Population Dynamics And Persistence Of An Invasive Species In Kelp Bed Ecosystems in the Northwest Atlantic Ocean

by

Danielle Elizabeth Denley

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ABSTRACT

The bryozoan *Membranipora membranacea* is an ecologically significant invasive species in the northwest Atlantic where it has facilitated substantial defoliation of kelp beds and dramatically altered rocky subtidal habitats. The main objectives of this thesis are to 1) identify life-history strategies and quantify critical demographic processes for *M. membranacea* in relation to physical and biological characteristics of its invaded habitat, and 2) incorporate this information into a population model to examine persistence and population growth in invaded habitats in response to near-future projections of ocean temperature and kelp bed community composition. Larvae of *M. membranacea* selectively settled within preferred regions of kelp blades, but did not exhibit preference for settling on specific kelp species or within kelp beds. Critical demographic processes were most strongly influenced by biological characteristics of the invaded habitat (algal substrate) or the colonies themselves (colony size). Rates of colony senescence increased with colony size and recovery capacity was related to the location of damage within the colony, while mortality rate varied with loss of kelp biomass and potential colony fecundity depended on the specific host algal substrate. In model simulations, increasing ocean temperature led to increased population growth of *M. membranacea*; however, temperature-dependent growth and persistence of the population depended on the species composition of invaded kelp bed communities. These results indicate that the persistence and abundance of this invasive bryozoan in the northwest Atlantic will depend on both the direct effects of climate change on its population dynamics, as well as indirect effects mediated though climate-driven changes to kelp bed ecosystems. The results provide evidence that impacts of climate change on ecosystem structure can influence invasion success, further complicating predictions of the ecological impact of invasive species under future climate conditions.

LIST OF ABBREVIATIONS AND SYMBOLS USED

Abbreviation/Symbol	Definition	Units
r^2	Coefficient of determination	
SL	*Saccharina latissima*	
LD	*Laminaria digitata*	
AC	*Agarum clathratum*	
Fu	*Fucus evanescens*	
SMB	St. Margarets Bay	
TL	The Lodge	
FS	Feltzen South	
PH	Paddy's Head	
SC	Sandy Cove	
Lun	Lunenburg	
FHL	Friday Harbour Laboratories	
NS	Nova Scotia	
T	Temperature	°C
t	Time	days
GR	Growth rate	mm d^{-1}
SA	Colony surface area	cm^2, m^2
GDD	Growing degree-day	°C d
D	Colony diameter	cm
M	Biomass (Ch. 3)	g
C	Choice (Ch. 3)	

NC	No choice (Ch. 3)	
N	Number of colonies (Ch. 4)	m^{-2} seabed
M	Instantaneous mortality rate (Ch. 4)	d^{-1}
s	Size class (Ch. 4)	cm
S	Colony size (Ch. 4)	mm
R	Rate of colony senescence (Ch. 4)	$cm^2\ d^{-1}$
B_{Loss}	Biomass loss (Ch. 4)	$kg\ m^{-2}$
S_i	Initial colony size (Ch. 4)	cm^2
n	Population vector (Ch. 7)	Colonies m^{-2} kelp
M_l	Larval mortality (Ch. 7)	
A	Population projection matrix (Ch. 7)	
k	Time index (Ch. 7)	
Δk	Time step (Ch. 7)	
μ	Instantaneous whole-colony mortality rate (Ch. 7)	% d^{-1}
λ_s	Stochastic population growth rate (Ch. 7)	yr^{-1}
G_i	Growth transition probability (Ch. 7)	
S_i	Senescence transition probability (Ch. 7)	
L_i	Stasis transition probability (Ch. 7)	
P_i	Survival probability (Ch. 7)	
P_{OW}	Over-winter survival probability (Ch. 7)	
F_i	Potential colony fecundity (Ch. 7)	Oocytes $colony^{-1}$
α	Regression intercept parameter (growth rate) (Ch. 7)	

β	Regression slope parameter (growth rate, diameter) (Ch. 7)	
χ	Regression slope parameter (growth rate, temperature) (Ch. 7)	
S	Initial colony size (Ch. 7)	mm, cm
SR	Senescence rate (Ch. 7)	cm d^{-1}
γ	Regression intercept parameter (senescence rate) (Ch. 7)	
δ	Regression slope parameter (senescence rate, diameter) (Ch. 7)	
R	Proportion of total settlers (Ch. 7)	

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CHAPTER 1

INTRODUCTION

1.1 Background and study system

Biological invasions are recognized as a significant driver of global change (Vitousek et al. 1997, Ricciardi 2007). There is evidence to suggest that the frequency of invasions has increased in recent decades (Carlton 1996, Ruiz et al. 1997, Ricciardi 2007) and may continue to increase in the future (Wonham & Carlson 2005). Increasing rates of invasion are often attributed to globalization creating more opportunities for human-assisted species introductions (Hulme 2009). These increases in the frequency of human-mediated introductions are occurring against a backdrop of rapidly changing environmental conditions associated with climate change and other anthropogenic disturbances, which, in turn, can enhance invasiveness of introduced species (Byers 2002, Marvier et al. 2004). Increasing temperature through climate change is a global phenomenon that alters environmental conditions at local and regional scales. Changing thermal regimes are expected to influence species invasions by 1) allowing introduced species to become established at sites that were previously outside their thermal optimum, 2) facilitating secondary spread into novel habitats by shifting or expanding ranges of previously introduced species the distributions of which were temperature-limited, and 3) affecting demographic rates (reproduction, growth, survival) and interspecific interactions of introduced species, which, in turn, can influence their distribution, abundance, and ecological impacts (Ruiz et al. 1997).

The northwest Atlantic has been identified as an ocean warming hotspot and is estimated to be warming at rates faster than 90% of the rest of the ocean (Hobday & Pecl 2014). Rising sea temperature in combination with population outbreaks of an invasive epiphytic bryozoan, *Membranipora membranacea*, have been proposed as the main drivers of a dramatic shift in the structure and function of subtidal ecosystems in the northwest Atlantic from highly productive kelp beds to less productive communities dominated by turf algae (Krumhansl et al. 2014, Filbee-Dexter et al. 2016, O'Brien

2018). *M. membranacea* was first observed off the southwestern shore of Nova Scotia in 1992 (Scheibling et al. 1999), and over the last 30 years has expanded its range to include the entire Atlantic coast of Nova Scotia (Watanabe et al. 2010), the west coast of Newfoundland (Caines & Gagnon 2012), and into the Gulf of St. Lawrence (Denley, unpublished data). Outbreaks of *M. membranacea* in its invaded habitat have been linked to warm seawater temperatures (Saunders & Metaxas 2007, 2008, Scheibling & Gagnon 2009, Saunders et al. 2010), and there is evidence to suggest that colder temperature conditions may have inhibited outbreaks of this non-native species had it been introduced prior to 1983 (Krumhansl et al. 2014). However, prior to my research, the effects of increasing ocean temperature on critical demographic rates (reproduction, mortality), and implications for interactions with native algal assemblages had not been examined for this bryozoan in its invaded habitat.

Quantitative data on demographic rates and interactions with native kelp species are lacking for *M. membranacea* in part because it is a colonial organism with a complex lifecycle. Like many benthic marine invertebrates, *M. membranacea* has a dispersive larval stage, complicating interactions with its host algae through the potential for selective settlement and differential recruitment among different algal substrates (Seed & O'Connor 1981). Studies of selective settlement by larvae of *M. membranacea* in its native habitat are inconclusive (Ryland 1962, Bernstein & Jung 1979, Yoshioka 1986, Stricker 1989, Matson et al. 2010). However, factors influencing larval settlement behavior are of particular interest in invaded habitats where the relationship between settlement and percent cover of *M. membranacea* on kelp is strong (Saunders & Metaxas 2007, Caines & Gagnon 2012) and can be used to predict impacts of *M. membranacea* on different kelp species early in the course of a bryozoan outbreak. In addition, demographic processes that are well defined for aclonal organisms (Cole 1954) cannot be direct applied to clonal organisms (Harper & White 1974). Modular construction of colonial invertebrates complicates their demography, since reproduction and mortality occur independently at the level of each semiautonomous module. Consequently, while relationships between temperature and colony growth rate (Saunders & Metaxas 2009a) and the timing of onset of settlement and overall settler abundance (Saunders & Metaxas 2007) have been established for *M. membranacea* in its invaded habitat, effects of

temperature on modular level demographic processes, such as mortality and fecundity, and the influence of selective settlement behavior by larvae of *M. membranacea* on loss of kelp species have not been explored to date.

1.2 Objectives

The overall objective of this thesis is to use invaded kelp bed ecosystems in the northwest Atlantic to investigate the impact of increasing ocean temperature related to climate change on the population dynamics of an ecologically significant invasive species and its interaction with native algal assemblages. To achieve this, I first identify and quantify key demographic processes through a combination of field sampling and manipulative experiments *in situ* and in the laboratory. I then incorporate these processes into a population model for *M. membranacea* in its invaded habitat and use this model to predict the persistence and ecological impacts of this invasive bryozoan under near-future climate scenarios. This document is arranged into 8 chapters, including this Introduction (**Chapter 1**). **Chapters 2-7** have been published (**Chapters 2-6**) or submitted for publication (**Chapter 7**) as independent manuscripts in the primary literature. In **Chapter 2,** I examine selective settlement of *M. membranacea*, as well as a similar native bryozoan *Electra pilosa*, along individual kelp blades. In **Chapter 3**, I examine selective settlement by larvae of *M. membranacea* among kelp species, and determine whether mixed kelp beds in invaded habitat provide a settlement cue for larvae. In **Chapter 4**, I quantify rates of whole-colony and partial mortality of *M. membranacea* in relation to temperature, colony size, and algal substrate, and compare methods for measure whole-colony and partial mortality of colonial organisms in general. In **Chapter 5**, I examine the recovery capacity of *M. membranacea* following partial mortality and the effects of temperature, location within the colony, and magnitude of modular loss on recovery. In **Chapter 6**, I quantify potential colony fecundity in relation to temperature, colony size, and algal substrate, and identify differences in the reproductive dynamics of invasive compared to native populations of *M. membranacea*. In **Chapter 7**, I incorporate empirically derived estimates of demographic processes (Chapters **3**, **4**, and **6**) into a stage-based matrix population model to examine the effects of projected increases in

ocean temperature and changes in the community composition of kelp beds on the persistence and population growth of *M. membranacea* in the northwest Atlantic. Finally, in **Chapter 8**, I provide a summary of the research and suggest directions for further investigation

CHAPTER 2

SELECTIVE SETTLEMENT BY LARVAE OF *MEMBRANIPORA MEMBRANACEA* AND *ELECTRA PILOSA* (ECTOPROCTA) ALONG KELP BLADES IN NOVA SCOTIA, CANADA[1]

2.1 Abstract

Many larval sessile marine invertebrates exhibit settlement preferences, and larval behavioral responses to cues at settlement can ultimately influence the distribution of adults and an individual's lifetime fitness. Two epifaunal bryozoans, the invasive *Membranipora membranacea* and the native *Electra pilosa*, commonly co-occur on kelp species in the subtidal habitats of Nova Scotia, Canada. Outbreaks of *M. membranacea* have been linked to mass defoliation of the kelp canopy; however, *E. pilosa* has not been associated with any significant effect on its host substrate. To examine whether larvae of *M. membranacea* and *E. pilosa* exhibit settlement preference for a particular location along the blades of the kelps *Saccharina latissima* and *Laminaria digitata*, abundances of newly settled colonies were quantified at different locations along the kelp blade. Algae were sampled at 2 sites on the southwestern shore of Nova Scotia (The Lodge and Feltzen South) from September 2009 to October 2010, over one complete cycle of the annual life cycle of *M. membranacea*, and thus over a wide range of bryozoan percent cover. Settlers of both bryozoans were significantly more abundant towards the younger, more proximal regions of blades of both kelps across all sampling periods. These patterns did not vary seasonally with increasing colony density. Both *M. membranacea* and *E. pilosa* larvae showed preferential settlement, suggesting that they can detect small-scale differences in habitat quality at the scale of a single kelp blade.

[1] Denley D, Metaxas A, Short J (2014) Selective settlement by larvae of *Membranipora membranacea* and *Electra pilosa* (Ectoprocta) along kelp blades in Nova Scotia, Canada. Aquat Biol 21:47-56.

My coauthor Dr. Anna Metaxas supervised the study design and analyses, and edited the manuscript. My coauthor Jessie Short collected and compiled the data, and edited the manuscript.

2.2 Introduction

Spatial patterns in the distribution and abundance of sessile marine invertebrates with planktonic larvae are controlled in part by the non-random settlement of recruiting larvae onto substrates suitable for adult life (Hadfield 1986, Chia 1989). Consequently, substrate selection by planktonic larvae is a key process in the life history of benthic invertebrates, strongly influencing critical population parameters, such as post-settlement mortality (Keough & Downes 1982) and, ultimately, adult population density (Minchinton & Scheibling 1991). Larvae of many benthic invertebrates display clear preferences for specific substrates (Burke 1983). However, the small size of planktonic larvae relative to the substrate onto which they are settling makes it possible for gradients in microhabitat to exist within a given substrate (Seed & O'Connor 1981). This small-scale variability in habitat quality becomes particularly interesting on biologically dynamic substrates such as macroalgae, where variation in habitat occurs not only spatially, but also temporally.

Blades of laminarian algae are continually growing from the proximal end and eroding from the distal end, and the blade tissue can be entirely recycled between 1 and 5 times a year depending on temperature (Mann 1973). Consequently, while a kelp bed may persist for decades, the available habitat to an epiphyte colonizing an individual kelp blade is constantly changing. The successful recruitment and overall lifetime fitness of epiphytes on such ephemeral habitats is often related to the location of settlement on the alga (Keough 1986, Durante & Chia 1991), and as a result, larvae of marine epiphytes on macroalgae exhibit a range of settlement preferences (Hayward & Harvey 1974, Bernstein & Jung 1979, Roland 1980, Durante & Chia 1991).

In the subtidal habitats of Nova Scotia, the 2 most abundant bryozoan epiphytes are the recent invasive *Membranipora membranacea* (Watanabe et al. 2010) and the native *Electra pilosa*. *M. membranacea* is an encrusting colonial bryozoan and was first observed in the Gulf of Maine (USA) in 1987, where it facilitated significant alterations of the local marine community, establishing itself as the dominant epiphyte on laminarian kelps in as little as 2 yr (Berman et al. 1992, Lambert et al. 1992). Off the southwestern shore of Nova Scotia, small colonies of *M. membranacea* were first found in 1992 and

had similar dramatic effects, as rapid growth resulted in major loss of kelp canopy within 1 yr (Scheibling et al. 1999). *M. membranacea* has an annual life cycle. In Nova Scotia, *M. membranacea* larvae typically settle between May and July with peak settlement occurring in September (Saunders & Metaxas 2007). Larvae of *M. membranacea* settle predominantly on kelp substrates (Yorke & Metaxas 2012), where they metamorphose into a pair of filter feeding zooids (ancestrula) from which additional zooids bud asexually, forming sheet-like colonies. These colonies rapidly encrust the kelp blade, and colony growth and reproduction continue throughout the summer months (June to August), with maximum colony abundance (percent cover) occurring in fall (September to October) (Saunders & Metaxas 2009b). Colonies begin to senesce in late fall and early winter (November and December), a process that is characterized by stasis and/or shrinkage, with <1% of the population persisting overwinter, presumably providing the larval supply for the following season.

Encrustation by *M. membranacea* reduces the survival of its kelp host by causing degradation of the meristoderm, resulting in the weakening of kelp blades and increasing their susceptibility to breakage during strong wave action in late fall and winter (Krumhansl et al. 2011). Settlement and recruitment of *M. membranacea* into the adult population is of particular interest in the northwestern Atlantic, where recurring outbreaks of this invasive species have catalyzed mass defoliation of kelp beds (Scheibling et al. 1999, Saunders & Metaxas 2008, Scheibling & Gagnon 2009).

In its native range in the northwestern Pacific, larvae of *M. membranacea* exhibit searching behavior by actively crawling from older distal regions to settle on younger more proximal regions along the blade of the kelp *Nereocystis leutkeana* (Matson et al. 2010). Seed (1976) provided limited evidence from 8 plants at a single location and time that these larvae may also settle in greater abundance towards the proximal end of blades of the laminarian *Saccharina latissima*. However, little is known about the distribution of settlers of *M. membranacea* outside of their native habitat, where interactions with native kelp and epiphyte species may affect larval settlement behavior.

The cheilostome *E. pilosa* is a native bryozoan, also epiphytic on kelp substrates in Nova Scotia. Although seasonal patterns of settler abundance for *E. pilosa* in this

region are unknown, *E. pilosa* is generally more abundant on *Fucus* species. This is in contrast to *M. membranacea*, which is relatively more abundant on kelps (Yorke & Metaxas 2011). *E. pilosa* is also characterized by slower growth rates and smaller colony size and colony cover on kelp than *M. membranacea* (Yorke & Metaxas 2011). While it is not known whether *E. pilosa* settles preferentially at a given location along the blade of its algal hosts, it has been suggested that larvae of *E. pilosa* settle gregariously (Ryland & Stebbing 1971).

In Nova Scotia, *M. membranacea* and *E. pilosa* co-occur interspersed on blades of 2 of the most numerically abundant kelp species, *S. latissima* and *Laminaria digitata* (Yorke & Metaxas 2011). Morphology and seasonal cycles of growth differ slightly between these 2 kelp species. *S. latissima* tends to have single, narrow blades with crenulated edges. It grows rapidly in spring (May), and slowly in fall (September to November) (Krumhansl & Scheibling 2011). *L. digitata* has broad digitated blades. It follows a similar seasonal cycle in growth to *S. latissima* except that growth rate begins to increase earlier in the late winter and early spring (February to May) (Krumhansl & Scheibling 2011). However, *L. digitata* grows considerably more slowly than *S. latissima*, with peak growth rates of the 2 species at optimal temperature (10°C) differing by as much as 10% d^{-1} (Bolton & Lüning 1982).

In this study, we examined whether larvae of *M. membranacea* and *E. pilosa* exhibit preference for settling at a particular location along kelp blades at 2 locations in Nova Scotia. Because algae grow from the proximal towards the distal end, we compared abundances of newly settled individuals of each species in algal segments along the long axis of the blades of *S. latissima* and *L. digitata*. To determine whether any observed patterns in settlement varied temporally with colony density, we also quantified total cover of each bryozoan (%) within each segment along the length of the kelp blade.

Our study extends that by Seed (1976) by capturing seasonal variation in the life cycles of both kelp and bryozoan species, as sampling occurs over the duration of an entire year. Our study was conducted in the northwest Atlantic where *M. membranacea* is invasive, and its recruitment may be limited to niches where resources are underused by the native epiphytes. To address potential effects on settlement, we examined patterns on

both dominant kelps (*S. latissima* and *L. digitata)* and for the most abundant bryozoans (*E. pilosa* and *M. membranacea*) in the region.

We hypothesized that, in the absence of a preference for settling at a particular location along the kelp blade based on tissue quality, settlers should be randomly distributed along the blade; any deviation from a random distribution may be related to other factors, such as colony density of *M. membranacea*. For *M. membranacea* specifically, colony density (percent cover) could influence patterns in the distribution of settlers in 1 of 2 ways: (1) gregarious settlement resulting in a positive relationship between abundance of settlers and percent cover of colonies; (2) conspecific avoidance or limiting space resulting in a negative relationship between settler abundance and percent cover of colonies. Conversely, if bryozoan larvae preferentially settle at a particular location along the kelp blade, abundance of settlers should be consistently high at this location, regardless of percent cover of colonies, and settlement preference should be detectable during periods of both high and low density of *M. membranacea* colonies.

2.3 Methods

2.3.1 Study area

We sampled 2 sites on the southwestern shore of Nova Scotia, Canada, both of which were characterized by extensive mixed kelp beds dominated by *Saccharina latissima* and *Laminaria digitata*, and an understory of turf algal species. The Lodge (44° 33′ 3″ N, 64° 01′ 9″ W) is located on the western shore of St. Margarets Bay and has a moderately steeply sloping granite substratum dominated by large boulders and cobbles, as well as beds of *Fucus evanescens* at depths <4 m. Feltzen South (44° 19′ 57″ N, 64° 17′ 13″ W) is located on the southwestern shore of Lunenburg Bay, 30 km southwest of St. Margarets Bay, and has a gently sloping bedrock substratum dominated by cobbles and extensive beds of *Fucus serratus*. At The Lodge, sampling was done along 4, 8, and 12 m depth contours, while at Feltzen South, sampling was done at 9 m depth. The 2 sites were chosen based on previous studies showing differences in the relative abundance of *Membranipora membranacea* and *Electra pilosa* across different bays (Yorke & Metaxas 2011).

2.3.2 Collection of bryozoan colonies on kelps

We sampled colonies of *M. membranacea* and *E. pilosa* on the kelps *S. latissima* and *L. digitata* once in October and November 2009, and September and December 2009 at The Lodge and Feltzen South respectively, and approximately bi-weekly from 25 June to 7 October 2010 at both sites. On each sampling date, ~5 blades (30 to 140 cm long) of each kelp species were haphazardly collected from each depth at each site, and transported to Dalhousie University in coolers, where they were frozen in plastic bags until processed.

2.3.3 Quantification of bryozoans on algal blades

For both kelp species, each blade was divided into 10 cm zones perpendicular to the long axis of the blade from the stipe to the tip in increasing age of algal tissue (0 to10 cm being the most proximal zone). In each 10 cm zone and on both sides of the blade, percent cover of *M. membranacea* and *E. pilosa* colonies visible to the naked eye (>6 to 10 zooid rows) was estimated in a haphazardly positioned circular area (diameter = 8.5 cm). To evaluate the accuracy of this method of estimation of percent cover, visual estimates of percent cover for a subset of 6 kelps sampled in June and July 2010, and 9 kelps sampled in September, October, and November 2009, were compared with measurements of total bryozoan cover obtained using SigmaScan Pro 5 imaging software. Visual estimates and measurements acquired using imaging software were highly correlated ($y = 1.09x$, $r^2 = 0.94$).

To measure the abundance of newly settled individuals, the number of colonies consisting of 1.0 to 1.5 zooid rows was recorded in each of 5 circular areas (diameter = 2.6 cm) positioned equidistantly from each other within each 10 cm zone on each of the ~5 (depending on availability of particular algal species in the field) individual blades collected for each kelp species on each sampling date, using a Nikon SMZ1500 stereomicroscope.

2.3.4 Estimation of the age structure of the algal blade

We used blade age to standardize the location of bryozoan colonies across species, depth, and sampling date. Estimates of blade age for *S. latissima* and *L. digitata* were calculated based on growth rates (cm d^{-1}) measured using a hole punch method, where a hole is punched near the basal meristem of the kelp blade and growth rate is determined based on the distance the hole is displaced towards the distal end of the blade over a given period of time. Growth rates were calculated for each species (n = 10 to 20) at 4 to 8 m depth at The Lodge in July, September, and November 2008, and in May and September 2009 (K. A. Krumhansl & R. E. Scheibling unpubl. data). Growth rates for *S. latissima* ranged from 0.27 cm d^{-1} in July 2008 to 0.91 cm d^{-1} in May 2009, while growth rates for *L. digitata* ranged from 0.08 cm d^{-1} in September 2008 to 0.37 cm d^{-1} in May 2009. Because the available data on growth rates did not encompass all depths, sites, or sampling dates, growth rates were averaged across all months for each species to account for spatial and/or temporal variation. As a result, growth rate may have been underestimated in early spring and overestimated in early fall, particularly for *S. latissima*, the growth rate of which was more variable among sampling months (SD = 0.26 and 0.14, for *S. latissima* and *L. digitata*, respectively). The age of the algal tissue in each 10 cm zone was then calculated by dividing the distance (cm) of the leading edge of that zone from the base of the blade by the species-specific average growth rate (cm d^{-1}).

2.3.5 Data analysis

To quantify settler abundance of *M. membranacea* and *E. pilosa* along the length of the kelp blade, the number of settlers was determined in each zone and then summed across zones and sides for each blade. The relative frequency of total settlers in each zone to the total number of settlers across all zones for all collected blades for each kelp species at each site was calculated for the following: (1) all sampling dates combined; and (2) for periods of high and low settler abundance and percent cover of *M. membranacea* (high settlement and percent cover: September 2010; low settlement and percent cover: combined June, July, and August 2010) and *E. pilosa* (high settlement: July 2010; low settlement: combined June and August 2010). Patterns in settler

abundance along the length of kelp blades were consistent among bi-weekly sampling dates within the specified periods of high and low settlement and percent cover for *M. membranacea* and *E. pilosa*. Therefore, bi-weekly sampling dates were pooled into periods of high and low settlement and percent cover to increase the total number of settlers within each period. In particular, in some cases during periods of low settler abundance and percent cover, settler abundance for a single sampling date was zero. If bryozoan larvae preferentially settle at a particular location along the blade regardless of colony density, any observed patterns in settler abundance along the length of the blade should be consistent during periods of both high and low settlement and percent cover of bryozoan colonies.

Chi-squared goodness-of-fit tests were used to compare observed distributions of settlers among zones of kelp blades of increasing age with those expected by a random distribution. Although we collected blades of *S. latissima* >100 d old and of *L. digitata* >300 d old, we only included blades of *S. latissima* and *L. digitata* that were 60–80 and 225–300 d old, respectively, in the analysis, to maximize the number of observations within each age category as well as the number of age categories. For September 2010 only, blades were included if they were 40 to 60 d old for *S. latissima* and 150 to 225 d old for *L. digitata*, because most plants collected at this time were younger or had experienced erosion of older blade tissue.

Sampling was conducted at 3 different depths at The Lodge to ensure representativity across the study site. However, because seasonal patterns in the abundance of *M. membranacea* settlers were consistent among depths (three-way ANOVA for unequal replication: $F_{2,218} = 0.0315, p = 0.969$), measurements were pooled across depths.

We quantified percent cover of colonies of *M. membranacea* along the length of the kelp blade by calculating the average percent cover in each zone. For blades of *S. latissima* and *L. digitata* <20 cm wide, the percent cover of *M. membranacea* colonies in each zone was averaged across sides of each blade. For blades of *L. digitata* >20 cm wide, the percent cover of *M. membranacea* in each zone was first averaged across sides of 2 (if blade was 20 to 40 cm wide) or 3 (if blade was >40 cm wide) segments of each

blade, and then across the segments of each blade. Juvenile kelps (<30 cm in length) were not included when quantifying percent cover, since percent cover of bryozoans on their blades was highly variable. Typically, percent cover of bryozoans on juvenile kelps was 0; however, it could occasionally be 100%, if a single colony of *M. membranacea* was established and grew to cover the entire surface area of the blade (D. Denley pers obs).

2.4 Results

2.4.1 Patterns in the abundance of settlers and colonies

Overall, the pattern in settlement of *Membranipora membranacea* exhibited the typical timing and magnitude previously observed for this species in Nova Scotia (Saunders & Metaxas 2007, 2009b). Abundance of settlers of *M. membranacea* was highest in September 2010 at both sites, but was 0.5 to 1.0 orders of magnitude greater at The Lodge than at Feltzen South (Figure 2.1). Abundance of settlers of *Electra pilosa* was highest slightly earlier, in July 2010, on both kelps at The Lodge and on *Saccharina latissima* at Feltzen South, but was higher in September 2010 on *Laminaria digitata* at Feltzen South (Figure 2.1).

The peak abundance of settlers of *M. membranacea* was an order of magnitude greater than that of *E. pilosa* at The Lodge, but only on *S. latissima* (Figure 2.1a). The opposite was observed at Feltzen South, where peak abundance of settlers of *E. pilosa* was an order of magnitude greater than that of *M. membranacea*, but only on *L. digitata* (Figure 2.1b).

Seasonal patterns in percent cover of *M. membranacea* colonies differed slightly between sites; however, percent cover of colonies tended to increase in fall 2010 (Figure 2.1). Cover of *E. pilosa* colonies was much lower than that of *M. membranacea* at both sites (Figure 2.1)

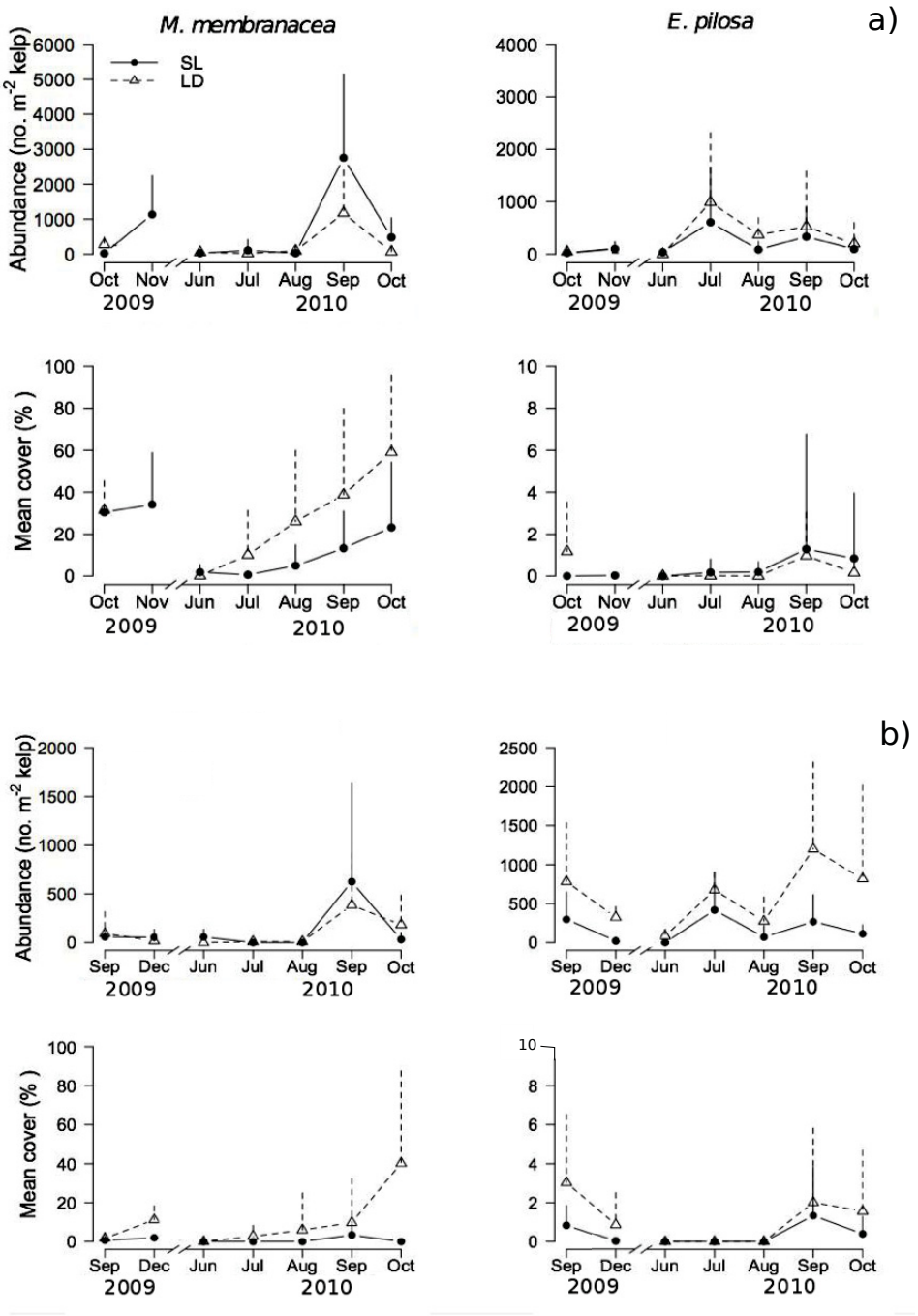

Figure 2.1 (a) *Membranipora membranacea* and *Electra pilosa* abundance (mean + SD, n = 5 to 30 kelps) of settlers (no. m^{-2} kelp) and percent cover (mean + SD, n = 4 to 29 kelps) of all colonies on *Saccharina latissima* (SL) and *Laminaria digitata* (LD) sampled at The Lodge in October and November 2009 and from June to October 2010. (b) *M. membranacea* and *E. pilosa* abundance (mean + SD, n = 4 to 10 kelps) of settlers (no. m^{-2} kelp) and percent cover (mean + SD, n = 4 to 10 kelps) of all colonies on *S. latissima* and *L. digitata* sampled at Feltzen South in September and December 2009 and from June to October 2010

2.4.2 Patterns in settler abundance with increasing blade age

Overall, when kelps of all ages were considered, there was a clear pattern of increasing relative abundance of settlers of *M. membranacea* and *E. pilosa* towards the younger, more proximal regions of the blade for both kelp species and at both sites over all sampling dates combined (Figure 2.2). When considering only blades of *S. latissima* and *L. digitata* that were 60–80 and 225–300 d old, respectively, settlers of *M. membranacea* were significantly more abundant on younger regions and less abundant on older regions of the blades than expected for a random distribution for both kelp species and all sampling periods examined at both sites (Figures 2.3 to 2.5, Table 2.1) except: (1) on *S. latissima* at Feltzen South for all dates combined (Figure 2.3, Table 2.1) and (2) during low settler abundance and percent cover (Figure 2.5, Table 2.1) and (3) on *L. digitata* at The Lodge during low settler abundance and percent cover (Figure 2.5, Table 2.1). In 2 of these 3 exceptions, the pattern was similar to that observed in the other cases, but was not detected statistically because of the low abundance of settlers.

The relative abundance of settlers of *E. pilosa* was greatest on blade tissue of young to intermediate age (20 to 40 d old for *S. latissima* and 75 to 150 d old for *L. digitata*) for both kelps and at both sites for the entire sampling period combined, and both during high and low settler abundance and percent cover (Figures 2.3 to 2.5, Table 2.1), except on *S. latissima* at Feltzen South during low settler abundance and percent cover (Figure 2.5, Table 2.1).

Percent cover of *M. membranacea* colonies did not vary consistently with blade age on either *S. latissima* or *L. digitata* at either site during peak settler abundance and cover of *M. membranacea*, except for *L. digitata* at Feltzen South where percent cover of *M. membranacea* appeared to decrease with increasing blade age (Figure 2.6).

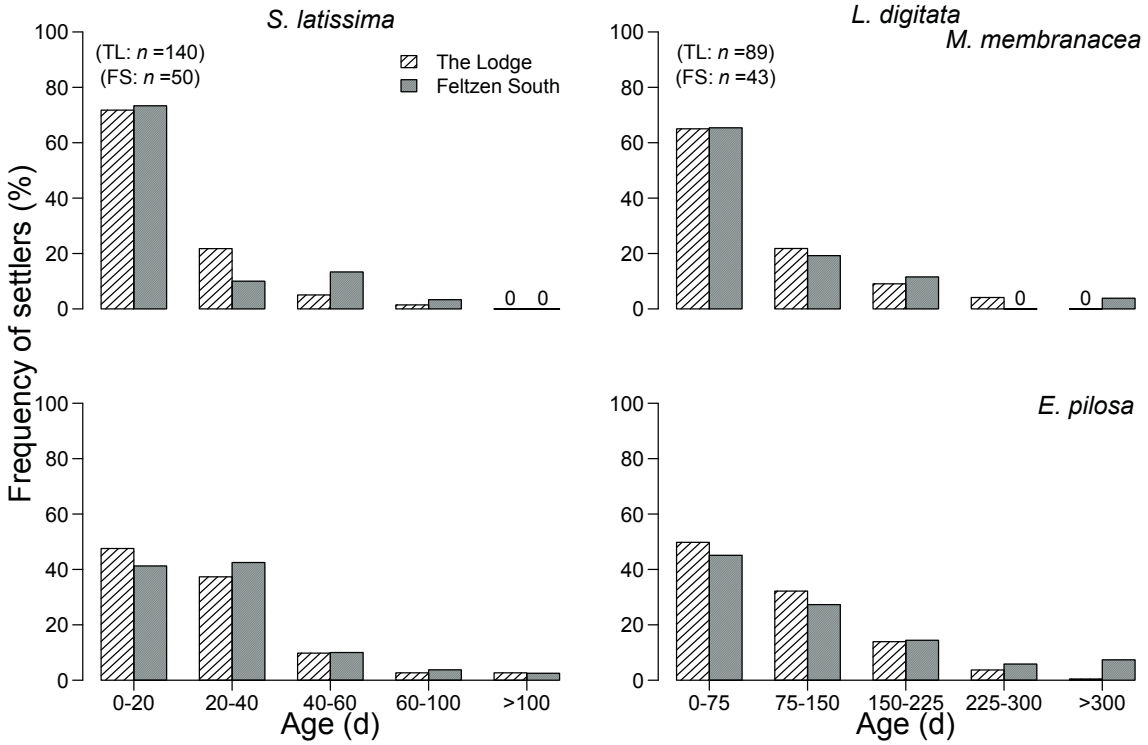

Figure 2.2 Proportional abundance of settlers of *Membranipora membranacea* and *Electra pilosa* on zones of increasing age on blades of *Saccharina latissima* and *Laminaria digitata* sampled at The Lodge (TL) in October and November 2009 and from June to October 2010, and at Feltzen South (FS) in September and December 2009 and from June to October 2010, and including all sampled kelps of all ages. For each kelp species, proportional abundance is calculated for each zone across all collected kelps and pooled across dates and depths (The Lodge only)

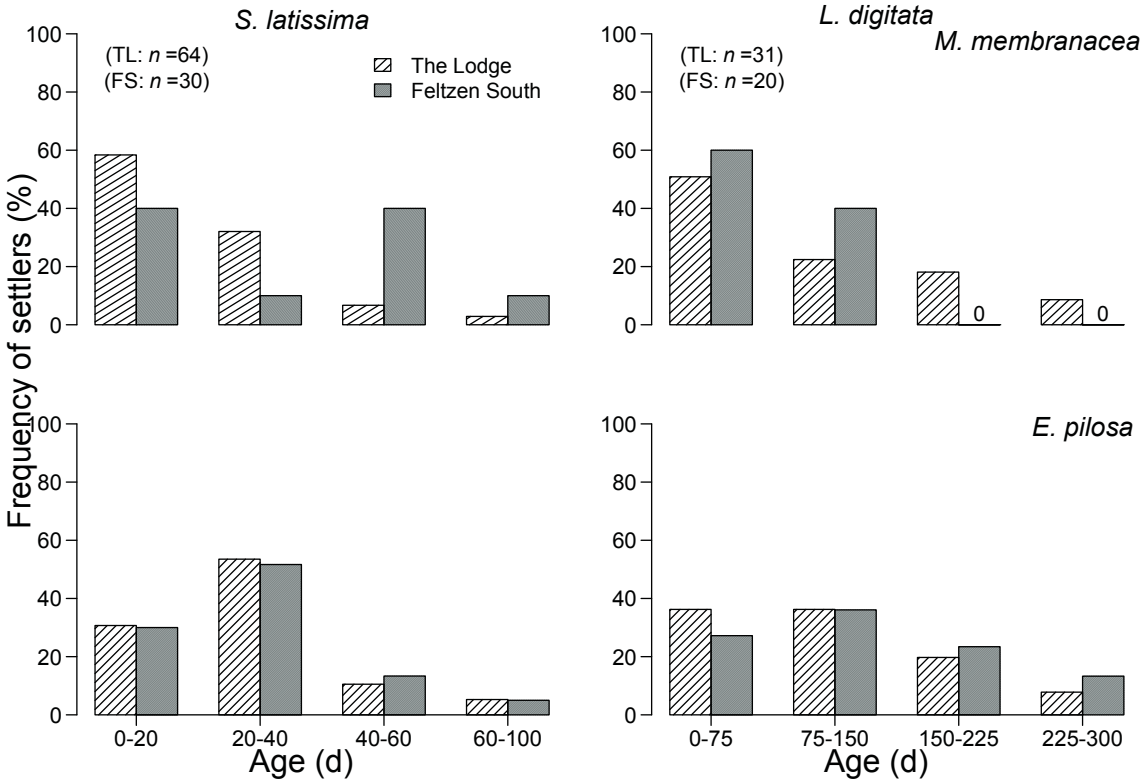

Figure 2.3 Proportional abundance of settlers of *Membranipora membranacea* and *Electra pilosa* on blade segments of increasing age for *Saccharina latissima* 60 to 80 d old and *Laminaria digitata* blades 225 to 300 d old at The Lodge (TL) and Feltzen South (FS) calculated for the entire sampling period

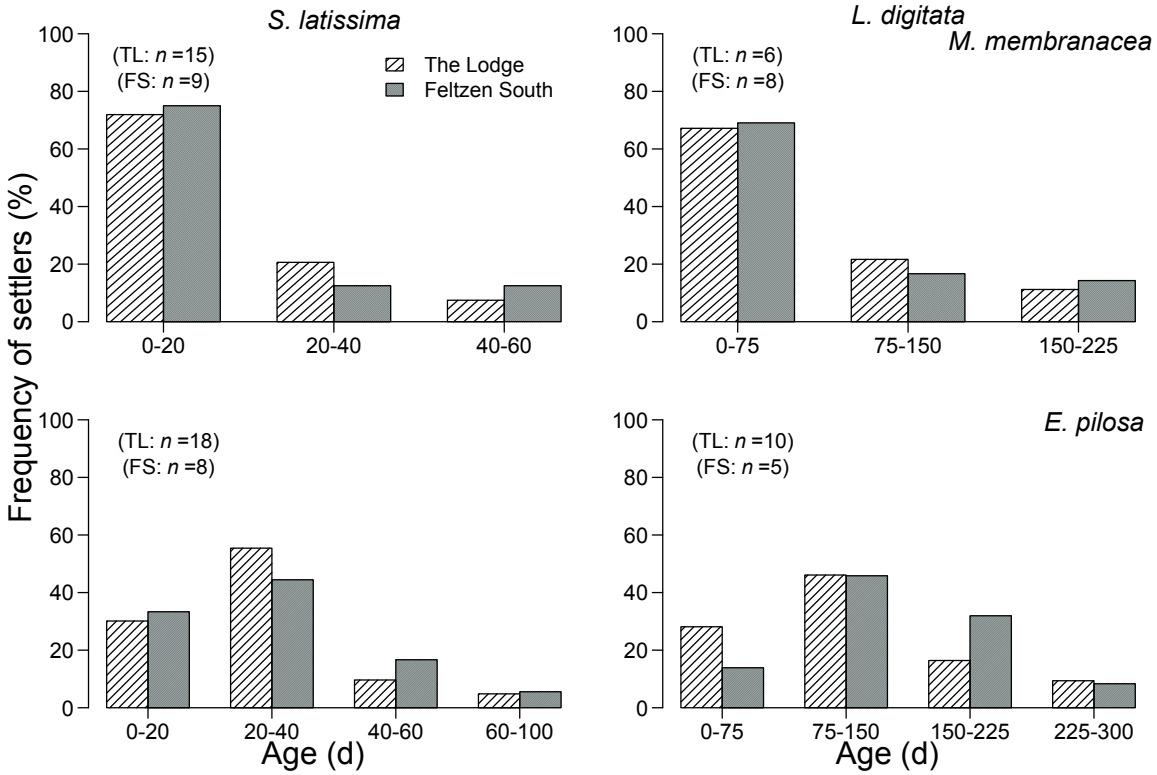

Figure 2.4 Proportional abundance of settlers of *Membranipora membranacea* and *Electra pilosa* on blade segments of increasing age for *Saccharina latissima* and *Laminaria digitata* sampled during high settler abundance and percent cover at The Lodge (TL) and Feltzen South (FS) (*M. membranacea*: September 2010; *E. pilosa*: July 2010). For *S. latissima*, we only used blades that were 40 to 60 d old for the analysis on *M. membranacea* and 60 to 80 d old for *E. pilosa*. For *L. digitata*, we only used blades that were 150 to 225 d old for the analysis on *M. membranacea* and 225 to 300 d old for *E. pilosa*. See 2.3 Methods for details

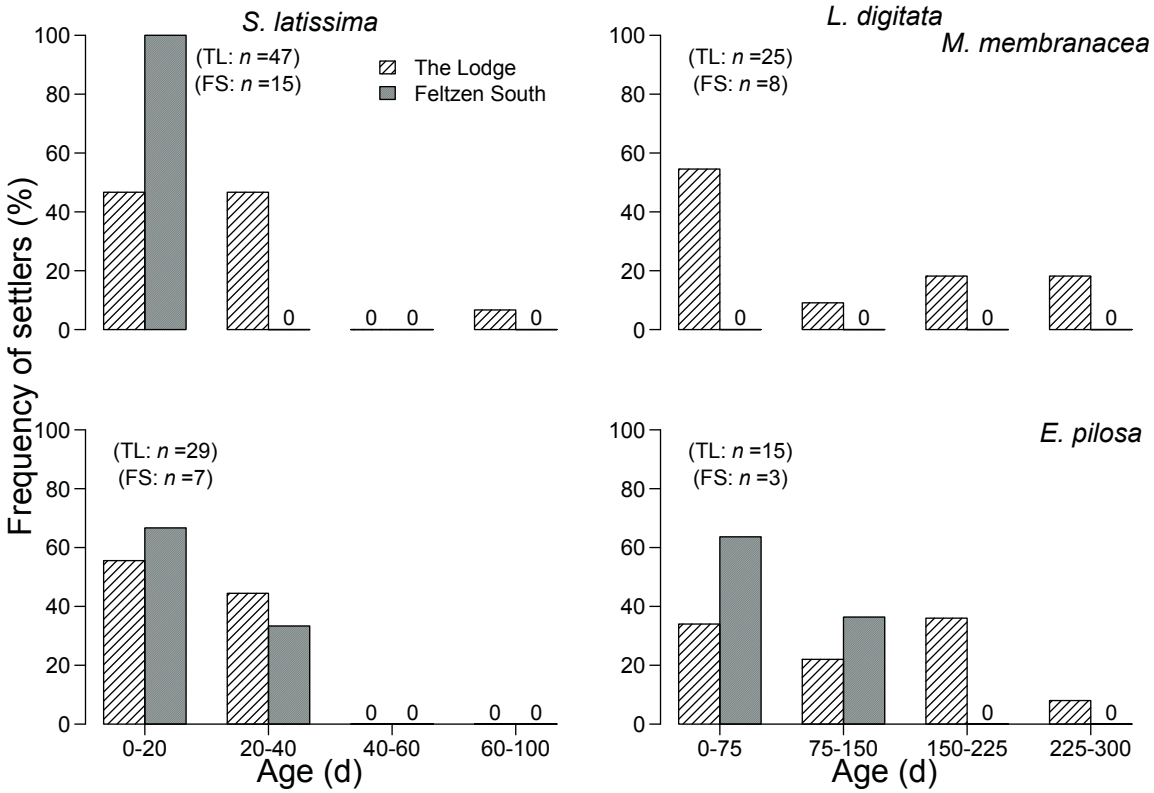

Figure 2.5 Proportional abundance of settlers of *Membranipora membranacea* and *Electra pilosa* on blade segments of increasing age for *Saccharina latissima* and *Laminaria digitata* sampled during low settler abundance and percent cover at The Lodge (TL) and Feltzen South (FS) (*M. membranacea*: pooled June, July, and August 2010; *E. pilosa*: pooled June and August 2010). For *S. latissima*, we used blades that were 40 to 60 d old for the analysis on *M. membranacea* and *E. pilosa*. For *L. digitata*, we used blades that were 225 to 300 d old for the analysis on *M. membranacea* and *E. pilosa*. See 2.3 Methods for details

Table 2.1 Results of chi-squared goodness-of-fit tests used to compare the observed distributions of settlers of *Membranipora membranacea* (Mm) and *Electra pilosa* (Ep) among zones of kelp blades of increasing age (4 categories) for *Saccharina latissima* (SL: 0–20, 20–40, 40–60, 60–80 d old) and *Laminaria digitata* (LD: 0–75, 75–150, 150–225, 225–300 d old) with those expected by a random distribution. Analyses were done for all sampling dates combined and for periods of high (Mm: September 2010; Ep: July 2010) and low (Mm: pooled June, July, and August 2010; Ep: pooled June and August 2010) settlement and percent cover. Significant *p*-values are shown in bold ($\alpha = 0.05$). NA: zero settler abundance

Sampling date	Bryozoan	Site	Kelp	$\chi^2_{(df)}$	*p*
All dates combined	Mm	TL	SL	$16.3_{(3)}$	**<0.001**
			LD	$45.0_{(3)}$	**<0.001**
		FS	SL	$3.60_{(3)}$	>0.05
			LD	$10.8_{(3)}$	**<0.025**
	Ep	TL	SL	$65.9_{(3)}$	**<0.001**
			LD	$50.3_{(3)}$	**<0.001**
		FS	SL	$30.5_{(3)}$	**<0.001**
			LD	$16.9_{(3)}$	**<0.001**
High settlement and percent cover	Mm	TL	SL	$158_{(2)}$	**<0.001**
			LD	$71.2_{(2)}$	**<0.001**
		FS	SL	$12.5_{(2)}$	**<0.001**
			LD	$24.1_{(2)}$	**<0.001**
	Ep	TL	SL	$53.0_{(3)}$	**<0.001**
			LD	$39.6_{(3)}$	**<0.001**
		FS	SL	$12.9_{(3)}$	**<0.001**
			LD	$25.5_{(3)}$	**<0.001**
Low settlement and percent cover	Mm	TL	SL	$11.4_{(3)}$	**<0.01**
			LD	$5.35_{(3)}$	>0.05
		FS	SL	$3.00_{(3)}$	>0.05
			LD	NA	NA
	Ep	TL	SL	$18.4_{(3)}$	**<0.001**
			LD	$10.0_{(3)}$	**<0.025**
		FS	SL	$3.66_{(3)}$	>0.05

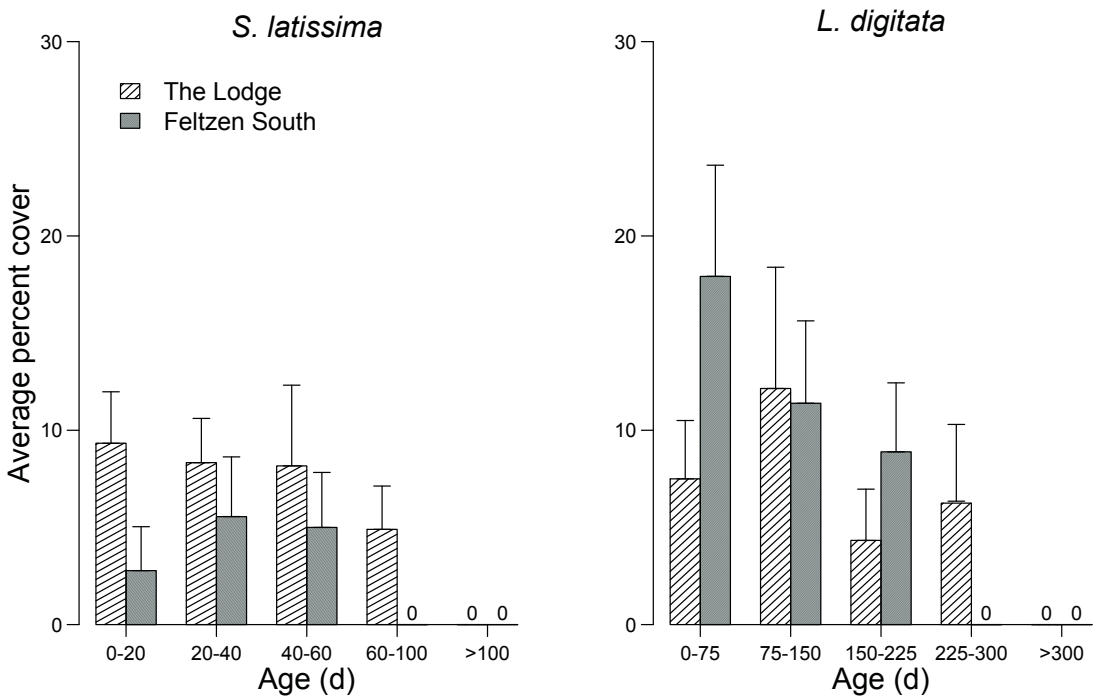

Figure 2.6 Percent cover (mean + SD, n = 18 to 36 kelps) of colonies of *Membranipora membranacea* on blade segments of increasing age for *Saccharina latissima* and *Laminaria digitata* >30 cm long, sampled during high percent cover and settler abundance of *M. membranacea* at The Lodge and Feltzen South (September 2010). Juvenile kelps (<30 cm in length) were not included since percent cover of bryozoans on their blades was highly variable. See 2.3 Methods for details

2.5 Discussion

Settlers of *Membranipora membranacea* were most abundant towards the proximal end of the blade, except when their abundance was low, likely preventing the detection of patterns. Settlers of *Electra pilosa* were also most abundant towards the proximal end of the blade; however, *E. pilosa* settlers were distributed across a wider age range of blade tissue than settlers of *M. membranacea*. Percent cover of *M. membranacea* colonies during high settler abundance and cover was fairly uniform along the length of the kelp blades. This suggests that the distribution of settlers towards the proximal end of kelp blades reflects a preference for settlement on younger algal tissue by bryozoan larvae, rather than space limitation or gregariousness. It is important to note when considering kelps of all ages, that although few collected kelps were >100 cm in length, the majority of kelps sampled were long enough to detect the transition from high to low

abundance of settlers, with settler abundance reaching zero well before the end of the blade.

High abundance of settlers near the base of fronds of *Saccharina latissima* and *Laminaria digitata* have also been observed for the bryozoan *Scrupocellaria reptans* in the northeastern Atlantic (Ryland & Stebbing 1971). In the laboratory, settlement preference experiments indicated that significantly more larvae of *S. reptans* settled on disks of *L. digitata* cut from younger regions of the blade than those from older regions when presented with a choice (Stebbing 1972). Similarly, larvae of the bryozoan *Lichenopora novae-elandiae* have been shown to preferentially settle on younger sections of the brown alga *Agarum fimbriatum* in Barkley Sound, Vancouver Island (Durante & Chia 1991).

By settling on younger, more proximal regions of kelp blades, larvae of *M. membranacea* have access to an intact, stable substrate for the longest period of time possible, thereby increasing the maximum colony size that can be attained. In turn, this will maximize colony fecundity (Yoshioka 1973, Harvell et al. 1990). However, the most common agent of obstructed colony growth for *M. membranacea* is crowding by conspecifics. Although mortality due to overgrowth among colonies of *M. membranacea* is rare, growth along margins of colony contact ceases, and obstructed growth during periods of high colony density can severely limit individual colony size (Harvell et al.1990). As a result, recruits of *M. membranacea* are smaller in high-density populations than those in low-density populations (Ellison & Harvell 1989). Although percent cover of *M. membranacea* colonies did not vary consistently with blade age, it is possible that the size distribution of colonies did. Growth of *M. membranacea* colonies is positively related to colony size (Saunders & Metaxas 2009a); therefore, larger colonies tend to dominate in competition for available space. If smaller, more recently established colonies are concentrated towards the proximal region of kelp blades, it would be advantageous for larvae of *M. membranacea* to settle preferentially on younger regions of the blade, where competition for space and crowding by conspecifics is reduced.

