Spatial and temporal patterns of fertilization in black abalone (*Haliotis cracherodii* Leach, 1814): Analysis of surrogate gamete spawning experiments with application towards populations on San Nicolas Island, California

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Abstract

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The endangered black abalone (*Haliotis cracherodii*) suffered devastating losses along the coasts of Southern California, primarily caused by the effects of withering syndrome. Subsequent recovery may be hindered by the Allee effect, which limits the reproductive output when population densities are below a critical threshold and broadcast spawners are too distant for their gametes to mix in sufficient concentrations to result in fertilized eggs. I conducted simulated spawning experiments in two types of habitat on San Nicolas Island (SNI) in California using surrogate gametes to examine how proximity of spawning black abalone affects the fertilization potential, based on concentration of particles over distance and time. I found that habitat type is an important factor contributing to particle retention. With the linear design of the simulated spawning experiments, fertilization can still occur between black abalone located as far as 4 m apart in the crevice habitat. Nearest neighbor data, collected since 2005, were compared with 8-years of data on abalone abundance and microhabitat type during annual surveys in nine sites around the periphery of SNI to determine potential causes for the disparities in recovery rates among the study sites. Abalone density was examined since 2001 to count the number of patches, defined as groups of at least five abalone within a 2 x 4 m^2 area along study transects. Annual population growth, number of patches, and the change in

nearest neighbor distances show a direct correlation, consistent with the hypothesis that the Allee effect is responsible for the inconsistent recovery around SNI.

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Table of Contents

List of Tables

Chapter 1

Chapter 2

List of Figures

Chapter 1

Chapter 2

Chapter 1

Simulated spawning experiments using surrogate gametes to measure spatial and temporal patterns of fertilization success in black abalone (*Haliotis cracherodii* Leach, 1814)

Abstract

Black abalone (*Haliotis cracherodii* Leach, 1814) are listed as endangered in their U.S. range due to disease and overexploitation. Abalone are dioecious broadcast spawners. As intertidal organisms exposed to oceanic surf, black abalone typically spawn in conditions of strong, turbulent water flow, which may quickly disperse gametes and limit fertilization. To determine the minimum proximity of male-female pairs necessary for successful fertilization, field experiments were conducted in two rocky intertidal habitats utilized by black abalone at San Nicolas Island (SNI), California: a tide pool habitat characterized by relatively calm, deep water subject to periodic wave surges; and a crevice habitat defined by deep a crack in the rock and complex rocky topography. I released gamete-sized particles at varying separation distances (0, 2, 4 m) in each habitat type. Post-release water samples were collected at -1, 0, 1, 2, 4, 8, and 12 m from surrogate sperm release location at 0, 1, 5, 10, 20, 30, 45, and 60 minutes post-release to measure densities of surrogate eggs and sperm in seawater. Samples were evaluated with microscopy to estimate densities of surrogate gametes. In studies of other abalone species, different ratios of sperm to egg were utilized to maximize fertilization

probabilities while minimizing polyspermy in laboratory settings. Due to the unknown fertilization requirements for black abalone and the effect of uncontrolled settings, four plausible alternative fertilization criteria (sperm to egg ratios of 100:1, 500:1, 1000:1, and 5000:1) were used to examine the potential patterns in fertilization success as influenced by habitat type and release distances. One- and two-way analyses of variance were applied to the data to test for significance in patterns. The high concentration of sperm to egg requirements indicates sperm concentration is the variable limiting fertilization. Although release distances were not significant in determining fertilization potential, particle retention was much higher in the crevice habitat, leading to increased probabilities for successful fertilization based on habitat type. Even for the strictest criterion (5000:1 sperm to egg ratio), fertilization potential was achieved at separation distances of up to 4 m in the crevice habitat.

Introduction

A sudden decline of the black abalone, *Haliotis cracherodii*, population along the exposed outer coasts of Southern California and the major California Islands began in the middle 1980s and early 1990s. The sharp decrease in population counts followed by a lack of recovery in subsequent decades was the primary risk factor leading to the listing of black abalone as "endangered" under the US Endangered Species Act of 1973 as amended (16 US Code §§ 1531-1543 *et seq*.) on January 14, 2009 (74 FR 1937). Factors recognized as contributing to the decline included overharvest, habitat degradation, and disease (Neuman et al., 2010).

Abalone have been an important food source and culturally significant for thousands of years. Black abalone, however, were not considered a commercially valuable species until red, pink, and green abalone were serially depleted before the 1970s. Harvest of black abalone began in 1956 to bolster declining harvests of other abalone species, peaked in 1973 at 868 metric tons, and closed in 1993 (VanBlaricom et al., 2009). Coinciding with the decline in commercial harvests was a change in oceanic conditions. Ocean conditions were severely affected by El Niño events in the 1980s and 1990s, which increased ocean temperatures and caused kelp abundance (a major food source for black abalone) to decline temporarily (Dayton and Tegner, 1984; Tegner et al., 2001; Vilchis et al., 2005).

While overharvest and habitat degradation may have contributed to the decline of black abalone, disease was later identified as the primary cause of the devastating loss in numbers throughout much of their range (Neuman et al., 2010). The disease, withering syndrome, was first documented on Santa Cruz Island in 1985 (Tissot, 1991, 1995; Haaker et al. 1992) and spread to the other California islands and the mainland by the early 1990's (Douros, 1987; Lafferty and Kuris, 1993; Richards and Davis, 1993; VanBlaricom et al., 1993). The disease first appeared on San Nicolas Island (SNI) in 1992 (VanBlaricom et al., 1993) where it caused catastrophic losses over the next decade. Withering syndrome is caused by a rickettsiales-like prokaryotic pathogen that invades the digestive gland disrupting the production of digestive enzymes (Friedman et al., 2000). The result is hindered absorption of materials from the gut lumen. Abalone

infected with withering syndrome have atrophied pedal muscles and lose their ability to tightly adhere to rocky substrata making them more susceptible to predation and dislodgement from their preferred substrata by breaking surf, which is common to their preferred habitats. Although withering syndrome infects all species of abalone in California waters, the degree to which it affects each species varies considerably (Moore et al., 2002), with black abalone among the more susceptible and incurring higher fatality rates than other California species (Moore et al., 2000, 2002; Friedman et al., 2002; Vilchis et al., 2005; Friedman et al., 2007).

A long-term study of black abalone populations was initiated at SNI in 1979, with the first quantitative surveys completed in 1981 (VanBlaricom, 1993; VanBlaricom et al., 1993). Surveys are focused at nine study sites distributed around the periphery of SNI (Figure 1.1) (VanBlaricom, 1993; VanBlaricom et al., 1993). Black abalone numbers at most SNI study sites suffered losses greater than 99 percent between 1991 and 2001, with summed counts for all sites falling from approximately 25,000 in 1991 to fewer than 200 in 2001. It is noteworthy that disease-induced mass mortalities were less severe at study site 8, located on the southwestern shore of SNI, with numbers dropping from approximately 4,000 in 1991 to 150 in 2001, a loss of approximately 95 percent (VanBlaricom, unpublished data). Since 2001, black abalone numbers have increased at several SNI study sites. However, trends were not consistent among sites. For example, populations at site 8 have quadrupled in size whereas counts at site 4, at the eastern end of SNI, have remained at fewer than 15 individuals for the past 13 years (VanBlaricom, unpublished data).

Abalone are dioecious broadcast spawners. Limited information is available specifically related to black abalone reproduction. Therefore, findings from studies focused on other species must be relied upon to form the basis for understanding black abalone reproduction dynamics. Abalone spawning is often stimulated by the presence of presence of spawn from the opposite sex (Morse et al., 1977; Suphamungmee et al., 2010), a strategy that may increase temporal synchronicity of gamete release and resultant improved rates of fertilization. Studies published on abalone spawning describe conflicting criteria for determining concentrations sufficient for successful fertilization. Some papers argue that concentrations of sperm per unit volume of seawater are the best indices of fertilization success rate (Babcock and Keesing, 1999; Baker and Tyler, 2001), while others link success rates to ratios of sperm to eggs in seawater following gamete release (Bryne et al., 2010; Suphamungmee et al., 2010; Bouma, personal communication). Eggs remain viable in the water for up to seven hours (Babcock and Keesing, 1999), however after only one hour, the outer membrane begins to harden and the probability of fertilization success decreases (Bouma, personal communication). Similarly, sperm are viable for several hours after initial spawning, but there is a significant decrease in fertilization success after one hour (Babcock and Keesing, 1999).

In the wild, population density and distance between individual broadcast spawners may limit fertilization success. Gamete dispersal, strongly influenced by hydrodynamic influences and habitat type, may also mediate fertilization potential. Depensation is the effect of a sudden decrease in reproductive individuals on a population, often resulting in

reduced survival or population recovery. The Allee effect (Allee and Bowen, 1932), analogous to depensation, is the positive relationship between a component of fitness (e.g. breeding success) and overall population size or density (Stephens et al., 1999). In the case of the broadcast spawners, the Allee effect is greatly influenced by the stochastic nature of spatial distribution of individuals (Lundquist and Botsford, 2011). Abalone at certain sites may be too far apart for their gametes to reach concentrations required for significant fertilization rates, either in terms of gamete densities relative to seawater, or distance from one another. A primary aim of this study is to determine the maximum distance between two black abalone in the California rocky intertidal zone at which successful fertilization of released gametes can occur. The criteria used to assess fertilization potential have not been determined for black abalone, and extreme variability exists among species. In laboratory settings, different ratios of sperm to eggs are considered for increasing the probability for successful fertilization in different species of abalone while minimizing polyspermy in conditions that were controlled and contained. Previous studies to determine maximum spawning proximity were based on generally accepted but largely untested perceptions, or on experiments in subtidal habitats which are only marginally applicable to black abalone, which appear to spawn exclusively in highly turbulent rocky intertidal habitats.

Attempts to induce spawning in captive black abalone have met with only minimal success to date, and a reliable method for spawning induction is not yet available. Working with an endangered species requires labor- and time-intensive processes associated with receiving the permits and permission for work with the endangered

species. The limited and unpredictable ability to acquire live black abalone gametes combined with the complicated permitting process, and the sensitive nature of potentially disturbing individuals of an endangered species led to development of simulated spawning experiments using neutrally buoyant micro-spherical particles as substitutes for live gametes. I used surrogate gamete-sized particles in abalone spawning simulations to determine spatial and temporal patterns of gamete distribution in two different habitat types (tide pool and crevice) where black abalone are commonly found.

This project pioneers a spawning simulation method in a species that cannot be reliably spawned in captivity. The results of this study will improve our understanding of linkages between spatial proximity patterns and fertilization rates of spawning in endangered black abalone populations. The conclusions from these experiments will inform planning for recovery actions, such as development of protocols for potential outplanting of the species along the mainland and island coasts of California. This chapter is written in anticipation of eventual publication in a peer-reviewed technical periodical.

Methods

Particle Design

Particles were used as surrogate gametes in simulated spawning experiments that represent the size and concentration of black abalone egg and sperm. The lack of

published reproductive information specific to black abalone requires exploration of similarities with other species of abalone for which published information is available on egg and sperm size and concentration.

Egg Particle Design

Female fecundity is measured by the amount of oocytes in the ovary. Many oocytes are not released during spawning and may be reabsorbed; therefore the total count of oocytes in the ovary is referred to as the "potential fecundity," and the number of eggs released during a spawning event is referred to as the "instantaneous fecundity" (Clavier, 1992). The relationship between shell size or body weight and fecundity in abalone is unclear. Grubert and Ritar (2005) found no significant relationship between shell length and egg production for *H. rubra* and *H. laevigata*, which corresponded with Babcock and Keesing's (1999) findings for *H. laevigata*. Other studies defined linear or exponential relationships between shell length or weight and fecundity. For example, Prince et al. (1987) modeled fecundity (F) as a univariate regression on maximum shell length (ML) for *H. rubra*:

$$F = (0.028 \times ML) - 2.415 \qquad \text{[equation 1]}$$

Peña (1986) found relationships between both shell length and body weight with fecundity for *H. coccinea canariensis*:

$F = 1.468^{0.074L}$ [equation 2]

where L is shell length in mm

$F = 2.723 + 2.963W$ [equation 3]

where W is body weight in grams

Clavier (1992) found a linear relationship between weight and potential fecundity for *H. tuberculata*:

$F = 2.35 \times 10^{4}W - 1.855 \times 10^{5}$ [equation 4]

Wilson and Schiel (1995) found that fecundity varied largely between species, and the effect of shell length on fecundity was greater in *H. iris* than *H. australis*. Actual potential and instantaneous counts vary significantly between individuals and between species (Table 1.1).

The size of unfertilized and fertilized eggs varies between species (Table 1.2). Eggs are measured pre-spawning to determine whether the gonad is mature and ready for spawning in many species (e.g. *H. cracherodii* - Webber and Giese, 1969; *H. laevigata*, *H. roei* - Shepherd and Laws, 1974; *H. rubra* - Huchette et al., 2004). Once spawned, the eggs swell as they take on water (Huchette et al., 2004), and increase in size further when they are fertilized (Huchette et al., 2004; De Vicose et al., 2007; Suphamungmee et al., 2010). The eggs remain at the fertilized size until they hatch (De Viçose et al., 2007).

