sites in the estuary, making up nearly the entire biomass of the community at Richmond during the late summer and fall.

Ciona intestinalis apparently prefers warmer temperatures and does not begin to recruit in high abundance until water temperature reaches 18° C, though it recruits in slightly cooler temperatures (16° C) in Tomales Bay. In the warm, saline waters of late summer and fall in San Francisco Bay, *C. intestinalis* grows rapidly and within three months, individuals can attain lengths of up to 9 inches (Chang, pers. obs.). While populations from the San Francisco Estuary have been shown to tolerate moderately lowered salinities (down to 15 psu as adults), *C. intestinalis* settles in highest abundances in warm, saline water (approaching 30 psu), such as during drought years. I have not found *C. intestinalis* in high abundances near the mouth of the bay (*i.e.* south of Loch Lomond or north of Oyster Point along the western shore of the bay). I suggest that this is due to the colder temperatures in this region of the Bay, predation (native marine or outer coast predators are more common around the mouth of the Bay), or some other factor.

***Ciona savignyi* (Herdman, 1882)**

KEY: p. 145, couplet 4

Ciona savignyi is a solitary tunicate introduced from Japan. The tip of the vas deferens is white (can be white or yellow in juveniles), and the pigment in folds of siphonal rims ranges from yellowish to orange to white and is more "patch-like" as opposed to the concentrated spots formed by ocelli. The tunic of *C. savignyi* is generally more flexible and slippery or slimy compared to that of *C. intestinalis* Type A. While large adult *C. intestinalis* Type A can eventually become fouled by other settlers, particularly around the base of the animal, *C. savignyi* generally appears to be less susceptible to such fouling. G. Miller-Messner (UC Davis) has investigated genetics and salinity tolerance in *C. savignyi* in several bays in this region.

Ascidia zara **(Oka, 1935)**

KEY: p. 145, couplet 5

Ascidia zara is a solitary tunicate apparently introduced from Japan. In Northern California, its distribution is limited to San Francisco Bay. Surveys of Tomales Bay and Bodega Harbor have only reported the native Pacific congener *Ascidia ceratodes*.

The recently introduced solitary tunicate *Corella inflata* has a similarly thin, translucent, colorless, tunic that is sometimes papillated, but almost never as bumpy as *A. zara*. *Corella* also has a distinctive mushroom shape with a relatively thin base of attachment, whereas *A. zara* is broadly attached, with nearly its entire body length cemented to the substrate. The oral siphon of *A. zara* is generally also distinct from the body, occasionally elongated as much as the entire length of the rest of its body (effectively doubling its body length). The siphons of *C. inflata* specimens found in San Francisco Bay are never distinct from the rest of its body.

Ascidia ceratodes **(Huntsman, 1912)**

KEY: p. 145, couplet 8

Ascidia ceratodes is a solitary tunicate native to the Pacific coast of North America. It appears to be limited to higher salinity waters and hence is found in Bodega Harbor, in the outer reaches of Tomales Bay, and probably only in the deepest, most saline parts of San Francisco Bay near the Golden Gate, if at all. No recent fouling studies, including this one, have found *A. ceratodes* in San Francisco Bay.

Ascidia zara is an introduced Japanese congener present in San Francisco Bay that looks extremely similar to *A. ceratodes* when smaller than about 2cm in length. Juvenile *A. ceratodes* are difficult to distinguish from *A. zara*. Adult *A. zara* usually have a translucent, colorless (unless silted or overgrown) bumpy, papillated tunic; degree of papillation varies, but tunic is always easily torn with forceps. Both have orange ocelli in the folds of the siphonal rims. Adult *A. ceratodes*, however, have tough, thick, wrinkly, orangish, opaque tunics that are extremely difficult to tear with forceps, in contrast to the translucent, thin tunic of *A. zara*.

To the best of my knowledge, *Ascidia ceratodes* has not been found landward of the Golden Gate in the past nine years. Historically, *A. ceratodes* may have been found occasionally at the mouth of the estuary, but it may be unable to withstand the decreased salinity of bay water. The California Academy of Sciences collections have occasional collections of *A. ceratodes*, generally from deep water, and none more recent than the 1970s. In Tomales Bay, *A. ceratodes* can seasonally be found in shallow waters throughout most of the estuary, with seasonally high settlement occurring in mid-bay between 8 km and 14 km from the mouth of the Bay. Occasionally high settlement occurs 16 km from the mouth, at Shell Beach.

Corella inflata (Huntsman, 1912)
KEY: p. 145, couplet 5

Corella inflata is a solitary tunicate apparently recently introduced from the Pacific Northwest. I first recorded it in the fall of 2008 in Sausalito and at San Francisco Marina (identification confirmed by G. Lambert). In about 2003–2004, *C. inflata* was introduced to Coos Bay and became locally abundant in the Inner Boat Basin region, but has not been seen recently. It is speculated that the heavy winter rains of 2006 wiped them out (R. Emlet and J. T. Carlton, pers. comm.)