In contrast, the slow growth rates and low colony abundance of *E. pilosa* on kelp suggests that intraspecific competition for space among conspecifics is weak for this

species. Settlement preference for younger parts of the blades of *S. latissima* and *L. digitata* may instead be driven primarily by the longevity of the substrate. The comparatively slower growth rate of *E. pilosa* may indicate a longer time to maturity for this species. If that is the case, the reproductive success of individual *E. pilosa* colonies may be strongly dependent on the duration of the substrate onto which they initially settle, which may explain the higher abundance of *E. pilosa* on more stable, slower-growing fucoid species than on faster-growing, more ephemeral kelp substrates in Nova Scotia (Yorke & Metaxas 2011).

The higher abundance of settlers of *E. pilosa* slightly further from the proximal end of the blade towards tissue of a young to intermediate age may be a result of out-competition by *M. membranacea* on the more favorable proximal blade tissue. *M. membranacea* has been shown to be competitively superior to *E. pilosa* on kelps in the northwest Atlantic (Berman et al. 1992); however, *M. membranacea* does not always overgrow *E. pilosa* upon encounter, and standoffs between the 2 species occur more frequently than would be expected if *M. membranacea* was competitively dominant (Yorke & Metaxas 2011).

The mechanisms by which competent larvae detect variations in microhabitat within the bounds of an individual kelp blade are unclear. Brown algae are known to produce secondary metabolites as a chemical defense against herbivory (Van Alstyne et al. 1999) and, in some cases, these or similar compounds have been shown to deter epiphytism by fouling organisms (Schmitt et al. 1995, but see Jennings & Steinberg 1997). For 21 species of laminarian kelps and rockweeds, Van Alstyne et al. (1999) found that meristematic tissue tended to have higher phlorotannin levels than non-meristematic vegetative tissues. In Nova Scotia, high concentrations of polyphenolic compounds have been detected in the meristem of *S. latissima*, where they were shown to deter grazing by the mesogastropod *Lacuna vincta* (Johnson & Mann 1986). However, this distribution of secondary metabolites, with higher levels towards the basal meristem of the blade, is inconsistent with chemical inhibition of *M. membranacea* and *E. pilosa*, for which settler abundance actually increases towards the proximal regions of *S. latissima* and *L. digitata*. It may be that their chitinous exoskeleton renders these

bryozoans more resistant to secondary metabolites. Anti-fouling metabolites extracted from the red alga *Dilsea carnosa* off the west coast of Sweden inhibited recruitment of some fouling organisms, but were ineffective against fouling by *M. membranacea* and *E. pilosa* (Nylund & Pavia 2005)

The distribution of microflora along the length of the blade may affect settlement of larval epibionts (Stebbing 1972). Since the spatial patterns in microflora along a kelp blade are likely correlated with its age, the presence or absence of a particular taxon may be used as a cue for younger tissue. For example, bacterial abundance and community composition were found to vary with tissue age on *L. digitata* (Corre & Prieur 1990); however, whether these variations are substantial enough to be detectable by bryozoan larvae is not known. Larvae of *M. membranacea* exhibit small-scale active searching behavior upon contact with a presumably high-quality habitat (Matson et al. 2010). Specifically, larvae of *M. membranacea* have been observed to move from older more distal regions to younger more proximal regions of the blades of *Nereocyctis luetkeana* in a flume in the laboratory (Matson et al. 2010). However, this may have been a response to the direction of flow along the blade rather than the physical characteristics of the algae. Although we did not measure flow either on the scale of the blade or at our study sites, kelp blades tend to be longer and broader at The Lodge and Feltzen South, morphologies indicative of kelps from low-energy environments (Gerard & Mann 1979). Under these conditions, flow velocity may be too weak and/or too variable to provide a consistent directional cue for searching larvae, particularly at the level of the kelp blade.

In conclusion, we clearly demonstrated that larvae of the dominant species of bryozoans in Nova Scotia, *M. membranacea* and *E. pilosa*, exhibit a preference for settling towards younger, more proximal regions on the blades of the numerically dominant kelps *S. latissima* and *L. digitata*. This preference appears to be independent of colony density, but may require a threshold level of settler abundance to be detected. While the mechanisms for settlement preference remain unclear, variation in the chemical composition of the substrate or the microfloral community along the blade are likely significant factors. Our findings suggest that post-settlement events, such as competition for space among conspecifics and colony longevity, as it relates to the duration of

substrate availability, can influence the evolution of selective settlement behavior in sessile marine epiphytes with planktonic larvae.

CHAPTER 3

LACK OF SUBSTRATE SPECIFICITY CONTRIBUTES TO INVASION SUCCESS AND PERSISTENCE OF *MEMBRANIPORA MEMBRANACEA* IN THE NORTHWEST ATLANTIC[2]

3.1 Abstract

Selective settlement by planktonic larvae plays a significant role in determining the distribution and abundance of many species of marine invertebrates. For non-indigenous species, larval settlement behavior can determine invasive potential by influencing initial invasion success, secondary spread, and persistence of species outside their native environments. *Membranipora membranacea* is an ecologically significant invasive in the northwest Atlantic, where settlers are most abundant on some but not all species of kelp. Whether the increased abundance of *M. membranacea* on select kelp species is the result of larval settlement preference remains unknown. In this study, we examine selective settlement by larvae of *M. membranacea* by 1) quantifying settlers in mixed kelp beds and determining whether larvae settle preferentially with respect to kelp species; 2) conducting laboratory settlement preference experiments using the most abundant kelp substrates in Nova Scotia; and 3) examining whether the presence of kelp beds provides a settlement cue for larvae by quantifying settlement of *M. membranacea* on plates deployed within and outside of kelp beds. Contrary to settlement behaviour described for native populations, our results suggest that larvae of *M. membranacea* in invaded habitats do not exhibit preference for settling on particular kelp species or within kelp beds. Instead, larvae settle on substrates extending furthest above the primary substratum. Lack of substrate specificity suggests that *M. membranacea* will continue to persist in the northwest Atlantic despite significant declines in regional kelp abundance.

[2] Denley D, Metaxas A (2017a) Lack of substrate specificity contributes to invasion success and persistence of *Membranipora membranacea* in the northwest Atlantic. Mar Ecol Prog Ser 580:117-129.

My coauthor Dr. Anna Metaxas supervised the development of the study design and analyses, and edited the manuscript.

Our results emphasize the importance of early life-history strategies in contributing to invasion success.

3.2 Introduction

The successful recruitment of marine invertebrates with planktonic larvae is strongly dependent on the location of larval settlement at scales ranging from millimeters (e.g. Lathlean et al. 2013) to kilometers (e.g. Hernández et al. 2010). Because of the strong association between the location of settlement and post-settlement survival, substrate selection by larvae of some marine invertebrates is thought to have evolved in response to specific cues affiliated with preferred juvenile or adult habitats (reviewed by Burke 1983). These cues may be directly related to characteristics of the substrate (e.g. macro-algae, Ryland 1959; crustose coralline algae, Harrington et al. 2004) or the local environment (e.g. cues released by adult conspecifics, Knight-Jones 1953; or preferred prey species, Morse & Morse 1984, Lambert & Todd 1994), but can also involve more general stimuli, such as light (reviewed by Thorson 1964) or gravity (Naylor 2006). Such stimuli or cues can elicit larval behavioural responses that increase the probability of a competent larva encountering preferred habitat.

Selective settlement of marine invertebrate larvae plays a critical role in predicting shifts in species distributions and abundances of adult populations in response to habitat alteration at local to regional scales. Quantifying the influence of specific cues on larval settlement is particularly important with respect to the establishment and spread of non-indigenous species, since invasion success is strongly related to conditions of the recipient region, including the availability of suitable substrates for settlement (Carlton 1996, e.g. Folino-Rorem et al. 2006). Differences in settlement behavior among ecologically similar invasive species can determine which species persists in the invaded habitat (e.g. Zabin 2009). However, larvae of many globally invasive species have been shown to successfully recruit onto a variety of substrates or under a wide range of environmental conditions (e.g. Creed & De Paula 2007, Rius et al. 2010). Consequently, lack of strong settlement preference may also enhance invasion potential.

Membranipora membranacea is a cosmopolitan invasive bryozoan that exhibits many of the life history characteristics common to successful invaders, including strong

competitive ability (Pratt 2008, Yorke & Metaxas 2011), rapid growth (Pratt 2008, Saunders & Metaxas 2009a, Yorke & Metaxas 2011), high recruitment peaks (Saunders & Metaxas 2007), and widespread dispersal of long-lived planktonic larvae (Yoshioka 1973). *M. membranacea* is native to the Pacific coast of North America and the Atlantic coast of Europe, and is an ecologically significant invasive species in the northwest Atlantic where periodic population outbreaks have been linked to massive defoliation of kelp beds (Lambert et al. 1992, Levin et al. 2002, Saunders & Metaxas 2008, Scheibling & Gagnon 2009). There is evidence to suggest that cyphonautes larvae from native populations of *M. membranacea* may be induced to settle by the presence of specific algal substrates (Ryland 1962, Stricker 1989, Matson et al. 2010). However, patterns in the distribution of settlers of *M. membranacea* within its native habitats have also been attributed to both passive filtration by the kelp canopy, and active behavior by the free-swimming larvae in response to ocean temperature (Bernstein & Jung 1979, Yoshioka 1986).

Off the coast of Nova Scotia, settlers of *M. membranacea* are generally most abundant on some kelps during periods of both early and peak settlement (Saunders & Metaxas 2009b, Yorke & Metaxas 2012). This may have significant implications for the persistence and spread of *M. membranacea* in the northwest Atlantic given its propensity to negatively impact kelps, its purported primary host substrate (Saier & Chapman 2004, Krumhansl & Scheibling 2011, Krumhansl et al. 2011). In particular, the 3 most abundant kelp species in Nova Scotia (*Saccharina latissima*, *Laminaria digitata*, and *Agarum clathratum*) differ in their susceptibility to defoliation following a *M. membranacea* outbreak. *S. latissima* appears to suffer the most from encrustation by *M. membranacea* (Saunders & Metaxas 2008, Saunders & Metaxas 2009b), whereas loss of *A. clathratum* following *M. membranacea* outbreaks is typically less severe and *L. digitata* is fairly resilient to high levels of encrustation (Saunders & Metaxas 2009b). Differential vulnerability to recurrent *M. membranacea* outbreaks may result in a shift in the distribution and abundance of kelp species within kelp beds in Nova Scotia, as described for the Gulf of Maine in the 1990s (Harris & Tyrell 2001).

Should kelp beds in the northwest Atlantic continue to decline, in part due to recurring outbreaks of *M. membranacea* (Filbee-Dexter et al. 2016), the persistence of *M.*

membranacea will depend on its capacity to colonize alternative substrates, both as stepping stones to facilitate spread between patchy kelp beds, as well as, refuges during periodic or persistent loss of kelp habitat. However, studies of settlement by larvae of *M. membranacea* in its native habitat are inconclusive (Ryland 1962, Bernstein & Jung 1979, Yoshioka 1986, Stricker 1989, Matson et al. 2010), and whether the increased abundance of *M. membranacea* on select kelp species in its invaded habitat is the result of selective larval settlement remains unknown.

In this study, we examine selective settlement by larvae of *M. membranacea* to determine whether larvae are capable of detecting and responding to settlement cues associated with specific kelp substrates, or more general cues affiliated with the presence of mixed kelp beds. To achieve this we address the following questions: 1) Do larvae of *M. membranacea* settle preferentially with respect to kelp species? 2) Can observed distributions of newly settled colonies in the field be explained by selective larval settlement? And 3) Does the presence of kelp beds provide a settlement cue for larvae of *M. membranacea*? We address these questions by (1) quantifying the distribution of newly settled colonies within mixed kelp beds in the field and comparing the observed distribution of newly settled colonies among the 3 dominant kelp species with that expected under a random distribution; (2) conducting laboratory settlement preference experiments; and (3) quantifying settlement of *M. membranacea* relative to that of other bryozoan epiphytes onto settlement plates deployed in locations within and outside of kelp beds.

Our study provides evidence that the behaviour of generalist settlement manifested by *M. membranacea* can contribute towards the persistence and spread of invasive marine invertebrates with planktonic larvae, emphasizing the importance of early life-history strategies in determining invasion success. Our results can be used to predict the response of invasive populations of *M. membranacea* to changing availability of substrates with implications for the recovery or continued decline of kelp beds throughout the northwest Atlantic.

3.3 Methods

3.3.1 Selective settlement by larvae of *Membranipora membranacea* within kelp beds

We collected individuals of the 3 most abundant kelp species in Nova Scotia (*Saccharina latissima*, *Laminaria digitata*, and *Agarum clathratum*) at 3 sites on the southwestern shore of Nova Scotia, Canada: The Lodge (44°33'3"N, 64° 01' 9" W) on the western shore of St. Margarets Bay, Paddy's Head (44°31'6"N, 63°57'2"W) on the eastern shore near the mouth of St. Margarets Bay, and Sandy Cove (44° 27' 6" N, 63° 42' 4" W) in Terence Bay, 20 km to the northeast of St. Margarets Bay, approximately every 6 weeks from Jun 2012 to Aug 2013 (for a map of the study sites see Saunders & Metaxas 2009b). At The Lodge and Paddy's Head, 10-15 kelps of each species were randomly collected along each of 4-, 8-, and 12-m depth contours, for a total of 90-135 kelps at each sampling time (Table 3.1). At Sandy Cove, we collected all kelps from 8-11 haphazardly-placed 0.5-m^2 quadrats at each of 4 and 8 m (the seabed becomes sandy beyond this depth), for a total of 84-184 kelps at each sampling time (Table 3.1, for detailed methods see Denley & Metaxas 2016). Collected kelps were transported to the Aquatron facility at Dalhousie University in plastic tubs without seawater, where they were maintained in aquaria with running ambient seawater.

For *M. membranacea,* we defined newly settled colonies as colonies <1 cm in diameter (<2 wk old). Post-settlement mortality is very low for *M. membranacea* (Yoshioka 1982, 1986) and size-specific mortality rates were negligible for small (<1 cm diameter) colonies in Nova Scotia (Denley & Metaxas 2016). Consequently, our definition of newly settled colonies (from here on referred to as settlers) accurately represents settlement of *M. membranacea* in the field.

We counted the number of settlers of *M. membranacea* on both sides of the blades of all collected kelps and then photographed each kelp blade to determine its surface area. We measured the surface area of individual kelp blades from photographs by tracing the

Table 3.1 Details of field sampling and experiments used in this study to measure a) selective settlement by larvae of *M. membranacea* within kelp beds, and b) the effect of understory kelp on bryozoan settlement

	Measurements	Location	Depth (m)	Collection method	Duration and frequency	Analysis	Comparison	Results
a)	Number of settlers m^{-2} kelp for each kelp species	TL PH	4, 8, 12 4, 8, 12	10-15 individuals of each kelp species randomly selected along a 30-m transect	18 Jun 2012 – 2 Aug 2013 ~every 6 weeks	Goodness of fit (G-tests)	Observed number of settlers versus random distribution	Table 3.2 Table 3.3 Fig 3.1
	Total surface area (m^2) of each kelp species							
	Total number of settlers on all kelp species	SC	4, 8	All individual kelps collected from within 8-11 haphazardly placed $0.5m^2$ quadrats		Binomial sign tests	Number of times kelp species is preferred or avoided versus expected by chance	
b)	Number of *M. membranacea* colonies	TL SC	8, 12 4, 8	Settlement collectors deployed within and outside of kelp beds	Deployed Sep 2012 Replaced 13 Nov 2012, 1 Jun, 2 Sep, 22 Nov 2013 Replaced 25 Nov 2012, 14 Jun, 5 Sep, 13 Nov 2013	Mixed effects models Zero-inflated negative binomial models	Effect of presence of kelp bed, distance above substratum, and depth on the number of colonies	Table 3.4 Table A.2
	Number of *M. membranacea*, E. *pilosa,* and *C. pallasiana* colonies	TL SC	8, 12 4, 8		2 Sep, 22 Nov 2013 5 Sep, 13 Nov 2013			

Note: Location codes are: TL, The Lodge; PH, Paddy's Head; SC, Sandy Cove

outline of each blade in ImageJ and multiplying the resulting area by 2 to yield the total surface area including both sides of the blade. Blades of *S. latissima* are highly crenulated; therefore, we corrected surface area measurements for this species using location- and depth-specific correction factors (Saunders & Metaxas 2007). For *A. clathratum*, we also measured the biomass of all collected thalli using a triple beam balance (accuracy, 0.005 g). The perforated nature of *A. clathratum* makes accurate measurement of its surface area using image analysis difficult. Thus, we were only able to obtain accurate measurements of surface area using ImageJ for a subset of the collected individuals (n = 172 kelps spanning all sites, depths and sampling times). To estimate the surface area of the remainder, we used the measurements of surface area and biomass from the subset of 172 kelps to generate a relationship between kelp biomass and surface area (Figure A.1). We then applied this relationship to convert our measurements of biomass to surface area for all remaining blades of *A. clathratum* for which surface area could not be measured accurately using image analysis.

From kelp collected at each depth, site and sampling time we calculated: 1) the number of settlers per m^2 kelp for each individual kelp, 2) the total surface area of each kelp species by summing individual surface areas, and 3) the total number of settlers on all kelp species combined (Table 3.1). To determine whether larvae of *M. membranacea* settle preferentially with respect to kelp species, we compared observed numbers of settlers (per m^2 kelp) with the number of settlers (per m^2 kelp) expected under a random distribution using goodness of fit tests (G-tests) (Table 3.1). If larvae settle randomly, we would expect the proportion of settlers on each kelp species to be equal to the proportion of the total surface area accounted for by that kelp species. We calculated the expected number of settlers for each depth, site and sampling time by multiplying the proportional surface area of each kelp species [e.g. proportional surface area of *S. latissima* (m^2) = surface area of all *S. latissima* collected (m^2) ÷ total surface area of all kelp collected (m^2)] by the total number of settlers. Within each site, only depths and sampling times at which all 3 kelp species occurred simultaneously were considered for analyses.

To reduce the probability of type I error, only combinations of depth, site, and sampling time for which the total number of settlers exceeded 25 ($n > 25$) were included in goodness of fit tests; in addition, the Williams' correction was applied for all cases

where $n < 200$ settlers (Sokal & Rohlf 1981). Within each site, there was significant heterogeneity among replicate combinations of depth and sampling times (The Lodge, $G_H = 826, df = 22, p < 0.0001$; Paddy's Head, $G_H = 109, df = 10, p < 0.0001$; Sandy Cove $G_H = 102, df = 6, p < 0.0001$). Therefore, we conducted separate G-tests for each depth and sampling time.

For each site, we also used binomial sign tests of the difference between observed and expected numbers of settlers on each kelp substrate at each depth and sampling time to determine whether any of the 3 kelp species were preferred (positive difference, scored as '+') or avoided (negative difference, scored as '−') more often than expected by chance (Table 3.1).

3.3.2 Selective settlement by larvae of *Membranipora membranacea* in the laboratory

We isolated competent larvae of *M. membranacea* from plankton samples collected from St. Margarets Bay in Oct-Nov 2012 and Oct 2013. Kelp (*S. latissima*, *L. digitata*, *A. clathratum*) was collected over the same periods from 8 m at The Lodge and/or Sandy Cove. We conducted settlement preference experiments in the laboratory using paired sample combinations of kelp substrates in 3 "choice" and 3 "no choice" treatments (Figure 3.1). In each paired sample combination, we randomly assigned two 2 x 2 cm kelp segments to 250-ml beakers filled with 1μm-filtered seawater. Kelp segments were excised from the central portion of each blade to ensure tissues were of similar intermediate age. Segments were cut 24 h prior to experiments and maintained in flow-through seawater tables to allow for removal of mucus exudates following cutting. We identified competent larvae of *M. membranacea* using a Nikon SMZ1500 stereomicroscope (20x magnification) based on the size and shape of the shell and the appearance of ornamentation along the basal edge of the shell, all indicators that larvae are ready to metamorphose (Atkins 1955). We introduced 30 competent larvae into each beaker and allowed larvae to settle for 72 h at ambient seawater temperature. Filtered seawater was manually replenished in each beaker daily to avoid anoxia. After 72 h, we examined the kelp under the microscope and counted the number of larvae that had settled on each segment. High temporal variability in larval developmental stage and

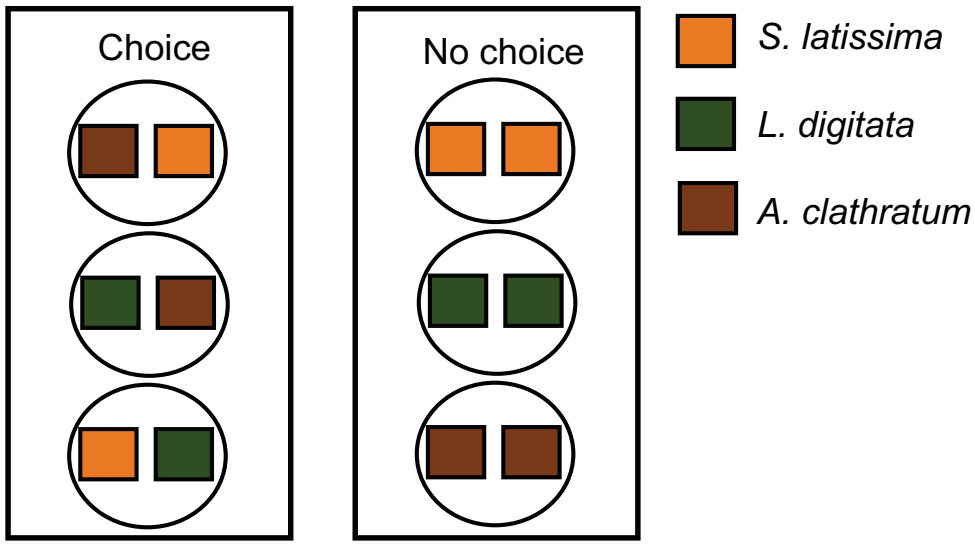

Figure 3.1 Schematic of experimental design for laboratory settlement preference experiments. Choice treatments include all paired combinations of kelp substrates. No choice treatments consist of paired samples of the same kelp species. For "no choice" treatments, a single kelp segment was randomly chosen from each beaker for analyses (LMM, *t*-tests, ANOVA) to maintain independence of replicates. See text for full description of experimental procedure and statistical analyses

abundance in the field prevented us from completing an entire replicate of all choice and no choice combinations in a single trial (3 choice treatments + 3 no choice treatments x 30 larvae per treatment = 180 competent larvae per replicate). Therefore, replicates were conducted over the course of 8 separate trials from Oct-Nov 2012 and in Oct 2013.

We used linear mixed effects models (LMMs) to compare the number of larvae that settled on each substrate when given a choice of substrates to the number of larvae that settled on that same substrate in the absence of choice. Treatment (choice, no choice) was included as a fixed factor in the model, with separate intercepts for the random effect of trial. The effect of trial was not significant for any kelp substrate (p = 0.396-0.794). Therefore, we compared the number of settlers between "choice" and "no choice" treatments for each paired combination of substrates pooled across all trials using 2-tailed independent samples *t*-tests. Lastly, we determined the effect of kelp substrate on the number of settlers over all trials with one-way ANOVA. Beakers in which no settlement occurred were excluded from all analyses.

3.3.3 The effect of understory kelp on bryozoan settlement

We deployed 20 settlement collectors at each of 8 and 12 m at The Lodge, and 4 and 8 m at Sandy Cove in Sep 2012 (Table 3.1). At each depth, 10 collectors were placed within a kelp bed and 10 collectors in areas naturally clear of kelp (density of kelp outside of kelp beds = 0 kelps per m^2, for details on the density of kelp within kelp bed treatments see Appendix A.1). Each collector held a pair of vertically suspended 10 x 15 cm plates cut from Sintra plastic PVC sheets, one at ~1 m above the substratum, thereby extending out of the kelp canopy, and the other within 10 cm of the substratum, well below the canopy. Plates were switched 4 times between Sep 2012 and Nov 2013 to capture seasonal variation in settlement (Table 3.1). Recovered plates were placed individually in labeled plastic bags and transported to the Aquatron facility at Dalhousie University, where they were maintained in aquaria with running ambient seawater until processing was completed (~1-2 days). The number of colonies of *M. membranacea* on each plate was recorded for all collection dates. Colonies of the bryozoans *Electra pilosa* and *Cryptosula pallasiana* were also recorded in Sep and Nov 2013 to allow us to distinguish settlement characteristics that may be unique to *M. membranacea* from those shared with the other 2 species (Table 3.1). We measured bryozoan settlement as the number of colonies of all sizes, rather than colonies <1 cm in diameter, because we believe this accurately represents total settlement of bryozoan larvae between collection dates. Settlement plates were switched frequently enough that 1) colonies on plates remained whole and unfragmented, as indicated by intact growing margins; 2) whole-colony mortality, which occurs primarily due to loss of kelp substrate (Denley & Metaxas 2016), was negligible on the artificial substrate; and 3) available space for settlement was not limited on any individual plate.

For each bryozoan species, we examined the main effects of understory kelp (treatment: within kelp bed, outside kelp bed), distance above the substratum (position: top plate, bottom plate), and depth (shallow, deep) on the number of colonies using mixed models (Table 3.1). Site and date of collection were considered as crossed random factors, with the random effect of individual settlement collector nested within site. For all 3 species of bryozoans, the number of colonies was $\log(x + 0.01)$-transformed to

better approximate a normal distribution. Residual plots indicated heterogeneity of variance for *M. membranacea* and *E. pilosa* that could not be alleviated through transformation. Heterogeneity of variance for these species was due to a high number of plates containing no colonies (zero-inflated data). Accordingly, we also analyzed the main effects on the number of settlers using zero-inflated negative binomial models (after Zuur et al. 2009, Table 3.1). Results were consistent with mixed models for *M. membranacea*, and similar for *E. pilosa* (Table A.2). Therefore, we chose to present results from mixed models and adopted a more conservative *p*-value ($\alpha = 0.01$) for analyses involving these two species.

3.4 Results

3.4.1 Selective settlement by larvae of *Membranipora membranacea* within kelp beds

Settlers of *M. membranacea* were not randomly distributed among kelp substrates, except at The Lodge and Sandy Cove at 8 m in Jul-Aug 2013 (Table 3.2). The observed number of settlers differed from the expected number of settlers for both *Saccharina latissima* and *Laminaria digitata* at all depths and sites for most dates when sampling occurred, although not in a consistent direction, i.e. sometimes showing preference while not at others (Table 3.2). However, when pooled across depths and sampling times, the ratio of the observed versus the expected number of settlers for each species did not differ significantly from 1, except for *S. latissima* which was preferred at The Lodge (Figure 3.2, Table 3.3). However, neither of these kelp species was preferred significantly more or less often than expected by chance based on available surface area (Table 3.3). Conversely, *Agarum clathratum* was preferred significantly less often than expected by chance at The Lodge and Paddy's Head (Table 3.3), and the ratio of the observed versus the expected number of settlers was significantly less than 1 at both sites for all depths and sampling times combined (Figure 3.2, Table 3.3). Although *A. clathratum* was never preferred at Sandy Cove (Figure 3.2, Tables 3.2 & 3.3), significant differences in preference may not have been detectable using sign tests due to the low number of total settlers at this site.

Table 3.2 Results of G-tests for goodness of fit comparing the observed distribution of settlers of *Membranipora membranacea* among the 3 most abundant kelp species in Nova Scotia with a random distribution. The expected number of settlers under a random distribution was calculated for each kelp species based on proportional surface area (see 3.3 Methods for details). For each site, only date and depth combinations where all 3 kelp species were present and the number of settlers of *M. membranacea* exceeded 25 ($n > 25$) are included. Significant *p*-values shown in bold ($\alpha = 0.05$); for $p < 0.05$, 'higher' indicates a greater number of settlers observed than expected under a random distribution, 'lower' indicates fewer settlers observed than expected under a random distribution. *: William's correction for $n < 200$

Date	Depth (m)	df	G	*p*	*S. latissima*	*L. digitata*	*A. clathratum*
The Lodge							
Jun-Jul 2012	8	2	32.3	**<0.0001**	higher	lower	lower
	12	2	122	**<0.0001**	higher	lower	lower
Jul-Aug 2012	8	2	55.3	**<0.0001**	higher	lower	lower
	12	2	278	**<0.0001**	higher	higher	lower
Sep 2012	8	2	514	**<0.0001**	higher	lower	lower
	12	2	69.7*	**<0.0001**	higher	lower	higher
Nov-Dec 2012	12	2	55.5*	**<0.0001**	higher	lower	lower
Mar-Apr 2013	12	2	40.6*	**<0.0001**	higher	higher	lower
Jun 2013	4	2	44.7	**<0.0001**	higher	lower	lower
	8	2	9.56	**0.008**	lower	higher	lower
Jul-Aug 2013	8	2	1.39*	0.499	-	-	-
Pooled		2	1042	**<0.0001**	higher	lower	lower
Paddy's Head							
Jun-Jul 2012	12	2	23.2*	**<0.0001**	lower	higher	lower
Sep 2012	8	2	49.8*	**<0.0001**	higher	higher	lower
	12	2	33.8*	**<0.0001**	higher	lower	lower
Nov-Dec 2012	12	2	190	**<0.0001**	higher	higher	lower
Jun 2013	12	2	93.1*	**<0.0001**	lower	higher	lower
Jul-Aug 2013	12	2	38.0*	**<0.0001**	higher	lower	lower
Pooled		2	327	**<0.0001**	higher	lower	lower
Sandy Cove							
Jun-Jul 2012	8	2	9.32*	**0.010**	lower	higher	lower
Sep 2012	4	2	25.4*	**<0.0001**	higher	lower	lower
Jun 2013	8	2	120*	**<0.0001**	lower	higher	lower
Jul-Aug 2013	8	2	1.73*	0.422	-	-	-
Pooled		2	43.6	**<0.0001**	lower	higher	lower

3.4.2 Selective settlement by larvae of *Membranipora membranacea* in the laboratory

Contrary to patterns of settlement preference observed in the field where *A. clathratum* was consistently less preferred, the laboratory experiments suggest that *S. latissima* may be less preferred than *L. digitata* by larvae of *M. membranacea* (Figure 3.3, Table 3.4). There was no effect of kelp substrate on the rate of settlement (one-way ANOVA: $F_{(2,15)} = 1.08$, $p = 0.36$; number of settlers cm^{-2} [mean ± SE]: *S. latissima* = 0.319 ± 0.056; *L. digitata* = 0.225 ± 0.115; *A. clathratum* = 0.156 ± 0.079); however, settler abundances were an order of magnitude greater than those observed in the field (e.g. 0.063 cm^{-2} on *S. latissima* during peak settlement at The Lodge in Sep 2016). To ensure that the lack of preference was not affected by the strength of the settlement cue (the concentration of which would be affected by the amount of kelp tissue present), we ran additional settlement preference experiments examining the effect of size of kelp segment (1 cm x 1 cm versus 2 cm x 2 cm) on settler abundance (Appendix A.2). We did not detect a significant effect, supporting the outcomes of the preference experiments.

3.4.3 The effect of understory kelp on bryozoan settlement

Settlement of *M. membranacea* did not vary between kelp and no kelp habitats (Figure 3.4, Table 3.5). Instead, settlement of *M. membranacea* increased significantly at deeper depths, and was significantly greater on top than bottom settlement plates at both depths and sites across all sampling dates (Figure 3.4, Table 3.5). In contrast, settlement of *Electa pilosa* did not vary with position of the settlement plate or among kelp treatments (Figure 3.5, Table 3.5). The number of settlers of *E. pilosa* tended to be greater at the shallow than deeper depths, however this difference was not significant at our more conservative $\alpha = 0.01$ (Figure 3.5, Table 3.5). The presence of kelp significantly affected settlement of *Cryptosula pallasiana*, but this effect depended on the position of the settlement plate. On bottom plates, the number of settlers was greater in the absence of kelp than within the kelp bed, but there was no difference in the number of settlers on top plates between within and outside kelp beds (Figure 3.5, Table 3.5). (For results using using zero-inflated negative binomial models, see Table A.2).

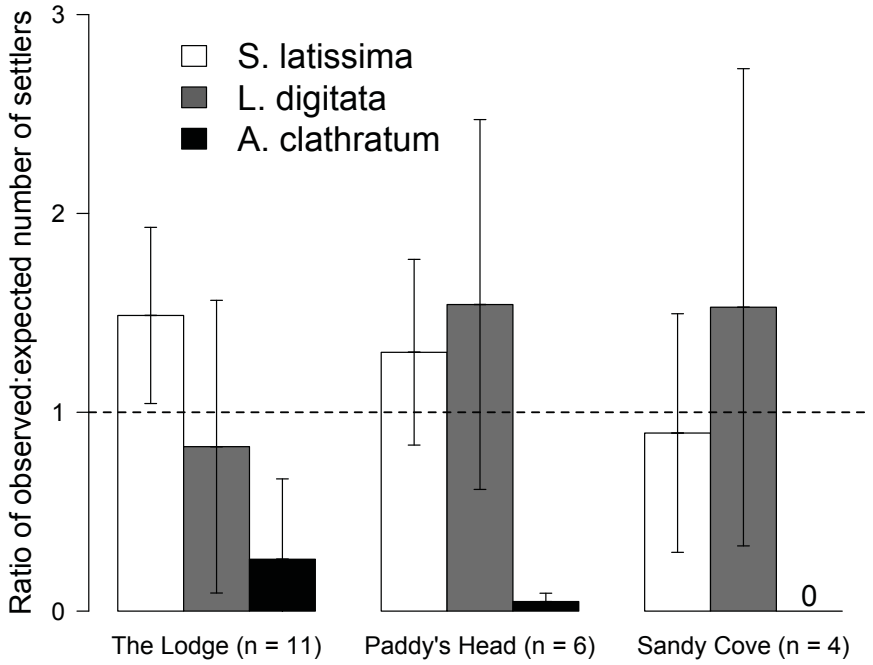

Figure 3.2 The ratio (mean ± SD) of observed versus expected number of settlers of *Membranipora membranacea* on the 3 most abundant kelp species in Nova Scotia collected at 3 sites and across 2-3 depths per site (4 and 8 m at Sandy Cove; 4, 8 and 12 m at The Lodge and Paddy's Head) approximately every 6 weeks from Jun 2012 to Aug 2013. A ratio of 1 (dotted line) indicates the observed number of settlers is equal to the expected number of settlers based on the available surface area of the kelp species; ratios <1 indicate fewer settlers were observed than expected, and ratios >1 indicate more settlers were observed than expected. Zero indicates no settlement of *M. membranacea* on *A. clathratum* at Sandy Cove. For each site, only date and depth combinations included in goodness of fit and binomial analyses are shown (see 3.3 Methods for details)

Table 3.3 Results of a) binomial sign tests of the difference between the observed and expected number of settlers, and b) one-sample *t*-test comparing the ratio of the observed versus the expected number of settlers to a value of 1 for each kelp substrate. For each site, only date and depth combinations where all 3 kelp species were present and the number of settlers of *M. membranacea* exceeded 25 ($n > 25$) are included. Significant *p*-values shown in bold ($\alpha = 0.05$)

a)	Number of times preferred	n	*p*	b)	$t_{(df)}$	*p*
The Lodge						
S. latissima	9	11	0.065		$3.65_{(10)}$	**0.004**
L. digitata	4	11	0.549		$-0.780_{(10)}$	0.454
A. clathratum	1	11	**0.012**		$-6.36_{(10)}$	**<0.0001**
Paddy's Head						
S. latissima	4	6	0.688		$1.58_{(5)}$	0.175
L. digitata	4	6	0.688		$1.43_{(5)}$	0.213
A. clathratum	0	6	**0.031**		$-56.1_{(5)}$	**<0.0001**
Sandy Cove						
S. latissima	2	4	1.00		$-0.348_{(3)}$	0.751
L. digitata	2	4	1.00		$0.882_{(3)}$	0.443
A. clathratum	0	4	0.125		NA	NA

Note: for *A. clathratum* at The Lodge only, the ratio of observed versus expected number of settlers was log(x+0.01)-transformed to fit the normal distribution (Shapiro-Wilk test, $p = 0.108$). NA indicates no settlement of *M. membranacea* on *A. clathratum* at Sandy Cove

Table 3.4 Laboratory settlement preference experiments. Results of 2-tailed independent samples *t*-tests comparing the number of settlers of *Membranipora membranacea* between choice and no choice treatments for paired combinations of kelp substrates. Beakers in which no settlement occurred are not included in the analysis. Mean differences between choice and no choice treatments and significance statistics are given. Significant *p*-values shown in bold ($\alpha = 0.05$)

Treatment	Alga	Mean Difference	$t_{(df)}$	*p*	
SL/LD	*SL*	-2.19	$-2.64_{(12)}$	**0.022**	**NC > C**
	LD	-0.75	$-0.577_{(8)}$	0.580	
SL/AC	*SL*	-1.40	$-1.64_{(14)}$	0.123	
	AC	0.11	$0.060_{(9)}$	0.954	
LD/AC	*LD*	0.09	$0.049_{(11)}$	0.962	
	AC	-0.31	$-0.362_{(10)}$	0.725	

Note: SL: *Saccharina latissima*, LD: *Laminaria digitata*, AC: *Agarum clathratum*

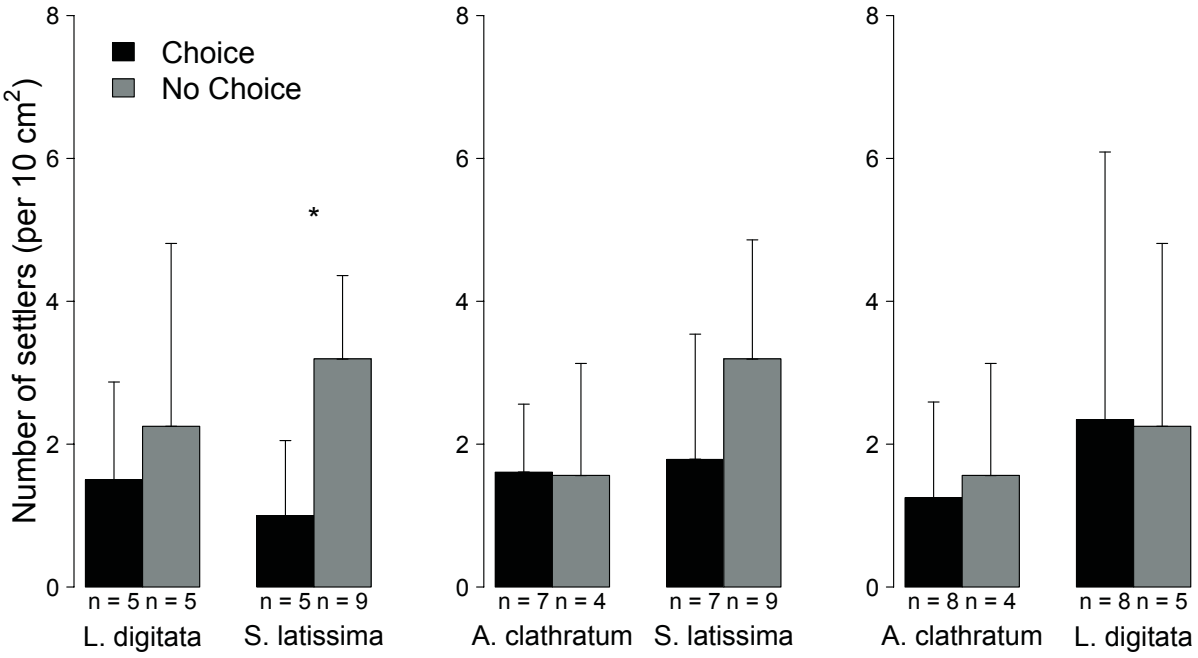

Figure 3.3 Laboratory settlement preference experiments. Settlement of larvae of *Membranipora membranacea* (mean ± SD) in 'choice' compared to 'no choice' treatments for all paired combinations of the 3 most abundant kelp species in Nova Scotia. For each kelp species in each paired combination: if number of settlers in the 'choice' treatment > number of settlers in the 'no choice' treatment, species is preferred; if number of settlers in the 'choice' treatment < number of settlers in the 'no choice' treatment, species is less preferred compared to the alternative; and if number of settlers in the 'choice' treatment = number of settlers in the 'no choice' treatment, species is equally preferable to the alternative. Data are pooled over 8 trials. * indicates a significant difference (at $\alpha = 0.05$) detected by 2-tailed independent samples *t*-tests (Table 3.4, see 3.3 Methods for details)

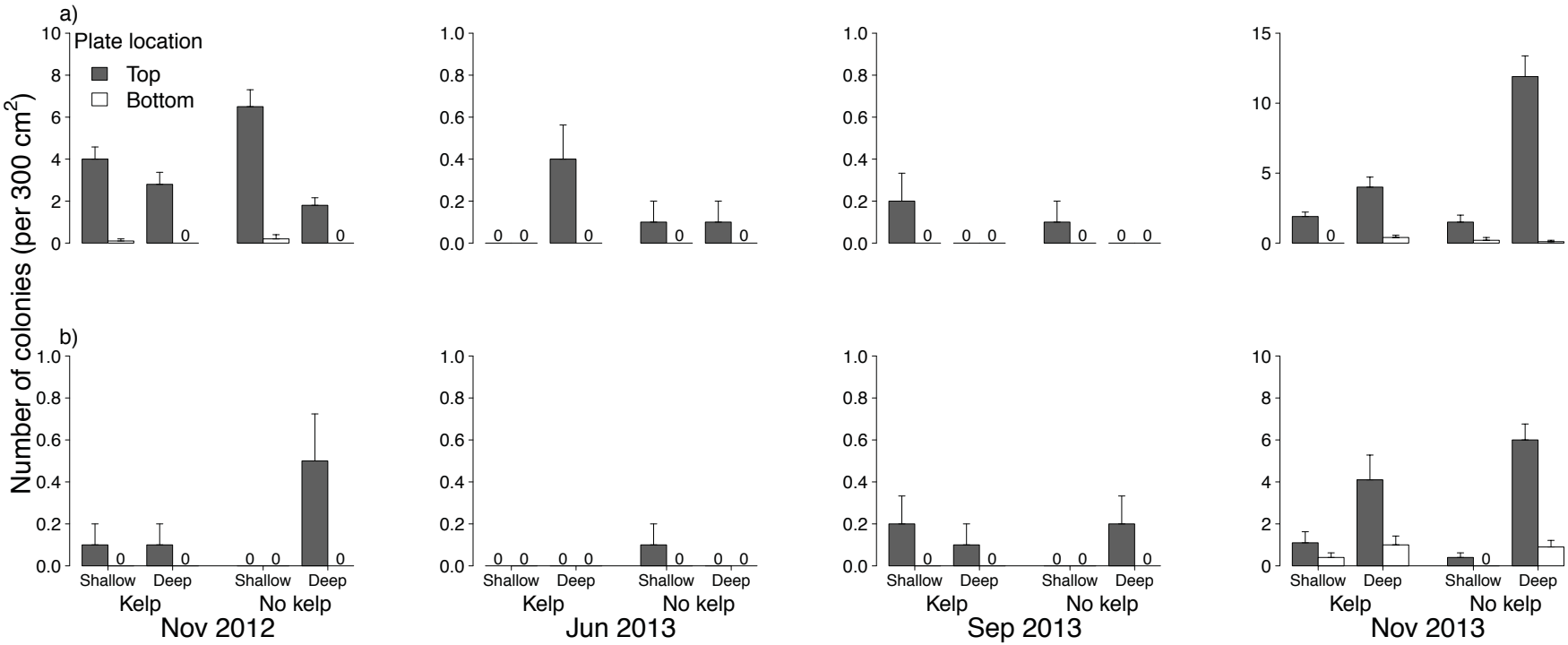

Figure 3.4 Settlement of *Membranipora membranacea* in the presence and absence of understory kelp canopies at a) The Lodge at 8 m (shallow) and 12 m (deep) and b) Sandy Cove at 4 m (shallow) and 8 m (deep) from Nov 2012 to Nov 2013. Data are the mean number of colonies (± SE) per 300 cm^2 settlement plate (n = 10 per treatment combination, see 3.3 Methods for details). Zeros indicate no settlement of *M. membranacea* over the time interval between deployment and collection of settlement plates. Note the difference in scale of the y-axes

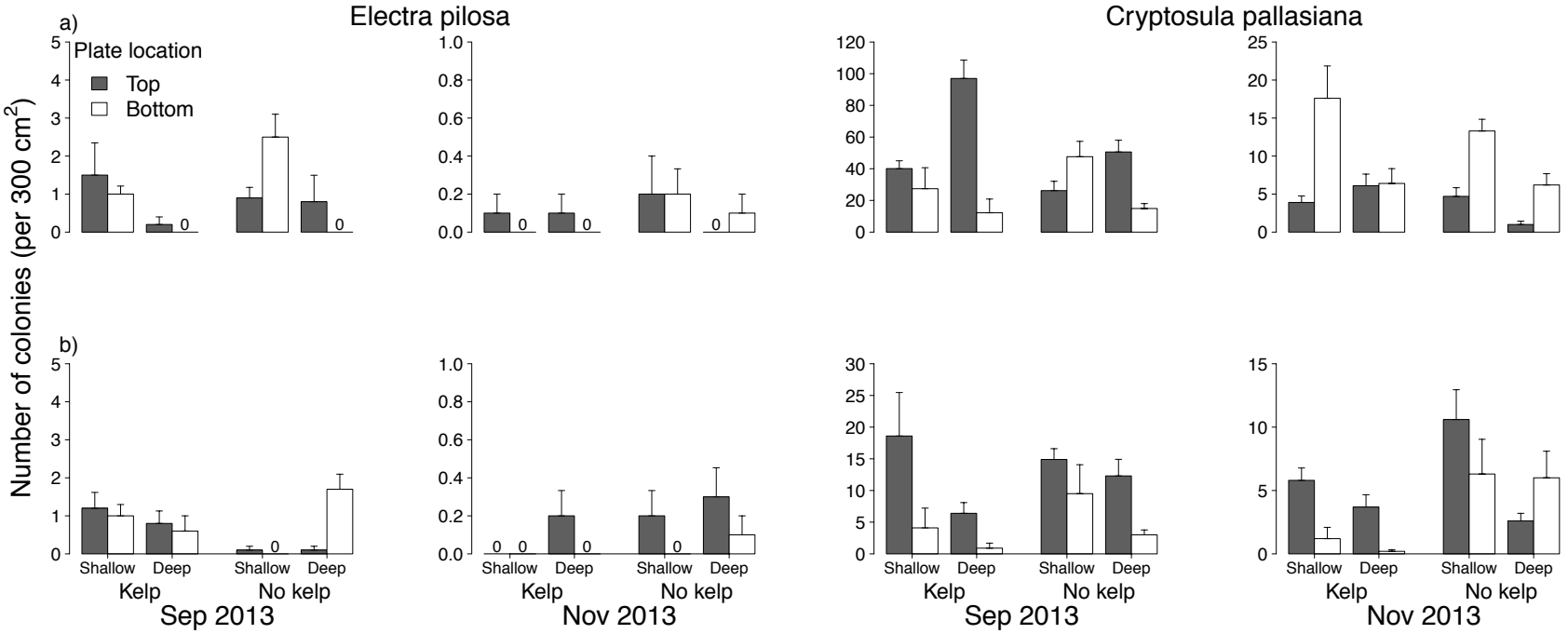

Figure 3.5 Settlement of *Electra pilosa* and *Cryptosula pallasiana* in the presence and absence of understory kelp canopies at a) The Lodge at 8 m (shallow) and 12 m (deep) and b) Sandy Cove at 4 m (shallow) and 8 m (deep) in Sep 2013 and Nov 2013. Data are the mean number of colonies (± SE) per 300 cm^2 settlement plate (n = 10 per treatment combination, see 3.3 Methods for details). Zeros indicate no settlement of *E. pilosa* over the time interval between deployment and collection of settlement plate. Note the difference in scale of the y-axes

Table 3.5 Results of mixed effects models examining the fixed effects of treatment (within kelp bed, outside kelp bed), position (top plate, bottom plate), and depth (shallow, deep), and random effects of site, date, and collector (nested within site) on settlement of invasive (*Membranipora membranacea*) and native (*Electra pilosa*, *Cryptosula pallasiana*) bryozoan larvae. Number of colonies was log(x+0.01)-transformed to better approximate a normal distribution. Significant *p*-values are shown in bold (*M. membranacea* and *E. pilosa*: $\alpha = 0.01$, *C. pallasiana*: $\alpha = 0.05$). See 3.3 Methods for specific sampling dates

	$\chi^2_{(df)}$	*p*	Tukey's HSD
Membranipora membranacea			
Fixed effects			
Treatment	$0.048_{(1)}$	0.827	
Position	$136_{(1)}$	**<0.0001**	Top > Bottom
Depth	$14.8_{(1)}$	**0.0001**	Deep > Shallow
Treatment x position	$0.361_{(1)}$	0.548	
Treatment x depth	$1.31_{(1)}$	0.253	
Position x depth	$3.04_{(1)}$	0.081	
Treatment x position x depth	$1.48_{(1)}$	0.223	
Random effects			
Site	$22.8_{(1)}$	**<0.0001**	
Date	$182_{(1)}$	**<0.0001**	
Collector	$0.00_{(1)}$	1.00	
Electra pilosa			
Fixed effects			
Treatment	$0.005_{(1)}$	0.946	
Position	$0.153_{(1)}$	0.696	
Depth	$5.56_{(1)}$	0.018	
Treatment x position	$2.04_{(1)}$	0.154	
Treatment x depth	$1.09_{(1)}$	0.292	
Position x depth	$0.645_{(1)}$	0.422	
Treatment x position x depth	$2.33_{11)}$	0.129	
Random effects			
Site	$0.00_{(1)}$	1.00	
Date	$36.2_{(1)}$	**<0.0001**	
Collector	$1.98_{(1)}$	0.160	
Cryptosula pallasiana			
Fixed effects			
Treatment	$19.4_{(1)}$	**<0.0001**	Top: Kelp = No Kelp Bottom: No Kelp > Kelp
Position	$23.4_{(1)}$	**<0.0001**	Kelp: Top > Bottom No Kelp: Top = Bottom
Depth	$14.0_{(1)}$	**0.0002**	Shallow > Deep
Treatment x position	27.2	**<0.0001**	
Treatment x depth	$0.164_{(1)}$	0.685	
Position x depth	$0.832_{(1)}$	0.362	
Treatment x position x depth	$0.808_{(1)}$	0.369	

	$\chi^2_{(df)}$	*p*	Tukey's HSD
Random effects			
Site	$33.3_{(1)}$	**<0.0001**	
Date	$16.7_{(1)}$	**<0.0001**	
Collector	$0.038_{(1)}$	0.846	

3.5 Discussion

3.5.1 Settlement by larvae of *Membranipora membranacea* in invaded habitat

In the field, larvae of *M. membranacea* settled preferentially on the kelps *Saccharina latissima* and *Laminaria digitata* over *Agarum clathratum*. This preference was consistent across three sites that differed substantially with respect to the bathymetric distribution and relative abundance of the 3 kelp species (Figures B.1-B.3). *Agarum* spp. have high phlorotannin concentrations (Steinberg 1985) which may inhibit fouling by marine epiphytes (reviewed by Amsler & Fairhead 2006). The absence of *M. membranacea* on *Agarum fimbriatum* in its native range off the coast of British Columbia has been attributed to toxicity of polyphenolic compounds (Durante & Chia 1991). However, blades of *A. clathratum* and *S. latissima* in the northeast Pacific contain similar phlorotannin content (Dubois & Iken 2012).

The distribution of settlers of *M. membranacea* in the field, where there were significantly fewer settlers on *A. clathratum* than expected, could not be explained by the results of laboratory settlement preference experiments in which neither *S. latissima* nor *L. digitata* were preferred over *A. clathratum*. Discordance between species distributions in the field and selective settlement in the laboratory can result from aspects of the natural environment that may act to obscure preference, such as the relative availability, and consequently the probability of encountering, preferred substrate. For example, larvae of *Bugula neritina* prefer to settle on surfaces coated with primary biofilms, but will settle in similar abundance on clean surfaces when their preferred substrate is not available (Miller et al. 1948). Although at The Lodge and Paddy's Head, kelp substrates were not necessarily sampled in proportion to their relative abundance in the field in our study, similar patterns in the distribution of settlers among algal substrates were observed at multiple sites which varied in both relative and absolute abundances of kelps. Alternatively, laboratory experiments can exaggerate preference for a particular substrate

beyond what is observed in the field, since the chemical cues released by algal substrates and the probability of a larva encountering a preferred substrate are often artificially amplified within the confines of the laboratory (Moore 1975). However, there was no effect of the area of algae used in settlement experiments, incorporating both strength of the cue and the probability of encounter, on the rate of settlement of *M. membranacea* larvae.

Interestingly, there was no effect of the presence of understory kelp beds on settlement of *M. membranacea* in the field. Nor was there any indication that passive filtration by the kelp canopy prevents larvae of *M. membranacea* from settling on under-canopy substrates, since the number of settlers was consistently greater on the top plates both within and outside of kelp beds. For comparison, the number of settlers of *Cryptosula pallasiana* was greater on top plates than on bottom plates within kelp beds, but did not differ between top and bottom plates outside of kelp beds, suggesting passive filtration of *C. pallasiana* larvae. Different patterns of settlement observed for the two other species of bryozoans examined, *Electra pilosa* and *C. pallasiana*, substantiate that these results are indicative of settlement characteristics unique to *M. membranacea* rather than being an artifact of the experimental manipulation.

Considering our results in their totality, we suggest that at scales larger than individual algal blades, settlement of *M. membranacea* larvae is not selective with respect to algal substrate in its invaded habitat. Instead, increased abundance of settlers on some kelp species may be the result of larval behavioral responses that are not directly related to algal substrate. Kelp beds create areas of weak circulation beneath the canopy and reduce mass transport at the substratum (Eckman et al. 1989). In previous field experiments in Washington, USA, patterns of settlement of *M. membranacea* were consistent with passive transport of larvae, with settlement being greater on plates in higher flow environments outside of kelp beds than within kelp beds (Duggins et al. 1990). However, settlement plates deployed by Duggins et al. (1990) were all located within 15 cm of the substratum, limiting measurements of settlement within kelp beds to below the kelp canopy without accounting for settlement of *M. membranacea* larvae onto overlying kelp fronds. In our study, consistently higher rates of settlement at ~1 m above the substratum, regardless of the presence of kelp beds, suggest that competent larvae

may settle on the first substrate they encounter as they descend from the overlying water column. This is consistent with the distribution of settlers of *M. membranacea* among kelp substrates in the field, where blades of *A. clathratum* lie just above the substratum (within ~10 cm) while *S. latissima* and *L. digitata*, extend their blades above the substratum, often projecting up to 1 m into the water column.