Differences in egg size within the same species may be attributed to the artificial induction of egg release (Wong et al., 2010), broodstock feeding and conditioning temperatures as well as broodstock size and age (De Viçose et al., 2007).

There is also conflicting information on the buoyancy of unfertilized eggs, which may vary between species. Unfertilized eggs of *H. varia* and *H. tuberculata* are neutrally or positively buoyant until they are fertilized and become negatively buoyant (Morris et al., 1980; Najmudenn and Victor, 2004; Manganaro et al., 2008)), while those of *H. rufescens* and *H. kamtschatkana* may be negatively buoyant immediately after spawning (S. Brombacker, unpublished observations). Other studies have reported that spawned eggs of *H. rubra* and *H. midae* remain in suspension for only a few minutes post-spawning before sinking (Prince et al., 1987; Genade et al., 1988; Huchette et al., 2004).

Black abalone are reported to have a peak spawning period primarily in the spring, and a secondary spawning period in autumn (Webber and Giese, 1969); however, little other general information on spawning attributes is available for black abalone, requiring inferences based on other abalone species for the purposes of planning surrogate gamete release experiments. The above information influenced decisions regarding the size and number of microspheres necessary to simulate spawning conditions in intertidal habitats. Species with the most published, relevant data include *H. laevigata* and *H. rubra*, both temperate species located in the intertidal and shallow subtidal that reach maximum shell lengths of approximately 200 mm and 220 mm, respectively. Thus, both species serve as plausible models for simulations involving black abalone. Pre-spawn egg size for *H.*

cracherodii was determined to be between 120 μm and 160 μm (Webber and Giese, 1969). This is similar to *H. rubra*, which has a pre-spawn egg size mean of 143 μm, and a spawned unfertilized size of 240 μm (Huchette et al., 2004). Instantaneous fecundity for *H. rubra* ranges between 100,000 and 2,110,000 eggs (Grubert and Ritar, 2006). Based on this information (summarized in Table 1.3), approximately 1,000,000 neutrally buoyant microspheres between 212 μm to 250 μm were used in simulated spawning experiments. While previous information indicates eggs may be more negatively buoyant, particles in the turbulent nature of surf-swept intertidal habitats would remain suspended longer than observed in laboratory settings or with subtidal species.

Sperm Particle Design

There is little published information available on sperm size in abalone. Bevelander (1988) measured the length of the head and mid-piece of sperm cells to be 10 μm, and the flagellum length up to 45 μm. During spawning, sperm are released in pulses driven by muscular contractions. A male abalone may have as many as 70 contractions associated with spawning over several hours (Clavier, 1992; Moss, 1998). Although sperm counts vary among species, the densities of sperm released during a typical spawn in controlled laboratory settings are approximately 10^5 to 10^6 sperm/mL (Baker and Tyler, 2001; Manganaro et al., 2008). The mean release rate of 10^7 sperm per second over the length of an hour, and a total release of 10^{11} to 10^{12} sperm cells (Babcock and Keesing, 1999; Grubert and Ritar, 2006). Based on attributes of surrogate gamete products available commercially (Cospheric, www.cospheric-microsphere.com, Santa Barbara, California

USA) and available data regarding sperm size, we used particles 10 μm to 45 μm. To simulate a single male spawning, an estimated a release rate of 10^7 particles/second was used in the simulated spawning experiments, with a total release concentration of approximately 1.8 x 10^{10} particles.

Sampling Bottle Design

To measure the dispersal of particles over time and space, a method was required for collection of water samples with minimal disturbance to water flow patterns. I designed a water sampler utilizing polyvinyl chloride pipe, Wilson Blue Bullet racquetballs (Wilson Sporting Good Co., Chicago, Illinois USA), surgical tubing (latex, 1/2 inch diameter, 1/16 inch thick), and zip-ties (Gardner Bender Cable Ties, Milwaukee, Wisconsin USA) (Figure 1.2), which allows continual water movement when open, and collects the water samples when closed. After collection, aliquots (n=3 per water sample, mean volume of approximately 40 mL) were transferred from the water samplers to three transportable tubes.

Simulated Spawning Project Design

I designed field experiments to simulate spawning black abalone in order to assess proximity requirements for successful fertilization. Experiments were intended to duplicate, to the maximum possible extent given limited available information, known

attributes of abalone species that spawn in shallow water on exposed temperate coastlines.

Simulated spawning experiments were run at SNI between December 2012 and March 2013. The locations chosen for the experiments are at SNI site 8 (Figure 1.1). As noted above, black abalone numbers at site 8 experienced a lesser mass mortality rate from withering syndrome than black abalone aggregations at other SNI study sites during the 1990s. In addition, black abalone at SNI site 8 have higher positive numerical trends and the highest densities overall compared to other monitored populations at SNI. Three simulated spawning experiments were conducted at each of two locations at the SNI study site.

Tide Pool Habitat. The first set of three experiments were conducted in a tide pool located at 33.231° N, 119.535° W (Figure 1.3). Approximately 50 black abalone were located within a 10 m radius of the surrogate sperm release site on the edge of the pool, based on enumerations done on several dates in December 2012, demonstrating that the pool was located in reasonably good black abalone habitat. In addition, the configuration of the pool facilitated a primarily unidirectional flow of water at low tide levels, minimizing effects of confounding variables and aiding in the interpretations of results. Water flow in the pool at low tide was driven by periodic surges associated with nearby breaking surf, and was highly variable in velocity over time during the experiments.

Crevice Habitat. The second set of three experiments was done along the mouth of a crevice located at 33.231° N, 119.533° W (Figure 1.4). The crevice was linear, horizontally oriented and parallel to the shoreline with the crevice opening facing landward, 13 m in length, with a mean vertical opening width of 150 cm and a mean depth of 15.2 cm. The crevice had a sufficient water supply at an ebbing tidal level between +1 and +1.5 feet MLLW for sample collection. Tidal heights were estimated as described above for preliminary experiments. The crevice segment contained approximately 180 individual black abalone with a corresponding estimated density of 13.8 individuals/m^2 (survey completed 10 January 2013), among the highest local densities known anywhere on SNI at the time of the field experiments. Water flow in the crevice at low tide was driven by periodic waves that entered either over the top of the entire crevice, or surged in from the end of the crevice most open to incoming seawater.

The experiments each had a total duration of 60 minutes. The procedure involved a surrogate sperm (SS) release at a fixed location in all experiments for 30 minutes, with a surrogate egg (SE) release beginning 10 minutes after SS release commenced at progressively greater distances (0, 2, and 4 m) downstream from the point of SS release in successive experiments. Sample collection continued for an additional 30 minutes following the cessation of SS release to determine the persistence of particles in the pool. To eliminate the risk of contamination from particles left over from previous experiments and control for environmental variables, successive experiments were separated by at least one tidal cycle, and all experiments were conducted during periods of similar tidal elevations (between -1.2 and -0.9 feet MLLW at the tide pool habitat, and between -0.8

and +0.5 feet MLLW at the crevice habitat, as inferred from NOAA tidal prediction tables published by the National Ocean Service, US National Oceanic and Atmospheric Administration, and "Tides and Currents™ 3.7" tidal prediction software [Nobeltec®, Inc., Beaverton, Oregon USA]). Control samples were collected before beginning each experiment to verify that no particles remained in the area from prior experiments.

Approximately 120 g of SS were mixed with 6 L of seawater in a 19 L bucket prior to release. A small quantity of detergent was added to the mixture prior to release to minimize entrapment of particles in the surface film during mixing, and to facilitate flow during release. A one-liter intravenous fluid bag (IV bag) of the type used in human medical applications (Baxter saline IV bags, manufactured in Deerfield, Illinois USA) was filled with the particle mixture. Plastic tubing (~1 mm diameter) extended from the bottom of the IV bag for a distance of ~1.5 m. The open end of the tube was anchored below the water surface at the upstream end of the tidal pool, and defined the point of release for SS. All valves, joints, and flow controls were removed from tubing to minimize the risk of clogging. The SS mixture was allowed to flow freely through the tube once mixture was added to the bag. The bag was filled frequently during each experiment to maintain continuous flow of the SS mixture over the thirty-minute release period. Based on the manufacturer's estimate of the quantity of particles per unit mass included in the mixture and the flow rate through the IV tube as determined in preliminary tests, it was possible to produce the desired flow rate of $\sim 10^7$ particles/second by regulating the mass of particles included in the SS mixture and the total fluid volume of the mixture.

Approximately 10 g of SE were mixed with 200 mL of seawater and a small volume of detergent, and were released over a time span of ~30 seconds using a turkey baster with 30 mL maximum fluid capacity (KitchenAid Classic Bulb Baster, manufactured in St. Joseph, Michigan USA), 10 minutes after SS release began. Samples were collected at -1 m, 0 m, 1 m, 2 m, 4 m, 8 m, and 12 m down current from the SS release location, at 0, 1, 5, 10, 20, 30, 45, and 60 minutes after the SS release began.

Counting Particles

SE were counted by hand (using a 2x magnifying glass) after pouring each water sample aliquot through a 180 μm screen. SS particles passed through the screen and the remaining water was retained for subsequent SS counts. Volumes of the total sample (all aliquots) were measured and recorded to allow computation of SS and SE concentrations per unit volume of seawater. SS counts were completed using three 1 mL subsamples from each aliquot of water that had been passed through the 180 μm screen, counted under an 4.2x dissecting microscope and averaged to determine the amount of sperm-sized particles per milliliter in a given water sample.

Data Analysis

I determined fertilization success by calculating the ratio of eggs to sperm in a water sample and compared the ratios to different parameters used to represent fertilization

criteria for other abalone species in controlled settings. Specifically, laboratory experiments have determined optimal sperm to egg ratios of 10:1 for *H. coccoridata* (Bryne et al., 2010), 100:1 for *H. asinina* (Suphamungmee et al., 2010), and 500:1 for *H. kamtschatkana* (Bouma, personal communication). In the wild, the greater gamete dilution and water movement may require higher concentrations for more probable fertilization; therefore more strict ratios, including 1000:1 and 5000:1 sperm to egg ratios were examined. Analyses of fertilization success between habitat types and release distances were conducted using one-way and two-way analysis of variance (ANOVA).

Results

Egg Density

In the six successful simulated spawning experiments, the distribution of SE was tracked by SE concentration over distance (m) and time (min) (Figures 1.5 through 1.10), with green bars indicating the concentration and black squares indicate 0 values. The experimental design allows a SS cloud to build for 10 minutes before the SE are released; therefore, the zero minutes, 1 minute, and 5 minutes values of the bar graph all indicate zero SE present. Data from samples collected immediately post-SE release (at the 10 minute sample collection) varied based on water flow. For example, the lack of water movement during the 4 m SE release in the tide pool resulted in little particle dispersal and abnormally high SE counts (18.83 SE/mL) when compared to other samples. Overall, SE concentrations were not significantly different among habitat types or release distances (Figure 1.11, $P = 0.4799$; Figure 1.12, $P = 0.205$).

Sperm Density

The distribution of SS was similarly tracked over distance (m) and time (min) (Figures 1.13 through 1.18). The black squares indicating zero values and light blue bars show concentration where no parameters for successful fertilization were met. Each of the different criteria for fertilization is indicated on the figures in different colors. For a maximum of 100:1 sperm to egg fertilization potential, the bar is orange; for a maximum of 500:1 sperm to egg fertilization potential, the bar is red; for a maximum of 1000:1 sperm to egg fertilization potential, the bar is blue; and for a maximum of 5000:1 sperm to egg fertilization potential, the bar is purple. The difference between SS concentrations is highly significant when compared over habitat types (Figure 1.19, $P = 0.0003$).

While each simulated spawning experiment was conducted with different release distances between the SE and SS, the SS release location, sample collection distances, and sample collection times all remained constant, resulting in three replicates of SS dispersal in each habitat type. The SS dispersal patterns vary among individual releases as well as habitat type (Figures 1.20 through 1.26, $P = 0.0004$). A two-way ANOVA revealed that although there is variability between each experimental release (Table 1.4), habitat remains a dominant factor influencing particle dispersal and retention in the area.

Fertilization Potential

Four different criteria (100:1, 500:1, 1000:1, and 5000:1 sperm to egg ratios) were applied to the sperm and egg concentrations in each sample, where each sample represents one distance and time unit in each run, and the amount of samples where fertilization could occur were totaled for each release distance and habitat type (Figures 1.27 through 1.31). The release distance did not influence potential fertilization success (Table 1.5) and habitat type was only significant in the strict 5000:1 criterion (Table 1.5).

Discussion

This study is to my knowledge the first to utilize surrogate gametes in a simulated spawning experiment to measure fertilization success of spawning abalone, and determine the relationships between spawning proximity and habitat type. Black abalone are commonly found in various habitat types in the rocky intertidal, but commonly occur in groups within crevice habitats. Aggregative behavior by black abalone may explain this distribution pattern and could be attributed to increased protection from wave activity (Shanks and Wright, 1986) and protection from predation by sea otters (Lowry and Pearse, 1973; Cooper et al., 1977; Fanshawe et al., 2003). Crevice habitat and clustering patterns may also be a strategy to improve potential fertilization between spawning pairs of black abalone.