The overall appearance and diagnostic features of *C. inflata* are described in Lambert *et al.* (1981). Briefly, *C. inflata* has a distinctive asymmetrical mushroom shaped body with a relatively narrow attachment to the substrate, forming an upside-down L shape in profile; tunic is thin, translucent, colorless, slightly papillated; siphons are not distinct from body, and oral siphon with inflated brood chamber; light yellow eggs, if present, can be seen in this brood chamber. In contrast, *Ascidia zara* has a thin, translucent, colorless, papillated tunic, but is shaped differently, having a more oval shape in top view and lying much lower to the substrate in profile compared to the inverse L shape formed by *C. inflata*. The south temperate and Antarctic species *C. eumyota* has an opaque, greyish tunic (G. Lambert, pers. comm.)

Based on the limited data available, *Corella inflata* appears to settle densely beginning in late spring (May/June) and continues to do so throughout the summer. Quarterly (3-month exposure time) panels placed at Sausalito Marine Harbor and San Francisco Marina during 2008 showed near complete cover by *C. inflata* during the summer months. In 2009, *C. inflata* showed near complete cover on quarterly panels by late spring.

Interestingly, *C. inflata* is the only solitary tunicate in the more marine portions of the San Francisco Estuary that appears to produce near complete cover of the substrate. Further upstream, up to Loch Lomond Marina in San Rafael, numerous solitary tunicates will seasonally settle in such numbers as to almost completely occupy the substrate. These include *Ascidia zara*, *Ciona intestinalis* (type A), and *Ciona savignyi* in the mesohaline regions of the bay (yearly salinity range generally 20–30 psu). Beyond Loch Lomond, in the Carquinez Strait, *Molgula manhattensis* will settle very densely in dry-to-moderately wet years. In wetter years, this region of dense *Molgula* recruitment is pushed downstream toward Loch Lomond and Richmond Marina.

***Styela clava* (Herdman, 1881)**
KEY: p. 145, couplet 7

Styela clava is a orangish-brown, club-like solitary tunicate native to the Northwest Pacific. It grows up to 6 inches in length in San Francisco Bay. Large individuals of *Styela clava* are often found on pilings and the sides of submerged floating docks at marinas around the Bay. On pilings, there is often a band of *S. clava* found 1–2 meters below mean lower low water.

***Molgula manhattensis* (DeKay, 1843)**
KEY: p. 145, couplet 8

This solitary tunicate is introduced from the Atlantic, where it appears to be native to Europe and introduced on the the North American Atlantic coast. The congener *Molgula ficus* was recently found in Ballena Isle Marina in Alameda (Lambert 2007).

***Botrylloides violaceus* (Oka, 1927)**
KEY: p. 146, couplet 13

This colonial tunicate is apparently introduced from the Atlantic, although there is some controversy surrounding its origin. *Botrylloides violaceus* is commonly found subtidally on docks and pilings. In some parts of the estuary, *B. violaceus* is also found in the low intertidal on suitable substrate, including rocks, twigs, and leaves.

Botrylloides diegensis **(Ritter and Forsyth, 1917)**
KEY: p. 146, couplet 13

Botrylloides diegensis has been suggested to be native to the eastern Pacific Ocean from San Diego south. *Botrylloides diegensis* is found subtidally on docks and pilings in San Francisco Bay, but is less common than *B. violaceus*.

Botryllus **sp. (Pallas, 1766)**
KEY: p. 146, couplet 12

Probably *B. schlosseri*. This colonial tunicate is apparently introduced from the Atlantic, although there is some controversy surrounding its origin. *Botryllus schlosseri* is commonly found subtidally on docks and pilings in San Francisco Bay.

Didemnum vexillum **(Kott, 2002)**
KEY: p. 146, couplet 15

Based on recent molecular analyses, this colonial tunicate appears to be introduced from the northwestern Pacific Ocean (Stefaniak 2009). Lambert (2009) has written an extensive review of *D. vexillum* taxonomy and its introduction history. G. Lambert first identified large, adult colonies of *D. vexillum* from Sausalito in 2003, and later analyses showed its presence in the Bay during previous years. *D. vexillum* became abundant at several sites in the Bay, particularly Richmond Marina, in 2005, but has declined since then.

Diplosoma listerianum **(Milne-Edwards, 1841)**
KEY: p. 146, couplet 14

This colonial tunicate is apparently introduced. Previous records of *D. macdonaldi* Herdman, 1886 refer to this species (Abbott *et al.* 2007). *Diplosoma listerianum* is a fairly "weedy" species, occurring in great abundance in the warmer parts of the year in the mesohaline regions of San Francisco Bay and the mid-to-back portions of Tomales Bay. This species shows a great predilection for overgrowing other organisms, even those that most others will not touch, including *Ciona* spp. Large colonies, especially those overgrowing other objects or organisms, frequently begin to grow off the substrate into lobes that fragment very easily and which will readily re-attach to other surfaces. This ready fragmentation and re-attachment no doubt facilitates its spread.

***Distaplia* sp.**
KEY: p. 146, couplet 15

Probably *D. occidentalis*. Its shiny, pearlescent, mushroom-shaped heads appear mostly in dry years. Individual colonies can become quite large (softball-sized) if left undisturbed for 3 or 4 months. Juveniles are difficult to distinguish from *Didemnum* at first glance. *Distaplia* appears inside the Bay as far north as Loch Lomond Marina in San Rafael and Richmond Marina Bay in dryer years when these areas experience higher salinities. I also have found *Distaplia* in the mid- to back portions of Tomales Bay with some frequency.

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