Settlement by larvae of *M. membranacea* onto substrates extending into the water column may be in response to increasing flow and thus food particle flux (Cancino & Hughes 1987, but see Okamura 1985, 1988, 1992). *M. membranacea* can effectively capture particles at higher flow velocities compared to other bryozoans (Pratt 2008), possibly conferring *M. membranacea* a competition advantage. In addition to increased particle flux, elevation above the primary substratum may also reduce rates of sedimentation, which negatively affect settlement (Ryland 1960, Duggins et al. 1990), growth (Eckman & Duggins 1991, Genovese & Witman 1999), and survival (Bak & Engel 1979, Keough 1986) of benthic marine invertebrates.

3.5.2 Invasive potential and implications for persistence and spread

The apparent lack of preference by larvae of *M. membranacea* for specific algal substrates likely contributed to its successful introduction and rapid spread in the northwest Atlantic. Similarly, the recent introduction and spread of two widely invasive bryozoans in the Netherlands, Belgium, and France differed based on their level of substrate specificity (De Blauwe & Faasse 2001). The generalist invader *Tricellaria inopinata* is now widespread and abundant throughout the southwestern Netherlands, also occurring in Belgium and France, and is expected to continue to spread northward. In contrast, the more selective *Bugula simplex* was found in abundance at only one location in the Netherlands. Consequently, *B. simplex* is not considered an ecologically significant invasive species in the region, and its spread throughout the northeast Atlantic is predicted to be limited due its preference for specific substrate.

Generalist settlement behavior by larvae of *M. membranacea* is also consistent with observations that *M. membranacea* populations do not appear to be in decline in Nova Scotia, despite concurrent declines in kelp (D. Denley, pers obs). Lack of suitable substrate can act as a dispersal barrier limiting secondary spread of even the most well-

established invasives (e.g. Bohn et al. 2015). However, this is not likely to be the case for *M. membranacea* in the northwest Atlantic Ocean, given its capacity to settle and grow on alternative natural (e.g. *Fucus* spp., Yorke & Metaxas 2012) and artificial (e.g. settlement plates, this study) substrates. While *M. membranacea* seems to prefer habitat within the water column, the occurrence of reproductive colonies on *A. clathratum* and *F. evanescens* (Denley & Metaxas 2017a) suggests other substrates that provide even minimal extension above the primary substratum are sufficient for persistence of the population. It is possible that extensive barrens may slow the spread of *M. membranacea* in the northwest Atlantic, particularly within the Gulf of St. Lawrence (Himmelman et al. 1983, Dumont et al. 2004, Gagnon et al. 2004), since colonies have yet to be observed on bedrock or crustose coralline algae, at least in our region (D. Denley, pers obs). However, artificial structures that provide submerged surface area above the substratum for colonization, such as pontoons, buoys, navigational markers, and floating or subtidal aquaculture structures, may facilitate the introduction and spread of *M. membranacea* into regions where natural substrates are sparse (e.g. Simons et al. 2016).

The ability to successfully colonize artificial substrate is widespread among invasive epiphytes (Dafforn et al. 2009). There is evidence that *Membranipora* spp. may benefit from their association with kelp substrates by absorbing kelp exudates as an additional source of nutrients (Burgh & Frankboner 1978, Manríquez & Cancino 1996), although the magnitude of this benefit likely varies depending on particulate-food concentrations. Further investigation into the growth and fecundity of *M. membranacea* on natural versus artificial substrates would be helpful in determining the significance of algal substrate as a potential food source.

Lack of a strong preference for settling on specific substrates appears to be another life history characteristic exhibited by *M. membranacea* that is common of marine invasive species (e.g. De Blauwe & Faasse 2001, Creed & De Paula 2007, Ruis 2010, Lezzi et al. 2016). Our results suggest more generalist settlement behavior of *M. membranacea* larvae in Nova Scotia compared to within *M. membranacea*'s native range in the northeast Pacific (e.g. Berstein & Jung 1979, Yokiosha 1986, Stricker 1989, Matson et al. 2010) and northeast Atlantic (e.g. Ryland 1962). Based on our findings, *M. membranacea* provides a key example of how variation in life-history strategies can alter

population dynamics of non-indigenous species outside of their native habitats, leading to potentially significant and unforeseen effects on the invasive potential of introduced species.

CHAPTER 4

QUANTIFYING MORTALITY OF MODULAR ORGANISMS: A COMPARISON OF PARTIAL AND WHOLE-COLONY MORTALITY IN A COLONIAL BRYOZOAN[3]

4.1 Abstract

Comprehensive studies on the population dynamics of colonial organisms require estimates of mortality from the level of the individual module (partial mortality) to the entire colony (whole-colony mortality), as well as, determining the factors that affect mortality at each level of organization. However, accurate measurements of whole-colony and partial mortality of modular organisms can be difficult to obtain, and few studies involve concurrent measurements of modular and colony mortality. We implemented multiple approaches to measure whole-colony and partial mortality of modular species using the colonial bryozoan *Membranipora membranacea* as a model organism. *M. membranacea* is a cosmopolitan species and of particular ecological significance in the northwest Atlantic, where it is the dominant epiphyte on laminarian kelps and the main driver for the defoliation of kelp beds. Rates of whole-colony mortality were measured in the field 1) indirectly through repeated subsampling cohort analyses, and 2) directly by tagging colonies *in situ*. Partial mortality of colonies was measured 1) in the field by quantifying the proportion of degenerated zooids per colony, and 2) in the laboratory by monitoring loss of colony surface area during seasonal senescence of colonies over winter. Temporal patterns differed substantially between partial and whole-colony mortality, suggesting that factors affecting mortality of individual modules differ from those affecting whole colonies. Our study indicated that the accuracy of common methods for measuring mortality of modular organisms depends on the species-specific life-history characteristics. However, we suggest that rates and

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mechanisms of whole-colony and partial mortality can be most accurately quantified by revisiting tagged individuals *in situ* and recording loss of entire colonies (whole-colony mortality) and loss of living colony area (partial mortality) between successive sampling times. For *M. membranacea*, measuring whole-colony or partial mortality in isolation would overestimate important demographic rates, and underestimate the influence of temperature on mortality, respectively. This study demonstrates the need to include mortality measurements from the level of the individual module to the entire colony when quantifying population dynamics of colonial organisms.

4.2 Introduction

Population dynamics are quantified predominantly with respect to four demographic rates: reproduction, mortality, immigration and emigration. Of these, instantaneous mortality remains one of the most difficult population parameters to estimate (Hewitt & Hoenig 2005). Mortality rates can vary spatially (e.g. Bythell et al. 1993), inter-annually (e.g. Hughes & Jackson 1985) and seasonally (e.g. Johnson & Eggleston 2010), as well as among populations (e.g. Vetter 1988) and individuals within the same population (e.g. Russo et al. 2002). Mortality estimates through repeated sampling, including using cohort analysis and catch curve analysis, generally do not provide information on the regulatory mechanisms of change (Vetter 1988). For motile species, it is often difficult to distinguish between mortality and emigration (e.g. de Pontual et al. 2013), whereas, for sessile species, determining specific agents of mortality requires frequent monitoring (e.g. Bythell et al. 1993).

While demographic processes are well-defined for aclonal organisms (Cole 1954), the same concepts cannot be directly applied to clonal animals and plants (Harper & White 1974), for which growth occurs through the iteration of repeated semi-autonomous units or modules (Winston 2010). Modular construction of colonial invertebrates significantly complicates their population dynamics, as growth, reproduction, and mortality can occur and be environmentally influenced independently at the level of each individual module. The ability of individual modules to survive and reproduce alone or in small groups allows colonial organisms to survive substantial modular loss due to fragmentation or senescence. As a result, mortality of colonial organisms must be

considered at both the colony-wide level (from here on referred to as whole-colony mortality), as well as at the level of the individual modules (from here on referred to as partial mortality).

Whole-colony mortality is important for measuring changes in population density and assessing patterns of species distribution and abundance (e.g. Rylaarsdam 1983, Keough 1986, Saunders et al. 2010). Estimates of whole-colony mortality can resolve the relative importance of other demographic processes, such as recruitment, in determining population size (e.g. Hughes 1990) or non-random patterns in population density (e.g. Keough & Downes 1982). However, relying solely on measurements of whole-colony mortality can often mask important demographic processes not necessarily reflected in changes of population density, such as colony fission, fusion, chronic tissue loss, and ramet turnover, and seemingly stable populations may in fact be highly dynamic (Cook 1983, Hughes & Jackson 1985, Bythell et al. 1993). Partial mortality is particularly important in estimating population parameters such as fecundity, which is related to the survival and reproduction of individual modules rather than colonies (Tuomi & Vuorisalo 1989). Moreover, partial mortality at the modular-level, either as a result of intrinsic colony senescence or damage to colonies from external agents, can often account for a larger proportion of tissue loss than whole-colony mortality (Hughes & Jackson 1985, Baird & Marshall 2002, but see also Bak & Luckhurst 1980).

Measuring rates and mechanisms of whole-colony and partial mortality can be challenging. Rates of whole-colony and partial mortality may be strongly dependent on colony size (Hughes 1984, Hughes & Jackson 1985). Because partial mortality can promote fragmentation of large colonies, field-derived rates of size-specific whole-colony mortality are often underestimated for small colonies and overestimated for large colonies (Hughes & Jackson 1980, Hughes 1984). Similarly, while partial mortality typically results in reductions in colony size, measuring partial mortality as negative colony growth (e.g Jackson & Winston 1981, Harvell et al. 1990) likely underestimates rates of partial mortality. This is particularly true for fast growing species, since net positive growth can still occur provided the rate of module production exceeds that of modular loss (Cook 1983). Partial mortality may also occur more frequently than is

evident from the number of colonies exhibiting signs of damage, since a single colony can be affected by more than one damaging event (Bythell et al. 1993).

Because of the difficulty in accurately measuring both whole-colony and partial mortality of colonial organisms, and in spite of the long-established recognition of the methodological shortcomings, many studies still rely on the use of size-class transition probabilities (e.g. Hughes 1990, Turon et al. 1998) or measurements of negative growth (shrinkage) (e.g. Harvell et al. 1990, Linacre & Keough 2003). Others consider measurements of either whole-colony (e.g. Rylaarsdam 1983, Keough 1986, Hughes & Connell 1987, Barnes & Lehane 2001) or partial mortality (e.g. Nugues & Roberts 2003) in isolation. Using these estimates as a best approximation may be acceptable in many cases; however, a conceptual understanding of the demographic properties of colonial organisms requires accurate measurements of both types of mortality.

4.2.1 The model system

We used *Membranipora membranacea,* an invasive colonial bryozoan with a complex life cycle, as a model organism to obtain concurrent estimates of whole-colony and partial mortality. *M. membranacea* is widely distributed throughout temperate oceans, and is an ecologically significant invasive species in the northwest Atlantic (Lambert et al. 1992, Scheibling & Gagnon 2009). Consequently, its population dynamics have been well studied, both in Nova Scotia and circumglobally (see Saunders & Metaxas 2007, 2008, 2009b, Denley et al. 2014). Unlike most corals (e.g. Pisapia & Prachett 2014), the sheet-like growth of *M. membranacea* makes it possible to visually measure partial mortality as a reduction in two-dimensional colony surface area, allowing the full extent of partial mortality to be quantified from a single photograph.

The seasonal progression of the bryozoan's annual life cycle does not occur across the entire colony. Individual colonies of *M. membranacea* are composed of zooids (semi-autonomous modular units), the polypides of which may be in one of four stages of a cycle: differentiating, active, reproductive, or degenerated. While in many species of bryozoans, polypides commonly undergo multiple cycles of differentiation, activity, and degeneration within the same zooid (e.g. Bayer & Todd 1997), in *M. membranacea* zooids with degenerated polypides are in effect "dead". Partial mortality of *M.*

membranacea primarily involves the degeneration of individual zooids within a colony while the colony as a whole persists. Whole-colony mortality occurs when an entire colony is lost, either as a result of external mechanisms, such as predation, overgrowth, or physical disturbance, or due to intrinsic senescence of the colony itself. External mechanisms of whole-colony mortality for *M. membranacea* in Nova Scotia are thought to be primarily related to substrate dynamics, particularly breakage and erosion of kelp blades, because predation and interspecific competition have little impact (Chapman et al. 2002, Yorke & Metaxas 2011).

4.2.2 Objectives

This study has four objectives: 1) to estimate rates of whole-colony mortality of *M. membranacea* in the field using a cohort analysis method by periodically sampling colonies over one complete cycle of *M. membranacea*'s annual life cycle; 2) to measure partial mortality of colonies in the field by quantifying the proportion of degenerated zooids per colony; 3) to quantify rates of whole-colony mortality and the relative contribution of external (kelp blade breakage and erosion) versus intrinsic (colony senescence) mechanisms to whole-colony mortality *in situ* by tagging and monitoring individual colonies of *M. membranacea* in the field; and 4) to estimate intrinsic rates of whole-colony and partial mortality during seasonal senescence of colonies over winter by monitoring colonies on settlement plates in the laboratory. Growth of *M. membranacea* colonies is positively related to temperature and colony size both in the laboratory and *in situ* (Saunders & Metaxas 2009a). Interannual variation in percent cover of *M. membranacea* colonies on kelps has been shown to be significantly related to the thermal integral over the preceding three months (Scheibling & Gagnon 2009). Although patterns have yet to be quantified for *M. membranacea*, polypide cycling has been shown to be distinctly seasonal in other bryozoans, possibly in response to variations in temperature (Barnes & Clarke 1998). For these reasons, rates of whole-colony and partial mortality both *in situ* (objectives 1 and 2) and in the laboratory (objective 4) were examined with respect to colony size and temperature.

These objectives allow us to differentiate measurements of mortality in colonial organisms at increasing scales, from the level of the individual zooid to the entire colony,

and determine how mortality rates at each level are affected by intrinsic (organismal) and environmental characteristics. The results of this study add to the growing body of research into the population dynamics of modular organisms, and can be used to identify patterns and processes of whole-colony and partial mortality consistent among colonial species.

4.3 Methods

4.3.1 Collection of *M. membranacea* colonies on kelp

We collected colonies of *M. membranacea* on the kelps *Saccharina latissima, Laminaria digitata,* and *Agarum clathratum* using SCUBA at three sites on the southwestern shore of Nova Scotia, Canada [The Lodge (44°33'3"N, 64° 01' 9" W) on the western shore of St. Margarets Bay, Paddy's Head (44°31'6"N, 63°57'2"W) on the eastern shore near the mouth of St. Margarets Bay, and Sandy Cove (44° 27' 6" N, 63° 42' 4" W) in Terence Bay, 20 km to the northeast of St. Margarets Bay] approximately every six weeks from 18 June 2012 to 2 August 2013 (Table 4.1, Appendix B.1.1, B.1.2). We measured temperature at each permanently marked sampling depth at each site throughout this study using HOBO® pendant loggers.

4.3.2 Estimating kelp biomass

For each kelp species at each site, we used kelp abundance (m^{-2}) measured during each sampling time (Appendix B.1.2) to convert individual biomass measurements (kg) to biomass per unit area seabed (kg m^{-2}) by multiplying the depth- and species-specific average biomass at each site by the corresponding depth- and species-specific densities for each sampling time (Appendix B.1.3, Figures B.1 – B.3).

4.3.3 Quantifying whole-colony and partial mortality of *M. membranacea* colonies on kelps

We categorized colonies of *M. membranacea* into one of five size classes (< 1 cm, 1-3 cm, 3-6 cm, 6-8 cm, >8 cm diameter) selected to to correspond roughly with two-week growth intervals, thereby encompassing the range in colony diameter while still maintaining sufficiently large sample sizes within each category. We determined the

number and size class of colonies on all collected algae during each sampling period, and calculated the rate of whole-colony mortality as instantaneous mortality rate (d^{-1}) using a cohort analysis model

[Eq. 4.1] $N_t = N_o e^{-Mt}$

where N_t is the number of colonies (m^{-2} seabed) of a given size class surviving to time t, N_o is the initial number of colonies (m^{-2} seabed) of a given size class, M is instantaneous mortality rate (d^{-1}), and t is the specified time interval (d) (after Ricker 1975). We modified this equation slightly to account for colony growth:

[Eq. 4.2] $M = [\ln (N_s/\{N_{(s-GR \bullet t)}\}]/-t$

where N_s is the number of colonies (m^{-2} seabed) in size class s observed at a given sampling date, t is the time between consecutive sampling dates (d), $N_{(s-GR \bullet t)}$ is the number of colonies (m^{-2} seabed) observed at the previous sampling date that are expected to have grown into size class s over the time interval t, and GR is the size- and temperature-dependent growth rate for *M. membranacea* colonies in the field (from Saunders & Metaxas 2009a):

[Eq. 4.3] $\log(GR) = -1.665 + 0.719 \bullet \log(S) + 0.072(T)$

where S is colony size (mm) and T is temperature (°C). Small fragments of colonies that were obviously the remains of once larger colonies were not included in calculations of whole-colony mortality rate. For The Lodge and Paddy's Head, we converted the abundance of colonies per individual kelp to abundance per m^2 seabed by multiplying the former by kelp density per m^2 seabed at the particular depth for each sampling time. For Sandy Cove, abundance of colonies was measured in 0.5-m^2 quadrats, and no conversion was necessary.

Table 4.1 Details of field and laboratory experiments used in this study to measure partial and whole-colony mortality of *Membranipora membranacea*

Measurement	**Effect**	**Substrate**	**Location**	**Duration**	**Analysis**	**Results**
Whole-colony mortality rate (d^{-1})	Depth, Sampling time	*S. latissima* *L. digitata* *A. clathratum*	TL SC PH	18 Jun 2012 – 2 Aug 2013	LMM	Fig 4.2
	Loss of kelp biomass	*S. latissima* *L. digitata* *A. clathratum*	TL SC PH	18 Jun 2012 – 2 Aug 2013	Linear regression	Table 4.2
	Temperature	*S. latissima* *L. digitata* *A. clathratum*	TL SC PH	18 Jun 2012 – 2 Aug 2013	Linear regression	Table 4.3
	Colony size	*S. latissima* *L. digitata* *A. clathratum*	TL SC PH	18 Jun 2012 – 2 Aug 2013	ANOVA	Fig 4.3
Whole-colony mortality (% of colonies)	Agent of mortality	*S. latissima*	TL SC	25 Jul – 7 Oct 2014 7 Oct – 16 Dec 2014	NA	Fig 4.5
Partial mortality (% degenerated zooids per colony)	Depth, Sampling time	*S. latissima* *L. digitata* *A. clathratum*	TL SC PH	18 Jun 2012 – 2 Aug 2013	GLMM	Fig 4.1
	Loss of kelp biomass	*S. latissima* *L. digitata* *A. clathratum*	TL SC PH	18 Jun 2012 – 2 Aug 2013	Linear regression	Table 4.2

Measurement	Effect	Substrate	Location	Duration	Analysis	Results
Partial mortality (% degenerated zooids per colony)	Temperature	*S. latissima* *L. digitata* *A. clathratum*	TL SC PH	18 Jun 2012 – 2 Aug 2013	Linear regression	Table 4.3
Frequency of occurrence of partial mortality (% of colonies)	Colony size	*S. latissima* *L. digitata* *A. clathratum*	TL SC PH	18 Jun 2012 – 2 Aug 2013	Chi-square test of homogeneity	Fig 4.4a
Level of partial mortality (median % degenerated zooids per colony)	Colony size	*S. latissima* *L. digitata* *A. clathratum*	TL SC PH	18 Jun 2012 – 2 Aug 2013	NA	Fig 4.4b
Relative senescence (% decrease in colony surface area)	Temperature, Level of initial partial mortality	Artificial	Laboratory	9 Jan 2013 – 12 Apr 2013	LMM ANOVA	Table 4.4
Rate of senescence ($cm^2 d^{-1}$)	Initial colony size	Artificial	Laboratory	9 Jan 2013 – 14 Mar 2013	Linear regression	Fig 4.6

Notes: Location codes are: TL, The Lodge; SC, Sandy Cove; PH, Paddy's Head

For each kelp species and each sampling time, we randomly selected one colony of each size class (when available) per kelp blade and classified a subset of zooids within each colony by polypide cycle using a Nikon SMZ1500 stereomicroscope (Appendix B.1.4) We then calculated partial colony mortality as the proportion of zooids per colony that had degenerated by dividing the number of degenerated zooids in a colony by the total number of zooids classified for that colony.

4.3.4 Data analysis: temporal variation in partial and whole-colony mortality of *M. membranacea*

We examined the effects of site, depth and sampling time on (1) instantaneous rate of whole-colony mortality and (2) the percentage of degenerated zooids per colony, using linear mixed models (LMM) and generalized linear mixed models (GLMM) with binomial error distribution, respectively (Table 4.1, Appendix B.2.1). Model selection was achieved using likelihood ratio tests. P-values for model selection were obtained using the chi-square distribution (Wilks 1938). Post hoc comparisons were conducted with Tukey's tests.

We examined the relationships of whole-colony mortality and of the percentage of degenerated zooids per colony with loss of kelp biomass (averaged across all kelp species) at each site using simple linear regressions (Table 4.1, Appendix B.2.1).

4.3.5 Data analysis: effects of temperature and colony size on partial and whole-colony mortality of *M. membranacea in situ*

We examined the relationship of instantaneous whole-colony mortality rate and the percentage of degenerated zooids with monthly-averaged temperature over the month preceding each sampling time at each site, using simple linear regressions for each size class of colonies (Table 4.1).

To examine the effect of colony size explicitly on whole-colony mortality of colonies in the field, the effect of colony size (fixed factor, five levels: < 1 cm, 1-3 cm, 3-6 cm, 6-8 cm, >8 cm diameter) on the instantaneous rate of whole-colony mortality was determined using one-way ANOVA for unequal replication (Table 4.1, Appendix B.2.2). Significant differences between means as detected by ANOVA were further examined with Tukey's HSD post-hoc tests. To examine the effect of colony size on partial colony

mortality, we calculated the frequency of occurrence of partial mortality, as well as the median level of partial mortality (% degenerated zooids), for each size class of colony collected over the seasonal cycle (Table 4.1). Differences in the frequency of occurrence of partial mortality among colonies of different size classes were tested using a chi-square test of homogeneity.

4.3.6 Quantifying whole-colony mortality of tagged *M. membranacea* colonies on kelps

We selected and labeled individuals of *S. latissima* at The Lodge (n = 32) and Sandy Cove (n = 20) by threading labeled cable ties through their holdfasts. We tagged one newly settled colony of *M. membranacea* on the basal half of each labeled blade by inserting cable ties through the kelp blade approximately 2-3 cm basally from the colony. We photographed colonies approximately every 1.5 to 2 weeks (Table 4.1), and recorded whole colony mortality as the loss of the entire colony by one of three agents of mortality: breakage of the entire kelp blade, erosion of the distal end of the kelp blade, and colony senescence. Breakage was defined as breakage of the kelp blade below the basal meristem resulting in loss of the entire blade, and was characterized by the remains of the labeled stipe and holdfast. Distal erosion was defined as erosion of the tagged colony off the distal end of the blade, and was characterized by the remains of the cable tie at the distal edge of the blade and the loss of the associated colony. Colony senescence was defined as intrinsic colony mortality and was characterized as the gradual loss of the entire colony leaving the underlying kelp tissue intact.

Following each sampling event, we analyzed photographs of tagged colonies and/or the remaining stipe or eroded kelp blade using ImageJ and determined the percentage of tagged colonies which had experienced whole-colony mortality, as well as the specific agent of whole-colony mortality. We calculated instantaneous rate of whole colony mortality for tagged colonies for each approximately 1.5-week sampling event using equation 4.1, where N_t is the number of live colonies observed during the sampling time; and N_o is the number of live colonies observed during the previous sampling time.

4.3.7 Quantifying partial mortality of *M. membranacea* colonies on settlement plates in the laboratory

Colonies collected on settlement plates (Appendix B.1.5) were acclimated in aquaria with running ambient seawater from collection on 20 November 2012 to 9 January 2013. Colonies of different initial mortality as quantified on 9 January 2013 (control colonies (< 25% senesced), 25% senesced, 50% senesced, and 75% senesced, Appendix B.1.5) were exposed to one of three temperature treatments in flow-through seawater tables: ambient seawater (~4°C), ambient +3°C (~7-8°C), and ambient +9°C (~14°C), from 9 January 2013 to 12 Apil 2013. During this time, colonies were photographed weekly to monitor partial mortality in the form of colony senescence (Table 4.1, Appendix B.1.5).

4.3.8 Data analysis: effects of temperature, colony size, and level of initial partial mortality on senescence of *M. membranacea* in the laboratory

We calculated relative senescence of colonies after 31, 62 and 92 days exposure to the same temperature treatments as a percentage of the initial colony size on 09 January 2013. Although plates containing colonies were photographed weekly, we chose to pool weekly measurements into monthly intervals, as weekly rates of colony senescence were often low (maximum rate of colony senescence ~ 0.5% wk^{-1}).

Linear mixed models (LMM) with separate intercepts for the random effect of plate were used to examine the effects of temperature (fixed factor, three levels: ambient, ambient +3°C, ambient +9°C) and initial partial mortality (fixed factor, four levels: control (< 25%), 25%, 50%, 75%) on the relative senescence of colonies after 30, 62, and 92 days in the laboratory (Table 4.1, Appendix B.2.3). Fixed effects were further investigated at monthly intervals using two-way ANOVAs for unequal replication, and Tukey's HSD post-hoc tests (Table 4.1, Appendix B.2.3).

The relationship between initial colony size (cm^2) and the rate of colony senescence ($cm^2\ d^{-1}$) over 62 days was examined using simple linear regressions for each combination of temperature and initial partial mortality (Table 4.1). The rate of colony senescence over 62 days was calculated as:

[Eq. 4.4] $R = (SA_{62d} - SA_{initial})/62$

where R is the rate of colony senescence ($cm^2\ d^{-1}$), SA_{62d} is colony surface area (cm^2) following 62 days exposure to temperature treatments, and $SA_{initial}$ is colony surface area (cm^2) on 09 January 2013 following colony senescence since 20 Nov 2012.

We chose to examine the rate of colony senescence at 62 days because, at that time, percent loss of surface area leveled off and rates of further senescence were negligible (average rate of senescence from 62 to 92 days ~ 0.004 $cm^2\ d^{-1}$; Figure B.4). Statistical analyses were conducted using R (version 3.0.2, the R Foundation for Statistical Computing 2013).

4.4 Results

4.4.1 Temporal variation in partial and whole-colony mortality of *M. membranacea*

Neither partial nor whole-colony mortality varied significantly among sites (standard deviation due to random effect of site = 0.00). Although there were significant interacting effects of sampling time and depth on the percentage of degenerated zooids ($\chi^2_{10} = 19.7, p < 0.032$), the percentage of degenerated zooids was greater in March 2013 than in July-August 2012 and September 2012 at all depths (Figure 4.1; Tukey's HSD test, $p < 0.001 – 0.008$). There was no significant interaction between sampling time and depth for instantaneous whole-colony mortality rate ($\chi^2_{10} = 6.97, p = 0.728$). Whole-colony mortality was greater in July-August 2012 than in March 2013 across all depths (Figure 4.2; $\chi^2_5 = 18.0, p = 0.003$ Tukey's HSD test, $p < 0.001$), and at 4 m than 8 m depth for all sampling times (Figure 4.2; $\chi^2_2 = 6.51, p = 0.039$ Tukey's HSD test, $p = 0.035$).

There was a significant positive relationship between whole-colony mortality rate and loss of kelp biomass at two of the three sites, as well as for all study sites combined (Table 4.2, Figure B.5). However, partial mortality (percentage of degenerated zooids)

was not significantly related to loss of kelp biomass at any study site (Table 4.2, Figure B.5).

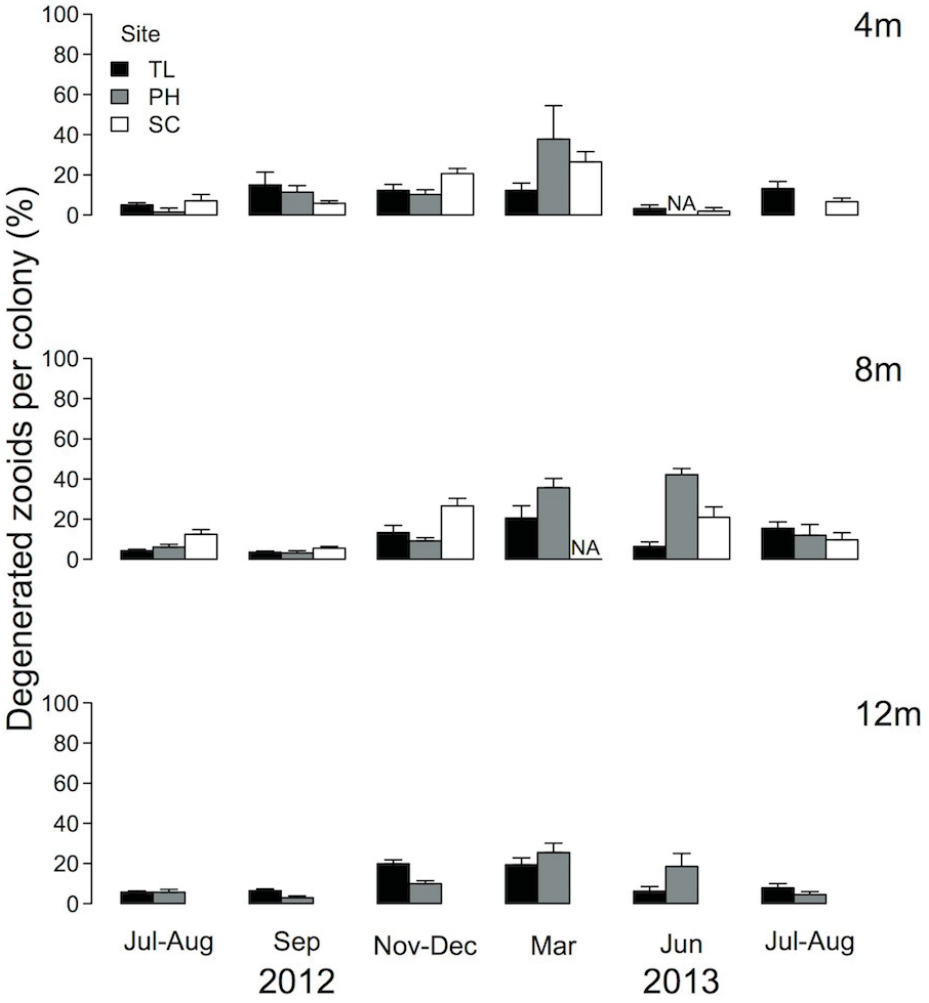

Figure 4.1 Percentage of degenerated zooids (mean + SE, n = 14 - 254) per colony on kelp species (*Saccharina latissima*, *Laminaria digitata*, and *Agarum clathratum*) collected at three sites (TL: The Lodge, PH: Paddy's Head, and SC: Sandy Cove) and 2-3 depths per site (TL and PH: 4, 8, and 12 m; SC: 4 and 8 m) over a seasonal cycle of the annual life cycle of *M. membranacea* from July 2012 to August 2013. NA indicates no colonies were present at the time of collection

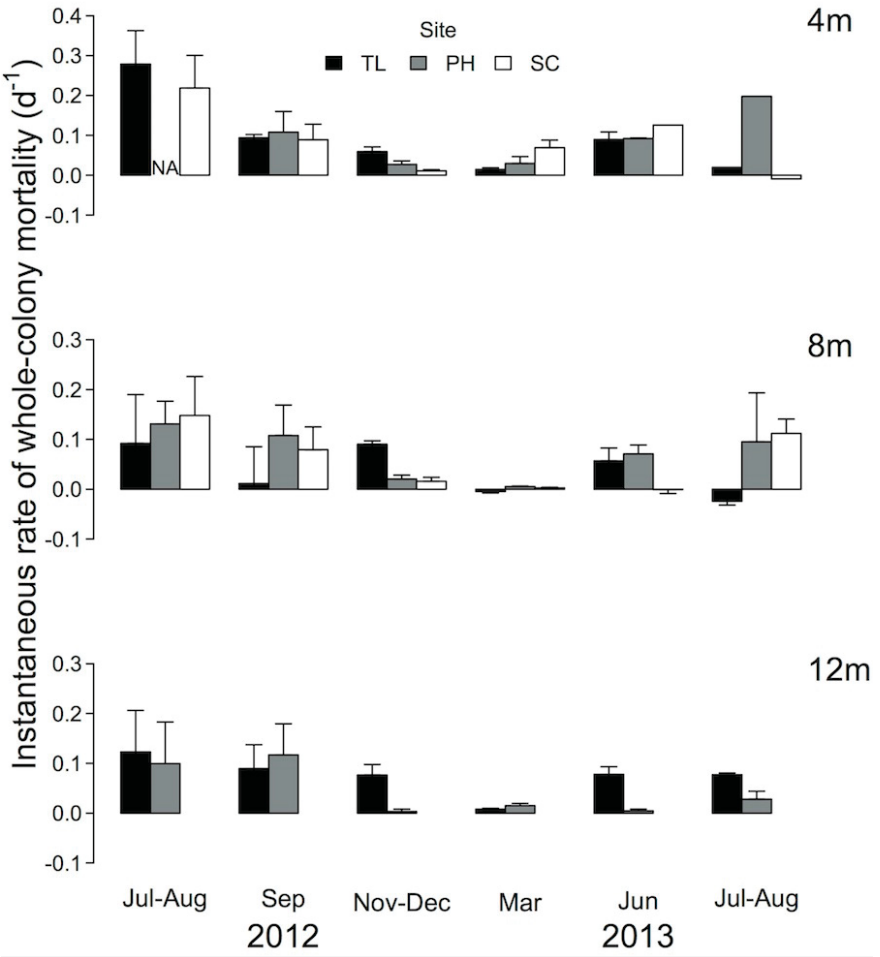

Figure 4.2 Instantaneous rate of whole-colony mortality (d^{-1}) pooled for all size classes of *Membranipora membranacea* colonies (mean + SE, $n = 5$). Colonies were collected (n = 23 - 2230) on kelp species (*Saccharina latissima*, *Laminaria digitata,* and *Agarum clathratum*) at three sites (TL: The Lodge, PH: Paddy's Head, and SC: Sandy Cove) and 2-3 depths per site (TL and PH: 4, 8, and 12 m; SC: 4 and 8 m) over a seasonal cycle of the annual life cycle of *M. membranacea* from July 2012 to August 2013. For negative values, a greater number of colonies was observed than expected based on the size-frequency distribution of colonies present at the previous sampling date and on size- and temperature-dependent growth rates for *M. membranacea* colonies in the field (see 4.3.3 Quantifying whole-colony and partial mortality of *M. membranacea* colonies on kelps). NA indicates no colonies were present at the time of collection

Table 4.2 Results of simple linear regression analyses examining the effect of loss of kelp biomass (B_{Loss}, kg m^{-2}) on the instantaneous rate of whole-colony mortality (d^{-1}) and partial mortality (percentage of degenerated zooids per colony) of *Membranipora membranacea* colonies at three different sites on the southwestern shore of Nova Scotia (The Lodge = TL, Paddy's Head = PH, Sandy Cove = SC)

	Relationship	r^2	$F_{(df)}$	p
Whole colony mortality				
TL	$1.41\ B_{Loss} + 0.502$	0.33	$7.90_{(1,16)}$	**0.013**
PH	$2.09\ B_{Loss} + 0.803$	0.33	$6.78_{(1,14)}$	**0.021**
SC	$0.574\ B_{Loss} + 0.262$	0.33	$4.81_{(1,10)}$	0.053
Overall	$0.706\ B_{Loss} + 0.191$	0.24	$13.7_{(1,44)}$	**
Partial mortality				
TL	$-2.34\ B_{Loss} + 2.48$	0.05	$0.895_{(1,16)}$	0.358
PH	$-7.91\ B_{Loss} + 8.01$	0.07	$0.974_{(1,14)}$	0.340
SC	$-0.156\ B_{Loss} + 1.49$	0.001	$0.011_{(1,10)}$	0.918
Overall	$-0.737\ B_{Loss} + 1.12$	0.01	$0.437_{(1,44)}$	0.512

Notes: Instantaneous rate of whole-colony mortality and the percentage of degenerated zooids per colony were arcsine-square-root-transformed and logit-transformed, respectively. Significant values shown in bold ($\alpha = 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

4.4.2 Effects of temperature and colony size on partial and whole-colony mortality of *M. membranacea in situ*

Partial mortality decreased significantly with increasing temperature (Table 4.3). In contrast, the rate of whole-colony mortality increased significantly with increasing temperature, but only for intermediately sized colonies (3-6 and 6-8 cm in diameter) (Table 4.3).

Instantaneous rates of whole-colony mortality were the same for small (<1 cm diameter) and large (>8 cm diameter) colonies; however, small colonies experienced significantly lower rates of whole-colony mortality than intermediate sized (1-3, 3-6, 6-8 cm diameter) colonies ($F_{4,181} = 9.94$, $p < 0.001$ Tukey's HSD test, $p < 0.001$ - $p = 0.003$; Figure 4.3). Overall, large (>8 cm diameter) colonies experienced partial mortality more frequently (chi-square test of homogeneity: $\chi^2_5 = 2098$, $p < 0.0001$) and at increased magnitude than smaller colonies (Figure 4.4).

4.4.3 Whole-colony mortality of tagged *M. membranacea* colonies on kelps

Whole-colony mortality of tagged colonies on *S. latissima* at The Lodge and Sandy Cove occurred primarily as a result of blade breakage and distal erosion, while

intrinsic colony senescence accounted for only a small proportion of whole-colony mortality and only at The Lodge (Figure 4.5). To compare instantaneous rates of whole-colony mortality calculated using cohort analyses with those measured *in situ*, rates of whole-colony mortality based on sampling of colonies at The Lodge and Sandy Cove from 18 June 2012 to 2 August 2013 were averaged across depths and colony sizes for each sampling period. Instantaneous rates of whole-colony mortality measured *in situ* were within the same order of magnitude as depth- and size-averaged estimates of whole-colony mortality calculated using cohort analyses for the corresponding sampling periods (The Lodge, Sep 2012: rate averaged across depths and colony sizes = 0.065 d^{-1}; Sandy Cove, Nov-Dec 2012: rate averaged across depths and colony sizes = 0.013 d^{-1}).

Table 4.3 Results of simple linear regression analyses examining the effect of temperature (T) on the instantaneous rate of whole-colony mortality (d^{-1}) and partial mortality (percentage of degenerated zooids per colony) of *Membranipora membranacea* colonies for five different size classes of colonies (<1, 1-3, 3-6, 6-8, >8 cm diameter).

	Relationship	r^2	$F_{(df)}$	*p*
Whole colony mortality				
<1 cm	-0.012 T + 0.008	0.05	$2.18_{(1,43)}$	0.147
1-3 cm	0.009 T + 0.008	0.03	$1.29_{(1,40)}$	0.263
3-6 cm	0.023 T + 0.006	0.28	$16.3_{(1,41)}$	***
6-8 cm	0.029 T + 0.006	0.38	$23.4_{(1,39)}$	***
> 8 cm	-0.004 T + 0.015	0.10	$0.08_{(1,13)}$	0.781
Overall	0.012 T + 0.004	0.05	$9.92_{(1,184)}$	**
Partial mortality				
<1 cm	-0.338 T + 0.141	0.01	$5.76_{(1,441)}$	**0.017**
1-3 cm	-1.22 T + 0.170	0.13	$51.2_{(1,347)}$	***
3-6 cm	- 1.23 T + 0.175	0.17	$49.6_{(1,236)}$	***
6-8 cm	-1.37 T + 0.251	0.12	$29.8_{(1,229)}$	***
> 8 cm	-1.07 T + 0.191	0.17	$32.6_{(1,149)}$	***
Overall	-0.720 T + 0.080	0.06	$82.0_{(1,1410)}$	***

Notes: Instantaneous rate of whole-colony mortality and the percentage of degenerated zooids per colony were arcsine-square-root-transformed and logit-transformed, respectively. Temperature was log-transformed for regression analyses with the percentage of degenerated zooids per colony only. Significant values shown in bold (α = 0.05, $^{**}p < 0.01$, $^{***}p < 0.001$)

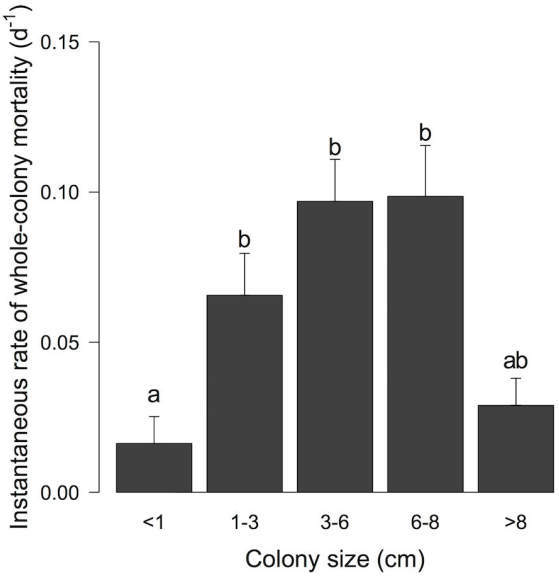

Figure 4.3 Instantaneous rate of whole-colony mortality (d^{-1}, mean + SE) of *Membranipora membranacea* for five different size classes of *M. membranacea* colonies (<1, 1-3, 3-6, 6-8, >8 cm diameter; n = 15 - 45) occurring on the three most numerically abundant kelp species off the coast of Nova Scotia (*Saccharina latissima*, *Laminaria digitata,* and *Agarum clathratum*) collected at three sites (TL: The Lodge, PH: Paddy's Head, and SC: Sandy Cove) and 2-3 depths per site (TL and PH: 4, 8, and 12 m; SC: 4 and 8 m) over one complete seasonal cycle of the annual life cycle of *M. membranacea* from July 2012 to August 2013. Bars with different letters are significantly different at α = 0.05 (Tukey's HSD test)

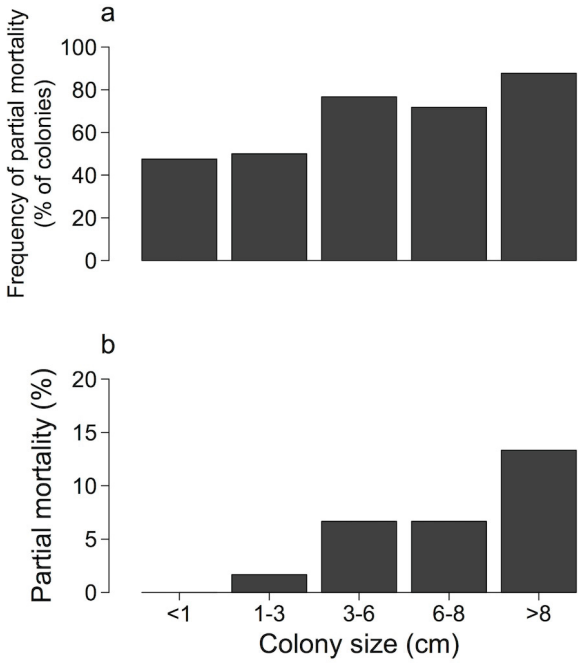

Figure 4.4 a) Frequency of partial mortality (≥ 1 degenerated zooid present) and b) median level of partial mortality (%) for five size classes of colonies (<1, 1-3, 3-6, 6-8, >8 cm diameter) pooled over the entire sampling period. n = 155-450 colonies

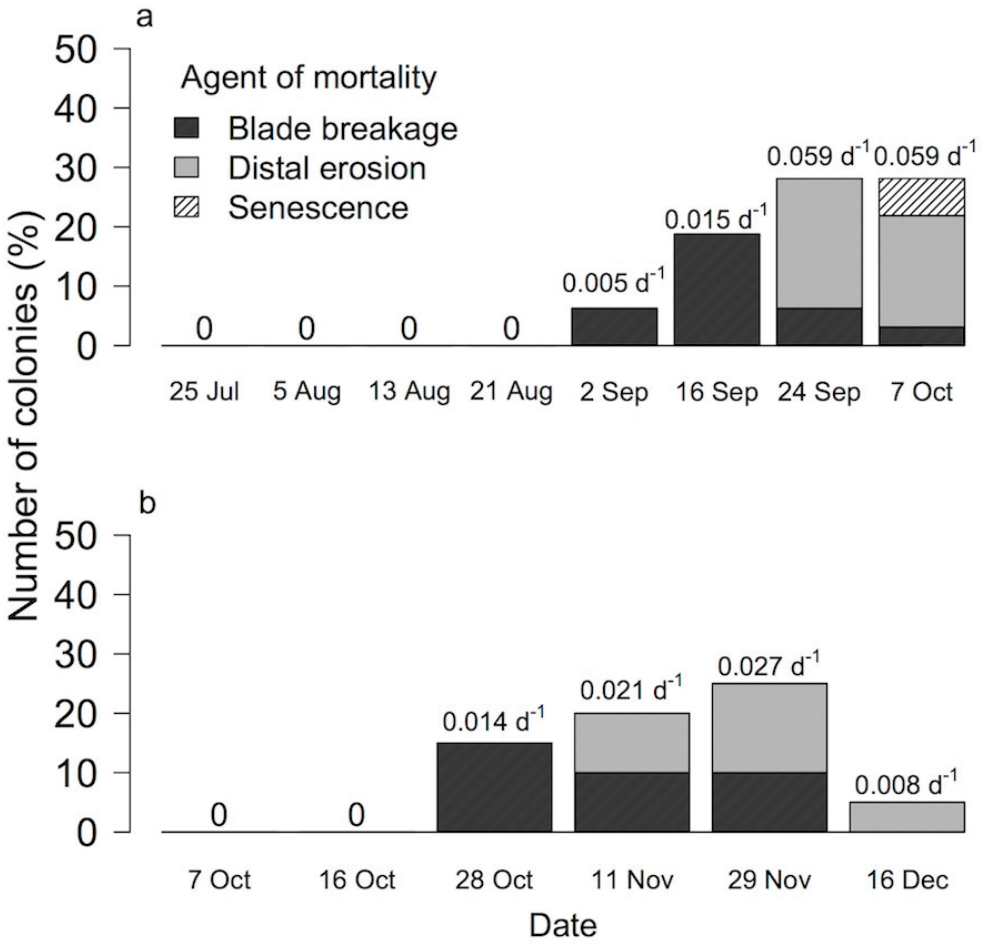

Figure 4.5 Percentage of *Membranipora membranacea* colonies experiencing whole colony mortality due to one of three agents of mortality (blade breakage, distal erosion, senescence) during a) approximately 1.5-week sampling periods at The Lodge from 25 July to 7 October 2014, and b) approximately biweekly at Sandy Cove from 7 October to 16 December 2014. Instantaneous rates of whole-colony mortality (d^{-1}) for each sampling date are indicated above bars

4.4.4 Effects of temperature, colony size, and level of initial partial mortality on senescence of *M. membranacea* in the laboratory

The effects of temperature and level of initial partial mortality on relative senescence (estimated as the percentage loss of colony surface area) of colonies were minimal (Table 4.4, Figure B.6). However, over 62 days, the rate of colony senescence was strongly related to initial colony size for all levels of temperature and initial partial mortality (temperature: $r^2 = 0.87\text{-}0.92$, $p < 0.0001$; mortality: $r^2 = 0.87\text{-}0.94$, $p < 0.0001$; Figure 4.6).

4.5 Discussion

For the encrusting colonial bryozoan *Membranipora membranacea*, temporal patterns of mortality differed substantially between partial and whole-colony mortality, suggesting differences in the agents of mortality between individual modules and whole colonies.

4.5.1 Effect of temperature on whole-colony and partial mortality of *M. membranacea*

Whole-colony mortality of colonial marine invertebrates is often related to extreme episodic disturbance events such as seasonal storms (Hughes & Jackson 1985, Cocito et al. 1998) or anomalous temperatures (Cerrano et al. 2000). Whole colony mortality of tagged *M. membranacea* colonies in the field occurred primarily as a result of disturbance in the form of breakage or erosion of the host kelp substrate. This effect is reflected in the seasonal variation in magnitude of whole-colony mortality and the positive relationship between whole-colony mortality and loss of kelp biomass. The positive relationship between whole-colony mortality and monthly average temperature may therefore be driven (1) primarily by the effect of increasing water temperature on erosion rates of *S. latissima* (Krumhansl & Scheibling 2011), and (2) secondarily by increased dislodgement of kelp and/or breakage of kelp blades during a period of increased extratropical storms (Lambert et al. 1992, Filbee-Dexter & Scheibling 2012).

Table 4.4 Results of ANOVA on the effects of temperature (ambient, ambient +3°C, ambient +9°C) and level of initial partial colony mortality (Control [<25%], 25%, 50%, 75%) on relative senescence of colonies after 31, 62 and 92 days

Effect	MS	$F_{(df)}$	p
31 d			
Temperature	4483	$5.592_{(2,138)}$	**0.01**
Mortality	1036	$1.292_{(3,138)}$	0.54
Temperature x Mortality	690.5	$0.8613_{(6,138)}$	0.37
Error	801.7		
62 d			
Temperature	487	$0.9037_{(2,132)}$	0.25
Mortality	1666	$3.094_{(3,132)}$	**0.02**
Temperature x Mortality	535.2	$0.9937_{(6,132)}$	0.14
Error	538.6		
92 d			
Temperature	255.5	$0.6838_{(2,132)}$	0.51
Mortality	1443	$3.861_{(3,132)}$	**0.01**
Temperature x Mortality	387.3	$1.036_{(6,132)}$	0.41
Error	373.9		

Notes: Significant values shown in bold (31 d and 62 d, $\alpha = 0.05$; 92 d, $\alpha = 0.01$). No significant differences between treatment levels were detected by Tukey's HSD post hoc tests (at $p < 0.05$)

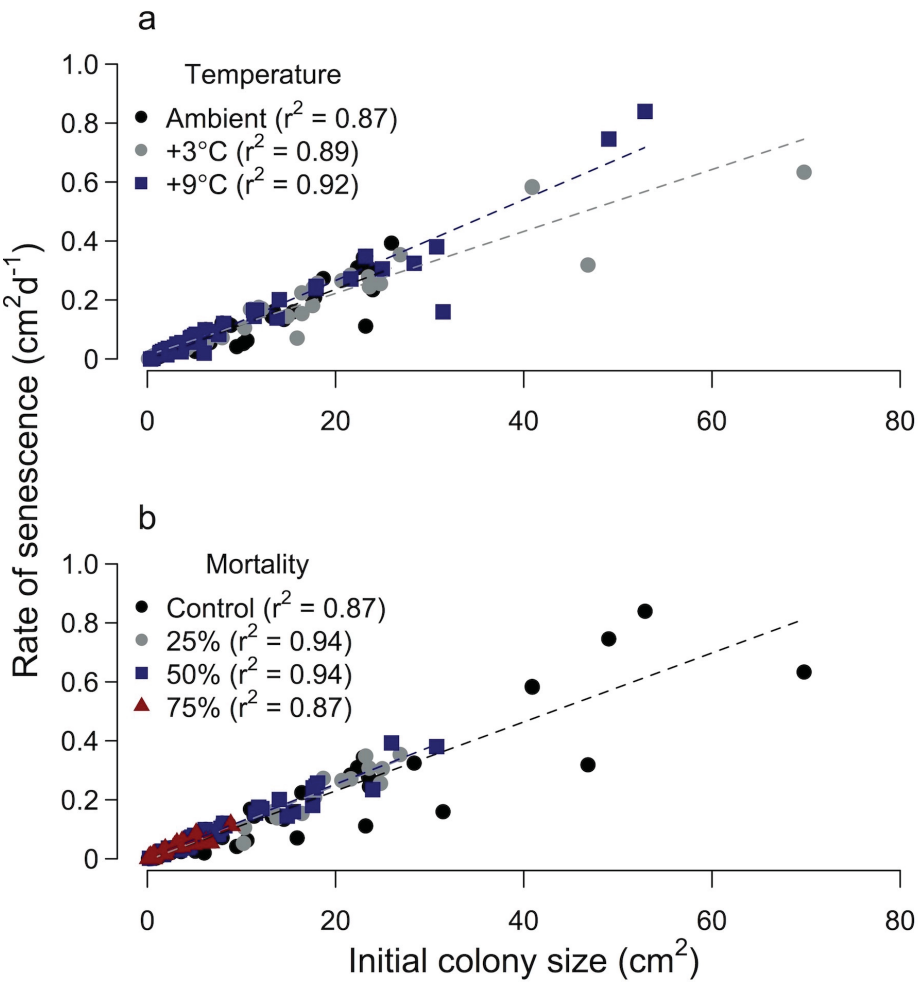

Figure 4.6 Rate of senescence of *Membranipora membranacea* colonies relative to initial colony size (S_i) under a) three levels of temperature (ambient, ambient +3°C, and ambient +9°C; n = 41-58 colonies) and b) four levels of initial partial mortality (<25%, 25%, 50%, 75%; n = 20-64 colonies) over a period of 62 days. Data are loss of colony surface area ($cm^2 d^{-1}$). Regression equation for all colonies pooled across three levels of temperature and four levels of initial partial mortality: rate of colony senescence ($cm^2 d^{-1}$) = 0.0119 S_i + 0.0008, $p < 0.0001$, $r^2 = 0.89$. Regression equation for ambient: rate of colony senescence ($cm^2 d^{-1}$) = 0.0120 S_i - 0.0035; ambient +3°C: rate of colony senescence ($cm^2 d^{-1}$) = 0.0105 S_i + 0.0122; and ambient +9°C: rate of colony senescence ($cm^2 d^{-1}$) = 0.0137 S_i - 0.0084. Regression equation for initial partial mortality <25% : rate of colony senescence ($cm^2 d^{-1}$) = 0.0117 S_i - 0.0038; 25% : rate of colony senescence ($cm^2 d^{-1}$) = 0.0127 S_i - 0.0062; 50% : rate of colony senescence ($cm^2 d^{-1}$) = 0.0126 S_i + 0.0020; and 75% : rate of colony senescence ($cm^2 d^{-1}$) = 0.0114 S_i + 0.0031

Modular degeneration leading to partial mortality of colonial organisms has been attributed to external factors including temperature (Menon 1972, Turon et al. 1998) or reduced food supply (Barnes & Clarke 1998, Shenkar et al. 2008), as well as intrinsic factors such as the age and position of individual modules within the colony (Bayer & Todd 1997) or genetically pre-determined module (Bayer & Todd 1997, Barnes & Clarke 1998) or colony (Rinkevich et al. 1992) lifespan. Partial mortality of *M. membranacea* was greater between November and March than July and September. Seasonal recession (degeneration of zooids) is not uncommon among colonial marine species (e.g. Greene et al. 1983) including *M. membranacea* (Harvell et al. 1990).

