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Marine Population Connectivity: Range Boundaries and Climate Change

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MARINE POPULATION CONNECTIVITY: RANGE BOUNDARIES AND CLIMATE
CHANGE

by

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DEDICATION

This dissertation is dedicated to my parents; Ralph Rognstad, Jr. and Judy Baldwin Rognstad.

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First, I would like to thank my committee members for all of their guidance, support, and generous use of equipment throughout my time at USC. Dr. Jesús Pineda provided valuable insights into the uses and limitations of models and support when collecting barnacles in New England. Dr. Richard Showman was an excellent and organized committee chair and provided helpful advice about the lab experiment. Dr. Joe Quattro provided insight into thinking about population connectivity on multiple scales. Dr. David Wethey was incredibly helpful with the modelling component, and I doubt I would have been able to complete this chapter without him. Finally, this work would not have been possible without the guidance and support of my advisor, Dr. Jerry Hilbish.

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ABSTRACT

Population connectivity, particularly in open systems, is an important metric for understanding population-level processes on both ecological and evolutionary timescales. In coastal marine systems, adults are typically sedentary and dispersal occurs primarily during a larval stage when individuals are transported in ocean currents. Because coastal marine populations exist as networks of interconnected subpopulations, variation in the magnitude and extent of population connectivity can have profound effects on population dynamics and species distribution limits. Connectivity is a complex process, affected by a multitude of factors, including adult inputs and physical dispersal, which operate at multiple scales and may interact. This dissertation describes work on three research questions, which examine how variation in temperature and dispersal patterns drive variation in connectivity and subsequently affect species range boundaries. The project integrated a combination of field surveys, laboratory experiments, and computer simulations and addresses the specific questions: (1) How does temperature-induced variation in adult input into the larval pool affect population dynamics and range limits? (2) What is the effect of temperature on reproductive success in intertidal organisms? and (3) How do potential connectivity patterns vary over time and can we use physical dispersal models to predict the rate of recolonization after a local extinction event?

This work uses the widely-distributed acorn barnacle *Semibalanus balanoides* as a study organism, and takes advantage of climate-induced variation in the fecundity of

this species to examine the role of adult input into the larval pool in the field and laboratory. Multi-year field surveys at a local southern limit of *S. balanoides* indicated that recruitment of this species was greater following cold winters than following warm winters and recent cold winters have led to a range expansion of this species at a local southern range limit. Climate-induced variation in population connectivity also explains decadal-scale oscillations in population abundance and geographic distribution of *S. balanoides* at this range limit. Laboratory experiments demonstrated that temperature did not significantly affect larval development rate, but brooding individuals reared at cold temperatures had significantly greater reproductive mass than individuals reared at warm temperatures. This mass difference is caused by an over three-fold larger number of embryos surviving in the coldest treatment (7°C) compared to the warmest treatment (13°C). Temperature-induced variation in number of surviving embryos likely contributes to differences in recruitment following cold winters vs. warm winters. Computer-simulated estimates of potential connectivity predicted recolonization following local extinction at a comparable rate to that observed in the field. This work highlights the importance of adult input into the larval pool and physical dispersal in controlling population connectivity, as well as the significant role of climate variation in determining the range limits of marine organisms.

TABLE OF CONTENTS

DEDICATION	iii
ACKNOWLEDGEMENTS.....	iv
ABSTRACT	vi
LIST OF TABLES	ix
LIST OF FIGURES	x
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: CONNECTIVITY AND POPULATION REPATRIATION: LIMITATIONS OF CLIMATE AND INPUT INTO THE LARVAL POOL.....	7
CHAPTER 3: TEMPERATURE-INDUCED VARIATION IN THE SURVIVAL OF BROODED EMBRYOS DRIVES DECADAL PATTERNS OF RECRUITMENT AND ABUNDANCE IN <i>SEMIBALANUS</i> <i>BALANOIDES</i>	27
CHAPTER 4: CONNECTIVITY MODELLING AND GRAPH THEORY ANALYSIS TO UNDERSTAND RECOLONIZATION IN TRANSIENT POPULATIONS	51
CHAPTER 5: CONCLUSION	104
REFERENCES	107
APPENDIX A – PERMISSION LETTER.....	122

LIST OF TABLES

Table 3.1 Developmental traits for staging <i>Semibalanus balanoides</i> embryos	46
Table 4.1 ID and location of modeling sites	78
Table 4.2 Spatial statistics for MANGA700 and ATLNE2000 models (2010-2013)	79
Table 4.3 Top sequential betweenness scores.....	80

LIST OF FIGURES

Figure 2.1 Map of study region and demography survey sites in Southwest England	24
Figure 2.2 <i>Semibalanus balanoides</i> adult and recruitment densities (2010-2012).....	25
Figure 2.3 Days below 10°C in Southwest England (1983-2012).....	26
Figure 3.1 Percent of <i>Semibalanus balanoides</i> larvae reaching stage 4b	47
Figure 3.2 <i>Semibalanus balanoides</i> standardized reproductive mass over time	48
Figure 3.3 <i>Semibalanus balanoides</i> standardized reproductive mass for five temperature treatments	49
Figure 3.4 Number of embryos vs. basal diameter of <i>S. balanoides</i> reared at three temperatures	50
Figure 4.1 Map of study region and modeling sites in Southwest England	81
Figure 4.2 ATLNE2000 dispersal matrices (2010-2012)	82
Figure 4.3 ATLNE2000 vertex and edge graph releasing larvae east of Start Point (2010-2012)	83
Figure 4.4 ATLNE2000 vertex and edge graph releasing larvae between The Lizard and Start Point (2010-2012).....	84
Figure 4.5 ATLNE2000 vertex and edge graph releasing larvae between Land's End and The Lizard (2010-2012)	85
Figure 4.6 ATLNE2000 vertex and edge graph releasing larvae north of Land's End (2010-2012).....	86
Figure 4.7 MANGA700 dispersal matrices (2010-2012)	87
Figure 4.8 MANGA700 vertex and edge graph releasing larvae east of Start Point (2010-2012)	88
Figure 4.9 MANGA700 vertex and edge graph releasing larvae between The Lizard and Start Point (2010-2012).....	89

Figure 4.10 MANGA700 vertex and edge graph releasing larvae between Land’s End and The Lizard (2010-2012).....	90
Figure 4.11 Number of steps in shortest dispersal path between all source and destination sites using the ATLNE2000 circulation model (2010-2012).....	91
Figure 4.12 Number of steps in shortest dispersal path between all source and destination sites using the MANGA700 circulation model (2010-2012).....	92
Figure 4.13 Sequential site removal shortest dispersal path matrices using the ATLNE2000 circulation model (year 2010).....	93
Figure 4.14 Sequential site removal shortest dispersal path matrices using the ATLNE2000 circulation model (year 2011).....	94
Figure 4.15 Sequential site removal shortest dispersal path matrices using the ATLNE2000 circulation model (year 2012).....	95
Figure 4.16 Sequential site removal shortest dispersal path matrices using the ATLNE2000 circulation model (year 2013).....	96
Figure 4.17 Sequential site removal shortest dispersal path matrices using the MANGA700 circulation model (year 2010).....	97
Figure 4.18 Sequential site removal shortest dispersal path matrices using the MANGA700 circulation model (year 2011).....	98
Figure 4.19 Sequential site removal shortest dispersal path matrices using the MANGA700 circulation model (year 2012).....	99
Figure 4.20 Sequential site removal shortest dispersal path matrices using the MANGA700 circulation model (year 2013).....	100
Figure 4.21 Community assignments using the Constant Potts Model community detection algorithm	101
Figure 4.22 Vertex and edge graph of sequential dispersal modeling using the ATLNE2000 circulation model	102
Figure 4.23 Vertex and edge graph of sequential dispersal modeling using the MANGA700 circulation model	103

CHAPTER 1

GENERAL INTRODUCTION

This dissertation describes the interplay of temperature and physical dispersal in generating variation in population connectivity, which subsequently affects the abundance and the distribution limits of coastal marine species. Additionally, models are used to make predictions about how future climate change could affect species distributions and abundance.

Climate change has been implicated as the driving force behind changes in species' distributions in both marine and terrestrial environments (Parmesan 1999, Thomas and Lennon 1999) and there is substantial interest in prediction of future changes in distribution, particularly from a conservation perspective (Guisan and Zimmerman 2000). Early modeling efforts focused on statistical relationships among species presence/absence and environmental variables, but researchers have recognized the limitations of this methodology and now strive to include functional relationships and ecological theory in the generation of species distribution models (Guisan and Zimmerman 2000, Austin 2002).

Particularly in the context of climate change, a mechanistic understanding of the forces influencing the local and regional maintenance or extinction of subpopulations is essential for understanding the ecology and evolution of the population as a whole. These changes often do not progress linearly, but are effected by extreme events that

promote species range extension or retraction (Crisp 1964, Wetthey et al. 2011).

Increased climate variability is forecast as a component of climate change in many areas (Karl and Trenberth 2003, Salinger 2005), so an understanding of the effects of such events on populations is essential for our ability to predict future species distributions.

As climate change can lead to local extinctions, connectivity among subpopulations may lead to resistance to climate change. Alternatively, dramatic and sudden shifts in species distributions may occur when connections among subpopulations collapse. The overarching goal of my dissertation is to understand the interplay of demographic and physical factors, which are partially controlled by temperature, on population connectivity. Connectivity has been shown to influence ecological and evolutionary processes including species persistence (Lowe and Allendorf 2010), species coexistence (Berkley et al. 2010), the spread of invasive species (Andrew and Ustin 2010), and the degree and rate of local adaptation (Epperson 2003, Sanford and Kelly 2010). In coastal systems, connectivity may be influenced heavily by adult population input into the propagule pool and transport during development, as described below.

In marine systems, many species are sedentary as adults, constraining connective processes to the early life stages. Adults in these populations are unlikely to be major contributors to the next generation at their particular location because larvae have the potential to be transported over wide geographic areas (Nathan 2001, Gilg and Hilbish 2003, Pineda et al. 2009). Consequently, understanding the decline, maintenance, or new establishment of subpopulations requires knowledge of both local conditions and regional patterns of connectivity. Most studies of marine population connectivity focus on post-spawning processes, especially physical circulation, that

describe the link between larvae and adults (Levin 2006). These include factors that determine whether larvae will be transported to a settlement site (Rumrill 1990), the number of settlers (Minchinton and Scheibling 1991), or the transition from settlers to recruits (McGuinness and Davis 1989, Menge 2000). Conversely, the reciprocal relationship, the link between adults and larval production, is much less well known because this requires enumerating the contribution of all adult populations that potentially contribute to the larval population at the geographic scale of larval dispersal (Hughes et al. 1999, 2000). In open marine systems with planktonic larvae this would entail enumerating the larval production of all adult populations across spatial scales of 10-100 km (Gilg and Hilbish 2003, Becker et al. 2007). Nonetheless the few studies that have explored the relationship between adults and larval pool size indicate that variation in fecundity of adults is responsible for a large amount of variation in recruitment (Hughes et al. 2000, Reed et al. 2009, Burrows et al. 2009) and metapopulation growth (Carson et al. 2011). This dissertation examines how temperature-driven variation in adult input into the larval pool and physical dispersal produce variation in population connectivity, affecting species abundances and range limits.

1.1 Study System:

As a study system for addressing questions about marine connectivity and demography, the acorn barnacle *Semibalanus balanoides* exhibits several important characteristics. It is a common, widely-distributed member of boreo-arctic communities that is relatively easy to rear in the laboratory and populations are easily manipulated in the field. Additionally, there is a wealth of literature on *S. balanoides* physiology (Crisp, 1959;

Barnes, 1963; Crisp and Patel, 1969) and historical distributions (Moore, 1936; Southward and Crisp, 1954; Southward, 1991; Southward et al., 1995; Hawkins et al., 2003), providing data for testing developed models and context for future work. *S. balanoides* is a cross-fertilizing hermaphrodite, which fertilizes in the late fall (Nov-Dec; Crisp, 1964) and broods its embryos outside the body but within the shell until the late winter/early spring (March; Crisp, 1964), releasing one brood of larvae per year.

Intertidal barnacles, including *Semibalanus balanoides*, are a classic study system for investigating questions about demography and zonation, including the roles of competition (Connell 1961a) and predation (Connell 1961b). *S. balanoides* in particular provides an example of the control of geographic limits by temperature, though the mechanism and the role of air vs. water temperature has long been debated (Hutchins 1947, Barnes 1958, Wells et al. 1960, Jones et al. 2012). Additionally, shifts in the geographic distribution of *S. balanoides* with temperature variation have been documented on both sides of the Atlantic (Woodin and Wethey 2008, Wethey et al. 2011, Jones et al. 2012). On the North American coast, high summer temperatures, which are outside the thermal tolerance of *S. balanoides* set the southern range limit (Jones et al. 2012), while the limitation of reproduction during warm winters (Barnes 1963, Crisp and Patel 1969) appears to set the southern limit in Europe (Wethey and Woodin 2008).

The goal of this dissertation was to further develop *Semibalanus balanoides* as a study system for examining the interplay of various demographic (e.g. adult input into the larval pool) and physical (e.g. temperature and physical dispersal) factors in generating variation in population connectivity.

1.2 Structure of Dissertation:

In **Chapter 2**, I describe the results of multi-year field surveys and use the results test the hypothesis: Adult propagule input has a measurable effect on population connectivity, which consequently affects species' distributions. Surveys of recruitment and adult densities were conducted in two cold years (2010, 2011) and one warm year (2012) in Southwest England, an area of historical transience for this species. These surveys span the southern geographic limit of *S. balanoides* in this region. We documented increased recruitment in cold years compared to the warm year and a range expansion filling in gap in *S. balanoides* range in Southwest England after three years.

Chapter 3 describes a laboratory-based study investigating potential mechanisms generating the observed relationship between recruitment and cold temperatures. Specific hypotheses tested are: (1) variation in brooding temperature produces differences in reproductive timing and (2) variation in brooding temperature produces differences in reproductive output, in terms of larval quality larval quantity, or both. Laboratory experiments demonstrated that temperature did not significantly affect larval development rate, but brooding individuals reared at cold temperatures had significantly greater reproductive mass than individuals reared at warm temperatures. This mass difference is caused by an over three-fold larger number of embryos surviving in the coldest treatment (7°C) compared to the warmest treatment (13°C). Temperature-induced variation in number of surviving embryos likely contributes to differences in recruitment following cold winters vs. warm winters.

Chapter 4 uses results of computer-based dispersal modeling to address the questions: (1) How do potential connectivity patterns of *Semibalanus balanoides* in

Southwest England vary over time? and (2) What is the predicted timescale of recolonization of Southwest England predicted by dispersal models and are these predictions supported by the data of actual recolonization events (described in Chapter 2)? Dispersal models indicated variation in potential connectivity estimates among years and generated predictions of recolonization of Southwest England that was more likely to proceed from the east than from the north and occurs in 2-4 years, consistent with field observations of 3-4 years.

The final chapter, **Chapter 5**, provides an overall conclusion for the dissertation.

CHAPTER 2

CONNECTIVITY AND POPULATION REPATRIATION: LIMITATIONS OF CLIMATE AND INPUT INTO THE LARVAL POOL¹

¹ Rognstad RL, Wethey DS, and TJ Hilbish. 2014. *Marine Ecology Progress Series*. 495: 175-183.

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2.1 Abstract:

We used climate induced variation in fecundity of the barnacle *Semibalanus balanoides* to test the hypothesis that variation in adult input to the larval pool is a driver of connectivity among adult populations of coastal marine invertebrates. We predicted that cold winters will lead to high reproductive output and high recruitment by *S. balanoides*, while warm winters will result in low reproductive output and low recruitment. Following the cold winter of 2009/2010 larval recruitment was exceptionally high ($>6/\text{cm}^2$) in Southwest England, resulting in a range expansion of over 100 km. The following winter was also cold and high recruitment again occurred. The winter of 2011/2012 was warm and recruitment was low ($\sim 1/\text{cm}^2$) throughout Southwest England. These data indicate that recruitment and population connectivity are strongly linked to adult input to the larval pool. We analyzed winter temperature variation over the past 3 decades and found that warm winter temperatures over the 13 years prior to this study likely led to frequent reproductive failure and the decline of *S. balanoides* in Southwest England. Continued warming in this region is likely to permanently exclude *S. balanoides* from this portion of its geographic range. This study illustrates an important role of adult input into the larval pool in controlling population connectivity in open marine ecosystems and also implicates climate variation in determining the range limit of a significant component of coastal ecosystems in Europe.

2.2 Introduction:

In marine systems, many species are sedentary as adults, constraining connective processes to the early life stages. Adults in these populations are unlikely to be major

contributors to the next generation at their particular location because larvae have the potential to be transported over wide geographic areas (Nathan 2001, Gilg and Hilbish 2003, Pineda et al. 2009). Consequently, understanding the decline, maintenance, or new establishment of subpopulations requires knowledge of both local conditions and regional patterns of connectivity. Most studies of marine population connectivity focus on post-spawning processes, especially physical circulation, that describe the link between larvae and adults (Levin 2006). These include factors that determine whether larvae will be transported to a settlement site (Rumrill 1990), the number of settlers (Minchinton and Scheibling 1991), or the transition from settlers to recruits (McGuinness and Davis 1989, Menge 2000). Conversely, the reciprocal relationship, the link between adults and larval production, is much less well known because this requires enumerating the contribution of all adult populations that potentially contribute to the larval population at the geographic scale of larval dispersal (Hughes et al. 1999, 2000). In open marine systems with planktonic larvae this would entail enumerating the larval production of all adult populations across spatial scales of 10-100 km (Gilg and Hilbish 2003, Becker et al. 2007). Nonetheless the few studies that have explored the relationship between adults and larval pool size indicate that variation in fecundity of adults is responsible for a large amount of variation in recruitment (Hughes et al. 2000, Reed et al. 2009, Burrows et al. 2010) and metapopulation growth (Carson et al. 2011).

In this study we exploit a feature of the reproductive biology of the barnacle *Semibalanus balanoides* that creates annual variation in reproductive output at the spatial scale of larval dispersal to test the hypothesis that adult fecundity appreciably influences the size of the larval pool and population connectivity in open marine ecosystems.

Reproduction in *S. balanoides* is inhibited by warm temperatures above 10-12°C (Barnes 1957, 1959, 1963, Crisp and Clegg 1960, Crisp and Patel 1969); laboratory studies indicate that a period of four to six weeks at or below 10°C is necessary for reproduction (Crisp and Patel 1969). These results suggest that greater reproductive output is expected when adult *S. balanoides* experience a cold winter than when warm conditions prevail. Sea surface temperature (SST) also varies at a broad spatial scale (25-100 km) and thus is expected to simultaneously affect the reproductive output of all adult populations within a region. In this study we test the hypothesis that annual variation in winter SST results in variation in adult reproduction, provisioning of the larval pool and population connectivity at regional scales. We conducted this study on the English Channel coast of Southwest England because the abundance of *S. balanoides* in this region oscillates through time (Southward et al. 1995, Hawkins et al. 2003) and this species has been rare or absent from much of the region prior to 2010. The cold winters of 2009-2010 and 2010-2011 exceeded the physiological threshold where *S. balanoides* is expected to have high larval production and the warm winter of 2011-2012 did not exceed the threshold and consequently low larval production is predicted in this year.

2.3 Methods:

2.3.1 Field Work:

During early June of 2010, 2011, and 2012, 15 intertidal sites were sampled at approximately 10 km intervals, encompassing over 250 km of the coast of Southwest England (Fig. 2.1). At each site, we randomly sampled barnacle populations within the mid-tidal range where *Semibalanus balanoides* are found if present. We photographed

barnacles attached to the substratum along with a metric tape for determining the size and density of barnacles in each sample. Sites selected for photographic sampling appeared to be representative of the location. If no individuals identifiable as *S. balanoides* could be located at a site after 30 minutes of searching, it was assumed the species was absent or very rare at the site and a density of zero was recorded.

2.3.2 Image Analysis:

A subset of at least five images (out of 10-30) was selected for analysis from each site during each year. These images were included based on focus quality, as barnacles outside the focal plane could not be identified. Because of variation in the flatness of the underlying rock, the size of the available space in focus in each image varied; the countable area ranged from 5cm² to 155cm², with a mean quadrat size of 32.4cm². Images were imported into the opensource software ImageJ (Abramoff et al. 2004) for subsequent analysis. *Semibalanus balanoides* individuals were identified based on plate number and shape as described by Southward and Crisp (1963). Individuals were then assigned to one of two age classes: young of the year (YOY) and adults. YOY are readily recognized by having white plates that are well defined whereas adults are more stained and brown in color and typically have significant fusion and weathering of the plates. The metric tape in each of the photographic quadrats was used to calibrate ImageJ's measurement tool, which was subsequently used to calculate the average density of *S. balanoides* individuals of each age class at each site.

2.3.4 Temperature and Statistical Analysis:

NOAA Optimum Interpolation 1/4 Degree Daily Sea Surface Temperature Analysis data (Reynolds OI V2 SST data) were obtained from the NOAA's NCDC webpage at <http://nomads.ncdc.noaa.gov/thredds/dodsC/oisst2/>. These data represent daily sea surface temperature estimates encompassing the entire study area at a spatial resolution of 0.25 °. The data were imported into R 2.9.2 (R Development Core Team) and a script was developed to identify the closest marine pixel to each sample site. Lima and Wetthey (2012, Supplementary tables) have shown the nearest pixel to intertidal sites in this dataset is highly correlated to SST measured onshore. The resulting data set was used to generate a table containing the number of days below 10°C at each sampling site during the predicted *S. balanoides* reproductive season for each year between 1983 and 2012. The reproductive season was estimated by subtracting the period between fertilization and larval release (110-120 days, Crisp 1964) for *S. balanoides* in the UK from the range of dates of release (9 March-24 March, Crisp 1964). The 10°C temperature threshold was selected based on the findings of Barnes (1963) and Crisp and Patel (1969) that *S. balanoides* requires a period of 4-6 weeks below this temperature for reproduction to occur. However, we don't expect this to be an all or nothing response; reproductive output may be inversely proportional to temperature so long as winter temperatures fall below the temperature (10-12°C) that inhibits reproduction (Abernot-Le Gac et al. 2011). In addition, the potential role of air temperature in regulating reproductive output in *S. balanoides* is poorly known and thus reproduction of some individuals within a population may be inhibited if they reside in warm microsites while others, in cold microsites, may reproduce. Accordingly we assigned each site to one of three categories;

if SST was $<10^{\circ}\text{C}$ for more than six weeks we concluded that it is probable that adult *S. balanoides* would have high reproductive output, if SST was below 10°C for 4-6 weeks, we concluded that it is possible that barnacles at this site spawned and reproductive output was expected to be modest, and if SST did not fall below 10°C for at least four weeks we concluded that it is likely that many adults at a site were inhibited from reproducing and reproductive output was expected to be low that year.

2.4 Results:

Start Point (Fig. 2.1) can be a formidable barrier to water and larval transport (Gilg and Hilbish 2003) and typically separates a region of comparatively cold water to the east from a region of warmer water to the west (see below). Consequently we report our results with respect to distance east and west of Start Point. In 2010 adult *Semibalanus balanoides* densities west of Start Point were low with most sites having fewer than 0.5 adult individuals per cm^2 . Adult densities were generally higher east of Start Point, where two populations had adult densities that exceeded 2.5 individuals per cm^2 . During the winter of 2009-2010 all but the two western-most sites experienced at least six weeks below 10°C and these populations were categorized as having high reproductive output (Fig. 2.2A). At the two western-most sites, SST was $<10^{\circ}\text{C}$ for between four to six weeks and populations at these sites were expected to have medium reproductive output. *S. balanoides* recruitment density in 2009-2010 was very high, averaging ~ 7 per cm^2 at sites east of Start Point and at sites up to 100 km west of Start Point. At sites 100-170 km west of Start Point recruitment density declined to ~ 3 per cm^2 and no recruits were observed at the two western-most sites (Fig. 2.2A).

During 2010-2011, temperatures were again cold and 10 of the 16 sites were classified as having high reproductive output while the six western-most sites experienced between 4-6 weeks $<10^{\circ}\text{C}$ and were categorized as having medium reproductive potential. *Semibalanus balanoides* recruitment ranged from 0 to 6.61 individuals per cm^2 (Fig. 2.2B). At sites 100-170 km west of Start Point, recruitment was again high during 2011 and was significantly greater than recruitment during 2010 (Fig. 2.2B; Wilcoxon rank sum test, $p<0.05$). The two western most sites, that did not receive recruits in 2010, both received recruits in 2011 albeit at low density. At sites within 100 km of Start Point, there is no clear pattern; some sites exhibited greater recruitment in 2011 than 2010, while others exhibited lower or similar recruitment density compared to 2010.

Adult density was significantly greater in 2011 than 2010 at all sites west of Start Point (Fig. 2.2B; Wilcoxon rank sum test, $p<0.05$), except for the westernmost site where adult density was zero in both years. At the four sites east of Start Point, adult density was significantly greater in 2011 than 2010 at three sites and significantly lower at one site (Fig. 2.2B; Wilcoxon rank sum test, $p<0.05$). It is probable that this increase in adults in 2011 is the result of high recruitment in 2010.

In contrast to the previous two years SST during the winter of 2011-2012 was comparatively warm; no location within the study region experienced >6 weeks of temperature below 10°C and only sites east of Start Point experienced 4-6 weeks below 10°C . Recruitment density was very low, averaging ~ 1 per cm^2 throughout the region. This low recruitment is in contrast to the increase in adult density that occurred at most sites as a result of high recruitment during the previous two years. *S. balanoides* may

mature within their first year if they experience rapid growth (Moore 1936, Southward 1967, Wethey 1985a) thus both one year old and older barnacles may contribute to adult reproduction if temperatures permit. Thus it is striking that recruitment fails just as recruits from 2010 and 2011 mature into reproductively active adults.

To determine whether the density of YOY was explained by adult density within the settlement site, we compared YOY density to the adult density at each site by performing Pearson rank-order correlation tests. YOY and adult densities were not significantly correlated in any of the three years of this study (2010, $r=0.128$, $p=0.517$; 2011, $r=-0.178$, $p=0.511$; 2012, $r=0.352$, $p=0.217$) indicating the density of local adults is insufficient to explain local recruitment patterns.

