POPULATION BIOLOGY AND REPRODUCTIVE ECOLOGY OF *CHLOROSTOMA* (*TEGULA*) *FUNEBRALIS,* AN INTERTIDAL GASTROPOD

by

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Title: POPULATION BIOLOGY AND REPRODUCTIVE ECOLOGY OF *CHLOROSTOMA* (*TEGULA*) *FUNEBRALIS*, AN INTERTIDAL GASTROPOD

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The trochid gastropod *Chlorostoma* (*Tegula*) *funebralis* is found in rocky intertidal habitats along the west coast of North America from Baja California to Vancouver Island. Size-frequency distributions of populations were analyzed along a latitudinal gradient from northern Oregon to Baja California. Populations in California and southern Oregon were dominated by individuals in the juvenile size classes (0.1g – 2g). Along the Oregon coast, populations dominated by juveniles were correlated with coastline topography, with protected areas having large numbers of juveniles and exposed areas dominated by larger size classes. The largest size classes (> 9g) were rarely present in southern populations.

The seasonality of reproduction was investigated in a southern California population and an Oregon population to determine whether continuous reproduction in southern populations increases recruitment success over the year and decreases inter-annual variation in reproductive success. Constant recruitment may cause populations to have a large number of juveniles, the observed pattern in southern populations. Although individuals capable of reproduction were found year-round in both populations, the southern population experienced multiple spawning events over the year, while the northern population experienced only one such event. Constant recruitment may be a strategy to compensate for shorter life spans and smaller sizes of individuals in southern California.

To investigate whether variations in predation rates on large adult *C. funebralis* affect the size structure of populations, the main predators and predation rates for different populations in Oregon were identified. All observed predation events were by the intertidal seastar *Pisaster ochraceus.* Predation pressure by *P. ochraceus* varied significantly with site and between sampling dates but did not remove enough *C. funebralis* from the adult population to have a significant effect on population size structure.

To determine the connectivity between populations and the maximum dispersal potential, the mitochondrial gene COI was sequenced from individuals from nine populations ranging from southern California to northern Oregon. Although haplotype diversity was high, no genetic structure was found between populations. Rather than an indication of high dispersal potential and a panmictic species, the lack of isolation by distance may be a result of range expansion following the last glacial maximum.

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For my grandfather, who literally showed me the stars.

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CHAPTER I

GENERAL INTRODUCTION

Population Structure of *Chlorostoma funebralis*

The intertidal gastropod *Chlorostoma* (*Tegula*) *funebralis* is common in the rocky intertidal of the western coast of North America (Figure 1). It ranges from Baja California to Vancouver Island (Morris et al 1980), and can occur in densities of more than 1000/m^2 (Paine 1969). As a member of the family Trochidae, *C. funebralis* free spawns gametes which develop into lecithotrophic larvae. These larvae are planktonic for five to eight days before metamorphosis. Populations of *C. funebralis* are known to vary in size-structure through its species range (Frank 1975, Wright 1975). In the southern portion of its range, from Baja to central California, individuals have a lifespan of five to eight years and remain small. In contrast, populations in Northern California, Oregon, and Washington have distributions that are not as heavily weighted towards juveniles. Populations frequently have bimodal distributions and individuals can live up

Figure 1: Adult *Chlorostoma* (*Tegula*) *funebralis*

to 30 years (Darby 1964). This is attributed to regular recruitment events in the southern portion of the species range, and high inter-annual variation along the Oregon and Washington coastlines (Frank 1975, Wright 1975). Although this pattern described in the 1970's, the factors causing these differences have not been explored.

Larval Transport

The planktonic larval stage is often cited as a source of variation in recruitment and adult distributions, because inter-annual variation in recruitment to a population will cause gaps in the adult distributions as those juveniles age (Ebert and Russell 1988, Roughgarden et al 1988, Menge et al 2004, McQuaid and Phillips 2006). Larval supply and dispersal may be largely determined by a combination of local hydrography, coastal oceanography, and larval behavior. *Chlorostoma funebralis* larvae are small (< 250 μm) and marine invertebrate larvae have long been expected to disperse with the prevailing oceanographic currents. However, the ability to migrate vertically through the water column may allow larvae to move through different bodies of water and mitigate the effects of dispersal in any one direction (Cronin and Forward 1979, Rothlisberg et al 1983, Shanks et al 2003a, Shanks 2009). Variation in hydrodynamic conditions and the biology of *C. funebralis* may therefore cause the variation in age structure observed by Frank (1975) and Wright (1975).

Variations in hydrographic conditions on a latitudinal scale are hypothesized to have a significant effect on population and community structure (Roughgarden et al 1988, Menge et al 2004). Upwelling moves surface waters offshore, and larvae are expected to move offshore with this water mass. In contrast, downwelling moves surface water onshore. Along the Oregon coast, upwelling occurs primarily in the summer and is intermittent. Periods of upwelling, which move larvae offshore, are divided by downwelling periods, which move larvae back to the inter- or sub-tidal environments where they can metamorphose. In contrast, central and Northern California have constant and strong upwelling, so larvae rarely get the opportunity to move back to appropriate adult habitat (Menge et al 2004). This hypothesis should lead to higher, more constant recruitment along the Oregon coast, and episodic recruitment in central and northern California with high inter-annual variation (Menge et al 2004). This pattern is in direct contrast to that observed for *C. funebralis* by Frank (1975) and Wright (1975), which indicated regular recruitment along the California coastline and high inter-annual variation in recruitment along the Oregon and Washington coastlines.

However, the ability of larvae to vertically migrate may allow larvae to stay nearshore even during periods of strong upwelling (Poulin et al 2002, Shanks and Brink 2005, Morgan et al 2009a, 2009b, Shanks and Shearman 2009). The larvae of many taxa reside in deeper waters which are not moved offshore by upwelling, and instead are able to maintain a constant depth through changing oceanographic conditions (Shanks and Brink 2005). The strength of upwelling as a factor driving the observed latitudinal differences in population structure of *C. funebralis* is dependent on the ability of *C. funebralis* to regulate dispersal and movement offshore through larval behavior.

The effects of hydrodynamic conditions on larval transport will also depend on the timing of reproduction. If larvae are not present in the water column during seasonal upwelling, the upwelling and downwelling patterns of the summer will have little effect on larval dispersal. The seasonality of reproduction and triggers for spawning may have a large effect on the conditions the planktonic stages encounter.

In addition to variation on a latitudinal gradient, the size-frequency distribution of some marine invertebrate species has been shown to vary with coastal topography and the degree of exposure of intertidal populations. The limited swimming ability of many invertebrate larvae may increase the importance of local hydrographic factors that can keep larvae close to shore or transport larvae offshore. Larvae may be retained within protected areas such as bays and coves by oceanographic fronts that form at the mouths (Shanks et al 2003a, 2003b , von der Meden et al 2008,). At exposed areas, particularly those near headlands, larvae may be transported by jets of water moving offshore (Ebert and Russell 1988). This offshore movement reduces larval supply and increases inter-annual variation in recruitment at these sites (Ebert and Russell 1988).

Community Structure

While hydrography and larval behavior only affects the early life stages, the interactions with other intertidal species become important for *Chlorostoma funebralis* after metamorphosis. It has been hypothesized that predation by *Pisaster ochraceus* may play an important role in limiting populations of large, adult *C. funebralis* in Washington populations (Paine 1969). In California, predation pressure from octopus and crabs is more prevalent than predation by *P. ochraceus*.

Due to the habitat selection of juvenile and adult *C. funebralis*, predation by *P. ochraceus* occurs predominately on the adult portion of the population. Juvenile *C. funebralis* settle in the high intertidal, beyond the physiological tolerance of sea stars. As individuals grow larger, they migrate into the mid intertidal, where they encounter predation pressure. If the pressure exerted by predators on *C. funebralis* populations differ between populations, it may be a source of variation in the size structure of populations of *C. funebralis*.

Using Molecular Markers to Study Size Structure and Population Connectivity

Molecular markers, particularly DNA sequences, have become a regular method of studying larval dispersal and population connectivity (Palumbi 2003, Marko 2004, Sotka 2004, Marko et al 2007). These methods can provide information on the degree of isolation between populations of varying distances from each other, and show patterns on a latitudinal scale (Sotka 2004). If populations in different areas of the species range are isolated from each other or have low connectivity, evolutionary divergence between the two regions may explain some of the differences in age structure in *Chlorostoma funebralis*, such as the shorter life span in the southern portion of the range. However, if the population is panmixic and no genetic isolation is apparent, the differences in age structure are likely to be ecological in nature.

Molecular markers can also test for differences in age structure caused by coastal topography (Nicastro et al 2008). If larvae are retained within protected areas, more private haplotypes and lower overall genetic diversity should be observed, in contrast to exposed populations, where larvae are more likely to be transported offshore and less likely to recruit to the parent population.

Scope and Objectives

The primary objectives of this dissertation are to document variation in size-frequency distributions of *Chlorostoma funebralis* and to explore some of the mechanisms that potentially cause this variation. A combination of laboratory and field techniques were used to determine the relative importance of various factors to the size-frequency and age structure of *C. funebralis*.

Chapter II includes size – frequency distributions collected from Baja California to northern Oregon, including many sites previously sampled 35 years ago (Frank 1975, Wright 1975). Previous studies had documented difference in size-structure along a latitudinal gradient, with larger numbers of smaller individuals in the southern portion of the species range and larger individuals in the northern portion of the range. The research presented in Chapter II shows a similar trend, but also increased the number of populations sampled from other studies, showing a previously undescribed pattern of the importance of coastal topography in population structure. Within the northern portion of the range, populations in physically protected areas such as bays and coves have a significantly higher percentage of juvenile individuals. In contrast, exposed populations have few juveniles and more large individuals. The remaining chapters address some of the potential reasons for the variations both in latitude and coastal topography.

Chapter III explores the role of seasonality of reproduction in the size-frequency distributions through the species range. If reproduction is annual and episodic in the northern portion of the species range, as proposed by Paine (1971), high inter-annual variation would not be unexpected, since some years would provide optimal conditions for larval survival and recruitment while other years would suffer high larval mortality. If, in contrast, southern populations reproduce multiple times throughout the year as proposed by Wright (1975), recruitment events may be smaller in magnitude but offer higher chances of successful recruitment at some point during the year. This may be one of the factors causing large numbers of juveniles in the southern portion of the range and higher variability in the number of juveniles in the northern portion of the range.

Chapter IV focuses just on the differences in the number of large individuals between populations along the Oregon coast. Because *C. funebralis* settle in the high intertidal and migrate into the mid intertidal as they grow larger, primary predators in the intertidal such as crabs and seastars are only encountered by adult *C. funebralis*. If predators are removing significant numbers of large adults from populations, we expect to see fewer large adult *C. funebralis* in populations that co-occur with high predator densities. To test the effects of predation, this chapter used field experiments to identify the important predators of *C. funebralis*, document the densities of those predators, and measure the predation rate at multiple sites along the Oregon coast.

In Chapter V, the mitochondrial sequence COI is used to study the degree to which populations of *C. funebralis* are reproductively isolated from each other, including populations from southern California to northern Oregon. Because *C. funebralis* has a relatively short larval period (five to eight days), some genetic differentiation may be observed along this gradient. Due to the longer time scales that affect the genetic differentiation between populations, this technique offers a different analysis than ecological studies.

CHAPTER II

LATITUDE AND COASTLINE SHAPE DETERMINE AGE-STRUCTURE OF *CHLOROSTOMA* (*TEGULA*) *FUNEBRALIS* POPULATIONS

Introduction

The size-frequency distributions and age structures of many marine invertebrates vary among populations (Ebert and Russell 1988, McQuaid and Phillips 2006). At times these attributes vary along a latitudinal gradient (Frank 1975, Ebert 1983, Menge et al 2004). These differences may be the result of larval supply and recruitment, since years of poor recruitment will result in gaps in the size-frequency distribution as the population ages (Ebert and Russell 1988, Roughgarden et al 1988, Menge et al 2004). Differences in recruitment, in turn, may be driven by hydrodynamics and coastal topography. Because larvae have limited swimming capacity, their movement may largely be determined by the movement of water masses, which can vary latitudinally as well as locally. Frank (1975) and Wright (1975) collected data on the size distribution of *Chlorostoma* (*Tegula*) *funebralis* along the west coast of North America. These distributions showed a latitudinal gradient in the number of adult individuals, with the largest number of adults in the northern populations and more juveniles in southern populations. However, populations for these studies were geographically disparate. To distinguish between latitudinal gradients that might be driven by gradients in coastal hydrodynamics and the effects of coastal topography, more sampling of populations on a larger latitudinal gradient and a range of coastal topography types is necessary.

The intertidal snail *C. funebralis* is an ideal organism to study variation in size-frequency distributions because it is common and often abundant in the rocky intertidal from Baja, California to British Columbia in both high and low energy environments (Morris et al 1980). The duration of the pelagic lecithotrophic larval stage is between five and eight days (Moran 1997, Guzmán del Próo et al. 2006). Individuals live up to 30 years in the northern portion of the range (Darby 1964). Growth rates have been calculated throughout the species range (Frank 1975, Wright 1975). *C. funebralis* grow throughout their lifespan, so it is possible, using size, to age individuals and use size-frequency distributions as an indication of age structure of populations (Frank 1975). The differences in age-structure of *C. funebralis* populations present an opportunity to study the mechanisms that determine population structure.

Larval supply can drive patterns in recruitment and adult distributions (Ebert and Russell 1988, Roughgarden et al 1988, Menge et al 2004). Populations with low larval supply have been shown to have low population densities. This relationship between larval supply and population structure has been seen, for example, in urchins (Ebert and Russell 1988), mussels (McQuaid and Phillips 2006) and barnacles (Menge et al 2004). The recruitment of many marine invertebrate larvae to adult populations is driven by hydrodynamics both at large scales, such as on a latitudinal gradient (Menge et al 2004) and local scales of bays and headlands (McQuaid and Phillips 2006). Species with a planktonic larval stage have a dispersal distance determined by hydrodynamics and larval swimming behavior, primarily vertical migration (Shanks et al 2003, Shanks 2009). Upwelling, which moves surface waters offshore, is hypothesized to move larvae away from benthic habitats where they must ultimately settle (Roughgarden et al 1988). When upwelling is relaxed, surface waters move back towards shore, carrying larvae back to the adult habitat. Larval capacity for vertical migration may be a confounding factor, enabling larvae to stay nearshore even during upwelling events (Poulin et al 2002, Shanks and Brink 2005, Morgan et al 2009a, 2009b, Shanks and Shearman 2009). Larvae may also have a preference for depths below the Ekman transport layer, which would minimize movement offshore during upwelling (Shanks and Brink 2005, Morgan et al 2009a, 2009b, Shanks and Shearman 2009).

On the west coast of North America, coastal upwelling varies on a latitudinal gradient. Summer wind-driven upwelling is intermittent on the Oregon Coast south to Cape Blanco, and relatively constant from Cape Blanco to Point Conception (Roughgarden et al 1988). Roughgarden et al (1988) hypothesized that the intermittent upwelling in central and northern Oregon should transport pelagic larvae to the rocky intertidal, reducing larval mortality and causing constant, annual larval supply. More constant upwelling in central and northern California should transport larvae offshore, resulting in delivery of larvae to the shore only under rare conditions (Menge et al 2004).

Coastal topography has also been shown to affect larval dispersal and settlement. Because many types of larvae have limited swimming capability, local hydrodynamic features may play a role in retaining larvae or moving them offshore. Ebert and Russell (1988) found a correlation between coastal headlands and larval recruitment. Sites with low recruitment were near coastal headlands, where regular coastal upwelling jets are hypothesized to move larvae offshore. In South Africa, protected bays and exposed headlands have different population structures of mussels, possibly related to recruitment patterns. Higher recruitment in bays in comparison with nearby exposed populations may be due to larval retention within the bay and larval advection from exposed sites (von der Meden et al 2008). The flushing times of bays is correlated with retention of cyprid larvae (Gaines and Bertness 1992). In one study, the presence of oceanographic fronts at the mouths of bays and coves was correlated with upwelling, and the fronts acted as a barrier to the shoreward movement of larvae into the bay (McCulloch and Shanks 2003).

The distribution of adult marine invertebrates is the cumulative effect of recruitment success, juvenile mortality, and adult mortality. Each of these factors may vary between populations independently of each other. Previous studies have shown that large *C. funebralis* are absent in the southern portion of the species range (Frank 1975, Wright 1975). This absence may be a reflection of a latitudinal gradient in adult mortality, but not enough populations have been studied to determine the potential effects of other factors. The presence of large adults in the population may have a significant impact on the reproductive capacity of the population (McQuaid and Phillips 2006).

Previous studies of *Chlorostoma funebralis* did not sample enough sites within each oceanographic region to separate the confounding effects of upwelling and coastal topography on population structure. In this study, a number of sites covering a wide latitudinal range and types of coastal topographies were sampled for the size-frequency distributions of populations of *C. funebralis* to separate the impact of upwelling and coastal topography. If upwelling, through its impact on larval dispersal, is driving patterns of population structure, differences in population structure should be observed in regions with different upwelling strength and duration. If population structure is caused by coastal topography affecting larval dispersal, differences within regions with similar upwelling regimes should vary with the type of coastline. Detailed description of these patterns will allow the formation and testing of hypotheses describing the mechanisms driving variation in population structure.

Methods

Site Classification

To investigate whether population structure changes with coastal topography, populations were sampled at "protected" and "exposed" sites. Sites identified as protected included bays and coves predominately enclosed on at least three sides. Exposed sites were exposed to the open ocean on two or more sides. The wave energy of sites may differ within exposure classifications due to changing hydrographic conditions along the coast of North America, so classification is an indication of the shape of the local coastline and not an indication of local wave energy.