Partial mortality of colonies in the field was inversely related to temperature over the month preceding colony collection, unlike in the laboratory experiments, during which there was no effect of temperature on colony senescence. The negative relationship between temperature and partial colony mortality of *M. membranacea in situ* may therefore be strictly correlative, driven by the seasonal timing of *M. membranacea*'s annual lifecycle in which other phases are temperature dependent.

4.5.2 Effect of colony size on whole-colony and partial mortality of *M. membranacea*

In our study, small colonies of *M. membranacea* did not experience whole-colony mortality more frequently than larger colonies, despite the widely accepted hypothesis to the contrary (Jackson 1979). Smaller colonies of *M. membranacea* were able to avoid whole-colony mortality, presumably as a result of selective settlement towards younger more basal regions on blades of host kelps (Denley et al. 2014). Selective settlement allows *M. membranacea* recruits the maximum amount of time to grow before becoming at risk of mortality due to breakage or erosion of the kelp substrate.

In contrast, as is common for colonial organisms (Hughes & Jackson 1980, 1985, Hughes & Connell 1987, Stocker 1991, Turon et al. 1998), larger colonies of *M. membranacea* experienced partial mortality more frequently and to a greater extent than smaller colonies, both in the field and on artificial substrates in the laboratory. The positive relationship between colony size and the rate of colony senescence may indicate increased senescence of older colonies. It appears that senescence may in fact be an

intrinsic property of the colonies themselves, with older (larger) colonies senescing more rapidly than younger (smaller) ones, irrespective of temperature.

4.5.3 Factors affecting whole-colony and partial mortality of colonial organisms

Based on our results, whole-colony mortality occurs predominantly in response to external agents. Except for extreme temperature anomalies (e.g. Cerrano et al. 2000) or in the case of coral reef bleaching (reviewed by Baker et al. 2008), the specific effects of temperature and size depend on complex interactions with other biotic and abiotic factors. For example, selective predation can result in size-specific mortality patterns in colonial plants (Cooke 1983, Young 1984). Similarly, size-specific whole-colony mortality rates in colonial marine invertebrates may arise due to competitive overgrowth interactions (e.g. octocoral *Alcyonium siderium*, Sebens 1982) or dislodgement due to physical disturbances (Hughes & Jackson 1985). Additionally, the effect of temperature on colony growth rates may indirectly affect whole-colony mortality by influencing the frequency of competitive overgrowth interactions. For colonial marine invertebrates on ephemeral substrata such as algae, whole-colony life span seems to be limited by substrate longevity for all but the shortest-lived species (McKinney & Jackson 1989).

In contrast, we found that partial mortality may be more strongly influenced by intrinsic factors. Models of leaf dynamics suggest that modular (leaf) senescence in plants may be primarily a function of leaf age (Kikuzawa 1991) or the position of the leaf on the shoot (Ackerly 1999). Although the contribution of external factors such as predation, infection, and fouling to partial mortality of colonial organisms cannot be ignored, there is evidence that susceptibility of individual modules to these factors is affected by modular age (e.g. Palumbi & Jackson 1983, Ward 2007). Distinct seasonality in the proportion of degenerated zooids per colony, in combination with senescence of colonies on artificial substrates, suggests that there is an intrinsically regulated component to partial colony mortality and eventual colony senescence.

4.5.4 Comparison of methods for quantifying whole-colony and partial mortality of colonial organisms

We estimated instantaneous rates of whole-colony mortality by repeated subsampling cohort analyses to calculate rates of size-specific colony mortality between successive sampling times, a method which involves relatively easy sampling. For populations where comprehensive growth data are available, estimating mortality rates using cohort analysis can be achieved by randomly sampling the size-frequency distribution of the population (Van Sickle 1997). However, for colonial organisms in particular, differences in size-frequency distributions between successive samples may over-or underestimate size-specific survivorship. Direct whole-colony mortality estimates obtained by tagging colonies *in situ* can resolve this issue. In our study, the two approaches provided similar rates for *M. membranacea*. However, fusion and fission events are uncommon for *M. membranacea,* and fragments of colonies generated through fission are usually short-lived and unlikely to survive from one sampling time to the next (Harvell et al. 1990). While repeated subsampling may be appropriate for estimating whole-colony mortality of *M. membranacea*, this method is less likely to provide accurate measurements of mortality for colonial species for which incidence of fission and fusion are comparatively higher (e.g. Bak et al. 1981, Hughes & Jackson 1985, Stocker 1991).

We measured partial mortality of *M. membranacea in situ* at the modular level on colonies collected from the field, and during seasonal colony senescence as loss of colony surface area over time. Results were consistent between methods, with larger colonies experiencing partial mortality more frequently and to a greater extent than smaller colonies *in situ*, and larger colonies experiencing increased rates of senescence than smaller colonies across all temperature treatments in the laboratory. For species such as *M. membranacea* that undergo distinct seasonal cycles of growth, stasis, and shrinkage (senescence), measuring partial mortality as change in colony surface area may be appropriate during periods when colony growth is negligible. However, we propose that

measurements of partial mortality encompassing all phases in the lifecycle of colonial organisms can be most accurately obtained by revisiting identified individuals *in situ* and measuring loss of living colony area as a percentage of the total live colony area at the time of initial sampling (after Bythell et al. 1993). Additionally, summing measurements of percent partial mortality relative to initial colony size across all colonies gives the total amount of living tissue lost from the sample population due to partial mortality over the specified sampling interval, which has important implications when estimating demographic parameters such as fecundity at the population level.

4.6 Conclusions

While seasonal mortality may not affect long-term population trends, identifying potential causative mechanisms is important for predicting future change. We suggest that whole-colony mortality in colonial invertebrates is primarily driven by external factors (e.g. competition, disturbance, substrate longevity) and that the effects of temperature and colony size on rates of whole-colony mortality are largely indirect. In contrast, partial mortality of colonial invertebrates appears to be more strongly related to intrinsic factors (e.g. colony size, module age, module position). The decision of which metric of mortality to consider in studies involving colonial organisms is not a trivial one, as we demonstrate using *M. membranacea*. For this bryozoan, measurements of mortality based on whole-colony mortality alone would overestimate important demographic rates, such as fecundity, during winter and early spring when rates of whole-colony mortality are minimal but mortality of individual zooids is high. Alternatively, relying solely on measurements of partial colony mortality would underestimate the influence of temperature on mortality of *M. membranacea* through the effect of temperature on its primary host substrate. Obviously, more comprehensive studies of the population dynamics of colonial organisms require measurements of mortality from the level of the individual module to the entire colony; however, this is not always logistically realistic. We propose that measurements of whole-colony and partial mortality can be used independently to provide useful and accurate information on community and population dynamics of colonial organisms, provided the limitations of each measurement are recognized and the results interpreted accordingly.

CHAPTER 5

RECOVERY CAPACITY OF THE INVASIVE COLONIAL BRYOZOAN *MEMBRANIPORA MEMBRANACEA* FROM DAMAGE: EFFECTS OF TEMPERATURE, LOCATION, AND MAGNITUDE OF DAMAGE[4]

5.1 Abstract

The survival and reproduction of individual or small groups of modules affords colonial organisms a great regenerative capacity. Consequently, modular loss due to fragmentation or senescence may not necessarily lead to colony mortality. This study, 1) examines *in situ* partial mortality for colonies of the invasive bryozoan *Membranipora membranacea* in Nova Scotia by quantifying the location, magnitude, and timing of partial mortality for colonies growing on kelp (*Saccharina latissima*) in the field, and 2) estimates the effects of temperature (5°C - 20°C), and location and magnitude of modular loss on the recovery capacity of experimentally damaged colonies in the laboratory. *In situ* zooid mortality was substantial, with 50 - 100% of colonies experiencing some level of partial mortality by the end of the growing season. Colonies with damage to older centrally located zooids maintained their capacity for growth and recovery, while colonies where younger peripheral zooids were removed showed no sign of recovery, and often experienced further loss of zooids. The effect of temperature depended on the location of colony damage, with increasing temperature resulting in increased loss of zooids for peripherally damaged colonies, but having no effect on the recovery of colonies with damage to central zooids. Variation in colony recovery may be related to the age distribution and reproductive maturity of zooids within a colony. Alteration of resource allocation between sexual and asexual reproduction may be adaptive in that it maximizes lifetime fitness in response to localized partial mortality.

[4] Denley D, Metaxas A (2015) Recovery capacity of the invasive colonial bryozoan *Membranipora membranacea* from damage: effects of temperature, location, and magnitude of damage. Mar Biol 162: 1769-1778

My coauthor Dr. Anna Metaxas supervised the study design and analyses, and edited the manuscript.

5.2 Introduction

The ability of colonial organisms to regenerate following damage as a result of injury or prolonged exposure to unfavorable conditions is arguably one of the primary advantages of their modular construction. Regeneration of colonial organisms relies on asexual, vegetative production of new modular units (Henry & Hart 2005), the ability of modular units to survive and reproduce individually or in small groups (Highsmith 1982, Hughes & Jackson 1985) and the sharing and/or reallocation of resources among modules in response to localized demand within a colony (Palumbi & Jackson 1983, Harvell & Helling 1993, Oren et al. 2001). The propensity of colonial organisms to persist and recover following dramatic reductions in colony size makes the accurate measurement of their demographic properties extremely complicated. This is particularly the case for mortality estimates, because modular losses due to fragmentation or senescence may not necessarily lead to colony-wide mortality.

Membranipora membranacea is a colonial cheilostome bryozoan that is native to the Pacific coast of North America and the Atlantic coast of Europe, and was introduced to Nova Scotia in the northwest Atlantic in the early 1990s (Scheibling et al. 1999). It is epiphytic, primarily encrusting laminarian kelps (Yorke & Metaxas 2012), and its colonies grow through the addition of new modules (zooids) to the colony edge. As a result, the age distribution of zooids within a colony is such that the oldest zooids are located at the center of the colony, with peripheral zooids being progressively younger. The age gradient within colonies is reflected in the timing of the onset of reproduction in individual zooids, which varies spatially within a colony. Typically, older more centrally located zooids begin to reproduce before younger more peripheral zooids; however, crowding by conspecifics and simulated predation damage have been shown to influence the timing and pattern of reproduction within experimentally manipulated *M. membranacea* colonies (Harvell & Helling 1993). Throughout this manuscript, we will refer to interior regions of *M. membranacea* colonies consisting of older zooids as central, and the outer regions consisting of younger zooids as peripheral.

In Nova Scotia, colonies of *M. membranacea* experience modular loss in one of three ways: 1) degeneration of older central zooids during seasonal colony senescence; 2)

removal of younger peripheral zooids due to physical abrasion, as well as, erosion and breakage of kelp blades, and 3) incidental rasping of zooids from central and/or peripheral regions of colonies during grazing of kelp by the herbivorous gastropod *Lacuna vincta*. Although attempts have been made to estimate mortality of *M. membranacea* in Nova Scotia (e.g. Saunders et al. 2010), these estimates are based solely on declining numbers of whole colonies; the frequency of occurrence of modular loss and the extent to which colonies are able to recover from this loss remains unknown.

The regenerative capacity of colonial marine invertebrates has been linked to colony size (Oren et al. 2001), the number (Oren et al. 2001) and location (Wahle 1983, Harvell 1984, Bone & Keough 2005) of damaged modules, the age distribution of modules throughout a colony (Palumbi & Jackson 1983, Meesters & Bak 1995), and the connectivity of individual modules within a colony (Harvell & Helling 1993, Bone & Keough 2005). In addition, temperature has been shown to affect colony growth rate, and consequently colony recovery, for several cheilostome bryozoans (Menon 1972, O'Dea & Okamura 1999, Amui-Vedel et al. 2007, Yorke & Metaxas 2011). For *M. membranacea* specifically, temperature is known to affect the timing and extent of population outbreaks (Saunders & Metaxas 2007, 2008, Scheibling & Gagnon 2009, Saunders et al. 2010) and has been shown to be positively related to colony growth rates under both field and laboratory conditions (Saunders & Metaxas 2009a). In this study, we: 1) quantified the location, magnitude, and timing of zooid mortality for *M. membranacea* colonies on kelp in the field; and 2) estimated the effects of temperature, and location and magnitude of modular loss on the recovery capacity (rate and extent) of damaged *M. membranacea* colonies in controlled experiments in the laboratory.

Estimates of modular loss and recovery are critical for understanding the population dynamics of colonial organisms like *M. membranacea,* for which important demographic characteristics, such as fecundity, are related to the survival and reproduction of individual modules as opposed to colonies as a whole (Tuomi & Vuorisalo 1989).

5.3 Methods

5.3.1 Zooid mortality *in situ*

To measure the location and magnitude of *in situ* zooid mortality, colonies of *Membranipora membranacea* growing on *Saccharina latissima* were collected from 4-8 m at two sites on the southwestern shore of Nova Scotia: The Lodge (44°33'3"N, 64° 01' 9" W) on the western shore of St. Margarets Bay, and Sandy Cove (44° 27' 6" N, 63° 42' 4" W) in Terence Bay, 20 km northeast of St. Margarets Bay. These sites were chosen based on previous studies showing consistently high abundance of *Membranipora membranacea* over multiple years (Saunders & Metaxas 2008, 2009b), and to account for variation in different bays. Blades of *S. latissima* (n = 15 - 30) with colonies of *M. membranacea* were collected haphazardly from each site approximately monthly from 25 July to 29 November 2014. Collected algae were immediately transported to the Aquatron facility at Dalhousie University in coolers without seawater, where they were maintained in aquaria with running ambient seawater until processing was completed, typically within 1-3 days. The location (central or peripheral) and magnitude (% of colony surface area) of zooid mortality was estimated visually for all colonies on the collected algae (n = 34 - 274). Mortality to central zooids was defined as the loss of colony surface area confined within the colony, leaving the entire circumference of the colony intact (Figure 5.1a). Mortality that affected any section of the outer growing edge of the colony was considered to be peripheral (Figure 5.1b). If zooid loss extended from the interior of the colony across the growing edge, mortality of both central and peripheral zooids was estimated for each region independently, with peripheral damage incorporating the loss of colony circumference only (Figure 5.1c). We could not distinguish visually between living zooids and the remaining exoskeleton of degenerated zooids. However, degenerated zooids tend to be more abundant within colonies over winter and in early spring (D. Denley, unpubl data) and typically slough off of the kelp substrate during periods of maximum colony settlement and growth from July to November. As a result, it is unlikely that incorporating degenerated zooids into our categorical estimates of percent damage would change the frequency distribution of colonies among damage categories.

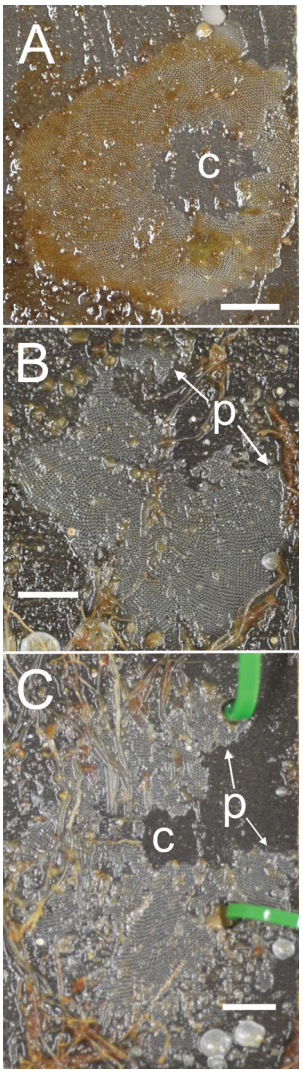

Figure 5.1 *Membranipora membranacea* colonies on settlement plates collected from ~ 8 m depth at The Lodge and Sandy Cove, Nova Scotia, Canada. Colonies exhibit mortality of: A) older central (c) zooids; B) younger peripheral (p) zooids; and C) both central (c) and peripheral (p) zooids. Scale bars indicate 1 cm

5.3.2 Recovery capacity of *Membranipora membranacea* colonies in the laboratory

Colonies of *M. membranacea* on individual blades of *Laminaria digitata* were collected from 4-8 m at Sandy Cove in September 2013 and were transported to the Aquatron facility at Dalhousie University in plastic tubs without seawater. Because growth rate of *M. membranacea* colonies varies with initial colony size (Saunders & Metaxas 2009a), collected colonies were selected to represent a wide range of sizes (~1 -

30 cm in length) in order to incorporate any variation in the rate or extent of colony recovery that may result due to differences in colony size. Colonies were randomly assigned to flow-through seawater tables maintained at 1 of 3 temperature treatments (5°C, 12°C, and 20°C) and allowed to acclimate for 1 week prior to experimental manipulation. Temperature treatments were chosen to represent seasonal variation in the region (Figure 5.2), with 5°C being typical of early winter (December-January) and early spring (April-May), 12°C of summer to autumn (July-October), and 20°C representing the maximum daily averaged temperature at 4 m, typically occurring in August (The Lodge: 20.92°C, Sandy Cove: 19.64°C).

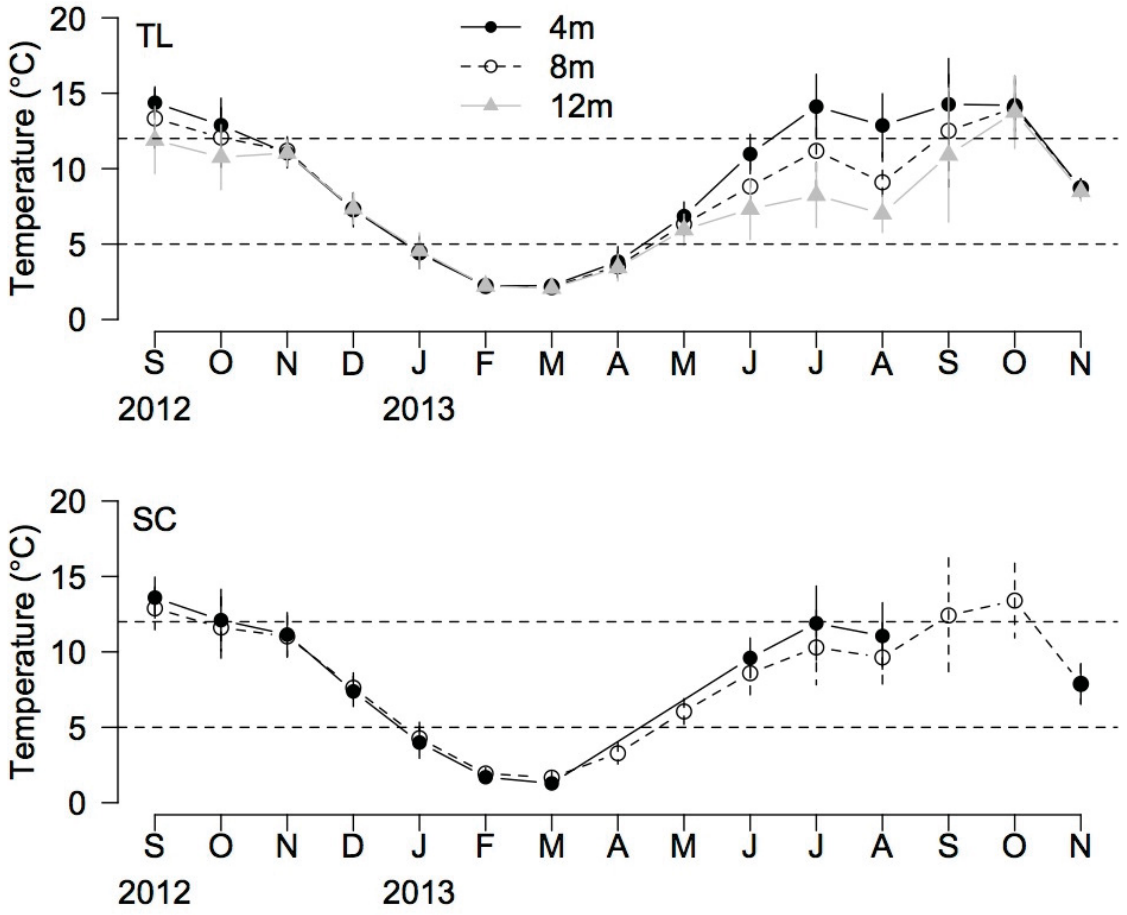

Figure 5.2 Monthly averaged temperature (mean + SD, *n* = 27-31) at 3 depths at The Lodge (TL: 4, 8, 12 m) and 2 depths at Sandy Cove (SC: 4, 8 m) from September 2012 to November 2013. Temperature treatments used in laboratory experiments (5°C, 12°C) are indicated by horizontal dashed lines; the highest temperature treatment used in laboratory experiments (20°C) exceeds the monthly averaged annual maximum temperature in the region

Following 1 week of acclimation to temperature, colonies were randomly assigned to 1 of 5 mortality treatments (n = 13-15 colonies per treatment): a control, and 4 levels of damage that were inflicted by scraping zooids off of the kelp substrate using a scalpel. Damage treatments orthogonally combined 2 levels of damage location (central zooids removed, peripheral zooids removed) and 2 levels of percent damage (50% of zooids removed, 75% of zooids removed). For the central damage location, 50 or 75% of zooids were removed from the interior of the colony, leaving a ring of younger peripheral zooids the total surface area of which was half or one quarter the size of the original intact colony, respectively (Figure 5.3a, c). Similarly, for the peripheral damage location, 50 or 75% of zooids were removed from the entire colony perimeter, and the remaining colony was half or one quarter the size of the original intact colony, respectively, and consisted only of older interior zooids (Figure 5.3b, d).

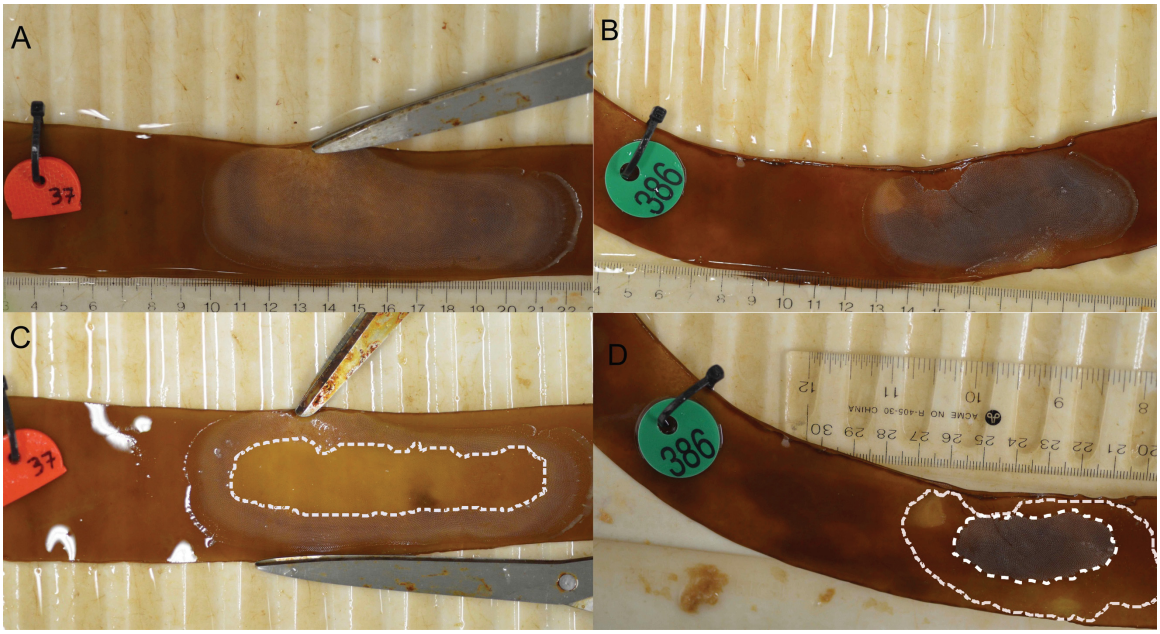

Figure 5.3 Colonies of *Membranipora membranacea* on *Laminaria digitata* collected from Sandy Cove, before (panels A and B), and after experimentally inflicted removal of C) central, and D) peripheral zooids. Dashed white lines indicate areas from which zooids were removed

Damage percentages were based on pilot studies where recovery of colonies was observed after removal of 50% of colony surface area. Control colonies were left intact for the duration of the experiment. All colonies were photographed prior to and immediately following experimentally inflicted damage, and initial and post-damage colony surface area was measured using ImageJ photo analysis. Colonies were subsequently photographed at 7 and 14 days after damage, and change in surface area was measured using ImageJ. To further account for any potential variation in growth rate among different sized colonies, change in surface area was standardized by dividing by initial colony size [change in colony surface area = (final surface area – initial surface area) ÷ initial surface area, see 5.3.3 Data Analysis]. Results of pilot studies confirmed that this method of standardization was appropriate for comparing growth and recovery of colonies within the size range examined. Overall, including both acclimation and experimental periods, colonies were maintained in the laboratory for 21 days. During this time, colonies were fed a combination of live microalgae three times a week at concentrations (~4.5 x 10^4 cells ml^{-1}) known to be sufficient for unlimited colony growth under laboratory conditions (Saunders & Metaxas 2009a).

5.3.3 Data Analysis

5.3.3.1 Zooid mortality *in situ*

To determine whether incidences of damage differed between central and peripheral zooids within a colony, the frequency of percent damage (categories: 0, <25%, 25%, 50%, 75%, >75%) was compared between central and peripheral locations of damaged colonies collected from each site in November 2014, when levels of colony damage were greatest, using chi-square tests of homogeneity. For colonies that experienced mortality of both central and peripheral zooids, the location of damage was randomly selected for the purpose of analysis to ensure independence. For example, if a colony exhibited 25% mortality of centrally located zooids and <25% mortality of peripherally located zooids, either central or peripheral mortality was considered for the analysis. This did not substantially affect the sample size or our results, as comparatively few colonies (n = 0 - 57) exhibited zooid mortality in both central and peripheral regions during each sampling period. In some instances, >1 colony was sampled per kelp blade

leading to potential non-independence of mortality. To account for this, we conducted the analysis on 50 subsamples randomly drawn from the full dataset for each site. Each of the 50 subsamples randomly selected 40% of the total number of colonies sampled in November 2014 from The Lodge ($n_{total} = 100$, $n_{subsample} = 40$) and 90% of the total number of colonies from Sandy Cove ($n_{total} = 34$, $n_{subsample} = 30$). We then calculated a mean χ^2 statistic and associated standard error (of the statistics yielded by the 50 random subsamples), which we present along with the associated range of *p*-values. The proportion of data included in each subsample was determined based on the maximum proportion of colonies that could have been sampled from the same kelp at each site (60% at The Lodge, 10% at Sandy Cove).

5.3.3.2 Growth of control colonies in the laboratory

Relative growth of control colonies was calculated as the percentage change in surface area over time relative to initial colony surface area on day 1 of the experiment (Harvell et al. 1990, Saunders & Metaxas 2009a, Bone & Keough 2010, Marzinelli et al. 2012). The effect of temperature (fixed factor, 3 levels: 5°C, 12°C, 20°C) on the relative growth of control colonies after 7 and 14 days was examined using two-way ANOVA with repeated measures on day (RM ANOVA). Significant differences between means as detected by RM ANOVA were examined with Tukey's HSD post-hoc tests. For control colonies, measurements of relative growth exhibited heterogeneity of variance as detected by Cochran's test that could not be alleviated by transformation of the data. To account for this, we adopted a more conservative α ($\alpha_{critical} = 0.01$) for the RM ANOVA; however, an α-value of 0.05 was maintained for all post-hoc tests, as Tukey's HSD is already fairly conservative (Crawley 2007). According to the Shapiro-Wilk test ($p < 0.05$), relative growth of control colonies was not normally distributed and normal distribution could not be attained through transformation; however, ANOVA is robust to deviations from normality (Zar 1999).

5.3.3.3 Relative recovery of damaged colonies in the laboratory

Relative recovery of damaged colonies through the budding of new zooids was calculated as the percentage change in surface area over time relative to the initial colony

surface area following artificially inflicted damage [relative recovery (%) = {[(final surface area – initial surface area post-damage) ÷ initial surface area post-damage] x 100}]. We examined the effects of temperature (fixed factor, 3 levels: 5°C, 12°C, 20°C), damage percentage (fixed factor, 2 levels: 50% of zooids removed, 75% of zooids removed), and damage location (fixed factor, 2 levels: central zooids removed, peripheral zooids removed) on the relative recovery of damaged colonies after 7 and 14 days using four-way ANOVA with repeated measures on day (RM ANOVA). Based on the results of RM ANOVA, the effects of temperature, damage percentage, and damage location (all fixed effects) on the relative recovery of damaged colonies were also examined separately for days 7 and 14, using three-way ANOVA. Significant differences between means as detected by ANOVA were further examined using Tukey's HSD tests. Measurements of relative recovery were arcsine-square root transformed to better approximate the normal distribution and to eliminate heterogeneity of variance as detected by Cochran's test. As with the control colonies, relative recovery of damaged colonies was not normally distributed (Shapiro-Wilk test, 7 days: $p = 0.02$, 14 days: $p = 0.01$), even after transformation.

For damage treatments where the relative recovery of damaged colonies was similar to the relative growth of control colonies, differences in relative growth between control and damaged colonies were examined using one-tailed Student's *t*-tests (when variances were equal) and Welch's *t*-tests (for unequal variances).

5.4 Results

5.4.1 Zooid mortality *in situ*

Damage of colonies on kelp increased during the growing season for both sites. In Nov 2014, when colony damage was greatest, there was no difference in the frequency of percent zooid mortality between central and peripheral locations of colonies (The Lodge: $\overline{X}^2_4 = 3.30 \pm 0.268$, $p = 0.084$ - 0.967; Sandy Cove: $\overline{X}^2_4 = 4.70 \pm 0.192$, $p = 0.059$ - 0.641, Figures 5.4 & 5.5). Analyses of randomly subsampled data yielded consistent results with those using the complete dataset (The Lodge: $X^2_4 = 1.28$, $p = 0.865$; Sandy Cove: $X^2_4 =$

5.70, $p = 0.223$). Most colonies experienced some level of damage *in situ*, with >50% of colonies at The Lodge and >80% of colonies and Sandy Cove experiencing >50% mortality at either the central or peripheral location (Figures 5.4 & 5.5).

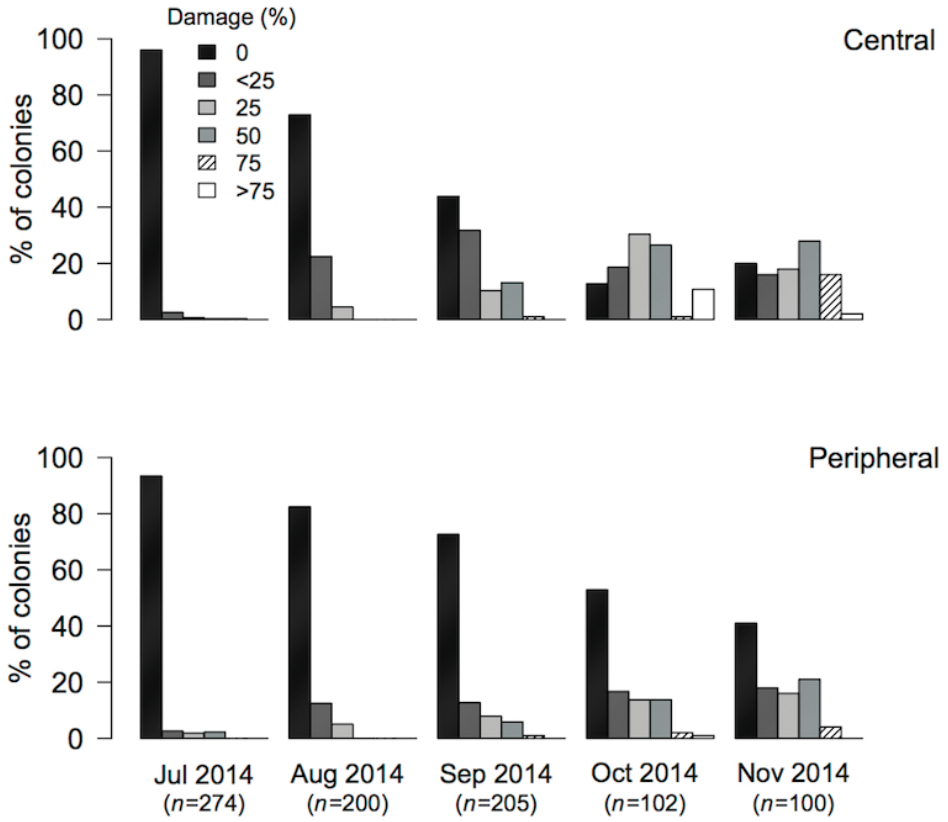

Figure 5.4 Frequency (%) of all *Membranipora membranacea* colonies collected on *Saccharina latissima* at The Lodge from July 2014 to November 2014 and showing different magnitudes of damage (0%, <25%, 25%, 50%, 75%, >75%) to central and peripheral zooids. A subsample ($n = 40$) was randomly drawn from this distribution in November 2014 for analysis (see 5.3.3.1 Zooid mortality *in situ*)

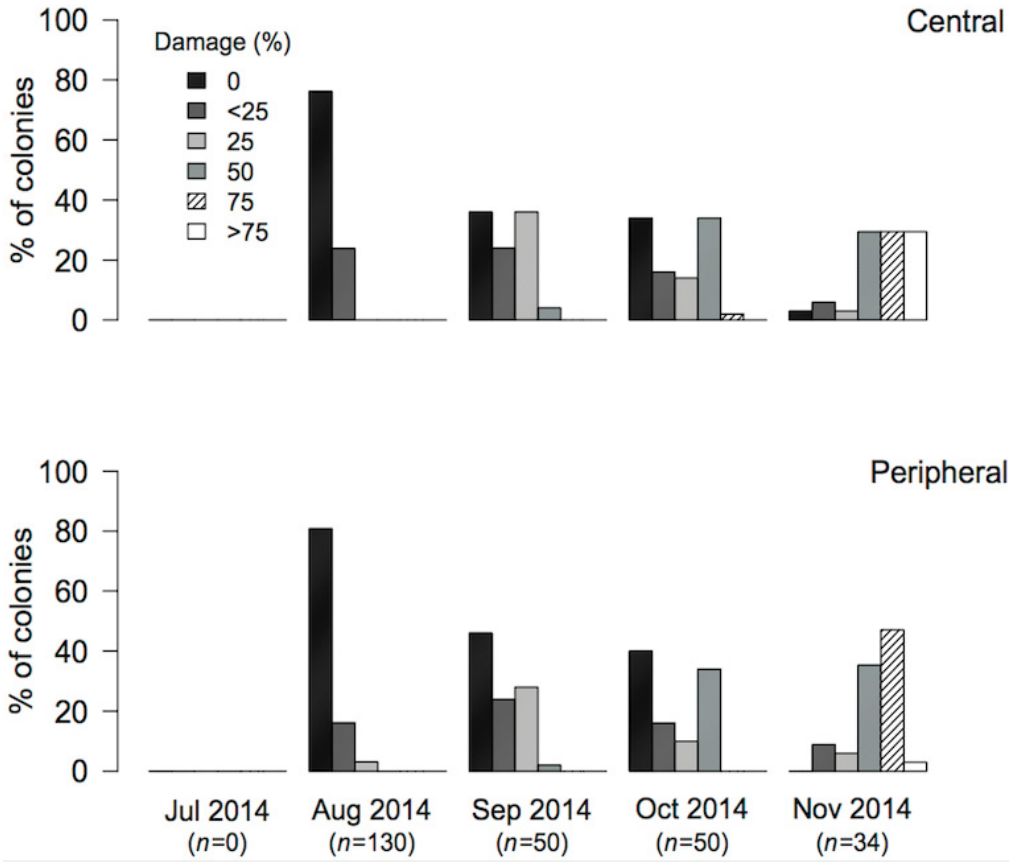

Figure 5.5 Frequency (%) of all *Membranipora membranacea* colonies collected on *Saccharina latissima* at Sandy Cove from July 2014 to November 2014 and showing different magnitudes of damage (0%, <25%, 25%, 50%, 75%, >75%) to central and peripheral zooids. A subsample (n = 30) was randomly drawn from this distribution in Nov 2014 for analysis (see 5.3.3.1 Zooid mortality *in situ*)

5.4.2 Recovery capacity of *Membranipora membranacea* colonies in the laboratory

5.4.2.1 Growth of control colonies

Growth of undamaged control colonies of *M. membranacea* occurred at 12°C and 20°C in the laboratory, and there was no significant difference in relative growth of colonies between these two temperature treatments after 7 or 14 days (Figure 5.6, Table 5.1). At 5°C, control colonies appear to decrease in size (Figure 5.6); however, growth of these colonies did not differ significantly from zero after 7 or 14 days (Welch's *t*-test, 7 days: $t_{14} = -1.57, p = 0.14$; 14 days: $t_{14} = -1.84, p = 0.09$). Both the observed growth rates

of control colonies and the effect of temperature on these rates are consistent with previous studies in Nova Scotia in both the field and the laboratory, where colony growth rates ranged from 0.01 - 12 mm d^{-1} (Saunders & Metaxas 2009a).

5.4.2.2 Relative recovery of damaged colonies

Relative recovery of damaged colonies differed between days, but only in magnitude and not in direction and these differences varied with temperature (Figure 5.7, Table C.1). The effects of temperature, damage percentage, and damage location on the relative recovery of damaged colonies were then examined separately for days 7 and 14, using three-way ANOVA. Colonies showed greater recovery after 7 days, and lower further loss of zooids after 14 days, when central zooids were removed than when peripheral zooids were removed for all temperature treatments (Figure 5.7, Table 5.2). Recovery of colonies from which central zooids were removed occurred by the addition of new zooids via asexual budding around the intact colony periphery, and not through the regeneration of damaged central zooids. Magnitude of damage (damage percentage) had no significant effect on the relative recovery of colonies after 7 and 14 days. Recovery of colonies did not vary among temperatures when central zooids were removed; however, when peripheral zooids were removed, further loss of zooids increased significantly with increasing temperature (Figure 5.7, Table 5.2).

Relative growth of control colonies was equal to or exceeded relative recovery of damaged colonies after 7 and 14 days at 12°C and 20°C. However, this pattern was not consistent at 5°C when 50% of central zooids were removed after 7 and 14 days, and 75% of central zooids were removed after 7 days (Figures 5.6 & 5.7, Table 5.3).

It should be noted that for colonies where central zooids were damaged, negligible relative growth after 14 days is the result of continued loss of central zooids and does not reflect lack of recovery at the growing edge. While the addition of new zooids through peripheral budding was observed for these colonies, simultaneous loss of central zooids resulted in net colony recovery that did not differ significantly from zero. No addition of new zooids was observed for colonies where peripheral zooids were removed.

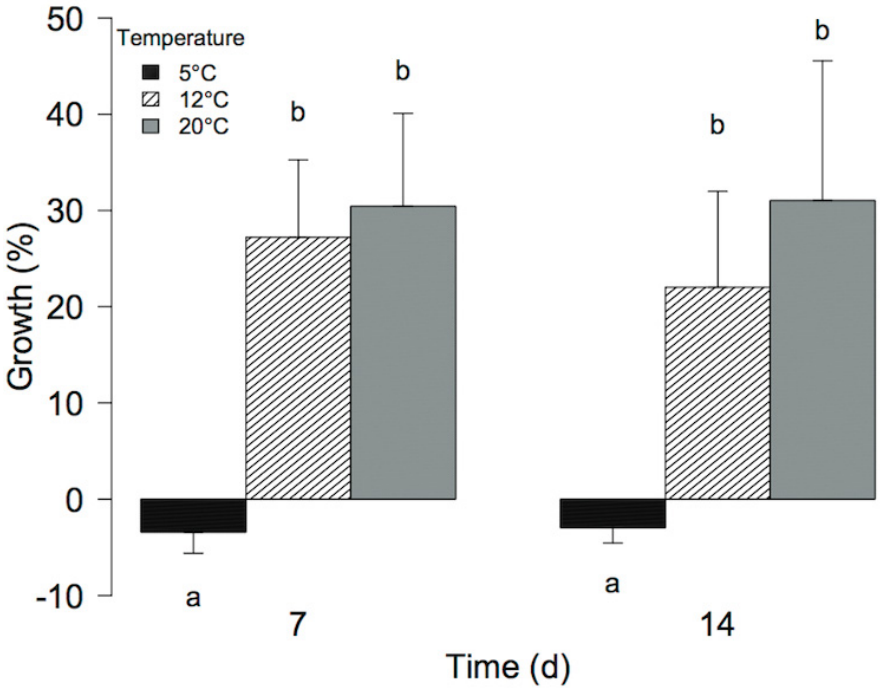

Figure 5.6 Relative growth (mean + SE, n = 13-15) of undamaged *Membranipora membranacea* colonies under 3 temperatures after 7 and 14 days in the laboratory. Relative growth was calculated as a percentage of the initial colony size [relative growth (%) = ((final surface area – initial surface area) ÷ initial surface area) x 100]. Negative values indicate partial mortality. Letters above bars indicate homogeneous subsets among temperature treatments, identified using Tukey's HSD test, $\alpha = 0.05$

Table 5.1 Results of two-way ANOVA examining the effect of temperature (5°C, 12°C, 20°C) on the relative growth of control colonies after 7 and 14 days (repeated measures). Relative growth was calculated as a percentage of the initial colony size. Significant values shown in bold ($\alpha = 0.01$). Only significant differences in post hoc tests are shown (at $\alpha < 0.05$)

Effect	df	MS	*F*	*p*	Tukey's HSD
Between subjects					
Temperature treatment	2	2.83	5.203	**0.010**	5°C < 12°C = 20°C
Error	36	0.544			
Within subjects					
Day	1	0.0110	0.3677	0.55	
Day x temperature treatment	2	0.0255	0.8528	0.44	
Error	36	0.030			

5.5 Discussion

Levels of partial mortality for *Membranipora membranacea* colonies in the field did not differ between central and peripheral locations within colonies; however, *in situ* zooid mortality was substantial, with >50% of colonies experiencing >30% partial mortality by the end of the growing season. In Nova Scotia, seasonal senescence of *M. membranacea* typically begins in late summer to early autumn (D. Denley, unpubl data), a pattern consistent with the observed increase in partial mortality of centrally located zooids later in the season. Similarly, increased mortality of peripheral zooids in autumn may be related to seasonal increases in temperature and wave action that occur during this time (Figure 5.2; D'Amours & Scheibling 2007). Erosion rates for the two most abundant kelp species in Nova Scotia, *S. latissima* and *L. digitata*, are positively related to water temperature and site exposure, respectively (Krumhansl & Scheibling 2011). Although levels of partial mortality did not differ between central and peripheral locations within *M. membranacea* colonies in the field, the location of modular loss significantly affected the recovery capacity of damaged *M. membranacea* colonies in the laboratory, irrespective of the level of damage inflicted. Lack of colony recovery following damage to peripheral zooids suggests that spatial and temporal variation in temperature and wave intensity, as they relate to increased breakage and erosion of kelp blades, will greatly influence recovery capacity, and consequently partial mortality of *M. membranacea* colonies, both seasonally and interannually.

Growth of *M. membranacea* colonies through the budding of new zooids at the colony edge generates an age gradient within the colony, with central zooids being the oldest and successively younger zooids being found more distally towards the colony periphery. Thus, it is possible that the inability of colonies to regenerate following removal of the peripheral growing edge reflects intra-colonial differences in zooid function associated with zooid age.

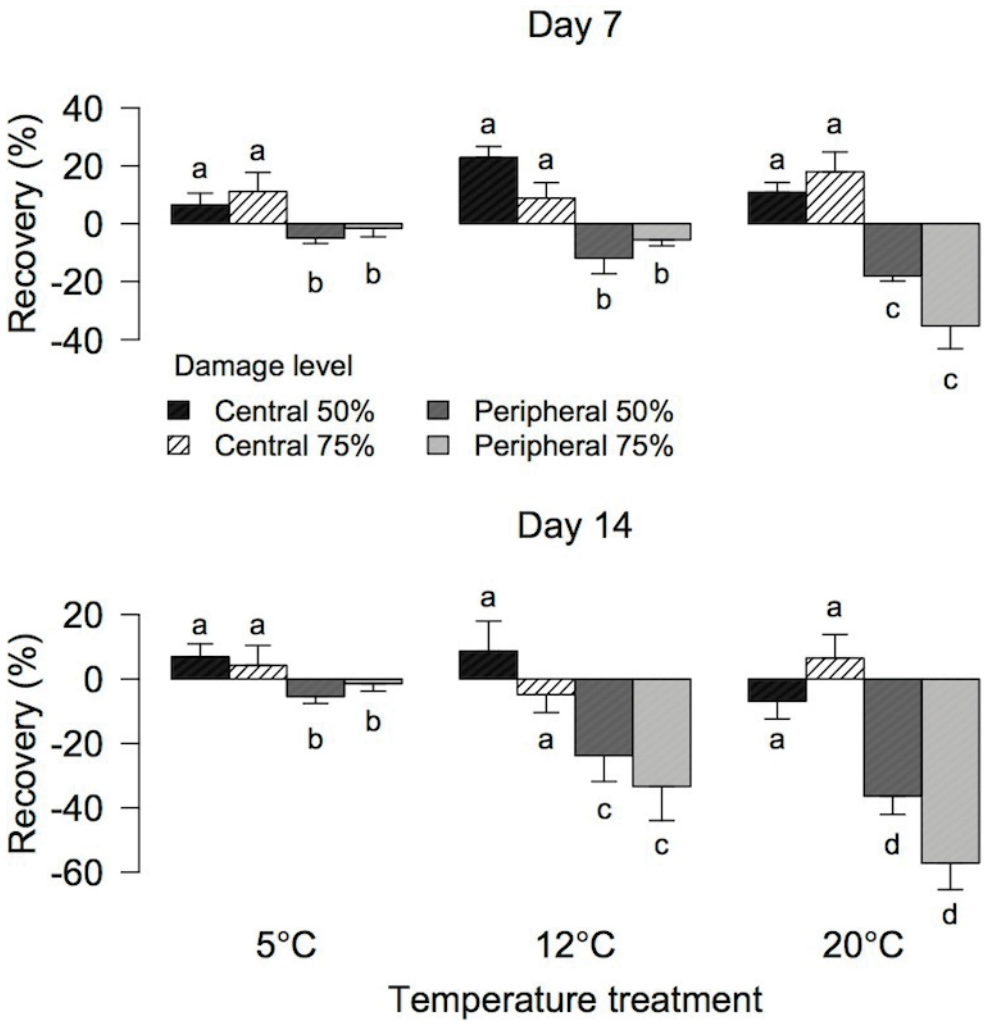

Figure 5.7 Relative recovery (mean + SE, n = 14) of *Membranipora membranacea* colonies after 7 and 14 days of 4 types of experimentally inflicted damage (50% of central zooids removed, 50% of peripheral zooids removed, 75% of central zooids removed, 75% of peripheral zooids removed) under each of 3 temperatures (5°C, 12°C, and 20°C). Relative recovery was calculated as a percentage of the initial colony size following artificially inflicted damage [relative recovery (%) = ((final surface area – initial surface area post-damage) ÷ initial surface area post-damage) x 100]. Negative values indicate negative growth or increased loss of zooids following damage. Bars with different letters are significantly different within each day at $\alpha = 0.05$ (Tukey's HSD test)

Table 5.2 Results of three-way ANOVA examining the effects of temperature (5°C, 12°C, 20°C), damage percentage (50%, 75%) and damage location (central zooids removed, peripheral zooids removed) on relative recovery of colonies after 7 and 14 days. Relative recovery was calculated as a percentage of the initial colony size following artificially inflicted damage. Significant values shown in bold ($\alpha = 0.05$)

Effect	MS	$F_{(df)}$	p
Day 7			
Temperature	0.5650	$4.755_{(2,156)}$	**0.0099**
Damage percentage	0.0047	$0.0481_{(1,156)}$	0.8267
Damage location	12.17	$124.4_{(1,156)}$	**<0.0001**
Temperature x damage percentage	0.1663	$1.670_{(2,156)}$	0.1861
Temperature x damage location	0.9733	$9.951_{(2,156)}$	**<0.0001**
Damage percentage x damage location	0.0734	$0.7502_{(1,156)}$	0.3877
Temperature x damage percentage x damage location	0.2288	$2.340_{(2,156)}$	0.0997
Error	0.0978		
Day 14			
Temperature	2.320	$13.09_{(2,156)}$	**<0.0001**
Damage percentage	0.1632	$0.9205_{(1,156)}$	0.3388
Damage location	10.44	$58.90_{(1,156)}$	**<0.0001**
Temperature x damage percentage	0.1612	$0.9092_{(2,156)}$	0.4050
Temperature x damage location	1.046	$5.901_{(2,156)}$	**0.0034**
Damage percentage x damage location	0.0004	$0.0020_{(1,156)}$	0.9641
Temperature x damage percentage x damage location	0.5100	$2.877_{(2,156)}$	0.0593
Error	0.1773		

Table 5.3 Results of one-tailed Welch's (W) and Student's (S) *t* tests comparing relative growth of control colonies with relative recovery of damaged colonies (50% or 75% of central zooids removed) for 3 different temperature treatments (5°C, 12°C, 20°C) after 7 and 14 days. Direction indicates whether growth or recovery was greater for control (c) or damaged (d) colonies. Relative growth and relative recovery were calculated as a percentage of the initial colony size and as a percentage of the initial colony size following artificially inflicted damage, respectively. Significant values shown in bold ($\alpha = 0.05$)

	t	df	*p*	Test	Direction
Day 7					
5°C					
50%	2.15	20.1	**0.022**	W	d > c
75%	2.08	15.8	**0.027**	W	d > c
12°C					
50%	-0.494	17.2	0.314	W	
75%	-1.94	25	**0.032**	S	c > d
20°C					
50%	-1.91	15.0	**0.038**	W	c > d
75%	-1.07	25.0	0.147	S	
Day 14					
5°C					
50%	2.28	17.1	**0.018**	W	d > c
75%	1.14	14.7	0.137	W	
12°C					
50%	-0.976	25	0.169	S	
75%	-2.40	25	**0.012**	S	c > d
20°C					
50%	-2.43	15.6	**0.014**	W	c > d
75%	-1.51	18.0	0.075	W	

Similarly, only young zooids of *M. membranacea* were competent to produce defensive spines in response to exposure to a predatory nudibranch extract regardless of their location within the colony, which had been experimentally manipulated (Harvell 1991). Regenerative capacity was also greater in younger peripheral regions compared to older more central regions of the cheilostome bryozoan *Dendrobeania lichenoides* (Harvell 1984). Regeneration rate has been negatively correlated with both the number of brown bodies per zooid in the damaged region, a proxy for zooid age, and the distance of the damaged region from the colony edge in *Steginoporella* (Palumbi & Jackson 1983). In contrast, central fragments of the encrusting bryozoan *Parasmittina delicatula* were capable of rapid regrowth and exhibited high survivorship following experimentally

inflicted damage in the field, suggesting this species may not be as susceptible to colony-wide mortality following partial zooid mortality (Bone & Keough 2010).

The difference in the relative regenerative capacity of central versus peripheral zooids among different species of bryozoans may be related to intracolonial transfer of resources among zooids. In cheilostome bryozoans, individual zooids are physiologically linked by perforated communication plates (Bobin 1977). The special cells in the pore plates are morphologically polar, orienting transport of lipids and other nutrients from central to peripheral zooids within the colony (Bobin 1977). In *M. membranacea*, metabolites and carbon are translocated in a distal direction only (Best & Thorpe 1985, Miles et al. 1995). In our study, the lack of recovery after 14 days when central zooids were damaged may be the result of the transfer of nutrients only from central to peripheral zooids. For these colonies, negligible relative growth does not reflect lack of recovery at the growing edge. Rather, the continued loss of central zooids coincided with the addition of new zooids through peripheral budding, resulting in net colony recovery that, however, did not differ significantly from zero.

Reallocation of resources in response to injury has been observed in *M. membranacea* (Harvell & Helling 1993). When growth of colonies was disrupted by removal of the growing edge from one half of the colony perimeter, the intact side of damaged colonies exhibited elevated rates of edge extension exceeding those of undamaged control colonies (Harvell & Helling 1993). This suggests colony-wide transfer of resources from damaged to undamaged regions of injured colonies. In our study, although the relative recovery of damaged colonies where central zooids had been removed exceeded growth of control colonies at 5°C after 7 days, this pattern was not consistent after 14 days, and growth of control colonies often exceeded recovery of damaged colonies at higher temperature treatments. Further, the level of damage, or alternatively the proportion of zooids remaining following damage, did not significantly affect recovery rate of colonies for either damage location. Similarly, colonies of the aborescent bryozoan *Bugula neritina* with an entire branch removed maintained an average growth rate similar to that of undamaged colonies and significantly greater than that of colonies from which half or all of the branching tips were removed (Bone & Keough 2005). In the encrusting *Watersipora subtorquata,* regeneration following

experimental removal of zooids was directional, occurring only along the remaining colony margin, and growth rate depended exclusively on the length of the remaining growing edge and not on the size of the colony (Hart & Keough 2009).

In our study, when peripheral zooids were damaged, further loss of zooids increased with increasing temperature. This result suggests that the effect of temperature on recovery of damaged *M. membranacea* colonies may be more complex than the positive relationship observed between temperature and growth of control colonies. Similar decoupling of growth and regeneration in response to temperature was observed in the coral *Montastrea annularis*, for which daily influxes of colder deep water inhibited regeneration of tissue lesions but not the linear growth of colonies (Lester & Bak 1985). Increased loss of zooids in peripherally damaged colonies at 20°C may have been a stress response resulting from prolonged exposure to a temperature that approximates the daily-averaged annual maximum. An increase of 3°C above ambient temperature significantly reduced growth of the bryozoan *Celleporaria nodulosa* in the laboratory during summer when ambient temperatures were already at their seasonal maximum, but had no significant effect on growth in the winter when ambient temperature was lower (Durrant & Clark 2013). However, thermal stress cannot explain the significant decrease in recovery after 14 days between 5°C and 12°C when 75% of peripheral zooids were removed, since 12°C represents an intermediate temperature in our region, to which *M. membranacea* is well adapted. More commonly, recovery of benthic marine invertebrates is associated with comparatively warmer temperatures (Menon 1972, Lester & Bak 1985, Kramarsky-Winter & Loya 2000), resulting in faster growth and regeneration of lost tissue through increased metabolic rate (eg. O'Dea & Okamura 1999, Kramarsky-Winder & Loya 2000). Conversely, it has been suggested that increased metabolic rate, while enhancing growth rate, may also lead to higher energy demands required for maintenance (Denis et al. 2011).