While it is clear that warm winter temperatures inhibit reproduction in *Semibalanus balanoides* it is not certain that 10°C is necessarily the threshold for inhibiting reproduction. It may be that inhibitory temperature thresholds occur either above or below 10°C. We compared days below 10°C with days below 9°C and below 11°C and they were all highly correlated (in both cases $r=0.78$, $p<0.0001$, $df=838$). Thus while we used days below 10°C in the analyses reported here, this temperature is not necessarily a threshold but it is correlated with other measures of winter temperature.

We analyzed winter SST from 1983 to 2012 (the extent of the Reynolds OI V2 SST dataset) to determine the frequency and distribution of high, medium and low reproductive output years for *S. balanoides* spawning in Southwest England (Fig. 2.3). At sites east of Start Point, high, medium and low reproductive output years represented 44%, 23%, and 33% of the total years, respectively. Sites west of Start Point were typically warmer, with low reproductive output occurring in 56% of the years and years

with high and medium reproductive output representing 26% and 17% of the total time period, respectively. The pattern of high, medium and low reproductive output years varies through time (Fig. 2.3). Prior to 1998, high and medium output years were separated by one or two low output years. Since 1998, high and medium output years have occurred less frequently (Fig. 2.3) both east and west of Start Point and were separated by several years in a row where spawning was expected to be low. If the four recent years (2009-2012) are removed, there is a significant negative correlation between number of cold days and time for sites east ($df=24$, $r=-0.47$, $p=0.016$) and west ($df=24$, $r=-0.46$, $p=0.018$) of Start Point.

2.5 Discussion:

The factors contributing to marine population connectivity are notoriously difficult to measure and adult input to the larval pool is perhaps the most intractable component because it requires knowledge of the contribution of all potential source populations over large spatial scales. We took advantage of the fact that warm winter temperatures inhibit reproduction in *Semibalanus balanoides* to test the hypothesis that variation in adult contribution to the larval pool significantly influences connectivity among adult populations. In Southwest England, annual variation in SST determines the reproductive potential of adult populations over broad spatial scales and determines the magnitude of potential connectivity over the same geographic scales. Consequently we expect adult contribution to the larval pool and subsequent larval recruitment to be much greater following cold winters where the threshold for reproduction has been met and low larval recruitment following warm winters where the threshold has not been met. This is

precisely what we observed; following the cold winters of 2009-2010 and 2010-2011 high levels of recruitment occurred throughout most of Southwest England. The magnitude of larval recruitment was exceptional, especially in 2010 where recruitment was $\sim 6-10/\text{cm}^2$ at sites 100 km west of Start Point. These are greater densities than have occurred in Southwest England for many years, especially at locations more than 50 km west of Start Point (Hawkins et al. 2003, Moore 1936, Southward 1991, Southward et al. 1995) and are comparable to high recruitment sites elsewhere in Europe near the center of the species range distribution with much higher adult population density (Connell 1985, Wetthey 1985b, Jenkins et al. 2000, 2001). In contrast, following the warm winter of 2011-2012 we observed low recruitment throughout Southwest England. The prediction that cold-winters that exceeded the reproductive threshold of 4-6 weeks below 10°C would result in increases in adult provisioning of the larval pool is therefore strongly supported.

Jenkins et al. (2000) described larval settlement and recruitment of *Semibalanus balanoides* at three sites 6-40 km west of Start Point. In 1997, following a cold winter that met the criterion for high reproduction (Fig. 2.3), they observed high levels of larval recruitment ($\sim 6-8/\text{cm}^2$) comparable to our observations in 2010. In 1998, following a warm-winter during which the reproduction was expected to be inhibited at sites west of Start Point (Fig. 2.3), they observed negligible recruitment. If we combine these results with ours we find that in three of the five years we predicted and observed high recruitment success and in the remaining two years we predicted and observed recruitment failure. The probability of obtaining this result in the correct sequence is 0.035 ($3/5 * 2/5 * 3/5 * 3/5 * 2/5$) and thus is it unlikely that we would have observed the

predicted sequence of recruitment success and failure by chance alone. The results of Jenkins et al. (2000) endorse the hypothesis the adult provisioning of the larval pool drives larval recruitment and this in turn depends upon temperature falling below the 10°C threshold for at least 4-6 weeks during the winter.

In this study we measured larval recruitment within two months of larval settlement, thus it is possible that post-settlement mortality affected the abundance of juvenile barnacles (Pineda et al. 2009, 2010) prior to our measuring young of the year densities. Jenkins et al. (2000) compared annual and spatial variation in settlement and recruitment at multiple spatial scales across Europe. They found settlement and recruitment to be highly correlated within 3 of 4 regions studied across Europe and in Southwest England 99% of the variation in recruitment among sites and between years could be explained by the level of settlement. Thus we believe our assessment of annual variation in recruitment accurately reflects variation in larval settlement rate.

Bertness et al. (1991), Leslie et al. (2005) and Burrows et al. (2010) report that regional primary production (as measured by the chlorophyll *a* concentration) contributes to variation in growth and reproductive output in barnacles. It is possible that the annual variation in recruitment observed in Southwest England is due to variation in primary production that is correlated with winter SST. We tested this possibility by comparing annual variation in primary production to the recruitment of *S. balanoides* observed by us and by Jenkins et al. (2000). Primary production was measured by *in situ* fluorometry at a station within 10 km of sample sites used in this study and by Jenkins et al. (2000) (<http://www.westernchannelobservatory.org.uk>). We averaged weekly total chlorophyll *a* concentration between May 1 and November 1 prior to the measurement of recruitment

as an estimate of the period over which *S. balanoides* accumulates reserves for growth and reproduction (between settlement in mid-April and egg deposition in November). Primary production and recruitment were not significantly correlated ($r=0.0005$); in fact the period of highest primary production corresponded to the year with lowest recruitment. We also compared recruitment to the average concentration of chlorophyll *a* in March of the year recruitment was measured to assess whether barnacle larvae in the plankton may survive better in years with high primary production. There was again no significant correlation ($r=0.09$) and the year with the highest primary production in March corresponded to the second lowest recruitment. Thus there is no evidence that annual variation in primary production contributes to the observed annual variation in recruitment in Southwest England.

Variation of larval input driven by variation in winter temperature provides a mechanism for the long-observed pattern of oscillating population size of *Semibalanus balanoides* in Southwest England (Moore 1936, Southward and Crisp 1954, Southward 1967, 1991, Southward et al. 1995, Hawkins et al. 2003). Prior to 2010, adult *S. balanoides* were rare or absent from locations west of Start Point (Fig. 2.2A) but this has not always been the case. *S. balanoides* was a dominant intertidal species in the 1930s and 1940s, declined to become rare in the late 1940s and early 1950s when the western English Channel warmed, enjoyed a rebound during a cold period from 1952-1957, declined again as SST warmed between 1958 and 1961, became abundant again during rapid cooling in the early 1960s, and then has continuously declined during the current warming trend that began in the 1980s (Moore 1936, Southward and Crisp 1954, Southward 1967, Southward et al. 1995, Hawkins et al. 2003). Thus the high levels of

larval recruitment observed in 2010 and 2011 and the resurgence of adults observed in 2011 and 2012 occur against a backdrop of regional rarity of *S. balanoides* that has persisted for several decades. Our data indicate that whether winter temperatures drop below the reproductive threshold for a sufficient duration is a key component of the reproductive success of *S. balanoides* and this in turn determines the size of the larval pool available for settlement and recruitment.

The origins of the larvae that recruited in 2010 and 2011 are unclear. In this study we observed a westward expansion of *Semibalanus balanoides* into Southwest England in 2010 and further to the west again in 2011. Southward (1967) also observed a westward expansion of *S. balanoides* during a cold period in the early 1960s. Southward (1967) reported the “main front” of *S. balanoides* moved an average 53 km/yr and a maximum of ~120 km/yr. Dense populations of adults only occurred east of Start Point in 2010 so it is possible that larvae produced by these populations were dispersed to the west. During the dispersal period for *S. balanoides* (March to April) near-shore currents in Southwest England are driven westward by strong easterly winds (Pingree and Griffiths 1980). In 2011 recruitment advanced further westward which may suggest that the larvae transported to these western sites were produced by adults that were established west of Start Point in 2010 (Fig. 2.2). These results all indicate that the western sites are connected to and depend upon recruitment from populations to the east, however, bulk transport of barnacle larvae over such large distances seems unlikely, especially given this would include transport across Start Point which has previously been shown to be a formidable barrier to larval dispersal (Gilg and Hilbish 2003). Alternatively westward expansion may originate from refuge populations in the west, as

small numbers of adults were indeed present west of Start Point prior to 2010.

Southward (1967) also suggested refuge populations may be important for repatriation of western sites. However, this is unlikely to be the sole explanation of the westward expansion; the temperature patterns were similar in 2010 and 2011, with all sites in the study region classified as having high or moderate reproductive output, but recruitment at the westernmost sites only occurred in 2011. If the recruitment patterns were based solely on cold temperatures allowing the reproduction of refuge populations, we would expect a similar recruitment pattern in both cold years, not the advancing front that was observed.

The observed pattern of permissive vs. non-permissive years of reproduction (Fig. 2.3) has population-wide implications for *Semibalanus balanoides* in Southwest England. The average lifespan of *S. balanoides* is three years (Southward and Crisp 1954, Wethey 1985a), so local extinction of *S. balanoides* will likely occur in areas that are not reseeded with larvae at least once every three years. Local extinction resulting from lack of larval settlement likely produced the dearth of *S. balanoides* in areas west of Start Point prior to 2010. Over the past two decades there has been a significant decline in the annual number of cold days (excluding the two recent cold winters) and an increase in the number of years in which reproduction should be inhibited separating years with high or modest reproductive output. Thus we predict that populations of *S. balanoides* in southwest England will remain transient. In addition, data from both Southward (1967) and our study indicate a single cold year is insufficient for larvae to colonize the far western sites. If the frequency of inhibitory years continues to increase, these areas may

permanently be outside of the range of *S. balanoides* and the species range will significantly contract.

Semibalanus balanoides populations in Southwest England highlight the combined roles of connectivity and adult input into the larval pool as controlling forces in population dynamics. In these populations, local recruitment is not significantly correlated with local adult density, indicating that their demography is a regional process mediated by population connectivity. Although input into the larval pool is an understudied component of connectivity, the few studies that have simultaneously measured recruitment and adult input indicate recruitment variation is strongly related to adult density (Smith et al. 2009) and adult fecundity (Hughes et al. 2000, Reed et al. 2009, Burrows et al. 2010). In contrast Leslie et al. (2005) found regional primary production influenced barnacle growth and reproductive output but had little apparent impact on recruitment. Together these examples indicate that variation in input into the larval pool can drive variation in connectivity and illustrate the need to incorporate variation in larval pool input into models of marine population connectivity. They also illustrate the need for a mechanistic understanding for the forces generating variation in adult input into the larval pool; in the study by Smith et al. (2009) recruitment was correlated with upstream adult biomass while in Hughes et al. (2000) adult abundance only explained 1.2% of the variation in recruitment, while fertility accounted for 72% of the variance.

A striking commonality between this study and those by Hughes et al. 2000, Smith et al. 2009, Reed et al. 2009, and Burrows et al. 2010 is that in all cases a significant relationship was found between adult inputs into the larval pool and

recruitment, even though these studies placed little emphasis on measuring, or even identifying, the other forces that potentially shape population connectivity between larval release and recruitment. Overall, input into the larval pool plays a key role in population connectivity, as it provides the initial starting conditions upon which other factors (physical transport, mortality, behavior) subsequently modify. Realistic variation in larval pool input needs to be incorporated into existing models of population connectivity to understand the magnitude of the signal generated by these other factors and their relative effect on the density of larvae inputted by adults and subsequent connectivity in populations.

2.6 Acknowledgments:

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2.7 Figures:

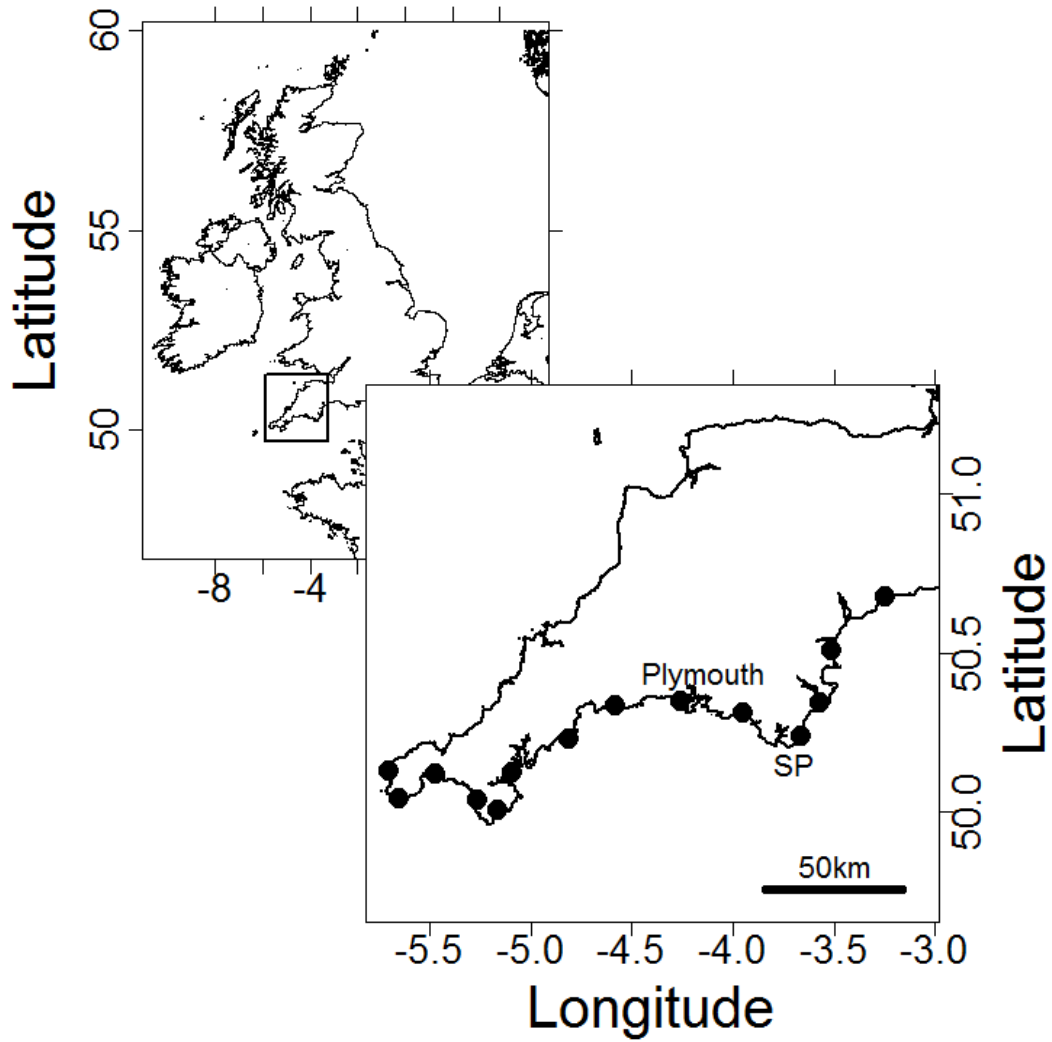


Figure 2.1: Map of study region and *Semibalanus balanoides* sampling sites in Southwest England. Photographic quadrats were taken at 15 sites (solid dots) located in the intertidal zone of Devon and Cornwall during 2010, 2011 and 2012. Start Point is designated by “SP”.

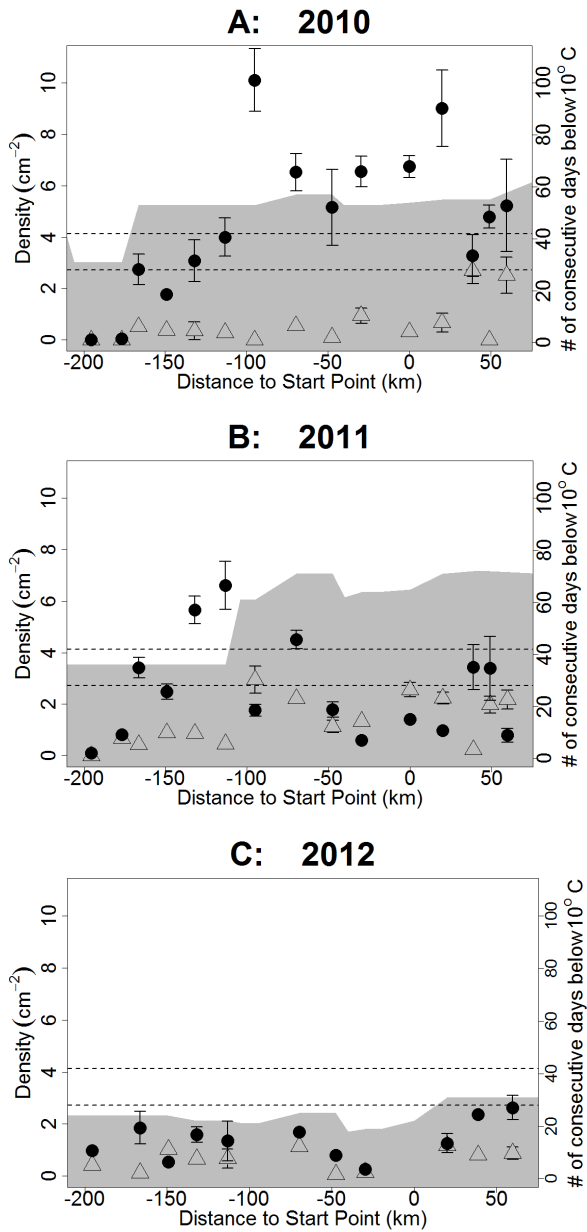


Figure 2.2: *Semibalanus balanoides* young of the year (solid circles) and adult (open triangles) densities (± 1 standard error) measured in 2010 (Panel A), 2011 (Panel B), and 2012 (Panel C) and number of consecutive days below 10°C (shaded area) as a function of distance from Start Point. Dashed lines represent thresholds at 28 and 42 days, delineating conditions under which high (>42 days), medium (28-42 days), and low (<28 days) reproductive output are predicted.

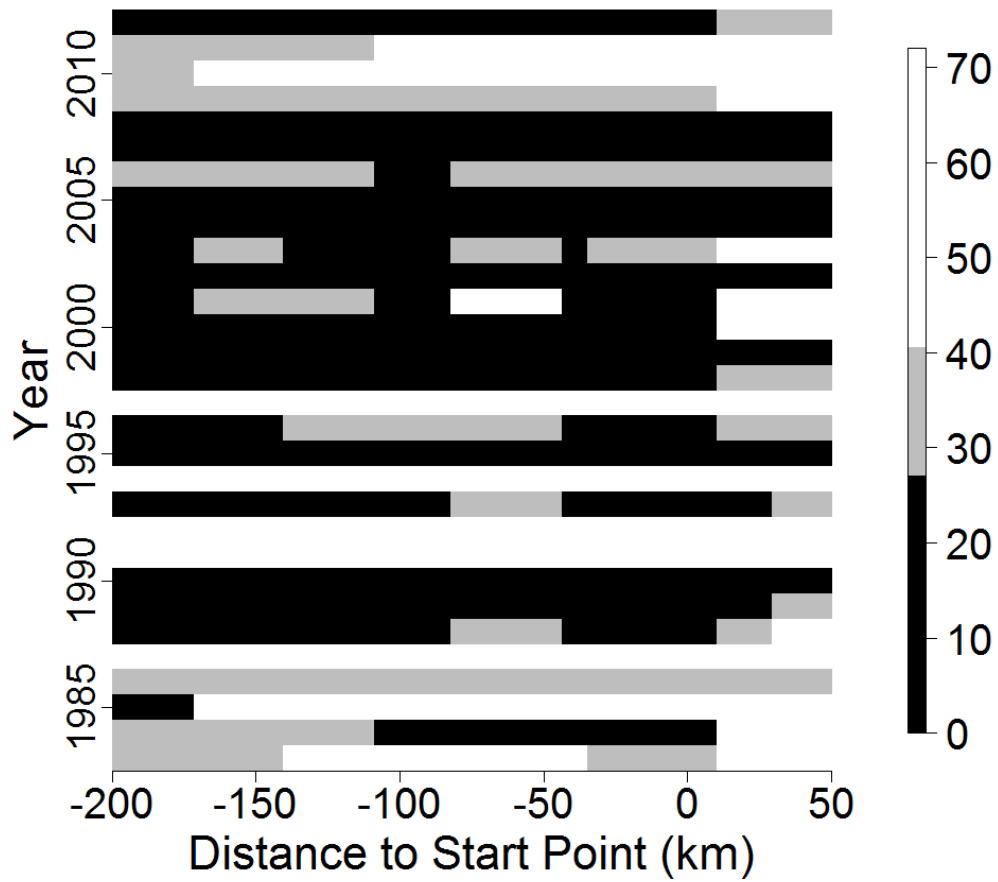


Figure 2.3: Number of consecutive days below 10°C as a function of time and distance from Start Point for 1983-2012. White, grey and black regions respectively represent the expectation of high, medium and low reproductive output.

CHAPTER 3

TEMPERATURE-INDUCED VARIATION IN THE SURVIVAL OF BROODED EMBRYOS

DRIVES DECADAL PATTERNS OF RECRUITMENT AND ABUNDANCE IN

*SEMIBALANUS BALANOIDES*²

² Rognstad RL and TJ Hilbish. Submitted to *Journal of Experimental Marine Biology and Ecology*. 7/2/2014

3.1 Abstract:

Geographic distributions of species are frequently influenced by temperature and as a result, there is considerable interest in predicting the effect of climate change on biogeography. Previous studies have found strong correlations between sea surface temperature and recruitment in the acorn barnacle *Semibalanus balanoides*, an important member of temperate intertidal communities in the Northern Hemisphere. The underlying mechanism that drives this relationship is, however, unknown. We tested the hypothesis that variation in recruitment is determined by temperature-induced variation in survival of brooded embryos.

Brooding adults were held under several environmentally-relevant temperature conditions from 7°C to 13°C until eggs were ready to hatch. There was no significant effect of temperature on embryonic development rate. Brooding individuals held at colder temperatures had more than twice the reproductive mass of those held at warmer temperatures. This mass difference is caused by an over three-fold larger number of embryos surviving in the coldest treatment (7°C) compared to the warmest treatment (13°C). Temperature-induced variation in number of surviving embryos likely contributes to differences in recruitment following cold winters vs. warm winters. Such temperature-dependent variation in recruitment has been shown to cause year to year fluctuations in the positions of the geographic limits of the species in Southwest England, France, and Spain. This study illustrates the key role of adult input into the larval pool in generating variation in recruitment and its influence on biogeography.

3.2 Introduction:

Climate change has been implicated as the driving force behind changes in species' distributions in both marine and terrestrial environments (Parmesan et al., 1999; Thomas and Lennon, 1999; Wetthey et al., 2011; Hilbish et al., 2012) and there is substantial interest in prediction of future changes in distribution, particularly from a conservation perspective (Guisan and Zimmerman, 2000; Newbold and Eadie, 2004; Bourg et al., 2005). Early modeling efforts focused on statistical relationships among species presence/absence and environmental variables, but as limitations to such approaches are identified (Guisan and Zimmerman, 2000; Austin, 2002), there is motivation to build mechanistic models describing the effects of climate change (and smaller scale temperature variation) on population dynamics.

The cold-water acorn barnacle *Semibalanus balanoides* (L.) provides an excellent study organism for parameterization of mechanistic species distribution models as it is a common, widely-distributed member of boreo-arctic communities that is relatively easy to rear in the laboratory and populations are easily manipulated in the field. Additionally, there is a wealth of literature on *S. balanoides* physiology (Crisp, 1959; Barnes, 1963; Crisp and Patel, 1969) and historical distributions (Moore, 1936; Southward and Crisp, 1954; Southward, 1991; Southward et al., 1995; Hawkins et al., 2003), providing data for testing developed models. *S. balanoides* is a cross-fertilizing hermaphrodite, which fertilizes in the late fall (Nov-Dec; Crisp, 1964) and broods its embryos outside the body but within the shell until the late winter/early spring (March; Crisp, 1964), releasing one brood of larvae per year.

Recruitment in populations of *Semibalanus balanoides* is strongly correlated with cold winters (Southward, 1991; Jenkins et al., 2000; Drévès, 2001; Abernot-Le Gac et al., 2013) and near its southern range boundary this variation results in range expansion and contraction that depends upon regional patterns of winter temperature (Southward, 1967; Wethey and Woodin, 2008; Wethey et al., 2011; Jones et al., 2012). Rognstad et al. (2014) showed that variation in recruitment and changes in geographic limits at the southern range of *S. balanoides* in Southwest England depends on whether sea surface temperature (SST) is less than 10°C during the 8-12 week period that adult barnacles incubate developing embryos. During cold winters that met this threshold larval recruitment was high and *S. balanoides* expanded its geographic range, whereas recruitment was very low following winters where SST was greater than 10°C (Rognstad et al., 2014).

Several mechanistic hypotheses have been proposed to explain the striking association between winter SST and recruitment in *Semibalanus balanoides*. First, it is possible that fertilization rate depends upon cold temperature in the autumn when *S. balanoides* copulate and that during warm years there is a low rate of fertilization. Barnes (1963), Tighe-Ford (1967), and Crisp and Patel (1969) found in experimental studies that *S. balanoides* must experience cold temperatures in the autumn for fertilization to be successful. Second, following fertilization developing embryos are incubated by the adult for several weeks and embryonic development may depend upon temperature. There are several mechanisms by which this may occur; embryos may fail to complete development during warm winters, the rate of development may depend upon temperature, or embryos may exhaust their metabolic reserves during warm winters

and die before release into the plankton (Crisp, 1959). Many investigators have speculated that temperature-dependent variation in developmental rate may cause a mismatch with the phytoplankton bloom that results in larval starvation and high mortality rates during warm winters (e.g. Barnes, 1956). It is also possible that variation in the timing of larval release may result in variation in the timing of settlement that produces high post-settlement mortality rates, either because settlement in warm winters is more likely to coincide with high temperature events following settlement (Kendall et al., 1985; Jarrett, 2003; Pineda et al., 2006; Poloczanska et al., 2008) or low quality larvae resulting from a mismatch with the phytoplankton bloom are less likely to survive once they have settled (reviewed by Pechenik et al., 1998).