Sites were classified as "north" or "south" based on the geographic range of the large (> 5 g) adult *Chlorostoma funebralis*. Initially, populations were identified as north or south of Cape Blanco, the traditional breakpoint between regions of intermediate (north of Cape Blanco) and constant upwelling (south of Cape Blanco) (Barth et al 2000).

However, the geographic distribution of distributions including large individuals has a southern limit of Brookings, OR, approximately 100 km south of Cape Blanco.

Site Selection

Twenty-two populations of *Chlorostoma funebralis* from northern Oregon to Baja, California were sampled (Figure 2, Table 1). Sites were selected by the presence of *C. funebralis* habitat, primarily mid to high rocky intertidal, and the ease of accessibility. Sites were selected throughout this range to test for a latitudinal gradient in size-frequency distributions.

In Oregon, 15 sites were sampled (seven protected, eight exposed). Five of these sites (Lighthouse Beach, Sunset Bay, Ezzy Cove, Middle Cove, South Cove) are found near Cape Arago, Oregon. In northern California, four sites were sampled (three exposed, one protected). Point Dume was the one exposed site sampled in Southern California. In Baja, two populations were sampled (one protected, one exposed) (Figure 2).

Sampling Populations

Because *Chlorostoma funebralis* settle into the high intertidal and migrate into the mid intertidal as they grow larger, sampling was conducted with a belt transect across tidal heights. The width of the transect was selected based on the density of individuals at that site (0.25, 0.5, or 1 m width). In populations with low densities, a wide transect was used (up to 1m) to ensure that an adequate number of individuals were sampled on

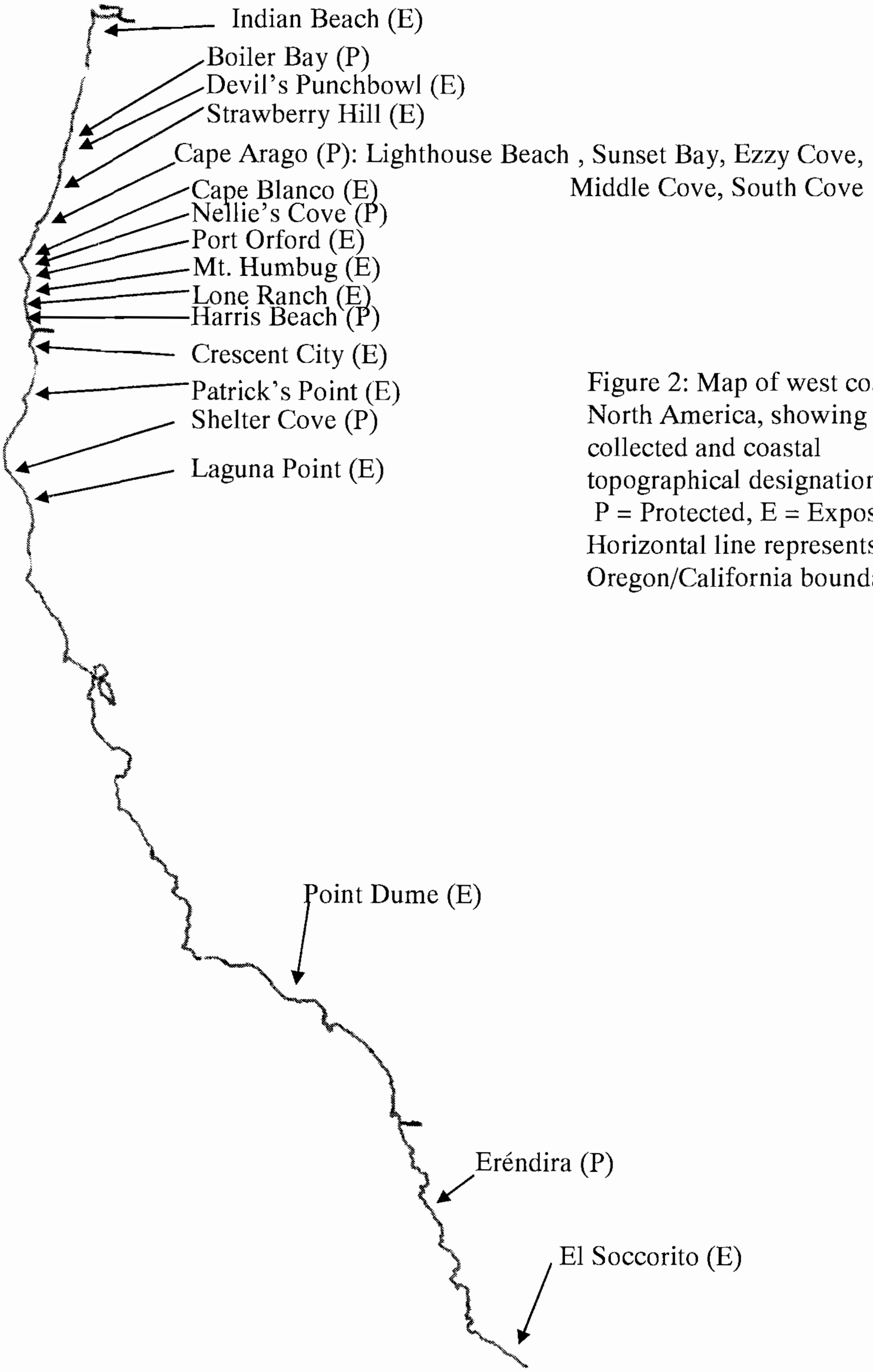


Figure 2: Map of west coast of North America, showing sites collected and coastal topographical designation. P = Protected, E = Exposed. Horizontal line represents Oregon/California boundary.

Table 1: Latitude and longitude for sampled populations of *Chlorostoma funebralis*. Protected populations were enclosed on at least three sides, while exposed sites were open to the open ocean on two or more sides. Coefficient of Variation measured as a percentage of size distribution of populations.

Site	Exposed or Protected	Latitude	Longitude	Coefficient of Variation (%)
Indian Beach	Exposed	45°55’05N	123°58’44W	40
Boiler Bay	Protected	44°49’47N	124°03’33W	96
Devil’s Punchbowl	Exposed	44°44’46N	124°03’50W	67
Strawberry Hill	Exposed	44°15’14N	124°06’43W	41
Lighthouse Beach	Protected	43°20’25N	124°22’30W	85
Sunset Beach	Protected	43°20’00N	124°22’32W	135
Ezzy Cove	Protected	43°19’56N	124°22’40W	123
Middle Cove	Protected	43°18’17N	124°24’02W	64
South Cove	Protected	43°18’12N	124°23’56W	108
Cape Blanco	Exposed	42°50’17N	124°33’37W	60
Nellie’s Cove	Protected	42°44’25N	124°33’37W	45
Port Orford	Exposed	42°44’17N	124°29’57W	40
Mt. Humbug	Exposed	42°42’51N	124°27’14W	8
Lone Ranch	Exposed	42°05’58N	124°20’42W	81
Harris Beach	Protected	42°03’57N	124°18’33W	132
Crescent City	Exposed	41°45’19N	124°13’12W	82
Patrick’s Point	Exposed	41°07’48N	124°09’53W	81
Shelter Cove	Protected	40°01’21N	124°04’00W	88
Laguna Point	Exposed	39°29’23N	123°48’18W	104
Point Dume	Exposed	34°00’11N	118°48’18W	150
Eréndira	Protected	31°17’23N	116°24’14	63
El Soccorito	Exposed	30°17’19N	115°48’31W	94

each transect. In areas with high densities, narrower transects (0.25m or 0.5m) were used. A minimum of three transects and 500 individuals were sampled at each site when possible. Transects were a minimum of 50 m apart to insure independent sampling. If after sampling three transects fewer than 500 individuals were sampled, additional transects were sampled. Indian Beach and Port Orford were sites too small to use more than three transect lines so fewer than 500 individuals were collected from those two sites. The smallest juveniles were collected by hand-sorting coarse sediment collected from under boulders in the high intertidal. Larger juveniles and adults were collected from under and on boulders, in tidepools, and under algae.

All individuals within the belt transect were collected and allowed to air dry. Initially, individuals were weighed and shell diameters were measured as the widest point across the umbilical region from the shell lip to opposite body whorl (Frank 1975). After weighing and measuring the width of 700 individuals, a power relationship was obtained between snail mass and shell diameter (Figure 3). These data were log-transformed and the regression was found to be significant ($p < 0.0001$, $R^2 = 0.916$). After these initial measurements, weight alone was used to measure the size of individuals as this was the more efficient method of quantifying size. The age of individuals was calculated using Frank's (1975) growth rates and size-age correlation models along a latitudinal gradient.

To test for differences in population structure between sites of differing coastal topography, the percentage of juveniles in each population was calculated after minimum size at reproduction was established from reproductive output data (see below). To test

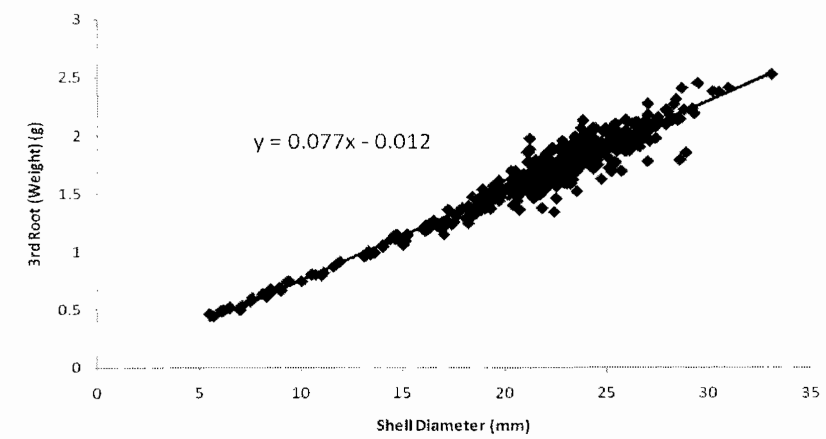


Figure 3: Regression ($p < 0.0001$, $R^2 = 0.916$) between mass and maximum shell diameter in *C. funebralis*.

for population differences on a latitudinal gradient and interaction with coastal topography, populations were tested using a 2-way ANOVA with latitudinal region and coastal topography as factors. Further analyses using 1-way ANOVA's were used to test differences within latitudinal regions.

To test for differences in size distributions between sites, I used the coefficient of variation (CV) (Ebert and Russell 1988). The CV was calculated for each population, arcsine transformed, and differences between factors were tested using ANOVA.

To test for differences in latitudinal gradient in the largest size classes, the percentage of the population ten years or older (approximately 9 g) was calculated using growth rates reported by Frank (1975). The percentage of these old individuals in each population was tested using the same statistical methods as the percentage of juveniles.

Reproductive Output

To quantify the potential reproductive consequences of differing size-frequency distributions, the number of eggs/female produced by *Chlorostoma funebralis* was calculated for individuals that were sexually mature, up to the largest individuals in the population. Individuals between 2 and 15 grams were collected from South Cove, Crescent City, Patrick's Point, Shelter Cove, and Laguna Point. Females smaller than approximately 2.0 grams (approximately 14 mm in diameter) had gonad tissue with very few eggs and were judged sexually immature.

After weight and shell diameter were established, the female gonad was removed, weighed, and displacement volume measured. Ovaries were macerated, diluted and suspended in sea water. Four 20 μL aliquots were sampled from each female and the number of eggs in each aliquot quantified. The aliquot was placed on a slide and gently pressed with a cover slip. A digital camera was used to photograph the sample under 4x magnification. ImageJ (Rasband 2008) was used to superimpose a grid over the image to increase the accuracy of enumeration of eggs in the sample. All of the eggs in the entire 20 μL aliquot were counted.

With few exceptions, replicate aliquots from females were not significantly different from each other and so were averaged. Total egg production was calculated from mean egg concentration in the 20 μL aliquots multiplied by the total ovary mass suspended in seawater. A linear regression was used to test the association of number of eggs in the gonad and the size of the individual. Sexually immature individuals (< 2 g) were excluded from this analysis.

Results

Size-Frequency Distributions

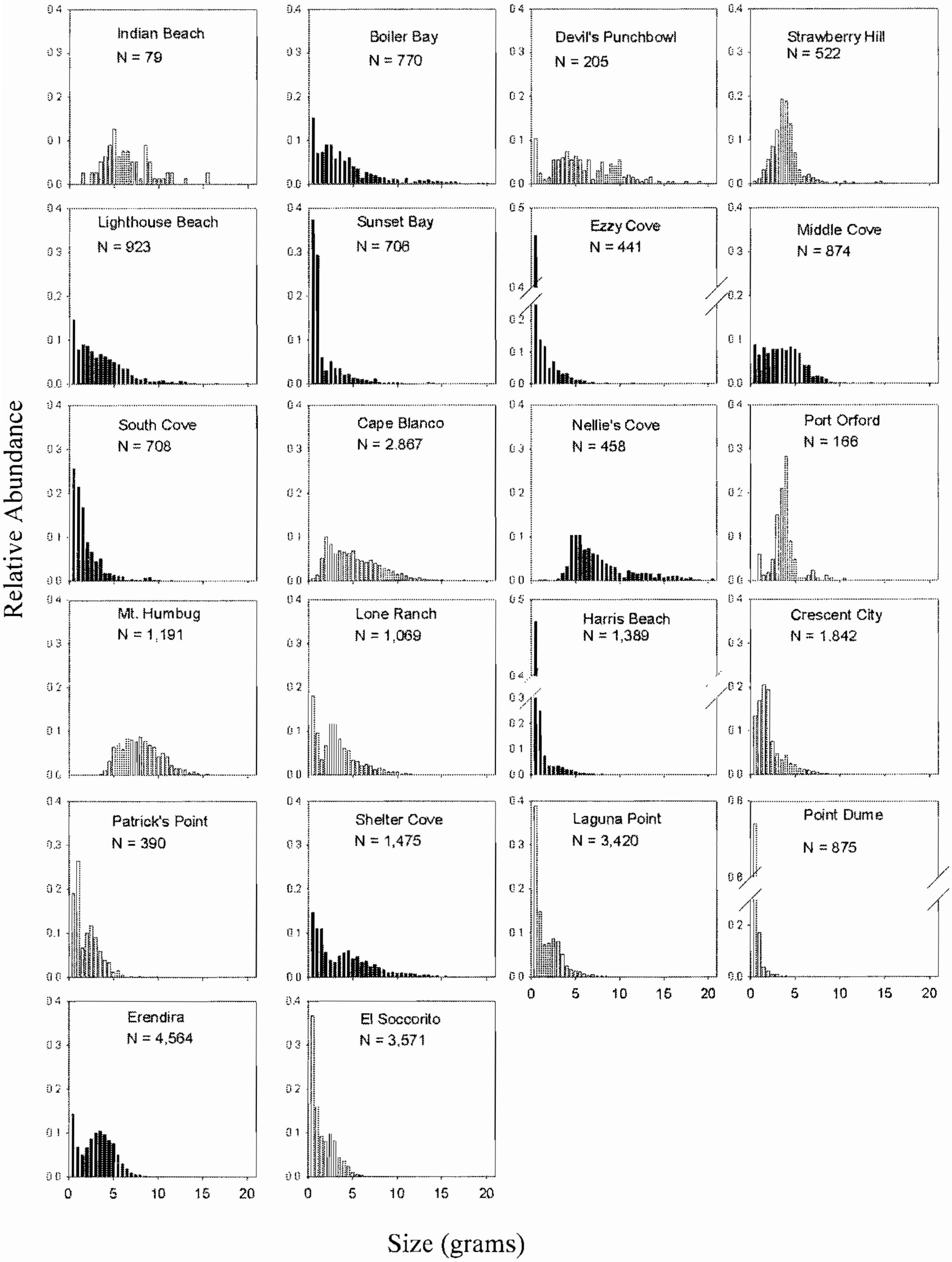
Size-frequency distributions were collected from 22 sites along the west coast of North America (Figure 4). The number of individuals measured from a population ranged from 75 to 4,564 and were collected from between three and five independent transect lines. Sites were grouped by coastal topography (protected or exposed) and latitudinal range (north or south of Brookings, OR (see methods)). Two size-frequency distributions, those weighted towards juveniles and those lacking large numbers of juveniles, were apparent from in populations north of Brookings. Populations also varied in the presence or absence of large individuals (> 5 g). In populations south of Brookings, all were weighted towards the juvenile size classes (< 2 g) and most populations had few individuals larger than 5 g.

Proportion of Juveniles

When a 2-way ANOVA was used with coastal topography and latitudinal region as factors, the differences based on coastal topography were not significant (F[1,18] = 1.19, p = 0.290). The difference between north and south was significant (F[1,18] = 10.57, p = 0.004) as was the interaction (F[1,18] = 5.98, p = 0.025).