The concentration of SS particles appears to be the main factor limiting fertilization. Due to this limiting potential, the increased retention of SS particles in the crevice habitat is

key to improved fertilization success. The three replicates of SS releases following the same criteria in each habitat type indicate there is a high degree of natural variability, however the crevice habitat shows higher particle retention over time and distance. During wave surges, the tide pool had fewer obstructions for dispersal, and particles could move more widely within the water and become diluted; however the crevice habitat had pockets and more complex topography which may create more eddies that I hypothesize are responsible for retaining the particles in the area more efficiently.

The dispersion of surrogate gamete particles (SE and SS) is highly dependent on wave activity, and although it varies considerably between experimental runs, the amount of particle retention is also highly dependent on habitat type. The crevice habitat shows a significantly higher degree of particle retention over the tide pool habitat, leading to an increased potential for fertilization. Based on the more strict 5000: 1 sperm to egg fertilization potential criterion, the tide pool fertilization potential was restricted to -1 m and 0 m from the SS release location, 20 to 30 minutes after SS release commenced and when surrogate gametes were released close together (0 m and 2 m apart). The crevice habitat contained more samples that met the criteria for potential fertilization. Samples as far as 12 m from the SS release location as long as 60 minutes after the initiation of simulated spawning activity, and at surrogate gamete release distances of up to 4 m exhibited high fertilization potential.

The increased fertilization potential seen in the crevice habitat counters hypotheses by Denny and Shibata (1989); in their study on external fertilization in intertidal habitats,

using the sea urchin *Strongylocentrotus purpuratus*, they model the effects of turbulence and mixing in intertidal habitats. Their results indicate fertilization was less likely in intertidal habitats exposed to high wave activities, and fertilization success had higher potential in tide pool habitats (Denny and Shibata, 1989). In contrast to their results, however, the researchers recognized that sea urchins were still aggregated in areas indicated by models to have lower probabilities for fertilization, and speculate this may be because their model may need further development, there are offsetting advantages to intertidal life that compensate for reduced fertilization success, or the organisms have no control over where they settle and have limited mobility (Denny and Shibata, 1989).

Intertidal turbulence is generated when the fluid approaches in a manner that creates eddies. Obstacles such as rocks create additional turbulence by adding wakes. The models created by Denny and Shibata (1989) hypothesize that particles in rocky intertidal encounter more eddies and will disperse and mix at a greater rate as eddies grow larger. Based on the turbulent nature of the surf zone and intertidal areas, previous conventional knowledge indicated abalone must be within close proximity to reproduce, but no distances have been defined. Results from simulated spawning experiments indicate concentrations of gametes from one spawning male and female pair can potentially achieve fertilization from 4 m apart in the crevice habitat. The number of obstacles may constrain the size of eddies, keeping them from expanding, and may work to retain more particles, counter to what the Denny and Shibata (1989) models suggest.

The simulated spawning experiments have several large assumptions that affect their interpretation. The basic linear trajectory is not applicable to each spawning abalone because the unidirectional flow in the experiment assumes the female spawning abalone is directly downstream from the spawning male. While this isn't applicable to all spawning abalone, it is relevant for black abalone inhabiting a large portion of crevice habitats and tide pool habitats. Approximately 180 black abalone were counted along the transect in the crevice habitat used in the simulated spawning experiments, and over 50 black abalone were counted within a 10 m proximity of the tide pool habitat. The experimental design also assumes the presence of at least one male and one female black abalone within the 4 m required spawning distance.

This work pioneers the use of surrogate particles in simulated spawning events to determine fertilization potential with no disturbance to the endangered black abalone. The high cost of each experiment and timing restrictions for finding accessible low tides limited the amount of simulated spawning events that could be conducted, resulting in a low statistical power. While this limited the amount of statistical significance that could be seen in the data, high significance was still seen between habitat types, with crevice habitat shown to retain particles at a much higher rate. Due to safety constraints involved with working in the intertidal, all the work was done at low tide, so there are no corresponding results for potential spawning events at high tide or with higher wave action. Additional experimentation is needed within both habitat types to confirm existing findings, as well as among other study sites.

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Tables

Table 1.1 Interspecific variation in abalone fecundity (not reported as potential or instantaneous).

Species	Egg Count	Citation
H. asinina	$1.5x10^5$ to $6.0x10^5$	Capinpin et al., 1998
H. australis	$2.0x10^3$ to $2.7x10^6$	Wilson and Schiel, 1995
H. diversicolor	$1.3x10^5$ to $2.0x10^5$	Chen, 1989
H. iris	$3.0x10^3$ to $1.8x10^6$	Wilson and Schiel, 1995
H. laevigata	$1.0x10^5$ to $8.2x10^6$	Babcock and Keesing, 1999
H. midae	$7.5x10^4$ to $1.2x10^6$	Genade et al., 1988
H. rubra	$2.0x10^5$ to $5.9x10^6$	Litaay and De Silva, 2001; Plant et al., 2002
H. rufescens	$8.5x10^5$ to $11.1x10^6$	Ault, 1985
H. tuberculata	$1.1x10^5$ to $1.6x10^6$	Peña, 1986; Clavier, 1992
H. varia	Mean = $7.6x10^4$	Najmudeen and Victor, 2004

Table 1.2 Pre-spawning egg size, spawned egg size, unfertilized egg size, fertilized egg size, fertilized egg size range, and fertilized egg size mean

Species	Egg size (µm)					Citation
	Pre-Spawn	Unfertilized	Fertilized	(Fert) Range	(Fert) Mean	
H.asinina		180	185		190	Singhagraiwan and Sasaki, 1991; Suphamungmee et al. 2010
H. australis					175	Wilson and Schiel, 1995
H. coccoradiata				150-250	175	Wong et al., 2010
H. cracherodii	143					Webber and Giese, 1969
H. discus hannai					230	Ino, 1952
H. iris					230	Harrison and Grant, 1971
H. laevigata	200-250					Shepherd and Laws, 1974
H. midae				212-222	214	Genade et al., 1988
H. roei	150-250					Shepherd and Laws, 1974
H. rubra	120-160	240		217-247	230	Huchette et al., 2004
H. rufescens				170-190		Leaf, 2005
H. sieboldii				270-280		Ino, 1952
H. sorenseni					200	Leighton, 1972
H. tuberculata		198	205		180	De Vicose et al., 2007
H. varia					180	Najmudenn and Victor, 2004

Table 1.3 Comparison of spawning statistics between *H. rubra* and *H. cracherodii*.

	H. rubra	***H. cracherodii***	**Citation**
Habitat	Temperate	Temperate	Huchette et al. 2004; VanBlaricom et al. 2009
Maximum Shell Length (mm)	220	200	Huchette et al. 2004; VanBlaricom et al. 2009
Pre-spawn Egg Size (µm)	120 to 160	143	Webber and Giese 1969
Spawned, Unfertilized Egg Size (µm)	240	**Unknown**	Huchette et al. 2004
Instantaneous Fecundity	$1x10^5$ to $2.2x10^6$	**Unknown**	Litaay and de Silva 2001; Plant et al. 2002

Table 1.4 Two-way ANOVA values for surrogate sperm (SS) concentration, testing significant differences in habitat type and run for variability between replicates

	df	F	P	Significance
Treatments	1	0.4547	0.5006	
Habitat	1	13.264	0.0003	***
Run	1	0.1848	0.5006	
Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1				

Table 1.5 Two-way ANOVA values for each of the four fertilization criteria (100:1, 500:1, 1000:1, and 5000:1 sperm to egg ratios), testing significant differences in habitat type and release distances.

	df	F	P	Significance
Criteria 100:1				
Treatment	1	5.333	0.1472	
Habitat	1	0.5	0.5528	
Release Distance	1	0.33333	0.622	
Criteria 500:1				
Treatment	1	4.9587	0.1559	
Habitat	1	7.438	0.1123	
Release Distance	1	0.4463	0.5729	
Criteria 1000:1				
Treatment	1	5.0116	0.1546	
Habitat	1	15.8266	0.0578	.
Release Distance	1	0.8497	0.4540	
Criteria 5000:1				
Treatment	1	0.8824	0.4467	
Habitat	1	39.5529	0.0244	*
Release Distance	1	0.0353	0.8683	
Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1				

Figures

Figure 1.1 Map of San Nicolas Island, located approximately 120 km WSW of Los Angeles, California. Numbers refer to the nine sites sampled by VanBlaricom. Simulated spawning experiments were conducted at site 8 (Figure courtesy of D. Witting).

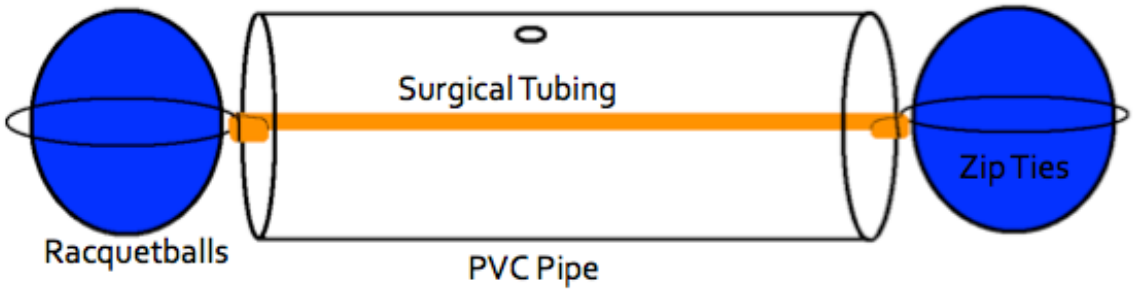

Figure 1.2 Construction of water samplers consisting of polyvinyl chloride tubes, racquetballs, zip-ties, and surgical tubing.

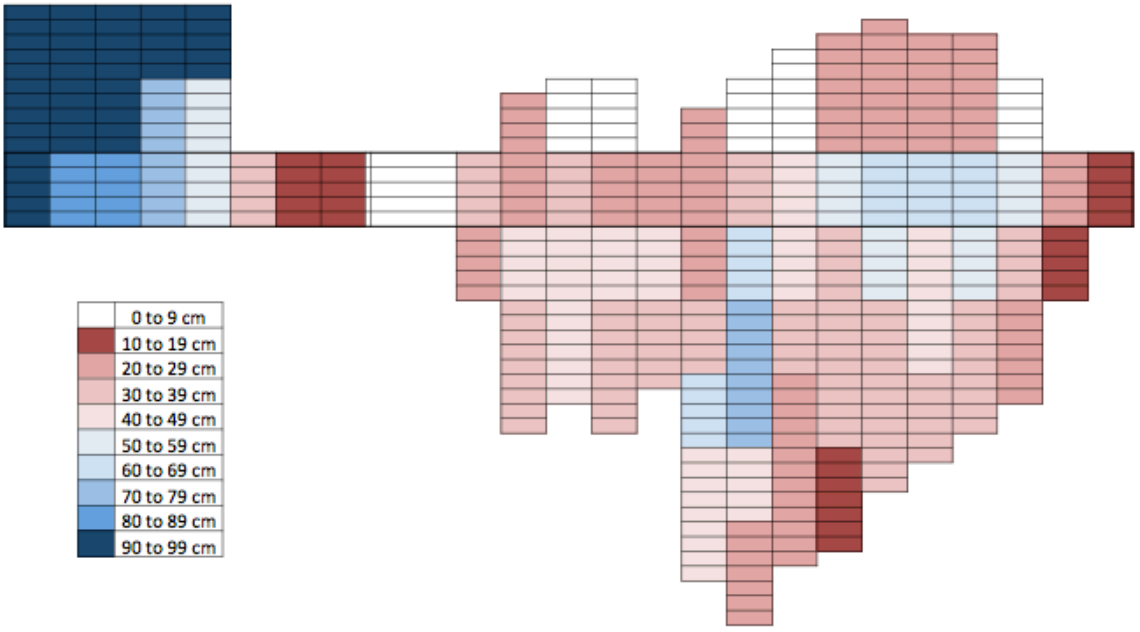

Figure 1.3 Dimensions of tide pool, located at 33.231° N, 119.535° W.