In this study we test the hypothesis that temperature influences embryonic development during incubation by their parent. We specifically test the hypotheses that 1) embryos fail to complete development under warm conditions and 2) the rate of embryonic development during incubation depends upon temperature. The design of this study separates hypotheses that pertain to the incubation phase of development from those that focus on the fertilization success and those that attribute variation in recruitment to processes that occur in the plankton or following settlement. We focus this study on temperature variation in the range of 7-13°C because this is the range of annual variation in winter SST that occur in Southwest England and other regions where *S. balanoides* reaches its southern limit, and because it encompasses the threshold temperature of 10-12°C that is required for the completion of successful development suggested by Crisp (1959), Barnes (1963), and Crisp and Patel (1969). Finally, previous studies did not distinguish between the effects of SST and air temperature on recruitment success of *S.*

balanoides so this study explicitly tests whether air or water temperature is of greater importance in determining success during embryonic development.

3.3 Material and Methods:

3.3.1. Experimental design:

Adult *Semibalanus balanoides* were collected from the beach at the end of Park Road in Woods Hole, Massachusetts, USA (41.532°N×70.671°W) on December 6, 2012.

Individuals were collected on cobbles that were small enough to be easily transported and were selected to have barnacles at intermediate densities. Barnacles were sparse enough to be cone-shaped rather than columnar yet were within one body length of at least one other individual so fertilization was possible. Individuals were transported to the University of South Carolina at Columbia where the cobbles were randomly assigned to one of five 52.9-L containers measuring 31.4cm × 58.4cm × 41.2cm. Treatment containers were placed within reach-in chambers with individual temperature and lighting controls. Barnacles were exposed to a simulated semi-diurnal tide cycle with the timing of both the tide and daylight schedule at Woods Hole. Seawater was pumped from the experimental chamber to a head tank and the barnacles were exposed to the air for 6 hours to simulate low tide and then submerged for 6 hours to simulate high tide. Seawater was vigorously aerated at all times. Every two weeks the timing of the tide and light cycle was adjusted to match that of Woods Hole. Treatment containers were covered with transparent Plexiglas to prevent evaporation but allowed the barnacles to be exposed to the light cycle. Barnacles were liberally fed every 2 days throughout the experiment by addition of Spat Formula (Innovative Aquaculture Products LTD), a

commercial aquaculture product containing the diatoms *Chaetoceros-B* and *Phaeodactylum tricornutum* and the flagellate *Nannochloropsis oculata*, at a final concentration of 10^8 cells l^{-1} . The seawater in the experimental chambers was maintained at 35 ppt and was replaced every two weeks. Feces and settled algal particles were removed weekly. The water changes occurred during low tide (when the water was in the head tank), allowing it to equilibrate to the treatment temperature before the animals were submerged.

In three of the treatments, barnacles were exposed to the same air and water temperature of 7°, 10°, and 13°C to represent a range of environmentally relevant temperatures spanning the temperature threshold proposed in the literature (Crisp, 1959; Barnes, 1963; Crisp and Patel, 1969). The temperatures were maintained using the internal temperature controls of the reach-in incubators and monitored using both iButton data loggers and thermocouple temperature sensors, which indicated the mean temperature of each treatment over the duration of the experiment was within 0.5°C of the intended temperature. Two additional, mixed-temperature treatments were also conducted, one with 7°C air temperature and 13°C water temperature, the other with 13°C air temperature and 7°C water temperature. These treatments were designed to differentiate among the effects of air temperature, water temperature, and mean temperature on reproduction.

All experimental treatments were conducted simultaneously over a total of 63 days and chambers were sampled at 8 time-points, 2, 9, 15, 27, 36, 46, 55, and 63 days after the start of the experiment. At each time-point, 10-20 individuals were selected from haphazardly chosen cobbles and carefully removed from the cobbles using a razor

blade. Efforts were made to sample a representation of available sizes of adults (5-13 mm basal diameter). Basal diameter at the widest point, operculum length at longest point, and height at tallest point was measured for each individual (± 0.1 mm). The developmental stage of the embryos was assessed under a dissecting microscope (50 \times) using the developmental scale described in Table 3.1 (J Pineda and V Starzcak, personal communication). Somatic and gonad tissue were dissected, dried at 55°C for at least 48 hours, and then weighed with a microbalance. At the final time-point (Day 63), a subsample of embryos (200-800) was taken from each individual and counted using a Sedgwick Rafting Counter, then dried and weighed to determine the average mass per embryo for each treatment.

3.3.2 Statistical analyses:

All statistical analyses were performed with R, version 3.0 (R Core Development Team, 2013). Analysis of Covariance (ANCOVA) was performed using the R package ‘HH’, version 2.3-42 (Heiberger, 2014). To determine the effect of temperature on development rate, we calculated the time to reach stage 4b for each of the temperature treatments. We chose to use stage 4b because at this stage, the embryos are fully developed (possessing all appendages, etc.) and could potentially survive outside of the adult (Crisp, 1959). We used linear regression equations to estimate the time at which 50% of the embryos had reached stage 4b and the time at which 100% of the embryos had reached stage 4b for each temperature treatment. Regressions were calculated using data from the period 0-35 days when less than 100% of the embryos were in stage 4b.

We regressed reproductive mass against each of the linear measures of body size and determined that basal diameter explained the greatest portion of variance. We then examined the effect of temperature on reproductive mass over time, by first regressing reproductive mass against basal diameter at each sample date in each experimental treatment to adjust reproductive mass to the mean basal diameter across all treatments (9.1 mm). For each treatment the adjusted mean reproductive mass (± 1 standard error) was then regressed against incubation time over the course of the experiment. The effect of temperature on reproductive mass at the last time point (63 days incubation time) was further assessed by ANCOVA using basal diameter as the covariate and temperature as the treatment effect. We used the same procedure to assess the effects of temperature on changes in somatic tissue weight.

3.4 Results:

3.4.1. Effect of temperature on development rate:

There was no significant difference among treatments in the rate at which embryos reached stage 4b of development (Fig 3.1; ANCOVA, intercept: $F_{2,9}=1.040$, $p=0.39$; slope: $F_{2,9}=0.093$, $p=0.91$). Using separate regression equations for each temperature, the mean time (± 1 SE) at which 50% of the embryos reached stage 4b was 12.7 ± 1.0 , 17.1 ± 2.9 , and 15.7 ± 1.3 days, for the 7°C , 10°C , and 13°C treatments, respectively. All embryos were estimated to have reached stage 4b by day 34.4, 34.9, or 35.1 for the 7°C , 10°C , and 13°C treatments, respectively.

3.4.2. *Effect of temperature on reproductive mass:*

Over the course of the experiment, there was a significant decrease in size-adjusted reproductive mass for individuals in the 13°C treatment ($F_{1,6}=16.02$, $p=0.007$) and a significant increase in size-adjusted reproductive mass for individuals in the 7°C treatment ($F_{1,6}=6.28$, $p=0.046$; Fig 3.2). There was no significant relationship between size-adjusted reproductive mass and time for the 10°C treatment ($F_{1,6}=0.01$, $p=0.924$, Fig 3.2). Additional analyses were conducted on the data collected on the last sample date (day 63), when the greatest difference among treatments was expected. At the last time-point, there was a significant negative effect of temperature on size-adjusted reproductive mass (Fig 3.3). ANCOVA, with basal diameter as the covariate, indicated that there was a significant effect of temperature on reproductive mass ($F_{2,56}=24.44$, $p<0.001$) and basal diameter on reproductive mass ($F_{1,56}=69.67$, $p<0.001$) and there was no significant difference among treatments in the slope of the relationship between reproductive mass and basal diameter ($F_{2,54}=2.72$, n.s.). Posthoc Tukey comparison of adjusted means indicated that all pairwise comparisons between the single temperature treatments were significant. In the single temperature treatments, mean size-adjusted reproductive mass ($\pm 1SE$) was largest for the 7°C treatment ($8.00\pm 0.43\text{mg}$), intermediate in the 10°C treatment ($5.70\pm 0.44\text{mg}$), and smallest in the 13°C treatment ($3.75\pm 0.45\text{mg}$) (Fig. 3.3). At day 63, we measured the relationship between reproductive mass and the number of embryos for 10 individuals from each treatment to determine if the difference in total reproductive mass among experimental temperatures were caused by variation in number of embryos, variation in mass per embryo, or both. ANCOVA, with basal diameter as the covariate, indicated that there was a significant effect of temperature treatment on

number of embryos ($F_{2, 26}=10.59$, $p<0.001$) and basal diameter on number of embryos ($F_{1, 26}=13.66$, $p=0.001$) and there was no significant difference among temperatures in the slope of the relationship between the number of embryos and basal diameter ($F_{2, 24}=1.99$, n.s.). Posthoc Tukey comparison of adjusted means (adjusted to the grand mean basal diameter, 9.1mm) indicated that there were significantly more embryos in the 7°C (3555±492 embryos) and 10°C (3280±534) treatments compared to the 13°C treatment (956±124; $p<0.01$, Fig 3.4), and the 7°C and 10°C treatments did not significantly differ in number of embryos. There was also significant difference among treatments in the average mass per embryo on day 63 (ANOVA, $F_{2, 27}=11.35$, $p<0.001$). Posthoc Tukey comparison of means indicated a significantly greater mass per embryo (3.46±0.18µg) in the 13°C treatment than in the 7°C treatment (2.30±0.16µg , $p<0.001$) or the 10°C treatment (2.41±0.21µg , $p=0.002$). The 7°C and 10°C treatments did not differ significantly in mass per embryo.

Somatic mass also differed among the treatments at the last time point. ANCOVA, with basal diameter as the covariate, indicated that there was a significant effect of temperature on somatic mass ($F_{2, 56}=4.28$, $p=0.018$) and basal diameter on somatic mass ($F_{1, 56}=69.30$, $p<0.001$) and there was no difference among temperatures in the slope of the relation between somatic mass and basal diameter ($F_{2,54}=0.96$, n.s.). Posthoc Tukey comparison of size-adjusted means (adjusted to the grand mean basal diameter, 9.1mm) indicated that somatic mass was significantly greater in the 7°C treatment (1.42±0.09mg) compared to the 13°C treatment (1.03±0.09mg), and the 10°C treatment (1.31±0.09mg) did not significantly differ from either of the other single temperature treatments.

3.4.3. Effects of air vs. water vs. mean temperature:

Mean reproductive mass of the animals at the last time point incubated in the mixed temperature treatments (Air 13°C –Water 7°C, 5.32 ± 0.74 mg; Air 7°C -Water 13°C, 5.53 ± 0.75 mg) did not differ significantly from those held at a constant temperature of 10°C (5.70 ± 0.44 mg) (Fig 3.3). ANCOVA, with basal diameter as the covariate, indicated that there was a significant effect of basal diameter on reproductive mass ($F_{1, 36} = 13.40$, $p < 0.001$), but there was no significant difference among temperature treatments in adjusted mean reproductive mass ($F_{2, 36} = 0.09$, n.s.) and there was no significant difference among temperature treatments in the slope of the relationship between reproductive mass and basal diameter ($F_{2, 34} = 1.43$, n.s.). The mixed temperature treatments also had no effect on development rate. When compared to all three single temperature treatments, there was no significant difference among treatments in the linear portion (first 5 time-points) of the relationship between time and percent of embryos reaching stage 4b (ANCOVA, intercept: $F_{4, 17} = 0.6621$, $p = 0.63$; slope: $F_{4, 15} = 0.1055$, $p = 0.98$; data not shown).

3.5 Discussion/Conclusion:

Semibalanus balanoides is circumpolar in the Northern Hemisphere and reaches its southern range limit in regions where winter temperatures exceed 10-15°C (Barnes, 1958). The recruitment and abundance of adult *S. balanoides* at its southern range boundary exhibits strong oscillations that are correlated with changes in sea surface temperature at annual and decadal time scales (Southward, 1967; Southward, 1991;

Jenkins et al., 2000; Drévès, 2001; Abernot-Le Gac et al., 2013; Wetthey and Woodin, 2008; Wetthey et al., 2011; Jones et al., 2012; Rognstad et al., 2014). Both field observations and experimental studies have suggested that successful reproduction depends upon photoperiod (Davenport et al., 2005) and experiencing a cold period during either the time of fertilization (typically in November) or during the incubation of embryos between fertilization and their release in the early spring (Barnes, 1963; Crisp and Patel, 1969). There are, however, two basic problems with interpreting these studies. First, is the difficulty of determining the appropriate measure of temperature since the mechanism(s) of temperature effects on reproduction success are not well known and different expressions of temperature (maximum, minimum, mean, duration, etc.) and the timing of these events are often highly correlated. For example, the mean temperature in February (during incubation of embryos) is correlated with the minimum and maximum temperature, and with the duration of cold events (e.g. days below a given value) and additionally is often correlated with temperature in November (during fertilization) and later in spring and early summer (during settlement) (Rognstad unpublished data). Consequently correlational studies may offer insight into potential hypothetical mechanisms for the influence of temperature on reproduction but cannot be definitive. Second, previous experimental studies have often confused the potential effects of temperature on fertilization success with effects on development and success during incubation (Barnes 1963) or only measured the frequency of successful fertilization but did not quantify the number of competent embryos released to the plankton (Barnes, 1963; Crisp and Patel, 1969). Consequently, without experimental analysis it is not

possible to disentangle the various potential roles of temperature on different components of the life history that may determine variation in reproductive success in *S. balanoides*.

In this study we have focused specifically on the effects of temperature on development following fertilization. Almost all adult barnacles used in this study were fertilized at the beginning of the experiment and the embryos were in the early stages of development; thus the effects of temperature on fertilization success is not conflated with the effects of temperature on the success of embryonic development during incubation.

3.5.1 Effect of temperature on development rate:

There was no evidence of a temperature-dependent blockage of development; in all treatments embryos completed development through stage 4b within 40 days (Fig 3.1). There was also no significant effect of temperature on development rate to stage 4b, which is ecologically relevant because embryos at this stage would be viable if eggs hatched and larvae were released into the plankton (Fig 3.1). Crisp (1959) found similar results; over the range of temperatures used in this study he found a small effect of temperature on very early development and no effect of temperature for the majority of development. Thus there is no evidence that indicates the elevated recruitment observed following cold winters (Southward, 19676; Southward, 1991; Jenkins et al., 2000; Drévès, 2001; Abernot-Le Gac et al., 2013; Wetthey et al., 2011; Rognstad et al., 2014) results from temperature-induced variation in development rate that subsequently produces a better match with the phytoplankton bloom (Barnes, 1956) or less-stressful settlement period (Kendall et al., 1985; Pineda et al., 2006). This study did not test

whether fertilization success depends on temperature since ~90% of the individuals had already been fertilized at the onset of the experiment. Thus a temperature “threshold” temperature above which reproduction is inhibited as proposed by Barnes (1963) and Crisp and Patel (1969) could possibly occur prior to fertilization.

3.5.2 *Effects of temperature on reproductive mass:*

There was a nearly three-fold larger reproductive mass in the 7°C treatment compared to the 13°C treatment by the conclusion of the experiment (Figs. 3.2 and 3.3) which was caused by a nearly 4-fold difference in the number of embryos between these two treatments (Fig. 3.4). It is possible that the difference in the number of embryos is the result of earlier release of some of the embryos in the warm treatment. We think this explanation is unlikely for two reasons: first, the membranes surrounding the mass of embryos were still intact on the final time point of the experiment and embryos cannot hatch and be released to the plankton without rupturing this membrane. Second, we observed the experimental chambers daily and in the relatively small volume of our experimental chambers (~45L), released larvae in any appreciable quantity would have been visible and this was not observed. Thus we conclude that a much greater number of embryos complete development at 7°C than when they experience 13°C.

The large difference in the number of embryos produced among the experimental temperatures is very compatible with the observed annual variation in recruitment. Rognstad et al. (2014) found on average a three-fold greater recruitment of *Semibalanus balanoides* in Southwest England following the cold winter of 2010 compared to recruitment observed following the warm winter of 2012. There is also a

strong inverse relationship between winter temperature and recruitment of *S. balanoides* in France (Drévès, 2001; Abernot-Le Gac et al., 2013). Thus both the magnitude of the variation in larval production and its inverse relationship with temperature are mirrored by the variation in recruitment observed in the field. These results strongly indicate that temperature-induced variation in successful incubation of embryos plays a major role in controlling input into the larval pool and subsequent variation in recruitment. Additional variation in recruitment is likely generated by variation in larval success in the plankton and settlement, but the influence of annual variation in temperature determining larval recruitment densities appears to be heavily influenced by the role of temperature in determining the success of adults in the incubation of their embryos and their subsequent contribution to the larval population.

There are at least two possible mechanisms to explain why reproductive output is substantially reduced when embryos are incubated at higher temperatures. The decrease in reproductive mass in the 13°C treatment may be indicative of an inability to meet the oxygen demand of late-stage embryos at this temperature. At the final time-point, 20% of the embryonic masses reared at 13°C contained embryos with purple guts (compared to 0% in all other treatments), a condition caused by protein-astaxanthin complex dissociation (Crisp, 1959) and associated with oxygen limitation (Crisp and Spencer, 1958). In the case of *S. balanoides* embryos, Crisp (1959) proposed a critical temperature above which O₂ cannot diffuse into the egg mass in sufficient quantities to meet the demand of the developing embryos, explaining the greater frequency of purple embryos he observed in large egg masses vs. small egg masses at warm temperatures. In our study, warm temperatures could result in a loss of reproductive mass as embryos near

the center of the egg mass are unable to meet their O₂ demand and subsequently die. A second possible mechanism is that embryos reared at 13°C may exhaust their energy reserves more rapidly than embryos reared at lower temperatures and those embryos that were initially provisioned with fewer reserves die. This hypothesis is supported by the observation that adult somatic tissue also declined during the experiment at 13°C, which may be indicative of a general energetic stress rather than a specific inability to deliver sufficient oxygen to meet the metabolic demand of all of the embryos. These two hypotheses are not mutually exclusive; excessive metabolic rate at 13°C may lead to both exhaustion of metabolic reserves and selective death of embryos that cannot obtain sufficient oxygen to meet their metabolic demand.

Unexpectedly, the embryos brooded at 13°C had greater mass than those brooded at 7°C or 10°C. This does not support the hypothesis that embryos brooded at 13°C would use up their energy reserves faster and consequently decrease their reproductive mass relative to the other temperature treatments. However, the change in the biochemical composition of barnacle embryos over the course of development during incubation is complex (Lucas and Crisp, 1987) and it is unclear as to why embryos would have a greater average mass at the warmest experimental treatment. Further biochemical analysis of the embryos is needed to interpret the mechanism responsible for this mass difference.

3.5.3 Effects of air vs. water vs. mean temperature:

We conducted two additional, mixed-temperature treatments, one with 7°C air temperature and 13°C water temperature, the other with the reciprocal 13°C air

temperature and 7°C water temperature to determine whether air or water temperature is more important in determining the success of embryos during incubation. In both cases the average temperature experienced by the barnacle over the course of a day was 10°C, comparable to the constant temperature treatment where air and water temperature were both maintained at 10°C. Neither the development rate nor the final reproductive mass of individuals brooding in the mixed temperature treatments differed from each other or from the constant 10°C temperature treatment (Fig 3.3). These results suggest that the rate of embryonic development and reproductive output in *S. balanoides* depend upon mean temperature rather than either air or water temperature per se, at least within the range of temperatures used in this study. Many previous studies have focused largely on SST as an ecological driver, but the results of this study indicate that efforts to model the influence of temperature on the physiology and ecology of intertidal organisms should consider the response mechanism of their study organism and potentially incorporate both air and sea surface temperature (as in Wetthey and Woodin, 2008).

3.5.4 Conclusion:

Here we demonstrate that relatively small changes in mean brooding temperature have a dramatic effect on the number of *Semibalanus balanoides* embryos reaching the final development stage before release, but that developmental rate remains the same. The over three-fold difference in the number of embryos could have equally large effects on population dynamics as the odds of successful recruitment are increased because of greater input into the larval pool, a life-stage with high mortality (Pineda et al., 2009; Vaughn and Allen, 2010). The large increase in embryo production at low temperatures

may help explain the dramatic expansions in geographic distribution of *S. balanoides* following cold winters (Southward, 1967, 1991; Wethey et al., 2011; Rognstad et al., 2014). We also demonstrate that this effect on embryonic development is produced by the mean temperature experienced by the animals, indicating that SST, which is often used alone in intertidal population models (e.g. Poloczanska et al., 2008; Wethey et al., 2011), may be insufficient to model the dynamics of intertidal organisms. Adult input into the larval pool is a driver of variation in recruitment in barnacles (Burrows et al., 2010; Rognstad et al., 2014) and other species (Hughes et al., 2000; Reed et al., 2009; Smith et al., 2009). This study provides a mechanism linking variation in temperature to variation in adult input into the larval pool via differential survival of embryos, explaining the relationship between shifts in biogeographic range limits and temperature.

3.6 Acknowledgements:

This work was funded by NSF (OCE1129401) and NASA (NNX11AP77G). We thank J. Pineda and V. Starczak of the Woods Hole Oceanographic Institution for development of the barnacle embryo staging system and assistance collecting in the field. We also thank D. Wethey and G. Macho for valuable discussions concerning the design of the laboratory experiments and interpretation of results. D. Wethey also provided helpful suggestions on an earlier version of this manuscript.

3.7 Tables and Figures

Table 3.1 Description of developmental traits used to stage barnacle embryos (J Pineda and V Starczak, personal communication)

Stage	Description
0	White tissue
1	Yellow tissue, gonad is stringy and not yet egg-shaped
2	Eggs are visible and ovoid, but not developed.
3	Eggs are translucent and yellow. They have brown eyespots, but no body structure
4	Visible naupliar body structure, including caudal spines and distinctive eyespot, developing gut sometimes visible. Eggs beginning to change color from yellow to white
4b	Fully-developed nauplii with appendages and two brown spots, embryos contained within egg case
5	Free-swimming or loose nauplii with appendages free from the membrane

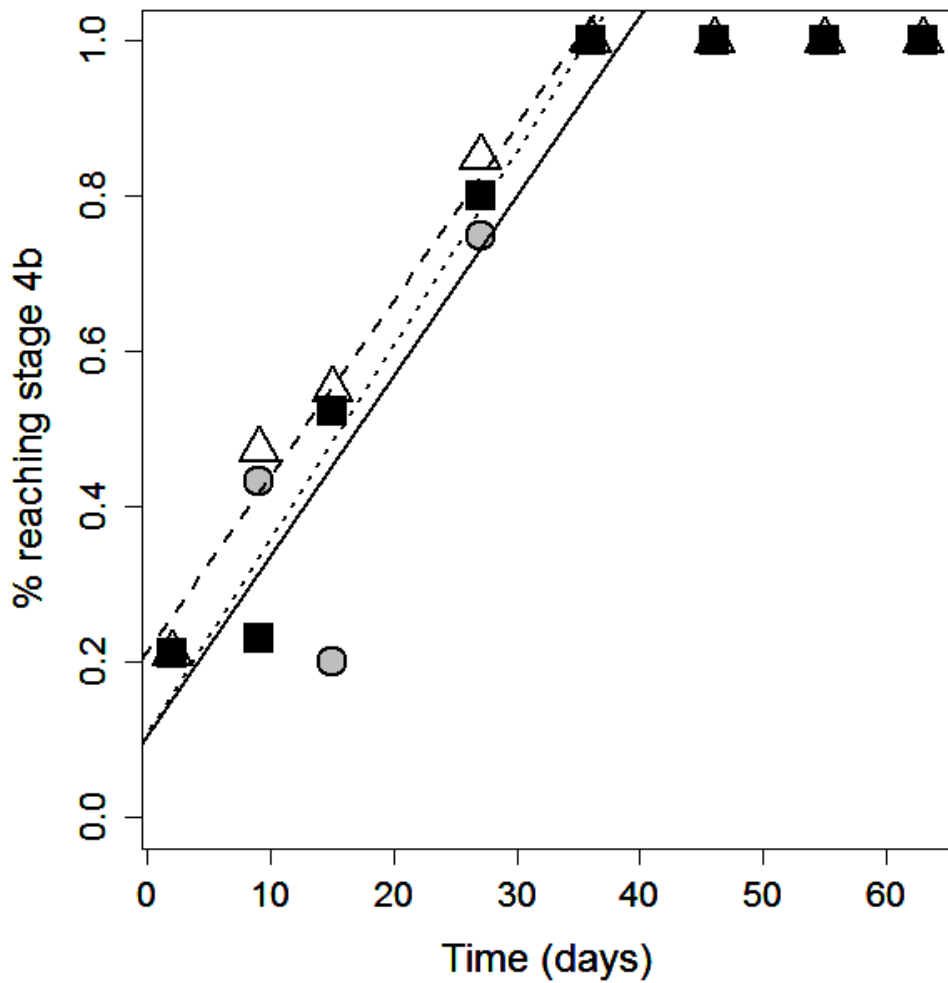


Figure 3.1 Percent of *Semibalanus balanoides* larvae reaching stage 4b over the course of the experiment for the three single temperature treatments: 7°C (open triangle, dashed line), 10°C (grey circle, solid line), and 13°C (black box, dotted line). Lines are linear regressions for linear portion of the curves.