For modular individuals, reproductive output is a function of the total number of modules and the average number of offspring produced per module (Tuomi & Vuorisalo 1989). This makes colony growth through the asexual budding of new modules an important component of lifetime fitness in colonial organisms. Since for *M. membranacea*, older centrally located zooids typically become reproductive before

younger more peripheral zooids (Harvell & Helling 1993), investing in peripheral growth following damage to central zooids may reflect a shift in energy investment from sexual to asexual reproduction following the loss of reproductive zooids. Similarly, fitness consequences associated with lack of recovery following damage to younger peripheral zooids may be partially offset by increased reproductive output of the remaining older zooids. Although not explicitly examined here, previous studies involving *M. membranacea* have shown that damage to peripheral zooids can trigger reproduction in adjacent more centrally located zooids (Harvell & Grosberg 1988, Harvell & Helling 1993). This shift in allocation of resources between sexual and asexual reproduction may be adaptive in that it maximizes lifetime fitness in response to localized partial mortality.

CHAPTER 6

EFFECTS OF INTRINSIC AND EXTRINSIC FACTORS ON REPRODUCTION OF AN ECOLOGICALLY SIGNIFICANT INVASIVE BRYOZOAN: IMPLICATIONS FOR INVASION SUCCESS[5]

6.1 Abstract

The capacity to predict changes in the distribution and abundance of sessile marine invertebrates depends on quantifying factors that affect spatial and temporal variation in propagule production. This is particularly important for invasive species, since both the timing and abundance of introduced propagules can determine invasion success. This study examines the role of reproductive dynamics in contributing to the invasion success of the highly invasive bryozoan *Membranipora membranacea* by quantifying temporal patterns in the sexual stage and potential fecundity of colonies, and examining the effects of intrinsic (colony size) and extrinsic (temperature, host substrate) factors on the reproductive potential of invasive populations. Colonies were collected on the 4 most abundant algal substrates in Nova Scotia at 2-3 sites approximately monthly from 2012 to 2015. Individual zooids within each collected colony were classified by sexual stage (immature, producing sperm only, producing sperm and oocytes, producing oocytes only), and the number of oocytes per colony was counted as a measure of potential colony fecundity (reproductive potential). There were significant seasonal patterns in colony fecundity, however, temperature and colony size accounted for only 12% and 7% of the observed variation, respectively. Instead, consistent differences in reproductive potential were observed among colonies on different algal substrates. Our results indicate how critical demographic processes can differ for invasive species outside of their native range. Our study underscores the need to quantify population dynamics of

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non-indigenous species within the invaded community to more accurately predict the long-term consequences for invaded ecosystems.

6.2 Introduction

For benthic marine invertebrates, abundance and distribution of adults are directly related to the recruitment of dispersive propagules (Underwood & Fairweather 1989); variation in the magnitude and/or temporal patterns of larval recruitment can alter community composition (Sams & Keough 2012a, b). In fact, spatial and temporal patterns of recruitment may be more strongly related to variations in fecundity than to adult abundance (Hughes et al. 2000). Therefore, the ability to predict future changes in the structure and function of marine invertebrate communities requires identification of the underlying factors driving reproductive dynamics. This is particularly relevant for invasive species, as reproductive capacity and phenology can contribute to initial invasion success (Carlton 1996, Ruiz et al. 1997, Edwards & Stachowicz 2010), as well as persistence within the invaded community post-establishment (Pöckl 2007, Lord 2016).

Membranipora membranacea is an encrusting colonial bryozoan and a highly successful invader that was introduced to the northwest Atlantic in the late 1980s and has since become the dominant epiphyte on laminarian kelps in its invaded habitat (Berman et al. 1992, Lambert et al. 1992). Zooids of *M. membranacea* are protandrous hermaphrodites, and transition sequentially from producing sperm only, to producing both sperm and oocytes, to producing only oocytes (Ryland 1976). Internally fertilized eggs are released into the water column where they develop into free-swimming planktotrophic larvae (cyphonautes) which remain in the plankton for approximately 4 weeks (Yoshioka 1973, Temkin 1994). Within its native range off the west coast of North America, colonies occur in dense populations on kelp blades (often occupying 100% of the surface area on distal portions of blades), and are seasonally abundant from May until September (Harvell 1985). In Nova Scotia, larvae typically begin to settle in June-July (Saunders & Metaxas 2007, Denley et al. 2014) and colonies begin to senesce in late autumn (October-November); however, a small proportion of the population (<1%) is

biennial, persisting over winter, presumably providing larvae for the following season (Saunders & Metaxas 2009).

In native populations of *M. membranacea*, onset of sexual maturity (production of oocytes) is triggered by both crowding by conspecifics and grazing by specialized nudibranch predators (Harvell & Grosberg 1998). In Nova Scotia, crowding by conspecifics does not occur until October-November, and percent cover of *M. membranacea* on kelp blades rarely reaches 100% (D. Denley, pers obs). Additionally, the predatory nudibranch *Onchidoris muricata* only exhibits low abundance in Nova Scotia, and its impact on *M. membranacea* is negligible (Chapman et al. 2002, Pratt & Grason 2007). Instead, we hypothesize that the reproductive dynamics of *M. membranacea* in its invaded habitat are primarily influenced by interacting effects of both organismal and environmental factors, specifically colony size, temperature, and algal substrate.

For colonial organisms such as *M. membranacea*, fecundity is related to survival and reproduction at the level of the module rather than the entire colony (Tuomi & Vuorisalo 1989). Consequently, indeterminate growth of colonial organisms allows fecundity to theoretically increase indefinitely with colony size (Ramirez-Llodra 2002). However, extrinsic factors that affect colony growth can influence reproductive phenology and fecundity (Harvell 1992, Harvell & Helling 1993, Van Veghel & Bak 1994). Furthermore, for epiphytic species growing on ephemeral substrates, seasonal cycles of growth and reproduction are often correlated with those of the host (e.g. Eggleston 1972). As for many wide-spread invasive fouling species (e.g. Stachowicz et al. 2002, Lord 2016), the timing of the onset of settlement of *M. membranacea* is strongly related to temperature (Saunders & Metaxas 2007, 2008, Saunders et al. 2010), suggesting that reproduction may also be seasonal, potentially related to variations in temperature. However, temporal patterns of reproduction have not been quantified for *M. membranacea* on natural (algal) substrates over multiple years in native or invaded habitats. In addition, the specific intrinsic (organismal) and/or extrinsic (environmental) factors affecting reproduction in invasive populations of *M. membranacea* in the northwest Atlantic have yet to be identified.

In this study, we quantify temporal patterns in the sexual stage (immature, sperm present, sperm and oocytes present, oocytes present) and potential fecundity (number of oocytes per colony) of colonies, and examine the effects of intrinsic (colony size) and extrinsic (temperature and algal substrate) factors on the reproductive potential of *M. membranacea* in its invaded habitat in Nova Scotia. We aim to relate variations in organismal and environmental conditions to observed shifts in the timing and magnitude of *M. membranacea* outbreaks in Nova Scotia, with implications for predicting the persistence and spread of *M. membranacea* within the northwest Atlantic. In addition, our results can be used to examine potential differences in reproductive dynamics between native and invasive populations of an ecologically significant invasive species, and to identify reproductive strategies that confer strong invasive potential in marine invertebrates.

6.3 Methods

6.3.1 Study Sites

We collected colonies of *Membranipora membranacea* from 3 sites on the southwestern shore of Nova Scotia, Canada: The Lodge (44°33'3"N, 64° 01' 9" W) on the western shore of St. Margarets Bay, Paddy's Head (44°31'6"N, 63°57'2"W) on the eastern shore near the mouth of St. Margarets Bay, and Sandy Cove (44° 27' 6" N, 63° 42' 4" W) in Terence Bay, 20 km to the northeast of St. Margarets Bay. All sites harbour mixed kelp beds, dominated by *Saccharina latissima*, *Laminaria digitata*, and *Agarum clathratum*, with fucoid species present at depths of <4 m (see Saunders & Metaxas 2009b for a detailed description of study sites). Temperature was measured at 10-min intervals at 4, 8, and 12 m at The Lodge and Paddy's Head, and at 4 and 8 m at Sandy Cove for the duration of the study (June 2012-November 2015) using HOBO® pendant loggers.

6.3.2 Collection of *M. membranacea* colonies

We sampled colonies of *M. membranacea* on *S. latissima*, *L. digitata*, *A. clathratum,* and *F. evanescens* using SCUBA at all 3 sites approximately every 6 weeks

from 18 June 2012 to 2 August 2013, and approximately monthly from May 2014 to November 2015 (when conditions allowed) at The Lodge (July 2014 - November 2014 and April 2015 - November 2015) and Sandy Cove (May 2014 - November 2014 and March 2015 - November 2015). During each sampling time from June 2012 to August 2013, we randomly collected 10-15 fronds of each kelp species at each depth (4 m and 8 m at all sites and 12 m at The Lodge and Paddy's Head), as well as all *F. evanescens* in 8-10 0.25-m^2 quadrats at <4 m depth. In 2014 and 2015, we collected haphazardly ~15-30 bryozoan colonies growing on each of the 3 kelp substrates by removing sections of kelp blades with colonies using dive knives. A single colony was collected per kelp frond, and collected colonies spanned the entire range of diameter present in each sampling month. In addition, we sampled colonies on *F. evanescens* by haphazardly collecting ~ 3 kg wet weight of *F. evanescens* during each sampling time. Collected algae were transported to Dalhousie University in coolers without seawater and maintained in aquaria with running ambient seawater until processing was complete (~3 days).

6.3.3 Processing of *M. membranacea* colonies

For all colonies collected from July 2012 to August 2013 (n = 2-211 colonies per algal substrate per sampling time), we measured colony diameter (length for colonies on *F. evanescens*, see below) and classified colonies as being in one of 5 size classes: <1 cm, 1-3 cm, 3-6 cm, 6-8 cm, and >8 cm diameter. For colonies collected in 2014 and 2015, we measured the diameter (length for colonies on *F. evanescens*) of each colony (2014, n = 1-90; 2015, n = 1-61 colonies per algal substrate per sampling time) to the nearest 0.5 cm. We also measured the area (mm^2) of 15 haphazardly selected zooids per colony under the microscope using stage and ocular micrometers for 10-26 colonies collected on each kelp substrate from The Lodge and Sandy Cove during each sampling time, and for 10 colonies collected on *F. evanescens* at The Lodge in September 2016.

To determine the total number of zooids per colony, we collected and photographed 100 colonies of *M. membranacea* on *L. digitata* from Sandy Cove on 14 August 2013, and measured colony diameter and surface area from the photographs using ImageJ. We used these measurements to generate an equation relating colony diameter to colony area (Figure D.1) and applied this equation to convert our measurements of

colony diameter to colony surface area for all colonies collected on kelp from 2012 to 2015. On fucoid substrates where space for colony growth is limited, colonies of *M. membranacea* are typically rectangular in shape, in correspondence with the morphology of individual *Fucus* spp. fronds. Therefore, for colonies collected on *F. evanescens*, colony length was substituted for colony diameter, and colony area was estimated as colony length multiplied by colony width. The average width of *F. evanescens* fronds is ~1 cm, and although fronds can be as wide as ~2.5 cm at their widest point, colonies grow along the entire length of the frond, which tapers basally to widths of <<1 cm. Consequently, colony area was estimated by multiplying colony length by 1 cm width, as colonies often extend to the margins of *F. evanescens* fronds. For colonies for which diameter and length were measured categorically (July 2012 to August 2013), the midpoint of categorical size ranges was taken as the colony diameter/length. To estimate the number of zooids per colony, we then divided colony area (mm^2) by the species-specific average zooid area (mm^2) for each algal substrate.

Zooid area was consistent across sampling times for each kelp substrate (mean + SD, *S. latissima*: 0.265 ± 0.062 mm^2, n = 1715; *L. digitata*: 0.245 ± 0.057 mm^2, n = 1645; *A. clathratum*: 0.261 ± 0.062 mm^2, n = 1146), and zooid areas were similar on kelp and fucoid (*F. evanescens*: 0.244 ± 0.037 mm^2, n = 150) substrates.

6.3.4 Quantifying temporal patterns in sexual stage and colony fecundity

For algae collected from July 2012-August 2013 we haphazardly selected one colony of each size class per kelp blade and 15-30 colonies of each size class on *F. evanescens* (when available) and classified a subset of zooids within each colony (15 zooids for colonies ≤3 cm in diameter, 30 zooids for colonies >3 cm in diameter) by sexual stage (immature, only sperm present, sperm and oocytes present, only oocytes present) using a Nikon SMZ1500 stereomicroscope. For bryozoan colonies collected in 2014-2015, we classified a subset of zooids within each measured colony (15-150 zooids depending on colony diameter) by sexual stage.

For all sampling times, when oocytes were present, we also counted the number of oocytes per zooid in zooids containing ≤10 oocytes. Oocytes could not be counted accurately in zooids with >10 oocytes, which were recorded as containing 10 oocytes.

Consequently, our calculations of colony fecundity underestimate actual colony fecundity, however only 40 colonies contained zooids with >10 oocytes across all sampling times.

We examined seasonal patterns in the sexual stage and reproductive potential of *M. membranacea* colonies using 2 metrics: 1) the proportion of zooids in each sexual stage, and 2) potential fecundity. We calculated the proportion of zooids per colony that were in each sexual stage by dividing the number of zooids in each stage by the total number of zooids classified for that colony. We calculated potential colony fecundity (number of oocytes per colony, from here on referred to as fecundity) as the average number of oocytes per zooid for zooids in the latter 2 sexual stages (i.e. producing oocytes), multiplied by the proportion of zooids sampled per colony that contain oocytes and by the total number of zooids per colony.

Colony diameter was not measured for colonies collected in June 2012; however, a subset of 30 zooids from each colony (n = 157) was classified as described for July 2012-August 2013. For these colonies, lack of data on colony diameter prohibited accurate estimates of colony fecundity, limiting our examination to the proportion of zooids in each sexual stage for colonies collected at this sampling date.

6.3.5 Data analysis

6.3.5.1 Temporal patterns and the effect of algal substrate on fecundity

We examined the effects of sampling month (random effect nested within site) and algal substrate (fixed effect, 4 levels: *S. latissima, L. digitata, A. clathratum, F. evanescens*) on the fecundity of colonies using linear mixed models (LMM) with separate intercepts for the random effect of site. For 2012 - 2013, when multiple colonies were collected from the same kelp frond, individual kelp frond was also included as a random factor nested within month and site. Fixed and random structures of the models were tested using maximum likelihood (ML) and restricted maximum likelihood (REML) estimations, respectively. *P*-values for model selection were obtained from likelihood ratios using the χ^2_p distribution and the 0.5 x χ^2_1 distribution when appropriate (Zuur et al.

2009). Post hoc comparisons were conducted with Tukey's HSD tests (significant at $\alpha = 0.05$).

6.3.5.2 Effects of temperature on fecundity

We examined the effect of two temperature indices (growing degree-day [GDD], and monthly average temperature) on colony fecundity using simple linear regressions, and also using quadratic regressions for GDD. For each site, thermal history (GDD) was calculated after Saunders and Metaxas (2007) by sequentially adding standardized daily average temperatures from 1 January of each year until each specific sampling day. In Nova Scotia, the coldest months of the year are January to March; therefore, we considered January to be the onset of the thermal season and calculated GDD from January of each year onwards. Monthly averaged temperature was calculated as the average of daily average temperatures for each month, in correspondence with approximately monthly sampling intervals. For both temperature indices, temperature was averaged across depths (The Lodge and Paddy's Head: 4, 8, and 12 m; Sandy Cove: 4, and 8 m), since colonies were collected over the entire depth range during each sampling time.

We identified spatial and temporal variation in winter/spring temperature regime (preceding peak reproduction of *M. membranacea*) using two-way ANOVA of the effects of site and year on GDD on June 30. We chose June 30 as the end of the spring temperature regime, as it precedes the period of most rapid increase in GDD from July to October (Figure D.2). Year was considered a fixed effect, and included all years where complete temperature records (January 01-June 30) were available for the current study (2013, 2014, 2015). We also examined the relationship between thermal history and the timing of the observed onset of reproduction of *M. membranacea* using simple linear regression of the day of year [1 (January 1)-365 (December 31)] of the onset of reproduction (determined as the earliest observation of zooids containing male and/or female gametes) and GDD (on the day of onset of reproduction).

Finally, we used cross-correlation analyses to identify potential correlations between the fecundity of colonies and temperature *n* months earlier (negative lag) by

relating fecundity with monthly average temperature as a function of a time lag (n, in months) for each site.

6.3.5.3 Effects of colony size on fecundity

We examined the effect of colony size (diameter) on fecundity of colonies for all colonies pooled across sites and sampling dates using simple linear regression. To determine whether the relationship between colony size and fecundity differed among algal substrates, the effect of alga on the fecundity of colonies was examined using one-way ANCOVA with colony size as the covariate. For colonies for which size was measured categorically (June 2012 to August 2013), the midpoint of categorical size ranges was taken as the colony diameter.

For all analyses, colony fecundity was log or log (x+0.01)-transformed to better approximate the normal distribution and to reduce heterogeneity of variance. Visual examination of residual plots for LMMs revealed deviations from normality that could not be completely alleviated through data transformation. Similarly, data transformation did not fully correct non-normal distribution for regression analyses (Shapiro-Wilk test, $p < 0.05$); however, regression analyses are robust to deviations from normality (Zar 1999).

6.4 Results

6.4.1 Temporal patterns and the effect of algal substrate on sexual stage and fecundity

Overall, temporal patterns in the sexual stage of colonies were fairly consistent across all 3 sampling periods (June 2012-August 2013, May 2014-November 2014, March 2015-November 2015). Over the reproductive season, the earliest occurrence of gametes within colonies was in March in 2013 and in May in 2014 and 2015, and was characterized by colonies producing male gametes (sperm) only (Figure 6.1). Although we only began sampling colonies in May of 2014, no collected colonies ($n = 7$) during preliminary sampling on May 9 contained zooids in any stage of reproduction.

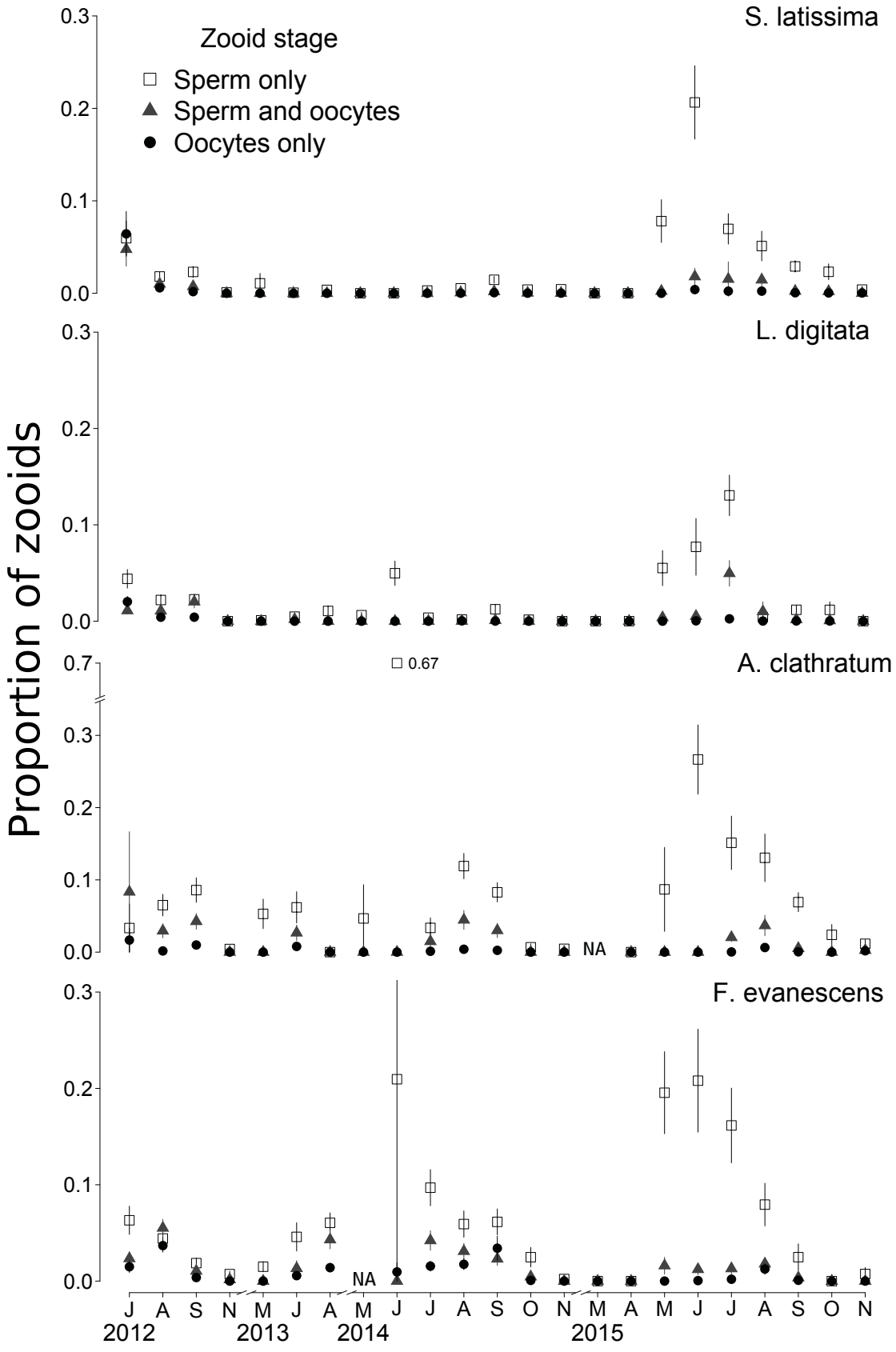
Zooid stage
Sperm only
Sperm and oocytes
Oocytes only
S. latissima
L. digitata
A. clathratum
F. evanescens
Proportion of zooids
0.67
NA
NA
J A S N M J A M J J A S O N M A M J J A S O N
2012
2013
2014
2015

Figure 6.1 Proportion of zooids per colony of *Membranipora membranacea* (mean + SE, n = 1-211) in each of 3 sexual stages (producing sperm only, producing sperm and oocytes, producing oocytes only) collected on the 4 most numerically abundant algal substrates in Nova Scotia (*Saccharina latissima*, *Laminaria digitata*, *Agarum clathratum*, and *Fucus evanescens*) at The Lodge, Sandy Cove, and Paddy's Head from June 2012 to August 2013; and at The Lodge from July 2014 to November 2015 and Sandy Cove from May 2014 to November 2015. The proportion of zooids is scaled differently for *A. clathratum* to accommodate the comparatively higher proportion of reproductive zooids in colonies on this substrate. Letters on the x-axis refer to the following months: 2012: J – Jun, A – Aug, S – Sep, N – Nov; 2013: M – Mar, J – Jun A – Aug; 2014: M – May, J – Jun, J – Jul, A – Aug, S – Sep, O – Oct, N – Nov; 2015: M – Mar, A – Apr, M – May, J – Jun, J – Jul, A – Aug, S – Sep, O – Oct, N – Nov. NA indicates no colonies were present at the time of collection

Additional colonies (n = 62) were collected on 20 May, and data were pooled across both sampling dates to generate a monthly average. Therefore, the occurrence of gametes in May 2014 reflects the true onset of reproduction for that year. Colonies produced predominantly sperm for the duration of the reproductive season, which ended in November in all 3 years. Female gametes (oocytes) were produced by colonies only during late spring and summer (May-September), and a comparatively small proportion of zooids within colonies produced exclusively oocytes and only during mid-late summer (July-September) (Figure 6.1). However, the seasonal timing and the relative proportion of zooids per colony in each sexual stage differed among algal substrates. A higher proportion of zooids contained gametes (sperm and/or oocytes), and zooids produced gametes for more months of the year, in colonies on *Agarum clathratum* and *Fucus evanescens* than on *Saccharina latissima* or *Laminaria digitata* (Figure 6.1). In 2014 in particular, gamete production occurred almost exclusively in colonies on *A. clathratum* and *F. evanescens*, and those colonies that did produce gametes on *S. latissima* and *L. digitata* produced only sperm (Figure 6.1).

Among site variability in colony fecundity was not significant for any sampling period, however, there was a significant effect of individual kelp frond on the fecundity of colonies collected in 2012-2013 (Table 6.1). There was a significant effect of sampling month within period on fecundity for all sampling periods (Table 6.1, Figure 6.2). The effect of algal substrate was also significant over all 3 sampling periods, with colony fecundity being consistently greater on *A. clathratum* and *F. evanescens* than on *S.*

latissima or *L. digitata*, except in 2015 when high variability in fecundity precluded the detection of significant differences among algal substrates (Table 6.1, Figure 6.2). Increased variability in fecundity in 2015 was due to a few highly fecund colonies (n = 12) in a particular patch of *L. digitata* at Sandy Cove.

Table 6.1 Results of linear mixed effects models examining the effects of sampling month, algal substrate, and site (and individual kelp frond for 2012-2013 only) on potential fecundity (number of oocytes per colony, log (x + 0.01)-transformed) of *Membranipora membranacea* collected on 4 substrates (SL: *Saccharina latissima,* LD: *Laminaria digitata,* AC: *Agarum clathratum,* and Fu: *Fucus evanescens*) at 2-3 sites (2012-2013: The Lodge, Sandy Cove, Paddy's Head; 2014 and 2015: The Lodge, Sandy Cove) from June 2012-November 2015. Significant p-values shown in bold ($\alpha = 0.05$)

	$\chi^2_{(df)}$	p	Tukey's HSD ($\alpha = 0.05$)
2012-2013			
Fixed effects			
Algae	$34.8_{(3)}$	**<0.0001**	SL < LD < AC =Fu
Random effects			
Site	$0.00_{(1)}$	1.00	
Month	$69.3_{(1)}$	**<0.0001**	
Kelp frond	$37.2_{(1)}$	**<0.0001**	
2014			
Fixed effects			
Algae	$139_{(3)}$	**<0.0001**	SL = LD < AC = Fu
Random effects			
Site	$0.00_{(1)}$	1.00	
Month	$54.4_{(1)}$	**<0.0001**	
2015			
Fixed effects			
Algae	$9.23_{(3)}$	**0.026**	No detectable difference
Random effects			
Site	$0.00_{(1)}$	1.00	
Month	$141_{(1)}$	**<0.0001**	

6.4.2 Effects of temperature on fecundity

There was a significant positive relationship between colony fecundity and monthly average temperature; however, monthly average temperature explained only a small amount of the variation in fecundity (log(Fecundity) = 0.254 ºC + 2.35, $r^2 = 0.12$, $p = 0.016$, $n = 43$; Figure 6.3). Instead, a significant quadratic relationship with GDD

accounted for a substantially larger amount of the variance in fecundity, with thermal history explaining 60% of the variation in fecundity of colonies ($\log(\text{Fecundity} + 0.01) = -4.7e^{-6}\,\text{GDD}^2 + 0.0172\,\text{GDD} - 9.10$, $r^2 = 0.60$, $p < 0.0001$, $n = 47$; Figure 6.3).

The winter/spring temperature regime (GDD from January 1 to June 30) was significantly lower in 2015 than in 2013 and 2014 (ANOVA, $F_{(2,4)} = 63.7$, $p = 0.001$), and there was no evidence of an interaction between site and year (Figure D.2); however, no significant relationship existed between thermal history (GDD) and the recorded timing of the onset of reproduction of *Membranipora membranacea* (Linear regression, $r^2 = 0.49$, $F_{(1,4)} = 5.75$, $p = 0.075$).

Cross-correlation analysis revealed a significant positive correlation between colony fecundity and monthly average temperature measured 1 month earlier (time lag -1) at The Lodge, and a significant negative correlation between colony fecundity and monthly average temperature measured 4-5 months earlier (time lag -4 to -5) at Sandy Cove (Figure D.3). However, there was no significant correlation between colony fecundity and monthly average temperature at Paddy's Head. Patterns of cross-correlation differed between Paddy's Head and the other two sites, presumably because of fewer samples in the former site (Figure D.3).

6.4.3 Effects of colony size on fecundity

There was a significant positive relationship between fecundity of colonies pooled across all algal substrates ($n = 4536$) and colony size (diameter), but colony size accounted for only a small proportion of the variation (Linear regression, $r^2 = 0.07$, $F_{(1,4534)} = 315$, $p < 0.0001$). The relationship between colony size and fecundity differed significantly among algal substrates (Table 6.2), with the slopes of the regressions being steepest for *A. clathratum* and *F. evanescens* (Figure 6.4).

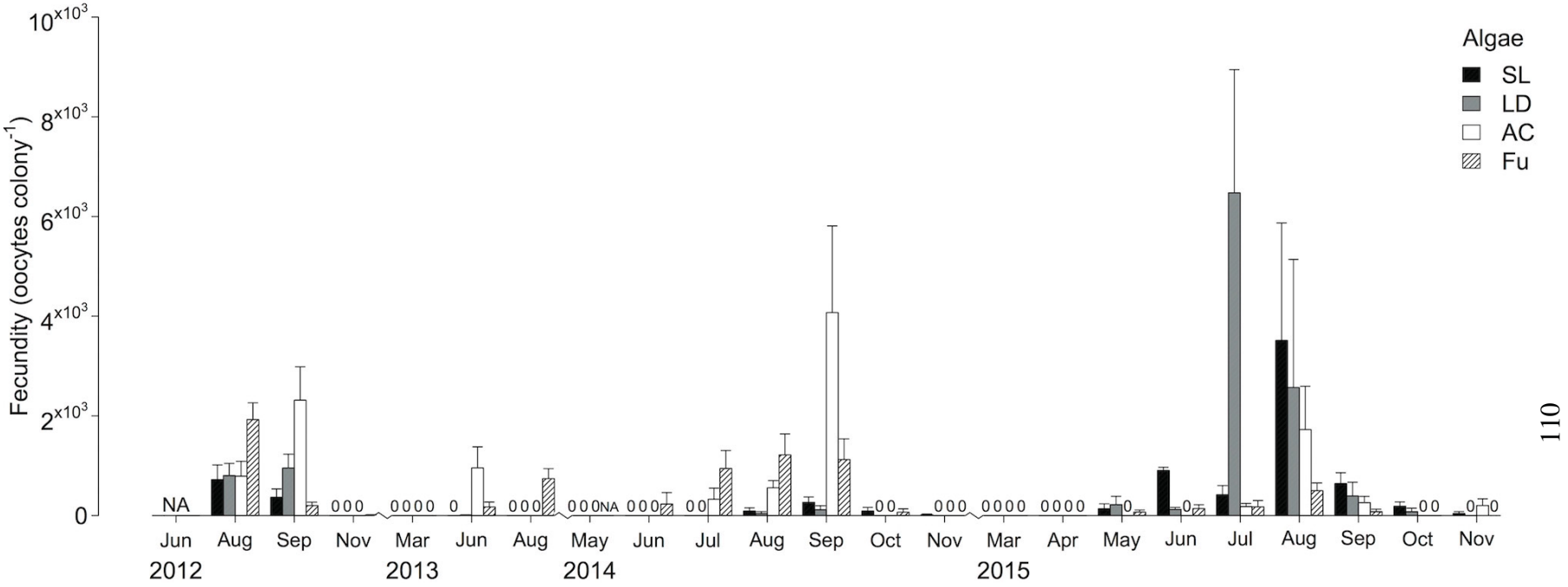

Figure 6.2 Potential fecundity (mean + SE, n = 1 - 211) of colonies of *Membranipora membranacea* collected on the 4 most numerically abundant algal substrates in Nova Scotia (SL: *Saccharina latissima*, LD: *Laminaria digitata*, AC: *Agarum clathratum*, and Fu: *Fucus evanescens*) at The Lodge, Sandy Cove, and Paddy's Head from June 2012 to August 2013; and at The Lodge from July 2014 to November 2015 and Sandy Cove from May 2014 to November 2015. *Zeros* indicate colonies were not fecund (fecundity = 0). *NA* indicates colony fecundity could not be accurately measured (June 2012, see 6.3.4 Quantifying temporal patterns in sexual stage and colony fecundity) or colonies were not present on the algal substrate (May 2014)

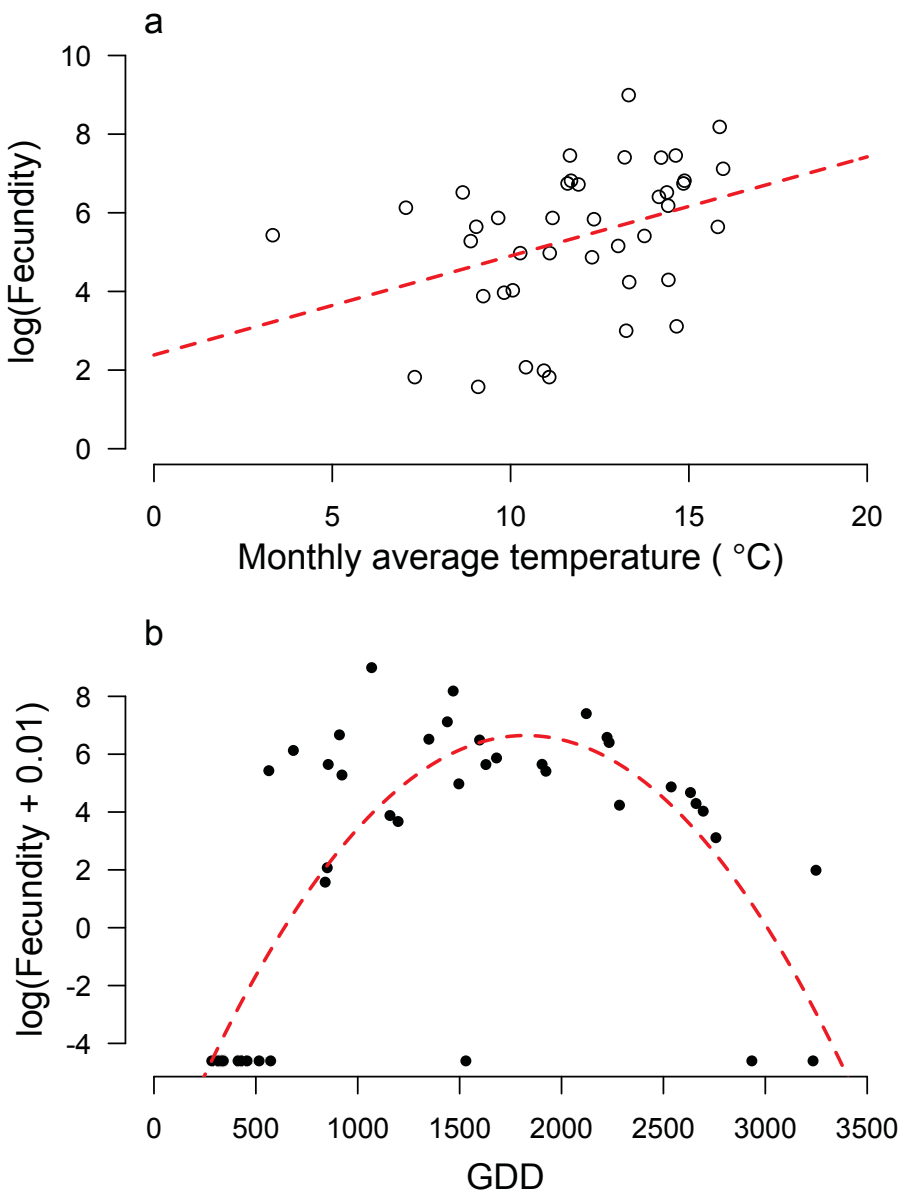

Figure 6.3 Relationships between a) monthly average temperature and potential fecundity [log(Fecundity) = 0.254 ºC + 2.35, $r^2 = 0.12$, $p = 0.016$, $n = 43$] and b) thermal history (GDD) and potential fecundity [log(Fecundity + 0.01) = $-4.7e^{-6}$ GDD^2 + 0.0172 GDD – 9.10, $r^2 = 0.60$, $p < 0.0001$, $n = 47$] of colonies of *Membranipora membranacea* collected on the 4 most numerically abundant algal substrates in Nova Scotia (*Saccharina latissima*, *Laminaria digitata*, *Agarum clathratum*, and *Fucus evanescens*) at The Lodge, Sandy Cove, and Paddy's Head from June 2012 to August 2013; and at The Lodge from July 2014 to November 2015 and Sandy Cove from May 2014 to November 2015. For the relationship with monthly average temperature, only months for which average colony fecundity >0 are shown ($n = 43$)

Table 6.2 Results of ANCOVA examining the effects of algal substrate (fixed factor, 4 levels: *Saccharina latissima, Laminaria digitata, Agarum clathratum, Fucus evanescens*) and colony size (diameter cm, covariate) on potential fecundity (number of oocytes per colony, log (x + 0.01)-transformed) of *Membranipora membranacea*

Factor	MS	*df*	*F*	*p*
Alga	753.1	3	67.50	<0.0001
Size	5039	1	451.6	<0.0001
Alga x size	1388	3	124.4	<0.0001
Error	11.20	4528		

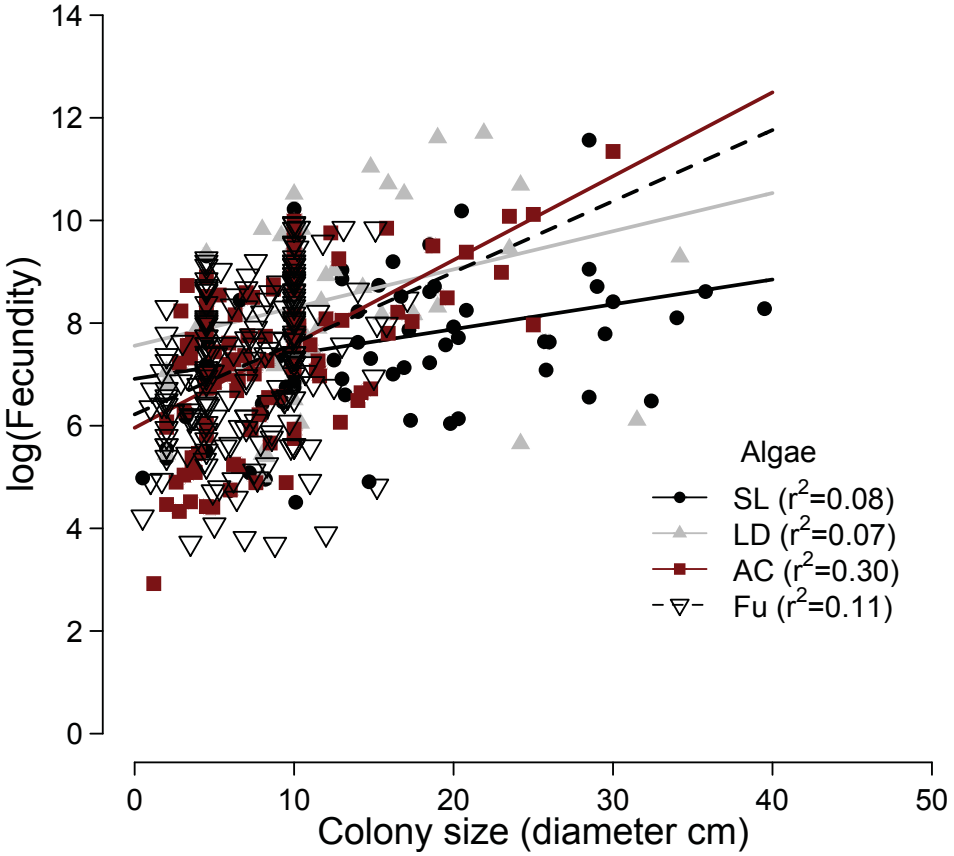

Figure 6.4 Relationships between potential fecundity and size [diameter (cm) = D] of colonies of *Membranipora membranacea* collected on the 4 most numerically abundant algal substrates in Nova Scotia (SL: *Saccharina latissima*, LD: *Laminaria digitata*, AC: *Agarum clathratum*, and Fu: *Fucus evanescens*) at The Lodge, Sandy Cove, and Paddy's Head from June 2012 to August 2013; and at The Lodge from July 2014 to November 2015 and Sandy Cove from May 2014 to November 2015. Only colonies for which fecundity >0 are shown (n = 462). Regression equations: SL: log(Fecundity) = 0.048 D + 6.91, p = 0.007; LD: log(Fecundity) = 0.074 D + 7.56, p = 0.016; AC: log(Fecundity) = 0.163 D + 5.96, p < 0.001; Fu: log(Fecundity) = 0.139 D + 6.17, p < 0.001

6.5 Discussion

6.5.1 Temporal patterns in sexual stage and the effects of intrinsic and extrinsic factors on reproductive potential

It is not uncommon in hermaphroditic marine invertebrates for initial sexual allocation to be predominantly towards sperm, with proportional investment in oocytes often increasing with increasing size and/or age (e.g. Rinkevich & Loya 1979, Cancino & Hughes 1987, Hall & Hughes 1996) or in response to more favourable environmental conditions (e.g. Hunter & Hughes 1995, Vizoso & Schärer 2007, but see Cancino & Hughes 1987). Zooids of *Membranipora membranacea* may invest primarily in male reproduction if they lack the resources required to produce oocytes. At the level of the individual zooid, producing sperm, on the off chance that other zooids are investing in female reproduction, affords greater potential for fitness gain than remaining sexually immature. It has been suggested that in its native range, gamete production by zooids is triggered when net carbon input exceeds threshold levels required for maintenance (Harvell & Helling 1993). Thus, the sexual stage of semi-autonomous zooids may be determined by their individual energy budgets.

There was considerable intra- and inter-annual variation in fecundity of *M. membranacea* that could not be fully explained by relationships with the temperature indices. While increased reproductive potential of colonies corresponded with higher monthly average temperatures, significant quadratic relationships between GDD and fecundity imply that relationships with temperature may primarily reflect similar seasonal patterns rather than a causative mechanism. Furthermore, although winter/spring temperature (GDD from January 1) can be a strong predictor of the timing of onset of larval settlement (Saunders & Metaxas 2008), significantly lower GDD in winter and spring of 2015 than in 2013 and 2014 had no corresponding effect on the timing of the onset of reproduction. This suggests that the relationship between ocean temperature over winter and the onset of settlement of larval *M. membranacea* may be driven primarily by the effect of temperature on rates of larval development (O'Connor et al. 2007), rather than a direct relationship between temperature and reproduction of over-wintering colonies.

For many species of colonial organisms, a minimum size at sexual maturity exists above which reproductive output typically increases with colony size (Rinkevich & Loya 1979, Hall & Hughes 1996, Ritzmann et al. 2009). This does not appear to be the case for *M. membranacea*, as gametes were produced by colonies spanning the entire size range (~0.5 – 45 cm diameter, Figure D.4) and colony size explained only a small amount of the variation in fecundity. For native populations, the onset of sexual maturity is thought to be related to external factors that restrict growth, which in turn can trigger production of oocytes in all but the smallest colonies (< 3-6 mm diameter) (Harvell & Grosberg 1988). In Nova Scotia, the overall fecundity of colonies, as well as the rate of increase in fecundity with colony size, was greater for colonies occurring on *Agarum clathratum* and *Fucus evanescens* than on *Saccharina latissima* and *Laminaria digitata*. The effect of algal substrate on reproductive potential may be related to the unique species-specific growth dynamics and morphology of the predominant kelp and fucoid substrates (see Saunders & Metaxas 2009b, Krumhansl & Scheibling 2011, Yorke & Metaxas 2011). Blades of *S. latissima* and *L. digitata* provide a large, continuous surface for growth and colonization by the bryozoan, and the comparatively fast growth rates of these kelps ensure that new unoccupied substrate is continually being produced at the basal end of the blade. In contrast, the limited surface area available on *F. evanescens* becomes rapidly occupied, and colony growth is often impeded, either by conspecifics or other epifauna. *A. clathratum* is perforated with holes that may act as barriers inhibiting or slowing growth of *M. membranacea*. We postulate that on *S. latissima* and *L. digitata*, the bryozoan colonies allocate available energy resources predominantly towards growth, leaving less energy remaining to invest in sexual reproduction. Conversely, on *A. clathratum* and *F. evanescens*, where colony growth is restricted, colonies redirect excess energy towards increased reproductive potential (Figure D.4).

6.5.2 Implications for invasive potential

The reproductive dynamics of *M. membranacea* in the northwest Atlantic differ from those of native populations. Off the coast of Washington, even when growth is unobstructed, large colonies (> 10 cm diameter) eventually stop growing and become reproductive (Harvell 1985). There was no evidence of limiting growth in favor of

reproducing for *M. membranacea* in Nova Scotia, as unimpeded colonies remained sexually immature to a maximum recorded size of 44.5 cm diameter. Also, the relationship between colony size and fecundity was negligible, challenging the assumption, based on native populations, that fecundity is directly proportional to colony size (Yoshioka 1973). Consequently, equating large colony size with increased reproductive potential (e.g. Caines & Gagnon 2012) may be inappropriate for invasive populations of *M. membranacea*. It is possible that release from specialist nudibranch predators in invaded habitats allows for continued colony growth in the absence of grazing damage, which targets actively dividing zooids along the colony periphery (Harvell 1985).

Rapid colony growth and the ability to reach large size has had profound consequences for the population dynamics of *M. membranacea* in northwest Atlantic. Orientated growth towards proximal regions of the blades of *S. latissima* and *L. digitata* (see Ryland & Stebbing 1971, Norton 1973) allows portions of large colonies to persist over-winter on perennial kelp tissue, such as the stipe and basal meristem (Saunders & Metaxas 2009b, Yorke & Metaxas 2012), providing a larval source for the following year. In native populations, the origin of spring larvae remains unknown (Harvell et al. 1990).

Unexpectedly, there was a minimal effect of temperature on fecundity, at least within the range examined in this study (0.36-16.0 °C, monthly averaged temperature), suggesting that observed relationships between temperature and settler abundance in invaded habitats (e.g. Saunders & Metaxas 2007, Caines & Gagnon 2012) may be driven primarily by pelagic processes affecting the larval stage (see Saunders & Metaxas 2010). Our results suggest that northward spread of the bryozoan in the northwest Atlantic will not be limited by the impact of temperature on reproductive potential. However, in Newfoundland and Labrador, near the northern limit of *M. membranacea* in the northwest Atlantic, thermal history over an interval that included the reproductive period was a better predictor of settler abundance than thermal integrals encompassing the larval stage duration alone (Caines & Gagnon 2012). Therefore, it is possible that temperature may have a stronger influence on fecundity of *M. membranacea* in colder marine environments.

One of the more consequential findings of our study is the influence of algal substrate on the reproductive potential of *M. membranacea*. The predominant algal substrates in Nova Scotia are differentially susceptible to loss and/or defoliation as a result of encrustation by *M. membranacea* (Saunders & Metaxas 2008, 2009b). In particular, *A. clathratum* and *Fucus* spp. seem to be less vulnerable than *S. latissima* to recurrent outbreaks of *M. membranacea*. If species-specific resistance to infestation by *M. membranacea* continues to result in changes in the relative composition of algal communities in the northwest Atlantic, the effect of algal substrate on colony fecundity may enhance the reproductive potential of *M. membranacea*. In addition to increasing the severity of outbreaks in regions where *M. membranacea* is already prevalent, increased fecundity of *M. membranacea* could also accelerate its spread by enhancing propagule size above the critical level (minimum propagule size) for successful establishment in newly invaded habitats (Carlton 1996).

Compared to native populations, the ability to reach large sizes, a prolonged reproductive cycle, and the capacity for portions of colonies to persist overwinter likely contributed to the successful invasion and continued persistence of *M. membranacea* in the northwest Atlantic. These characteristics, coupled with high fecundity when conditions allow, small size/age at sexual maturity (production of oocytes), and rapid growth of unobstructed colonies, add to the invasive potential of *M. membranacea*, making the introduction and establishment of this species a threat to temperate epiphytic communities world-wide.

CHAPTER 7

COMMUNITY COMPOSITION INFLUENCES THE PERSISTENCE AND ECOLOGICAL IMPACTS OF INVASIVE SPECIES IN RESPONSE TO CLIMATE CHANGE[6]

7.1 Abstract

Predicting long-term impacts of introduced species is challenging, since stressors related to global change can influence species-community interactions by affecting both demographic rates of invasive species and the structure of the invaded ecosystems. Invasive species can alter ecosystem structure over time, further complicating interactions between invasive species and invaded communities in response to additional stressors. Few studies have considered how cumulative impacts of species invasion and global change on the structure of invaded ecosystems may influence persistence of introduced species. Here, we present an empirically based population model for an invasive bryozoan that can dramatically alter the structure of its invaded kelp bed ecosystems. We use this model to predict the response of invasive species to climate change and associated changes to the invaded community. Contrary to our expectations, the community composition of invaded kelp beds determined the persistence and population growth of the bryozoan under near-future projections of increasing ocean temperature. Our results suggest that direct effects of climate change on invasive species may have less of an impact on invasiveness than indirect effects mediated through changes to the structure of the invaded habitat. However, our results were sensitive to propagule supply. Even a small external source of propagules was sufficient to override the negative effects of changes in community composition on population growth of the bryozoan. Our findings have important implications for management of invasive species, as modifying

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invaded habitats at local to regional scales may be more logistically feasible than addressing stressors related to global climate change.

7.2 Introduction

Predicting which species have a high probability of becoming invasive and which ecosystems are most vulnerable to invasion has been a long-time goal of invasion science (Pimm 1989, Carlton 1996, Kolar & Lodge 2001). However, although some generalizations relating patterns of invasion success to characteristics of introduced species and recipient regions have emerged (Kolar & Lodge 2001, Dick et al. 2017), our ability to predict the success of individual invaders and the resulting consequences for invaded ecosystems remains limited (Williamson 1996, Hulme et al. 2013). This limitation is compounded by the occurrence of concurrent anthropogenic stressors related to global change, which further complicate general patterns of species invasions (Strayer 2012). However, our limited capacity to predict which species will invade where has led to an increased recognition of the specific role of species-community interactions in determining invasion success, including feedbacks between invaders and invaded habitats (Vitousek 1990, Lodge 1993a, b, Ricciardi et al. 2013). For example, model simulations have shown that equilibrium abundance of invasive species varies non-linearly with the availability of suitable habitat within the invaded landscape (Barlow & Kean 2004). These model outcomes suggest that small changes in the amount of suitable habitat may substantially impact the abundance, and consequently the invasiveness, of introduced species.

Invasive species themselves can also impact ecosystem structure, increasing or decreasing the resilience of the ecosystem to invasion (MacDougall & Turkington 2005, Simberloff 2011). This is particularly true for invasive ecosystem engineers, the capacity of which to alter ecosystems directly can result in cascading effects for resident species (Crooks 2002). However, invasion dynamics involving ecosystem engineers are complex, and model simulations predict that overexploitation of native habitat by an invasive engineer can lead to population collapse of the invader when its intrinsic rate of population increase is high (Gonzalez et al. 2008). Therefore, stressors related to global change can theoretically influence invasion success through both direct effects on the

demography of invaders and indirect effects on the structure of invaded ecosystems (Occhipinti-Ambrogi 2007, Hellmann et al. 2008, Rahel & Olden 2008). However, few studies consider impacts of species invasions and habitat modification simultaneously (1.2% of studies on species invasions published between 2002 and 2007, Didham et al. 2007), and empirical data that explicitly test model predictions are lacking (but see Kean & Barlow 2000).

Climate change and invasive species are major contributors to global change (Vitousek et al. 1997), and rank among the greatest threats to rocky reef ecosystems (Halpern et al. 2007). In general, effects of climate change are expected to favour invasive species over native species, enhancing the impact of species invasions on ecosystems globally (Dukes & Mooney 1999, Hellmann et al. 2008). However, the responses of marine biological invasions to global climate change are highly variable (Carlton 2000). Climatically driven changes in ocean temperature and physiochemical conditions can enhance or depress new invasions by altering dispersal mechanisms and competitive interactions at local scales (Occhipinti-Ambrogi 2007). For example, in marine epiphytic communities, increasing ocean temperature can facilitate local shifts to dominance by introduced species (Stachowicz et al. 2002, Sorte et al. 2010); however, at regional scales, the proportion of introduced species showed a parabolic relationship with summer sea surface temperature (Lord et al. 2015).

In an ocean warming hotspot in the northwest Atlantic, rising sea temperature in combination with population outbreaks of an invasive epiphytic species have been proposed as the main drivers of a dramatic ecosystem shift from highly productive kelp beds to less productive communities dominated by turf algae (Krumhansl et al. 2014, Filbee-Dexter et al. 2016, O'Brien 2018). Warm ocean temperatures have been linked to both a decline in kelp growth (Filbee-Dexter et al. 2016) and to population outbreaks of the invasive bryozoan *Membranipora membranacea* in this region (Saunders & Metaxas 2007, Scheibling & Gagnon 2009). Should ocean temperatures continue to increase, indirect effects on kelp beds resulting from enhanced epiphytism by *M. membranacea* may lead to even greater loss of kelp and, importantly, inhibition of kelp bed recovery, than predicted based on direct effects of temperature alone (Saunders et al. 2010). Differential impacts of both increased temperature and the invasive epiphyte on the

different kelp species (Saunders & Metaxas 2008, 2009b, Simonson et al. 2015) may confer a new competitive advantage to the competitive inferior kelp *Agarum clathratum* and alter the community composition of regional kelp beds. Thus, *M. membranacea* can be considered an invasive ecosystem engineer that interacts with and modifies its invaded habitat.

In this study, we use the unique dynamics between the invasive bryozoan and its algal hosts in the northwest Atlantic as a model system to test predictions of the response of invasive populations to climate change and associated changes in the invaded communities. To achieve this, we incorporate relationships between temperature, kelp substrate, and demographic rates previously quantified for *M. membranacea* (Saunders & Metaxas 2009a, Denley & Metaxas 2016, 2017a,b) into a matrix population model for this bryozoan in its invaded habitat. We use the model to predict the persistence and intensity of outbreaks of this invasive species in the region over the next 20 years in response to near-future projections of increasing ocean temperature and potential changes in the community composition of kelp beds. We expect that warming ocean temperature will lead to increased abundance (Saunders et al. 2010) and consequently enhanced ecological impacts of *M. membranacea* in Nova Scotia. However, we hypothesize that effects of temperature on bryozoan abundance will vary spatially in relation to the total abundance and species composition of the algal hosts, and temporally in response to projected changes in available kelp substrate. Our study is unique in that it incorporates both direct effects of climate change on an invasive species and the cumulative impacts of climate change and species invasion on the invaded ecosystems into predictive models of persistence of introduced species.