The results of the 2-way ANOVA potentially mask differences within latitudinal region. A one-way ANOVA testing the impact of coastal topography on populations limited to the northern portion of the range (Brookings, OR to Indian Beach, OR) shows a significant

Figure 4: Size frequency distributions from populations of *Chlorostoma funebralis*. Populations in black are enclosed on at least 3 sides. Populations shown in gray are exposed to the open ocean on at least two sides. IB = Indian Beach; BB = Boiler Bay; DP = Devil's Punchbowl; SH = Strawberry Hill; LH = Lighthouse Beach; SB = Sunset Bay; EC = Ezzy Cove; MC = Middle Cove; SC = South Cove; CB = Cape Blanco; NC = Nellie's Cove; PO = Port Orford; MH = Mount Humbug; LR = Lone Ranch; HB = Harris Beach; CC = Crescent City; PP = Patrick's Point; ShC = Shelter Cove; LP = Laguna Point; PD = Point Dume; ER = Erendira; ES =El Socorrito


Indian Beach
N = 79
Boiler Bay
N = 770
Devil's Punchbowl
N = 205
Strawberry Hill
N = 522
Lighthouse Beach
N = 923
Sunset Bay
N = 706
Ezzy Cove
N = 441
Middle Cove
N = 874
South Cove
N = 708
Cape Blanco
N = 2,867
Nellie's Cove
N = 458
Port Orford
N = 166
Mt. Humbug
N = 1,191
Lone Ranch
N = 1,069
Harris Beach
N = 1,389
Crescent City
N = 1,842
Patrick's Point
N = 390
Shelter Cove
N = 1,475
Laguna Point
N = 3,420
Point Dume
N = 875
Erendira
N = 4,564
El Soccorito
N = 3,571
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
5
10
15
20
Relative Abundance
Size (grams)

(F[1,11] = 9.18, p = 0.021). Protected populations were heavily weighted towards juveniles, with populations averaging 59% juveniles, ranging from 30% to 77%, excluding Nellie's Cove. Exposed populations had few individuals in the juvenile size classes, averaging just 14% (ranging from 0% - 17%) of individuals. In the northern portion of the range, the only protected site that was not skewed towards juveniles was Nellie's Cove, near Port Orford, OR. This site had no juveniles and a large percentage of older individuals. When this site was removed from the one-way ANOVA, the difference in the relative abundance of juveniles between protected and exposed sites increases (F[1,11]= 25.27, p = 0.001).

In the southern portion of the range, Brookings OR to Baja California, there was no significant difference between protected and exposed sites in the proportion of the population that were juveniles (one-way ANOVA : F[1,7] = 0.96, p = 0.360). All sites were comprised largely of juveniles with very few sexual mature individuals. Protected populations had an average of 69% juveniles (ranging 32% - 82%), compared to exposed populations with 87% juveniles (ranging from 40% -97%).

When Nellie's Cove was removed from the 2-way ANOVA, the significance in coastal topography increases, but was still not statistically significant (F[1,17] = 3.94, p = 0.064), however, the strength of the interaction effect increases (F[1,17] = 13.72, p = 0.002).

Coefficient of Variation

The coefficient of variation (CV) is a measure of the dispersion of the size-frequency distribution (Table 1). When the CV's of populations were analyzed in a 2-way ANOVA, the results were similar to the percentage of the population in the juvenile size range. The difference between north and south was significant (F[1,18] = 5.01, p = 0.038), the difference between protected and exposed sites was not significant (F[1,18] = 3.41, p = 0.081), and the interaction between latitudinal range and coastal topography was significant (F[1,18] = 4.79, p = 0.042).

When analyses are separated by latitudinal region and the effects of coastal topography are tested with one-way ANOVA's, the results are again similar to the percentage of juveniles in populations. In the northern portion of the range, the difference in CV between exposed and protected sites was significant (F[1,11]= 11.27, p = 0.006). Exposed sites, which had a range of sizes of large individuals, had large CV's in comparison to protected sites, which had primarily small individuals (Table 1). In the southern portion of the range, CV's were not significantly different between protected and exposed sites (F[1,7] = 0.04, p = 0.841).

As with the percentage of the population in the juvenile size range, the significance of the coefficient of variation changes with the exclusion of Nellie's Cove. When Nellie's Cove was removed from the 2-way ANOVA, the latitudinal difference was no longer significant (F[1,17] = 4.18, p = 0.057), coastal topography becomes significant (F[1,17] = 5.35, p = 0.034), and the interaction term increased in significance (F[1,17] = 7.17, p = 0.016). In a one-way ANOVA testing coastal topography in the northern portion of the range, the significance of coastal topography increases with the removal of Nellie's Cove (F[1,10] = 19.28, $p = 0.001$).

Proportion of Large Adults

The distribution of individuals ten years (> 9g) and older has the same pattern as percentage of juveniles and CV in the 2-way ANOVA. There is no significant difference in the percentage of older individuals by exposure (F[1,18] = 0.09, $p = 0.765$), but there is a significant difference between north and south (F[1,18] = 9.31, $p = 0.007$), with a non-significant interaction factor (F[1,18] = 1.63, $p = 0.218$). However, there is no difference between protected and exposed in the northern portion of the study, tested with a one-way ANOVA (F[1,11] = 1.14, $p = 0.308$). When northern and southern sites are compared with a one-way ANOVA without coastal topography as a factor, there are significantly more large individuals in the northern portion of the range (F[1,18] = 10.69, $p = 0.004$).

Reproductive Output

Females with a mass ≤ 2 grams, or about 14 mm in diameter, did not have fully developed gonads. In *Chlorostoma funebralis* females larger than 2 grams, gonad mass increased linearly with increasing snail mass ($p < 0.0001$, $R^2 = 0.832$, Figure 5). Snail mass and diameter are strongly correlated, so snail diameter also has a significant regression with gonad mass ($p < 0.0001$, $R^2 = 0.730$). The concentration of eggs within gonads did not vary significantly with size, so the number of eggs produced by a female (extrapolated from gonad mass) increases linearly with snail weight ($p < 0.0001$, $R^2 = 0.337$, Figure 6).

To quantify potential egg production for populations, the number of eggs produced by each size class was calculated. The relative abundance of each size class in a population was calculated from the size-frequency distribution of the population and then standardized for 1000 individuals (Figure 7). The sum of egg production for an average representation of 1000 individuals was used as the relative egg production for each population. Because the relationship between size and egg production is linear, the number of eggs produced per 1000 individuals is derivative of the size-frequency distribution. However, statistical tests of the number of juveniles and large adults does not address the full distribution of sizes of adults and their egg production. Comparisons of egg production between populations tests the possibilities of differences due to the size-distribution of adults.

Larger individuals have the potential to produce more eggs than smaller individuals. Populations with a higher percentage of large individuals should produce more eggs per 1000 individuals than populations skewed towards the smaller size classes. In a 2-way ANOVA, exposure was not significant ($F[1,18] = 0.55$, $p = 0.469$) while latitudinal region was ($F[1,18] = 11.41$, $p = 0.003$) and the interaction between factors was significant ($F[1,18] = 5.06$, $p = 0.037$).

Egg production was not significantly different between coastal topographies in the north ($F[1,11] = 4.23$, $p = 0.064$) or in the south ($F[1,7] = 2.30$, $p = 0.174$). However, when Nellie's Cove was excluded from this analysis, the difference between exposed and

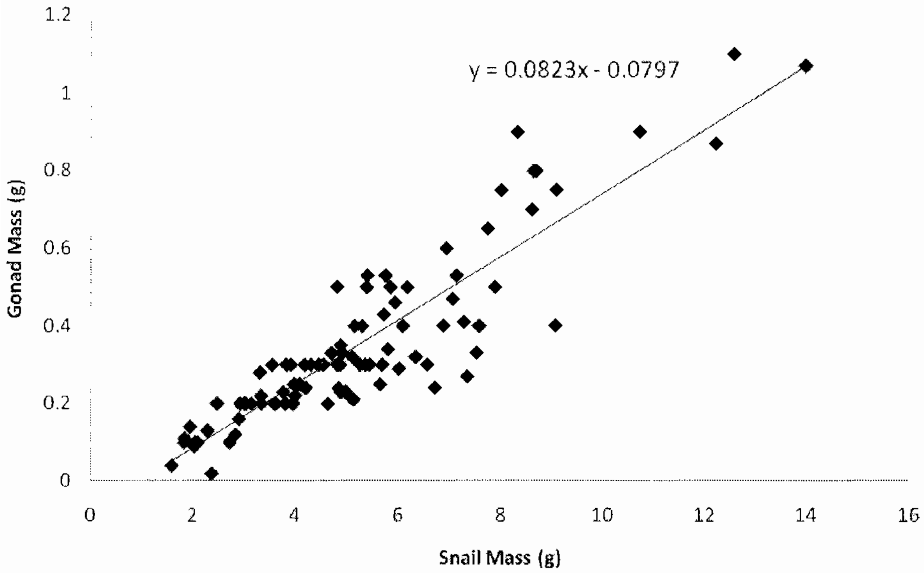


Figure 5: A significant linear regression was found between the total mass of a female and the mass of the gonad of *Chlorostoma funebralis* ($p < 0.0001, R^2 = 0.832$).

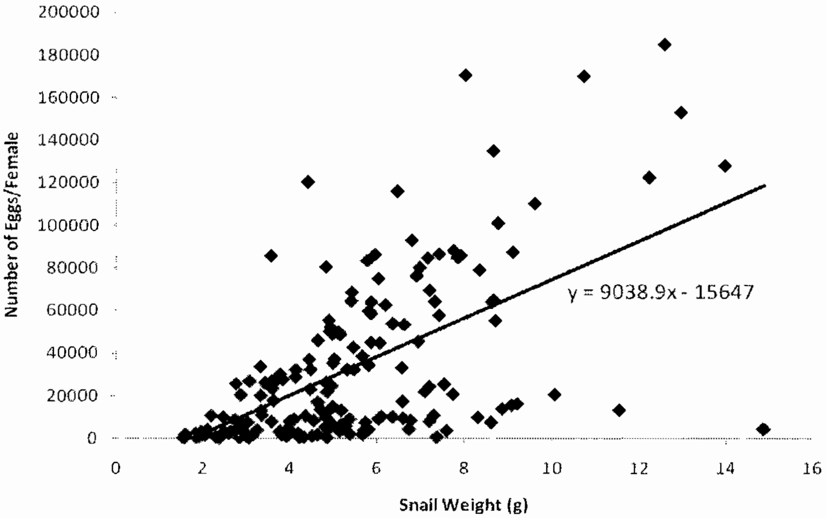


Figure 6: A significant linear regression was found between the total mass of a female and the total number of eggs produced ($p < 0.0001, R^2 = .337$).

protected sites in the north becomes highly significant (F[1,10] = 12.98, p = 0.005). In the northern portion of the study, exposed populations had high percentages of large individuals that contribute to the relative egg production of the population. Protected sites in the northern portion of the study are skewed towards the juvenile size classes but some populations, such as Boiler Bay and South Cove, also have a small percentage of larger individuals which will produce large numbers of eggs. The average egg production per 1000 individuals in the northern portion of the study was 2.75 x 10^7.

In the 2-way ANOVA without Nellie's Cove, latitudinal region was significant (F[1,17] = 13.49, p = 0.002) while coastal topography was not (F[1,17] = 2.51, p = 0.132). The interaction in the 2-way ANOVA was more significant than when Nellie's Cove was included (F[1,17] = 12.09, p = 0.003, from p = 0.037). This was because removing Nellie's Cove has increased the overall difference between exposed and protected sites in the northern portion of the range.

Comparing northern and southern regions without using coastal topography as a factor, egg production was significantly higher in the northern populations (F[1,18] = 10.74, p = 0.004). In the southern portion of the study, populations were heavily skewed towards pre-reproductive juveniles and small adults, which contribute little to the total egg production of the population. The average egg production per 1000 individuals in the southern portion of the study was 9.72 x 10^6, lower than the average egg production of populations in the north.

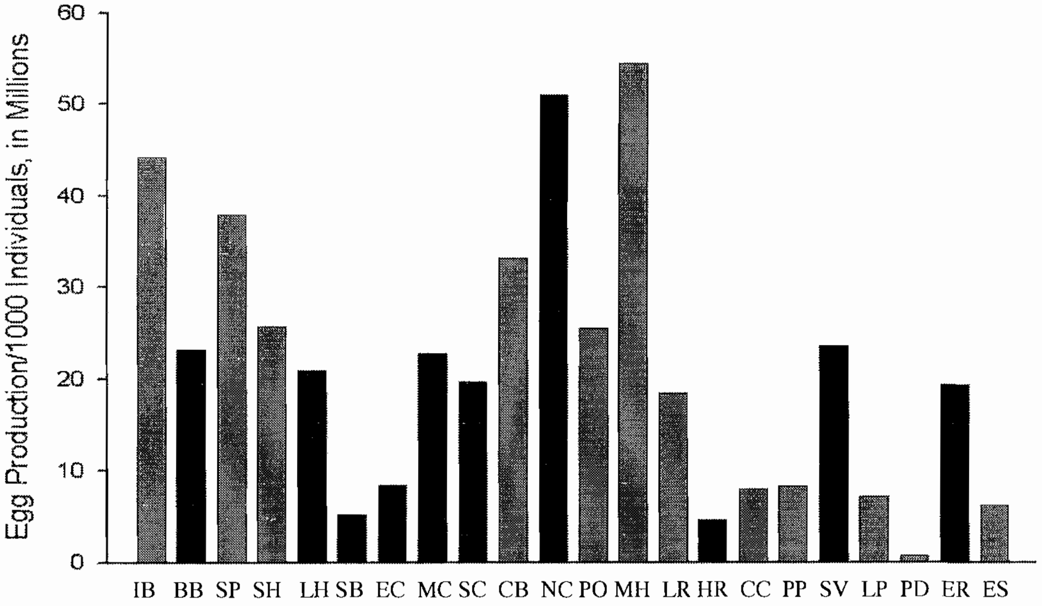


Figure 7: Egg production (in millions) per 1000 individuals of populations of *C. funebralis* , displayed from highest to lowest latitude, left to right. Shaded populations are exposed, black populations are protected. Egg production is calculated by gonad size and population size-frequency distribution. Northern sites are defined as north of Lone Ranch (LR). Protected sites are identified as (P), exposed sites by (E). IB = Indian Beach (E); BB = Boiler Bay (P); DP = Devil's Punchbowl (E); SH = Strawberry Hill (E); LH = Lighthouse Beach (P); SB = Sunset Bay (P); EC = Ezzy Cove (P); MC = Middle Cove (P); SC = South Cove (P); CB = Cape Blanco (E); NC = Nellie's Cove (P); PO = Port Orford (E); MH = Mount Humbug (E); LR = Lone Ranch (E); HB = Harris Beach (P); CC = Crescent City (E); PP = Patrick's Point (E); SV = Shelter Cove (P); LP = Laguna Point (E); PD = Point Dume (E); ER = Erendira (P); ES =El Socorrito (E)

Discussion

The structure of populations of *Chlorostoma funebralis* vary with both coastal topography and latitude. Coastal topography was a significant factor only in the northern portion of this study. In the northern region, populations in protected areas show a much higher percentage of juveniles than exposed populations, which are skewed toward large adults. In the southern portion of the study, population structure did not vary with coastal topography, and all populations were primarily composed of juveniles.

These observed patterns are most likely due to the combination of several factors. One component of size-frequency distributions, the presence or absence of juveniles, is due to factors affecting recruitment such as larval supply and post-settlement mortality. In contrast, the distribution of adults in populations is due to the sum of numerous factors. The numbers of adults in a population is a reflection of recruitment events in past years, as well as mortality due to predation and physical stress that has occurred during the intervening years.

An absence of juveniles in exposed populations may be caused by low and/or irregular larval supply, a failure of larvae to survive metamorphosis (settlement), or high mortality rates of newly metamorphosed juveniles. Protected sites may have had more juveniles because larval supply is high and populations experience high settlement with little inter-annual variation. Exposed sites may have low larval supply due to movement of larvae offshore, punctuated with occasional years of high settlement. This pattern of high inter-annual variation would result in populations where juveniles are rare except during certain years. This is one potential mechanism that would create the size-frequency distributions described by this study.

Larval supply may drive patterns of recruitment. Several studies have shown relationships between protected bays and coves and larval retention (Archambault et al 1998, Archambault and Bourget 1999, Shanks et al 2003, McQuaid and Phillips 2006). The hydrography of protected areas may retain larvae for long enough periods that larvae are unable to disperse outside of the protected area on a large scale. Many studies of larval dispersal and retention have focused on species with larval periods of weeks, such as mussels and barnacles (Menge et al 2004, Shanks and Brink 2005, McQuaid and Phillips 2006). The shorter larval period of *C. funebralis* (five to eight days) should amplify the effects of retention since there will be less time and opportunity for hydrographic conditions to change and for larvae to move offshore. Topographically generated fronts at the mouth of protected bays and coves can last for days (Shanks et al 2003), long enough to retain *C. funebralis* for the entirety of its larval period. Larval retention would increase larval supply, and the resulting recruitment rate should be higher and have lower inter-annual variation.

Studies with mussels show a positive correlation between high recruitment and gamete production within a protected population (McQuaid and Phillips 2006). Larval retention within this protected area results in high larval supply. This previously described pattern is unlikely to be mechanistically similar to patterns observed in *Chlorostoma funebralis*. In this study, the highest reproductive effort was observed in exposed, northern populations with the fewest juveniles. If reproductive effort was directly tied to larval supply and recruitment as in McQuaid and Phillips (2006), exposed populations with the largest individuals would also have large numbers of juveniles.

If large numbers of juveniles in protected habitats north of Brookings, Oregon was due to larval retention within bays and coves, the lack of juveniles in most exposed populations may be due to larval movement offshore. Populations at exposed sites have few individuals in the juvenile size classes, indicating poor recruitment in the previous years. Several exposed sites, such as Indian Beach and Devil's Punchbowl, have multiple peaks in the size-frequency distribution (Figure 4). If these peaks reflect years of high recruitment separated by gaps of poor recruitment, these populations have high inter-annual variation in recruitment. Exposed sites experience more wave energy, which may wash larvae away from the parental site, and larvae are only moved onshore at these sites during years when hydrographic conditions move larvae onshore during the spawning period. Many gastropods are known to spawn during wave events (Orton and Southward 1961, Bowman and Lewis 1977, Thompson 1979, Creese and Ballantine 1983). Some species in the genus *Tegula* spawn during major wave events in Japan (Sasaki and Shepherd 1995) and return to shore, potentially by remaining within local wave generated circulation cells. If *C. funebralis* larvae have similar methods for remaining close to shore, larval supply may remain high annually, and inter-annual variation in recruitment may be due to inter-annual variation in post-settlement mortality.