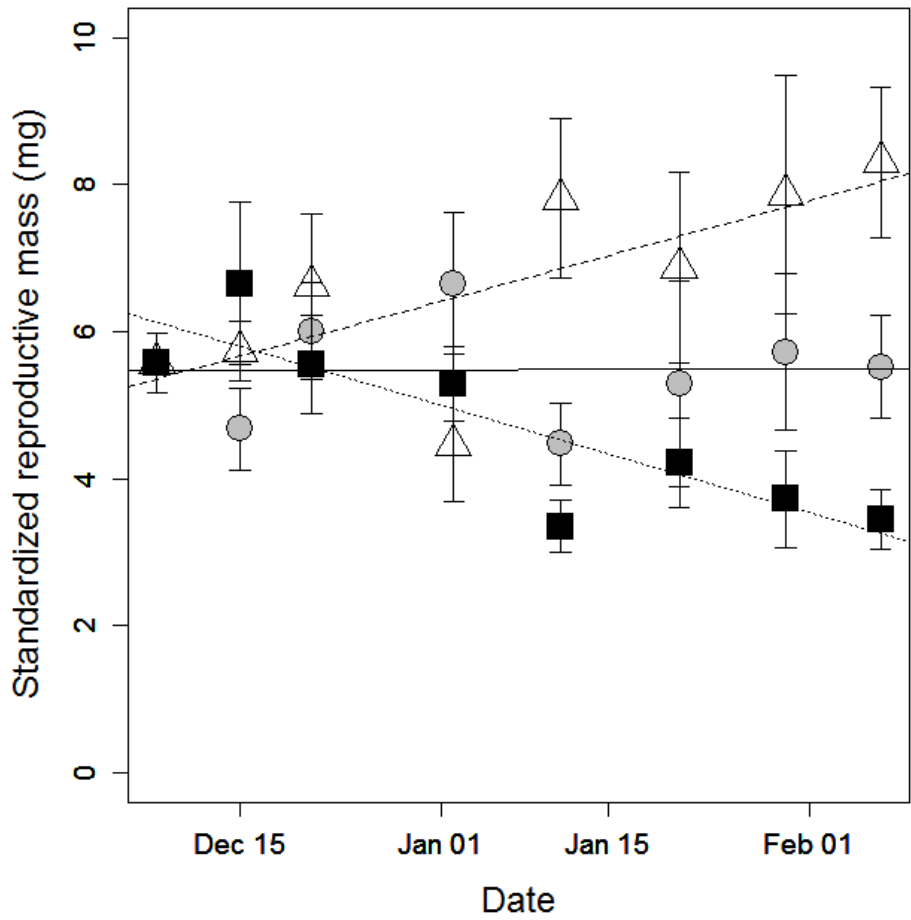


Figure 3.2 *Semibalanus balanoides* reproductive mass standardized to the grand mean of basal diameter (9.14mm) and linear regressions for the three single temperature treatments: 7°C (open triangles, dashed line), 10°C (grey circles, solid line), and 13°C (black boxes, dotted line). Adjusted means from ANCOVA ± 1 standard error.

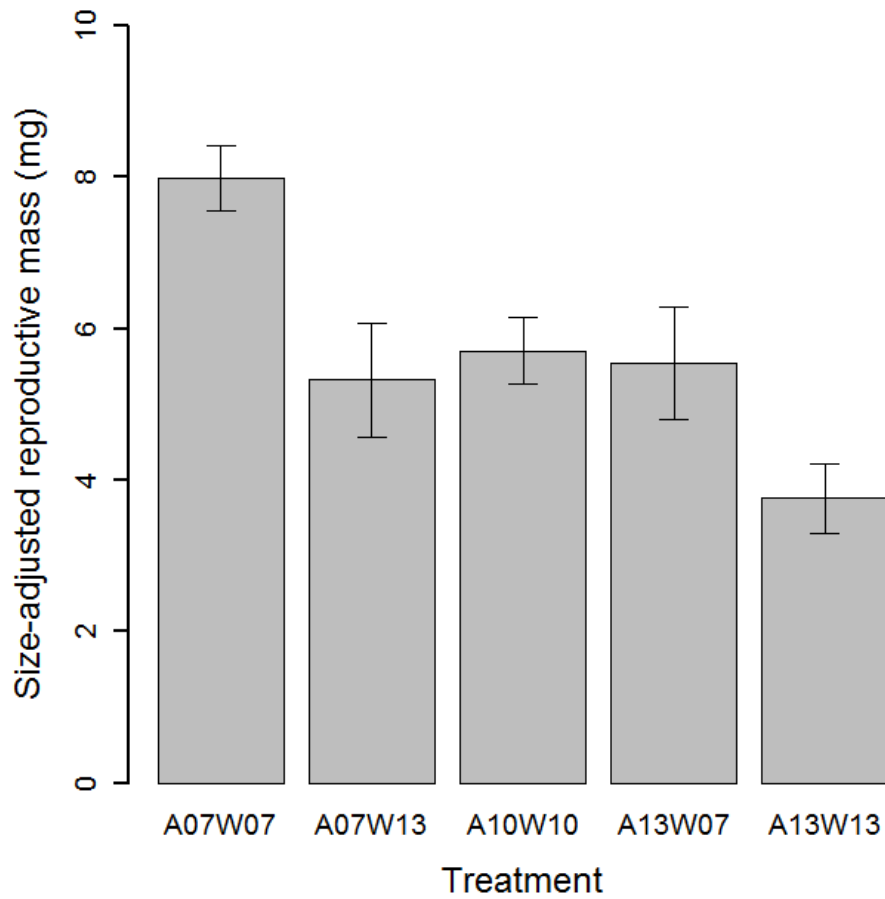


Figure 3.3 *Semibalanus balanoides* size-adjusted mean reproductive mass (adjusted to grand mean basal diameter of 9.1mm) for the three single temperature treatments (A07W07, A10W10, and A13W13) and the two mixed temperature treatments (A07W13 and A13W07). Treatment codes have “A” followed by the air temperature and “W” followed by the water temperature. Bar indicates mean and error bars are standard error.

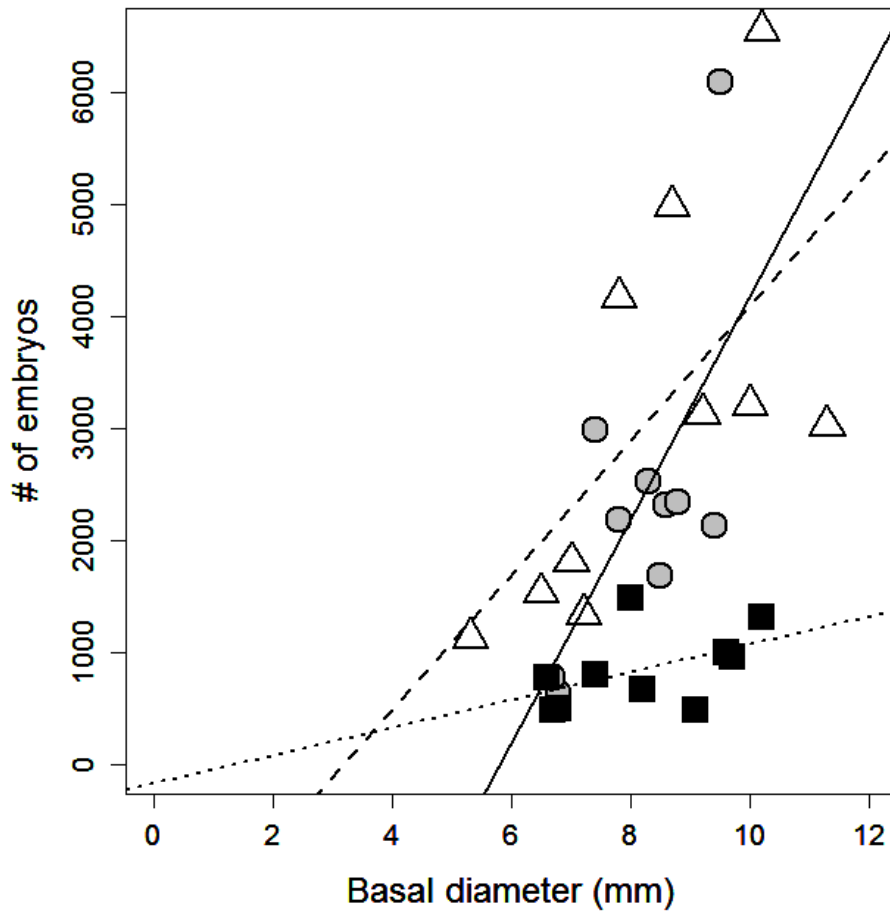


Figure 3.4 Number of eggs vs. basal diameter of *Semibalanus balanoides* reared at three temperature treatments: 7°C (open triangle, dashed line), 10°C (grey circle, solid line), and 13°C (black box, dotted line). Each point represents one individual (N=10 per treatment); lines are linear regressions.

CHAPTER 4

CONNECTIVITY MODELING AND GRAPH THEORY ANALYSIS TO UNDERSTAND

RECOLONIZATION IN TRANSIENT POPULATIONS³

³Rognstad RL, Wethey DS, Oliver H, and TJ Hilbish. Connectivity modeling and graph theory analysis to understand recolonization in transient populations, *In preparation*

4.1 Introduction:

In coastal marine systems, physical dispersal via transport in ocean currents is a major driver of population connectivity. Compared to the magnitude of ocean currents, larvae are often passive or weak swimmers, so ocean circulation patterns during the dispersal phase usually set the outside limits of dispersal. Consequently larval dispersal may contribute to the maintenance of adult populations in regions where they have limited reproductive or demographic success. Thus, circulation patterns contribute to setting the range limits of intertidal species (Lett et al. 2010, Sponaugle et al. 2012, lots of others). In variable environments, range limits also vary over short timescales as local extinction events may be followed by recolonization and such events are mediated by population connectivity (Levin 1974) and larval dispersal. Thus, an understanding of the magnitude and variability of connectivity among populations that are vulnerable to extinction and those that may serve as refugia is necessary to predict the effects of local extinctions on the whole metapopulation and identify the likelihood of recolonization if extinctions occur. Here we distinguish between demographic connectivity, which occurs at a level sufficient to subsidize low fecundity or survival at destination populations, from genetic connectivity, which occurs at a lower level that is sufficient to prevent genetic divergence, but not high enough to sustain a subpopulation without other inputs (Lowe and Allendorf 2010). The question of how much connectivity between two populations is necessary for them to be demographically connected is an important consideration for modeling recolonization dynamics and this value likely varies based on the specifics of the metapopulation studied (Lande et al. 1994). Waples and Gaggiotti (2006) suggest a transition at 0.1, or populations are demographically independent when the connection

between them is less than 10%, though this criterion is based on a relatively simple model (Hastings 1993). It is unlikely, however, that there is a simple critical level of connectivity required to maintain a metapopulation as this depends upon the local success of adult populations and of the geography of the connections among them. This requires the development of connectivity models that can incorporate these factors to assess the durability of metapopulations, especially near species range edges.

Recent advances in the availability and precision of ocean circulation models have produced a multitude of studies using these models to predict the dispersal of marine larvae in a diversity of taxa (Ellien et al. 2004, Siegel et al. 2003, Ayata et al. 2009). This is one of few techniques that can be used to estimate population connectivity on a demographic scale, as genetic estimates (Palumbi 2003, Allendorf and Luikart 2007, other citations) are often only useful to test hypotheses on a genetic level (but see Gilg and Hilbish 2003 for an exception). Mechanistic based models of connectivity are invaluable because they allow the test of hypotheses regarding changes in the physical forces that determine circulation, which is especially valuable in predicting changes in connectivity over annual, decadal, and centennial time-scales. Likewise they can be used to evaluate the outcome of altering habitat or exploitation patterns that influence the demographics of local or regional populations.

Regardless of their ultimate use, it is vital to validate the model by demonstrating it reflects realized connectivity. Most marine taxa, especially those with planktonic larval dispersal, are too small and diffuse too quickly to be tracked directly. There are three basic approaches that have been used to assess realized connectivity on a demographic scale. Elemental fingerprinting has been used to identify natal populations

and to estimate relative connectivity (Thorrold et al. 2007, Levin citations) in some organisms that retain a calcified structure that “records” the signal. A second approach uses genetic signatures that distinguish populations such that the movement of larval from one population to another (Gilg and Hilbish 2003, Jolly et al. 2009 others?). As is also the case with elemental fingerprinting genetic approaches are only valuable at detecting dispersal among distinct patches and cannot assess connectivity within a patch. Accordingly both of these methods are restricted to situations where the necessary “patchiness” of the signal is serendipitously available. Finally, the recolonization of expatriated or novel habitat can be used to test the efficacy of connectivity models. While several investigators have used recolonization to assess the spatial scale of larval dispersal(citations), rarely have such data been used to validate connectivity models (Higgins et al. 2001). However the opportunity to do so abounds as climate change and species introductions unfortunately provide multiple opportunities to assess the predictions of connectivity models. We utilize this approach in this study to utilize the repatriation of habitat at a southern range limit of the barnacle *Semibalanus balanoides* to validate models of population connectivity.

In many cases where empirical measures of connectivity are unavailable, especially with marine taxa that produce minute larvae that do not retain either genetic or elemental fingerprints, biophysical modeling is a key technique to estimate population connectivity at a demographic level. Biophysical modeling produces connectivity matrices, which are representations of the strength of the connection from each source location to each destination location. After connectivity matrices are generated, multiple methods can be used to extract useful information from them. An approach that has

recently gained momentum is to use graph theory methods, which have been used to analyze networks in a diversity of fields (Watts 2004) and used to analyze connectivity patterns in both terrestrial (Cantwell and Foreman 1993, Urban and Keitt 2001) and marine (Treml et al. 2008, Nilsson Jacobi et al. 2012, Thomas et al. 2014) systems. Such techniques can be powerful tools for identifying ecological subpopulations (Nilsson Jacobi et al. 2012, Thomas et al. 2014) and key transition sites in the system (Treml et al. 2008), and subsequently used to inform conservation decisions (Treml et al. 2008), including marine protected area design (Thomas et al. 2014).

The approach described here uses population connectivity models to predict the progress of recolonization after local extinction events, using the barnacle *Semibalanus balanoides* in Southwest England as a case study. Southwest England is a local southern limit of *S. balanoides* and populations in this region are transient (Moore 1936, Southward and Crisp 1954, Southward 1967, 1991, Southward et al. 1995, Hawkins et al. 2003, Rognstad et al. 2014). Connectivity matrices were generated by releasing simulated larvae into velocity fields using two circulation models of differing spatial scales. Using a graph theory approach, the resulting connectivity matrices were used to (1) classify sites into regions with low connectivity among regions, identifying potential barriers to dispersal, and thus recolonization, (2) examine the number of generations (steps) needed to recolonize sites within and between regions, and (3) identify central sites in the connectivity matrix that represent areas that if became inhabitable or inaccessible, would prevent recolonization. Predictions generated by the connectivity matrix analysis were then compared to observed recolonization events by *S. balanoides* in this region (Southward 1967, Rognstad et al. 2014).

4.2 Methods:

4.2.1 Study organism:

The acorn barnacle *Semibalanus balanoides* is a widely-distributed intertidal species found on both sides of the North Atlantic and the eastern North Pacific. This species produces one brood of larvae per year, fertilizing in the late fall (November-December, Crisp 1964) and brooding its embryos outside the body, but within the shell, until larval release in the late winter/early spring (March-April, Crisp 1962; March, Crisp 1964). Larvae are then released into the plankton where they are transported by ocean currents for approximately 20 days (Harms 1984) until they become competent to metamorphose. Larvae may continue to be dispersed in the plankton for another 3-4 weeks following the acquisition of metamorphic competence (Lucas et al. 1979) before settling. Within a region, there can be substantial variation in the timing and magnitude of larval release and settlement (Jenkins et al. 2000), which is associated with variation in temperature (Southward 1967, Jenkins et al. 2000, Rognstad et al. 2014) and/or variation in the spring phytoplankton bloom (Barnes 1956, Barnes 1962, Leslie et al. 2005, Burrows et al. 2010).

4.2.2 Dispersal model:

Larval dispersal was modeled using the Ichthyop software package (version 3, Lett et al. 2008). Two hydrodynamic models were used here, MANGA700 and ATLNE2000, which are both Model for Applications at Regional Scales (MARS) models that encompass portions of the Eastern North Atlantic, including the English Channel (Bailly du Bois and Dumas 2005, Lazure and Dumas 2008). These hydrodynamic models have

been validated in the English Channel region with radionuclide tracers (Bailly du Bois and Dumas 2005, Lazure et al. 2009) and been previously used to model dispersal of larval fish (Allain et al. 2007, Huret et al. 2010) and invertebrates (Ayata et al. 2010). The boundary conditions for these hydrodynamic models are based on the Mercator Ocean model and the wind forcing is from the ARPEGE numerical weather model (Météo France). There are two major differences between the MANGA700 and ATLNE2000 models; model region and resolution. The MANGA700 model is bounded by 43.2828°N and 52.0061°N and 5.7256°W and 4.1790°E and has a grid resolution of 700m, while the ATLNE2000 model covers the region of 39.9820°N to 64.9827°N and 20.0295°W to 14.9874°E and has a grid resolution of 2km.

4.2.3 Dispersal simulations:

Dispersal was modeled by releasing “larvae” simulated as passive Lagrangian particles from 34 (ATLNE2000) or 28 (MANGA700) sites in Southwest England (Table 4.1) over the typical larval release dates for *Semibalanus balanoides* in Southwest England (March 01-April 29). These dates were chosen to represent the extent of the larval period in the region, based on literature data of the timing and variation in larval release and settlement in the United Kingdom (Barnes 1956, Crisp 1962, Jenkins et al. 2000). Sites were spaced at intervals of 10 kilometers. On each release date, 500 simulated larvae were released uniformly-distributed in patches with five-kilometer diameters centered on each release location. Larval trajectories were followed for 45 days, with the simulated larvae becoming competent to settle on day 20 (Harms 1984, Barnes 1956). Connectivity between source and destination sites was determined by recording the number of

simulated larvae that passed within five kilometers of each destination site within the competency period and standardizing this value by dividing by the number of larvae released. Separate connectivity matrices were generated for each larval release date yielding 60 matrices for each site of origination in each season. Connectivity matrices for each dispersal season were then constructed by summing each matrix element over all of the release dates. Dispersal simulations were conducted over four years (2010-2013) which correspond to the years in which Rognstad et al. (2014) reported larval recruitment rates during the recolonization of expatriated habitat in Southwest England.

4.2.4 Connectivity statistics:

Connectivity matrices were described using the spatial statistics described by Thomas et al. (2014). Briefly, the statistics are: (1) **Weighted connectivity length:** the sum of all connection strengths (proportion of larvae released from a particular location that settle at another particular location) multiplied by the corresponding connection length (shortest geographic distance between the two locations without crossing land), divided by the sum of all connection lengths. (2) **Proportion of self-recruitment:** the fraction of all dispersal paths that start and end at the same location, and (3) **Average plume length:** the furthest distance from each source location where connectivity occurs, averaged over all of the source locations (Thomas et al 2014). Distances between sites were calculated in R (version 2.15.3, R Core Development Team, 2013) using the *gdistance* package (van Etten 2014) which uses the Dijkstra (1959) algorithm to find the shortest route between points, then quantifying the length of the resulting path.

4.2.5 Recolonization analysis:

To determine the ecological impact of annual variation in connectivity matrices, the connectivity matrices were converted to graphs and the shortest paths between each pair of sites were determined. These paths were determined using the Dijkstra (1959) algorithm and are not the shortest in geographic distance, but the shortest in the number of steps it take to get from one site to another. For example, every pair of sites that is connected by a non-zero value in the connectivity matrix has a shortest path distance of one, meaning larvae released at the source location can reach the destination location, and potentially recolonize that site, if it is empty, in a single step. The length of the path corresponds to the number of steps it would take recolonization to occur between any pair of source and destination points. Since *S. balanoides* can reproduce once per year, including, under ideal circumstances, the year following recruitment, the steps in these paths correspond to the number of years it would take to recolonize a given site from a given site of origin. This analysis represents a best case scenario for recolonization potential with a particular dispersal regime described by the connectivity matrix. Based on the physical geography of the region, it was hypothesized that three geographic features could potentially represent physical barriers for dispersal: Start Point, The Lizard, and Land's End. Start Point has previously been demonstrated to be a formidable barrier to larval transport in mussels (Gilg and Hilbish 2003) although mussels reproduce later in the year than does *S. balanoides* and consequently dispersal in the two taxa occurs under different wind regimes. To examine the larval transport within and between these regions, the standardized connectivity was plotted for each source location in regions bounded by these points.

4.2.6 Betweenness calculations and effects of site removal:

To identify key sites in the network that may be important corridors for dispersal, and thus recolonization, node betweenness was calculated for each site. Betweenness is the likelihood that a shortest path passes through a particular site (Newman 2005, Treml et al. 2008). To calculate betweenness, the shortest path between each possible pair of sites was identified and the proportion of these paths passing through each site was recorded. This analysis was conducted using the igraph package in R (Csardi and Nepusz 2006). The site with the highest betweenness was then removed from the matrix and the number of steps analysis (described above) was then repeated to examine the effect of removal of that site on recolonization. This process was repeated for each model for the sites with the five highest betweenness values.

4.2.7 Spatial structure analysis:

To identify spatial patterns in the connectivity matrices, the clustering method described by Thomas et al. (2014) and Traag et al. (2011) was used to group sites into communities. Briefly, this method uses the Constant Potts Model (CPM) to cluster sites into groups based on comparing the density of connections within the groups and among the groups to a resolution parameter. The clustering was performed using the Traag et al (2011) C++ implementation of the modified Louvain Method (Blondel et al. 2008) algorithm to optimize the CPM. Community assignment was performed over a range of values for the resolution parameter values and the sensitivity analysis described by Thomas et al. (2014) was conducted to identify community assignments that gave low proportions of

intercommunity connectivity, which could represent communities that are demographically independent and may be used to identify barriers to dispersal.

4.3 Results:

We modeled dispersal of the acorn barnacle *Semibalanus balanoides* over its local southern limit in Southwest England. Simulated larvae were released daily over potential source sites spaced at 10-km intervals around the coast during four years (2010-2013) and their trajectories and potential settlement locations were recorded and used to generate connectivity matrices.

4.3.1 Spatial statistics and general patterns:

Connectivity matrices were generated for both of the models in each year. Figure 2 gives the results for the coarser-scale ATLNE2000 model. In all four years the model predicts that dispersal has a generally westward bias. In all four years Start Point (site 270) appears to provide a barrier to connectivity between populations of *Semibalanus balanoides* east and west of the Point while populations within these regions appear to be highly interconnected (Fig. 4.2). Land's End (site 250) also appears to be a strong barrier to dispersal from northern Cornwall (sites 245-250) to southern Cornwall (sites 251-278). However, there does appear to be some level of dispersal across Land's End from southern Cornwall to northern Cornwall. It is not apparent from the data presented in Fig. 4.2 that the Lizard represents a significant barrier to dispersal.

To assess these results in greater detail we converted the connectivity matrices to vertex and edge graphs in which the nodes are the sites and the edges in the graph are

weighted with the values from the connectivity matrix. Each graph represents a region of larval origin and all prospective destinations. These graphs allow an exploration of the results of connectivity matrices. Using the connectivity matrices generated with the ATLNE2000 circulation model, we constructed graphs for larvae originating in the regions east of Start Point (Fig. 4.3), between Start Point and The Lizard (Fig. 4.4), between The Lizard and Land's End (Fig. 4.5) and northern Cornwall (Fig. 4.6). There was high connectivity within each of these regions in all four of the annual matrices (Figs. 4.3-4.6) but the regions were isolated from each other to varying degrees. In all four years larvae originating among sites east of Start Point exhibit weak but regular dispersal to sites west of Start Point (Fig. 4.3). In 2010 larvae dispersed to sites up to 30 km west of Start Point while in 2012 they potentially dispersed to sites up to 110 km west of Start Point. In 2011 and 2013 dispersal distances were intermediate (60 and 70 km, respectively). Conversely, when larvae are released in the region between Start Point and The Lizard (Fig. 4.4) transport to sites east of Start Point was weak and in no case extended more than 50 km east of the Point. Westward transport, however, was great; in all four years larvae release between Start Point and The Lizard resulted in significant dispersal to the sites located west of The Lizard and occasionally to sites in Northern Cornwall (Fig. 4.4). When larvae were released from sites between The Lizard and Land's End (Fig. 4.5) they routinely were transported to sites in Northern Cornwall but were rarely transported in the opposite direction to sites east of The Lizard. When larvae were released from sites in Northern Cornwall (Fig. 4.6) there is only occasional transport to sites located between The Lizard and Land's End. Thus the ATLNE2000 model indicates that Start Point, The Lizard and Land's End all present significant, but

highly asymmetric barriers to larval dispersal. Populations within the regions bounded by these geographic features appear to be highly interconnected and separated from one another albeit some level of transport to the west or north is found at each barrier.

The results of the connectivity matrices for the finer scaled MANGA700 model are given in Figure 7. Connectivity is generally more symmetrical in this model than in the ATLNE2000 model (Figs. 4.2 and 4.7). The results of this model also suggest there is a barrier to dispersal at Start Point and that The Lizard may also restrict transport of larvae to sites to the west. The spatial extent of the MANGA700 model does not allow the analysis of connectivity between these sites and those in Northern Cornwall. Graphical analyses of the results from the connectivity matrices generated by the MANGA700 model are similar to those obtained with the ATLNE2000 model. In all four years transport from sites in the region east of Start Point to sites west of the Point is very weak (Fig. 4.8). Like the previous model, larvae released from sites in the region between Start Point and The Lizard are highly connected to sites west of The Lizard and can be transported as far as Land's End (Fig. 4.9). Transport east of Start Point is weak but occurs regularly albeit stronger in some years (2011 and 2013) than others (2010 and 2012). Finally, larvae released from sites located west of The Lizard are typically transported westward and are only weakly transported east of The Lizard (Fig. 10). The dispersal estimates generated using the ATLNE2000 and MANGA700 models agree that Start Point and The Lizard represent significant but asymmetric barriers to connectivity and bound regions of highly interconnected sites.

The results of the spatial statistics analysis are similar for the connectivity matrices generated by the two circulation models (Table 4.2). The ATLNE2000 model

typically predicts transport further from source locations than the MANGA700 model, as indicated by higher weighted connectivity length (WCL) and average plume length values, though these differences are small. The weighted connectivity length, which describes the typical extent of larval transport over the entire modeled region, differs by ~10km between the two models (24.48-33.16km for ATLNE2000, 15.44-21.89km for MANGA700). Similarly, the average plume length, which is the furthest distance at which dispersal occurs, averaged over all of the source locations, is ~15km further in the ATLNE2000 model (65.18-85.95km) compared to the MANGA700 model (59.33-69.82km). The MANGA700 model consistently predicts slightly greater proportions of self-recruitment (16.4-22.3%) than the ATLNE2000 model (10.3-15.4%). The statistics for 2012 also describe greater WCL and average plume length, indicating both greater mean and maximum transport from source locations in 2012 than the other modeled years in both models.

4.3.2 Recolonization analysis:

The lengths (number of steps) of the shortest path between all source and destination site pairs were calculated to examine recolonization potential. These lengths represent a best case scenario and do not incorporate the strength of the connections between sites. If any dispersal paths extend to a destination location, the site is reachable from the source site in one year, regardless of the strength of the connection. This analysis asks the question: how far can larvae release at a particular location travel in one reproductive event? and assuming those larvae mature and reproduce the next year, how far could the potentially recolonize in a second year, third year, etc.?