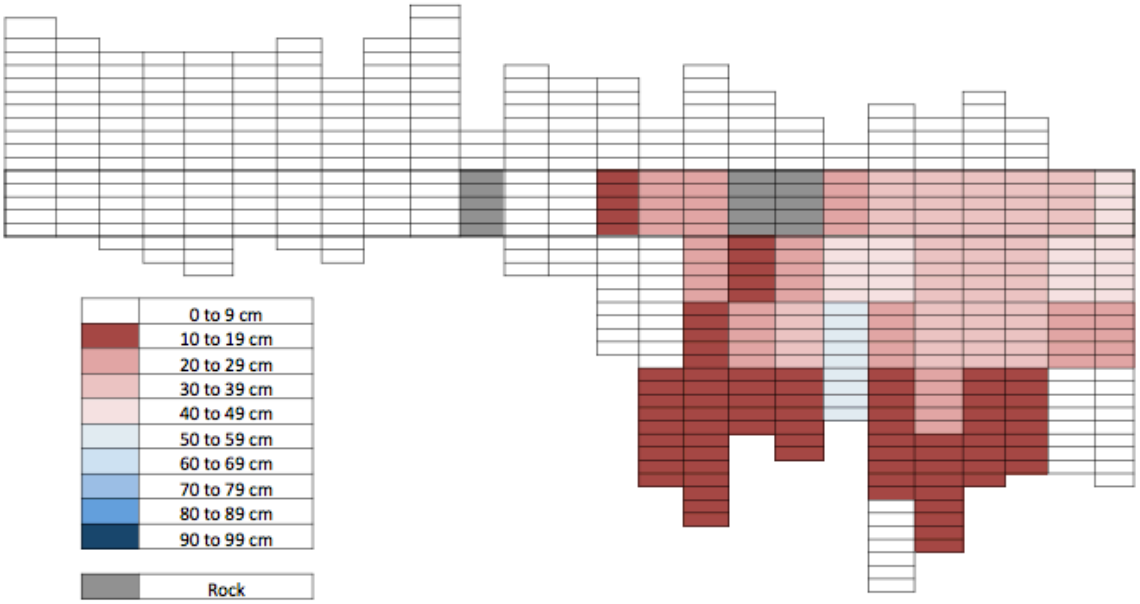

Figure 1.4 Dimensions of the crevice habitat, located at 33.231° N, 119.533° W.

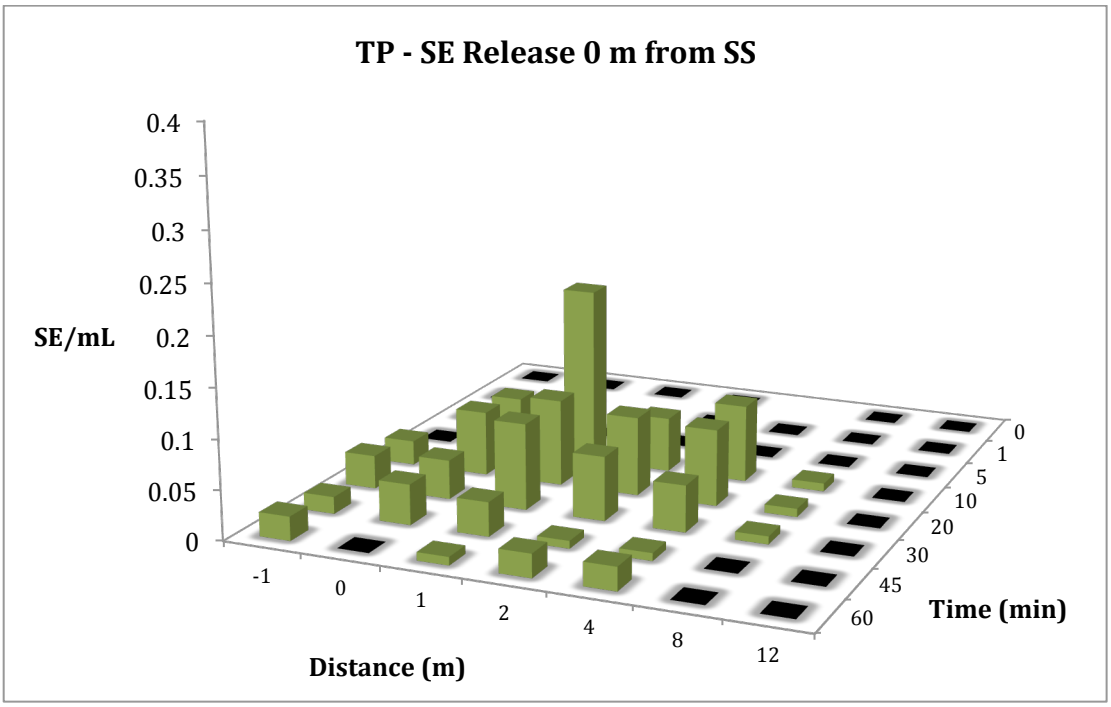

Figure 1.5 Amount of surrogate eggs (SE) per mL recovered at varying distnances (m) and time (min) in the tide pool (TP) habitat, relative to the SS release site. SE were released 0 m from surrogate sperm (SS) release location. Black indicates 0 values. SE are released at 10 minutes following SS release, therefore the 0 minute, 1 minute, and 5 minute values are pre-SE release and 0 values.

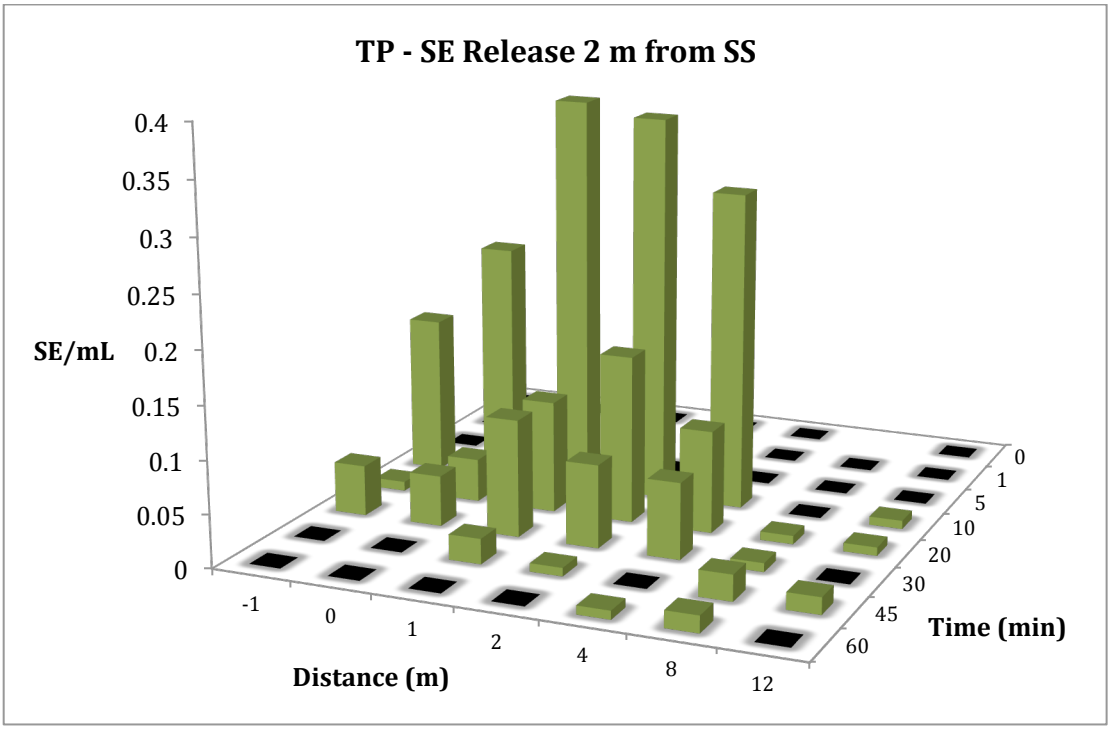

Figure 1.6 Amount of SE per mL over distance (m) and time (min) in TP habitat, with SE released 2 m from SS release location (black indicates 0 values).

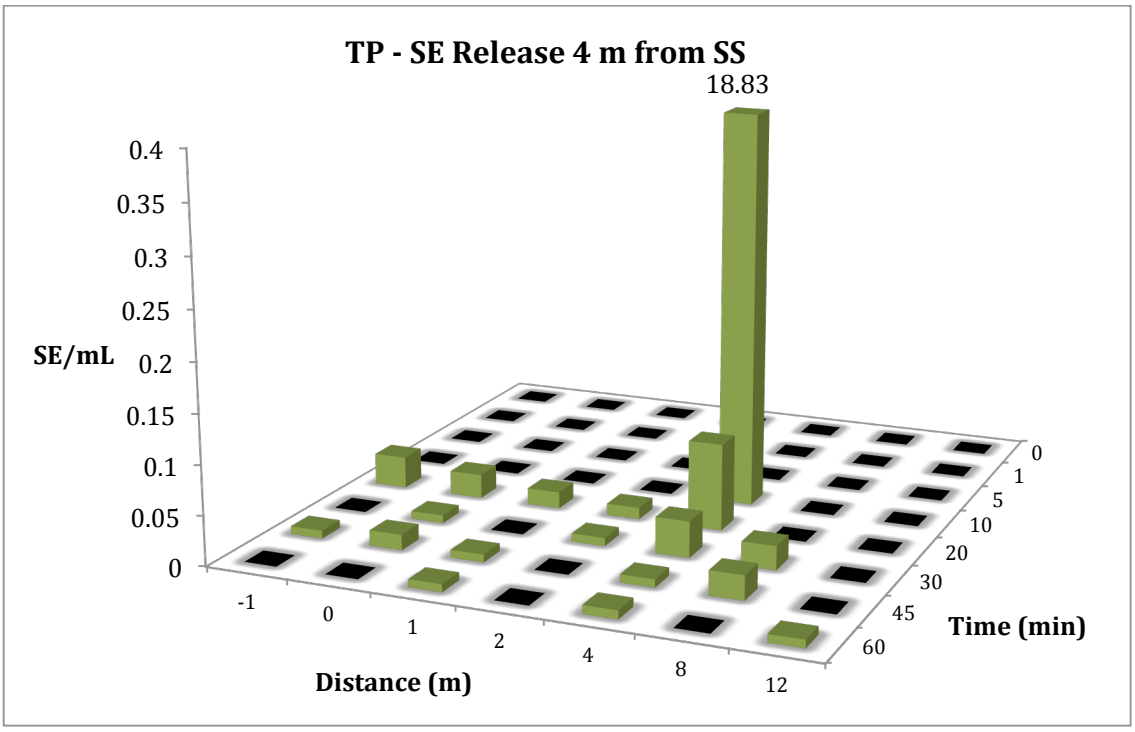

Figure 1.7 Amount of SE per mL over distance (m) and time (min) in TP habitat, with SE released 4 m from SS release location (black indicates 0 values).

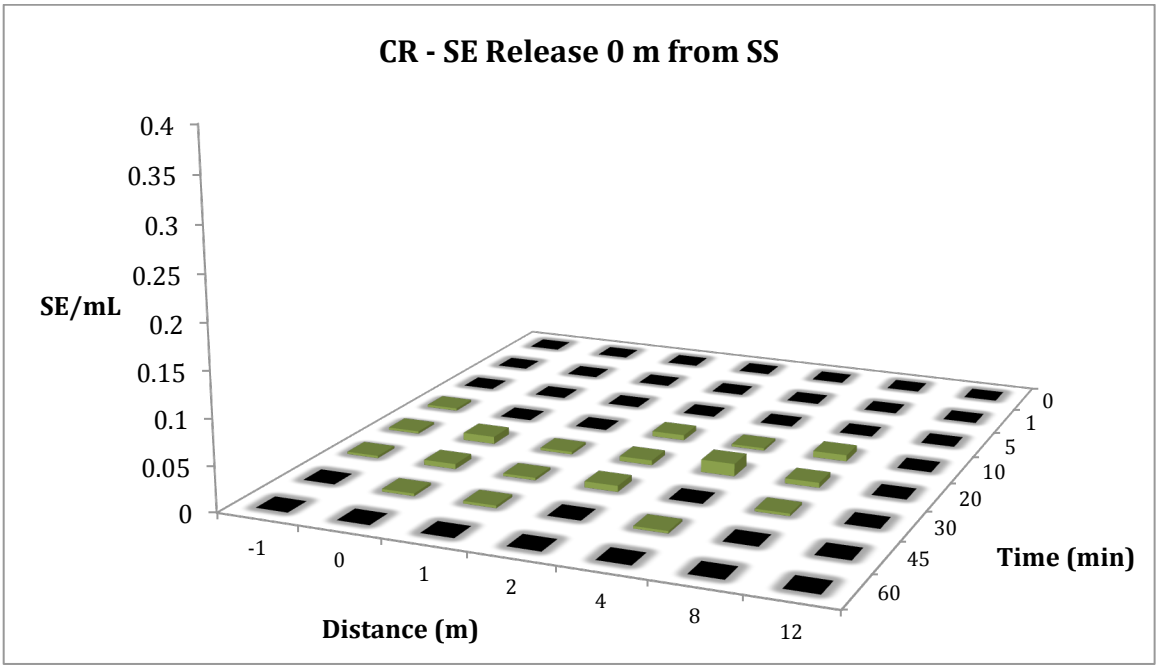

Figure 1.8 Amount of SE per mL over distance (m) and time (min) in crevice (CR) habitat, with SE released 0 m from SS release location (black indicates 0 values).