The population in Nellie's Cove was different from all other protected sites in the north. Other protected populations in the northern portion of this study had a large portion of the population in the juvenile size classes, and few large adults. Nellie's Cove, in contrast, is a protected population with no juveniles and a high percentage of large adults. Nellie's Cove is a south-facing cove on the south face of Port Orford Headlands. This site is protected during the summer, when wave energy is primarily from the north (Shanks et al 2003), but is exposed to storm waves from the south in the fall and winter; it is the only protected site in this study that is exposed to storms from the south in the fall. *C. funebralis* larvae are expected to be in the water column between August and October in northern populations (Belcik 1965, Paine 1971, Moran 1997), hence, the period of storm waves may overlap with the spawning window preventing larval retention in an otherwise protected site.

Differences in recruitment between protected and exposed populations may also be due to post-settlement mortality. The small size of recently metamorphosed individuals and the likelihood of early post-settlement mortality make the youngest juveniles difficult to enumerate. The size of *C. funebralis* at metamorphosis is between 240 and 260 μm (Moran 1997, Guzmán del Próo et al 2006) and the smallest individuals collected in this study were approximately 500 μm. Using juvenile growth rates observed in the laboratory, the smallest juveniles collected in this study may be between two and six months old (Moran 1997, Guzmán del Próo et al 2006). The smallest individuals sampled had settled months before they were counted, hence, we cannot differentiate between the effects of larval supply and variation in mortality in the first few months after metamorphosis on the population structure.

Variation in post-settlement mortality may be caused by differences in juvenile habitat between protected and exposed sites that exist only within the northern portion of the population. These differences may include wave energy, which may cause differences in temperature, oxygen levels, sedimentation, food availability and sediment movement. However, juveniles are found in the high intertidal, exclusively in coarse sediment under boulders and very protected cracks on rocks (personal observation). There is no immediately obvious biotic or abiotic factor within this micro-habitat that would differ systematically between protected and exposed populations.

South of Brookings, Oregon, all populations sampled showed similar size-frequency distributions; the distributions were heavily skewed toward juvenile *C. funebralis*. No difference is apparent between northern and southern California and Baja California. This pattern was also observed by Frank (1975), who proposed that in the southern portion of the species range, individuals have shorter life spans, faster growth rates, and reproduce throughout the year rather than annually spawning as in the north. In southern California, a conjoining species, *Chlorostoma* (*Tegula*) *eiseni*, spawns and recruits throughout the year (Wolf 1991). In Oregon and Washington, *C. funebralis* spawns in late summer or fall (Belcik 1965, Paine 1971, Moran 1997); spawning of *C. funebralis* has not been studied in California and the species may show variation in reproductive strategy in different latitudinal regions. If, like *C. eiseni*, *C. funebralis* in California reproduce throughout the year, they may be using a reproductive strategy which allows for more opportunities to spawn during hydrographic conditions that allow larvae to remain close to shore, regardless of local coastal topography.

Non-seasonal reproduction may be necessary for *Chlorostoma funebralis* in the southern portions of its range. By reducing inter-annual variation in recruitment success, *C. funebralis* populations in the south may be sustained despite the shorter life span of individuals indicated by growth rate data (Frank 1975) and the size-frequency distributions from populations sampled in California. Individuals in central and southern California rarely live longer than seven years (Frank 1975), so inter-annual variation in recruitment must be dampened in comparison to populations in Oregon, where individuals may live up to 30 years (Frank 1975) and so have many more years to attempt reproductive success. Further studies of the reproductive and recruitment seasonality on a latitudinal gradient are necessary to determine if *C. funebralis* reproduce year-round in the southern portion of the species range and annually in the northern portion of the range.

Differences in population structure may also be due to differences in predation rates. Juvenile *C. funebralis* are found in the high intertidal (Paine 1971, Frank 1975, Moran 1997), generally above the tidal height of the adult predators, so it is unlikely that these predators have an effect on the number of juveniles in a population. The main predators through the species range of *C. funebralis* are the seastar *Pisaster ochraceus* and crabs of the genus *Cancer*. In southern California, intertidal octopus (*Octopus bimaculoides* and *O. bimaculatus*) are also important predators (Fawcett 1984). *P. ochraceus* and *Cancer spp.* abundances do not vary on a latitudinal gradient (Fawcett 1984), but the added predation pressure of *Octopus spp.* in southern California and Baja California may cause the absence of larger *C. funebralis* in those populations. Predation pressure of *P. ochraceus* and *Cancer spp.* may vary along the Oregon coast, which could explain why some populations, such as Devil's Punchbowl and Cape Blanco, have many large individuals, while other populations, such as Sunset Bay have very few large individuals. No studies have identified potential predators on the juvenile stages of *C. funebralis*, which will be necessary to fully understand the effects of predation on population structure.

Although two latitudinal regions are clear from the data collected in this study, they do not conform to the predictions of population structure on a latitudinal gradient predicted by upwelling regions. The intermittent upwelling during summers north of Cape Blanco has been hypothesized to move larvae offshore during upwelling and transport them back toward shore during relaxation of upwelling (Roughgarden et al 1988, Menge et al 2004). It has been hypothesized that constant upwelling south of Cape Blanco offers few opportunities for larvae to return to shore, leading to low recruitment with high inter-annual variation. In contrast, the data collected in this study show high recruitment success in California and southern Oregon where upwelling is more persistent. North of Brookings, Oregon, where upwelling is more variable, population structure varies with coastal topography. *C. funebralis* may not be as affected by upwelling patterns as other species with longer larval periods. Surviving larvae of *C. funebralis* may be retained close to shore and metamorphose before they are moved far offshore. If the larvae are transported offshore by any mechanism, the larval period will not last long enough to allow a return to shore.

Population structure of *C. funebralis* varies with both coastal topography and latitude. Numerous factors such as local hydrodynamics, post-settlement mortality, and predation may all contribute to the observed patterns. Local, small-scale processes may be important in determining larval settlement and population dynamics, but this spatial scale is often ignored in favor of large scale observations on a latitudinal gradient. Without further studies that use both meso- and large-scale observations, we will not be able to understand the relative importance of these factors.

Bridge I

Size-frequency distributions of *Chlorostoma funebralis* vary between the northern and southern portions of the species range, with the divide just north of the Oregon-California border. In the northern portion of the range, coastal topography plays a significant role in determining the presence or absence of juveniles. Size-frequency distributions in protected areas are weighted heavily towards the juvenile size classes, but exposed populations have more adults. In contrast, populations in the southern portion of the range are smaller and populations are dominated by juveniles, regardless of coastal topography.

One potential source of variation on a latitudinal gradient is differences in the timing of reproduction. If populations in the southern portion of the range reproduce throughout the year, this behavior may increase the probability of having at least one reproductive event each year. In contrast, if northern populations spawn in one episodic event each year, this may increase the variability in reproductive success each year, causing gaps in the size-frequency distributions. This hypothesis is tested in Chapter III using a time series of reproductive stage in San Diego, California and Cape Arago, Oregon.

CHAPTER III

VARIATION IN SEASONALITY OF REPRODUCTION AND REPRODUCTIVE STRATEGIES OF *CHLOROSTOMA* (*TEGULA*) *FUNEBRALIS* ON A LATITUDINAL GRADIENT

Introduction

The supply of larvae has long been hypothesized as a driving factor in structuring intertidal communities (Ebert and Russell 1988, Roughgarden et al 1988, Menge et al 2004). Because space is often a limiting factor in the intertidal (Connell 1961, Stachowicz et al 1999), the timing and success of larval supply and recruitment events may have important repercussions for population and community ecology in the intertidal. Marine invertebrates have been shown to spawn in response to a number of environmental cues, including but not limited to temperature, tidal cycle, and wave energy (Giese and Kanatani 1987). These cues may signify optimal conditions for larval survival or dispersal, or in species with external fertilization may increase the chances of synchronous spawning and increased probability of fertilization.

Populations of marine invertebrates show several types of reproductive cycles. Spawning of individuals can be synchronized and seasonal, in which case individuals will all be at the same stage of gametogenesis and females will produce mature oocytes at the same time. In contrast, populations that reproduce continuously can be produced in two ways: individuals that spawn only once but asynchronously with other individuals in the population, or individuals that spawn continuously throughout the year (Eckelbarger and Watling 1995). Investigation of oocytes can distinguish between these two patterns – if the number of oocytes in the ovary of one female but not others decreases precipitously at one time point, it indicates that the female is spawning in one event but asynchronously with the rest of the population. If females have multiple stages of oocytes present in the ovary continuously, it is likely that individuals are "dribbling", or releasing a few eggs throughout the year. However, it can be difficult to distinguish this pattern from individuals that maintain mature oocytes for long periods and spawn on a non-seasonal schedule that cannot be determined by irregular sampling methods (Tyler and Young 1992).

The timing of spawning may have important implications for larval survival and dispersal. Spawning during high wave energy events can reduce the time larvae spend in the surf zone, which may reduce the chances of predation (Shanks 1998). These events can co-occur with periods of downwelling, which pushes surface waters onto shore and may limit the distance larvae are transported offshore (Sasaki and Shepherd 1995, Shanks 1998). However, models suggest that fertilization success for broadcast spawners should be limited during high energy, turbulent periods (Denny and Shibata 1989).

The intertidal gastropod *Chlorostoma (Tegula) funebralis* ranges from Baja California to Vancouver Island (Morris et al 1980). *C. funebralis* are broadcast spawners with external fertilization and a larval period of five to eight days (Moran 1997, Guzmán del Próo et al 2006). The size-frequency distributions of *C. funebralis* populations differ significantly along a latitudinal gradient (Chapter I). In the southern portion of the range, populations consist mainly of small juveniles (Frank 1975, Wright 1975, Chapter I). In the northern portion of the species range, coastal topography is correlated with size distributions, with protected populations containing juveniles and exposed populations with few juveniles. This division between southern and northern populations occurs in southern Oregon (Chapter I).

In Washington populations of *C. funebralis,* spawning is hypothesized to occur synchronously between May and September, as indicated by a significant drop in the energy content of females (Paine 1971). Spawning in the laboratory has been observed in Oregon in mid-September (Moran 1997). The presence of juveniles in the field indicates recruitment between May and December in Oregon (Frank 1975, Moran 1997) but potentially year-round in California (Frank 1975, Wright 1975). *Chlorostoma eiseni*, a conjoining species to *C. funebralis* in southern California, spawns year-round (Wolf 1991).

If *C. funebralis* populations in the southern portion of the species range spawn continuously or multiple times throughout the year, this may increase the probability that there will be successful recruitment each year, increasing the number of juveniles if populations are limited by larval supply. In contrast, if populations in the northern portion of the species range reproduce seasonally and episodically, successful recruitment may not occur every year, creating high inter-annual variation in the size-frequency distributions. This study tests this hypothesis by examining the timing of oogenesis throughout an annual cycle in two populations, one in San Diego, California and the other in Charleston, Oregon.

Methods

Sample Collection and Embedding

From August 2008 – September 2009, 20 females were collected once a month from Cape Arago, Oregon (43°18'12N, 124°23'56W), excepting November 2008. Twenty females each month were collected from San Diego (32°48'50N, 117°16'23W) from April 2009 - March 2010, excepting May, September and November 2009.

The ovaries of *Chlorostoma funebralis* are distinct in the adult body due to their green color (Moran 1997) and were easily dissected from the snail body and preserved in 6 – 8 % recycled formalin. After 48 - 72 hours, samples were transferred to 70 % Ethanol for long term storage. A dehydration series in ethanol was followed by toluene for 24 hours, followed by overnight in melted paraffin wax (mp = 52°) and then embedded.

Sections of 5 μm thickness were cut and mounted on glass slides. At least three slides were made for sections of each individual. Slides were stained with Harris modified Hematoxylin, counterstained with Eosin, and glass cover slips were permanently fixed to slides with Permount. Pictures were taken of each slide under 20X magnification for later analysis.

Analysis

Two methods were used to assess the reproductive stage of each female. The sizes of 100 eggs were measured from each individual using ImageJ (Rasband 2008). Because oocyte shape may be irregular due to tight packing in the ovary, oocyte feret diameters were calculated (Feret diameter = $((4 \text{ x area})/\pi)^{1/2}$) to standardize oocyte size (Tyler et al 2008). Only individuals with a visible nucleolus were sampled to avoid the resampling of individual oocytes over several sections. Individual females were analyzed using an ANOVA, with individuals nested within dates (Grant and Tyler 1983b). Diameters were log-transformed to equalize variances.

Because of the high variance in individual oocyte diameters, a categorical method of estimating reproductive stage, a maturity index, was also used for each specimen (Grant and Tyler 1983a). After examination of stained specimens, five stages of reproductive status were designated, modified from Patent (1969), Grant and Tyler (1983a), and Tyler et al (2008). Individual specimens were assigned a reproductive stage number of one through five, dependent on the ratio of vitellogenic to previtellogenic oocytes within each female. Previtellogenic oocytes were identified as those that did not stain pink in the Eosin counter-stain, indicating a lack of yolk within the egg. Reproductive stages were analyzed with a single-level ANOVA with dates as a fixed factor (Grant and Tyler 1983a) and a Tukey HSD for comparisons between consecutive months. Maturity index data were square root transformed to make variances more equal.

Maturity index stages were identified as follows:

Stage 1, Post-spawning: Nearly all oocytes are previtellogenic, with relict oocytes very rare (Patent 1969). Oocytes are not tightly packed (Figure 8A). *Stage 2, Early Growth:* Most area in ovary (> 50) consists of previtellogenic oocytes, with 2 – 5 % of area consisting of small vitellogenic oocytes. Oocytes are not tightly packed (Figure 8B). *Stage 3, Mid – Cycle:* 6 – 50 % of ovary consists of previtellogenic oocytes, with both small and full-sized vitellogenic oocytes present. Some oocytes are packed tightly together (Figure 8C). *Stage 4, Pre-Spawning:* Less than 5 % of ovary is previtellogenic oocytes. Oocytes are tightly packed (Figure 8D). *Stage 5, Spawning:* All oocytes are large and vitellogenic and packed tightly within the ovary (Figure 8E).

Results

Oocyte Size

Oocytes ranged in size from 50 μm for previtellogenic oocytes to 250 μm for the largest mature oocytes. Diameters of spawned eggs have been estimated at 200 μm (Moran 1997). Spawning is rarely complete as mature, vitellogenic oocytes were seen in nearly all females. Previtellogenic oocytes were observed in most, but not all, individuals.

In the Oregon population, oocyte size-frequency distributions did not show seasonal variation (Figure 9A), as evidenced by the lack of significant differences among dates in a nested ANOVA (F[12,247] = 0.25, p = 0.99). If individuals were spawning continuously throughout the sampling period, all females should have a wide

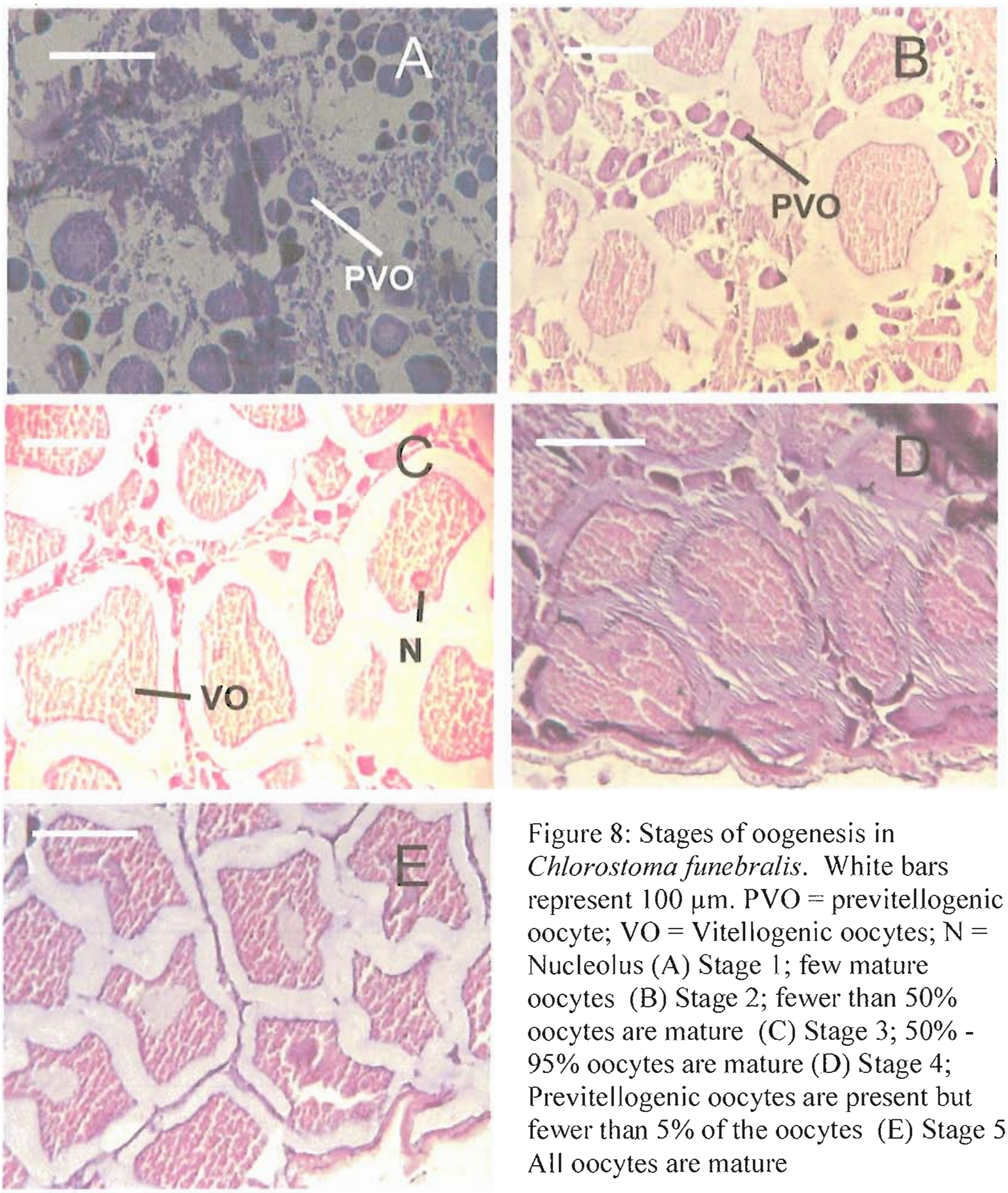


Figure 8: Stages of oogenesis in *Chlorostoma funebralis*. White bars represent 100 µm. PVO = previtellogenic oocyte; VO = Vitellogenic oocytes; N = Nucleolus (A) Stage 1; few mature oocytes (B) Stage 2; fewer than 50% oocytes are mature (C) Stage 3; 50% - 95% oocytes are mature (D) Stage 4; Previtellogenic oocytes are present but fewer than 5% of the oocytes (E) Stage 5; All oocytes are mature