The ATLNE2000 model consistently predicts a recolonization time of 1-2 years for sites east of Start Point to reach all other sites east of Start Point and sites within 30-70km west of Start Point (Fig. 4.11). Sites east of Start Point can potentially recolonize sites as far as the Lizard, 140km west of Start Point in two years and sites as far as Land's End (190km west of Start Point) and North Cornwall in two (2012 and 2013) to three (2010 and 2011) years. Recolonization in the opposite direction, from west to east, is much slower as predicted by the ATLNE2000 model. Sites between Land's End and the Lizard take 3-4 years to reach sites near to, but west of Start Point and 5-10 years to reach sites east of Start Point. Analysis of the 2012 matrix indicates eastward transport is impossible in many cases.

The MANGA700 model predicts slightly longer recolonization times from east to west than the ATLNE2000 model (Fig. 4.12). Sites east of Start Point can reach other sites east of Start Point in one year, sites between Start Point and the Lizard in 2-4 years, and sites between the Lizard and Land's End in 3-5 years. As expected from the more symmetrical nature of the connectivity matrices generated by this model, the recolonization time in the reverse direction is similar to that in the east-west direction, except in 2012 when transport is predominantly to the west (Fig. 4.7) and recolonization from west to east is predicted to take 7-9 years. Because of the limitation of the spatial extent of the MANGA700 model, no conclusions can be drawn about the ability of larvae originating in sites in North Cornwall to recolonize areas south of Land's End.

4.3.3 Betweenness calculations and site removal analysis:

Node betweenness, or the proportion of the shortest paths that pass through a particular site, was calculated for each release site in the mean matrices. The sites with the five highest betweenness values (Table 4.3) were sequentially removed from the connectivity matrix and the number of steps for recolonization analysis was repeated to examine the effects of removal of these nodes on time to recolonization (Figs. 4.13-4.22, left column). A difference matrix was generated by subtracting the new number of steps matrix (with site(s) removal) from the original number of steps matrix to visualize the change in the number of steps (4.13-4.22, right column).

The sites with the highest betweenness values in the matrices generated using the ATLNE2000 model are consistently sites near Land's End (250, 251) and sites in the region 0-60km west of Start Point (sites 264-268). When sites near Land's End are removed, connectivity from North Cornwall to south and east of Land's End is completely cut off and recolonization is no longer possible via this route (Fig. 4.13, row 3; Fig. 4.14, row 1). Interestingly, removal of this site has no effect on transport in the opposite direction. The second group of sites with high betweenness values is clustered just west of Start Point (sites 264-268). Removal of these sites individually has little effect on the overall recolonization potential, though the number of steps to reach a particular area often increases by one step. However, removal of 2-4 of these sites may completely inhibit transport from areas east of Start Point to areas west of Start Point (Fig. 4.13).

The sites with the highest betweenness value in the matrices generated using the MANGA700 model are consistently located in the region 0-60km west of Start Point

(sites 264-268, Table 4.3). Removal of these sites individually may increase the recolonization time from sites east of Start Point to those 60-120km west of Start Point by one step (Fig. 4.17-4.20). Removal of more than one of these key sites causes sites further than 60km west of Start Point become inaccessible from sites to the east (Figs. 4.17-4.20), . Recolonization in the opposite direction (west to east) is sometimes still possible, though the number of steps may be increased (Fig. 4.20).

4.3.4 Community detection:

The Constant Potts Model community detection method described by Thomas et al. (2014) was used to partition the geographic sites into communities based on different strengths of connection within and between groups. Sensitivity analysis was conducted to determine the proportion of intercommunity connectivity, or the proportion of connections between groups, corresponding to the different community assignments identified. The community assignments corresponding to intercommunity connectivity closest to 5% and 10% for the mean matrices of each model are reported here (Fig. 4.17). These thresholds were selected because they represent relatively low values of intercommunity and can be used to identify geographic features separating regions with low levels of exchange that may not be demographically connected (Waples and Gaggiotti 2006).

Community partitioning analysis of connectivity matrices generated by the ATLNE2000 model consistently identified sites at Land's End and 20 km west of Start Point as boundaries between communities with low levels of intercommunity connectivity. These divisions are present in the community assignments at both 7 and

9% intercommunity connectivity levels when using the mean connectivity matrix (Fig. 4.7, top row). Additional communities identified when intercommunity connectivity is 9% are sites 250 and 253, each of which becomes its own isolated community.

Analysis of connectivity matrices generated by the MANGA700 model consistently identified sites near Start Point as breakpoints between communities at low levels of intercommunity connectivity. Using the mean connectivity matrix, sites east and west of Start Point are grouped into two communities at the 5% intercommunity connectivity level (Fig. 4.7, bottom row). At the 8% intercommunity connectivity level, a new community is formed by the sites at Start Point and within 20km to the east of Start Point.

4.4 Discussion:

An understanding of the dynamics of local extinction and recolonization events is essential for the understanding of metapopulation dynamics and can be directly applied to conservation and management of species living in variable environments (Treml et al. 2008, Thomas et al. 2014). The increasing availability and resolution of physical circulation models has made them important tools for predicting marine population connectivity on multiple scales (citations). Here we combine biophysical dispersal modeling using circulation models at two spatial scales with graph theory methods to predict the scale and rate of recolonization following local extinction of *Semibalnaus balanoides* at its local southern limit in Southwest England.

There were some qualitative and quantitative differences between the connectivity matrices generated by the two circulation models and among matrices generated by the same model in different years. Connectivity matrices generated by the

ATLNE2000 model consistently predict more westward transport than those generated by the MANGA700 model (Figs. 4.2 and 4.7) and the average plume length and weighted connectivity length (Table 4.2) of the ATLNE2000-generated matrices were larger, indicating greater transport distances, on average. Both models predicted greater westward transport in 2012 relative to the other modeled years (Figs. 4.2 and 4.7) and the largest WCL for each model occurred in 2012, indicating greater average transport in 2012 compared to the other years. The circulation models are identical in most respects, so the different results are generated by (1) variation in pixel size and/or (2) variation in the spatial extent of the model or proximity to the edge of the model boundary.

Our analysis of the predicted connectivity patterns, indicates there are three potential barriers to dispersal, and thus recolonization in the Southwest England population of *S. balanoides*: Start Point, The Lizard, and Land's End. Three analyses using connectivity matrices generated by both circulation models indicate the region 0-50km west of Start Point is an important region for connectivity in this metapopulation. The regional release analyses indicate low transport across Start Point from east to west (Figs. 4.3 and 4.8), particularly when using the MANGA700 circulation model (Fig. 4.8). In simulations with both models, this barrier is asymmetric and the magnitude of transport across Start Point varies among years. The simulations using the ATLNE2000 model show transport extending further to the west from sources east of Start Point than the simulations using the MANGA700 model. The MANGA700 model predicts greater permeability of Start Point from west to East (Fig. 4.9 vs. Fig. 4.8), while the inverse is true for the ATLNE2000 simulations, with transport usually occurring further from Start Point in an east to west direction (Fig. 4.3) than the west to east direction (Fig. 4.4). The region

around Start Point is also consistently identified as an important region for dispersal pathways in the betweenness and site removal analysis. Sites within 50km west of Start Point (265-269) are consistently in the top five of the ranked betweenness scores (Table 4.3) for both models, indicating many shortest dispersal paths pass through these sites. Removal of two (Figs. 4.13, 4.17, 4.20) or three (Figs. 4.18 and 4.19) of these sites can be sufficient to prevent recolonization of sites west of Start Point from sites east of Start Point. In the community partitioning analysis, a separation occurs between sites east and west of Start Point at low proportions of intercommunity connectivity when using both models (7% intercommunity connectivity for ATLNE2000, 5% intercommunity connectivity for MANGA700). The Lizard is also identified as a potential asymmetric barrier to connectivity in the regional release analysis using both circulation models. It does not appear to be a strong barrier for transport from east to west in either model (Figs. 4 and 9), but transport from west to east is weak in both models (Figs. 4.5 and 4.10), particularly in 2012. The third barrier, Land's End, is apparent in the regional release analysis (Figs. 4.5 and 4.6), where transport occurs readily from the region between The Lizard and Land's End to North Cornwall (Fig. 4.5), but is weak or does not occur at all in the opposite direction (Fig. 4.6). This barrier is also apparent in the site removal analysis in 2010, where removal of a single site prevents transport from South Cornwall to North Cornwall and this transport does not occur at all in 2012. The community partitioning analysis separates North and South Cornwall into two regions at a low intercommunity connectivity threshold (7%), indicating little exchange of larvae between these regions.

Thus, while there were some differences between the models their major results were highly consistent. Both models predict that the transport of barnacle larvae across Southwest England will generally be westward and that migration in the opposite direction is considerably more restricted, they identify the same three regions within which populations are expected to be highly interconnected, they identify the same two barriers that separate these regions and they both predict that transport across these barriers, while restricted is asymmetric. Both models also predict the same annual variation in these results, specifically, that 2012 would have greater westward transport and the barriers at Start Point and the Lizard would be more permeable to westward transport than in the other three years of in the study. In addition, simulations using the ATLNE2000 model consistently predicted that Northern Cornwall is a region of highly interconnected populations and that this region is strong isolated by restricted transport around Land's End from the regions in Southern Cornwall. Because of their similarity in identifying regions of high population connectivity and the barriers that separate these regions the models also consistently predicted the same "communities" when connectivity fell below ~10% and identified populations in the same areas, especially populations just west of Start Point, as critical to recolonization. The number of steps for recolonization of the area between the Lizard and Land's End from sites east of Start Point is relatively consistent between the two models, varying by only 1-2 years (Figs. 4.11 and 4.12). This difference is the same scale of variation among years of recolonization time estimates for the same model; estimates generated using the ATLNE2000 model are three years for recolonization in 2010 and 2011 and two years in 2012 and 2013 (Fig. 4.11), while MANGA700-derived recolonization estimates for the

same region are five years in 2010, four years in 2011 and 2013, and three years in 2012 (Fig. 4.12). Estimates of potential recolonization in the reverse direction, from North Cornwall to South Cornwall and Devon using the ATLNE2000 model are more variable; to recolonize regions between the Lizard and Start Point from North Cornwall would require 5-7 years in 2010 and 2011, 3-5 years in 2013, and is impossible in 2012 (Fig. 4.11). Recolonization time estimates generated by the ATLNE2000 model consistently predict longer recolonization times from west to east than the reverse. Conversely, recolonization estimates generated using the MANGA700 model predicted shorter potential recolonization times from west to east in 2010, 2011, and 2013, with recolonization of the region east of Start Point from the region between the Lizard and Land's End taking 2-5 years (Fig. 4.12). In 2012, when much more westward transport is apparent from the connectivity matrix (Fig. 4.7), recolonization from west to east is predicted to take 7-9 years.

Two range expansions of *Semibalanus balanoides* in Southwest England have been previously described (Southward 1967, Rognstad et al. 2014) and in both cases repatriation appeared to originate from sites east of Start Point and the expansion occurred step-wise over several years. Both of these results are consistent with the outcomes of this connectivity analysis. The connectivity analyses presented here predict that the repatriation of Southwest England is much more likely to originate from sites east of Start Point than from Northern Cornwall and that it would require multiple years to recolonize Southwest England, both because many populations are simply beyond the range that larvae can disperse in one year and because of the presence of semipermeable barriers that will restrict transport. Southward (1967) documented very low densities of

S. balanoides in Southwest England prior to the extremely cold winter of 1963, and an advancing front of adults (adult density $>1/\text{cm}^2$) permeating from the east following the extreme winter. The front reached Lyme Bay in 1963 (~70km east of Start Point), Plymouth by 1964 (west of Start Point), St. Austell by 1965 and the Fal Estuary (~20km east of The Lizard) by 1966, with an average westward expansion of 53 km/yr. Southward's (1967) observed expansion, taking 3-4 years to expand from east of Start Point to Land's End, is consistent with the rate predicted here by the physical circulation models (2-3 years predicted using the ATLNE2000 model, 3-5 years predicted using the MANGA700 model).

The predictions of the connectivity analyses were also very consistent with repatriation of Southwest England by *Semibalanus balanoides* documented by Rognstad et al. (2014) between 2010 and 2012. In 2010, adults were very rare in most of Southern Cornwall and present at densities greater than $1/\text{cm}^2$ east of Start Point and up to 40km west of Start Point. Following the cold winter of 2010 newly recruited *S. balanoides* were observed in high abundance at sites up to 100km west of Start Point. Recruitment was substantially lower at sites further to the west. By 2011, the front of adults had advanced to about 100 km west of Start Point and high levels of larval recruitment occurred to the Lizard, about 150 km west of Start Point. The winter of 2011/2012 was warm and there was low larval recruitment throughout Southwest England in 2012. The following winter was again cold and high levels larval recruitment occurred at sites west of the Lizard (unpublished data). In this expansion, repatriation of Southwest England was consistent with the results of connectivity models in several important respects. First, connectivity estimates indicate that larvae originating from sites in North

Cornwall will only rarely disperse to Southern Cornwall (Fig. 4.6), consistent with the appearance that the re-expansion of *S. balanoides* originated in the east from sites east of or just west of Start Point (Southward 1967, Rognstad et al. 2014). This is took about three years from just east of Start Point to Land's End, again consistent with recolonization time estimates generated by both physical circulation models. Figures 4.22 and 4.23 illustrate this point; the repatriation of Southwest England was simulated using connectivity estimates generated with both the ATLNE2000 and MANGA700 circulation models by allowing dispersal generated from sites east of Start Point to successively feed the dispersal pattern predicted by each of the following years (Figs. 4.22 and 4.23, top row). Additional simulations used sites within 20km west of Start Point (sites 268 and 269), identified by the site removal analysis as critical to recolonization, in addition to sites east of Start Point as sources for recolonization (Figs. 4.22 and 4.23, bottom row).

Repatriation of Southwest England took three steps in simulations using matrices generated with the ANLNE2000 model if started with larvae originating sites east of Start Point (Fig. 4.22, top row) and only two steps if larvae also originated from the critical sites just west of Start Point (sites 268 and 269; Fig. 4.22, bottom row). The simulation using MANGA700 model, which generally had lower westward transport, produced the same results with one additional step in the time to complete the observed repatriation (Fig. 4.23). In 2010 the simulation using the MANGA700 circulation models and the critical sites just west of Start Point as source locations, resulted in larval dispersal to the same distance west as was observed by Rognstad et al (2014) for the same year (Fig. 4.23, bottom row). By 2011 both versions of this simulation (with and

without starting trajectories at the critical sites) predicted larval dispersal to the Lizard in agreement with the recruitment observed that year. Interestingly, Rognstad et al. (2014) also observed that in 2011 there was a dearth of larval recruitment in the region 50 km west of Start Point and this feature develops in the simulations produced using both the ANTNE700 and MANGA700 circulation models (Figs. 4.22 and 4.23). Thus these connectivity models agree both generally and in many of the details of the repatriations of *S. balanoides* in Southwest England documented by Southward (1967) and Rognstad et al. (2014).

The presence of and asymmetrical character of these barriers has repercussions for recolonization in this region. The area of Southwest Cornwall, between Start Point and Land's End, historically has transient populations of *Semibalanus balanoides* (Moore 1936, Southward and Crisp 1954, Southward 1967, 1991, Southward et al. 1995, Hawkins et al. 2003, Rognstad et al. 2014), with periods of absence or low densities alternating with period of high densities. In this species, range boundary expansion and contraction is correlated with winter temperatures (Southward 1967, Wetthey and Woodin 2008, Wetthey et al. 2011, Jones et al. 2012) and recruitment in *S. balanoides* is correlated with cold winters (Southward 1991, Jenkins et al. 2000, Drévès 2001, Abernot-Le Gac et al. 2013, Rognstad et al. 2014). In Chapter 3 of this dissertation I showed that at colder temperature *S. balanoides* has substantially greater success at incubating their embryos than they do when temperatures exceed 10°C. Other mechanisms may also contribute to an inverse relationship between temperature and recruitment including a relationship between temperature and fertilization (Barnes 1963, Tighe-Ford 1967, Crisp and Patel 1969), timing of the release of larvae (Barnes 1956), and/or post-settlement mortality

(Kendall et al. 1985, Jarrett 2003, Pineda et al 2006, Poloczanska et al. 2008).

Regardless of the mechanism, recolonization of South Cornwall requires larval dispersal from outside of the region from areas where *S. balanoides* persists (North Cornwall or east of Start Point). Our analyses indicate it is extremely unlikely for Southwest Cornwall to be recolonized from North Cornwall (Fig. 4.6) and that recolonization from east of Start Point does not occur in a single step, but requires 2-5 years to completely fill in the region between Start Point and Land's End (Figs. 4.11 and 4.12).

The predictions for repatriation potential represent a best-case scenario as successful larval recruitment is driven by more than just ocean currents. Other factors that also play a role include adult fecundity (Hughes et al 2000, Reed et al. 2009, Burrows et al. 2010, Rognstad et al 2014), mortality in the plankton (), onshore transport success (Rilov et al. 2008, Pineda et al. 2009, Shanks et al. 2010), availability of a suitable settlement habitat (), and post-settlement mortality (Kendall et al. 1985; Jarrett 2003; Pineda et al. 2006; Poloczanska et al. 2008). For example, while the connectivity modeling indicates that 2012 was a year where westward larval transport was expected to be especially high this was also a year where temperature during the preceding winter was warm and not conducive to larval production (Rognstad et al. 2014) and low levels of recruitment were observed throughout Southwest England.

The success of the connectivity models in describing the repatriation of Southwest England between 2010-2013 suggest they may be valuable in understanding changes in connectivity over longer time-scales. Large scale climatic patterns, such as the North Atlantic Oscillation (NAO), are known to alter the magnitude westerly winds on decadal time scales. An increase in westerly winds during a positive phase of the

NAO (Pingree 2002), would be expected to increase connectivity across Southwest England similar to that observed in the modeling results from 2012 when there was an increase in westerly transport compared to the other three years. If true, this suggests that barriers to transport will weaken and isolation among the communities dissipate during NAO positive years. The models produced here provide a means to incorporate decadal or longer-term climatic patterns into understanding changes connectivity and recolonization potential.

This study demonstrates the use of biophysical modeling and graph theory methods to predict the rate of recolonization following a local extinction. Though this model incorporates relatively few biological parameters (timing of larval release, time to competency), we predict a time to recolonization (2-5) consistent with the timing of observed recolonization events (2-4 years, Southward 1967, Rognstad et al. 2014). Other factors, both biological (adult fecundity, mortality) and physical (availability of suitable settlement habitat, onshore delivery), undoubtedly also play a role in successful recolonization and could be incorporated into a more detailed model. Our approach provides a framework for analysis of dispersal matrices to estimate the potential for recolonization and identify barriers to this process.

4.5 Tables and Figures:

Table 4.1: Model Sites

Site #	Distance to Start Point	Model
245	-240	ATLNE2000
246	-230	ATLNE2000
247	-220	ATLNE2000
248	-210	ATLNE2000
249	-200	ATLNE2000
250	-190	ATLNE2000
251	-180	ATLNE2000, MANGA700
252	-170	ATLNE2000, MANGA700
253	-160	ATLNE2000, MANGA700
254	-150	ATLNE2000, MANGA700
255	-140	ATLNE2000, MANGA700
256	-130	ATLNE2000, MANGA700
257	-120	ATLNE2000, MANGA700
258	-110	ATLNE2000, MANGA700
259	-100	ATLNE2000, MANGA700
260	-90	ATLNE2000, MANGA700
261	-80	ATLNE2000, MANGA700
262	-70	ATLNE2000, MANGA700
263	-60	ATLNE2000, MANGA700
264	-50	ATLNE2000, MANGA700
265	-40	ATLNE2000, MANGA700
266	-30	ATLNE2000, MANGA700
267	-20	ATLNE2000, MANGA700
268	-10	ATLNE2000, MANGA700
269	0	ATLNE2000, MANGA700
270	10	ATLNE2000, MANGA700
271	20	ATLNE2000, MANGA700
272	30	ATLNE2000, MANGA700
273	40	ATLNE2000, MANGA700
274	50	ATLNE2000, MANGA700
275	60	ATLNE2000, MANGA700
276	70	ATLNE2000, MANGA700
277	80	ATLNE2000, MANGA700
278	90	ATLNE2000, MANGA700

Table 4.2: Spatial Statistics

Model	Year	Weighted connectivity length (km)	Proportion self-recruitment	Average Plume length (km)
ATLNE2000	2010	24.65	0.154	65.18
ATLNE2000	2011	25.84	0.135	72.26
ATLNE2000	2012	33.16	0.103	85.95
ATLNE2000	2013	24.48	0.154	84.09
ATLNE2000	mean	28.14	0.129	90.90
MANGA700	2010	16.96	0.209	60.03
MANGA700	2011	15.44	0.223	59.33
MANGA700	2012	21.89	0.192	61.43
MANGA700	2013	19.36	0.150	69.82
MANGA700	mean	17.03	0.181	71.92

Table 4.3: Top Sequential Betweenness Scores

Rank	ATLNE 2010	ATLNE 2011	ATLNE 2012	ATLNE 2013	MANGA 2010	MANGA 2011	MANGA 2012	MANGA 2013
1	267	250	267	267	264	266	267	267
2	268	267	266	268	263	265	265	268
3	251	259	265	265	266	267	266	266
4	266	265	264	266	265	276	264	265
5	265	263	268	268	269	269	259	269

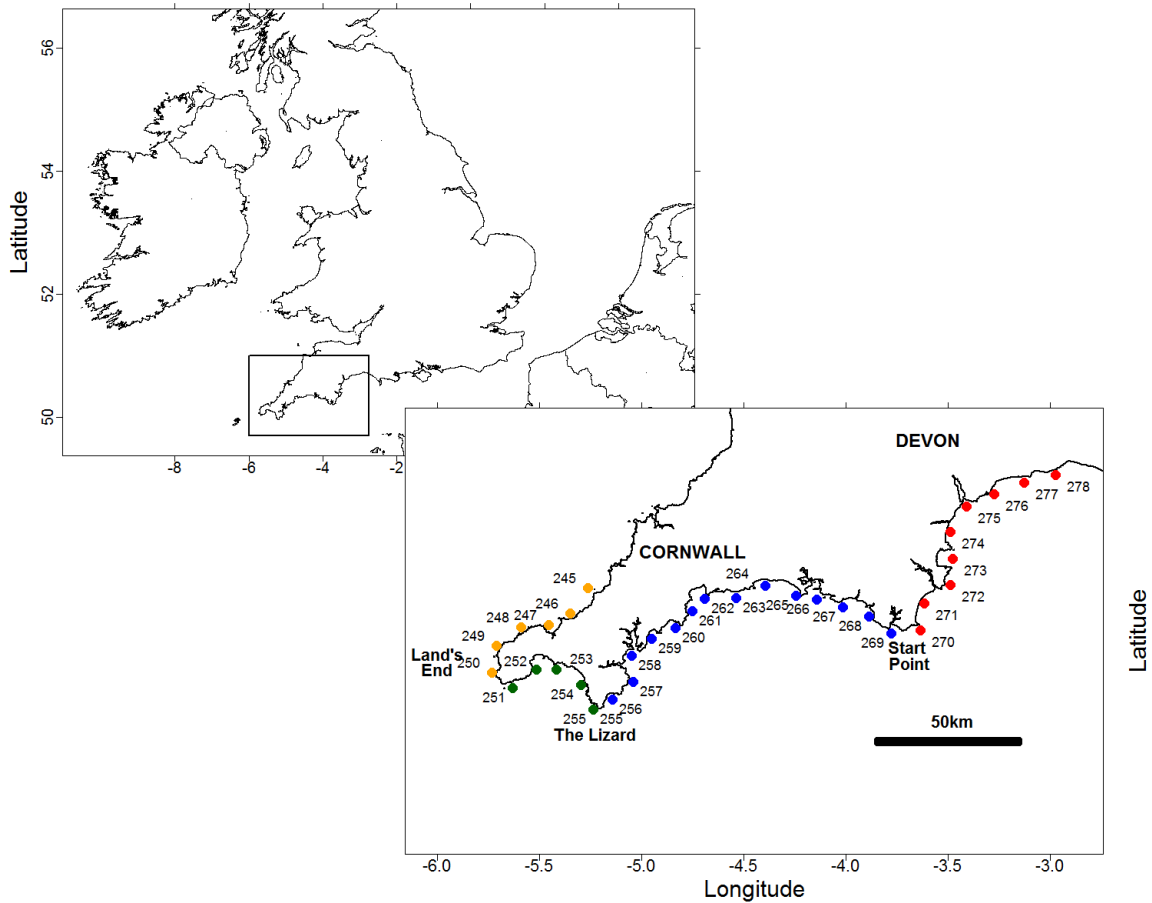


Figure 4.1: Map of model region and source/destination locations from modeled larval trajectories. Sites 215 through 278 are included in both the ATLNE2000 and MANGA700 simulated trajectories, sites 245 through 250 are included only in the ATLNE2000 model. Sites are divided into regions separated by major geographic features: East of Start Point (sites 270-278, red), between The Lizard and Start Point (sites 256 to 269, blue), between Land's End and The Lizard (sites 251 to 255, green), and North Cornwall (sites 245-250, orange).