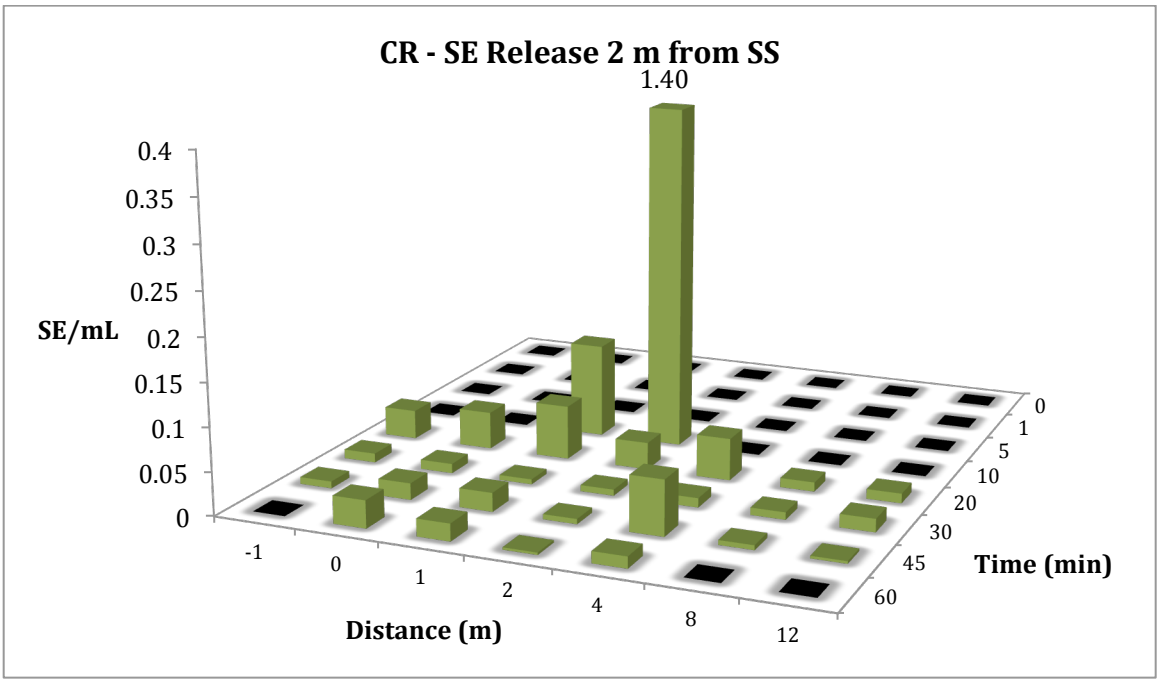

Figure 1.9 Amount of SE per mL over distance (m) and time (min) in CR habitat, with SE released 2 m from SS release location (black indicates 0 values).

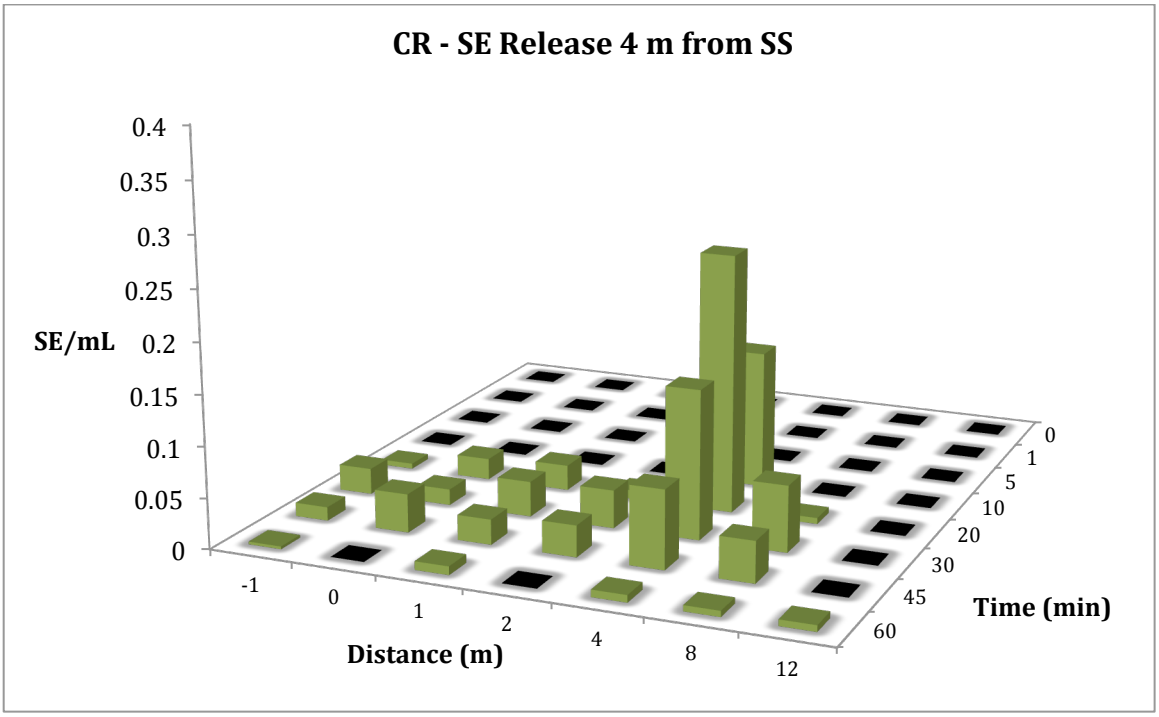

Figure 1.10 Amount of SE per mL over distance (m) and time (min) in CR habitat, with SE released 4 m from SS release location (black indicates 0 values).

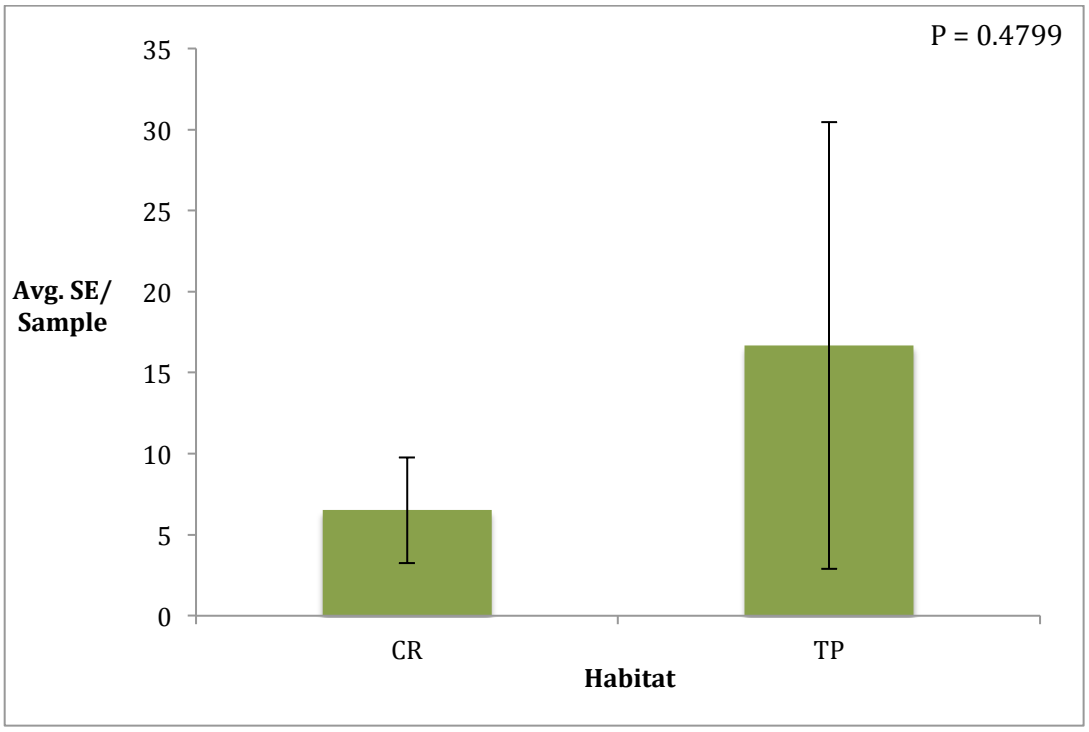

Figure 1.11 Average SE concentration in each sample compared between habitat types (P = 0.4799), with error bars indicating 95% confidence.

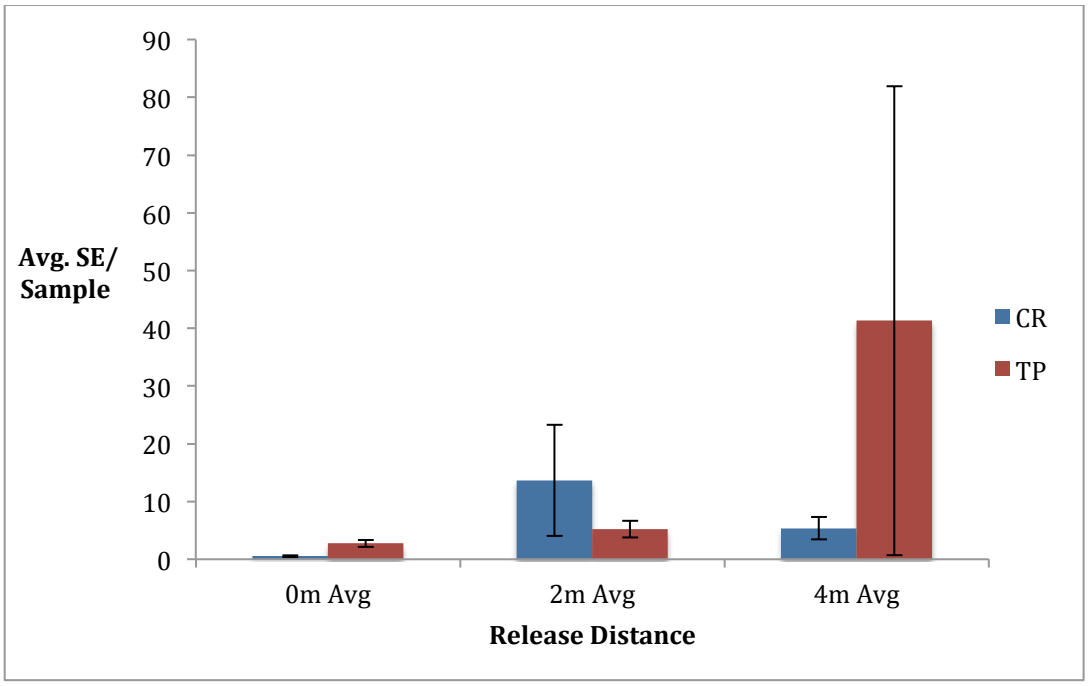

Figure 1.12 Average SE concentration difference between release distances (P=0.2082) and habitat type (P=0.4767). Error bars indicate 95% confidence.

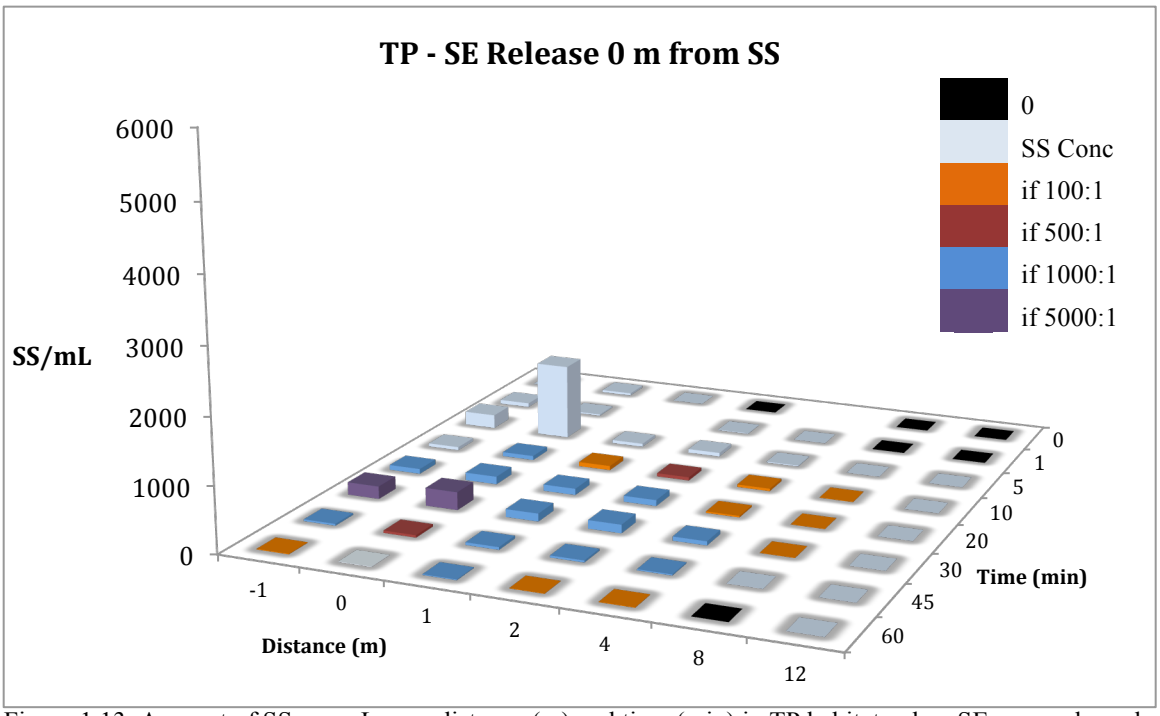

Figure 1.13 Amount of SS per mL over distance (m) and time (min) in TP habitat, when SE were released 0 m from SS release location (black indicates 0 values, light blue indicates surrogate sperm concentration (SS Conc) where the fertilization criteria were not met. The bar color indicates maximum fertilization criterion met by sperm to egg ratio.