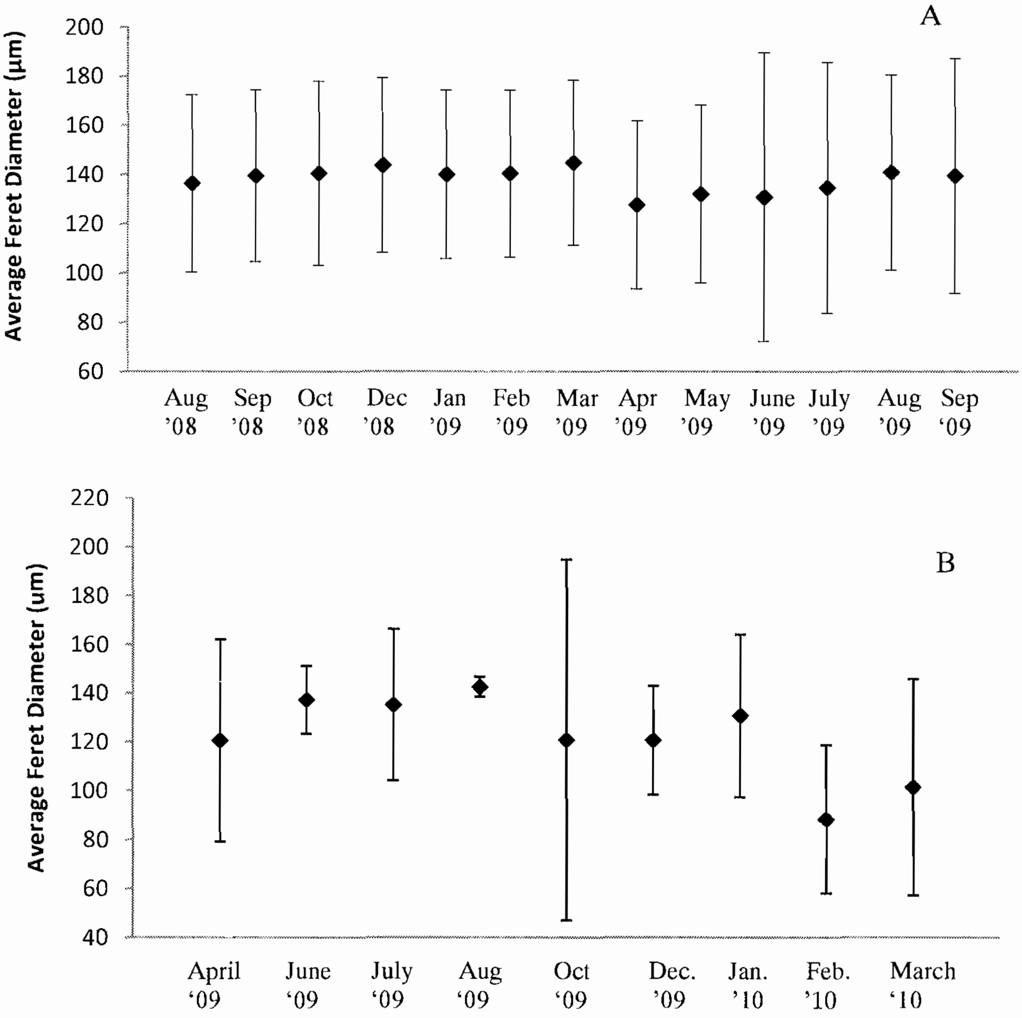


Figure 9: Average oocyte feret diameter of female *C. funebralis*. Vertical error bars represent 95% confidence intervals. A. Individuals collected from Cape Arago, Oregon. B. Individuals collected from San Diego, California.

range of oocytes present in the ovary and no differences will be observed between individuals. This is the case as shown by individuals, nested within dates ($F[247,25740] = 0.36$, $p = 0.99$).

A similar pattern was observed for the southern California population (Figure 9B). No difference was detected among dates ($F[8,171] = 0.70$, $p = 0.6952$) or among individuals nested within dates ($F[171,17820] = 0.63$, $p = 0.999$).

Maturity Index

The maturity index offers a more general overview of each female's reproductive stage than oocyte size-frequency distributions. Although individuals of all stages were observed, most individuals were between stages three and four (Figure 10). In the Oregon population, dates were significantly different from each other ($F[12,247] = 5.686$, $p < 0.001$). Tukey's post-hoc comparisons show a significant drop in average maturity index between March 2009 and April 2009 ($p = 0.009$, Figure 10A, Figure 11). This drop in maturity index is also observed as a decrease in mean oocyte diameter (Figure 9A), although the difference in oocyte diameter between March and April 2009 is not statistically significant. A significant increase in maturity index was observed between July 2009 and August 2009 ($p = 0.001$).

In the southern California population, most individuals were also between stage three and stage four throughout the year (Figure 10B, Figure 12). There were significant differences among sampling dates ($F[9,171] = 8.545$, $p < 0.001$). Significant drops in the maturity index were observed between August 2009 and October 2009 ($p < 0.001$) and

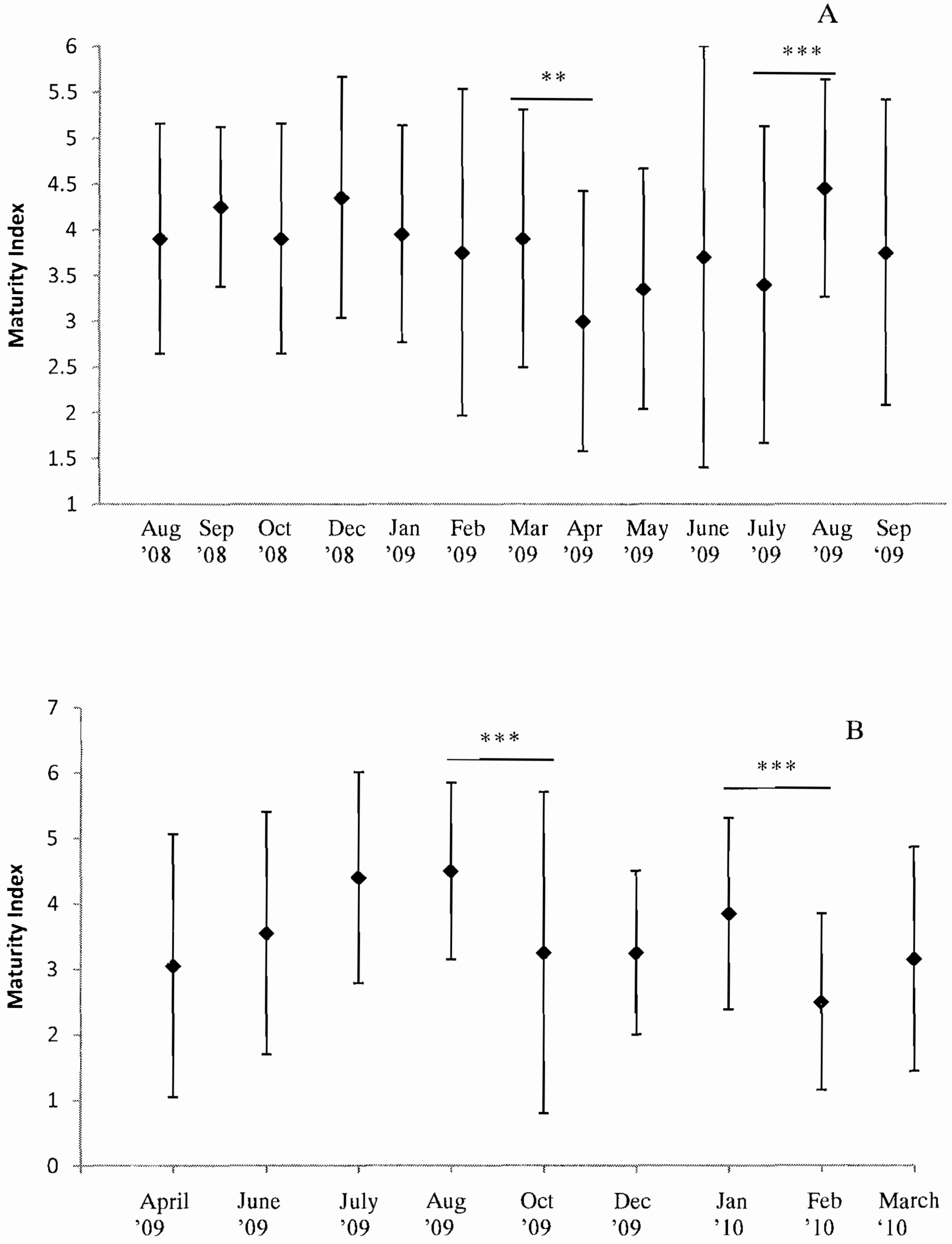


Figure 10: Average of maturity indices of individual *C. funebralis*. Vertical bars represent 95% confidence intervals. Horizontal bars represent monthly pairs with a significant change in average maturity index. (A) Cape Arago, August 2008-September 2009. (B) San Diego, April 2009-March 2010. ** = ($p < 0.01$); *** = ($p < 0.001$)

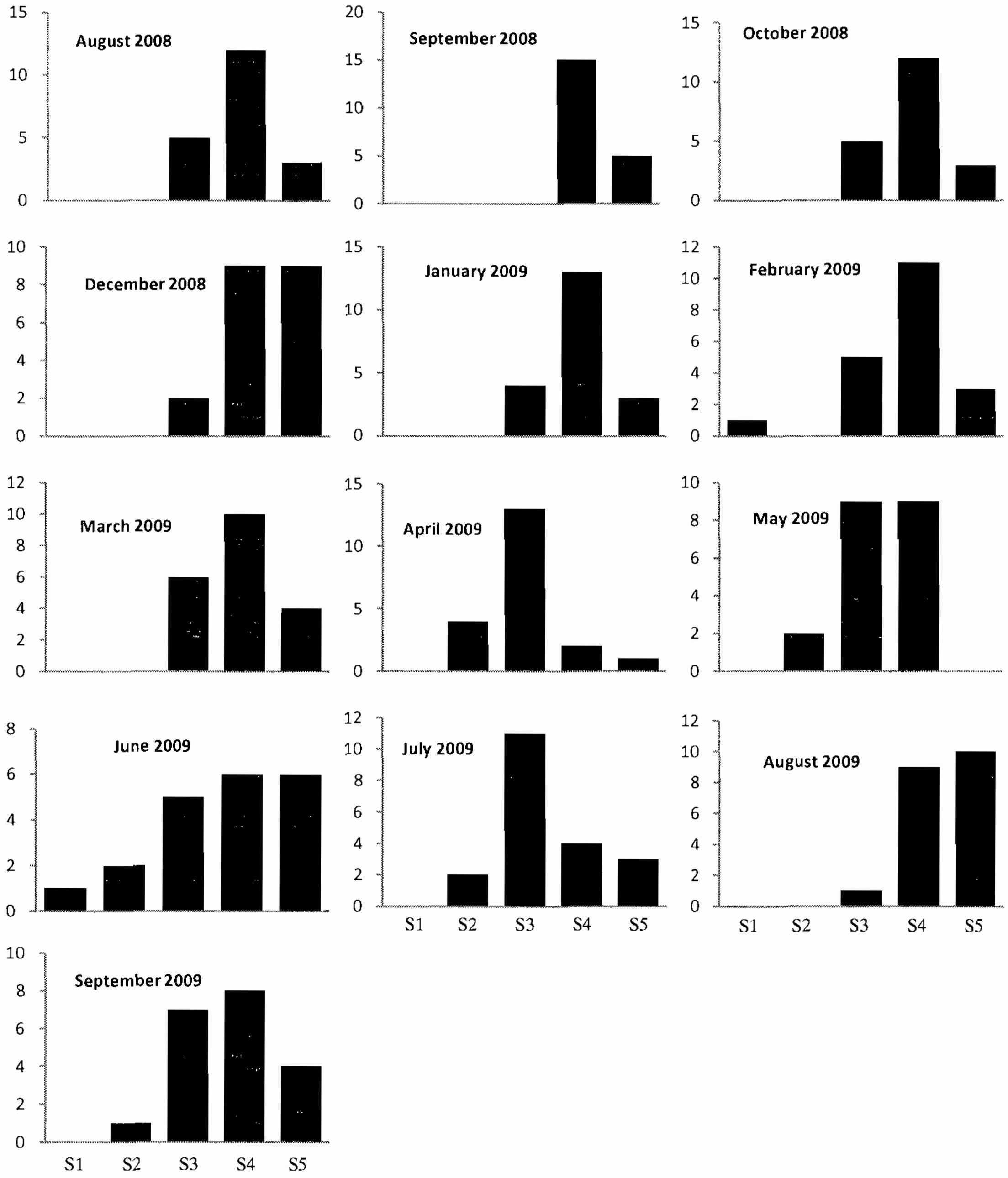


Figure 11: Distribution of maturity indices of *C. funebralis* from Cape Arago, Oregon collected August 2008 through September 2009. Maturity index was assigned on a scale of 1 (no mature oocytes) through 5 (fully mature oocytes).

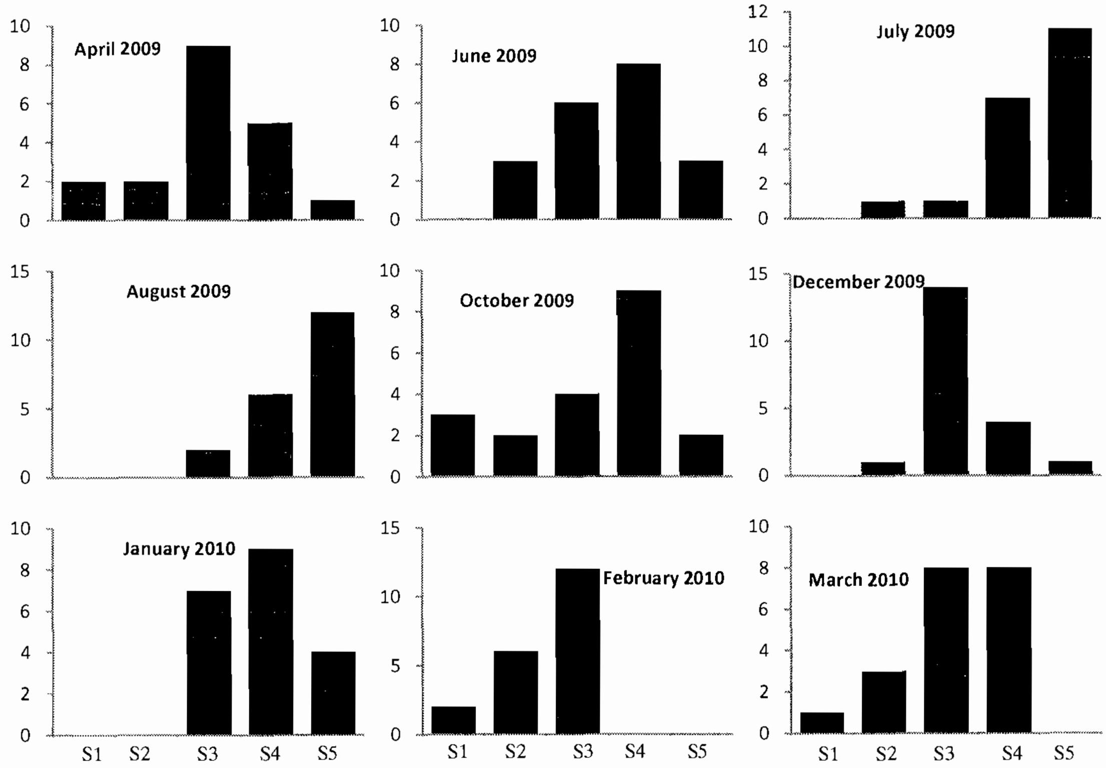


Figure 12: Distribution of maturity indices of *C. funebralis* from San Diego, California collected April 2009 through March 2010. Maturity index was assigned on a scale of 1 (no mature oocytes) through 5 (fully mature oocytes).

January 2010 and February ($p < 0.001$, Figure 10B, Figure 12). No significant increases in maturity index were observed between consecutive months.