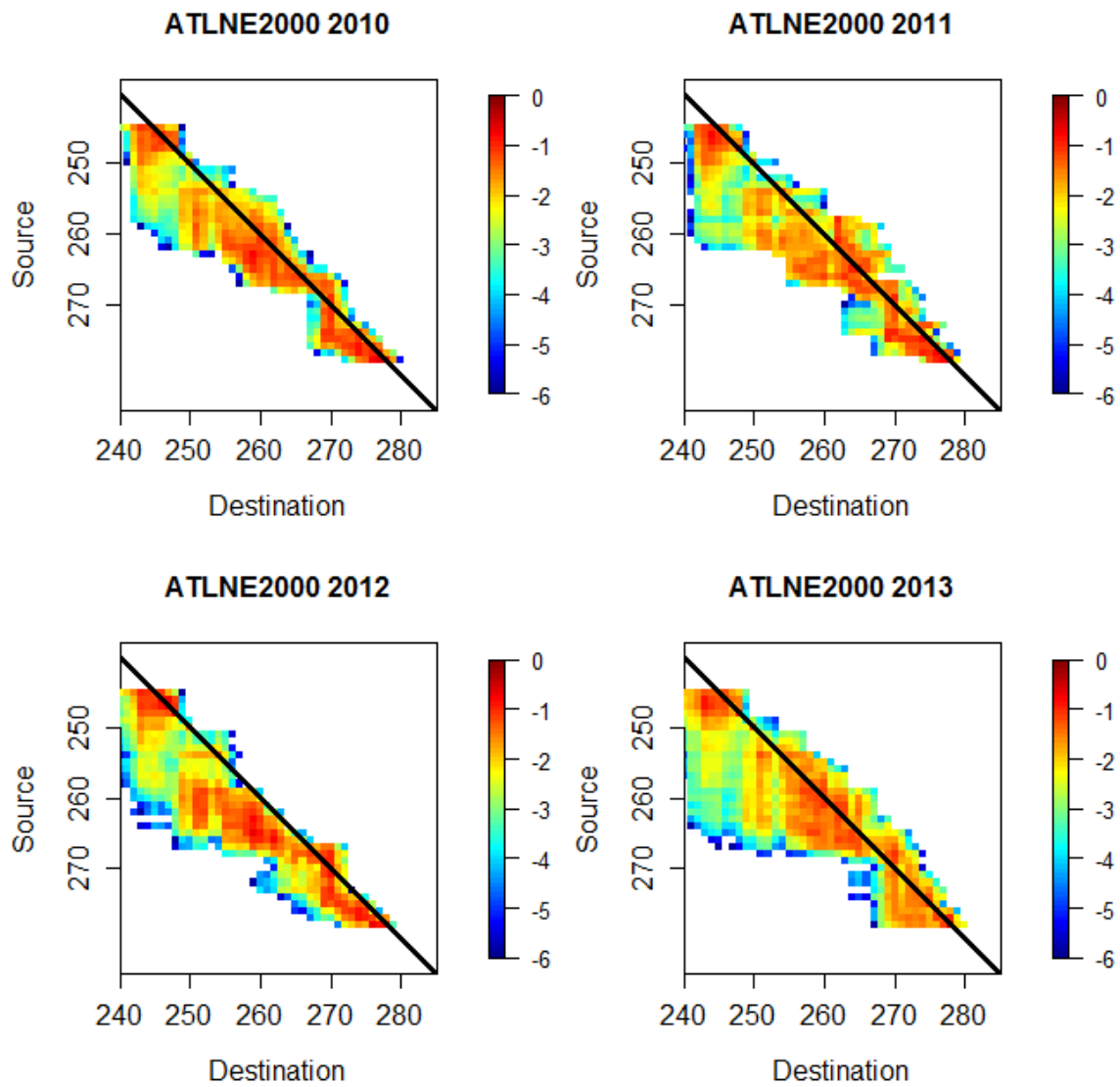


Figure 4.2: Dispersal matrices summed over all release dates (March 01- April 29) for dispersal simulations using the ATLNE2000 circulation model of the four study years. Color bar is the log of the number of larvae reaching the destination site from the source site, standardized to the total number of larvae released. The solid line is the 1:1 line, representing self-recruitment.

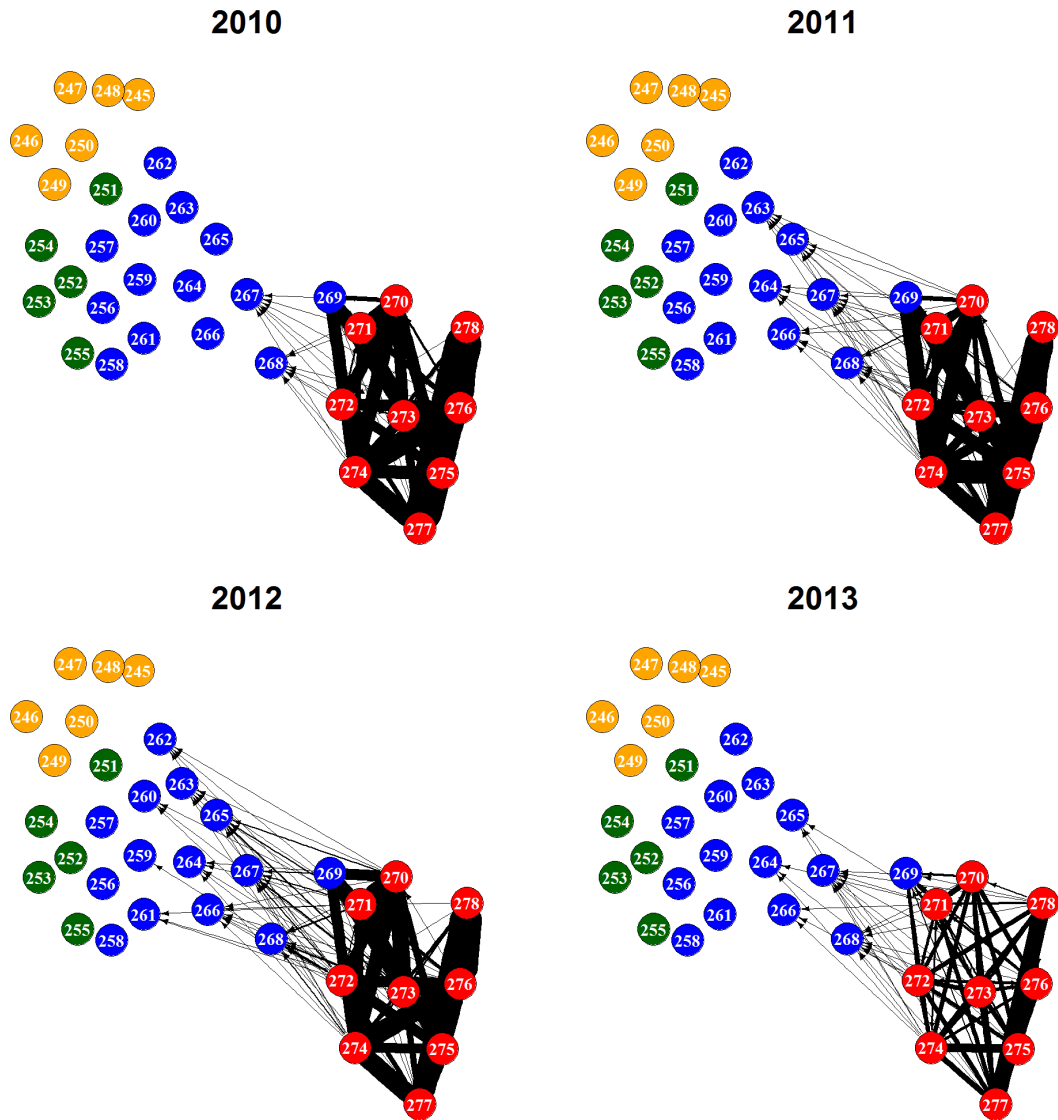


Figure 4.3: Vertex and edge graph of dispersal modeling using the ATLNE2000 circulation model and releasing larvae from the region east of Start Point (red points) in the four study years. Width of arrows is relative strength of connection between source and destination sites. Self-recruitment arrows were omitted. Colors represent the location of the sites: East of Start Point (sites 270-278, red), between The Lizard and Start Point (sites 256 to 269, blue), between Land's End and The Lizard (sites 251 to 255, green), and North Cornwall (sites 245-250, orange).

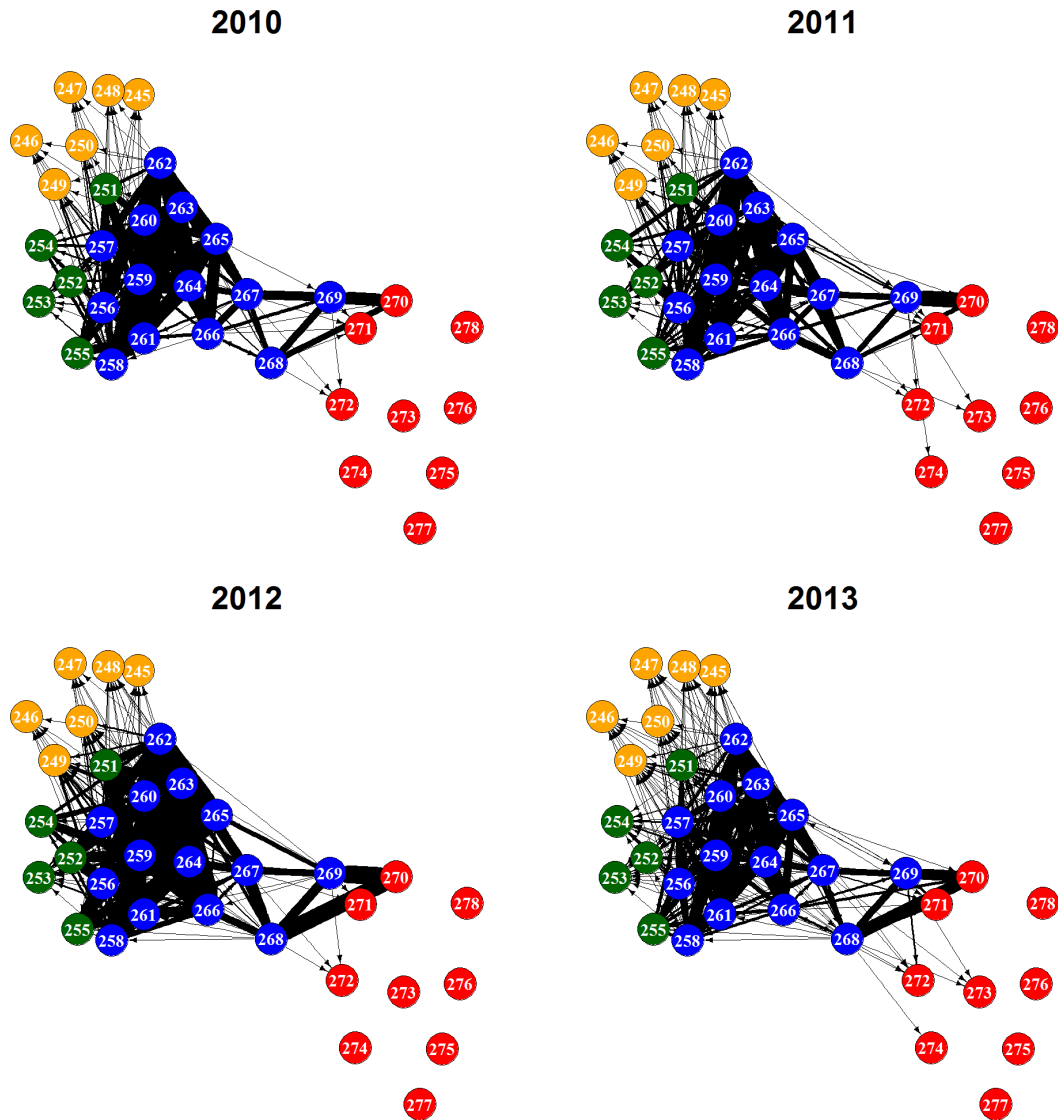


Figure 4.4: Vertex and edge graph of dispersal modeling using the ATLNE2000 circulation model and releasing larvae from the region between The Lizard and Start Point (blue points) in the four study years. Width of arrows is relative strength of connection between source and destination sites. Self-recruitment arrows were omitted. Colors represent the location of the sites: East of Start Point (sites 270-278, red), between The Lizard and Start Point (sites 256 to 269, blue), between Land's End and The Lizard (sites 251 to 255, green), and North Cornwall (sites 245-250, orange).

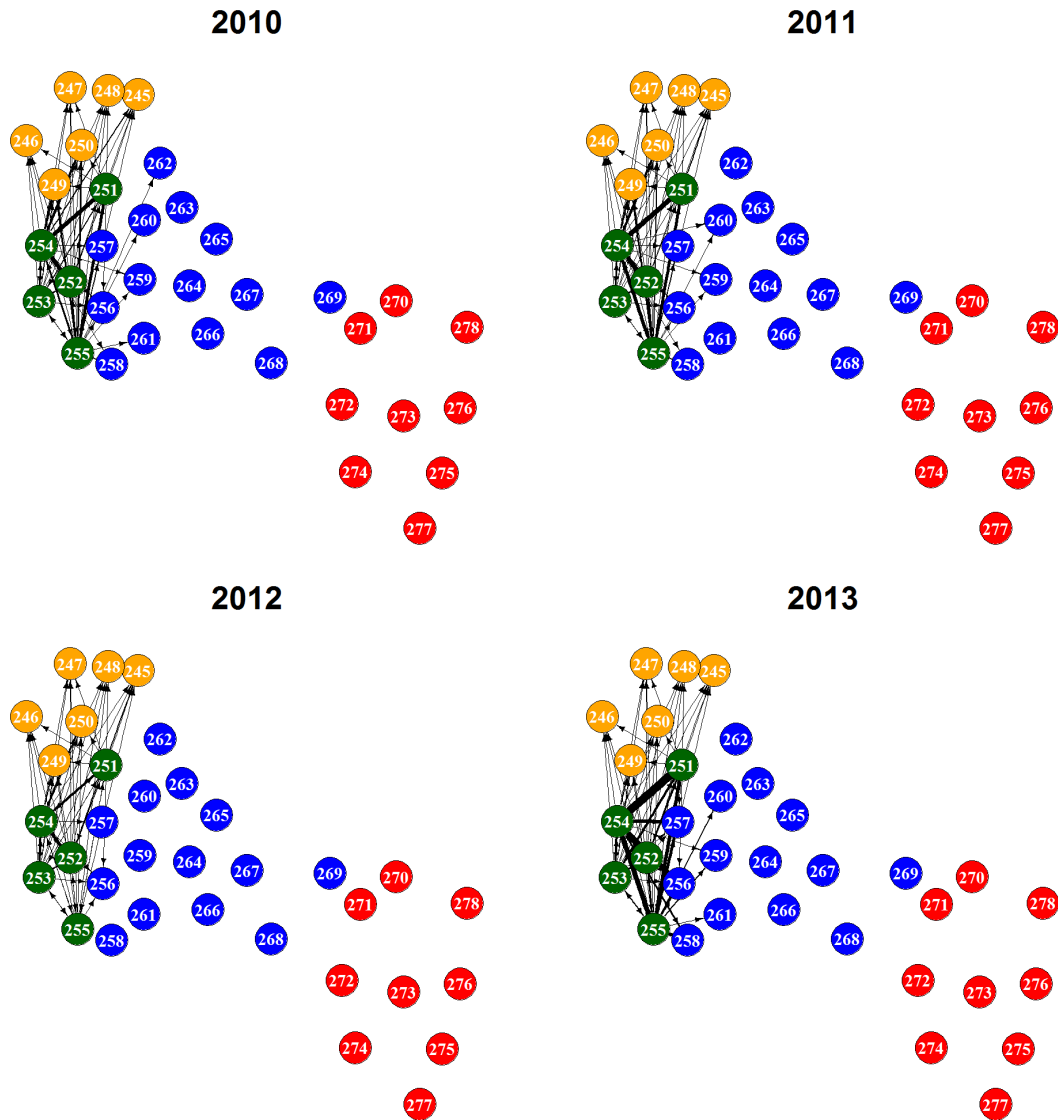


Figure 4.5: Vertex and edge graph of dispersal modeling using the ATLNE2000 circulation model and releasing larvae from the region between Land’s End and The Lizard (green points) in the four study years. Width of arrows is relative strength of connection between source and destination sites. Self-recruitment arrows were omitted. Colors represent the location of the sites: East of Start Point (sites 270-278, red), between The Lizard and Start Point (sites 256 to 269, blue), between Land’s End and The Lizard (sites 251 to 255, green), and North Cornwall (sites 245-250, orange).

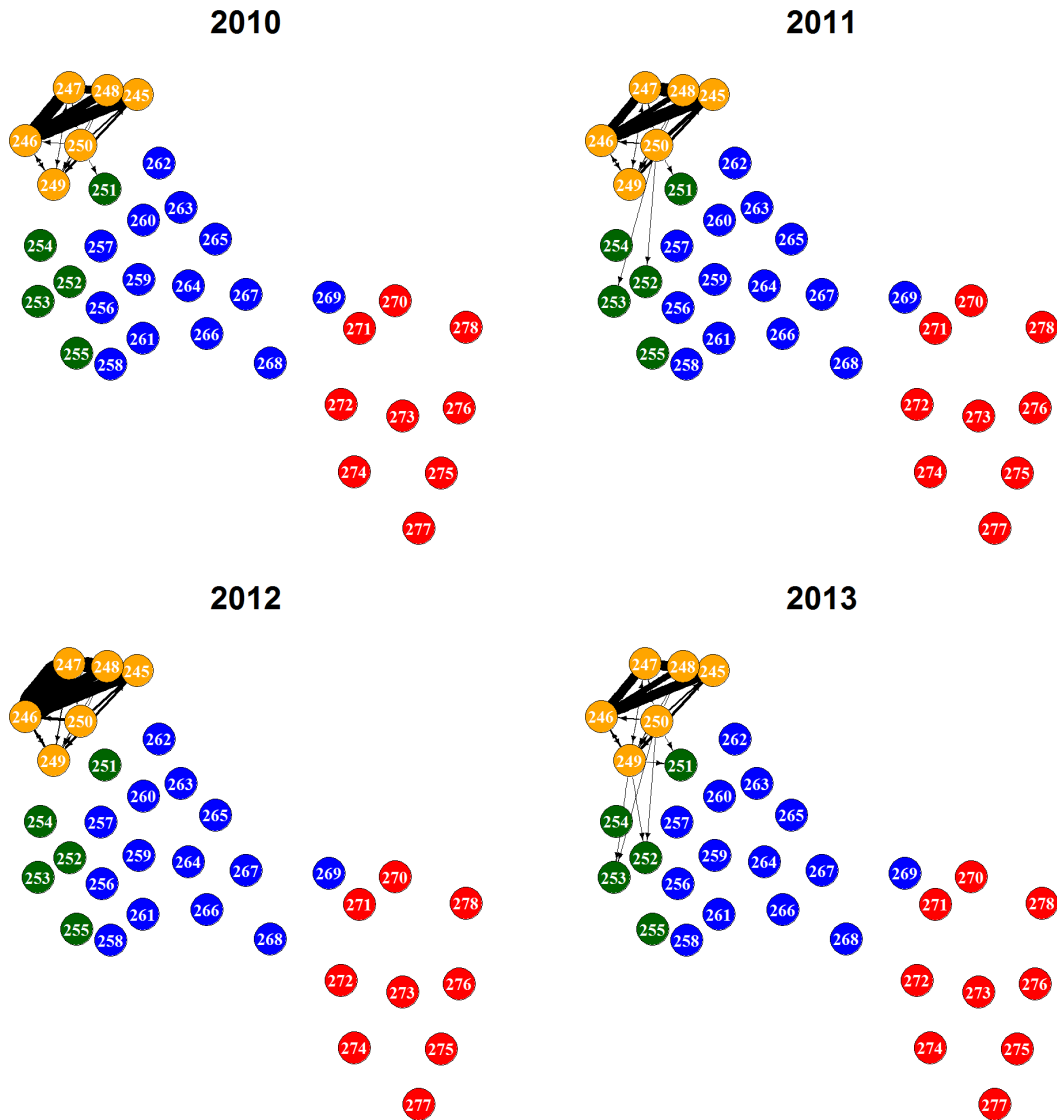


Figure 4.6: Vertex and edge graph of dispersal modeling using the ATLNE2000 circulation model and releasing larvae from North Cornwall (orange points) region in the four study years. Width of arrows is relative strength of connection between source and destination sites. Self-recruitment arrows were omitted. Colors represent the location of the sites: East of Start Point (sites 270-278, red), between The Lizard and Start Point (sites 256 to 269, blue), between Land's End and The Lizard (sites 251 to 255, green), and North Cornwall (sites 245-250, orange).

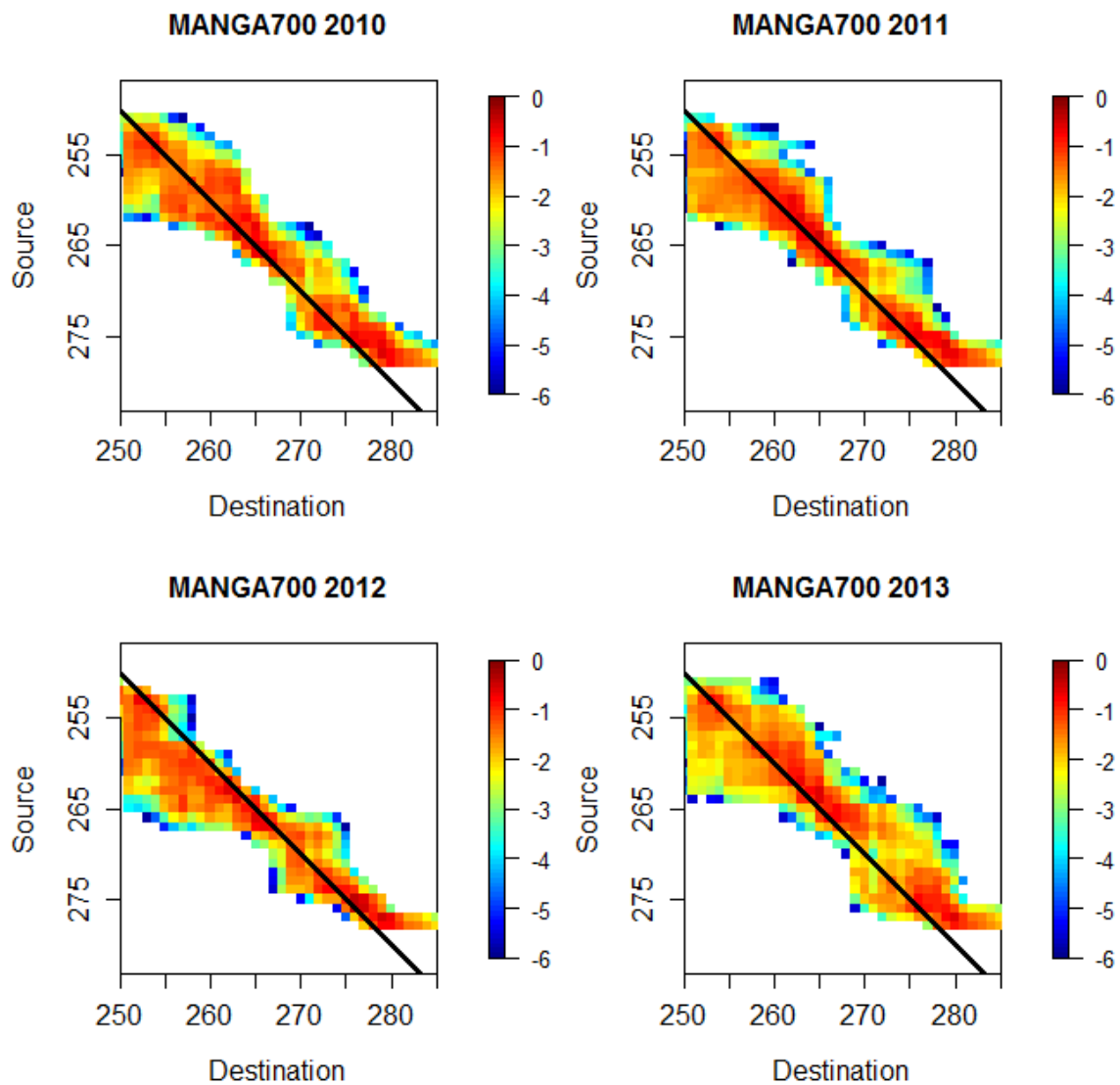


Figure 4.7: Dispersal matrices summed over all release dates (March 01- April 29) for dispersal simulations using the MANGA700 circulation model of the four study years. Color bar is the log of the number of larvae reaching the destination site from the source site, standardized to the total number of larvae released. The solid line is the 1:1 line, representing self-recruitment.

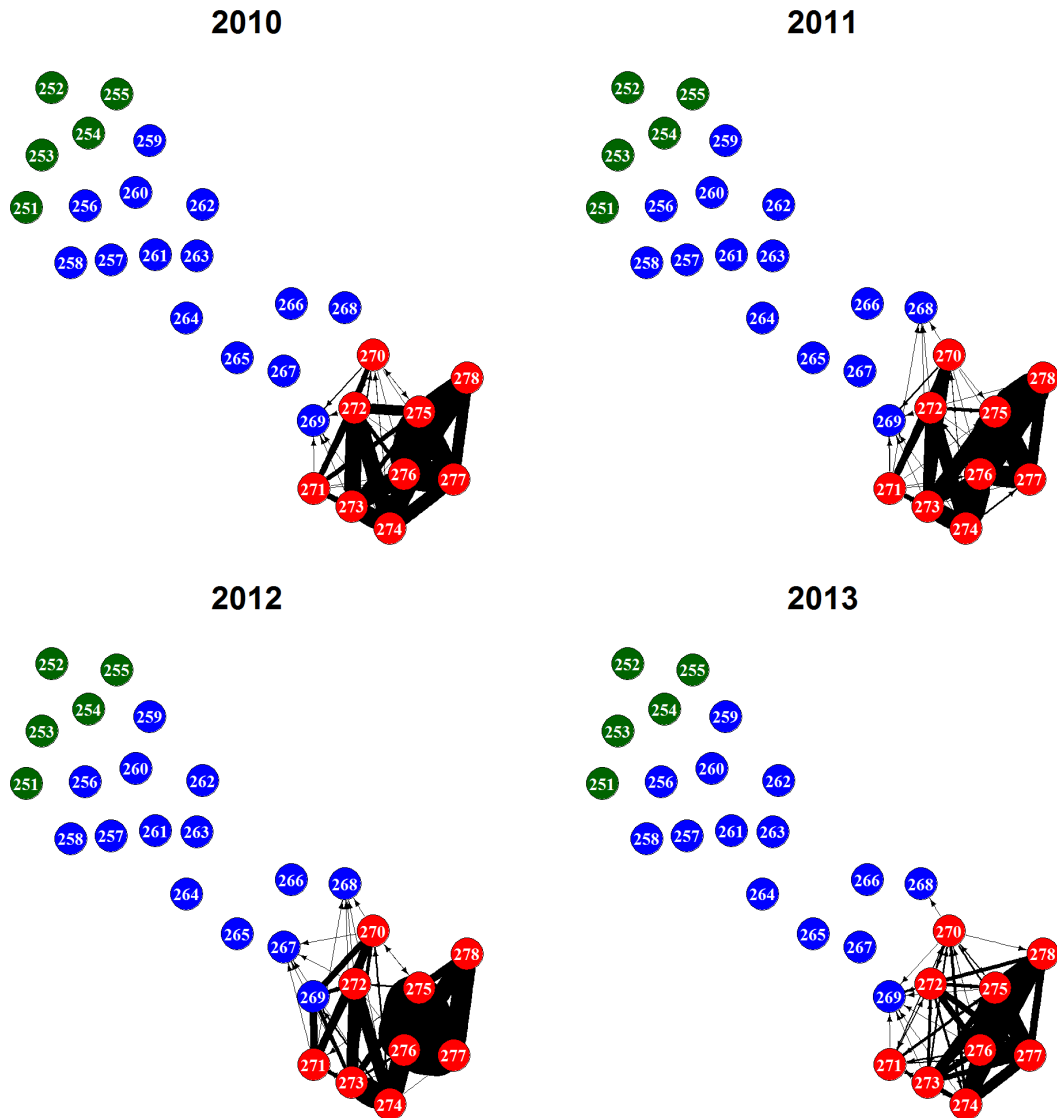


Figure 4.8: Vertex and edge graph of dispersal modeling using the MANGA700 circulation model and releasing larvae from the region east of Start Point (red points) in the four study years. Width of arrows is relative strength of connection between source and destination sites. Self-recruitment arrows were omitted. Colors represent the location of the sites: East of Start Point (sites 270-278, red), between The Lizard and Start Point (sites 256 to 269, blue), and between Land's End and The Lizard (sites 251 to 255, green).