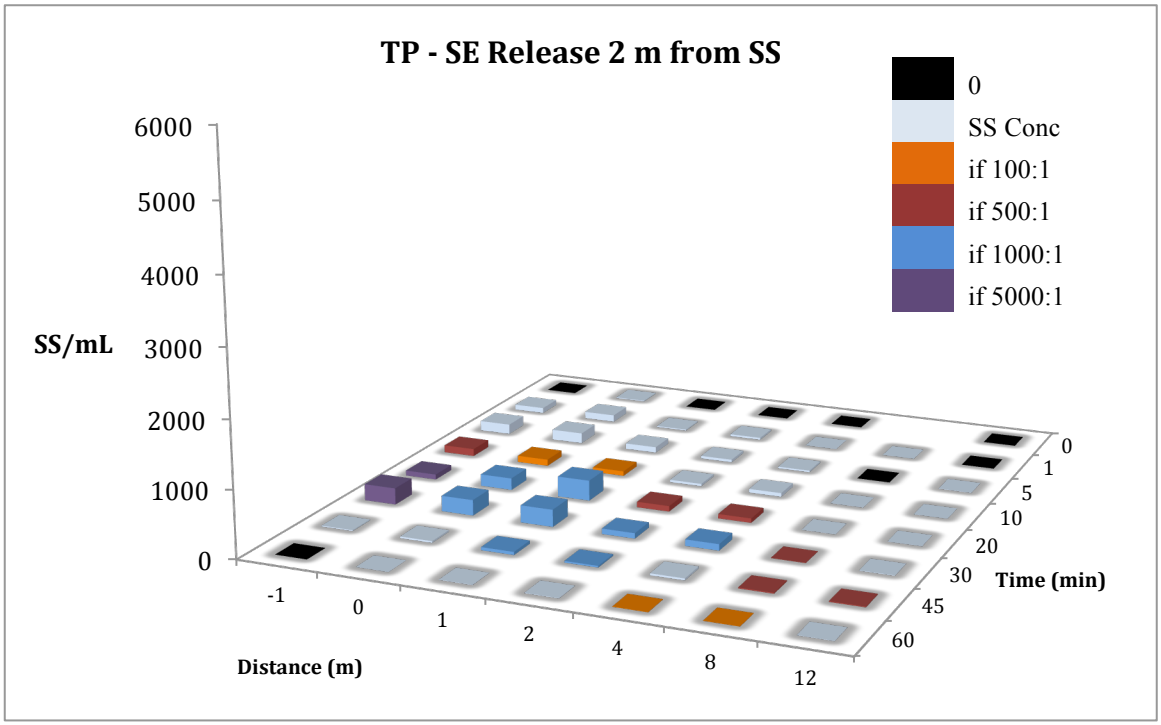

Figure 1.14 Amount of SS per mL over distance (m) and time (min) in TP habitat, when SE were released 2 m from SS release location (black indicates 0 values, light blue indicates surrogate sperm concentration where the fertilization criteria were not met. The bar color indicates maximum fertilization criterion (minimum ratio value) met by sperm to egg ratio.

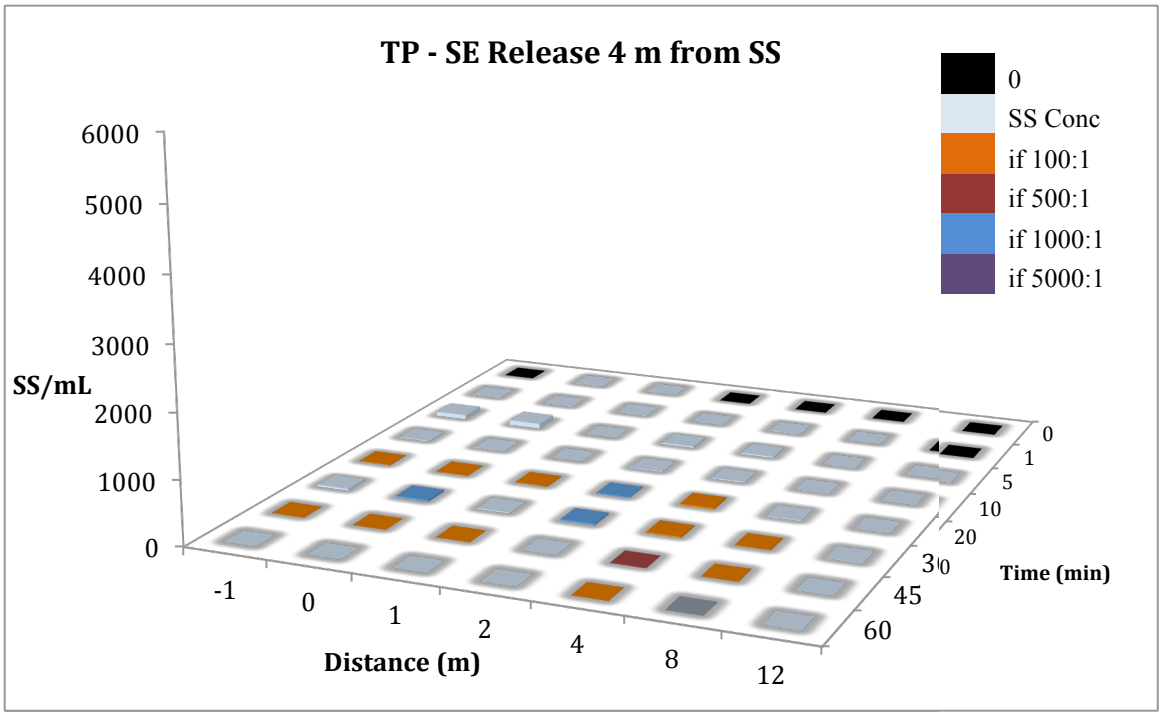

Figure 1.15 Amount of SS per mL over distance (m) and time (min) in TP habitat, when SE were released 4 m from SS release location (black indicates 0 values, light blue indicates surrogate sperm concentration where the fertilization criteria were not met. The bar color indicates maximum fertilization criterion (minimum ratio value) met by sperm to egg ratio.

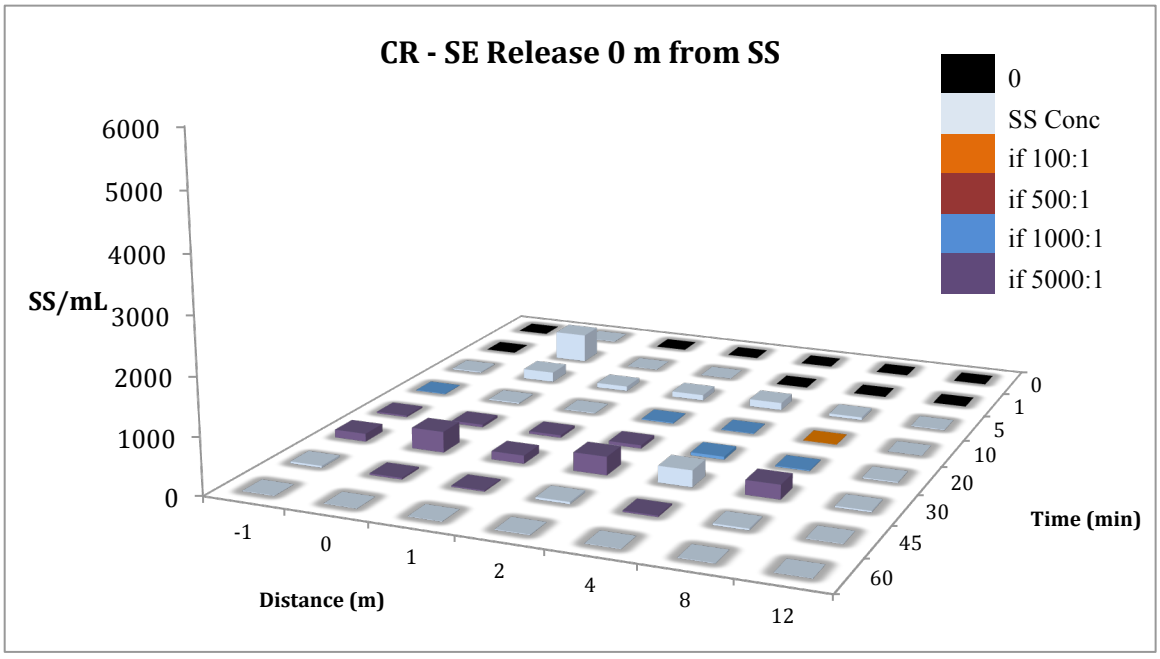

Figure 1.16 Amount of SS per mL over distance (m) and time (min) in CR habitat, when SE were released 0 m from SS release location (black indicates 0 values, light blue indicates surrogate sperm concentration where the fertilization criteria were not met. The bar color indicates maximum fertilization criterion (minimum ratio value) met by sperm to egg ratio.

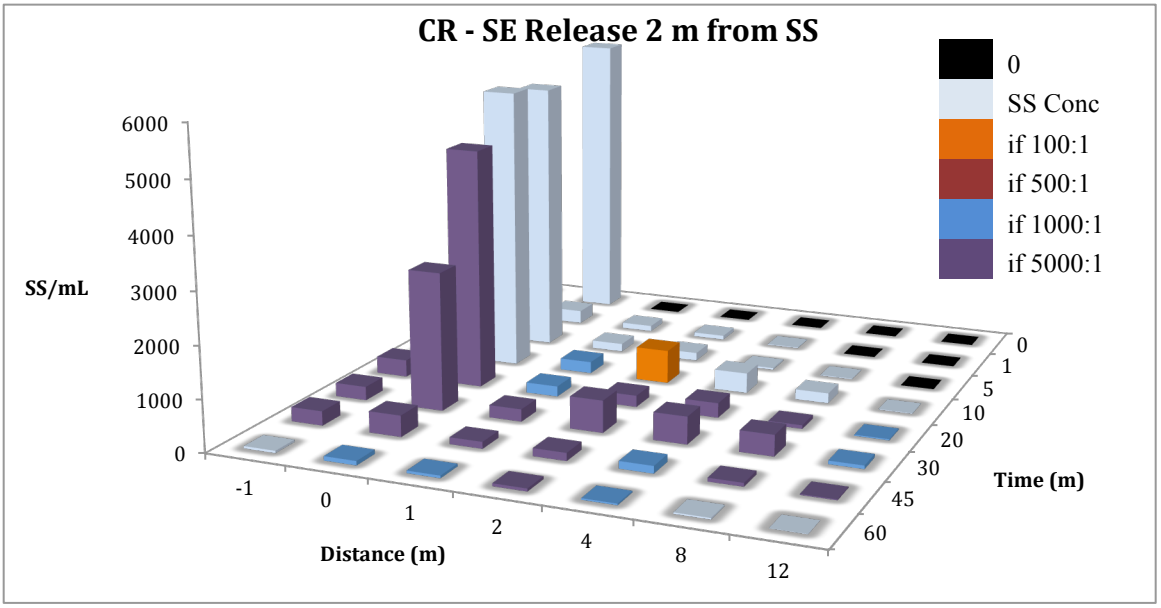

Figure 1.17 Amount of SS per mL over distance (m) and time (min) in CR habitat, when SE were released 2 m from SS release location (black indicates 0 values, light blue indicates surrogate sperm concentration where the fertilization criteria were not met. The bar color indicates maximum fertilization criterion (minimum ratio value) met by sperm to egg ratio.

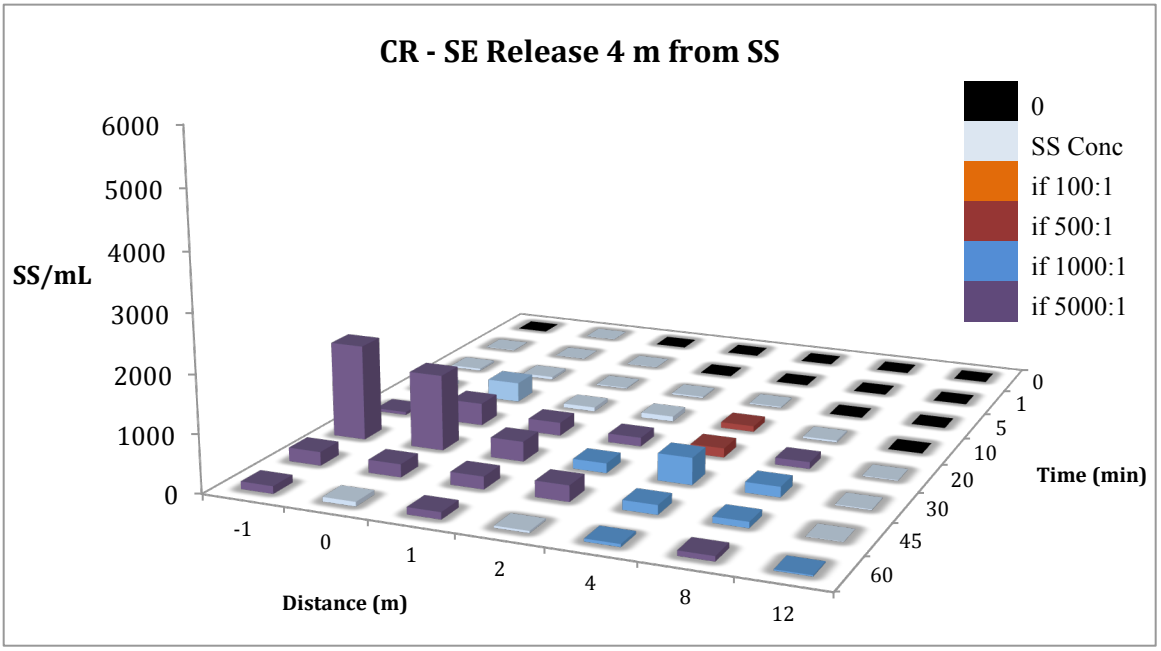

Figure 1.18 Amount of SS per mL over distance (m) and time (min) in CR habitat, when SE were released 4 m from SS release location (black indicates 0 values, light blue indicates surrogate sperm concentration where the fertilization criteria were not met. The bar color indicates maximum fertilization criterion (minimum ratio value) met by sperm to egg ratio.