Discussion

Both populations sampled in this study had synchronous spawning events where a number of females in the population shed mature eggs simultaneously, as indictaed by a large drop in the average maturity index. Females likely to spawn have ovaries packed with large, mature oocytes and were identified in stages four and five of oogenesis. One spawning period occurred in the Oregon population during the observed 13 months, between March and April, when a large decrease occurred in the number of females in the fourth and fifth stages of oogenesis. This coincides with a decrease in the average feret diameter of oocytes between March and April, although this decrease was not statistically significant. Even after spawning, at least a few females ready to spawn were present in the Cape Arago population throughout the year. This may allow for some continuous spawning of a few mature oocytes throughout the year, or a population-level readiness for another spawning event. Continuous monitoring of the population, with multiple samples taken every month, will be necessary to distinguish between these two patterns.

The spawning event observed in this study is earlier in the year than those observed by Paine (1979) in Mukkaw Bay, Washington, where *Chlorostoma funebralis* spawned from June to September. This spawning event was also earlier than the spawning period estimated from juvenile growth rates and size in the field by Moran

(1997), in a study that estimated larval settlement between July and December. Recruitment of *C. funebralis* juveniles has been observed at Cape Arago in September and October (Cooper, unpublished data).

The population from San Diego had the largest proportion of mature females in July and August, followed by a spawning event shown in the drop in maturity index. The number of females ready to spawn recovered by January, and another spawning event occurred between January and February, again indicated by a drop in maturity index. Between October and December, there was a drop in the number of individuals in stages four and five, but this difference is not statistically significant and may not represent a third spawning event over the observed year.

At least two spawning events were observed over one year in San Diego, in contrast a single spawning event at Cape Arago. By spawning multiple times in a year, *C. funebralis* in San Diego may increase the probability of a successful recruitment event every year. *C. funebralis* south of central California are smaller than those in Oregon or Washington (Frank 1975, Wright 1975) and produce fewer eggs due to smaller gonads (Chapter I). Spawning multiple times in one year may maximize the number of potential recruits within the size limitations of individuals in southern populations. Northern populations may delay reproductive effort in favor of attaining larger body size. Body size correlates with the number of eggs produced (Chapter I) so a delay in reproduction may ultimately lead to a larger reproductive effort. Southern populations may have limited body size due to increased reproductive effort, limiting the scope of growth remaining to increase body size.

Chlorostoma funebralis in Oregon and Washington can live up to 30 years (Darby 1964). In contrast, individuals in southern California live only five to seven years (Frank 1975). Reproducing several times throughout the year in the southern portion of the species range may be a shift in reproductive strategy that allows populations to compensate for a shorter life span and smaller maximum size. The number of eggs produced by a female increases with size (Chapter II), so southern populations weighted towards small individuals will not produce as many gametes as populations with large individuals in a single spawning event. While populations in the northern portion of the species range can be self-sustaining with high inter-annual variation in recruitment (Chapter I), the shorter lived individuals of California utilize multiple reproductive events in a year to hedge against environmental conditions that may cause larval or juvenile mortality.

Because samples were taken approximately one month apart, this study cannot determine if spawning occurred on a single tidal event or over the course of several days or weeks. However, for broadcast spawners with external fertilization, there is a significant advantage for a large portion of the population to spawn at the same time. Where distances between a spawning male and female are significant, eggs are less likely to be fertilized (Pennington 1985, Levitan et al 1992, Sewell and Levitan 1992). *Chlorostoma funebralis* can naturally occur in densities of more than $1000/m^2$ (Paine 1969) so may not need to further aggregate if spawning is synchronous. However, naturally high densities may mean that populations need to spawn synchronously only within a very limited area for successful fertilization. If local aggregates spawn synchronously with each other but asynchronously with the rest of the population, the resulting data would not show these local effects.

Although collecting samples on a monthly basis allows for the identification of mass spawning events, the temporal scale is not fine enough to identify potential cues for spawning. Along the Oregon coast, the spring transition occurs between March and June and marks the start of the seasonal upwelling (Barth et al 2000). Prior to the spring transition, downwelling conditions dominate along the Oregon coast, keeping surface waters close to shore. Following the spring transition, downwelling periods are separated by periods of strong upwelling (Barth et al 2000). Upwelling may have significant effects on larval mortality by moving surface waters offshore (Roughgarden et al 1988, Menge et al 2004). Some gastropods have been documented to spawn during conditions that indicate downwelling (Sasaki and Shepherd 1995, Shanks 1998). However, the larvae of many invertebrate taxa reside well below surface waters and so are not moved offshore during upwelling (Shanks and Brink 2005, Morgan et al 2009, Shanks and Shearman 2009), presumable reducing larval mortality due to offshore movement. *C. funebralis* may be cuing to downwelling conditions; however, further sampling will be necessary to test this hypothesis.

As predicted by Frank (1975) and Wright (1975), *C. funebralis* in southern California spawned multiple times throughout the year. In contrast, *C. funebralis* at Cape Arago, Oregon, spawned only once, similar to populations in Washington (Paine 1971). These differences in reproductive strategy may be one mechanism driving decreased inter-annual variation in recruitment in populations at the southern portion of the species range when compared to northern populations. Further data on a finer temporal spatial scale used in this study may be able to identify specific cues that induce spawning in *C. funebralis*.

Bridge II

Chapter III explored how the reproductive strategies of *Chlorostoma funebralis* vary along a latitudinal gradient. The oldest individuals with the largest capacity for reproduction are most common in the northern portion of the species range, but their prevalence varies between locations. Unlike the presence of juveniles explored in Chapter II, adult populations are not correlated with coastal topography. Chapter IV examines predation as a potential cause of differences in adult populations of *C. funebralis* along the Oregon coastline.

CHAPTER IV

EFFECTS OF PREDATION ON SIZE STRUCTURE OF *CHLOROSTOMA FUNEBRALIS* POPULATIONS IN OREGON

Introduction

The size-frequency distributions of populations of marine invertebrates can vary along a latitudinal gradient (Frank 1975, Ebert 1983, Menge et al 2004) as well as between geographically close populations (Ebert and Russell 1988, McQuaid and Phillips 2006). These differences may be caused by inter-annual variation in larval supply and recruitment that create gaps in the size structure of the population (Menge et al 2004). Adult mortality factors such as predation may be important in regulating the densities and longevity and, hence, the maximum size of adults (Paine 1969, Fawcett 1984). The intertidal gastropod *Chlorostoma* (*Tegula*) *funebralis* is an interesting species to study since size distributions differ both on a latitudinal gradient (Frank 1975, Wright 1975, Chapter I) and with coastal topography (Chapter I).

Chlorostoma funebralis is common in the rocky intertidal zone along the west coast of North America from Vancouver Island to Baja California (Morris et al 1980). Juvenile *C. funebralis* settle in the high intertidal and migrate to the mid-intertidal as they grow larger (Paine 1969, 1971), a size gradient that is recreated even after individuals are relocated to different tidal heights (Doering and Phillips 1983). This behavior generates a spatial refuge from predation for juvenile and small *C. funebralis* since most predators occur in the mid- to low- intertidal (Paine 1969).

Populations of *C. funebralis* differ in the age structure of populations (Frank 1975, Wright 1975, Chapter I). Along the Oregon coast, populations in relatively protected areas have more juvenile *C. funebralis* than populations that are in more exposed habitats (Chapter I). In California and Baja California, all populations are heavily weighted towards the juvenile size classes (Frank 1975, Chapter I). Large *C. funebralis* produce more gametes than small *C. funebralis* (Chapter I), so populations dominated by juveniles that have fewer adults may have a lower reproductive potential than populations with many adults (Chapter I). While the presence or absence of juveniles in a population is correlated with the exposure of the population, the number of adults is not related to coastal topography (Chapter I). The additive effects of selective pressures on juveniles and adults may result in differences in size-structure between populations.

The age structure of marine invertebrates is often interpreted as reflecting historical recruitment to that population (Ebert and Russell 1988, Roughgarden et al 1988, Menge et al 2004). However, predation may be a major source of mortality for adult *C. funebralis* and play an important role in shaping the size structure of populations of *C. funebralis*. Paine (1969) directly measured predation rates by *Pisaster ochraceus* on *C. funebralis* and calculated that 16-31% of adults are consumed by *P. ochraceus* annually. Higher mortality of *C. funebralis* has been observed at sites with higher densities of predators, including *P. ochraceus*, octopuses of the genus *Octopus,* and crabs of the genus *Cancer* (Fawcett 1984). All of these predators are found in the sub- to mid-intertidal zone. Larger adult *C. funebralis* occur in the mid intertidal zone while smaller, younger individuals tend to be found high in the intertidal zone. The vertical limits of these predators create a refuge for small *C. funebralis* in the high intertidal and creates a narrow zone of overlap in the mid-intertidal where predation on larger *C. funebralis* may occur (Paine 1969, Fawcett 1984).

Pisaster ochraceus is known to exert strong predation pressure on mussels, changing the community structure in areas where predation rates and densities are high (Paine 1966, Menge et al 1994). The strength of predation rate as a factor in determining community structure is dependent on exposure (Robles 1987, Menge et al 1994). Predation rates by *P. ochraceus* are also temperature dependent, with higher densities in the intertidal and higher predation rates on intertidal mussels correlated with higher water temperatures (Sanford 1999, 2002). *C. funebralis* is the second or third preferred prey item of *P. ochraceus* behind mussels of the genus *Mytilus*, but is a highly preferred item in the absence of mussels.

If predation pressure affects the number of adult *C. funebralis* in a population, populations with higher predation rates will have fewer adults in comparison to populations with lower predation pressure. This study was undertaken to understand the relationship between predation pressure and the size structure of populations of *C. funebralis*.

Methods

Snail Tethering Experiment

To compare predation rates, I leashed six sets of ten snails each at five sites during July 2008. Cable ties were attached to the rocks and snails were tethered to the cable ties with 10 cm of monofilament line. All snails used in the experiment were larger than 6.00 g, placing them in size classes that would encounter *Pisaster ochraceous* naturally. To avoid variation in predation rates on a latitudinal scale (Fawcett 1984), predation rates were only studied at populations considered in the northern portion of the species range (Frank 1975, Chapter I). Snails were tethered at the following sites: Boiler Bay (44°49'47N, 124°03'33W), Strawberry Hill (44°15'14N, 124°06'43W), South Cove (43°18'12N, 124°23'56W), Cape Blanco (42°50'17N, 124°33'37W), and Mt. Humbug (42°42'51N, 124°27'14W).

After 11 or 12 days, the numbers of alive and dead *Chlorostoma funebralis* were counted and probable cause of death noted. Empty shells that remained tethered were counted as predation by *P. ochraceous*. Shells that were cracked or peeled were counted as predation by intertidal crabs. Shells that disappeared from the tethering experiment were counted as unknown. Differences in predation rates between sites were analyzed with ANOVA and a post-hoc Power Test with G*Power (Buchner et al 1997).

Pisaster Surveys

The density of predators is potentially directly related to the predation pressure experienced by populations of *Chlorostoma funebralis*. To determine the density and predation rate of *Pisaster ochraceus*, a modified methodology from Paine (1969) was used. Parallel transect lines were laid out in the mid-intertidal overlap zone of *P. ochraceus* and *C. funebralis* vertical distributions. Populations were sampled monthly from June through August, the most active months of *P. ochraceus* predation (Paine 1969). A random number generator was used to select locations along transect lines to lay out 1 m^2 quadrats. A total of 100 m^2 were sampled at each of the following populations: Boiler Bay (44°49’47N, 124°03’33W), Otter Rock (44°44’46N, 124°03’50W), Sunset Bay (43°20’00N, 124°22’32W), South Cove (43°18’12N, 124°23’56W), Cape Blanco (42°50’17N, 124°33’37W), and Mt. Humbug (42°42’51N, 124°27’14W). Within each quadrat, the number of *P. ochraceus* was counted. Each *P. ochraceus* was also removed from the rocks and the taxon and number of prey items held in the arms or stomach were recorded. Differences in densities of *P. ochraceus* among populations were tested using ANOVA after an arcsine transformation, with dates nested within sites.

The water temperature can affect the feeding rate and density of *P. ochraceus*. Sea surface temperatures for the dates and locations sampled were obtained from satellite images from NOAA Coastwatch (2010). Although several satellite images are archived from each day, cloud cover prohibits obtaining sea surface temperature for each location in each picture. For each sampling day, the satellite image with the information closest to the location of sampling at the time of sampling was used.

P. ochraceus are capable of feeding on each low tide, and can catch and fully digest a *C. funebralis* in that time period (Paine 1969). This gives *P. ochraceus* two feeding periods each day to prey on *C. funebralis*. Predation rates on *C. funebralis* were calculated using the number of *C. funebralis* found in the arms or stomach in *P. ochraceus* for each date and site. The number of *C. funebralis* consumed/m^2 was multiplied by the number of feeding periods in each day. This was then multiplied by the number of days in the month, which gave an estimate of the number of *C. funebralis* consumed/m^2 for each of the three sampled months.

To investigate the hypothesis that *P. ochraceus* predation determines the number of adult *C. funebralis* in a population, the number of adult *C. funebralis* was correlated against both *P. ochraceus* density and predation rates.

Results

Tethering Experiment

During the 11 or 12 days that tethered snails were left in the intertidal, shells became unattached from the experimental setup and were lost. Many of these snails were later found alive in the intertidal. However, the fate of all lost snails cannot be explicitly determined, so they are not included in the analysis. The loss of snails varied between sites from 16% to 73% (Table 2).

The only evidence of predation was empty shells, indicating predation by *Pisaster ochraceus*. No broken (crab predation) or drilled (octopus predation) shells were found attached to the experimental setup. Predation by *P. ochraceus* varied between 0% (Boiler Bay) and 40% (Cape Blanco) of the recovered snails at each site (Table 2).

Table 2: *Chlorostoma funebralis* recovery and predation rates for tethering experiment, July 2008. Snails recovered includes all snails, dead or alive, still tethered to experimental setup after 11-12 days. Percent consumed is percent of recovered snails that were empty shells, indicative of *Pisaster ochraceus* predation.

Site	Snails Tethered	Snails Recovered	% Consumed
Mt. Humbug	60	26	15
Boiler Bay	60	50	0
Cape Blanco	60	25	40
South Cove	60	48	10
Strawberry Hill	60	16	6

To investigate if high predation rates are associated with the number of adults in the population, correlation was run between the percentage of tethered *Chlorostoma funebralis* consumed by *P. ochraceus* and the percentage of the population over 6 grams, the approximate size at which individuals migrate low enough in the intertidal where they may encounter *P. ochraceus*. All percentages were arcsine transformed. There was no relationship between predation rate as estimated from this tethering experiment and the percentage of older individuals in the population ($r = 0.403$, df = 3, $p > 0.2$).

Pisaster Density

Because tethering studies showed only predation by *Pisaster ochraceus* at the sites studied, surveys were conducted to measure *P. ochraceus* density and excluded other potential predators. *P. ochraceus* surveys were carried out once a month at each of six sites during the summer of 2009. Water temperatures during sampling days were 10-11°C for all sampling in July and August, and 10-12°C for dates sampled in July. No temperatures exceeding 12°C were observed. *P. ochraceus* densities were calculated for each month (Table 3). In a nested ANOVA, with dates nested within sites, density of *P. ochraceus* varied significantly between sites (F[5,12] = 4.12, p = 0.021) as well as dates (F[12,1728] = 2.44, p = 0.004).

A correlation was used to investigate the relationship between densities of *P. ochraceus* and the number of adults in a population. Because sampling dates at the same site are significantly different from each other, each date plotted independently against the percentage of the *Chlorostoma funebralis* population that is larger than 6 grams (Figure 13). A negative correlation would indicate fewer adult *C. funebralis* in locations with higher densities of *P. ochraceus*. The correlation was negative, but not significant (r = -0.357, df = 16, p = 0.146).

Table 3: Density of *Pisaster ochraceus* at six sites in Oregon and predation rates on *Chlorostoma funebralis*.

Site	Month	% of *C. funebralis* population > 6 g	*Pisaster*/m^2	# *C. funebralis* consumed	*C. funebralis* consumed/month*m^2
Boiler Bay	June	20	.19	0	0
Boiler Bay	July	20	.18	0	0
Boiler Bay	August	20	.25	0	0
Otter Rock	June	41	.05	0	0
Otter Rock	July	41	.02	0	0
Otter Rock	August	41	.01	0	0
Sunset Bay	June	5	.22	0	0
Sunset Bay	July	5	.41	0	0
Sunset Bay	August	5	.31	0	0
South Cove	June	3	.22	1	0.6
South Cove	July	3	.14	0	0
South Cove	August	3	.10	0	0
Cape Blanco	June	32	.32	3	1.8
Cape Blanco	July	32	.24	8	4.96
Cape Blanco	August	32	.27	2	1.2
Mt. Humbug	June	26.7	.23	2	1.2
Mt. Humbug	July	26.7	.25	0	0
Mt. Humbug	August	26.7	.35	4	2.48

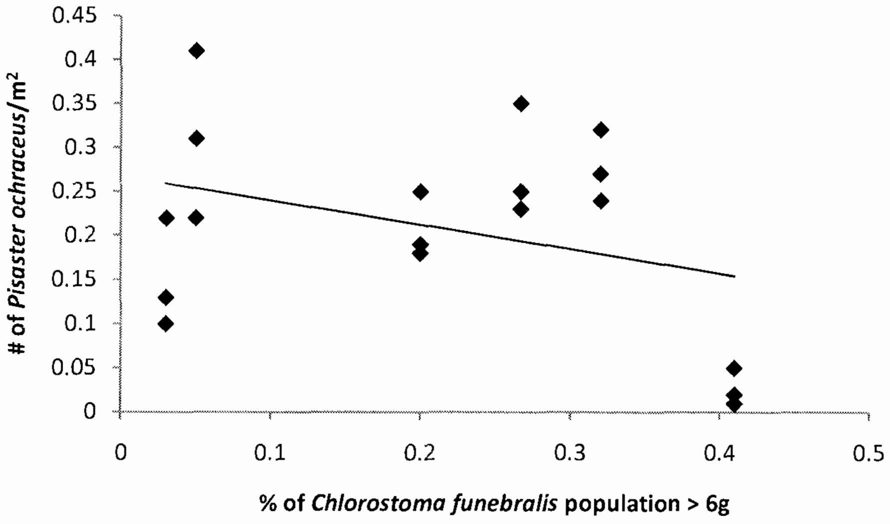


Figure 13: Correlation between percentage of *C. funebralis* population > 6 g and density of *P. ochraceus*. *P. ochraceus* densities were sampled once a month during June, July, and August 2009. Correlation is negative but not significant (r = -0.357, df = 16, p = 0.146).