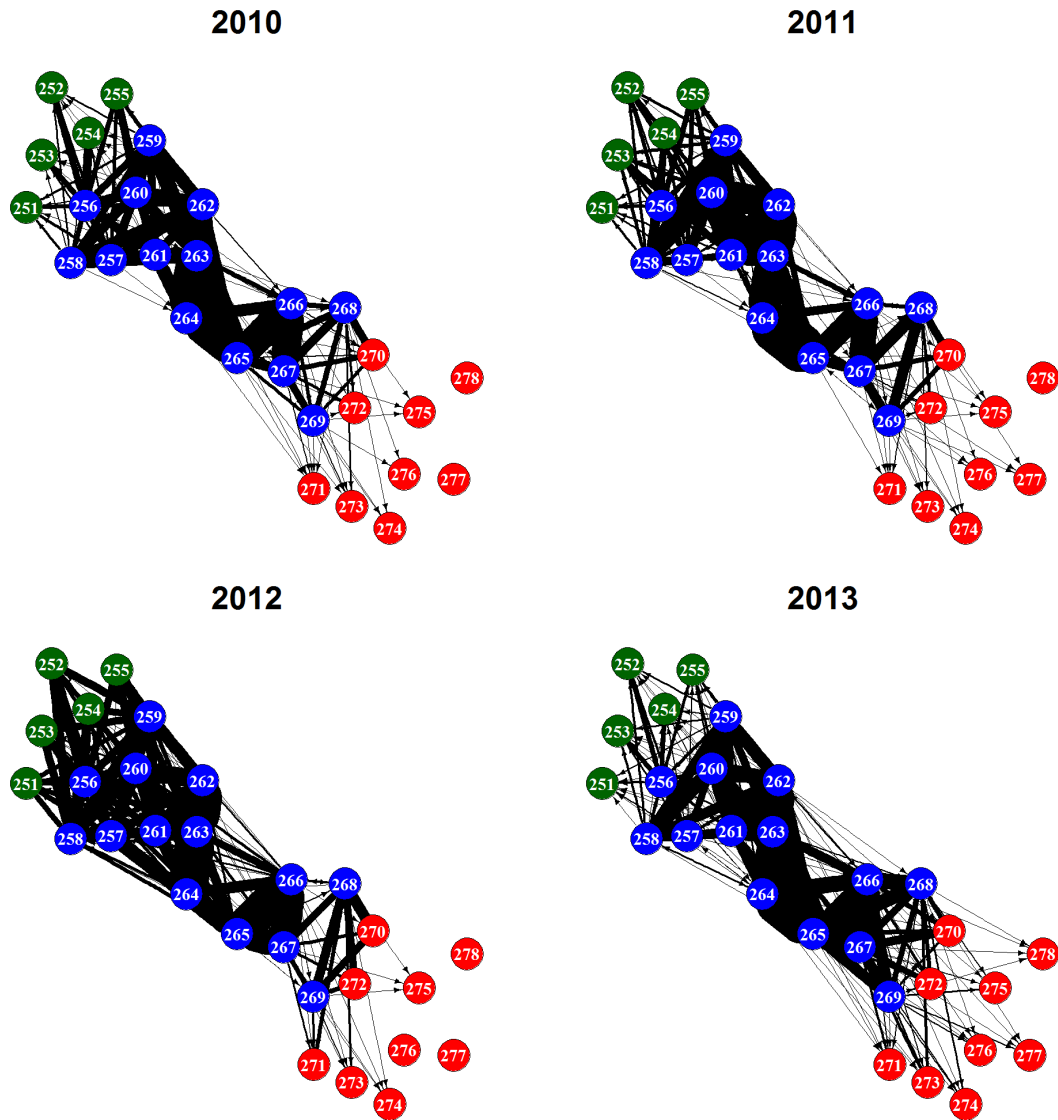


Figure 4.9: Vertex and edge graph of dispersal modeling using the MANGA700 circulation model and releasing larvae from the region between The Lizard and Start Point in the four study years. Width of arrows is relative strength of connection between source and destination sites. Self-recruitment arrows were omitted. Colors represent the location of the sites: East of Start Point (sites 270-278, red), between The Lizard and Start Point (sites 256 to 269, blue), and between Land’s End and The Lizard (sites 251 to 255, green).

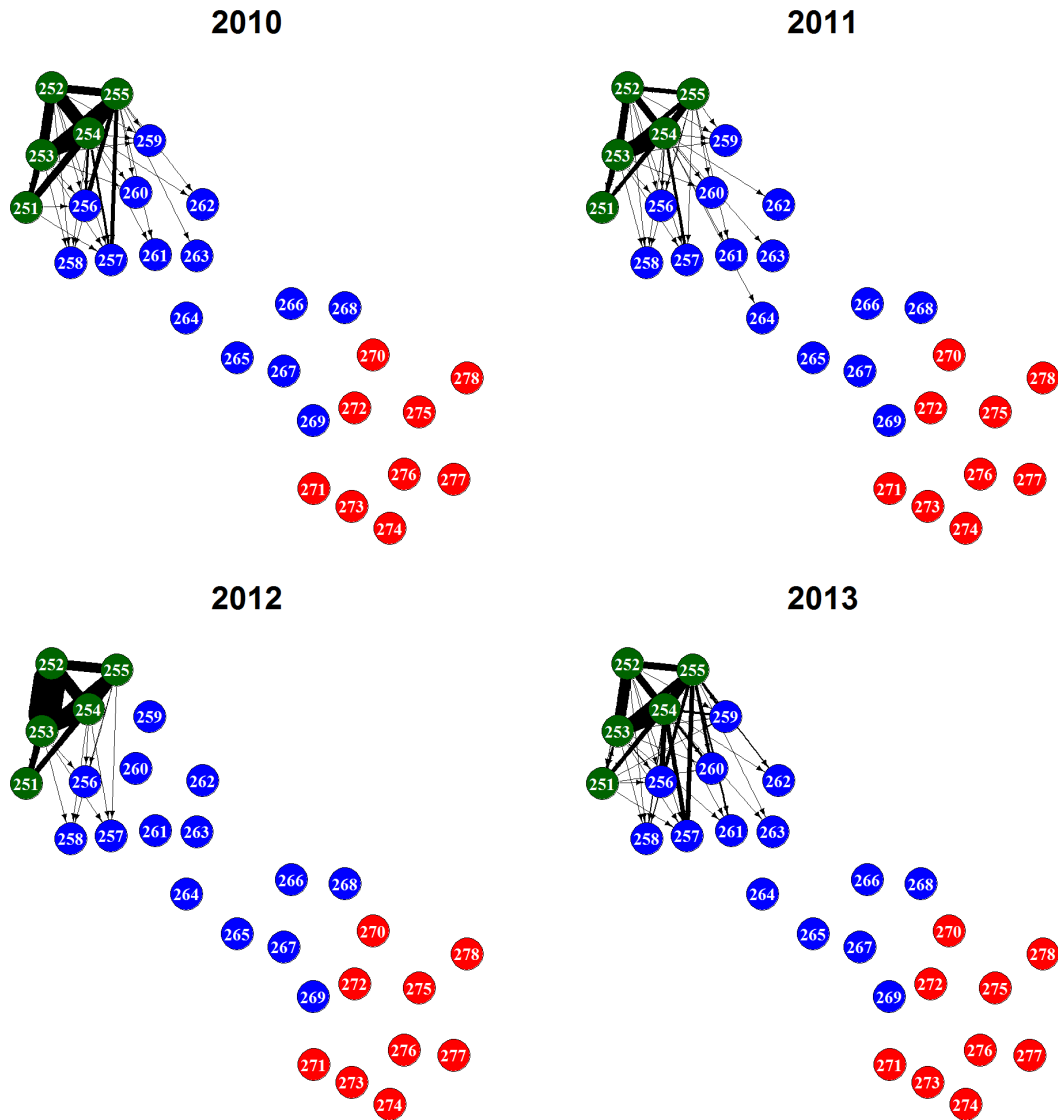


Figure 4.10: Vertex and edge graph of dispersal modeling using the MANGA700 circulation model and releasing larvae from the region between Land’s End and The Lizard in the four study years. Width of arrows is relative strength of connection between source and destination sites. Self-recruitment arrows were omitted. Colors represent the location of the sites: East of Start Point (sites 270-278, red), between The Lizard and Start Point (sites 256 to 269, blue), and between Land’s End and The Lizard (sites 251 to 255, green).

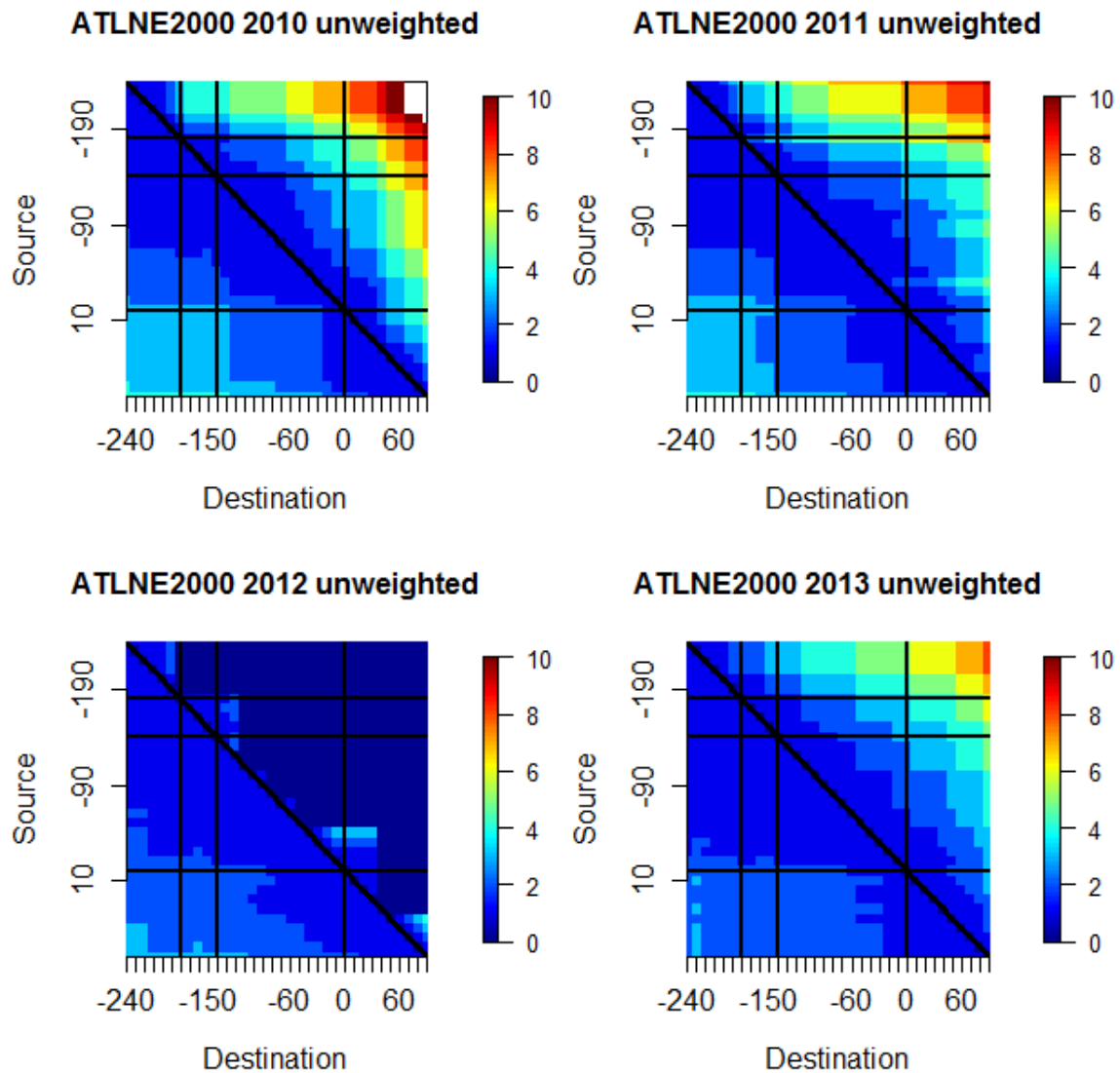


Figure 4.11: Number of steps in the shortest dispersal path between all source and destination sites using paths generated using the ATLNE2000 circulation model in the four study years. Colors represent the number of steps, in this case years. The x and y-axes are in units of kilometers from Start Point, a major geographic feature in the region. The diagonal line is the 1:1 line, representing self-recruitment. The horizontal and vertical lines are the locations of major geographic features (Start Point, 0km; The Lizard, -140km; Land’s End, -180km). White color indicates a number of steps greater than 10.

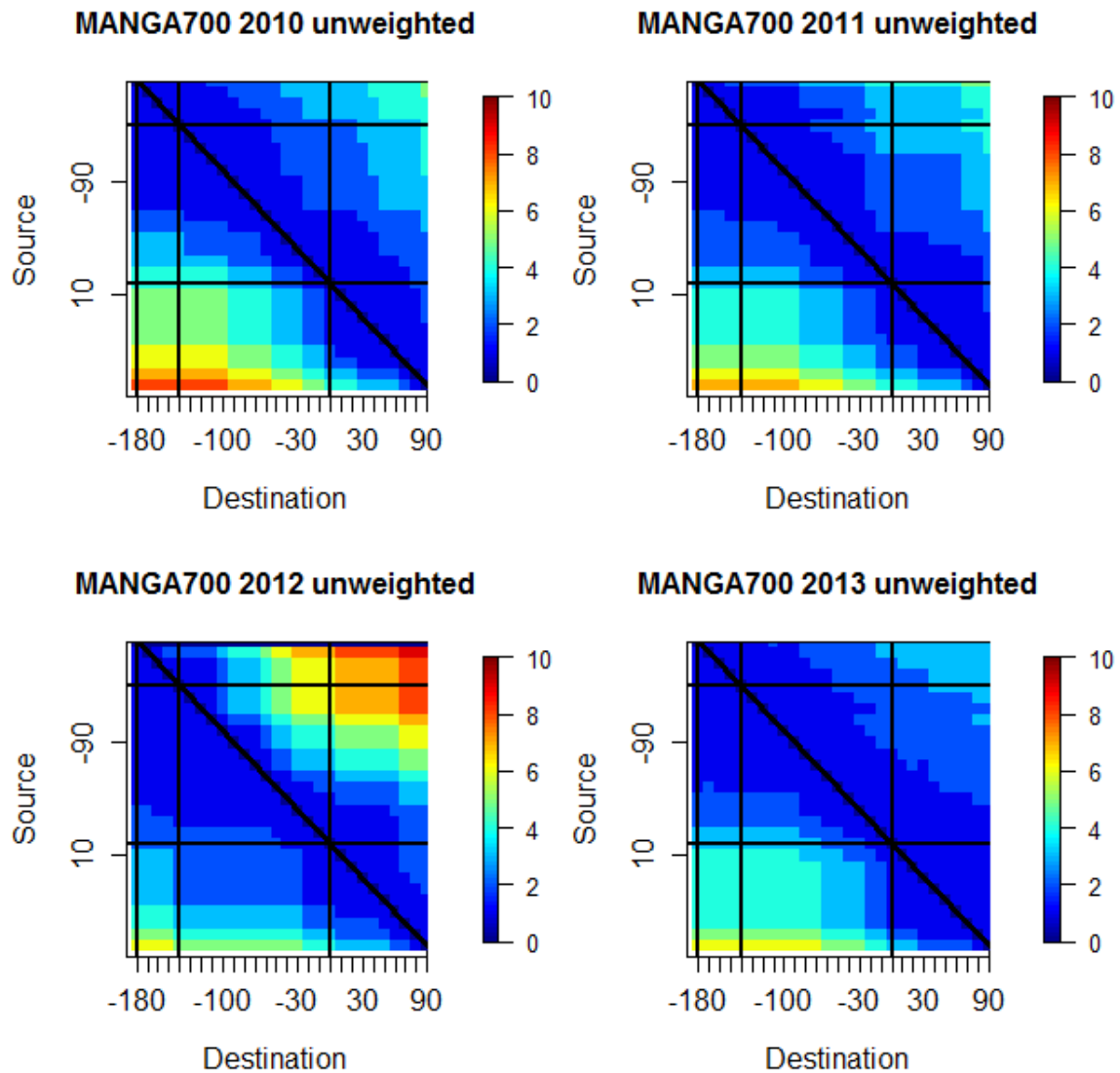


Figure 4.12: Number of steps in the shortest dispersal path between all source and destination sites using paths generated using the MANGA700 circulation model in the four study years. Colors represent the number of steps, in this case years. The x and y-axes are in units of kilometers from Start Point, a major geographic feature in the region. The diagonal line is the 1:1 line, representing self-recruitment. The horizontal and vertical lines are the locations of major geographic features (Start Point, 0km; The Lizard, -140km; Land's End, -180km).

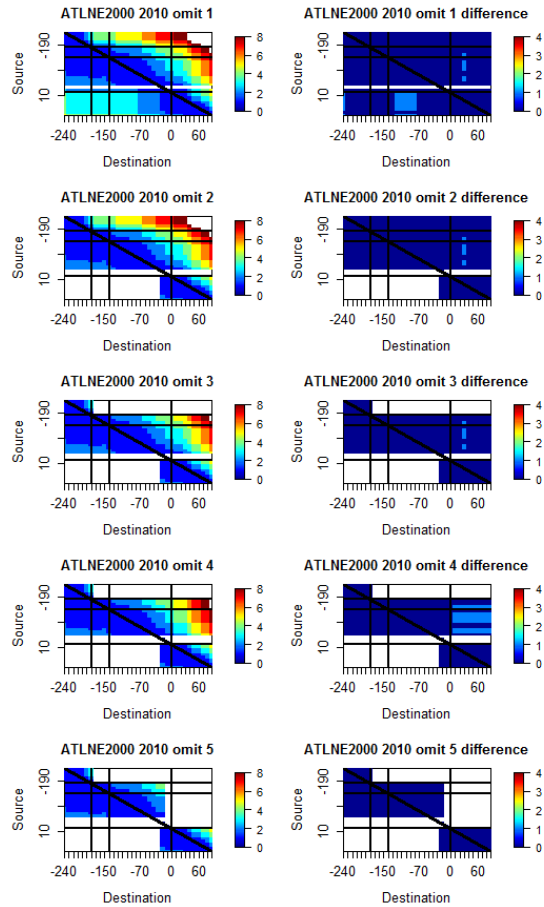


Figure 4.13: Number of steps in the shortest dispersal path between all source and destination sites using paths generated using the ATLNE2000 circulation model in 2010. Sites are omitted sequentially based on their betweenness score, beginning with the site with the top betweenness score (top row), and removing the site with the next highest betweenness score in each successive row. Colors represent the number of steps, in this case years. Locations indicated in white are not accessible. The x and y-axes are in units of kilometers from Start Point, a major geographic feature in the region. The diagonal line is the 1:1 line, representing self-recruitment. The horizontal and vertical lines are the locations of major geographic features (Start Point, 0km; The Lizard, -140km; Land’s End, -180km).

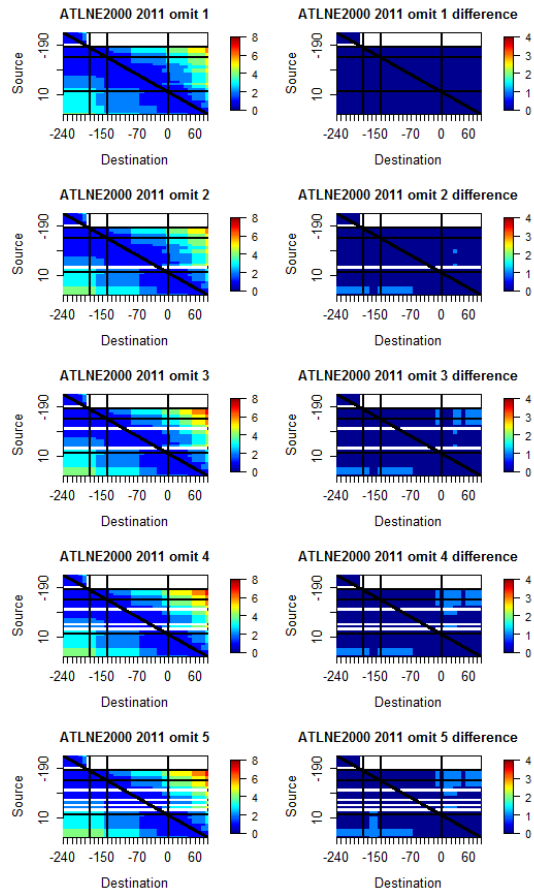


Figure 4.14: Number of steps in the shortest dispersal path between all source and destination sites using paths generated using the ATLNE2000 circulation model in 2011. Sites are omitted sequentially based on their betweenness score, beginning with the site with the top betweenness score (top row), and removing the site with the next highest betweenness score in each successive row. Colors represent the number of steps, in this case years. Locations indicated in white are not accessible. The x and y-axes are in units of kilometers from Start Point, a major geographic feature in the region. The diagonal line is the 1:1 line, representing self-recruitment. The horizontal and vertical lines are the locations of major geographic features (Start Point, 0km; The Lizard, -140km; Land’s End, -180km).

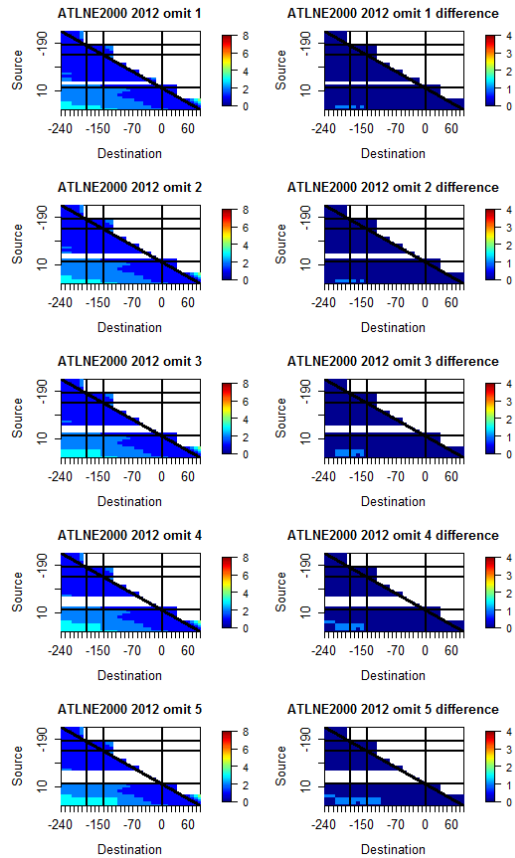


Figure 4.15: Number of steps in the shortest dispersal path between all source and destination sites using paths generated using the ATLNE2000 circulation model in 2012. Sites are omitted sequentially based on their betweenness score, beginning with the site with the top betweenness score (top row), and removing the site with the next highest betweenness score in each successive row. Colors represent the number of steps, in this case years. Locations indicated in white are not accessible. The x and y-axes are in units of kilometers from Start Point, a major geographic feature in the region. The diagonal line is the 1:1 line, representing self-recruitment. The horizontal and vertical lines are the locations of major geographic features (Start Point, 0km; The Lizard, -140km; Land’s End, -180km).

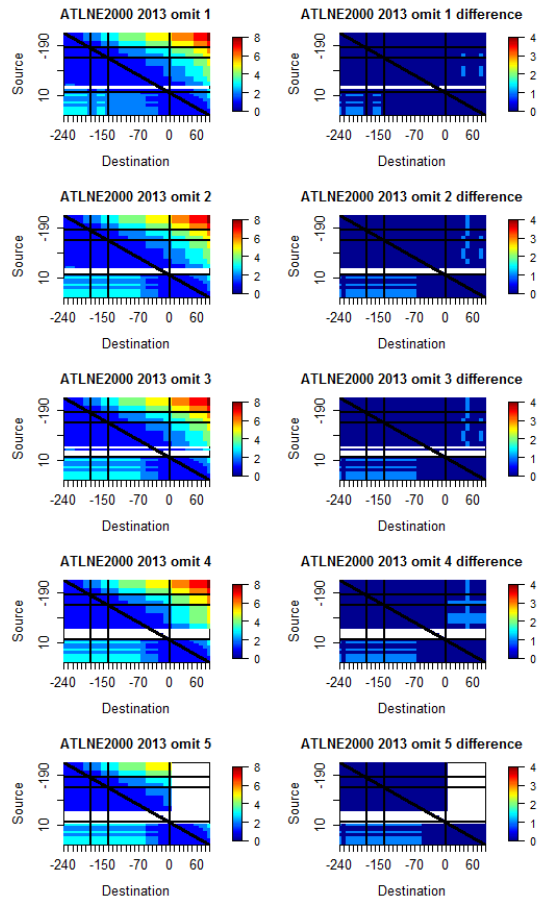


Figure 4.16: Number of steps in the shortest dispersal path between all source and destination sites using paths generated using the ATLNE2000 circulation model in 2013. Sites are omitted sequentially based on their betweenness score, beginning with the site with the top betweenness score (top row), and removing the site with the next highest betweenness score in each successive row. Colors represent the number of steps, in this case years. Locations indicated in white are not accessible. The x and y-axes are in units of kilometers from Start Point, a major geographic feature in the region. The diagonal line is the 1:1 line, representing self-recruitment. The horizontal and vertical lines are the locations of major geographic features (Start Point, 0km; The Lizard, -140km; Land’s End, -180km).