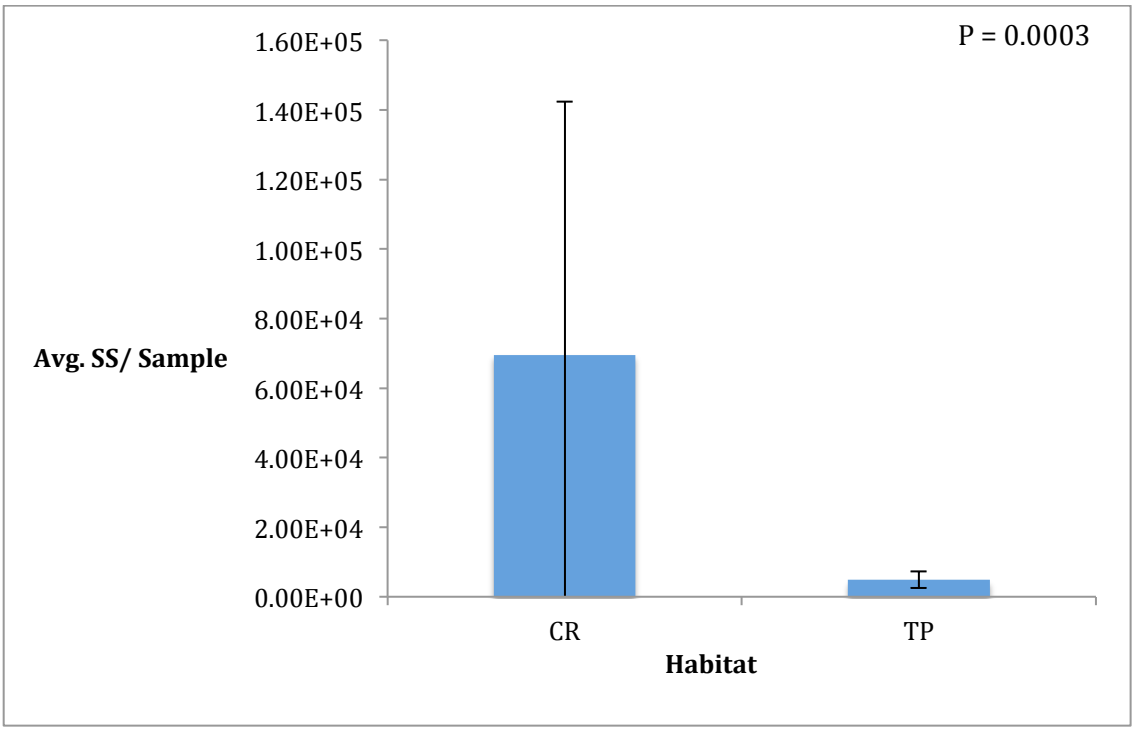

Figure 1.19 Average SS per sample concentration difference between habitat types (P = 0.0003).

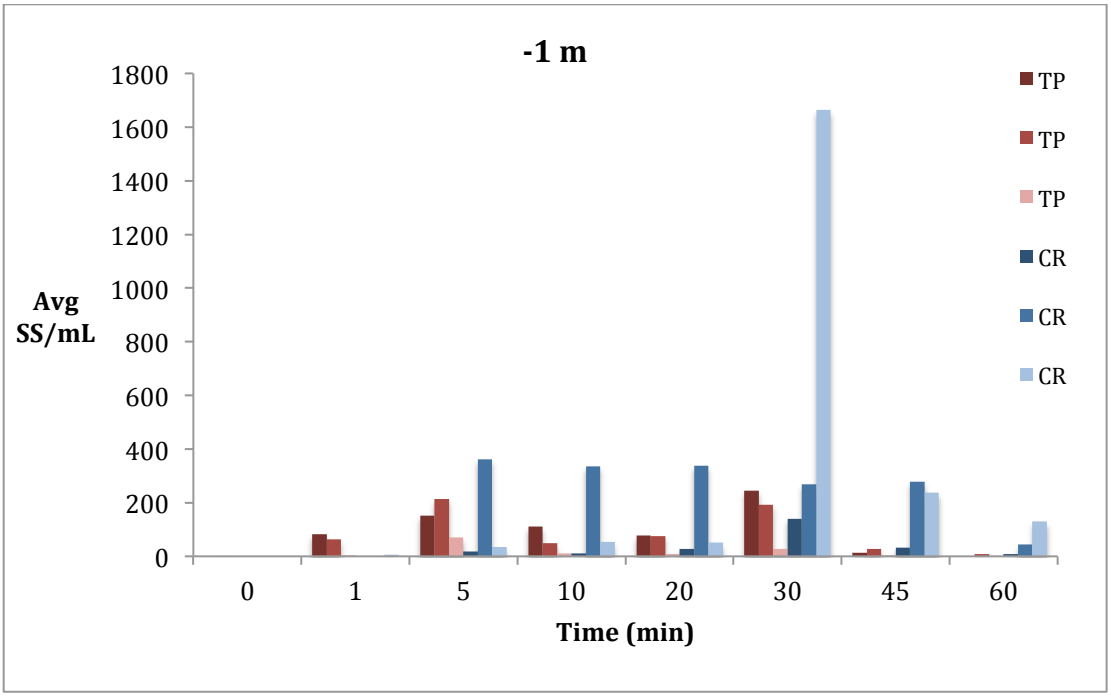

Figure 1.20 Average SS/mL for each trial collected at -1 m from SS release location over time, with tide pool (TP) trials in shades of pink, and crevice (CR) trials in shades of blue.

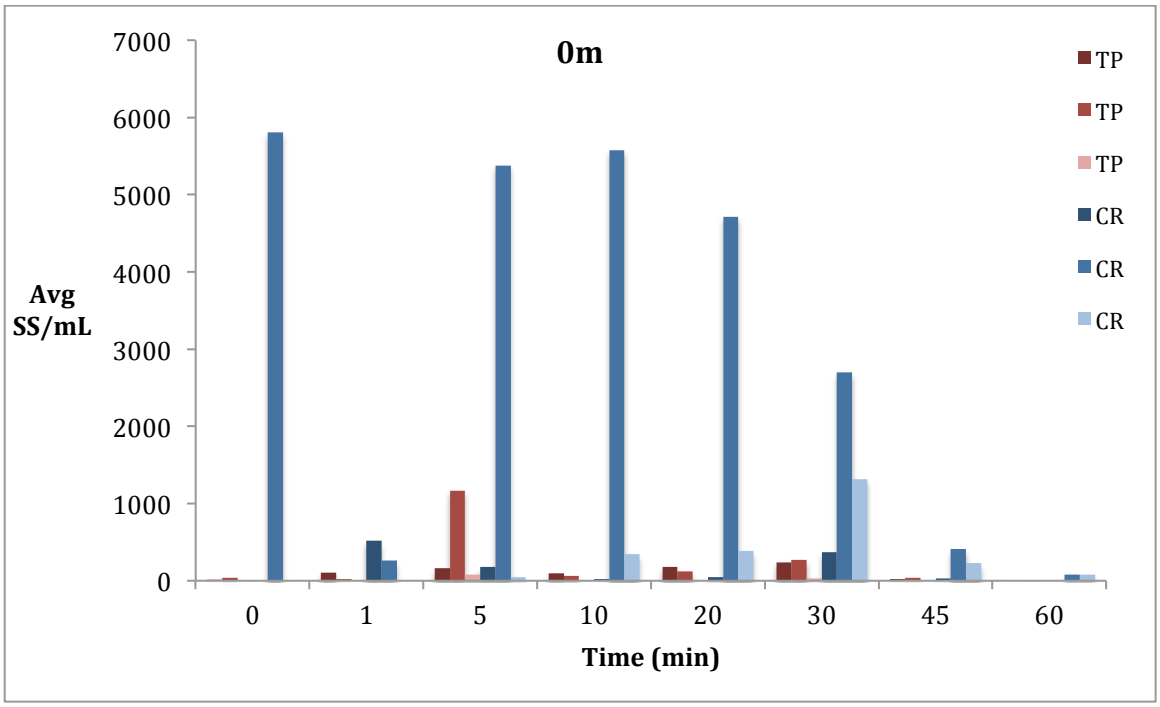

Figure 1.21 Average SS/mL for each trial collected at 0 m from SS release location over time, with tide pool (TP) trials in shades of pink, and crevice (CR) trials in shades of blue.

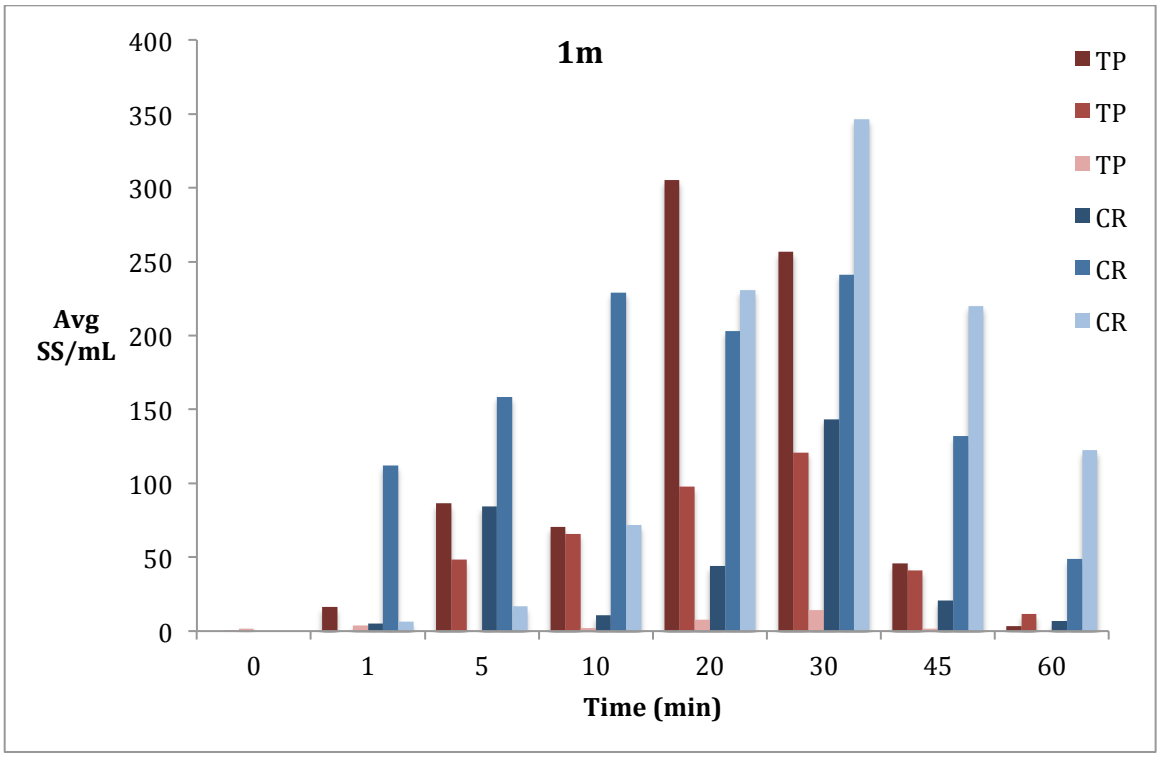

Figure 1.22 Average SS/mL for each trial collected at 1 m from SS release location over time, with tide pool (TP) trials in shades of pink, and crevice (CR) trials in shades of blue.