Predation Rates

A total of 376 *Pisaster ochraceus* were counted during summer surveys at 6 locations. Of these, 136, 36%, had prey items either in the stomach or arms. Of feeding *P. ochraceus*, *Chlorostoma funebralis* was the third most common prey item (20%), behind acorn barnacles (51%) and mussels (24%). Less common prey items included juvenile *Lottia* sp, *Lacuna sp.*, *Nucella sp.*, small chitons, and bivalves.

At four of the six sites, one or no *C. funebralis* were observed being consumed by *P. ochraceus* (Table 3). The highest predation rate was at Cape Blanco in July, where eight *C. funebralis* were found in sea star stomachs or arms, an estimated 4.96 *C. funebralis* may have been consumed/m^2 over the course of the month.

The predation rate was calculated for each site and month and correlated against the density of predators. A correlation between the density of *P. ochraceus* and the predation rate on *C. funebralis* was not significant ($r = 0.235$, $df = 16$, $p > 0.1$), suggesting that the predation rate was not simply a function of the density of predators. If the predation rate is related to the availability of *C. funebralis*, higher predation rates will correlate with more *C. funebralis* larger than 6 g in the population. This correlation is also non-significant ($r = 0.310$, $df = 16$, $p > 0.1$). At Otter Rock, the population with the highest percentage of large *C. funebralis* (41% of the population over 6 g), no *C. funebralis* were observed at any point in the stomach or arms of *P. ochraceus*.

Discussion

Previously documented predators throughout the species range of *Chlorostoma funebralis* are octopus, crabs, and sea stars. The tethering experiment indicated that *Pisaster ochraceus* was the major predator of *C. funebralis* in Oregon, as documented in previous studies (Fawcett 1984, Paine 1969). The effects of octopus and crabs on the population may be stronger in the southern portion of the species range including Baja California and Southern California, with their effect decreasing with increasing latitude (Fawcett 1984). However, mobile predators such as crabs and octopus may have the ability to remove prey from the tethering experiment and consume them elsewhere. If this was the case, the tethering experiment will have falsely underestimated the predation rates of these mobile predators. Although studies of potential predation rates are not difficult to conduct in the lab, measuring the consumption of actual prey items in the field presents challenges that may not be met with this tethering experiment.

Along the Oregon coastline, the age structure of *C. funebralis* populations differs significantly among locations (Chapter I). Populations dominated by juveniles are associated with the coastal topography of the population. In contrast, the presence of adult *C. funebralis* at a location is not associated with coastal topography (Chapter I). If these differences in adult populations were due to predation, we would expect to observe fewer adult *C. funebralis* in populations exposed to high predation rates. Because only adult *C. funebralis* occur low enough in the intertidal to encounter predators, predation is a mortality source that affects only the adult population. Both the tethering experiment and *P. ochraceus* surveys indicate that there was no relationship between the predation rate, the density of predators, and the prevalence of adult *C. funebralis*. Otter Rock, which has large numbers of adult *C. funebralis*, had the lowest rate of predation and *P. ochraceus* density. In contrast, Cape Blanco, which also has a large number of adult *C. funebralis*, had the highest rate of predation and *P. ochraceus* density in this study.

In the intertidal zone where *C. funebralis* and *P. ochraceus* are both present, *C. funebralis* can occur in densities that exceed $1000/m^2$ (Paine 1969, personal observation). Given their high densities and the fact that the two species are often found within centimeters of each other, I expected that consumption by *P. ochraceus* would have been higher. *C. funebralis* do exhibit an escape response in the laboratory when exposed to predatory sea stars, which has been used to explain lower than expected predation rates (Yarnall 1964). However, the predation rates calculated by Paine (1969) were much higher than those found in this study, and it is unlikely that differing escape responses can account for this difference when *P. ochraceus* and *C. funebralis* were found in such close association in this study.

Low predation rates on *C. funebralis* may be a function of other available food sources. *Chlorostoma funebralis* was not the major prey item of *P. ochraceus*. Small barnacles of the genus *Balanus* were numerically the most common prey item and are a preferred food source (Mauzey 1966) although they do not have as high a caloric content as molluscan prey items (Mauzey 1966). Gastropods in general were consumed significantly less by *P. ochraceus* than would be predicted from their intertidal densities (Feder 1959).

There are a number of sources of variation in *P. ochraceus* density and predation rates that this study did not sample. *P. ochraceus* are sensitive to changes in water temperature of 3-5°C (Sanford 1999) and consume more prey in higher temperatures. Water temperatures were relatively similar for all sampling periods in this study, and all cool enough to represent periods of upwelling. During downwelling events in the summer of 2009, near-shore sea surface temperatures along the Oregon coast exceeded 15°C (NOAA Coastwatch 2010). The observed predation rates upon *C. funebralis* may have been significantly higher if sampled during these periods of warmer waters, and therefore this study may underestimate the total effects of predation during the summer months.

Densities of *P. ochraceus* in the intertidal also vary seasonally. Intertidal densities are highest from June through August in both California (Feder 1959) and Washington (Mauzey 1966, Paine 1969). If *P. ochraceus* densities are highest in the summer in Oregon as well, predation rates calculated during the summer will overestimate annual predation. However, if seasonal variation is dependent solely on temperature, predation rates along the Oregon coast may increase in September and early October, months not sampled in this study, due to summer upwelling and fall relaxation.

Chlorostoma funebralis is a species that can live up to 30 years (Darby 1964, Frank 1965), and the age structure of populations is necessarily the sum of recruitment and multiple sources of mortality. Adult *C. funebralis* have a high physiological tolerance to temperature changes (Stenseng 2005) and desiccation (personal observation), so predation may be the main source of mortality for adults. If adult mortality is low, the age structure of populations may reflect the recruitment history of the population. Periods of high recruitment followed by years of low recruitment would create the size frequency distributions observed in Oregon populations (Chapter I). Variation in recruitment may be due either to larval supply or post-settlement mortality, the relative effects of which are difficult to assess due to the small and ephemeral nature of newly settled *C. funebralis*. Historic variations in recruitment larger than those previously observed for *C. funebralis* may cause the observed distribution of adults. If the recruitment and mortality of juveniles is the primary source of variation of adult densities, long term studies of age structure and recruitment will be necessary to observe juvenile populations as they mature into adult *C. funebralis*.

Seasonal and inter-annual variation in *P. ochraceus* density may mean that the long-term effects of predation on the size-structure of *C. funebralis* are more significant than those calculated in this study. However, predation may not be a consistent and strong driving factor in the abundance of large, adult *C. funebralis*. Long term monitoring of *C. funebralis* populations will be necessary to further study the causes of mortality in adults.

Bridge III

Chapter IV documented the limited effects of predation on *Chlorostoma funebralis* populations over a single summer season. In Chapter V, I used the mitochondrial gene COI to study longer-term patterns of population structure in *C. funebralis* populations. The mitochondrial genome was used to explore dispersal potential integrated over both the larval and adult life stages.

CHAPTER V

POPULATION STRUCTURE AND CONNECTIVITY IN *CHLOROSTOMA* (*TEGULA*) *FUNEBRALIS:* NO GENETIC STRUCTURE DESPITE LOW DISPERSAL POTENTIAL

Introduction

The connectivity of marine invertebrate populations and the dispersal of larval stages is a factor of much concern in marine ecology and conservation (Roughgarden et al 1988, Underwood and Fairweather 1989, Menge 1991, Shanks et al 2003). The larvae of marine invertebrates are expected to disperse with the prevailing ocean currents due to their poor swimming capacity. Therefore, the dispersal potential of marine invertebrates has often been modeled using larval duration and oceanographic conditions (Cowen and Sponaugle 2009). However, this method will estimate the near-maximum dispersal potential, which may not accurately represent the ecological consequences of dispersal (Palumbi 2003, Shanks 2009). Larval behavior such as vertical migration may limit the realized distance that larvae travel (Poulin et al 2002, Shanks and Brink 2005, Morgan et al 2009a, 2009b, Shanks and Shearman 2009). Other indirect methods of measuring population connectivity, such as molecular markers, provide a different method for estimating the exchange of individuals between populations. Genetic differentiation has been used to estimate dispersal rates in many marine taxa, some of which have large dispersal distances (Sokta et al 2004). However, many species have shown far less dispersal than expected given the duration of the larval period (Taylor and Hellberg 2003, Jones et al 2005, Marko et al 2007).

The intertidal gastropod *Chlorostoma (Tegula) funebralis* is a common member of the rocky intertidal along the west coast of North America. The genus *Chlorostoma* dates to the mid-Miocene, and the species *C. funebralis* first occurred in the early Pliocene (Hellberg 1998). Although it occurs in densities of up to 1000's /m^2 (Paine 1969) and ranges from Baja California to Vancouver Island, little is known about its reproductive ecology or larval dispersal. Dispersal is expected to be limited because the lecithotrophic larval stage is estimated between five and eight days (Moran 1997). However, no studies of population connectivity have been conducted.

The limited larval duration of *C. funebralis*, limited adult dispersal potential, and expanses of unsuitable habitat along the coastline indicate that it may have limited population connectivity and strong genetic structure. With less expected dispersal potential, populations are more likely to be isolated from each other and show stronger genetic structure. Cape Blanco has been proposed to act as a dispersal barrier for pelagic larvae (Menge et al 2004). In this study, the mitochondrial gene COI was used to describe existing population structure in *C. funebralis* using populations that are separated both by short distances (< 20 km) and longer distances (> 1000 km) on both sides of Cape Blanco.

Methods

Sampling, DNA Extraction, Amplification, and Sequencing

Chlorostoma funebralis were collected from nine sites along the California and Oregon coastlines (Figure 15). Three sites were protected (Boiler Bay, South Cove, San Diego) (Chapter I), and five were exposed (Otter Rock, Indian Beach, Cape Blanco, Mount Humbug, Crescent City). Twenty to 50 individuals were collected from each site.

Tissue was non-destructively collected from the cephalic tentacles or the foot from live individuals and DNA was extracted using the Qiagen DNEasy kit. After extraction, samples were precipitated with ethanol, washed and resuspended in water at 1:10 or 1:100 dilutions of the original extraction concentration. Cytochrome oxidase I (COI) was amplified with primers LCOI4190 (5' –GGT CAA CAA ATC ATA AAG ATA TTG - 3') and HCO2198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA- 3'; Folmer et al 1994) in PCR reactions. Amplification was carried out in 20 μl solutions containing 2 μl of diluted genomic DNA, 1 mM of each primer, 5 μl of Green Go-*Taq* buffer (Promega), and 1U Go-*Taq* (Promega). PCR cycling consisted of an initial denaturation at 95°C for 3 min; 15 cycles of denaturation at 95°C for 40 sec, annealing at 60°C for 30 sec, extension at 72°C for 45 sec; decreasing 0.3°C each cycle; followed by 31 cycles of denaturation at 95°C for 30 sec, annealing at 54.5°C for 30 sec, extension at 72°C for 1 min; final extension at 72°C for 10 min.

Successfully amplified sequences were purified using the Promega Wizard PCR Clean-up System and quantified with either a Low-Mass-Ladder or Quant-iT DNA Assay Kit. Sequences were amplified with the LCOI4190 primer and sequenced using a Big

Dye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems) and sequenced on an Automatic sequencer 3130XL Genetic Analyzer (Applied Biosystems).

Analysis

Sequence data from 266 individuals were aligned and proofread using Geneious (Drummond et al 2007). All sequences of low quality or shorter than 530 bp were discarded. TCS 2.1.1 was used to generate a haplotype network and identify the ancestral haplotype (Clement et al 2000). Haplotypes that had only one individual and differed in only one nucleotide polymorphism were combined with the closest haplotype to create a conservative measure of haplotypes in case of sequencing error. Clade groups were identified for related groups of haplotypes and used for further haplotype analysis. ARLEQUIN 3.5 (Excoffier et al 2005) was used to calculate population parameters. AMOVA and the Mantel test were used to test isolation by distance. Populations were grouped as Northern (Indian Beach, Boiler Bay, Otter Rock, South Cove, Cape Blanco), Mid (Mount Humbug, Crescent City, Fort Bragg) and Southern (San Diego) to investigate if Cape Blanco acts as a dispersal barrier (Menge et al 2004). Populations were also sorted into two groups, Northern and Southern, with only San Diego in the Southern group, to test for connectivity of populations outside of the Southern California Bight.

Results

Sequence Characteristics

A 530-bp segment of COI was sequenced in 266 individuals in nine populations. In total, 60 polymorphic sites were identified in 58 unique haplotypes (Table 4). Few private haplotypes for any population were identified (Figure 14). Eleven clade groups were identified from the haplotype network and labeled A through K (Figure 14). Tajima's D was negative in all populations but only significantly so in four populations (Table 4). Over the entire sampling range, Tajima's D was negative and highly significant (D = -2.12, p < 0.0001), indicating a large number of low frequency haplotypes.

Table 4: COI nucleotide diversity, haplotype diversities and Tajima's D among populations of *C. funebralis*.

Collection Site	N	No. Polymorphic Sites	Nucleotide Diversity	No. Haplotypes	Tajima's D (p value)
Indian Beach	25	16	0.00494 ± 0.00304	15	-1.311 (0.077)
Boiler Bay	24	16	0.00517 ± 0.00316	14	-1.281 (0.099)
Otter Rock	24	17	0.00559 ± 0.00337	14	-1.251 (0.099)
South Cove	57	35	0.00496 ± 0.00298	22	-2.16 (0.002)
Cape Blanco	25	14	0.00440 ± 0.00279	12	-1.268 (0.094)
Mt. Humbug	44	22	0.00405 ± 0.00255	17	-1.891 (0.009)
Crescent City	20	16	0.00473 ± 0.00297	11	-1.647 (0.038)
Fort Bragg	24	15	0.00542 ± 0.00329	13	-1.003 (0.175)
San Diego	23	19	0.00538 ± 0.003320	14	-1.631 (0.042)
Total	266	60	0.00492 ± 0.00292	58	-2.121 (< 0.001)

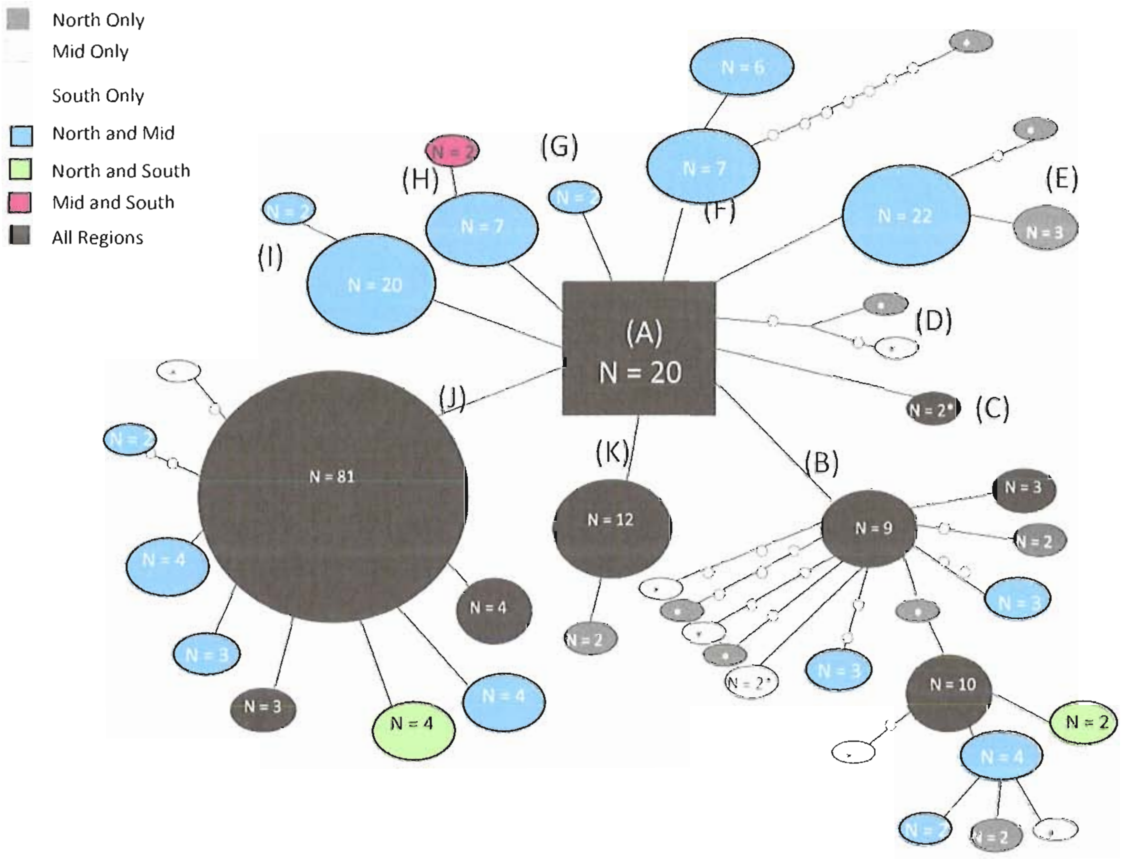


Figure 14: Haplotype network created using statistical parsimony for COI in *Chlorostoma funebralis*. Size of ovals is roughly proportional to the number of individuals possessing that haplotype, also indicated by the N value for each haplotype. Private haplotypes are signified with an *. Haplotypes with only one individual are by definition private and so N values are not printed. Branches correspond to single mutations and white circles along branches indiciate one additional mutation between haplotypes. The square haplotype is the inferred ancestral haplotype. Shading indicates regions in which those haplotypes are found.