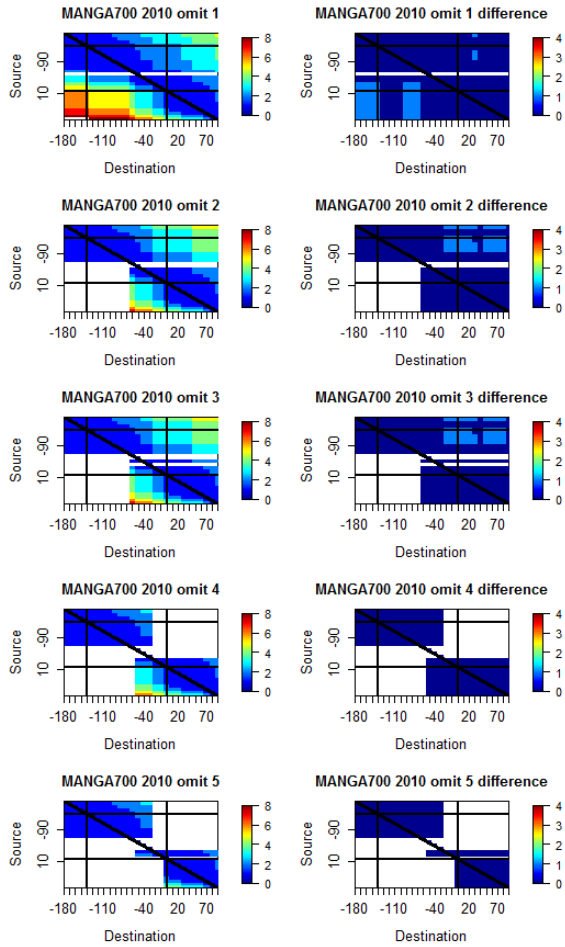


Figure 4.17: Number of steps in the shortest dispersal path between all source and destination sites using paths generated using the MANGA700 circulation model in 2010. Sites are omitted sequentially based on their betweenness score, beginning with the site with the top betweenness score (top row), and removing the site with the next highest betweenness score in each successive row. Colors represent the number of steps, in this case years. Locations indicated in white are not accessible. The x and y-axes are in units of kilometers from Start Point, a major geographic feature in the region. The diagonal line is the 1:1 line, representing self-recruitment. The horizontal and vertical lines are the locations of major geographic features (Start Point, 0km; The Lizard, -140km).

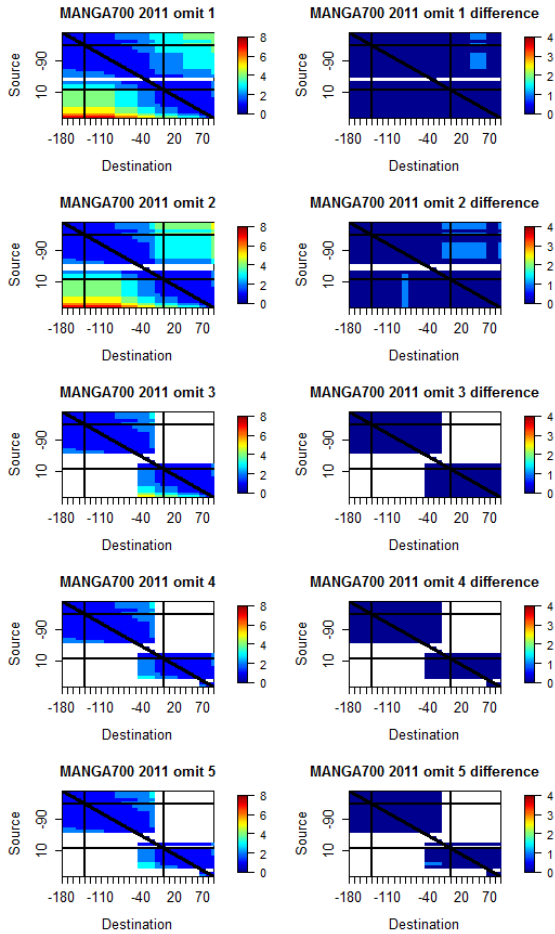


Figure 4.18: Number of steps in the shortest dispersal path between all source and destination sites using paths generated using the MANGA700 circulation model in 2011. Sites are omitted sequentially based on their betweenness score, beginning with the site with the top betweenness score (top row), and removing the site with the next highest betweenness score in each successive row. Colors represent the number of steps, in this case years. Locations indicated in white are not accessible. The x and y-axes are in units of kilometers from Start Point, a major geographic feature in the region. The diagonal line is the 1:1 line, representing self-recruitment. The horizontal and vertical lines are the locations of major geographic features (Start Point, 0km; The Lizard, -140km).

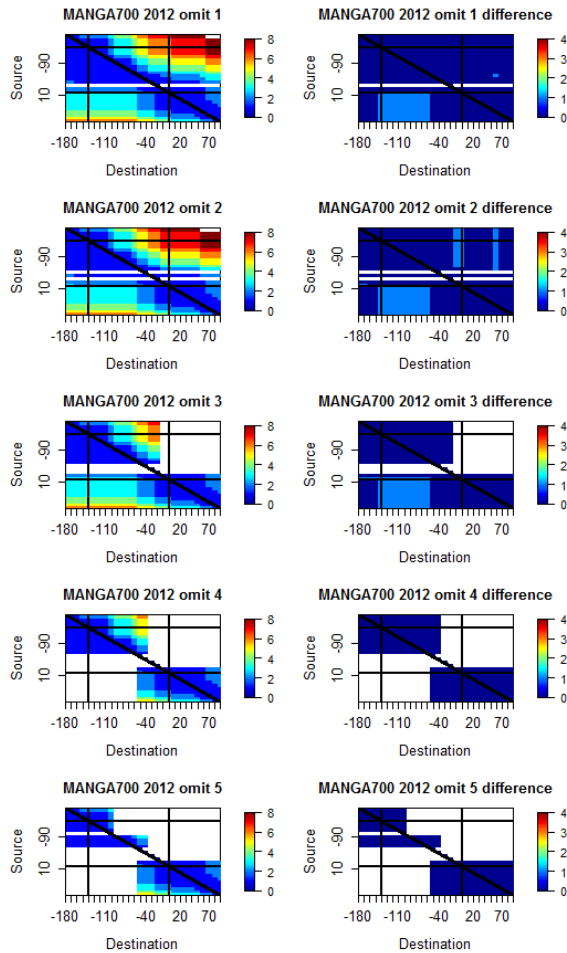


Figure 4.19: Number of steps in the shortest dispersal path between all source and destination sites using paths generated using the MANGA700 circulation model in 2012. Sites are omitted sequentially based on their betweenness score, beginning with the site with the top betweenness score (top row), and removing the site with the next highest betweenness score in each successive row. Colors represent the number of steps, in this case years. Locations indicated in white are not accessible. The x and y-axes are in units of kilometers from Start Point, a major geographic feature in the region. The diagonal line is the 1:1 line, representing self-recruitment. The horizontal and vertical lines are the locations of major geographic features (Start Point, 0km; The Lizard, -140km).

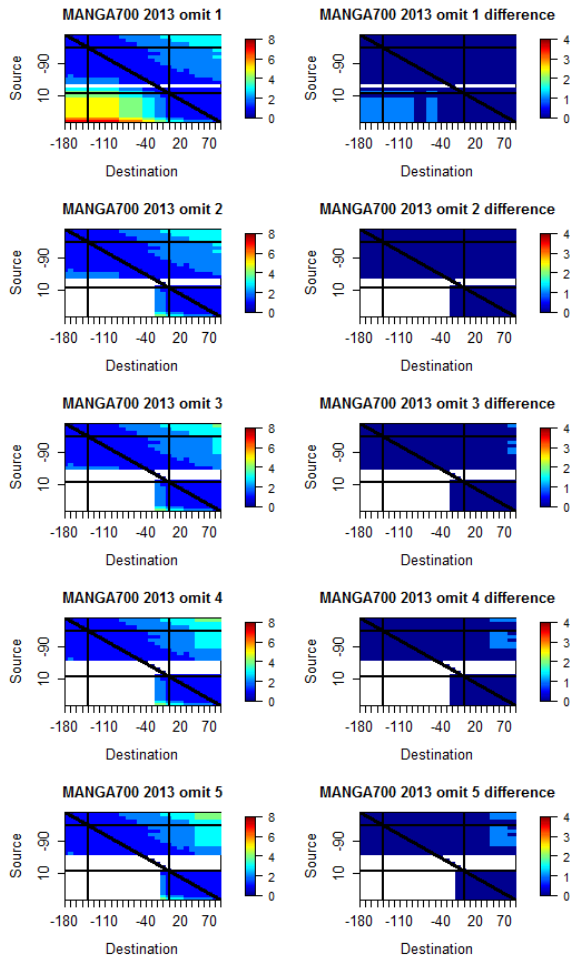


Figure 4.20: Number of steps in the shortest dispersal path between all source and destination sites using paths generated using the MANGA700 circulation model in 2013. Sites are omitted sequentially based on their betweenness score, beginning with the site with the top betweenness score (top row), and removing the site with the next highest betweenness score in each successive row. Colors represent the number of steps, in this case years. Locations indicated in white are not accessible. The x and y-axes are in units of kilometers from Start Point, a major geographic feature in the region. The diagonal line is the 1:1 line, representing self-recruitment. The horizontal and vertical lines are the locations of major geographic features (Start Point, 0km; The Lizard, -140km).

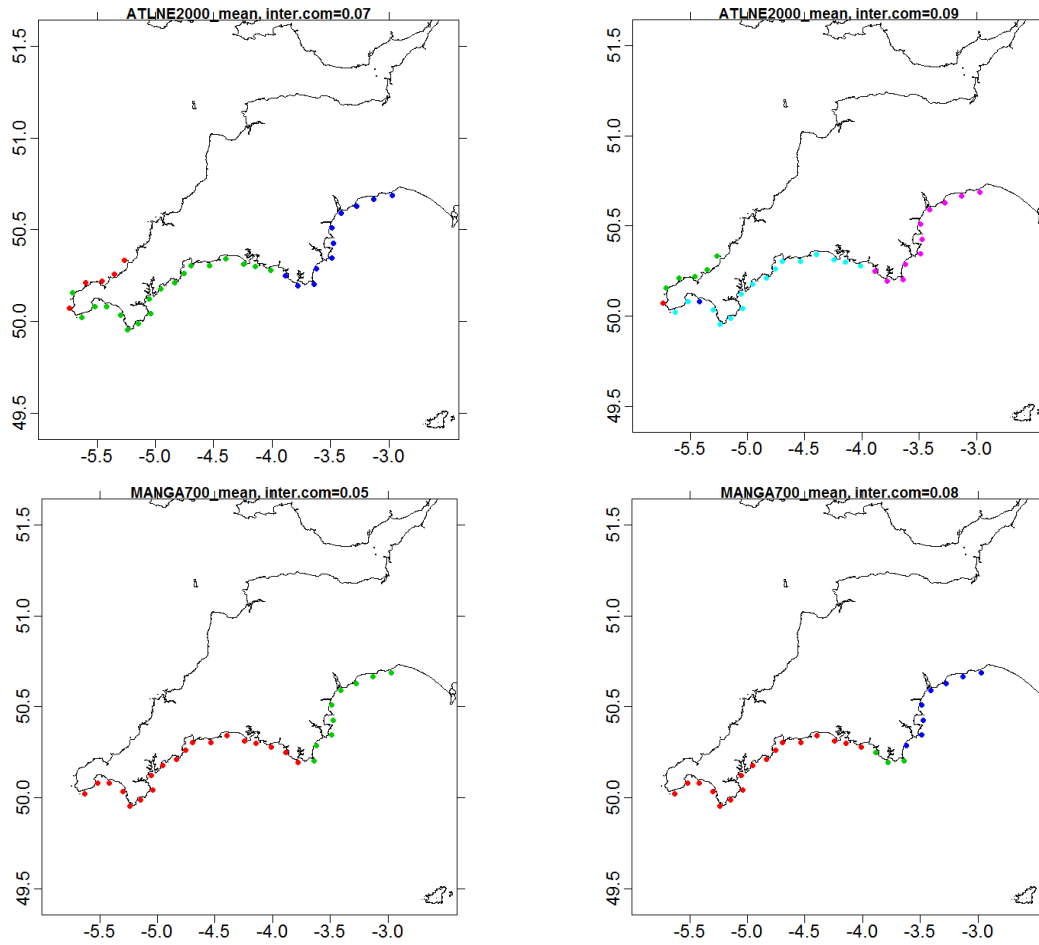


Figure 4.21: Community assignments using the Constant Potts Model community detection algorithm. The proportion of simulated larvae crossing the boundaries of the communities is 7% and 9% for the ATLNE200 model (top row) and 5% and 8% for the MANGA700 model (bottom row). Sites plotted in the same color are assigned to the same community.

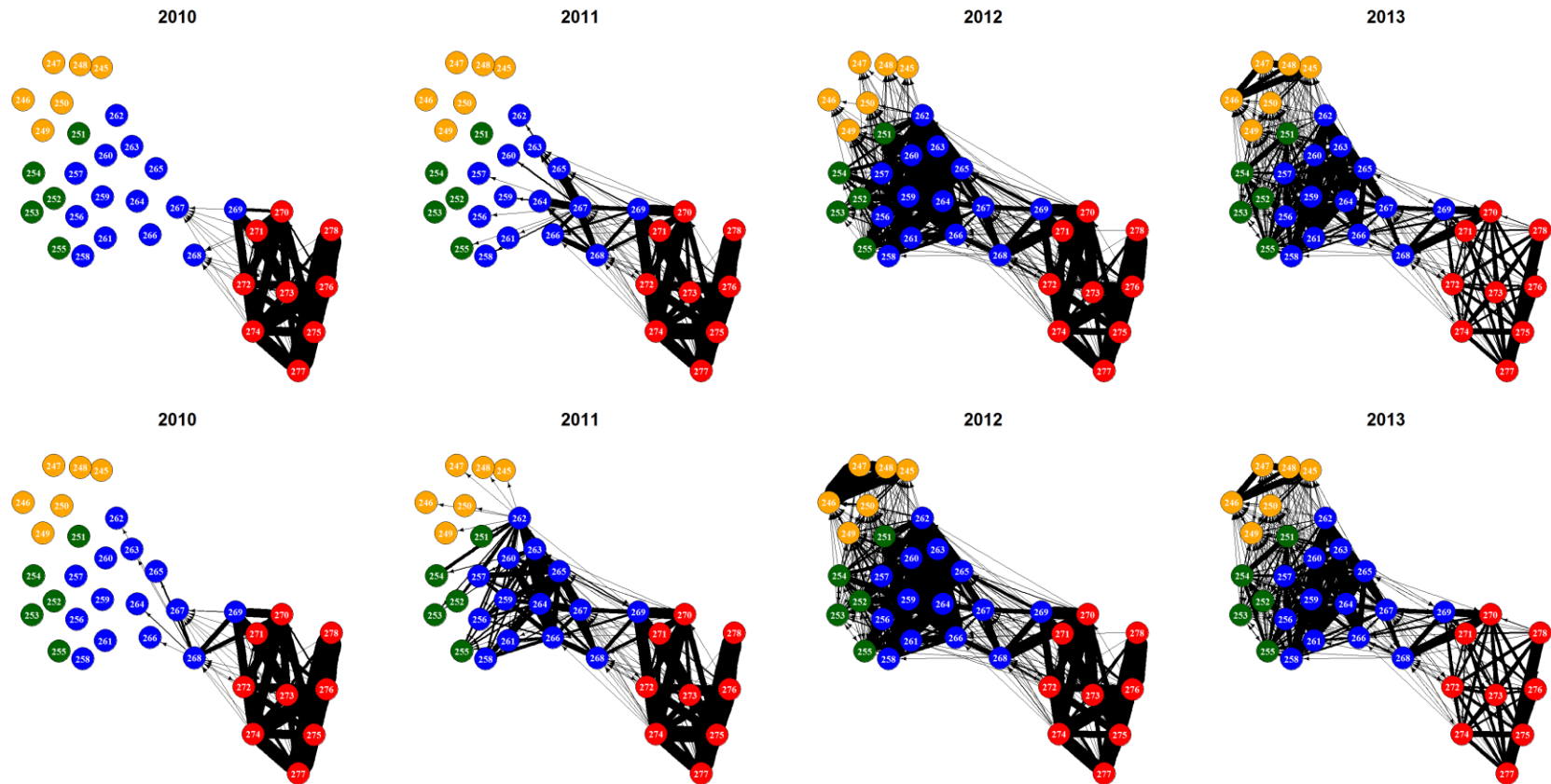


Figure 4.22: Vertex and edge graph of sequential dispersal modeling using the ATLNE2000 circulation model. Larvae were initially released from sites in the region east of Start Point (top row) and east of Start Point and within 20km west of Start Point (sites 268 and 269, bottom row) in 2010. The second release pattern is a more accurate representation of measured adult distributions in 2010. Width of arrows is relative strength of connection between source and destination sites. Self-recruitment arrows were omitted. Colors represent the location of the sites: East of Start Point (sites 270-278, red), between The Lizard and Start Point (sites 256 to 269, blue), between Land's End and The Lizard (sites 251 to 255, green), and North Cornwall (sites 245-250, orange).

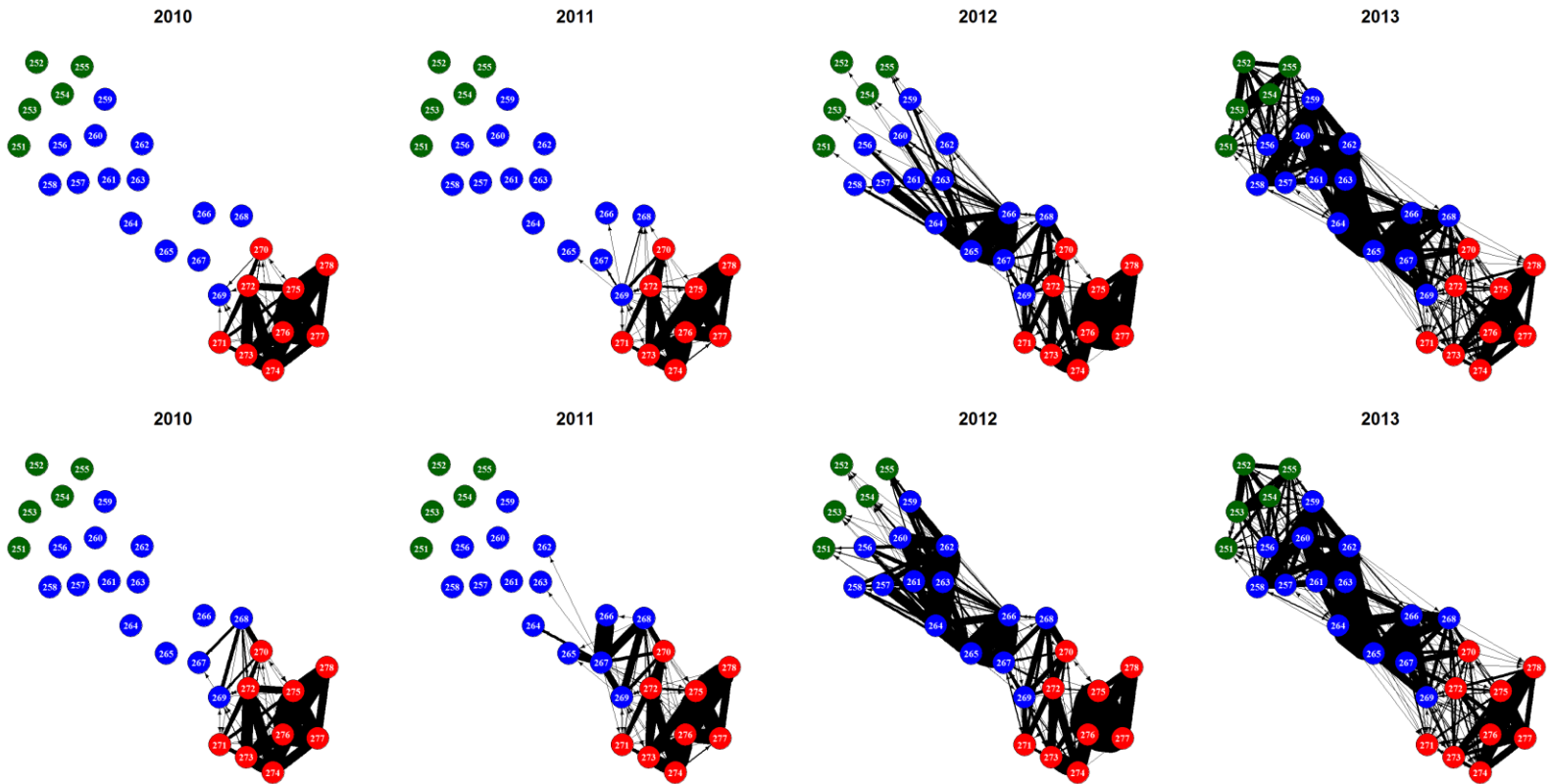


Figure 4.23: Vertex and edge graph of sequential dispersal modeling using the MANGA700 circulation model. Larvae were initially released from sites in the region east of Start Point (top row) and east of Start Point and within 20km west of Start Point (sites 268 and 269, bottom row) in 2010. The second release pattern is a more accurate representation of measured adult distributions in 2010. The destinations of simulated larval paths were used as the origins of larvae in 2011 and the process was repeated sequentially for 2012 and 2013. Width of arrows is relative strength of connection between source and destination sites. Self-recruitment arrows were omitted. Colors represent the location of the sites: East of Start Point (sites 270-278, red), between The Lizard and Start Point (sites 256 to 269, blue), and between Land's End and The Lizard (sites 251 to 255, green).

CHAPTER 5

GENERAL CONCLUSION

Individuals of a particular species are often not grouped together in a single location, but are distributed in space in multiple subpopulations. Individuals may be exchanged among subpopulations, a process called population connectivity. The degree of population connectivity depends on a multitude of factors, including: the size of the subpopulations, the fecundity of individuals in the subpopulations, and transport processes that inhibit or promote transport in particular directions. These processes vary of time and space, thus connectivity also varies over time and space. Variation in connectivity can lead to local extinctions as deaths and exports of individuals outweigh local births and imports, thus population connectivity contributes to the maintenance of existing subpopulations and establishment of new subpopulation. This dissertation focused on factors that control the number of larvae adults input into the larval pool and physical dispersal and the population wide repercussions of this variation.

Chapter 2 described the results of multi-year field surveys and tested the hypothesis: Adult propagule input has a measurable effect on population connectivity, which consequently affects species' distributions. Surveys of recruitment and adult densities were conducted in two cold years (2010, 2011) and one warm year (2012) in Southwest England, an area of historical transience for this species. These surveys span the southern geographic limit of *S. balanoides* in this region. We documented increased

recruitment in cold years compared to the warm year and a range expansion filling in gap in *S. balanoides* range in Southwest England after three years.

In **Chapter 3**, the results of a laboratory-based study investigating potential mechanisms generating the observed relationship between recruitment and cold temperatures were described. Specific hypotheses tested were: (1) variation in brooding temperature produces differences in reproductive timing and (2) variation in brooding temperature produces differences in reproductive output, in terms of larval quality larval quantity, or both. Laboratory experiments demonstrated that temperature did not significantly affect larval development rate, but brooding individuals reared at cold temperatures had significantly greater reproductive mass than individuals reared at warm temperatures. This mass difference is caused by an over three-fold larger number of embryos surviving in the coldest treatment (7°C) compared to the warmest treatment (13°C). Temperature-induced variation in number of surviving embryos likely contributes to differences in recruitment following cold winters vs. warm winters.

Chapter 4 used results of computer-based dispersal modeling to address the questions: (1) How do potential connectivity patterns of *Semibalanus balanoides* in Southwest England vary over time? and (2) What is the predicted timescale of recolonization of Southwest England predicted by dispersal models and are these predictions supported by the data of actual recolonization events (described in Chapter 2)? Dispersal models indicated variation in potential connectivity estimates among years and generated predictions of recolonization of Southwest England that was more likely to proceed from the east than from the north and occurs in 2-4 years, consistent with field observations of 3-4 years.

Overall, this work provides a striking example of the non-lethal effects of climate change. Chapters 2 and 3 demonstrated the role of temperature in controlling the reproductive output of *Semibalanus balanoides*, with the number of larvae produced increasing dramatically (~3-fold) with decreasing temperatures during larval brooding. This increase in inputs into the larval pool produced an increase in recruitment during cool winters compared to warm winters and a range expansion following sequential cold winters. Physical dispersal also plays an essential role in population connectivity (Chapter 3), but, as larvae usually cannot be tracked directly, computer-based modeling is often used to generate hypotheses about the mechanisms of larval transport. Physical transport modeling indicated recolonization of Southwest England was more likely to proceed from the east than from north, and would take 2-4 years, consistent with the observed recolonization of the region (Chapter 2) in three years. Both input into the larval pool and physical dispersal are key components to population connectivity, and variation in these factors should be incorporated into models of population dynamics.

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APPENDIX A – PERMISSION LETTER

8/25/2014

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Rhiannon Rognstad <rrrogstad@gmail.com>

permission to use Rognstad et al. 2014 in PhD dissertation

Ian Stewart <ian.stewart@int-res.com>

Mon, Aug 25, 2014 at 7:15 AM

To: Rhiannon Rognstad <rrrogstad@gmail.com>

Dear Rhiannon

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With best wishes

Ian Stewart
Inter-Research

[Quoted text hidden]

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1/1