7.3 Methods

7.3.1 Model construction

We used a stage-based matrix model (Lefkovitch 1965) to explore the population dynamics of *M. membranacea* on each of the three numerically dominant kelp species (*Saccharina latissima*, *Laminaria digitata*, and *Agarum clathratum*) in its invaded habitat in the northwest Atlantic. The model is of the form:

[Eq. 7.1] $n^{k+1} = \mathbf{A}n^{k}$

where n^{k} is a vector of the abundance of individuals (per m^2 kelp) in each stage of the population at time index k, $k + 1 = k + \Delta k$ with time step Δk, and **A** is a population projection matrix (specific to each kelp substrate). Five stage classes were defined based on colony size (<1 cm diameter, 1-3 cm diameter, 3-6 cm diameter, 6-8 cm diameter, >8 cm diameter). Size classes were selected to correspond with approximately 2-week growth intervals (Saunders & Metaxas 2009a) based on the water temperature at the time of initial field sampling in June 2012. The elements of **A** represent: 1) familiar demographic processes of unitary organisms: growth, sexual reproduction, and mortality; and 2) a demographic process unique to colonial organisms: partial mortality of individual zooids (colony shrinkage/senescence) (Appendix E.1).

We generated model parameters and variables used to calculate transition probabilities based on empirically derived relationships between demographic rates (colony growth, whole-colony mortality, colony senescence, potential colony fecundity) and intrinsic (colony size) and environmental (temperature, kelp substrate) factors measured at The Lodge (44° 33′ 3″ N, 64° 01′ 9″ W) on the western shore of St. Margarets Bay, Nova Scotia (Table 7.1, Appendix E.2).

The model evolves at bi-weekly time steps, representing the minimal time required for colonies in size class i to grow to size class $i + 1$, from 01 January to 31 December of each year. The first size class consists entirely of new recruits, with 100% of colonies in this size class either growing into a larger size class or experiencing mortality due to senescence during each time step. For the first year for which the model is run, the model is initiated on 11 March with an initial population vector based on empirical data of the number of colonies on each of the three kelp substrates in November-December at three sites on the southwestern shore of Nova Scotia (The Lodge, Paddy's Head, and Sandy Cove) in 2005, 2006 (Saunders & Metaxas 2009b) and 2012 (Table E.1). For each kelp substrate, the number of colonies per m^2 kelp was recorded in 2005 and 2006, and the number and size-category of colonies per m^2 kelp in 2012. We calculated the average number of colonies per m^2 kelp in November-December across all

years, as well as the size-distribution of colonies for 2012. We used the relative proportion of colonies in each size class in November-December 2012 to partition the average number of colonies per m^2 kelp among the five size classes. To estimate the initial population vector on 11 March, we multiplied the number of colonies per m^2 kelp in each size class for each kelp substrate by an over-winter survival probability of 0.005 or 0.5%. We calculated the proportion of colonies surviving over winter (P_{OW}) from 30 December to 11 March (70 d) as

[Eq. 7.2] $$P_{OW} = e^{-\mu t}$$

where μ is the instantaneous rate of mortality (0.075 $\%d^{-1}$, Denley & Metaxas 2016) and t is the time in days (70 d). Each successive year is initiated on 01 January with a population vector equal to the final population vector on 31 December of the previous year.

7.3.2 Model Projections

Because the data used to construct the model were obtained from The Lodge, we forced the model using independent data from two other sites: Paddy's Head and Sandy Cove. There is no evidence that demographic rates (mortality, fecundity) vary among sites (Denley & Metaxas 2016, 2017a), therefore, data from the population at The Lodge can be used to model the populations at the other two sites. However, the temperature regimes do vary slightly among sites, and the mixed kelp beds are characterized by different absolute and relative abundance of kelps (Denley & Metaxas 2016, 2017a). Site-specific temperature was used to force the model by incorporating depth-averaged daily average temperatures (Paddy's Head: 4, 8, 12 m; Sandy Cove: 4, 8 m) for each year into the equation for colony growth rate (Eq. E.1).

Table 7.1 Summary of model parameters included in a matrix population model for *Membranipora membranacea* in Nova Scotia.

Model parameter	Measurement	Location	Duration	Dependent on	Source
G	Rate of colony growth (mm d^{-1})	SMB, NS FHL, WA Lun, NS	Aug 2005, Aug-Sep 2006 May 2006 Jul-Aug 2007	Colony size Temperature	Saunders & Metaxas (2009a)
S	Rate of colony shrinkage (cm d^{-1})	TL	25 Jul – 7 Oct 2014	Colony size	Denley & Metaxas (2016)
P	Rate of whole colony mortality (%d^{-1})	TL	2012: Jun, Aug, Sep, Nov 2013: Mar, Jun, Aug	Colony size Kelp Substrate	Denley & Metaxas (2016)
F	Oocytes $colony^{-1}$	TL	2012: Jun, Aug, Sep, Nov 2013: Mar, Jun, Aug 2014: May, Jun, Jul, Aug, Sep, Oct, Nov 2015: Mar, Apr, May, Jun, Jul, Aug, Sep, Oct, Nov	Colony size Kelp substrate	Denley & Metaxas (2017a)

Note: Locations are: St. Margarets Bay (SMB), Friday Harbor Laboratories (FHL), Lunenburg (Lun), and The Lodge (TL)

Kelp abundances at each site were estimated from field sampling conducted approximately every six weeks from June 2012 to August 2013 (Denley & Metaxas 2016). For each month at each site, we generated normal distributions of kelp abundance using the mean and standard deviation of the surface area of each kelp species per m^2 seabed pooled across depths and years. We then randomly sampled abundance for each month from these distributions, and used linear interpolation to generate kelp abundances for each time index *k*. We restricted the maximum abundance of kelp to within the range observed in the field for each kelp species (D. Denley unpubl data, *S. latissima*: 2.4 m^2 per m^2 seabed, *L. digitata*: 1.2 m^2 per m^2 seabed, *A. clathratum*: 0.67 m^2 per m^2 seabed). Site-specific kelp abundances were used to convert model output (colonies per m^2 kelp) to colonies per m^2 seabed by multiplying the abundance of colonies (per m^2 kelp) at each point in time with the corresponding surface area of each kelp species (m^2) per m^2 seabed.

7.3.2.1 Response of *M. membranacea* to projected increases in ocean temperature

We initiated the model on 11 March 2016 using daily averaged temperatures from that year and ran the model consecutively for 19 years (from 2016 to 2035) under three scenarios of projected increases in ocean temperature by the year 2035: +0.5°C, +1°C, and +3°C (Kirtman et al. 2013). The reliability of regional climate change projections for the northwest Atlantic remains uncertain (Loder et al. 2015). Therefore, we selected increasing ocean temperature scenarios to encompass the globally averaged near-term changes in ocean temperature projected under the range of Representative Concentrations Pathways (RCP2.6 – RCP8.5) for emissions scenarios considered by the Intergovernmental Panel on Climate Change in their Fifth Assessment Report (Kirtman et al. 2013). For each temperature scenario, we divided the projected increase in ocean temperature by the year 2035 by the number of years between 2016 and 2035 (e.g. 2035-2016 = 19 yrs; 0.5°C ÷ 19 yrs = 0.026 °C yr^{-1}) and added the resulting value to each daily averaged temperature for each consecutive year the model was run. This resulted in a cumulative increase in daily averaged ocean temperature of +0.5°C, +1°C, or +3°C by 2035. Kelp abundances for each year were generated as described previously, and bi-weekly relative abundances of each kelp species were re-calculated for each consecutive

year to reflect interannual variation in kelp bed community composition. To reduce any artifacts of the initialization, we allowed the model to spin up from 11 March 2016 to 31 December 2016 and determined the mean annual maximum number of colonies of *M. membranacea* per m^2 seabed and 95% bootstrap percentile intervals based on model output from January 1 2017 to December 31 2035 for 2000 iterations (Caswell 2001). We calculated the stochastic population growth rates and corresponding 95% confidence intervals for each site and temperature scenario after Caswell (2001), using the mean annual maximum number of colonies of *M. membranacea* per m^2 seabed.

7.3.2.2 Response of *M. membranacea* to changes in the community composition of kelp beds

We ran the model from 11 March 2012 to 31 December 2013 for each species of kelp separately to compare seasonal population dynamics of *M. membranacea* (colonies per m^2 seabed) among mono-specific stands of each of the three kelp species. We chose to run the model for mono-specific stands rather than mixed kelp beds with various relative abundances of each kelp species because shifts in the relative abundance of kelp species within kelp beds are difficult to predict and will likely vary spatially in response to local conditions. Thus, we used the model to predict near-future responses of *M. membranacea* to extreme changes in the species composition of kelp beds. The mono-specific model was initiated with the same starting vector of *M. membranacea* abundance for each kelp species, which was calculated as the sum of all initial substrate-specific population vectors in the mixed kelp bed model. Similarly, abundances were summed across all three kelp species, and total kelp abundance (m^2 kelp per m^2 seabed) was the same for mixed and mono-specific kelp beds. This assumption allows us to isolate the effect of kelp species on the population dynamics of *M. membranacea* rather than confounding effects of available kelp species with effects of total available substrate. For the mono-specific model, oocytes produced by colonies on each substrate entered the first size class on that same substrate according to Eq. E.4, where, F_{total} is now the total number of oocytes produced by colonies of all size classes on a specific substrate and R is the proportion of the total number of settlers occurring per m^2 kelp summed across all three kelp species.

7.3.2.3 Response of *M. membranacea* to the combined effects of projected increases in ocean temperature and community composition of kelp beds

To examine the combined impact of projected increases in ocean temperature and changes in kelp bed community composition on *M. membranacea* in the northwest Atlantic, we ran the model for mono-specific stands of *S. latissima*, *A. clathratum* and *L. digitata* while increasing daily averaged temperatures annually from 11 March 2016 to 31 December 2035 for each temperature scenario.

Although *M. membranacea* occurs predominantly on kelps, it is also found on alternative algal substrates, primarily *Fucus* spp. (Yorke & Metaxas 2012). *Fucus* beds occur in shallow waters (< 4m depth) and are spatially separated from mixed kelp beds; however, they may provide an additional supply of allochthonous propagules. To explore the effect of this potential contribution, we estimated monthly larval supply (number of oocytes) from colonies on *F. evanescens*. We based this estimate on the potential fecundity of colonies (oocytes colony^{-1}) sampled on *F. evanescens* at Paddy's Head from 2012 to 2015 (Denley & Metaxas 2017a), and the number of colonies per m^2 seabed on *F. evanescens* measured at Paddy's Head approximately monthly from June 2012 to August 2013 (D. Denley, unpubl data). At Sandy Cove, *F. evanescens* is restricted to within a small sheltered embayment devoid of kelp, while mixed kelp beds occur along exposed bedrock ridges outside the bay. There are fewer colonies of *M. membranacea* on *F. evanescens* at Sandy Cove than at The Lodge and Paddy's Head where *F. evanescence* can be found immediately shoreward of mixed kelp beds (D. Denley, unpubl data). Therefore, we used the model to examine larval contribution ranging from a patch of *F. evanescens* (3 m^2) to a bed of *F. evanescens* spanning the entire shallow subtidal adjacent to mixed kelp beds (estimated to be ~30 m^2, Denley & Metaxas 2016) at Paddy's Head only. We added the monthly larval supply from colonies on *F. evanescens* for a patch (3 m^2) and a bed (30 m^2), multiplied with the corresponding monthly larval mortality, to F_{total} for each bi-weekly time step of the mono-specific model.

For mono-specific stands of each kelp species, we determined the mean annual maximum number of colonies of *M. membranacea* per m^2 seabed under each projected increase in ocean temperature from 2017 to 2035, with and without additional propagule supply from *F. evanescens* for Paddy's Head, for 2000 iterations. We calculated the

stochastic population growth rates and corresponding 95% confidence intervals for all combinations of kelp species and temperature scenario after Caswell (2001).

7.4 Results

7.4.1 Model validation

For Paddy's Head and Sandy Cove, 95th percentiles for modeled populations overlapped with 95% confidence intervals for sampled populations for each of two population indices, total number of colonies per m^2 seabed and total surface area of colonies (cm^2) per m^2 seabed, during both early and peak seasonal abundance of *M. membranacea* (Figure 7.1, Appendix E.3). For The Lodge, modeled and sampled percentile and confidence intervals overlapped for colony abundance (colonies m^{-2} seabed) during peak seasonal abundance in 2012 and early in the season in 2013, but not in early 2012 (Figure 7.1, Appendix E.3). Additionally, modeled and sampled percentile and confidence intervals did not overlap for colony surface area early in the season or during peak seasonal abundance of *M. membranacea* in 2012, but did overlap for early 2013 (Figure 7.1, Appendix E.3). We attribute this discrepancy between model estimates and sampled data to anomalously warm water temperatures at The Lodge in the spring of 2012 (Figure E.5), resulting in increased colony fecundity and earlier seasonal occurrence (prior to June 2012) of fecund colonies that were not measured in the field. Increased fecundity of colonies earlier in the season may lead to increased abundance and earlier onset of settlers, resulting in increased number of colonies early in the season and a larger size distribution (increased surface area) of colonies during peak colony abundance (Appendix E.4).

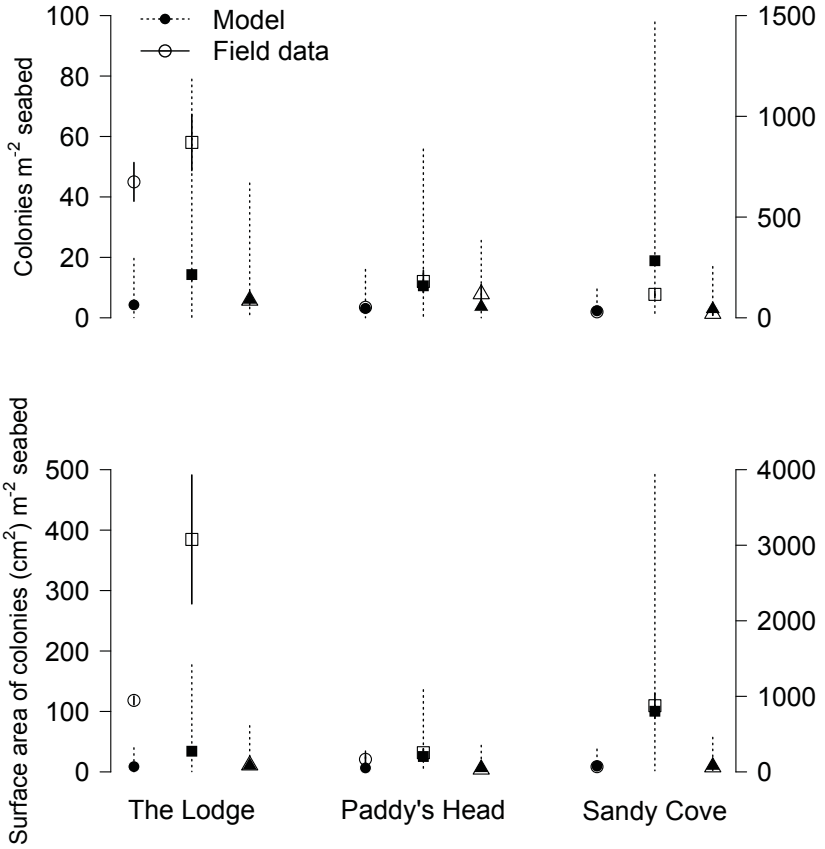

Figure 7.1 Model validation. Modeled estimates (mean ± 95% percentile intervals of 2000 model runs) and field data (mean ± 95% confidence intervals) of two population indices: the number of colonies per m^2 seabed (top panels) and the surface area of colonies (cm^2) per m^2 seabed (bottom panels) during the early (July-August 2012: circles, July-August 2013: triangles) and peak (September-October 2012: squares) stages of the seasonal occurrence of *Membranipora membranacea* at 3 sites on the southwestern shore of Nova Scotia: The Lodge (source of data used in constructing the model), Paddy's Head, and Sandy Cove (sites used for validating the model). Data for The Lodge and Paddy's Head are with respect to the left y-axis, data for Sandy Cove are with respect to the right y-axis

7.4.2 Model projections

7.4.2.1 Response of *M. membranacea* to projected increases in ocean temperature

Abundance of *M. membranacea* was consistently greater at Sandy Cove than at Paddy's Head (Figure 7.2); however, for mixed kelp beds at both Sandy Cove and

Paddy's Head, stochastic population growth rate of *M. membranacea* increased, and variability in population growth rate tended to decrease, with increasing ocean temperature (Table 7.2, Figure 7.2). Interestingly, the population only increased substantially in response to the most extreme near-future climate scenario (+3°C), and remained fairly stable under more conservative projected increases in ocean temperature (+0.5°C, +1°C) (Table 7.2, Figure 7.2).

7.4.2.2 Response of *M. membranacea* to changes in the community composition of kelp beds

The abundance and seasonal dynamics of *M. membranacea* differed among mono-specific stands of the three kelp species, and these differences were consistent among sites (Figure 7.3). Most notably, colony abundance peaked earlier in the season for mono-specific stands of *S. latissima* than *L. digitata* and *A. clathratum*, and peak colony abundance was greater for mono-specific stands of *A. clathratum* than the other two species (Figure 7.3). Additionally, colony abundance was low during most of the season for mono-specific stands of *A. clathratum*, peaking abruptly in October and declining again in November (Figure 7.3).

Table 7.2 Stochastic population growth rate ($\log\lambda_s$, $yr^{-1} \pm 95\%$ confidence intervals) for *Membranipora membranacea* in the northwest Atlantic in response to projected increases in ocean temperature. Stochastic population growth rates and corresponding 95% confidence intervals are calculated after Caswell (2001) based on model projections of the annual maximum number of colonies of *M. membranacea* per m^2 seabed for mixed kelp beds at Paddy's Head and Sandy Cove under projected increases in ocean temperature for the northwest Atlantic of +0.5°C, +1°C, and +3°C by the year 2035 (Figure 7.2). $\log\lambda_s > 0$ indicates exponential population growth, $\log\lambda_s = 0$ indicates population stability, $\log\lambda_s < 0$ indicates exponential population decline.

Site	Projected temperature (°C)	$\log\lambda_s \pm 95\%$ CI	Range in $\log\lambda_s$
Paddy's Head	+0.5	-0.045 ± 0.041	-0.218 – 0.109
	+1.0	-0.007 ± 0.045	-0.199 – 0.181
	+3.0	0.089 ± 0.036	-0.082 – 0.215
Sandy Cove	+0.5	-0.039 ± 0.046	-0.274 – 0.159
	+1.0	0.001 ± 0.035	-0.162 – 0.104
	+3.0	0.105 ± 0.030	-0.023 – 0.191

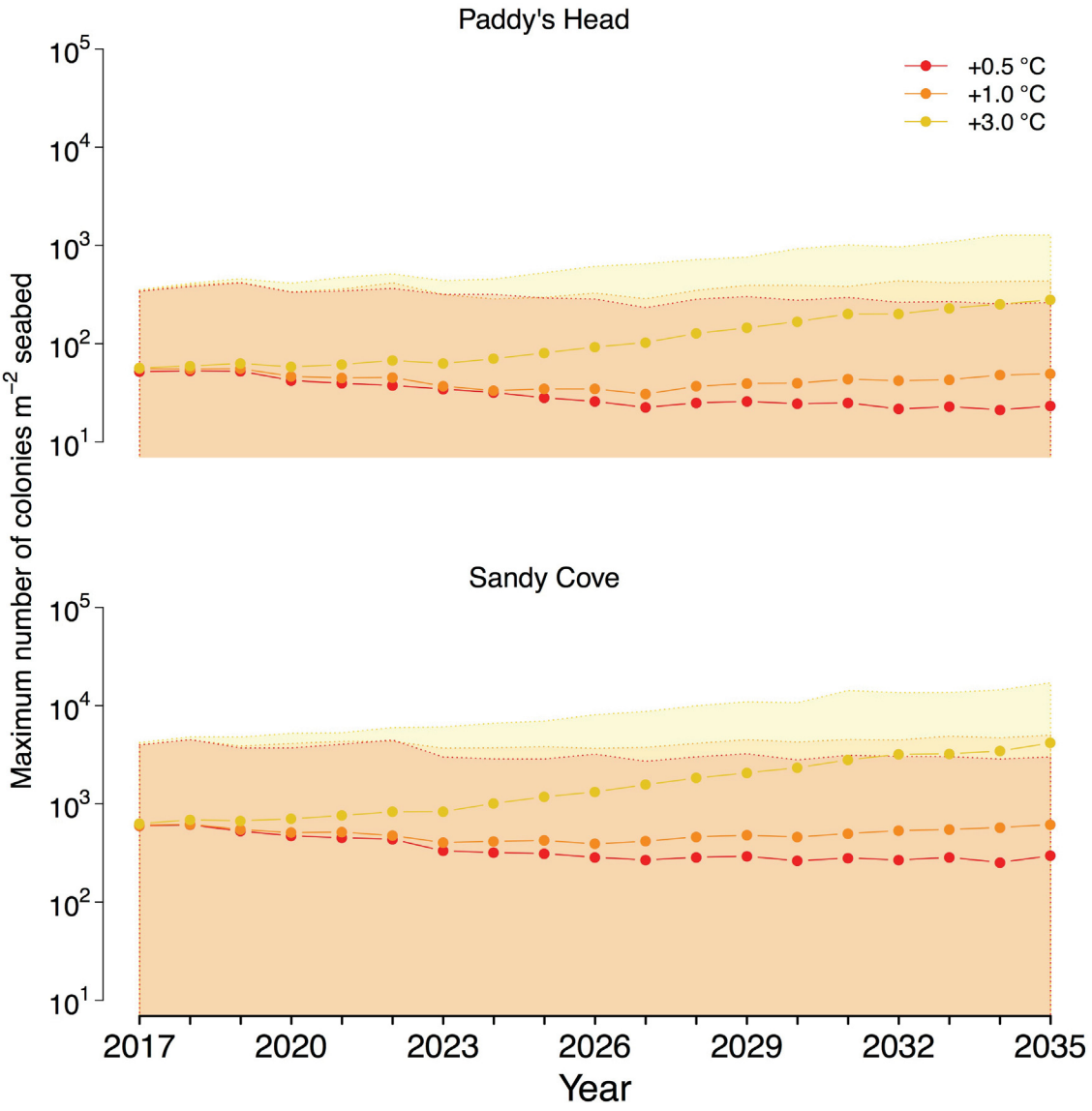

Figure 7.2 Model projections of the annual maximum number of colonies of *Membranipora membranacea* per m^2 seabed for mixed kelp beds at Paddy's Head and Sandy Cove under projected increases in ocean temperature for the northwest Atlantic of +0.5°C, +1°C, and +3°C by the year 2035. The model was initiated using temperature data from 2016 and daily average temperatures were increased annually from 2017-2035 by 0.026°C, 0.053°C, and 0.158°C respectively. Points are the means of 2000 iterations, shaded areas indicate the 95% percentile intervals

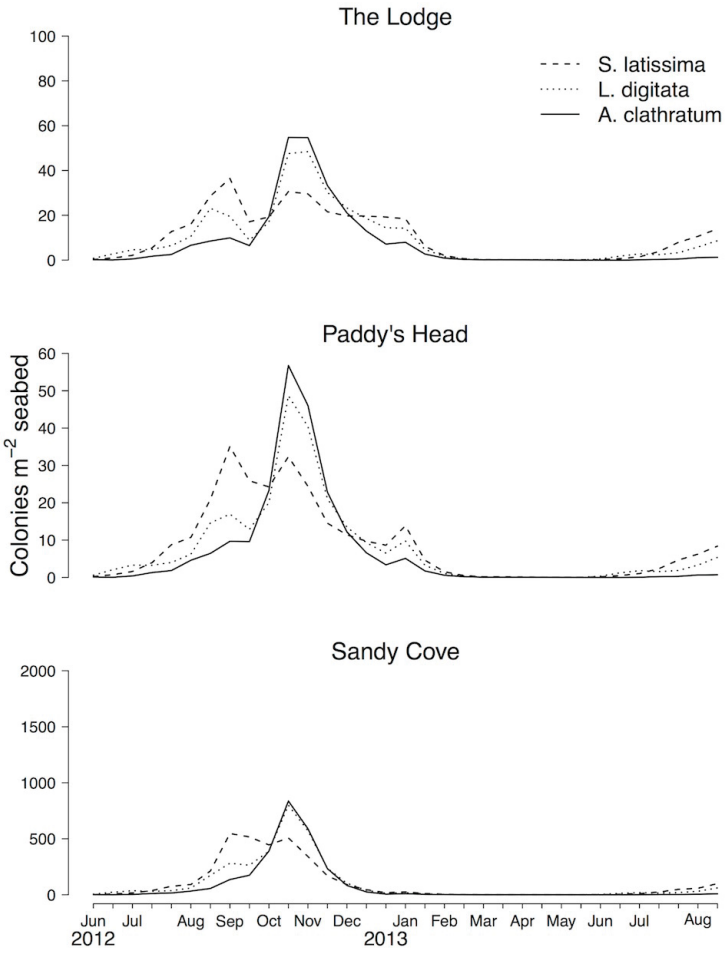

Figure 7.3 Model projections (means of 2000 iterations) of the seasonal dynamics of the population of *Membranipora membranacea* (colonies per m^2 seabed) from June 2012 to August 2013 at 3 sites on the southwestern shore of Nova Scotia: The Lodge (source of data used in constructing the model), Paddy's Head, and Sandy Cove (sites used for validating the model) for mono-specific stands of *Saccharina latissima*, *Laminaria digitata*, and *Agarum clathratum*. Tickmarks on the x-axis correspond to bi-weekly time indices. The model was initiated on 11 March 2012, resulting in 2 time indices in each of Jun, Aug, Sep Oct, and Nov, and 3 time indices in Jul and Dec (see 7.3.1 Model construction for details)

7.4.2.3 Response of *M. membranacea* to the combined effects of projected increases in ocean temperature and community composition of kelp beds

Differences in the seasonal dynamics of *M. membranacea* among kelp substrates were reflected in near-term projections of the population in response to increases in ocean temperature and changes in the community composition of kelp beds. The initial abundance of *M. membranacea* in 2017 was greater for mono-specific stands of all kelp

species at Sandy Cove than at Paddy's head (Figure 7.4). However, unlike for mixed kelp beds, the bryozoan population declined exponentially for all mono-specific stands at both sites (Table 7.3, Figure 7.4). In fact, the population declined to extinction (0 colonies per m^2 seabed) for mono-specific stands of all kelp species under +0.5 °C and +1.0 °C temperature scenarios for Paddy's Head, and for mono-specific stands of all kelp species under a projected increase of +0.5°C and mono-specific stands of *L. digitata* and *A. clathratum* under a projected increase +1.0°C at Sandy Cove (Figure 7.4). Rates of population decline tended to decrease with increasing temperature, resulting in small but extant populations of *M. membranacea* for mono-specific stands of *S. latissima* after 19 years under projected temperature increases of +1.0 °C at Sandy Cove, and mono-specific stands of *S. latissima* and *L. digitata* after 19 years under projected temperature increases of +3.0 °C at Paddy's Head and Sandy Cove (Table 7.3, Figure 7.4). The rate of population decline was greatest for mono-specific stands of *A. clathratum*, and the population of *M. membranacea* reached extinction by 2035 for mono-specific stands of *A. clathratum* under all temperature scenarios at both sites (Table 7.3, Figures 7.4).

Additional propagule supply from colonies on the alternative substrate was sufficient to override the decline in the population of *M. membranacea* at Paddy's Head. Under these conditions, population growth was limited by available kelp substrate, with annual maximum cover of *M. membranacea* on kelp reaching ~100% in each year, resulting in population stability for mono-specific stands of all kelp species under all scenarios of projected increases in ocean temperature (Table 7.3). Larval contribution from a patch of *F. evanescens* resulted in fewer colonies per m^2 seabed that from a bed of *F. evanescens*; however, the fewer colonies grew to larger sizes resulting in comparable surface area of *M. membranacea* per m^2 seabed for both magnitudes of external propagule supply (Table 7.4).

Table 7.3 Stochastic population growth rate ($\log\lambda_s$, $yr^{-1} \pm 95\%$ confidence intervals) for *Membranipora membranacea* in the northwest Atlantic in response to projected increases in ocean temperature. Stochastic population growth rates and corresponding 95% confidence intervals are calculated after Caswell (2001) based on model projections of the annual maximum number of colonies of *M. membranacea* per m^2 seabed for mono-specific stands of *Saccharina latissima, Laminaria digitata,* and *Agarum clathratum* at Paddy's Head (with and without additional propagule supply from alternative algal substrate *Fucus evanescens*) and Sandy Cove (without additional propagule supply only) under projected increases in ocean temperature for the northwest Atlantic of +0.5°C, +1°C, and +3°C by the year 2035 (Figure 7.4). $\log\lambda_s > 0$ indicates exponential population growth, $\log\lambda_s = 0$ indicates population stability, $\log\lambda_s < 0$ indicates exponential population decline. NA indicates model projections were not run with additional propagule supply for Sandy Cove (see 7.3.2 Model projections for details).

Site	Projected temperature (°C)	$\log\lambda_s \pm 95\%$ CI		
		No additional propagule supply	Additional propagule supply (*Fucus* bed)	Additional propagule supply (*Fucus* patch)
Paddy's Head				
S. latissima	+0.5	-0.388 ± 0.317	0.0000 ± 0.001	0.0018 ± 0.004
L. digitata		-0.544 ± 0.404	0.0001 ± 0.001	0.0003 ± 0.002
A. clathratum		-1.32 ± 0.308	0.0001 ± 0.000	0.0004 ± 0.003
S. latissima	+1.0	-0.252 ± 0.458	0.0001 ± 0.001	0.0032 ± 0.004
L. digitata		-0.434 ± 0.334	0.0001 ± 0.001	0.0007 ± 0.002
A. clathratum		-1.16 ± 0.344	0.0001 ± 0.000	0.0012 ± 0.003
S. latissima	+3.0	-0.136 ± 0.239	0.0005 ± 0.001	0.0067 ± 0.005
L. digitata		-0.128 ± 0.120	0.0002 ± 0.001	0.0029 ± 0.003
A. clathratum		-0.533 ± 0.522	0.0002 ± 0.000	0.0009 ± 0.004
Sandy Cove				
S. latissima	+0.5	-0.360 ± 0.470	NA	NA
L. digitata		-0.510 ± 0.473	NA	NA
A. clathratum		-1.222 ± 0.356	NA	NA
S. latissima	+1.0	-0.198 ± 0.521	NA	NA
L. digitata		-0.340 ± 0.386	NA	NA
A. clathratum		-1.02 ± 0.405	NA	NA
S. latissima	+3.0	-0.095 ± 0.311	NA	NA
L. digitata		-0.141 ± 0.161	NA	NA
A. clathratum		-0.392 ± 0.594	NA	NA

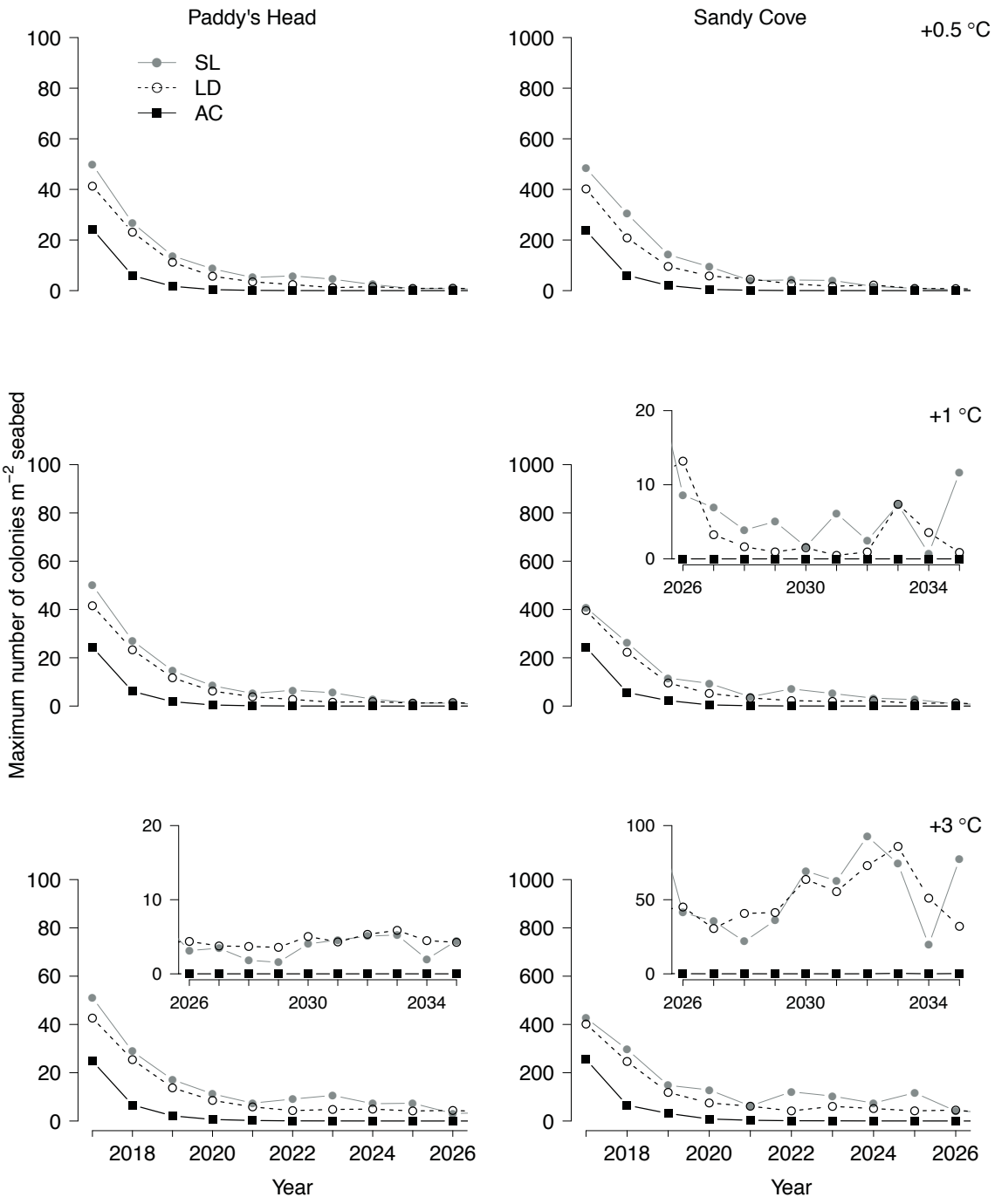
Paddy's Head
Sandy Cove
+0.5 °C
+1 °C
+3 °C
SL
LD
AC
Maximum number of colonies m^{-2} seabed
Year

Figure 7.4. Model projections (means of 2000 iterations) of the annual maximum number of colonies of *Membranipora membranacea* per m^2 seabed for mono-specific stands of *Saccharina latissima*, *Laminaria digitata*, and *Agarum clathratum* at Paddy's Head and Sandy Cove under projected increases in ocean temperature for the northwest Atlantic of +0.5°C, +1°C, and +3°C by the year 2035. The model was initiated using temperature data from 2016 and daily average temperatures were increased annually from 2017-2035 by 0.026°C, 0.053°C, and 0.158°C respectively. The annual maximum number of colonies does not recover following 2026 except for mono-specific stands of *S. latissima* under projected temperature increases of +1.0 °C at Sandy Cove, and mono-specific stands of *S. latissima* and *L. digitata* under projected temperature increases of +3.0 °C at Paddy's Head and Sandy Cove (shown in insets)

Table 7.4 Average annual maximum number and surface area of colonies (m^2) of *Membranipora membranacea* per m^2 seabed for mono-specific stands of *Saccharina latissima, Laminaria digitata,* and *Agarum clathratum* at Paddy's Head with additional propagule supply from alternative algal substrate *Fucus evanescens* under projected increases in ocean temperature for the northwest Atlantic of +0.5°C, +1°C, and +3°C by the year 2035.

Paddy's Head	Projected temperature (°C)	Maximum number (surface area, m^2) of colonies per m^2 seabed	
		Fucus bed (30 m^2)	*Fucus* patch (3 m^2)
S. latissima	+0.5	2557 (0.37)	1200 (0.29)
L. digitata		2570 (0.35)	1137 (0.29)
A. clathratum		2554 (0.34)	1260 (0.22)
S. latissima	+1.0	2655 (0.40)	1219 (0.31)
L. digitata		2661 (0.37)	1143 (0.30)
A. clathratum		2651 (0.37)	1270 (0.23)
S. latissima	+3.0	2665 (0.49)	1281 (0.37)
L. digitata		2667 (0.46)	1166 (0.37)
A. clathratum		2656 (0.46)	1298 (0.31)

7.5 Discussion

Our study supports previous studies linking range expansion and increased abundance of non-native marine epiphytes to warming seawater temperature at local and regional scales (Stachowicz et al. 2002, Sorte et al. 2010, Sorte & Stachowicz 2011, Rius et al. 2014). Projected increases in ocean temperature of ~1°C to 3°C were predicted to enhance population growth of the invasive *M. membranacea*, increasing the probability of persistence and the ecological impacts of this species in the northwest Atlantic. The prediction was consistent across two sites (Paddy's Head and Sandy Cove) that differ in

both total abundance and species composition of available kelp substrate. Increasing temperature related to climate change may increase or decrease the impact of non-native species depending on their thermal optima (Iacarella et al. 2015) or the seasonal timing of the temperature increase (Mech et al. 2018). Annually averaged projected increases in ocean temperature for the northwest Atlantic are within the range at which colony growth rate of *M. membranacea* was enhanced in the field (5.7°C-16.2°C, Saunders & Metaxas 2009a). Although maximum daily average temperature under the most extreme warming scenario (+3°C) in our region was 22°C, colony growth of *M. membranacea* is not retarded at this temperature (Yoshioka 1973). The effect of increasing ocean temperature on *M. membranacea* in our region can already be evidenced during anomalously warm seawater temperatures in the field (Appendix E.5). For example, model projections for The Lodge under near-future temperature scenarios that approximate the 2012 temperature anomaly successfully captured the increased colony abundance observed in July to September of that year (Appendix E.5).

Growth of the population of *M. membranacea* was sensitive to variations in whole colony mortality rate driven by loss of kelp substrate. Erosion is the primary mechanism of loss of kelp tissue in Nova Scotia, and rates of erosion are predicted to increase by ~4% over the next 20 years in response to increases in ocean temperature of 0.022°C yr^{-1} (Krumhansl et al. 2014), which approximately corresponds to our +0.5°C temperature scenario (+0.026°C yr^{-1}). Enhanced mortality rates related to temperature-induced loss of kelp tissue may lessen the effect of increasing ocean temperature on population growth of *M. membranacea*. Although kelp dynamics (growth, erosion, mortality, dislodgement) are not directly included in our population model, coupling our model with existing models of kelp substrate dynamics (e.g. Krumhansl et al. 2014) could be used to more explicitly examine the effects of changing climate conditions (temperature, significant wave height) on the interaction between the invasive bryozoan and its host kelp.

Interestingly, direct effects of climate change on the demography of *M. membranacea* had a smaller impact on the persistence of invasive populations than indirect effects of climate change mediated through changes to the structure of the invaded ecosystem. The community composition of kelp beds determined the persistence of *M. membranacea* in its invaded habitat independent of total kelp abundance or

increasing ocean temperature. Exponential population decline in response to mono-specific stands of equivalent total kelp abundance suggests that persistence of invasive populations of *M. membranacea* may be maintained through differences in the timing and magnitude of demographic rates (fecundity and mortality) among kelp substrates. In heterogeneous mixed kelp bed habitats, variation in the timing of the onset and seasonal peak in fecundity of colonies on different kelp substrates ensures a near continuous larval supply, while differences in seasonal rates of colony mortality among different kelp substrates act to maintain a year-round population of mature colonies.

Substantial loss of kelp has been observed in our region over the past ~30 years, corresponding to an increase in the average sea temperature at 2-6 m of 1.58°C (Filbee-Dexter et al. 2016). Total kelp abundance is expected to continue to decline in response to projected increases in ocean temperature that exceed historical observations, however rates of decline are likely to differ among the three kelp species. In laboratory experiments, *A. clathratum* exhibited limited tissue damage at increased temperature treatments (14°C-21°C), and was less susceptible to temperature-induced tissue loss than *S. latissima* or *L. digitata* (Simonson et al. 2015). Consequently, near-future kelp bed ecosystems may be characterised by reduced overall abundance of kelp and increased relative abundance and depth distribution of *A. clathratum*, resembling mono-specific stands of this species in our model.

Native geographic range size, degree of phenotypic plasticity, and ability to use multiple habitat types have all been positively associated with invasion success, implying that invasive species tend to be habitat generalists (Rejmánek 1995, Rosecchi et al. 2001, Blackburn et al. 2009). However, our results suggest that generalist introduced species that persist by taking advantage of a diverse range of resources may not benefit from this life-history strategy, and may even become competitively inferior to more specialist native species, should heterogeneity of the invaded habitat decrease (e.g. Radford & Cousens 2000). At broad scales (≥ 1 km^2), habitat heterogeneity can be positively related to invasive species richness (Fridley et al. 2007) due to reduced strength of competitive interspecific interactions with increasing resource availability (Byers & Noonburg 2003). In fact, homogenous habitats can act as a dispersal barrier for non-native species that are more abundant in mixed substrata habitat types (Bohn et al. 2015).

Propagule supply has emerged as one of the few general predictors for successful establishment, persistence, and impact of invasive species (Kolar & Lodge 2001, Drake et al. 2005, Ricciardi et al. 2011, 2013). Our results demonstrate the importance of allochthonous propagule supply in contributing to the persistence of invasive species in response to changes in the invaded habitat. For *M. membranacea*, even a small external source of larvae was sufficient to maintain a stable population following dramatic changes in kelp bed community composition when the population would not have persisted otherwise. Similarly, for invasive terrestrial plants, propagule supply from seedbanks can rescue small or disturbed populations from extinction, allowing invasive populations to persist or even increase in size following eradication efforts (Drayton & Primack 1999). Our findings emphasize the importance of limiting propagule reservoirs for introduced species to minimize the risk of establishment and persistence in invaded communities. For invasive marine epiphytes in particular, anthropogenic structures can act as external sources of propagule supply, facilitating persistence in adjacent natural habitats (Lambert 2003, Dafforn et al. 2009, Forrest et al. 2013, Simons et al. 2013).

Our study provides evidence for the role of interactions between introduced species and recipient communities in determining invasiveness under climate change scenarios. Under near-future climate conditions, habitat modification through the combined impacts of climate change and epiphytism by the invasive bryozoan had a stronger effect on its persistence and abundance (impact) on kelp bed ecosystems than increasing temperature alone. This finding has important implications for management of species invasions, since modification of invaded habitats at local to regional scales may be a more rapid and logistically feasible response to mitigate impacts of introduced species than addressing effects of global climate change.

CHAPTER 8

DISCUSSION

It is widely recognized that characteristics of both introduced species and recipient communities contribute towards invasion success. However, prior to this research, information on critical life-history and demographic processes, and the impact of novel algal hosts on these processes, was lacking for an ecologically significant invasive bryozoan in the northwest Atlantic. Using a combination of field, laboratory, and modeling approaches I have demonstrated that interactions with native algal substrates substantially influence the population dynamics of *M. membranacea* in its invaded habitat. Consequently, the persistence and ecological impacts of this bryozoan will depend on both direct effects of changing climatic conditions on *M. membranacea* and on the structure of future kelp bed ecosystems.

I examined selective settlement by larvae of *M. membranacea* at increasing scales from individual kelp blades to mixed kelp beds. Settlers of *M. membranacea* were consistently most abundant towards younger more proximal regions of blades of the kelps *Saccharina latissima* and *Laminaria digitata* (**Chapter 2**), suggesting that larvae exhibit preferential settlement and can detect differences in habitat quality at the scale of a single kelp blade. However, larvae of *M. membranacea* did not exhibit preference for settling on particular kelp species or within kelp beds, and instead settled in greatest abundance on substrates that extended furthers above the primary substratum (**Chapter 3**). Scale-dependent settlement behavior manifested by *M. membranacea* in its invaded habitat likely contributed to its invasion success by increasing colony longevity and decreasing competition for space on individual kelp blades, while allowing colonies to establish and persist on alternative substrates in the absence of mixed kelp beds.

I quantified demographic processes unique to colonial organisms such as colony senescence (**Chapter 4**), partial colony mortality (**Chapters 4** and **5**), recovery of colonies following partial (zooid) mortality (**Chapter 5**), and colony fecundity based on the reproductive potential of individual zooids (**Chapter 6**). Rates of colony senescence were independent of temperature and increased with colony size in the laboratory

(**Chapter 4**), suggesting that senescence may be an intrinsic property related to colony age. *In situ* partial mortality was substantial, with >50% of colonies experiencing some level of partial mortality (**Chapter 5**), and was also positively related to colony size (**Chapter 4**). In contrast, whole-colony mortality was greatest for intermediate sized colonies and varied with loss of kelp biomass related to kelp substrate dynamics (**Chapter 4**). The location of zooid mortality within colonies determined their capacity to recover following damage (**Chapter 5**). Colonies with damage to older centrally located zooids maintained their capacity for growth and recovery independent of temperature, while colonies with damage to younger peripheral zooids where unable to recover, and showed increased loss of zooids with increasing temperature (**Chapter 5**). Temperature and colony size explained minimal amount of variation in colony fecundity (**Chapter 6**). Instead, consistent differences in reproductive potential were observed among colonies on different algal substrates, with overall fecundity being greatest for colonies on *Agarum clathratum* and *Fucus evanescens* (**Chapter 6**). The minimal effect of temperature on potential fecundity of *M. membranacea* suggests that previously quantified relationships between temperature and settler abundance in its invaded habitat may be driven by pelagic processes affecting the larval stage.

By incorporating empirically derived relationships between organismal and environmental variables and life-history strategies and demographic rates of *M. membranacea* into a population model I was able to show that projected increases in ocean temperature will likely lead to increased population growth and enhanced ecological impacts of this bryozoan in the northwest Atlantic (**Chapter 7**). My findings support previous modeling results by Saunders and colleagues (2010), where increasing temperature by +2°C in winter/spring and summer resulted in increased abundance and cover of colonies on kelp blades, respectively. However, I also demonstrated that the persistence of *M. membranacea* in its invaded habitat is strongly influenced by the species composition of kelp bed communities and the existence of allochthonous propagule supply. My research shows that although warming seawater temperatures can enhance population outbreaks of *M. membranacea* through positive effects of increasing temperature on settler abundance and colony growth, other critical demographic rates of

mortality, senescence, and fecundity are not strongly related to temperature, and instead vary in response to characteristics of the invaded habitat or of the colonies themselves.

There remain several key aspects of *M. membranacea*'s complex lifecycle that could contribute towards an increased understanding of its invasion dynamics, and thus warrant further investigation. Firstly, incorporating direct impacts of increasing temperature on growth and erosion of kelps into population projections for *M. membranacea*, for example by coupling the model in this thesis with existing models of kelp substrate dynamics, could further elucidate effects of climate change on interactions between epiphytes and their algal hosts. In addition, quantifying rates of colony growth and mortality on alternative algal substrates (e.g. *Fucus* spp.) would shed light on the capacity of alternative substrates to contribute towards the persistence of *M. membranacea* in its invaded habitat should kelp continue to decline. Lastly, quantifying larval development time and mortality rates in relation to temperature could more conclusively link observed relationships between increasing temperature and increased abundance of settlers to factors affecting larvae of *M. membranacea*. Studies examining effects of climate change on pelagic life-stages would also have implications for larval dispersal and the spread of *M. membranacea* in the northwest Atlantic under conditions of warmer seawater temperature.

This thesis provides evidence that demographic processes and life-history stages of introduced species can be governed by different combinations of biotic and abiotic factors within invaded habitats, and that these factors can drive changes in the population dynamics and life-history strategies of introduced species outside of their native environments. Effects of climate change on ecosystem structure can influence invasion success, further complicating predictions of the ecological impact of invasive species under future climate conditions. Thus, it is necessary to quantify demographic rates and life-history processes for introduced species within their invaded habitat and in response to projected changes in the invaded habitat to most accurately predict long-term impacts of species invasions. Unfortunately, this may not always be logistically feasible at the spatial and temporal scales necessary for affectively managing populations of introduced species to prevent establishment or further spread during the early stages of invasion. However, identifying specific ecological impacts of introduced species in response to

global change may allow for negative effects of biological invasions to be mitigated indirectly through the management of other anthropogenic stressors that similarly impact ecosystem function.

APPENDIX A

CHAPTER 3

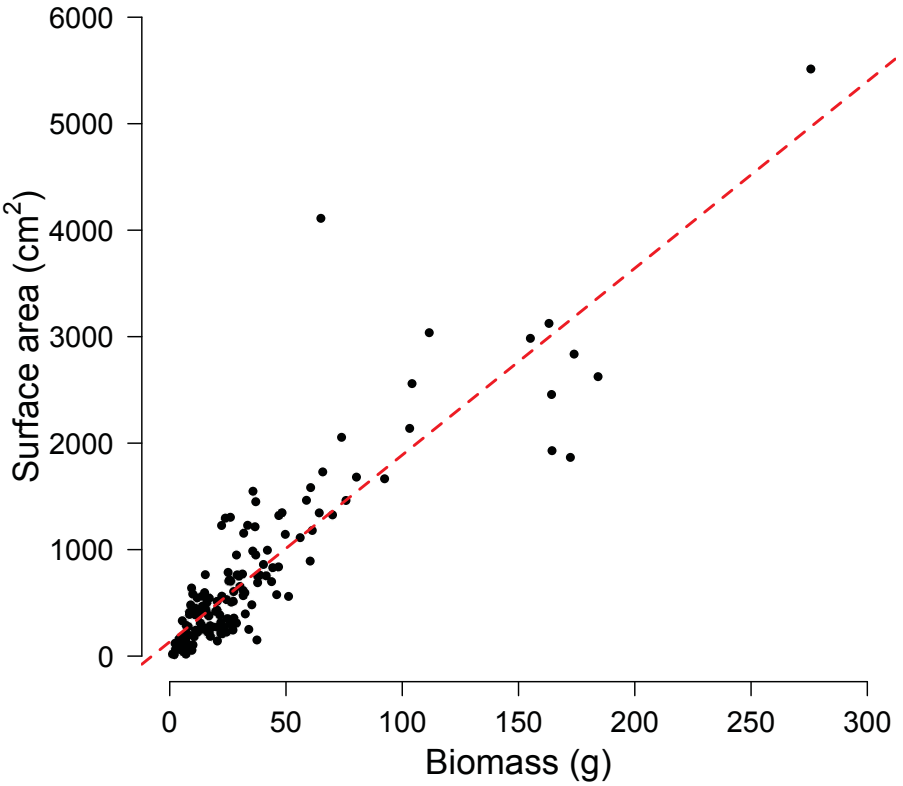

Figure A.1 The relationship between surface area (SA) and biomass (M) for 172 blades of *Agarum clathratum* collected at 3 sites (The Lodge, Paddy's Head and Sandy Cove) and 2-3 depths per site (4 and 8 m at Sandy Cove; 4, 8 and 12 m at The Lodge and Paddy's Head) from June 2012 to August 2013. [SA = 17.54 M + 134.2, $r^2 = 0.78$, $p < 0.0001$]

A.1 The Effect Of Understory Kelp On Bryozoan Settlement: Density Of Kelp Within Kelp Bed Treatments

At Sandy Cove, kelp density within the kelp bed did not vary substantially over the study period (Table A.1) and was significantly greater than for areas clear of kelp (4 m: One-sample *t*-test: $t = 8.45$, $df = 4$, $p = 0.001$; 8 m: One-sample *t*-test: $t = 9.97$, $df = 4$, $p = 0.0003$). At The Lodge, the abundance of kelp declined in 2012-2013, resulting in low kelp densities within "kelp beds", particularly at shallow depths (Table A.1). However, the density of kelp within kelp beds remained significantly greater than for areas clear of kelp at both depths (8 m: One-sample *t*-test, $t = 2.16$, $df = 4$, $p = 0.048$; 12 m: One-sample *t*-test, $t = 6.05$, $df = 4$, $p = 0.002$). At both sites, but particularly at The Lodge, the collectors were placed to ensure that the bottom plate was completely covered by individual kelp plants, and within the immediate proximity of any cue that the kelp may have provided.