Population Structure

No cline of haplotype diversity or clade dominance is apparent between populations along the coastline (Figure 15). Clades A, B, and J are found in all populations, with the other clade groups distributed through the populations with no trends along the coastline axis. When estimated from haplotype frequencies, no significant genetic differentiation was observed either between groups (North, Mid, and South, p = 0.60) or between populations (p = 0.13, Table 5). Variation within populations accounted for almost all of the observed variation (99.4 %). When groups are redefined into two groups with only San Diego in the Southern population, results are similar (Table 6). Differences between groups are insignificant (p = 0.22) as are differences between populations within groups (p = 0.18). Most variation is due to variation within populations (98.5 %).

Table 5: AMOVA table, population structure based on haplotype frequencies of populations of *Chlorostoma funebralis*. Populations are divided into three groups: North (Indian Beach, Boiler Bay, Otter Rock, South Cove, Cape Blanco), Mid (Mt. Humbug, Crescent City, Fort Bragg) and South (San Diego).

Source of Variation	df	Sum of Squares	Variance Component	% of Variation	P
Among groups	2	2.81	-0.0028	-0.21	0.602
Within groups, among populations	6	9.63	0.0106	0.81	0.131
Within Populations	258	334.86	1.2979	99.40	

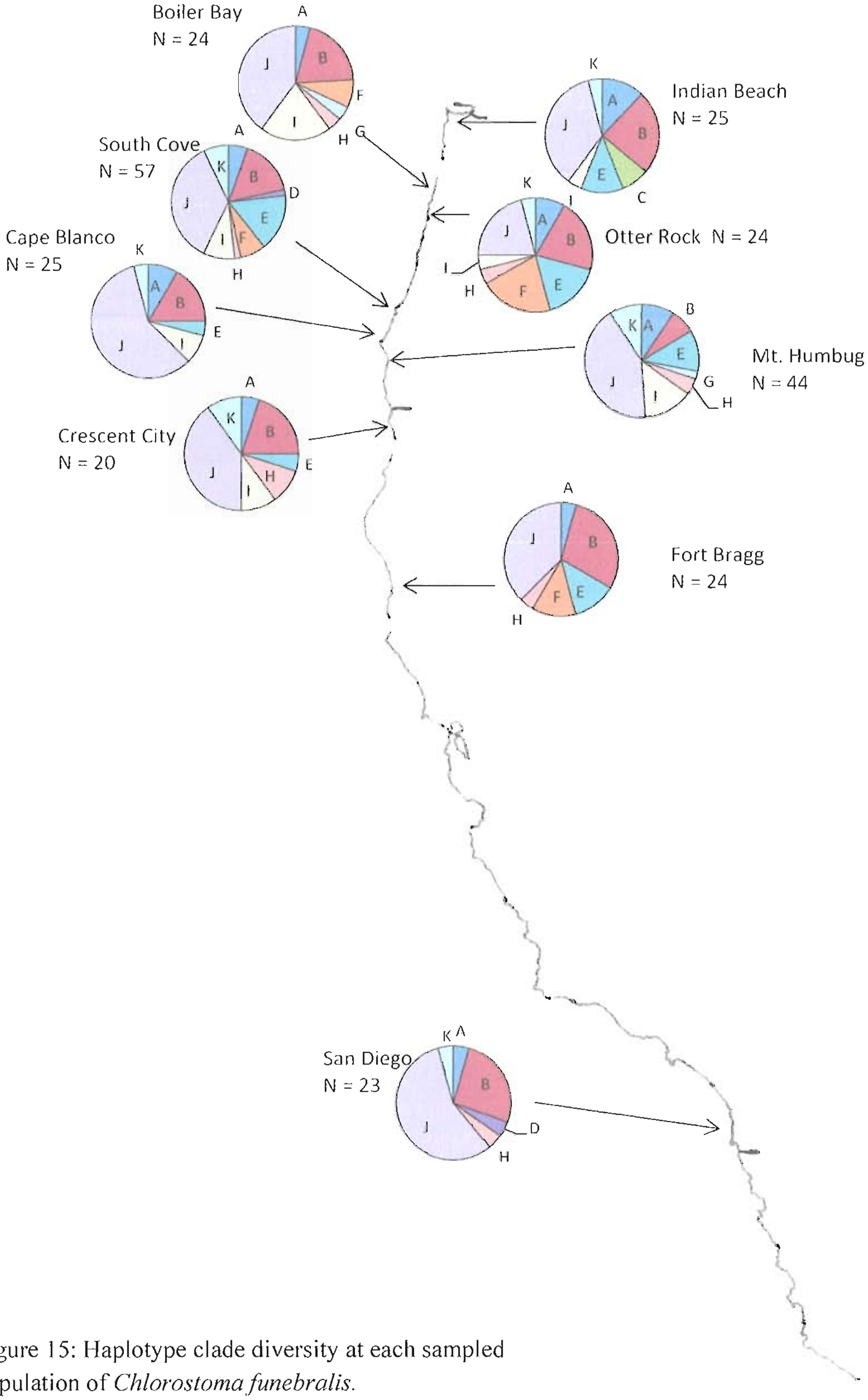


Figure 15: Haplotype clade diversity at each sampled population of *Chlorostoma funebralis*.

Table 6: AMOVA table, population structure based on haplotype frequencies of populations of *Chlorostoma funebralis*. Populations are divided into two groups: North (all populations excluding San Diego) and South (San Deigo).

Source of Variation	df	Sum of Squares	Variance Component	% of Variation	P
Among groups	1	1.97	0.0121	0.92	0.222
Within groups, among populations	7	10.48	0.0667	0.51	0.185
Within Populations	258	334.86	1.2979	99.57	

Isolation by Distance

A Mantel Test, using distance as measured along the coastline, found no correlation between genetic differentiation and geographic distance between populations ($r = 0.132$, $p = 0.265$). Likewise, no relationship was found between pairwise F_{ST} values and geographic distance ($r = 0.057$, $df = 35$, $p = 0.738$, Figure 16A). A Mantel Test using distance as measured in straight line between sites also shows no correlation between genetic differentiation and geographic distance ($r = 0.089$, $p = 0.353$). Pairwise F_{ST} values and geographic distance also show no relationship ($r = 0.052$, $df = 35$, $p = 0.7599$, Figure 16B).

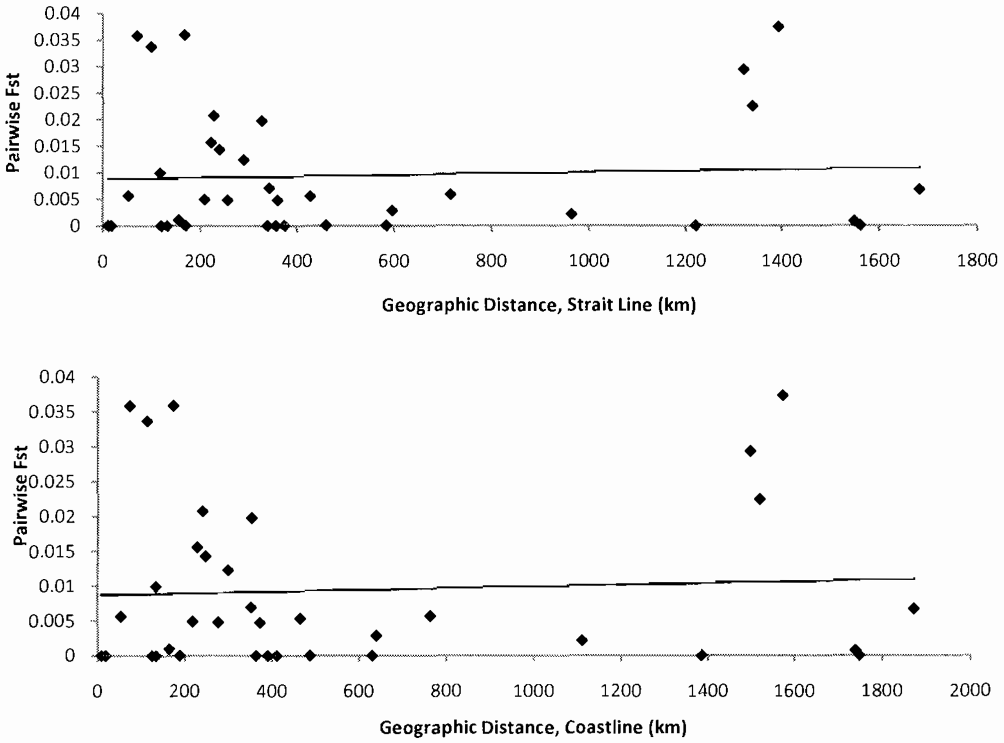


Figure 16: Plot of pairwise F_{ST} vs. geographic distance measured two ways. (A) Distance measured along the coastline. (B) Distance measured in a straight line between populations.

Discussion

Surprisingly, these data do not conform to expectations based on other work in marine species with a pelagic dispersal stage. The strong results of the AMOVA analysis indicate that there is little, if any, genetic structure between populations of *Chlorostoma funebralis* between northern Oregon and southern California. Marine invertebrates with short larval durations are expected to have limited dispersal and therefore are more likely to show strong genetic structure (Bohonak 1999). However, the larval period of *C. funebralis* is shorter than other species used in similar studies that show some genetic structure, such as barnacles (Sotka et al 1994) with a larval period of two to four weeks (Strathmann 1987) and show a genetic break in central California. Bryozoans with larval periods of weeks to days show significant genetic structure throughout England (Porter et al 2002). Lingcod have a larval period of three months and show limited connectivity of populations within Washington (Marko et al 2007). These species have a higher dispersal potential than *C. funebralis* as expected strictly from the pelagic larval duration but show significantly more genetic structure.

Although unexpected, the strong results of the AMOVA indicate that the results reflect the actual genetic variation between and within populations. An initial hypothesis is that sample sizes were not large enough to detect population structure given the variation on the mitochondrial genome. However, significant variation occurs on the COI gene, with nucleotide diversities of 0.00492 that can be clearly partitioned among different individuals. It is clear in the AMOVA analyses that this genetic variation is at the level of the individual, rather than populations or regions. This is corroborated by the negative values of Tajima's D for all populations, indicating a large number of rare haplotypes. Larger sample sizes may reveal some subtle genetic structure, but it is unlikely that more specimens from some populations will affect the lack of strong differentiation between populations separated by large distances. Therefore, other hypotheses for the lack of genetic structure need to be explored.

Chlorostoma funebralis occur in high densities in large populations in many locations (Paine 1969). This large population size may make the effects of genetic drift negligible and limit the number of differences that can accumulate between reproductively isolated populations. However, other marine invertebrates found in similar habitats, such as the intertidal barnacle *Balanus glandula*, have similarly large populations sizes and yet show genetic differentiation along a similar geographic range (Sotka et al 2004).

Population connectivity due to larval dispersal may be the cause of the lack of variation between populations. However, there are large expanses of sandy beaches, unsuitable for adult *C. funebralis,* separating some of the populations sampled for this study. Using the simplistic dispersal model of a passive particle moving at 10 cm/s (Shanks et al 2003), a *C. funebralis* larva could disperse approximately 70 km in eight days. Cape Arago and Strawberry Hill, two populations in Oregon, are separated by approximately 110 km of sandy beach habitat. For larvae to disperse from one population to another, optimal conditions of high dispersal such as high current speeds and extended larval duration must be met. Adult dispersal is also an unlikely hypothesis, as adult *C. funebralis* occur only in the mid- to high rocky intertidal, and are very rarely found in the low or subtidal. Sandy beaches and steep zones such as coastal cliffs are impassable barriers to adult dispersal since they are unlikely to survive a move to the lower intertidal to pass such areas. Rafting is a potential dispersal mode for adults, but adult *C. funebralis* are heavy (6g -30g) and would require large rafts of macroalgae to move from one population to another. Although these rafts can exist for more than 100 days (Thiel 2003), *C. funebralis* dispersal on such rafts has not been directly observed. Because eggs are shed directly into the water column, rafting of egg cases is not a mode of dispersal as in other gastropods (Marko 2004).

Mitochondrial DNA in *C. funebralis* is maternally inherited. A consequence of using only mitochondrial genes for a dispersal study is that it reflects only female dispersal. Differences in actual male and female dispersal distances will not be apparent in these data. However, difference in dispersal due to sex is unlikely since both males and females start the larval stage as free-spawned gametes. As adults, dispersal events are random (as described above) and unlikely to differ between male and females. Population analysis using nuclear DNA will be necessary to confirm there is no sex-dependent dispersal mechanism.

Another hypothesis that may account for a lack of variation between populations is a relatively rapid range expansion. The lack of genetic structure in gastropods, echinoderms, and intertidal fish, has been attributed to extinction in northern populations and recolonization from populations in California following the last glacial maximum (LGM) (Marko 2004, 2010). Range expansion following the LGM is expected to have occurred in the last 20 thousand years. In a range expansion event, the source population is expected to have higher haplotype diversity, while younger populations will have limited diversity due to the founder effect. Although species in the low or subtidal may have had a habitat refuge from glacial ice, species that are obligate inhabitants of the mid to high intertidal are significantly more likely to show a lack of genetic diversity in northern populations (Marko 2004). This study does not show reduced diversity in northern populations when compared to the southern portion of the species range, although too few southern samples were used and therefore cannot asses varying levels of diversity between regions. Larger sample sizes from more populations along the California coast will provide the data necessary to distinguish between high population connectivity and a recent range expansion event.

The lack of genetic structure over a large geographic area was unexpected given the short dispersal time of *C. funebralis*. As a measure of dispersal and population connectivity, genetic data may overestimate dispersal potential for species with dispersal longer than a week (Shanks 2009). More loci, including nuclear DNA, in more populations covering the entire species range should be sampled to explore hypotheses of range expansion. However, if future studies also indicate high population connectivity, the ecology of species with short pelagic larval durations should be reconsidered.

CHAPTER VI

GENERAL CONCLUSIONS

Understanding geographic variations in age and population structure of marine invertebrates requires the integration of both geographic and temporal scales. Throughout a species range, the physical and biological pressures differ. For intertidal species along the west coast of North America, these differences occur roughly on a latitudinal gradient. Additionally, many marine invertebrates have a pelagic dispersal stage, during which the habitat and selection pressures are significantly different from those experienced by juveniles and adults. Both life stages must be incorporated into any analysis of variations in population structure between populations in different locations. The work presented in this dissertation was undertaken to explore the mechanisms driving variation in population structure of *Chlorostoma funebralis*, previously documented in the mid-seventies by Frank (1975) and Wright (1975).

The structure of populations of the intertidal gastropod *C. funebralis* varies along a latitudinal gradient. Populations in the northern portion of the species range are large and long-lived (Darby 1964), while individuals in the southern portion of the range are smaller and have shorter life-spans (Frank 1975). The data I collected, 30 years after both Frank and Wright's studies, corroborated this pattern of variation on latitudinal gradient. However, I additionally observed a difference in population structure, specific to the Oregon coastline, between populations in exposed and protected locations. Populations along exposed coastlines had few juveniles and small individuals, and were dominated by older and larger *C. funebralis*. Populations in protected areas, such as bays and coves, were heavily weighted towards juveniles and had relatively fewer adults.

The potential reproductive output of individuals is directly correlated with their size, and consequently the size distributions of populations may have a significant impact on their reproductive capacity. Populations in the southern portion of the species range produce significantly fewer eggs than populations in the north, and protected populations in the north produce fewer eggs than exposed populations.

C. funebralis may use different reproductive strategies to along the latitudinal gradient. A population in southern California, dominated by small individuals, was observed to synchronously spawn several times throughout the year. In contrast, a population in Oregon had only one spawning event during the year studied. Spawning multiple times in a year may allow smaller individuals in the southern portion of the range to maximize reproductive output despite their small size. Additionally, multiple spawning events may increase the probability of at least one successful reproductive event each year, thereby increasing the probability of at least one successful reproductive event over an individual's lifetime. In the northern portion of the species range, individuals live much longer and it may be more advantageous to spawn only once a year.

Predation has been previously documented as a major force in limiting the adult population of *C. funebralis* (Paine 1969, Fawcett 1984). Variation in the number of adults *C. funebralis* between different populations along the Oregon coast may be due to differences in predation rates. In this study, I identified the major predator, *Pisaster ochraceus*, and observed no predation by other documented predators such as octopus and crabs (Fawcett 1984). *P. ochraceus* did not remove as many adult *C. funebralis* from populations as had been observed in previous studies (Paine 1969) and while long-term effects are still unstudied, predation does not appear to be a constant and strong influence on the size-structure of *C. funebralis* populations in Oregon.

The genetic structure of *C. funebralis* was studied to explore the levels of population connectivity and the potential effects of coastal topography on larval dispersal. Although samples were collected over a large geographic range, spanning from northern Oregon to San Deigo, California, no genetic structure was observed using the mitochondrial gene COI. Although this may be due to high levels of population connectivity, this is unlikely due to the limited dispersal period of larval *C. funebralis* of five to eight days (Moran 1997). An alternative hypothesis is a rapid range expansion event following the last ice age, although more data will be necessary to explore that possibility.

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