Table A.1 Density of mixed kelp beds consisting of *Saccharina latissima, Laminaria digitata,* and *Agarum clathratum* at 8 m (shallow) and 12 m (deep) at The Lodge and at 4 m (shallow) and 8 m (deep) at Sand Cove in areas adjacent to (within ~ 5 m) settlement collectors placed in "kelp" treatments. During each sampling date at the Lodge, the abundance of all established kelps (>20 cm in length) was measured within a 2 x 30 m transect oriented parallel to the shore and following the specified depth contour (8 or 12 m). Kelp density was calculated for each sampling date by dividing the number of kelps by the corresponding sampling area (60 m^2). At Sandy Cove, the abundance of all established kelps was measured within 8-11 haphazardly-placed 0.5-m^2 quadrats at each of 4 and 8 m. Kelp density per 0.5 m^2 was converted to kelp density per m^2 by multiplying the abundance of kelp in each quadrat by a factor of 2. Kelp density (m^{-2}) at Sandy Cove presented for each sampling date is the average over all quadrats sampled at each depth on that date (n = 8-11). Kelp density in the corresponding areas clear of kelp was zero for all sampling dates. Settlement collectors were deployed from September 2012 to November 2013; mean kelp density is the average over all sampling dates (n = 5)

	Depth (m)	Density (m^{-2})
The Lodge		
13 Sep 2012	8	0.85
	12	3.2
20 Nov 2012	8	0.03
	12	1.3
27 Mar 2013	8	0.18
	12	2.7
01 Jun 2013	8	0.4
	12	1.5
09 Aug 2013	8	1.6
	12	2.1
Mean ± SD	8	0.612 ± 0.633
(n = 5)	12	2.16 ± 0.799
Sandy Cove		
25 Sep 2012	4	17.9
	8	16.3
29 Nov 2012	4	9.5
	8	12
15 Mar 2013	4	12.3
	8	10.25
5 Jun 2013	4	10.25
	8	14.0
01 Aug 2013	4	12.22
	8	9.5
Mean ± SD	4	12.43 ± 3.29
(n = 5)	8	12.41 ± 2.78

Table A.2 The effects of understory kelp (treatment: within kelp bed, outside kelp bed), distance above the substratum (position: top plate, bottom plate), and depth (shallow, deep) on settlement of *Membranipora membranacea* and *Electra pilosa* larvae. Results of model selection using zero-inflated negative binomial models (ZINB). ZINB was chosen over zero-inflated Poisson (ZIP) to account for overdispersion in the count data. The mean (μ_i) for the count data and the probability (π_i) for the binomial distribution are modelled in terms of the fixed (treatment, position, depth) and both the fixed and random (site, date, collector) variables, respectively. Significant *p*-values shown in bold at α = 0.05

Dropped term	df	AIC	Likelihood ratio test
Membranipora membranacea			
π_I = modelled in terms of Treatment, Position, Depth, Site, and Date			
None	17	970.6	
Treatment from μ_i	12	968.3	$\chi^2 = 3.18$ (df = 1, $p = 0.075$)
Position from μ_i	**10**	**1017**	**$\chi^2 = 36.2$ (df = 1, $p < 0.0001$)**
Depth from μ_i	**11**	**982.9**	**$\chi^2 = 16.6$ (df = 1, $p < 0.0001$)**
Treatment x position from μ_i	13	967.1	$\chi^2 = 2.58$ (df = 1, $p = 0.108$)
Treatment x depth from μ_i	14	966.5	$\chi^2 = 0.722$ (df = 1, $p = 0.395$)
Position x depth from μ_i	15	967.8	$\chi^2 = 0.942$ (df = 1, $p = 0.332$)
Treatment x position x depth from μ_i	16	968.8	$\chi^2 = 0.264$ (df = 1, $p = 0.607$)
Treatment from π_i	16	968.7	$\chi^2 = 0.124$ (df = 1, $p = 0.725$)
Position from π_i	**15**	**984.3**	**$\chi^2 = 17.6$ (df =1, $p < 0.0001$)**
Depth from π_i	14	984.3	$\chi^2 = 2.20$ (df = 1, $p = 0.138$)
Site from π_i	**13**	**1016**	**$\chi^2 = 33.9$ (df =1, $p < 0.0001$)**
Date from π_i	**10**	**1170**	**$\chi^2 = 159$ (df =1, $p < 0.0001$)**
π_I = modelled in terms of Collector			
None	89	1242	
Collector from π_i	17	1172	$\chi^2 = 74.6$ (df = 72, $p = 0.395$)
Electra pilosa			
π_I = modelled in terms of Treatment, Position, Depth, Site, and Date			
None	15	505.3	
Treatment from μ_i	10	503.0	$\chi^2 = 0.228$ (df = 1, $p = 0.633$)
Position from μ_i	8	508.7	$\chi^2 = 1.83$ (df = 1, $p = 0.176$)
Depth from μ_i	**9**	**508.8**	**$\chi^2 = 7.78$ (df = 1, $p = 0.005$)**
Treatment x position from μ_i	11	504.8	$\chi^2 = 5.43$ (df = 1, $p = 0.020$)
Treatment x depth from μ_i	12	501.4	$\chi^2 = 1.92$ (df = 1, $p = 0.165$)
Position x depth from μ_i	13	501.5	$\chi^2 = 0.169$ (df = 1, $p = 0.681$)
Treatment x position x depth from μ_i	14	503.3	$\chi^2 = 0.006$ (df = 1, $p = 0.939$)

Dropped term	df	AIC	Likelihood ratio test
Treatment from π_i	**14**	**508.1**	**$\chi^2 = 4.89$ (df = 1, $p = 0.028$)**
Position from π_i	13	509.2	$\chi^2 = 3.03$ (df =1, $p = 0.082$)
Depth from π_i	12	508.9	$\chi^2 = 1.75$ (df = 1, $p = 0.186$)
Site from π_i	11	507.2	$\chi^2 = 0.260$ (df =1, $p = 0.611$)
Date from π_i	**10**	**546.8**	**$\chi^2 = 41.6$ (df =1, $p < 0.0001$)**
π_I = modelled in terms of Collector			
None	89	608.2	
Collector from π_i	17	550.2	$\chi^2 = 86.0$ (df = 72, $p = 0.125$)

A.2 The Effect Of Strength Of The Settlement Cue On The Rate Of Settlement Of *Membranipora membranacea* Larvae In Laboratory Experiments

Saccharina latissima was chosen as the algal substrate for these experiments based on observations of consistently high settler abundance on this substrate in the field (D. Denley pers obs). Competent larvae were isolated from plankton samples collected from St. Margarets Bay in September-October 2016. Blades of *S. latissima* were collected during the same period from 8 m at The Lodge and/or Sandy Cove. Thirty competent larvae were introduced into 250-ml beakers of 1μm-filtered seawater containing a single small (1 cm x 1 cm) or large (2 cm x 2 cm) segment of *S. latissima*. We allowed larvae to settle over 72 h, after which *S. latissima* segments were examined for settlers. Replicates of the settlement cue experiment were conducted over the course of 4 separate trials from September-October 2016.

We used linear mixed models to determine whether the rate of settlement (number of settlers 72 h^{-1}) was affected by the strength of the settlement cue. Settlement cue (2 levels: strong cue, weak cue) was a fixed effect, with separate intercepts for the random effect of trial and random slopes for the interaction between trial and settlement cue. The number of settlers was log(x+0.01)-transformed to better approximate a normal distribution. There was no effect of strength of the settlement cue on the rate of settlement (mean number of settlers ± SD, large cue: 2.8 ± 2.9; small cue: 2.2 ± 2.7; LMM likelihood ratio test: $\chi^2_{(1)} = 0.454, p = 0.50, n = 15$).

APPENDIX B

CHAPTER 4

B.1 Detailed Methods

B.1.1 Study sites

All three sites were characterized by mixed kelp beds, dominated by *Saccharina latissima* and *Laminaria digitata*, with *Agarum clathratum* being more abundant at greater depths (Saunders & Metaxas 2009b). At The Lodge and Paddy's Head, sampling was conducted along 4-, 8-, and 12-m depth contours, while at Sandy Cove, sampling was done at 4- and 8-m depths only, because the seabed becomes sandy at greater depths. Sampling depths were chosen to represent differences in algal composition and decreasing wave intensity with increasing depth at each site.

B.1.2 Collection of *M. membranacea* colonies on kelp

At The Lodge and Paddy's Head, during each sampling time we measured the abundance of all established kelps (>20 cm in length) within a 2 x 30 m transect oriented paralleled to the shore and following the specified depth contour (4, 8 or 12 m) at each depth. The starting position of each transect was determined haphazardly and, following measurements of kelp abundance, we collected 10-15 individuals of each kelp species at random within the 60-m^2 sampling area. If fewer than 10 individuals of any species were encountered within the 60-m^2 sampling area, the remaining kelps were collected upon encounter outside the sampling area, but within the specified depth contour. At Sandy Cove where the mixed kelp bed is considerably denser, we quantified the abundance of kelp by collecting all thalli from 8-11 haphazardly-placed 0.5-m^2 quadrats at each of 4 and 8 m. Collected specimens were immediately transported to the Aquatron facility at Dalhousie University in plastic tubs without seawater, where they were maintained in

aquaria with running ambient seawater until processing was completed, typically within 3-4 days.

B.1.3 Estimating kelp biomass

For The Lodge and Paddy's Head, we photographed the 10-15 individuals of *S. latissima* and *L. digitata* collected at each depth and determined the surface area of each kelp using ImageJ. Blades of *S. latissima* are highly crenulated; therefore, we corrected surface area measurements for this species using location- and depth-specific correction factors (Saunders & Metaxas 2007). For Sandy Cove, we photographed and measured the surface area of 2-5 *S. latissima* and *L. digitata* from each 0.5-m^2 quadrat. Kelp surface area (m^2) was converted into biomass (kg) using species-specific simple linear regressions (Yorke & Metaxas 2011). The perforated nature of *A. clathratum* makes it difficult to accurately measure its surface area using image analysis. Therefore, for this species, we measured the biomass of all individuals collected from all three sites directly, using a triple beam balance (accuracy, 0.005 g). For each site, we calculated seasonal loss of kelp biomass at each depth by subtracting the average biomass (kg m^{-2}) of each kelp species during a sampling time from that in the previous sampling time. A net gain in kelp biomass between sampling dates is indicated by a negative value, or a negative loss in biomass.

B.1.4 Quantifying whole-colony and partial mortality of *M. membranacea* colonies on kelps

We randomly selected 5-15 colonies per size-class and classified a subset of zooids within each colony (15 zooids for colonies ≤ 3 cm in diameter, 30 zooids for colonies > 3 cm in diameter) by polypide cycle (differentiating, active, reproductive, or degenerated). For colonies containing < 15 individual zooids (< 0.25 cm in diameter), all zooids within the colony were classified and the proportion of degenerated zooids was calculated by diving the number of zooids that had degenerated by the total number of zooids making up the colony.

B.1.5 Quantifying partial mortality of *M. membranacea* colonies on settlement plates in the laboratory

We deployed settlement collectors consisting of four vertically suspended 10 x 15 cm settlement plates cut from Sintra plastic PVC sheets at 8 m depth at The Lodge using SCUBA in August 2012. Colonies of *M. membranacea* were allowed to settle and grow undisturbed on the settlement plates from August 2012 to November 2012. We numbered and photographed plates upon collection, and measured the surface area (cm^2) of all colonies on each plate from the photographs using ImageJ. Every colony on each plate was labeled with a number. During acclimation (20 November 2012 to 9 January 2013), as well as for subsequent experimental treatments beginning on 9 January 2013, colonies were fed a combination of live microalgae three times a week at concentrations (~4.5 x 10^4 cells ml^{-1}) that are known to be sufficient for unlimited colony growth under laboratory conditions (Saunders & Metaxas 2009a) and are greater than the range of phytoplankton concentrations typically observed off the coast of Nova Scotia at this time of year (10^0 - 10^3 cells ml^{-1}) (Metaxas & Scheibling 1996).

We photographed colonies and measured colony surface area (cm^2) again on 9 January 2013. The percentage partial mortality that had occurred as a result of colony senescence during acclimation was determined for each colony as the difference in surface areas between 9 January 2013 and 20 November 2012. Based on these differences, we divided colonies into four levels of initial partial mortality: control colonies (< 25% senesced), 25% senesced, 50% senesced, and 75% senesced.

B.2 Detailed Analyses

B.2.1 Temporal variation in partial and whole-colony mortality of *M. membranacea*

To account for any variation associated with site, depth and sampling time were entered as fixed effects in LMM and GLMM with separate intercepts for the random effect of site. Random effects in both LMM and GLMM were evaluated at each hierarchical level of sampling time within depth within site. Sampling time was included as a fixed effect because sampling times were chosen to correspond with the seasonal

timing of events in the annual life cycle *M. membranacea* (Saunders & Metaxas 2009b). For the percentage of degenerated zooids, algal blade was also included as a random effect (nested within sampling time), to account for any potential correlation among colonies collected from the same kelp blade. Visual inspection of residual plots did not reveal deviations from normality or homoscedasticity for the percentage of degenerated zooids. Instantaneous mortality was arcsine square root transformed to eliminate heterogeneity of variance as detected by Levene's test; however, this transformation did not completely alleviate non-normal distribution (Shapiro-Wilk test, $p = 0.04$).

Because kelp biomass varies with depth (Figures B.1 – B.3), measurements of mortality and loss of kelp biomass for regressions were calculated separately for each of the 2-3 depths sampled per site (TL and PH: 4, 8, and 12m; SC: 4 and 8m) during each sampling period. Instantaneous rates of whole colony mortality and the percentage of degenerated zooids were arcsine square-root transformed and logit transformed, respectively, to achieve normality (Shapiro-Wilk test, instantaneous mortality, $p = 0.07$; percentage of degenerated zooids, $p = 0.72$).

B.2.2 Effects of temperature and colony size on partial and whole-colony mortality of *M. membranacea in situ*

For ANOVA, instantaneous mortality rate was arcsine square-root transformed to eliminate heterogeneity of variance (Levene's test, $p = 0.122$). For regression analyses, instantaneous mortality and the percentage of degenerated zooids were arcsine square-root and logit transformed, respectively. Data transformation did not correct for non-normal distribution (Shapiro-Wilk test, $p<0.05$), however, ANOVA and regression statistics are robust to deviations from normality (Zar 1999).

B.2.3 Effects of temperature, colony size, and level of initial partial mortality on senescence of *M. membranacea* in the laboratory

Model selection was achieved using likelihood ratio tests, and p-values for model selection were obtained using the chi-square distribution. The effects of temperature and level of initial partial mortality on the relative senescence of colonies varied between months, however, there was no significant effect of plate after 30, 62, or 92 days (p = 0.242-0.341). For all analyses, percent decrease in surface area was arcsine transformed

to successfully remove heterogeneity of variance, except day 92, for which a more conservative $\alpha_{critical}$ (0.01) was adopted for ANOVA.

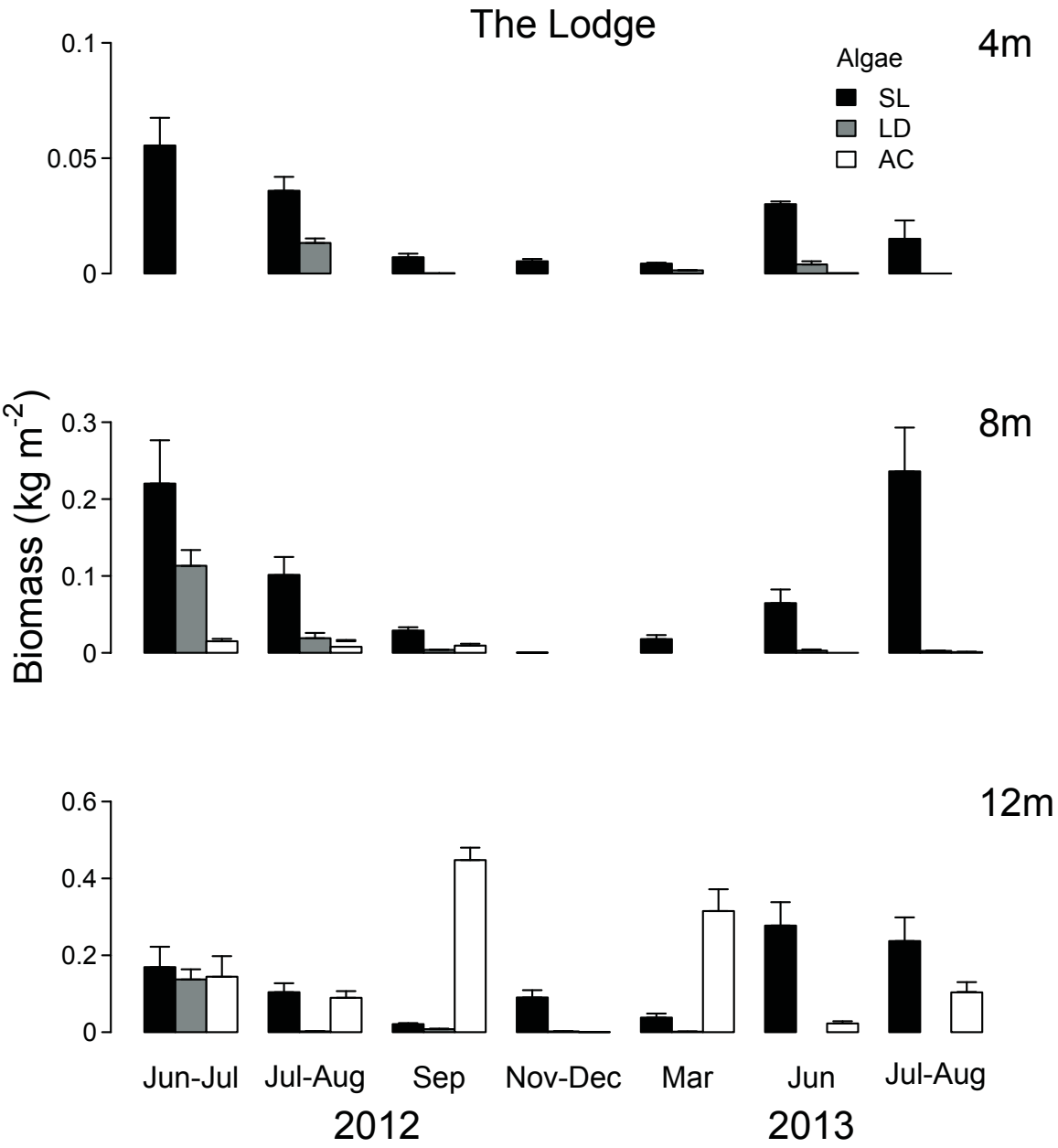

Figure B.1 Biomass (mean + SE, n = 1-14) of the three most numerically abundant kelp species off the coast of Nova Scotia (*Saccharina latissima*: SL, *Laminaria digitata*: LD, *Agarum clathratum*: AC) across three depths at The Lodge over one complete seasonal cycle from June 2012 - August 2013

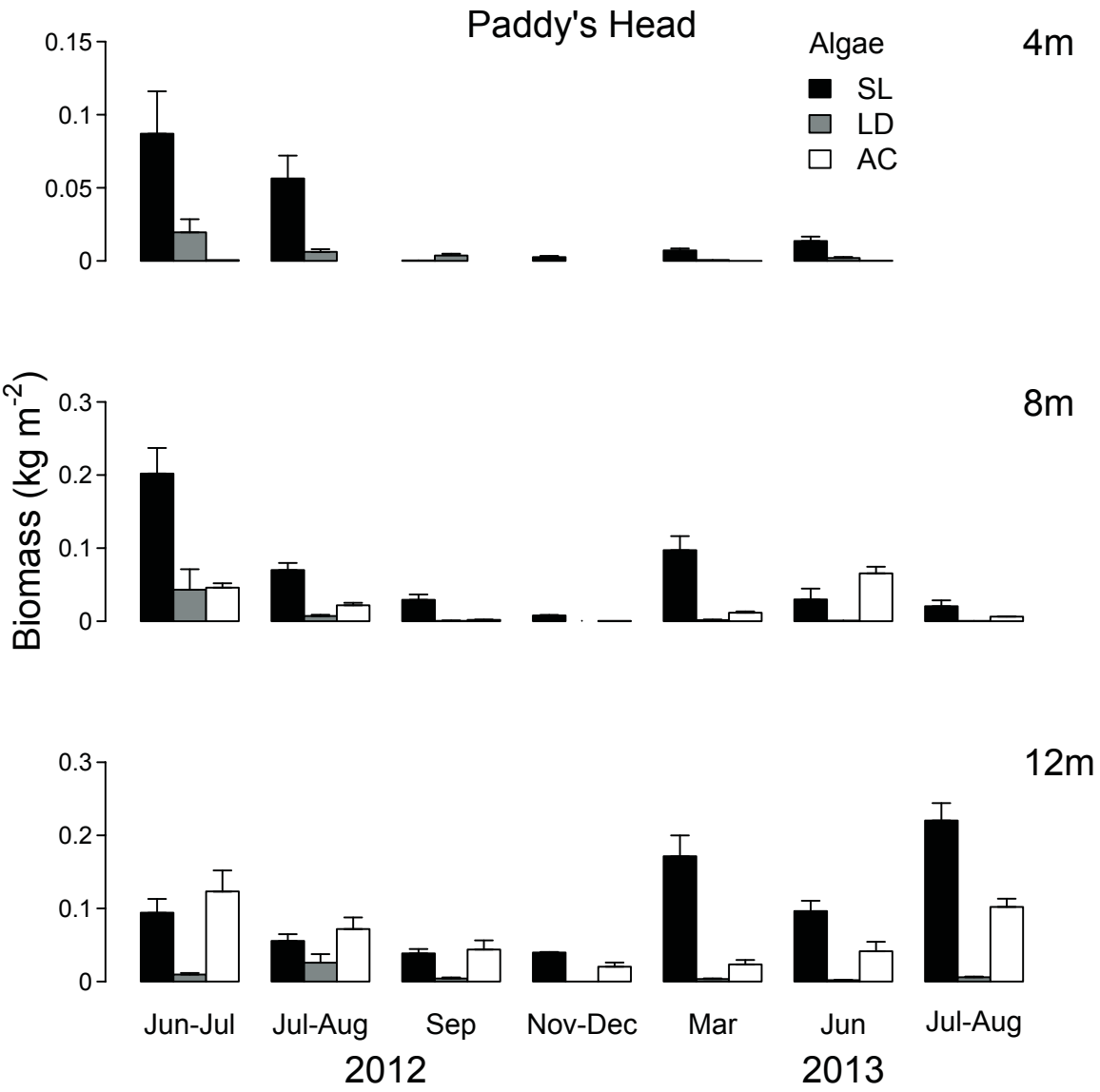

Figure B.2 Biomass (mean + SE, *n* = 1-14) of the three most numerically abundant kelp species off the coast of Nova Scotia (*Saccharina latissima*: SL, *Laminaria digitata*: LD, *Agarum clathratum*: AC) across three depths at Paddy's Head over one complete seasonal cycle from June 2012 - August 2013

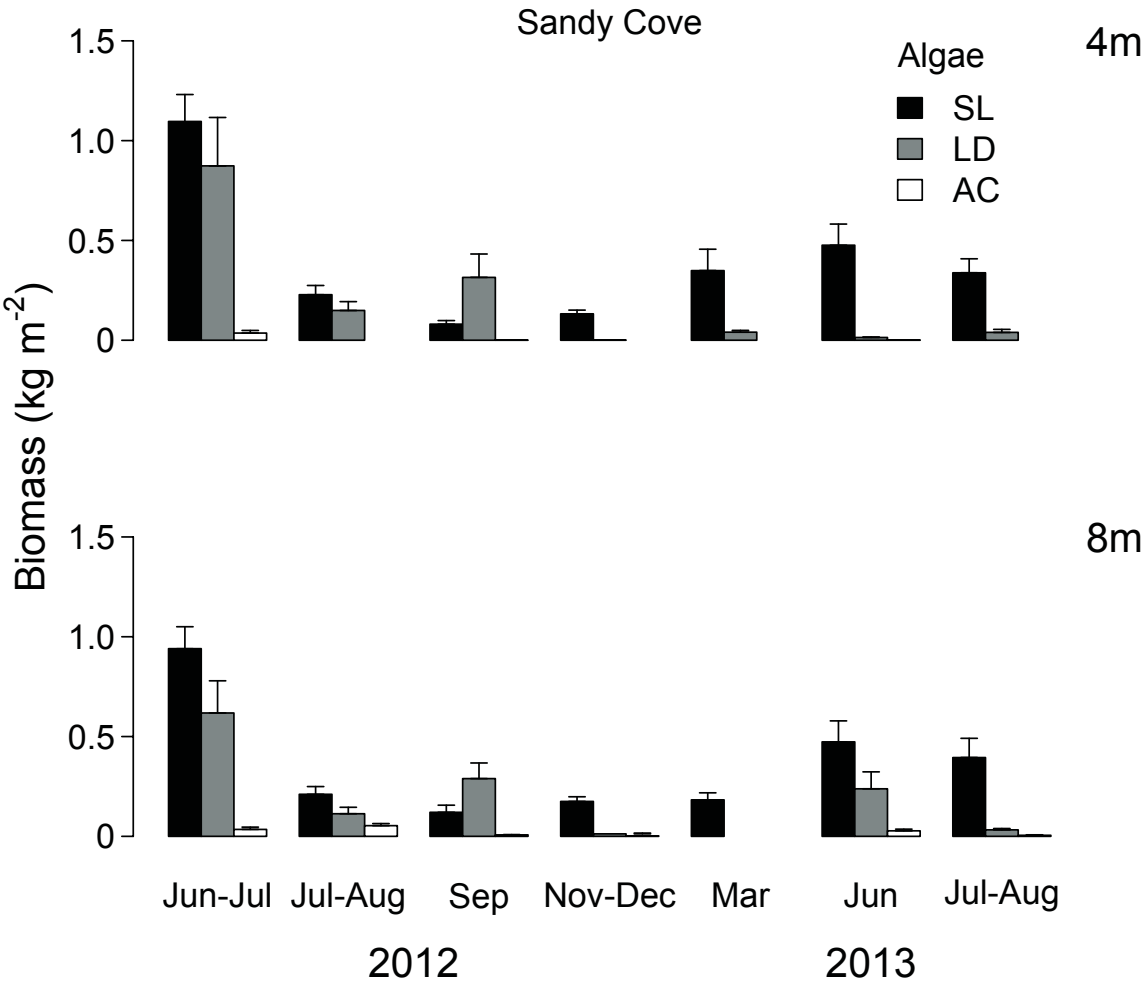

Figure B.3 Biomass (mean + SE, n = 1-64) of the three most numerically abundant kelp species off the coast of Nova Scotia (*Saccharina latissima*: SL, *Laminaria digitata*: LD, *Agarum clathratum*: AC) across two depths at Sandy Cove over one complete seasonal cycle from June 2012 - August 2013

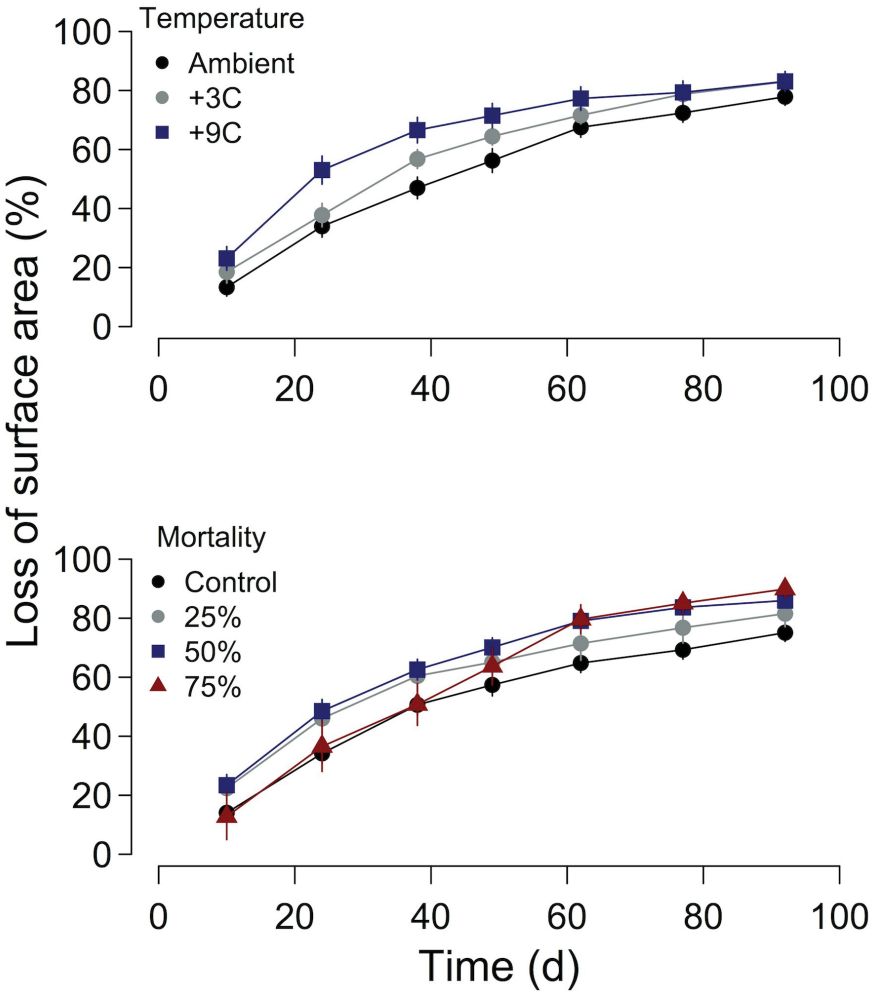

Figure B.4 Percent loss of surface area (mean + SE, n = 20 - 64 colonies) as a result of senescence for *Membranipora membranacea* colonies under three levels of temperature (ambient, ambient +3°C, and ambient +9°C) and four levels of initial partial mortality (control [<25%], 25%, 50%, and 75%) measured bi-weekly over a period of 92 days

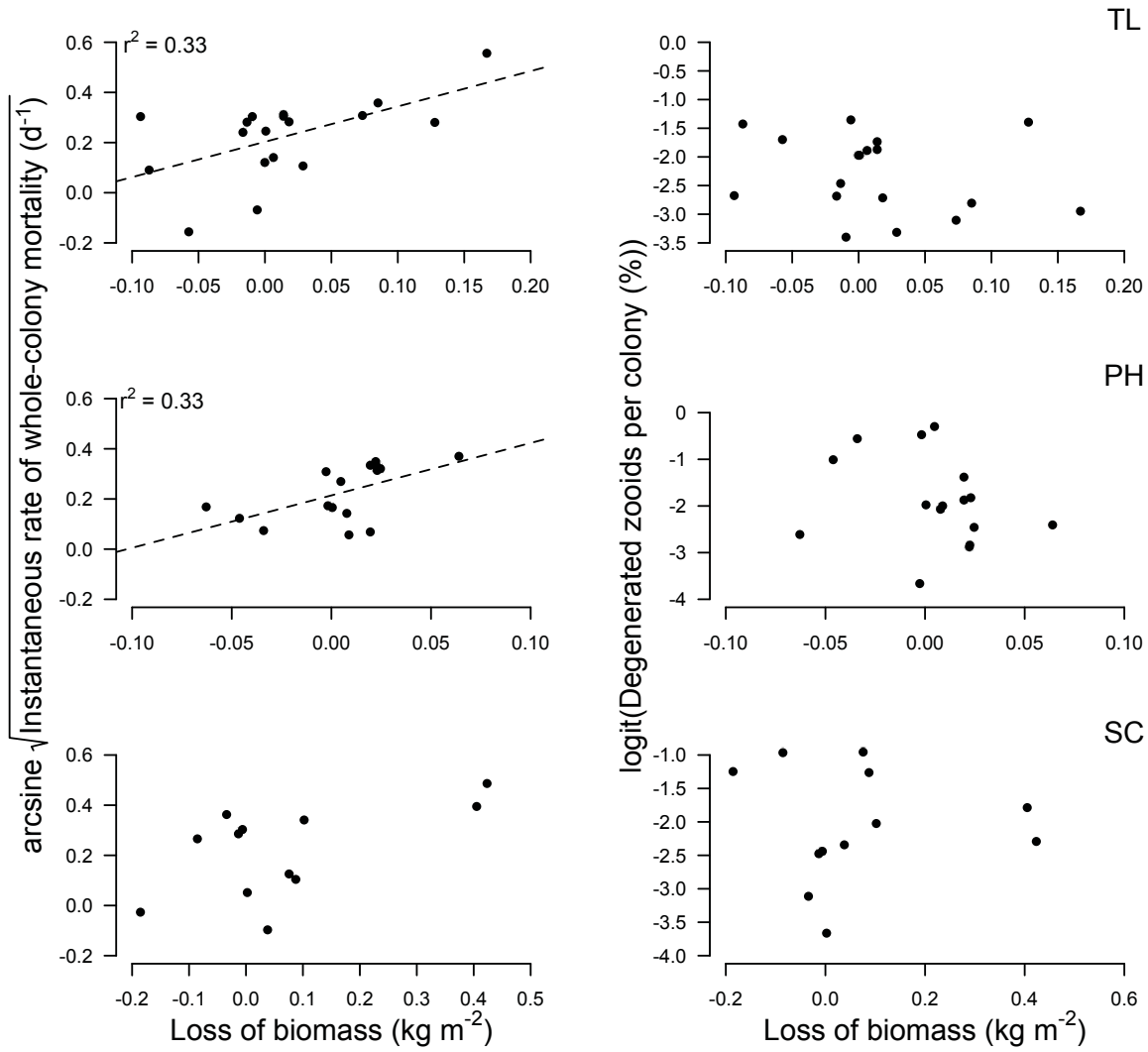

Figure B.5 Linear regressions of the instantaneous rate of whole-colony mortality (d^{-1}) of *Membranipora membranacea* colonies (arcsine square-root transformed) and the percentage (%) of degenerated zooids per colony (logit transformed) with increasing loss of kelp biomass (kg m^{-2}) for three different sites on the southwestern shore of Nova Scotia (The Lodge: TL, Paddy's Head: PH, Sandy Cove: SC) over a seasonal cycle of the annual life cycle of *M. membranacea* from July 2012 to August 2013

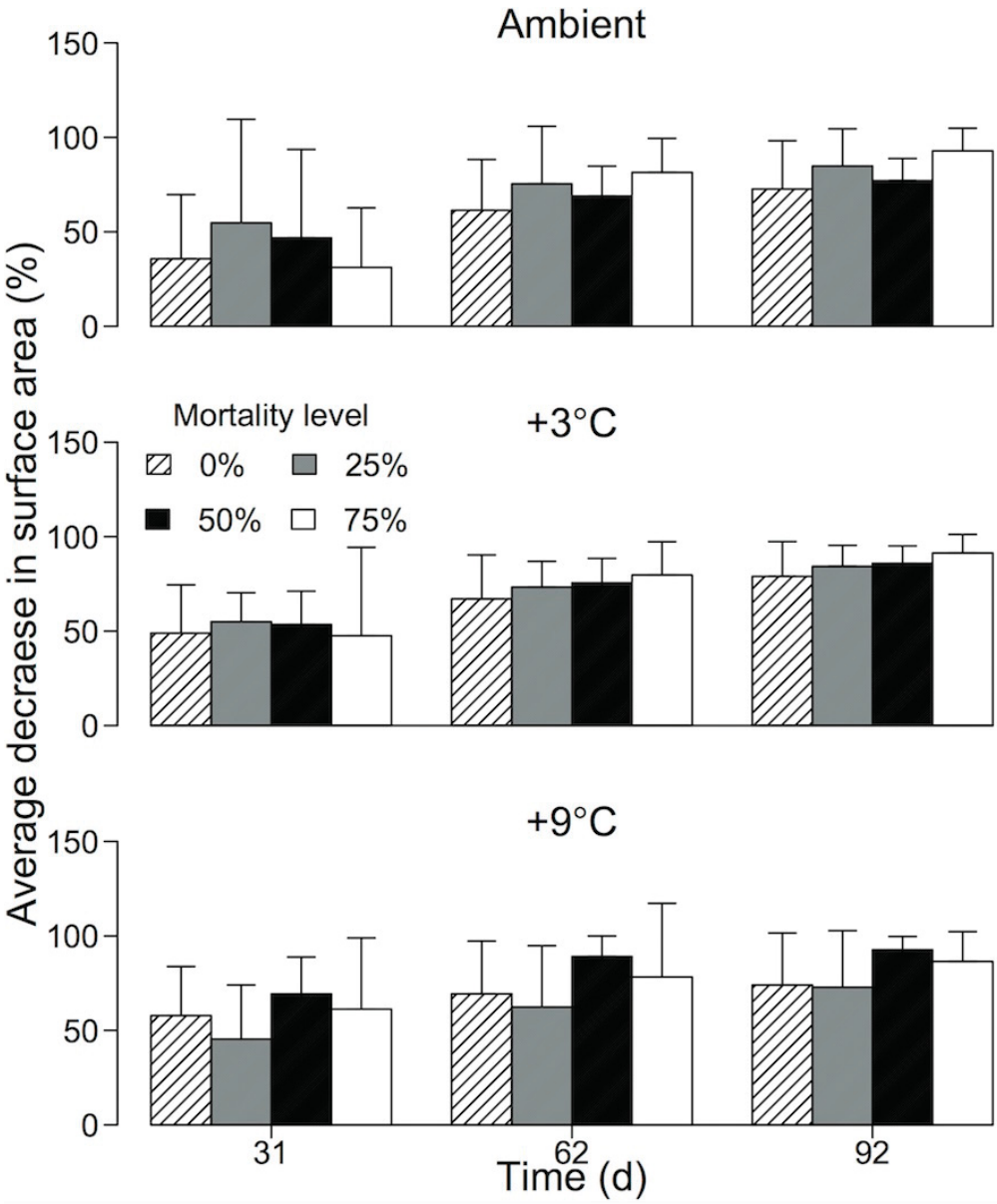

Figure B.6 Senescence of *Membranipora membranacea* colonies at three levels of temperature (ambient, ambient +3°C, and ambient +9°C) and four levels of initial partial mortality (<25%, 25%, 50%, and 75%), measured monthly over a period of 92 days. Data are mean percent loss of colony surface area + SD, n = 6-30 colonies

APPENDIX C

CHAPTER 5

Table C.1 Results of four-way ANOVA examining the effects of temperature (5°C, 12°C, 20°C), damage percentage (50%, 75%) and damage location (central zooids removed, peripheral zooids removed) on relative recovery of colonies after 7 and 14 days (repeated measures). Relative recovery was calculated as a percentage of the initial colony size following artificially inflicted damage. Significant values shown in bold ($\alpha = 0.05$)

Effect	df	MS	*F*	*p*
Between subjects				
Temperature	2	2.16	9.44	**0.0001**
Damage percentage	1	0.112	0.489	0.486
Damage location	1	22.6	98.8	**<0.0001**
Temperature x damage percentage	2	0.252	1.10	0.335
Temperature x damage location	2	1.20	8.73	**0.0003**
Damage percentage x damage location	1	0.032	0.139	0.710
Temperature x damage percentage x damage location	2	0.654	2.86	0.060
Error	156	0.288		
Within subjects				
Day	1	3.36	72.1	**<0.0001**
Day x temperature	2	0.627	13.5	**<0.0001**
Day x damage percentage	1	0.056	1.21	0.274
Day x damage location	1	0.033	0.707	0.402
Day x temperature x damage percentage	2	0.076	1.63	0.200
Day x temperature x damage location	2	0.024	0.511	0.601
Day x damage percentage x damage location	1	0.042	0.901	0.344
Day x temperature x damage percentage x damage location	2	0.085	1.83	0.164
Error	156	0.047		

APPENDIX D

CHAPTER 6

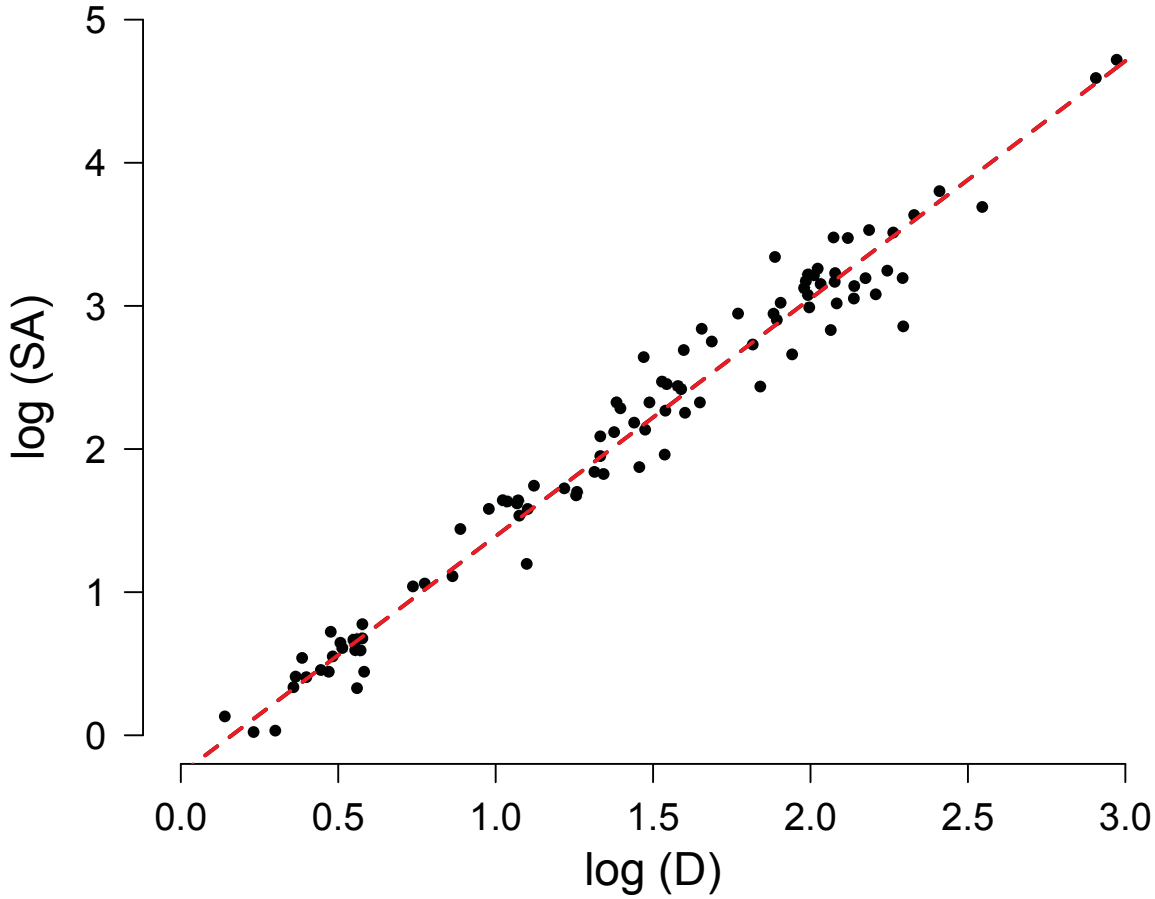

Figure D.1 The relationship between colony surface area (SA) and diameter (D) for 100 colonies of *Membranipora membranacea* collected on *Laminaria digitata* at Sandy Cove on 14 August, 2013. [log (SA) = 1.66 log (D) – 0.269, $r^2 = 0.97$, $p < 0.0001$]

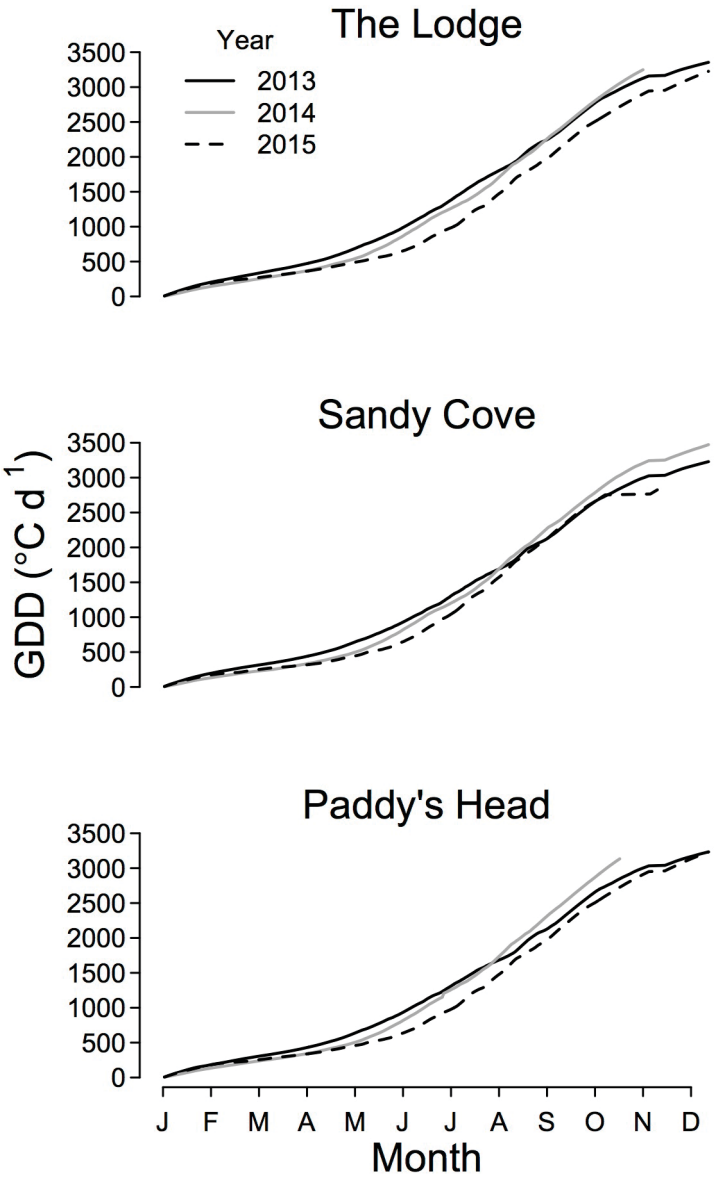

Figure D.2 Interannual differences in GDD for 3 sites on the southwestern shore of Nova Scotia (The Lodge, Sandy Cove, and Paddy's Head). GDD is depth-averaged across 2-3 depths for each site (The Lodge and Paddy's Head: 4, 8 and 12 m; Sandy Cove: 4 and 8 m). The winter/spring temperature regime (GDD from January 01 to June 30) was significantly lower in 2015 than in 2013 and 2014

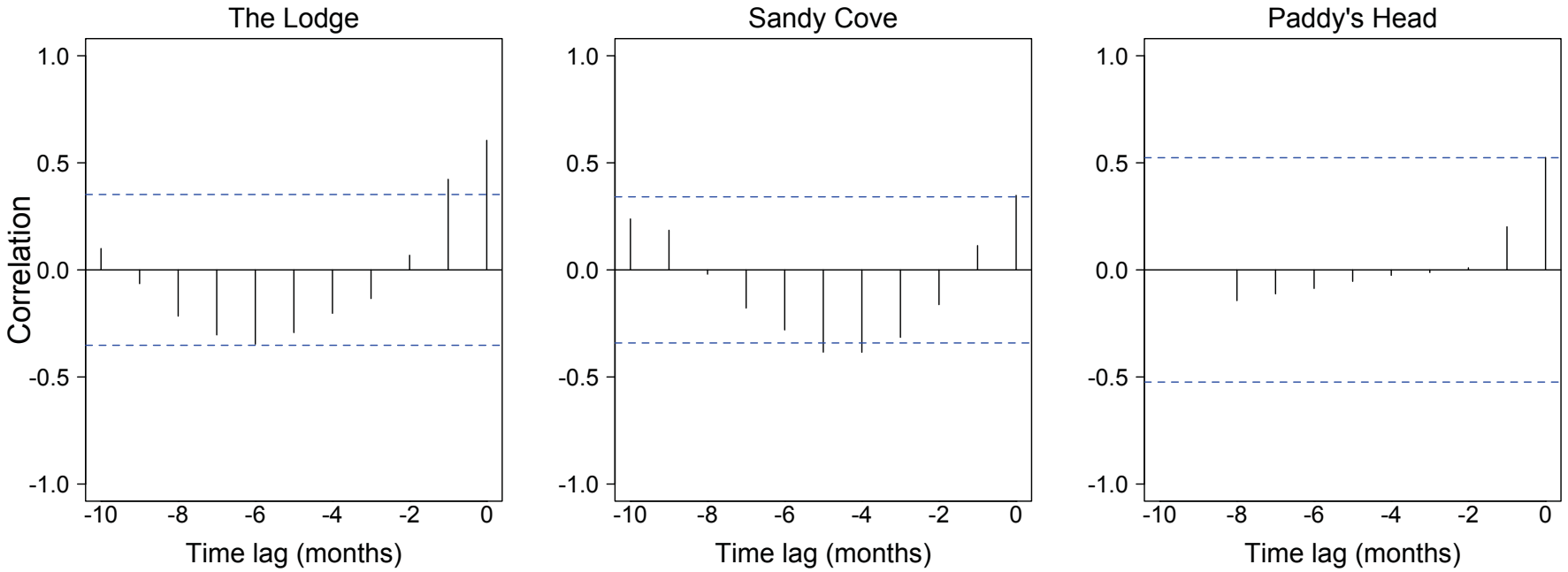

Figure D.3 Cross-correlation analysis between potential fecundity of *Membranipora membranacea* and monthly average temperature at The Lodge and Sandy Cove from June 2012 to November 2015, and at Paddy's Head from June 2012 to August 2013. Dotted lines indicate significant positive or negative correlation

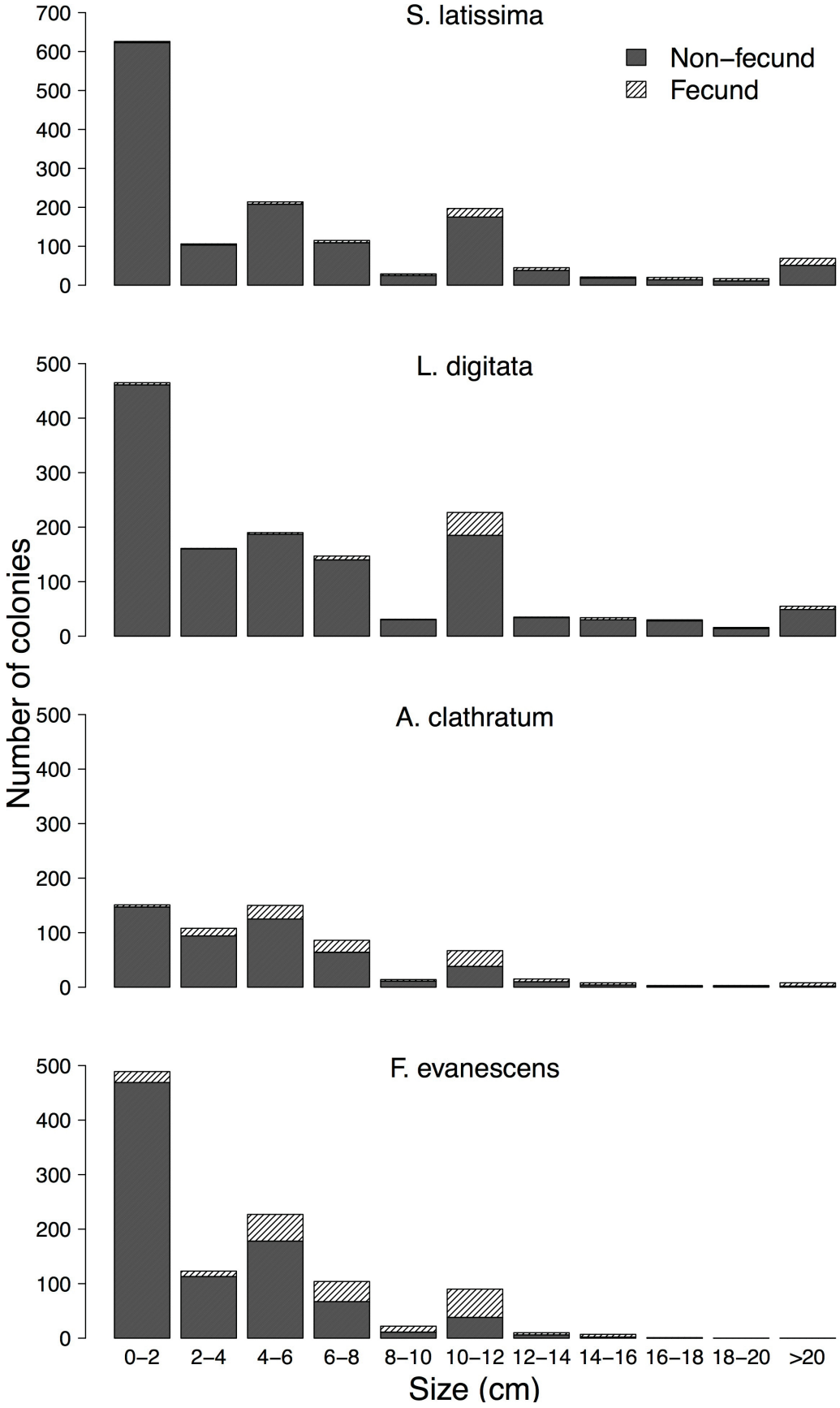
S. latissima
Non−fecund
Fecund
L. digitata
A. clathratum
F. evanescens
Number of colonies
0
100
200
300
400
500
600
700
0−2
2−4
4−6
6−8
8−10
10−12
12−14
14−16
16−18
18−20
>20
Size (cm)

Figure D.4 Size (diameter, cm) frequency distribution and the number of fecund (producing oocytes) colonies of *Membranipora membranacea* on the 4 most numerically abundant algal substrates in Nova Scotia collected at The Lodge, Sandy Cove, and Paddy's Head from June 2012 to August 2013; and at The Lodge from July 2014 to November 2015 and Sandy Cove from May 2014 to November 2015. N: 4377 (*Saccharina latissima*), 4173 (*Laminaria digitata*), 1839 (*Agarum clathratum*), 3219 (*Fucus evanescens*). There was a significant effect of algal substrate on colony size: one-way ANOVA, $F_{(3,4532)} = 26.4$, $p < 0.0001$; *S. latissima*, mean colony size = 5.8 cm, maximum colony size = 44.5 cm; *L. digitata*, mean colony size = 6.4 cm, maximum colony size = 44.5 cm; *A. clathratum*, mean colony size = 5.4 cm, maximum colony size = 30.0 cm; *F. evanescens*, mean colony size = 3.9 cm, maximum colony size = 7.1 cm

APPENDIX E

CHAPTER 7

E.1 Model Construction

Rates of growth, senescence, and mortality are incorporated into **A** as:

$$\mathbf{A} = \begin{bmatrix} L_1 + F_1 & P_2S_{12} + F_2 & P_3S_{13} + F_3 & P_4S_{14} + F_4 & P_5S_{15} + F_5 \\ P_1G_{21} & L_2 & P_3S_{23} & P_4S_{24} & P_5S_{25} \\ P_1G_{31} & P_2G_{32} & L_3 & P_4S_{34} & P_5S_{35} \\ P_1G_{41} & P_2G_{42} & P_3G_{43} & L_4 & P_5S_{45} \\ P_1G_{51} & P_2G_{52} & P_3G_{53} & P_4G_{54} & L_5 \end{bmatrix}$$

where P_iG_{ji} is the probability of an individual surviving P_i and growing G_i into the next stage class, P_iS_{ji} is the probability of an individual surviving and shrinking S_i into the previous stage class, and L_i is the probability of an individual surviving and remaining in the same stage class, $L_i = P_i - G_{ji} - S_{ji}$. Reproductive output via sexual reproduction is incorporated as potential fecundity (F_i, oocytes colony^{-1}) of colonies within each stage class (Figure E.1).

Table E.1. The abundance of colonies of *Membranipora membranacea* in each size class (mean per m^2 kelp) on each of the three most numerically abundant kelp species in Nova Scotia pooled across three sites (The Lodge, Paddy's Head, and Sandy Cove) in November-December 2012 used to estimate the initial population vector for the mixed kelp bed model

Kelp	Colony size (diameter, cm)	Mean number of colonies per m^2 kelp
Saccharina latissima	<1	94.6
	1-3	46.1
	3-6	19.2
	6-8	16.9
	>8	11.5
Laminaria digitata	<1	157
	1-3	101
	3-6	79.2
	6-8	48.7
	>8	69.0
Agarum clathratum	<1	7.65
	1-3	16.2
	3-6	4.41
	6-8	3.50
	>8	5.77

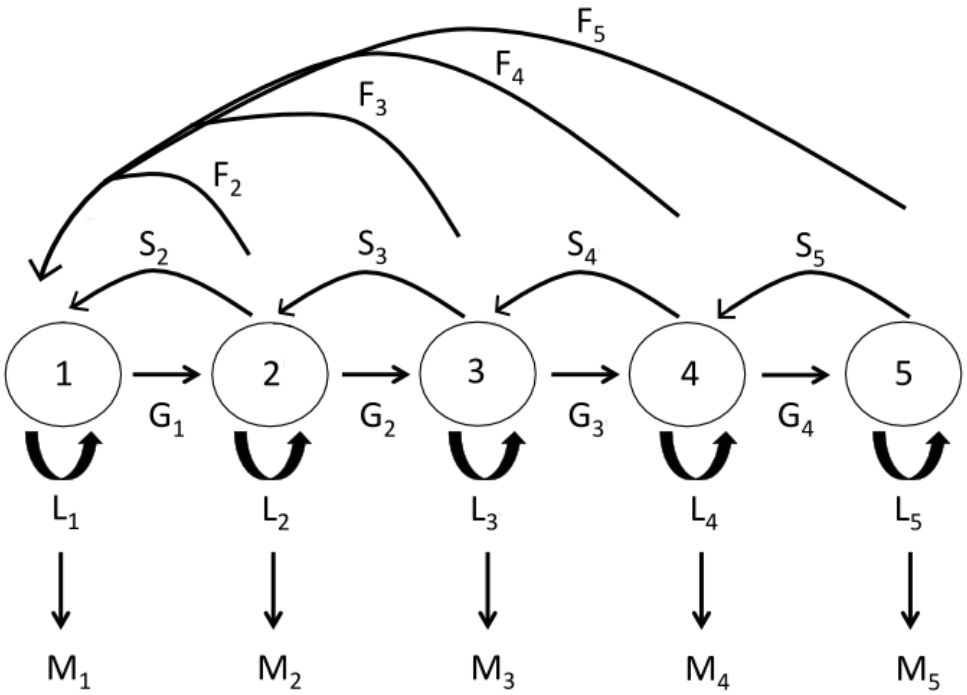

Figure E.1 Generalized life-cycle diagram for a size-classified colonial organism (increasing size classes from 1 - 5). Individuals in each size class may grow (*G*), remain in the same size class (*L*), transition into smaller size classes (*S*), or experience whole-colony mortality (*M*). Sexual recruits are contributed to the smallest size class by each size class according to size-specific fecundity (*F*). For *M. membranacea*, *M* and *F* are kelp substrate specific; *G* is temperature dependent

E.2 Model Parameterization

E.2.1 Colony growth (G_i)

The probability of a colony in size class *i* transitioning to size class *i*+1 is calculated based on temperature- and size-dependent growth rates obtained from field measurements of individual colonies (Table 7.1, Saunders & Metaxas 2009a):

[Eq. E.1] $\log(GR) = \alpha + \beta\log(S) + \chi(T)$

where *GR* is colony growth rate in mm d^{-1}, *S* is initial colony size (mm), *T* is temperature (°C), and α, β and χ are the regression parameters. For each year, daily average temperature data from

4, 8, and 12 m at The Lodge (sampled at 10-min intervals and averaged over 24 h) were averaged across depths and over 14 d intervals (beginning with 01 January - 14 January) to generate bi-weekly temperature values in correspondence with a 2-week time step. We generated normal distributions of each regression parameter using their mean and standard error from Saunders & Metaxas (2009a) ($\alpha = -1.665 \pm 0.086$; $\beta = 0.719 \pm 0.032$; $\chi = 0.072 \pm 0.008$). For each bi-weekly temperature interval (corresponding with the start of a 14-d time step), we randomly selected values for each regression parameter from their normal distributions 10,000 times (with replacement) and used these values to generate 10,000 growth rates for each size class of colony. We restricted the maximum growth rate for colonies of all sizes to 12 mm d^{-1} to ensure colony growth remained within the range observed in the field (Saunders & Metaxas 2009a). Growth rates calculated at the start of each time step were multiplied by 14 d and added to initial colony size to generate 10,000 final colony sizes for each size class of colony. Using the 10,000 calculations of final colony size, the probability of a colony in size class *i* transitioning to a larger size class $i + s$ was calculated for each time step as the number of colonies of initial size class *i* reaching size class $i + s$ divided by 10,000 ($0 \leq s \leq 2$). For colonies in the first size class, it was assumed that 100% of colonies transition to a larger size class for all time steps from 26 February to 4 November. During these months, any remaining proportion of colonies in size class 1 were included in size class 2. This assumption allowed us to incorporate selective settlement by larvae of *M. membranacea* into the model (see E.2.5 Selective settlement by larvae of *M. membranacea*) by ensuring that during each time step the first size class consisted entirely of new settlers.

E.2.2 Colony shrinkage/senescence (S_i)

In Nova Scotia, senescence of *M. membranacea* occurs during autumn/winter (October to March). From 4 November to 26 February, the probability of a colony in size class *i* transitioning to size class $i - 1$ was calculated based on size-dependent rates of senescence obtained from field measurements of tagged colonies at The Lodge:

[Eq. E.2] $\log_e(SR) = \delta(S) + \gamma$

where *SR* is the rate of colony senescence (cm d^{-1}), *S* is initial colony size (cm), and δ and γ are the regression parameters. Laboratory experiments indicate that rates of colony senescence are independent of temperature (Denley & Metaxas 2016). We generated normal distributions for each regression parameter using their mean and standard error ($\delta = 0.0474 \pm 0.009$; $\gamma = -3.452 \pm 0.696$) based on a log-linear model (Eq. E.2) of the rate of colony senescence (cm d^{-1}) relative to initial colony size (cm) for tagged colonies on *S. latissima* at The Lodge (Table 7.1). The probability of a colony in size class *i* transitioning to a smaller size class *i - s* was calculated as for colony growth. Size-specific probabilities of senescence are held constant for the duration of an annual cycle of the model and are re-sampled from the normal distributions at the beginning of each successive year. Colonies in the first size class that experienced mortality due to senescence were removed from the population for all time steps from 4 November to 26 February. For each time step during these months, any remaining colonies in size class 1 were included in size class 2. As for colony growth, this assumption allowed us to incorporate selective settlement by larvae of *M. membranacea* into the model (see E.2.5 Selective settlement by larvae of *M. membranacea*), again by ensuring that during each time step the first size class consisted entirely of new settlers.

E.2.3 Colony mortality (P_i)

We estimated instantaneous whole-colony mortality rates (μ, % d^{-1}) for each size class of colony at approximately monthly intervals using cohort analyses based on repeated sampling of the size frequency distribution of colonies at The Lodge from June 2012 to August 2013 (Table 7.1). Whole-colony mortality is primarily related to substrate dynamics (loss of kelp substrate due to breakage and erosion of kelp blades; Denley & Metaxas 2016), and the three predominant kelp species in Nova Scotia differ in terms of their rates of growth and erosion (Krumhansl & Scheibling 2011), as well as their susceptibility to breakage following encrustation by *M. membranacea* (Saunders & Metaxas 2008) and their thermal sensitivity (Simonson et al. 2015). Thus, we calculated unique size-specific rates of instantaneous whole-colony mortality for colonies occurring on each kelp substrate. Mortality rates for small (<1 cm diameter) colonies are negligible (Denley & Metaxas 2016), and we assumed 100% survival. For all size classes >1 cm in diameter on each kelp substrate, we generated log-normal distributions using the mean and

standard deviation of log(μ + 1) pooled across depths (n = 3; 4, 8, 12 m) for each sampling time (Table 7.1). We randomly sampled size- and substrate-specific rates of whole-colony mortality from the generated distributions for each sampling time, and used linear interpolation to generate bi-weekly size- and substrate-specific whole-colony mortality rates for each two-week time step from March to December. We then calculated size- and substrate-specific survival probabilities (P_i) over the two-week time step (Δk = 14 d) using Eq. 7.2, where P_{OW} is now P_i and t = 14 d. To project whole-colony mortality from 01 January to 26 February when empirical data are lacking, we used the depth-averaged instantaneous mortality rate at The Lodge in November 2012 (Denley & Metaxas 2016) to calculate P_i for all size classes of colonies for each two-week time step.

At the beginning of each successive year for which the model is run, we re-sampled monthly mortality rates used for linear interpolation from the log-normal distributions generated for each colony size class on each kelp substrate to reflect interannual variation in colony mortality.

E.2.4 Colony fecundity (F_i)

For invasive populations of *M. membranacea*, kelp substrate significantly affects colony fecundity (Denley & Metaxas 2017a). Therefore, we estimated potential colony fecundity (oocytes colony^{-1}) for each size class of colony on each kelp substrate based on approximately monthly sampling of colonies at The Lodge from 2012-2015 (Table 7.1). To capture seasonal patterns (intra-annual variation) in colony fecundity, we generated normal distributions using the mean and standard deviation of potential fecundity of colonies on each kelp substrate for each size class of colony during each sampling month (Table 7.1), pooled across depths (n = 3) and years (n = 2-4). We randomly sampled size- and substrate-specific colony fecundity for each month from the generated distributions, and used linear interpolation to generate bi-weekly size- and substrate-specific colony fecundity for each two-week time step. To account for the ~4-week larval duration of *M. membranacea* larvae (Yoshioka 1973, Temkin 1994), we offset bi-weekly fecundity values by a "lag time" of -2 time-steps (-28 d). This ensures that oocytes produced by fecund colonies enter the population as new settlers in a biologically realistic timeframe. At the beginning of each successive year for which the model is run, we re-sampled monthly fecundity

values used for linear interpolation from the normal distributions generated for each colony size class on each kelp substrate to reflect interannual variation in the reproductive output of colonies.

E.2.5 Selective settlement by larvae of *M. membranacea*

Although larvae of *M. membranacea* do not exhibit preference for any one of the three kelp species in Nova Scotia, there is evidence to suggest that larvae settle on substrates that extend furthest above the substratum (Denley & Metaxas 2017b). This settlement behavior results in a disproportionate distribution of settlers among kelp substrates, with fewer settlers on lower-lying *A. clathratum* than expected based on its relative abundance within mixed kelp beds (Denley & Metaxas 2017b). To account for this, at each time-step, the total number of oocytes (F_{total}) produced by colonies of all size classes across all three kelp substrates is calculated as:

[Eq. E.3] $$F_{total} = \Sigma F_i\, S.\ latissima + \Sigma F_i\, L.\ digitata + \Sigma F_i\, A.\ clathratum$$

The total number of oocytes produced at each time-step Δk is then re-distributed across the three kelp substrates as the number of colonies entering the first size class ($i = 1$) according to the relative abundance of settlers observed on kelp species within mixed kelp beds in the field (Denley & Metaxas 2017b).

e.g. For *S. latissima*

[Eq. E.4] $$n_1^{k+1} = (R_{Sl} \times F^{k}_{total} \times M_l)$$

where n_1 is the number of colonies in size class 1, R_{Sl} is the proportion of the total number of settlers occurring per m^2 *S. latissima*, and M_l is larval mortality.

E.2.6 Larval mortality

We assume 100% post-settlement survival (see E.2.3 Colony Mortality). For 6 May to 21 October, we selected larval mortality based on comparisons of colony fecundity (oocytes $colony^{-1}$, Denley & Metaxas 2017a) and adult (≥1 cm diameter) colony abundance (m^{-2} kelp) with the

abundance (m^{-2} kelp) of settlers (<1 cm diameter) ~4 weeks later (D. Denley, unpubl data). We multiplied colony fecundity with adult colony abundance to yield the number oocytes per m^2 kelp, and divided the number of settlers per m^2 kelp (measured ~4 weeks later to account for larval development time) by the number of oocytes per m^2 kelp as an estimate of the percentage of larval mortality. Mean larval mortality (± SD) pooled across all sampling months ($n = 6$) was 96.25 ± 4%, and was greatest in September (99.76%), and lowest in June (90.1%). Therefore, we adopted a larval mortality of 90% from 6 May to 29 July, 96% from 29 July to 9 September, and 99% from 9 September to 21 October. We assumed 100% larval mortality beginning in mid-October (22 October) of each year. This is consistent with empirical observations of substantial declines in the abundance of new settlers on kelp beginning in November (Saunders & Metaxas 2009b, Denley et al. 2014).

E.2.7 Density dependence

Colony density (number of colonies per m^2 kelp) was restricted such that the total surface area of colonies did not exceed 1 m^2 of kelp. To achieve this, for each kelp substrate at each time index k, if the total surface area of colonies at time index $k - 1 \geq 1$ m^2, colony growth (G_i) and the proportion of new settlers (R) were set to zero, while colony stasis (L_i) and senescence (S_i) continued. Total colony surface area (SA_{total}) at each time index was calculated by multiplying n^k, the vector of the abundance of individuals (per m^2 kelp) in all size classes, by a vector containing the average surface area of colonies within each size class (SA_i):

[Eq. E.5] $$SA_{Total} = [SA_1\ SA_2\ SA_3\ SA_4\ SA_5] \times \begin{bmatrix} n_1(k) \\ n_2(k) \\ n_3(k) \\ n_4(k) \\ n_5(k) \end{bmatrix}$$

We determined the average surface area of colonies within each size class based on 100 colonies we collected and photographed on *L. digitata* from Sandy Cove on 14 August 2013. We measured colony diameter and surface area from the photographs using ImageJ, and used these measurements to generate an equation relating colony diameter to colony area (Denley & Metaxas 2017a). We applied this equation to convert colony diameter (cm) to colony surface area (m^2) using the midpoint as the diameter of categorical size classes.

E.3 Model Validation

E.3.1 Methods

We validated the model by comparing 1) model projections of the seasonal dynamics of the population of *M. membranacea* (colonies per m^2 seabed) from June 2012 to August 2013 and 2) modeled estimates of two population indices, total number of colonies per m^2 seabed, and total surface area of colonies (cm^2) per m^2 seabed, early in the season (July to August 2012 and 2013, Figure 7.1) and during peak colony abundance (September to October 2012, Figure 7.1) with field data collected from mixed kelp beds over the same time periods (Denley & Metaxas 2016). The data used to construct the model were obtained from The Lodge, therefore, in addition to The Lodge, data from two other sites (Paddy's Head and Sandy Cove) were used to validate the model. Model validation was forced using site-specific temperatures and kelp abundances (see 7.3.2 Model projections).

We ran the model from 11 March 2012 to 31 December 2013 and calculated bootstrap percentile intervals for 2000 model runs (Caswell 2001) based on the proportion of the corresponding field data that fell within one standard deviation of the mean. For example, if 80% of the sampled data were within one standard deviation of the mean, we used bootstrap percentile intervals to generate 80% confidence intervals for model output. We then compared modeled estimates of the total number of colonies per m^2 seabed from June 2012 to August 2013 with sampled data (mean ± SD, pooled across size classes and summed across kelp substrates). We also compared modeled means (± 95% bootstrap percentile intervals) and sampled means (± 95% confidence intervals, obtained from Denley & Metaxas 2016) for each of the population indices for all sites (Figure 7.1). For all sites and each sampling date, we determined 1) the total number of colonies per m^2 seabed as the sum of the mean number of colonies per m^2 seabed on each kelp species, and 2) the total surface area of colonies per m^2 seabed as the sum of the mean surface area of colonies (cm^2) per m^2 seabed on each kelp species. We calculated the mean surface area of colonies per m^2 seabed on each kelp species by multiplying the mean number of colonies of each size class per m^2 seabed on each kelp species by the average surface area of colonies within each size class (as in Eq. E.5). We identify 95% confidence intervals for sampled data as

±1.96SE, were SE is the standard error in the number of colonies per m^2 kelp for each kelp species collected on a given sampling date propagated through the summation across all kelp species.

We further validated the model using data on the number of *M. membranacea* colonies per m^2 kelp (Saunders & Metaxas 2009b) and the surface area of *M. membranacea* per m^2 kelp (Yorke & Metaxas 2012) collected from The Lodge and Paddy's Head in 2005-2006 and 2008, respectively. We ran the model from 11 March 2005 to 31 December 2008, using daily average temperature data available for The Lodge from 2005-2008, and for Paddy's Head from 2006-2008. For Paddy's Head, for 2005 only, daily average temperature from The Lodge was used to force the model. Since kelp abundance data were not available for all years from 2005-2008, and available field data for validating the model were collected per unit area kelp, we forced the model using temperature data only for hindcasting. As before, we generated 95% confidence intervals of 2000 model runs using bootstrap percentile intervals (Caswell 2001), and compared modeled estimates with sampled data.

E.3.2 Results

Percentile intervals for modeled estimates of the seasonal dynamics of *M. membranacea* overlapped with sampled data for all sites except The Lodge from June to September 2012 (Figure E.2, see E.4 Sensitivity analysis). For Paddy's Head and Sandy Cove, mean model estimates overlapped with sampled data for all sampling dates, except for November 2012 at Sandy Cove (Figure E.2, see E.4 Sensitivity analysis). The model was able to successfully predict the abundance of *M. membranacea* in previous years based on corresponding temperature data (Figures E.3 & E.4).

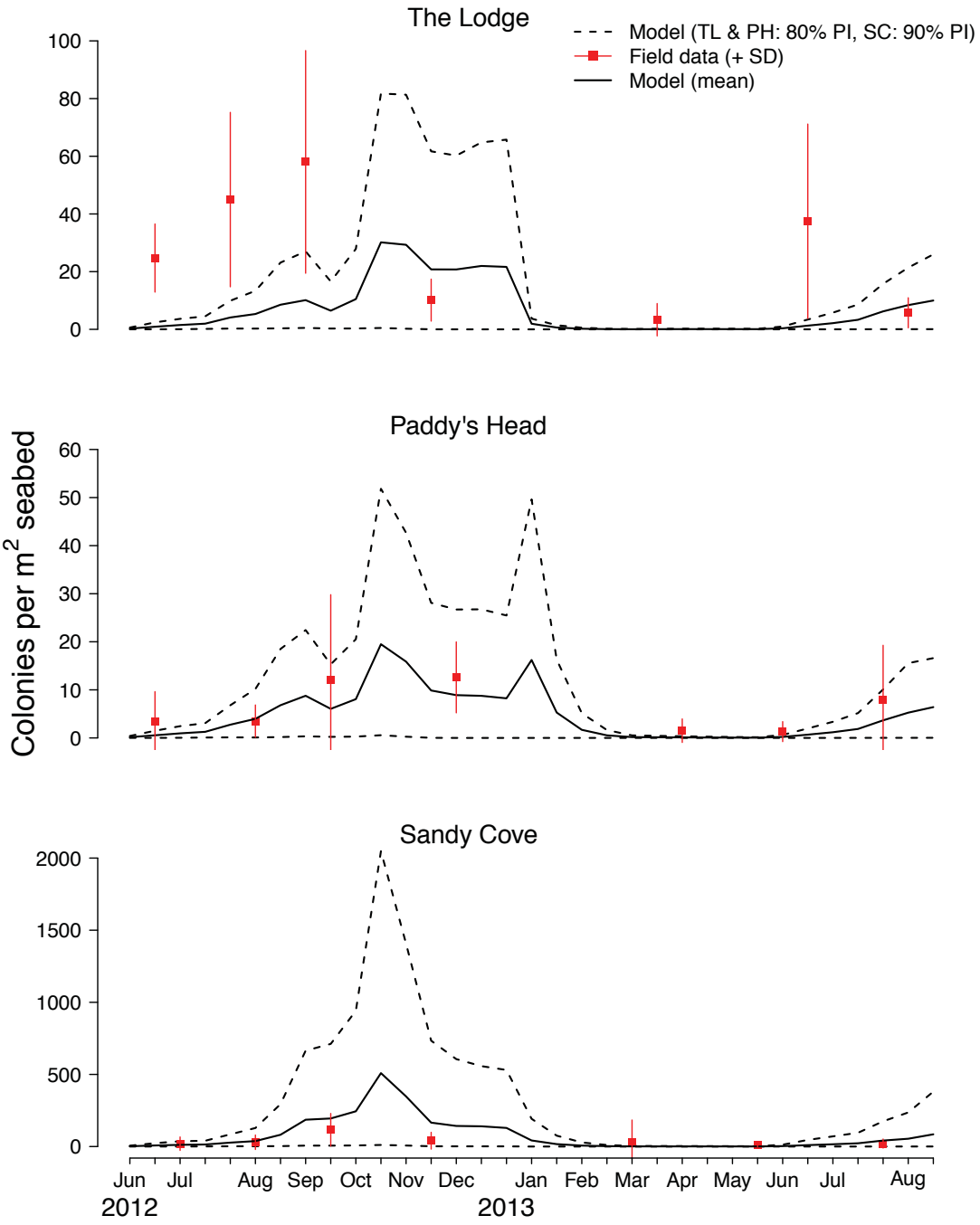

Figure E.2 Model validation. Model projections (mean ± upper and lower percentile intervals for 2000 model runs) and field data (mean ± SD) of the seasonal dynamics of the population of *M. membranacea* (colonies per m^2 seabed) from June 2012 to August 2013 at 3 sites on the southwestern shore of Nova Scotia: The Lodge (source of data used in constructing the model), Paddy's Head, and Sandy Cove (sites used for validating the model). Tickmarks on the x-axis correspond to bi-weekly time indices. The model was initiated on 11 March 2012, resulting in 2 time indices in each of Jun, Aug, Sep Oct, and Nov, and 3 time indices in Jul and Dec (see 7.3.1 Model construction for details)

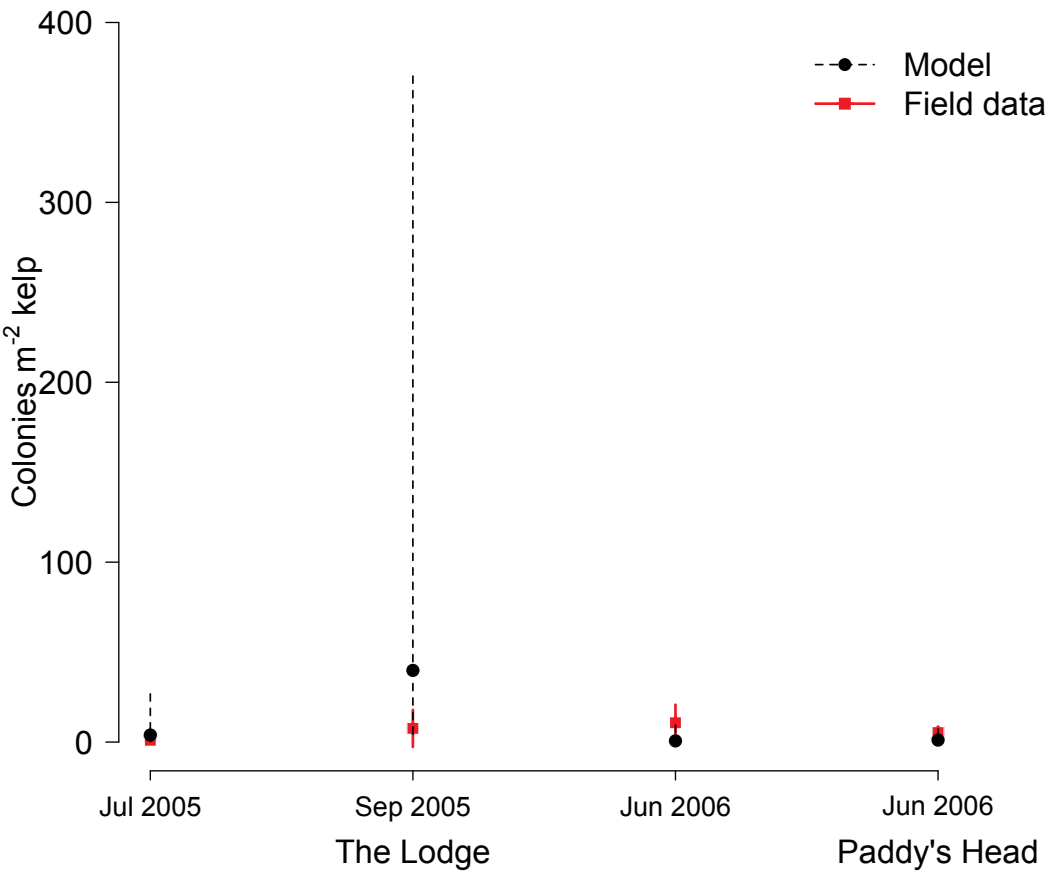

Figure E.3 Model validation. Modeled estimates (mean ± 95% percentile intervals of 2000 model runs) and field data (mean ± SD, Saunders & Metaxas 2009b) of the number of colonies of *M. membranacea* per m^2 kelp for mixed kelp beds in Nova Scotia in July 2005, September 2005 (The Lodge), and June 2006 (The Lodge, Paddy's Head)

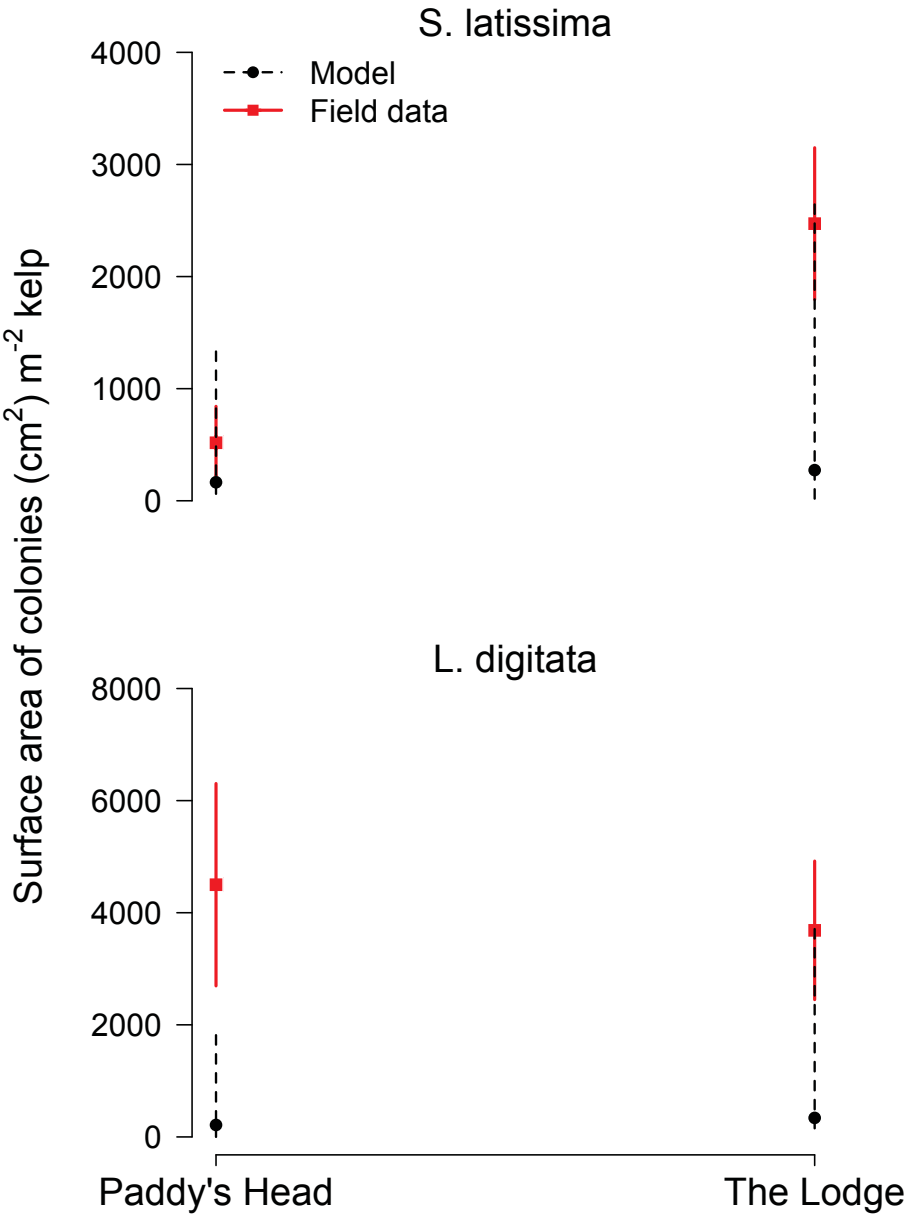

Figure E.4 Model validation. Modeled estimates (mean ± 95% percentile intervals of 2000 model runs) and field data (mean ± SD, Yorke & Metaxas 2012) of the surface area (cm^2) of *M. membranacea* per m^2 kelp on *Saccharina latissima* and *Laminaria digitata* in November-December 2008 at The Lodge and Paddy's Head

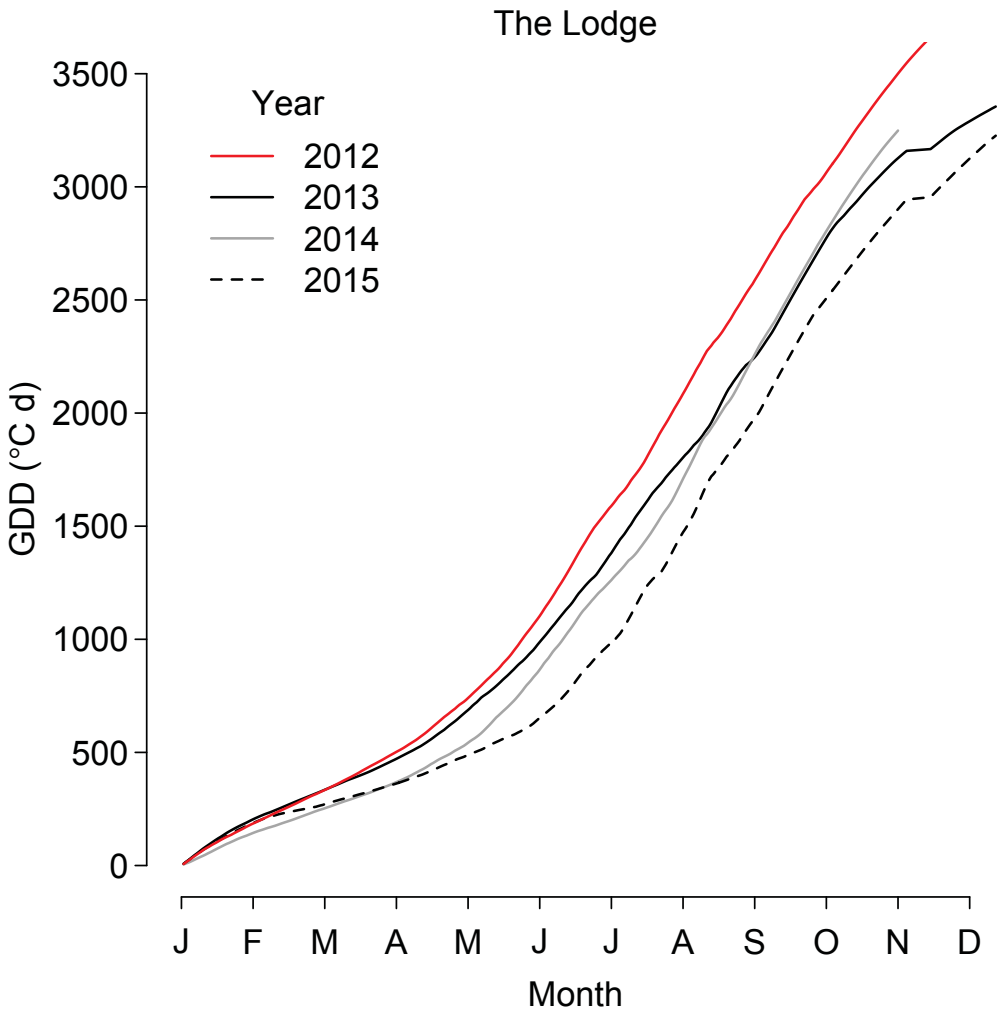

Figure E.5 Interannual differences in depth-averaged (n = 3: 4, 8 and 12 m) growing degree day (GDD) for The Lodge

E.4 Sensitivity Analysis

E.4.1 Methods

To examine sensitivity of the model, we maintained all model parameters and variables as described previously, and individually varied each parameter used to estimate colony growth (α, β, χ) and senescence (δ, γ), as well as mean size- and substrate-specific mortality and fecundity values used to generate bi-weekly survival and fecundity variables, by +10% and -10%. We ran the model from 11 March 2012 to 31 December 2013 and compared model projections of the seasonal dynamics of the population (colonies per m^2 seabed) from June 2012 to August 2013 for each scenario.

Temperature data were not available for Paddy's Head or Sandy Cove prior to June 2012, however, at The Lodge spring of 2012 was anomalously warm (Figure E.5). At the time of initial field sampling in June 2012, ~40% of colonies collected at The Lodge were already fecund, possibly related to warm water temperatures. Fewer colonies were fecund at Paddy's Head in June 2012 (~20% of collected colonies), and colonies did not become fecund at Sandy Cove until July 2012 (Denley & Metaxas 2017a). In addition, the mean abundance of settlers of *M. membranacea* in June 2012 was greater at The Lodge than at Paddy's Head or Sandy Cove (The Lodge: 29 settlers per m^2 kelp, Paddy's Head: 4 settlers per m^2 kelp, Sandy Cove: 2 settlers per m^2 kelp, D. Denley, unpubl data). Therefore, for The Lodge only, we ran additional sensitivity analyses examining the sensitivity of the model to the timing of the onset of settlement and the timing of occurrence of fecund colonies. To achieve this, we ran the model from 11 March 2012 to 31 December 2013 and examined the impact of advancing the timing of onset of settlement and occurrence of fecund colonies by: 1) advancing the onset of settlement by one month by assigning a larval mortality of 90% from 8 April to 29 July; and 2) advancing the onset of fecundity to 8 April for size classes of colonies that were observed to be fecund in June 2012. To advance the onset of fecundity, we randomly sampled size- and substrate-specific colony fecundity from the generated distributions for the earliest seasonal occurrence of fecund colonies (May or June depending on colony size) and applied these values for the months of April and May. Increased ocean temperature over winter is associated with earlier and more abundant settlers of *M. membranacea* (Saunders & Metaxas 2007, 2008), while increased temperature during the growth period in summer and fall is significantly related to the percent cover of colonies on kelp (Scheibling & Gagnon 2009). Increased abundance and earlier onset of settlement, in combination with increased colony growth, may result in availability of bryozoan-free kelp substrate becoming limited earlier in the season, possibly affecting seasonal patterns of settlement. To test the sensitivity of the model to changes in the seasonal timing and abundance of settlers, we advanced seasonal larval mortality by one month to reflect a seasonal advancement in the proportion of larvae that successfully settled on un-encrusted kelp substrate. This resulted in larval mortality of 90% (or settlement success of 10%) from 8 April to 1 July, larval mortality of 96% (or settlement

success 4%) from 1 July to 12 August, larval mortality of 99% (or settlement success of 1%) from 12 August to 23 September, and 100% larval mortality (or 0% settlement success) beginning in mid-September (23 September) as available kelp surface area for settlement approaches zero. We ran the model from 11 March 2012 to 31 December 2013 under the previously described advanced onset of fecundity while simultaneously advancing seasonal settlement success by one month. We further examined the impact of increased abundance of settlers by running the model under conditions of advanced occurrence of reproductive colonies and seasonal settlement success while varying the magnitude of fecundity by +10%, +25% and +50%.

E.4.2 Results

Model results were most sensitive to variations in growth parameters and the mortality variable (Figures E.6 & E.7). For Sandy Cove, increasing mortality by 10% improved the correspondence between model estimates and sampled data, particularly in November 2012 (Figure E.7). Although mortality rates are similar among sites (Denley & Metaxas 2016), this is consistent with observations of accelerated onset of whole-colony mortality at Sandy Cove compared to The Lodge (The Lodge: whole colony mortality observed ~5 weeks post-settlement, SC: whole colony mortality observed ~2 weeks post settlement, Denley & Metaxas 2016).

For The Lodge, simultaneously advancing the occurrence of fecund colonies and seasonal settlement success improved the correspondence between model predictions and empirical data, most substantially when the magnitude of colony fecundity was also increased by 50% (Figure E.8). This supports our hypothesis that the failure of our model to capture the early seasonal dynamics of *M. membranacea* at The Lodge in June to September 2012 is related to anomalously warm water temperatures at The Lodge at that time (Figure E.5), resulting in increased colony fecundity and earlier seasonal occurrence of fecund colonies that were not measured in the field prior to June 2012 and therefore not included in bi-weekly estimates of potential colony fecundity incorporated into our model.

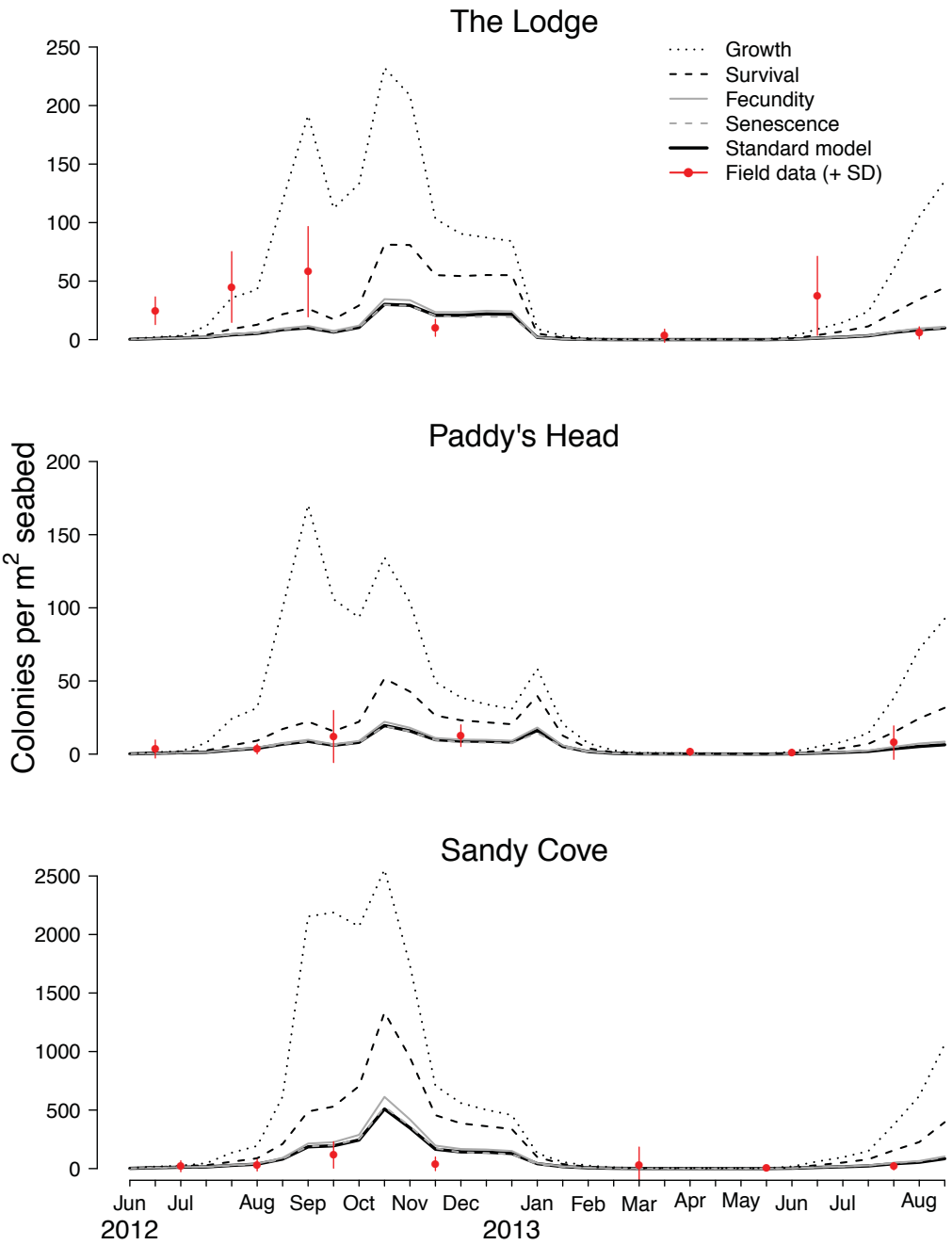

Figure E.6 Sensitivity analysis. Model projections (means of 2000 model runs) and field data (mean ± SD) of the seasonal dynamics of the population of *M. membranacea* (colonies per m^2 seabed) from June 2012 to August 2013 at 3 sites on the southwestern shore of Nova Scotia: The Lodge (source of data used in constructing the model), Paddy's Head, and Sandy Cove (sites used for validating the model). Model projections show results of individually varying each model parameter and variable by +10% compared to the standard model. Tickmarks on the x-axis correspond to bi-weekly time indices. The model was initiated on 11 March 2012, resulting in 2 time indices in each of Jun, Aug, Sep Oct, and Nov, and 3 time indices in Jul and Dec (see 7.3.1 Model construction for details)

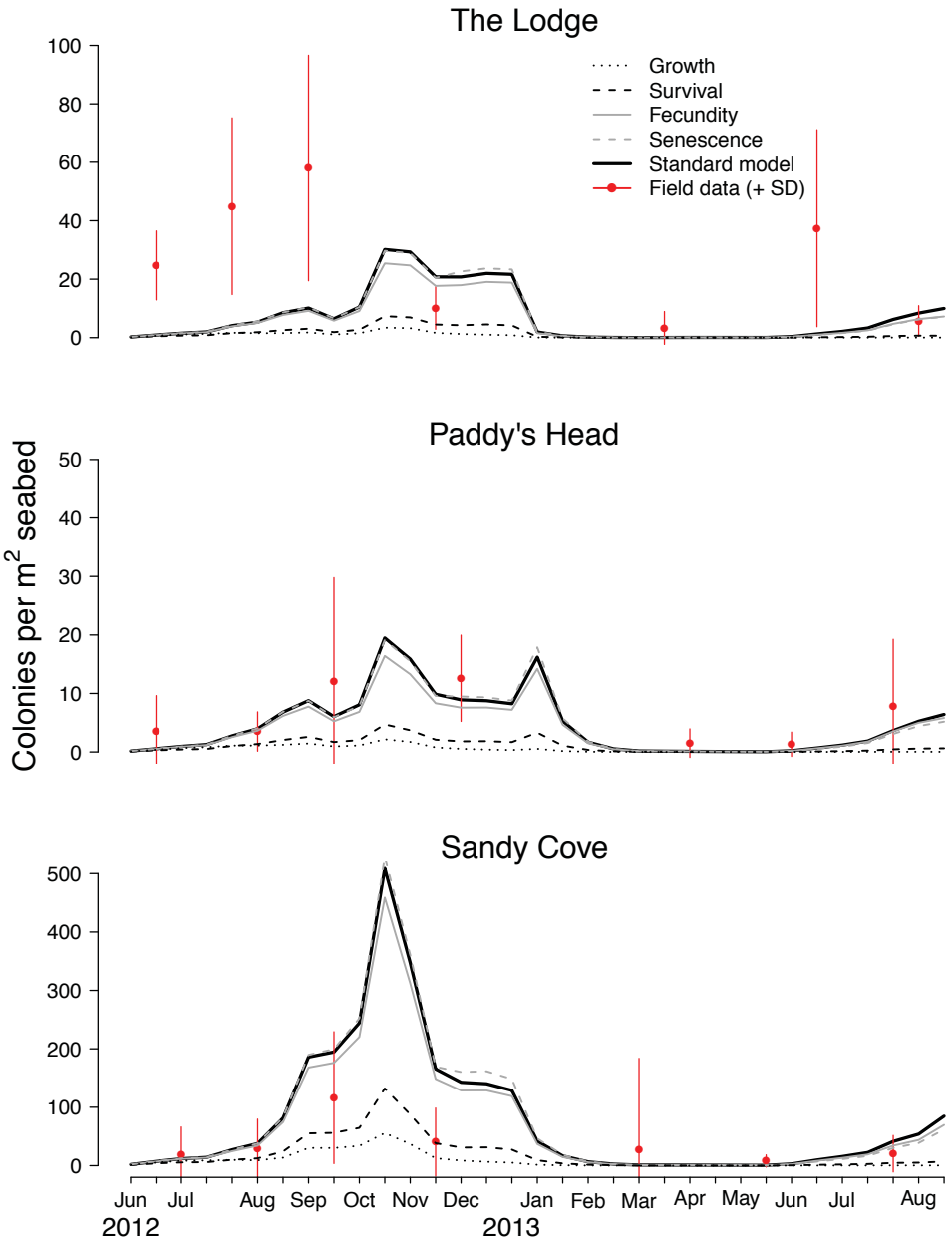

Figure E.7 Sensitivity analysis. Model projections (means of 2000 model runs) and field data (mean ± SD) of the seasonal dynamics of the population of *M. membranacea* (colonies per m^2 seabed) from June 2012 to August 2013 at 3 sites on the southwestern shore of Nova Scotia: The Lodge (source of data used in constructing the model), Paddy's Head, and Sandy Cove (sites used for validating the model). Model projections show results of individually varying each model parameter and variable by -10% compared to the standard model. Tickmarks on the x-axis correspond to bi-weekly time indices. The model was initiated on 11 March 2012, resulting in 2 time indices in each of Jun, Aug, Sep Oct, and Nov, and 3 time indices in Jul and Dec (see 7.3.1 Model construction for details)

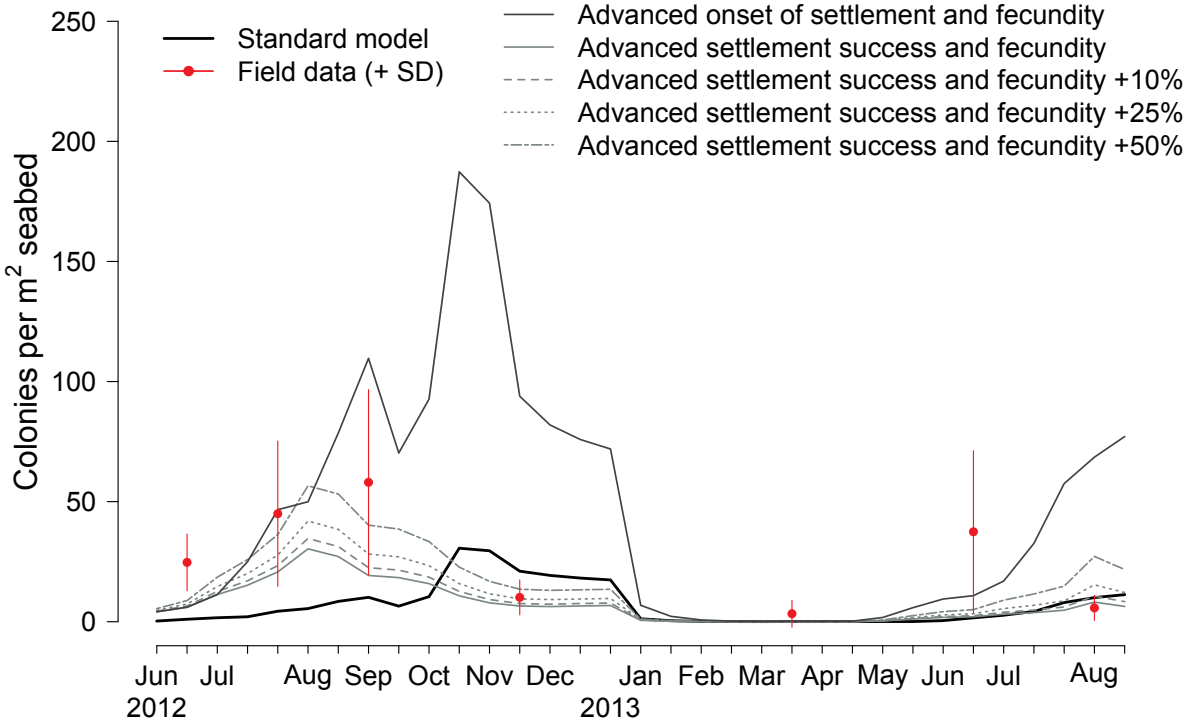

Figure E.8 Sensitivity analysis. Model projections (means of 2000 model runs) and field data (mean ± SD) of the seasonal dynamics of the population of *M. membranacea* (colonies per m^2 seabed) from June 2012 to August 2013 at The Lodge (source of data used in constructing the model). Model projections show the results of 1) advancing the onset of settlement and the occurrence of fecund colonies by 1 month (from May to April) and 1-2 months (from May or June to April), respectively (Advanced onset of settlement and fecundity), 2) advancing seasonal settlement success by 1 month and the occurrence of fecund colonies by 1-2 months (Advanced settlement success and fecundity), and 3) advancing seasonal settlement success by 1 month and the occurrence of fecund colonies by 1-2 months while varying the magnitude of fecundity by +10, +25, and +50% compared to the standard model (Advanced settlement success and fecundity +10, 25, and 50%). See E.4.1 Methods for detailed explanation. Tickmarks on the x-axis correspond to bi-weekly time indices. The model was initiated on 11 March 2012, resulting in 2 time indices in each of Jun, Aug, Sep Oct, and Nov, and 3 time indices in Jul and Dec (see 7.3.1 Model construction for details)

E.5 Comparing Model Projections For Near-Future Temperature Scenarios With Temperature Anomalies In The Field

E.5.1 Methods

To examine whether projected increases in ocean temperature could explain the increased abundance (colonies per m^2 seabed) of *M. membranacea* at The Lodge in June to September 2012, we ran the model for mixed kelp beds at The Lodge under projected increases in ocean temperature of +1°C and +3°C by the year 2035 (see 7.3.2.1 Response of *M. membranacea* to projected increases in ocean temperature). We initiated the model on 11 March 2016 using daily averaged temperatures at The Lodge from that year. We then ran the model consecutively for 6 and 17 years increasing the daily averaged temperature by +0.158°C and +0.053°C, respectively, for each consecutive year until the annual temperature regime was representative of the 2012 temperature anomaly (2022 for the +3°C scenario, 2033 for the +1°C scenario). We then compared model projections of the seasonal dynamics of the population of *M. membranacea* (colonies per m^2 seabed) from June to September 2012 with field data collected from The Lodge during the 2012 temperature anomaly (see E.3.1 Methods).

E.5.2 Results

Percentile intervals for modeled estimates of the seasonal dynamics of *M. membranacea* following both temperature projection scenarios overlapped with sampled data at The Lodge from June to September 2012 (Figure E.9). Correspondence between empirical data for a warm seawater anomaly and model projections under comparable increases in ocean temperature support our hypothesis that increased colony fecundity earlier in the season is driving increases in the abundance of *M. membranacea* in response to warming ocean temperature. In our model, increased colony fecundity earlier in the season is the result of temperature dependent growth of colonies into larger more fecund size classes.

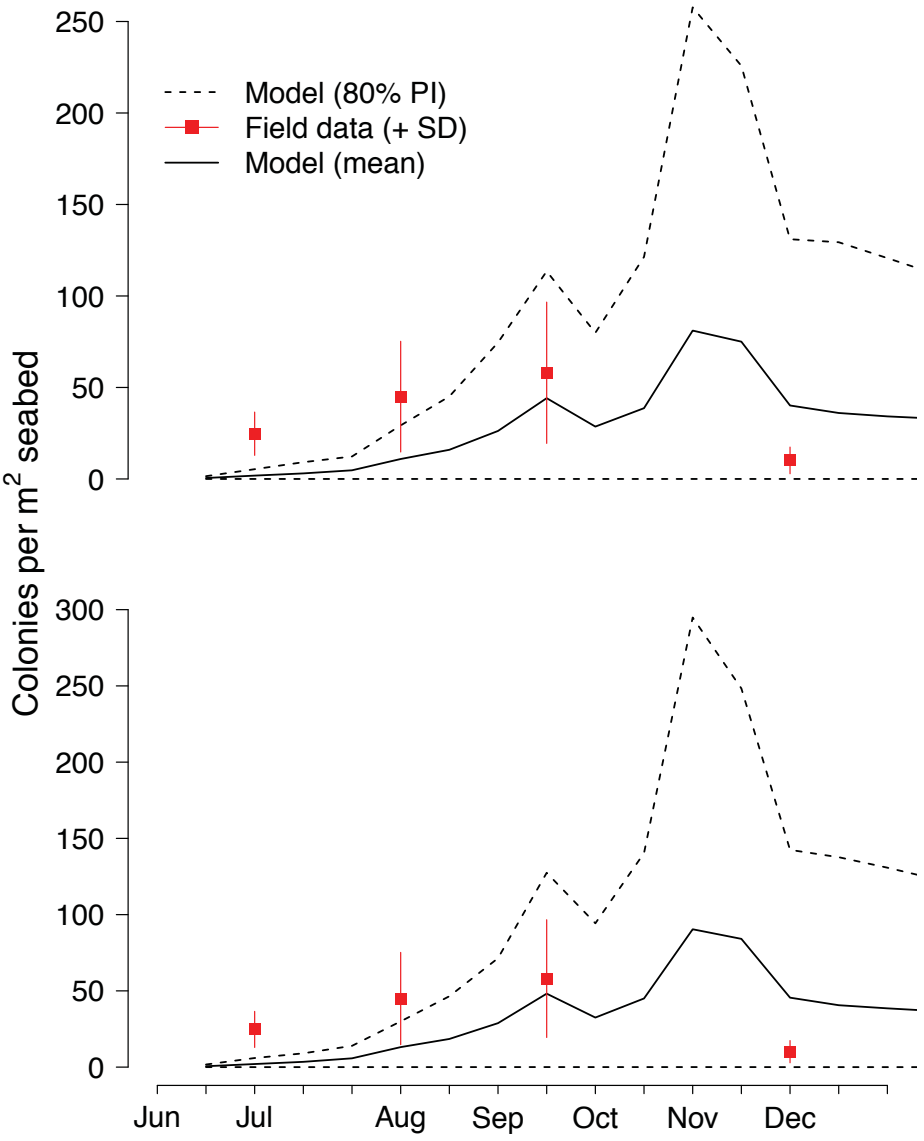

Figure E.9 Model projections (mean ± upper and lower percentile intervals for 2000 model runs) and field data (mean ± SD) of the seasonal dynamics of the population of *M. membranacea* (colonies per m^2 seabed) from June to December at The Lodge. Field data are from 2012 during anomalously warm seawater temperatures (~ +0.875°C compared to 2014, Figure E.6). Model projections are for the years 2022 and 2033 under projected increases in ocean temperature for the northwest Atlantic of +1°C (top panel) and +3°C (bottom panel) by the year 2035, respectively. The model was initiated using temperature data at The Lodge in 2016 and daily average temperatures were increased annually by 0.053°C (+1°C scenario) or 0.158°C (+3°C scenario) until the annual temperature regime was representative of the 2012 temperature anomaly (2033 for the +1°C scenario, 2022 for the +3°C scenario). Tickmarks on the x-axis correspond to bi-weekly time indices. The model was initiated on 11 March 2012, resulting in 2 time indices in each of Jun, Aug, Sep Oct, and Nov, and 3 time indices in Jul and Dec (see 7.3.1 Model construction for details)

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