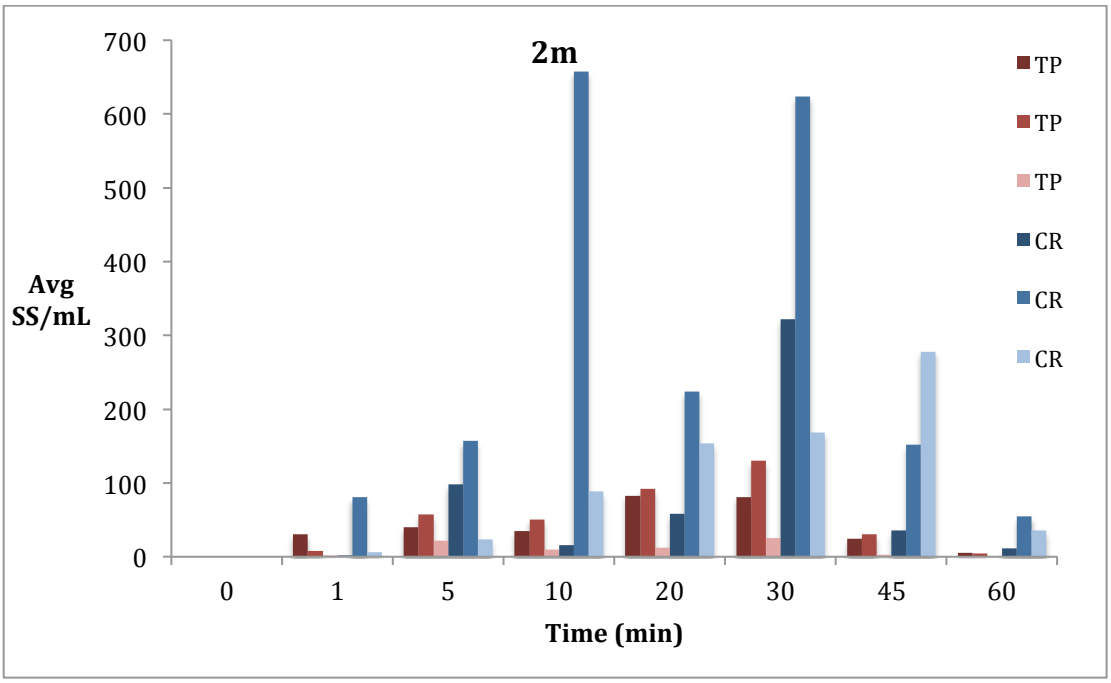

Figure 1.23 Average SS/mL for each trial collected at 2 m from SS release location over time, with tide pool (TP) trials in shades of pink, and crevice (CR) trials in shades of blue.

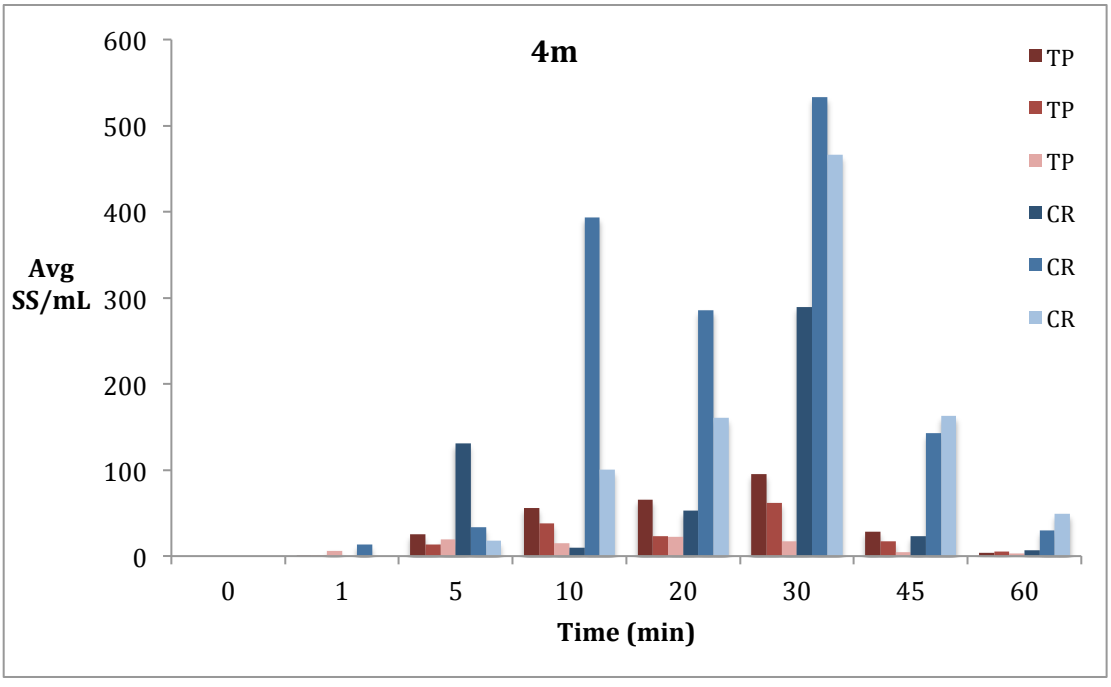

Figure 1.24 Average SS/mL for each trial collected at 4 m from SS release location over time, with tide pool (TP) trials in shades of pink, and crevice (CR) trials in shades of blue.

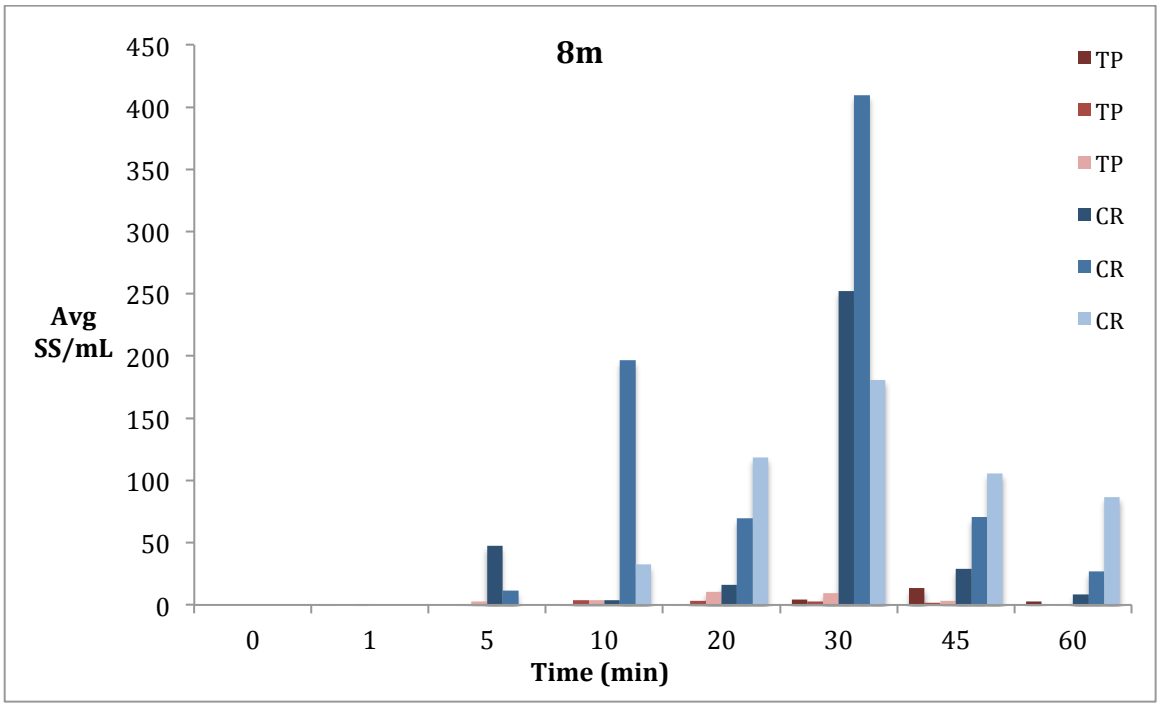

Figure 1.25 Average SS/mL for each trial collected at 8 m from SS release location over time, with tide pool (TP) trials in shades of pink, and crevice (CR) trials in shades of blue.

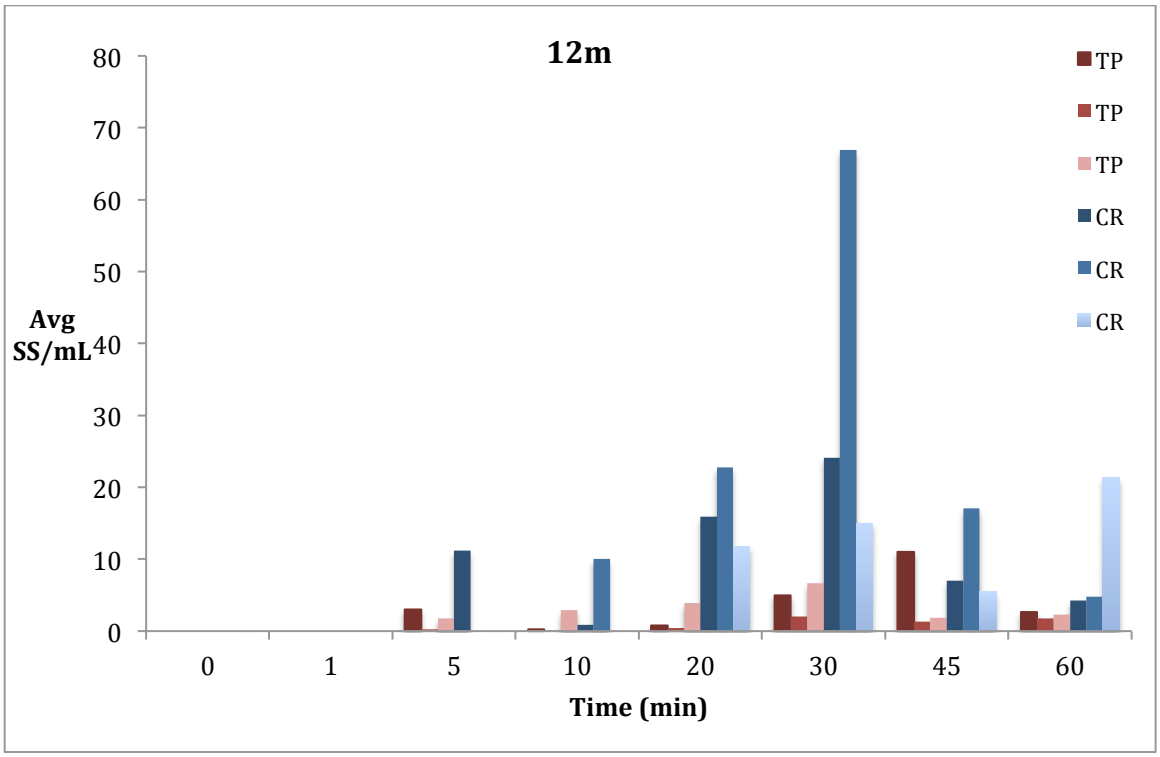

Figure 1.26 Average SS/mL for each trial collected at 12 m from SS release location over time, with tide pool (TP) trials in shades of pink, and crevice (CR) trials in shades of blue.

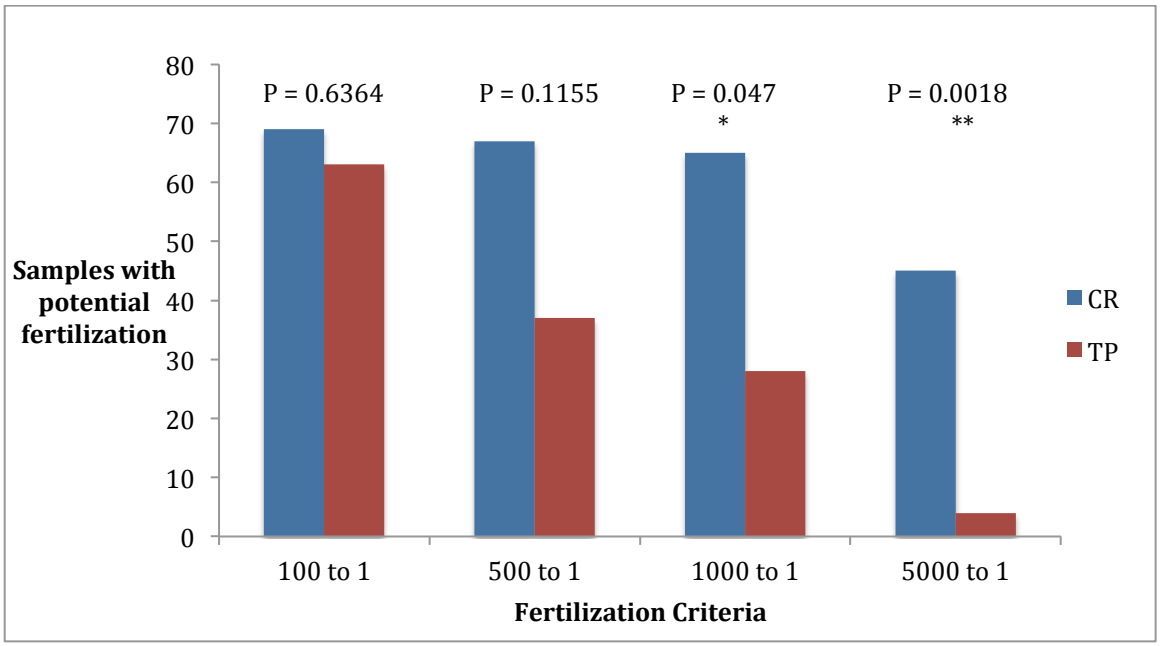

Figure 1.27 Total samples with potential fertilization based on increasingly strict criteria (100:1, 500:1, 1000:1, and 5000:1) for determining fertilization potential, separated by habitat type. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1.

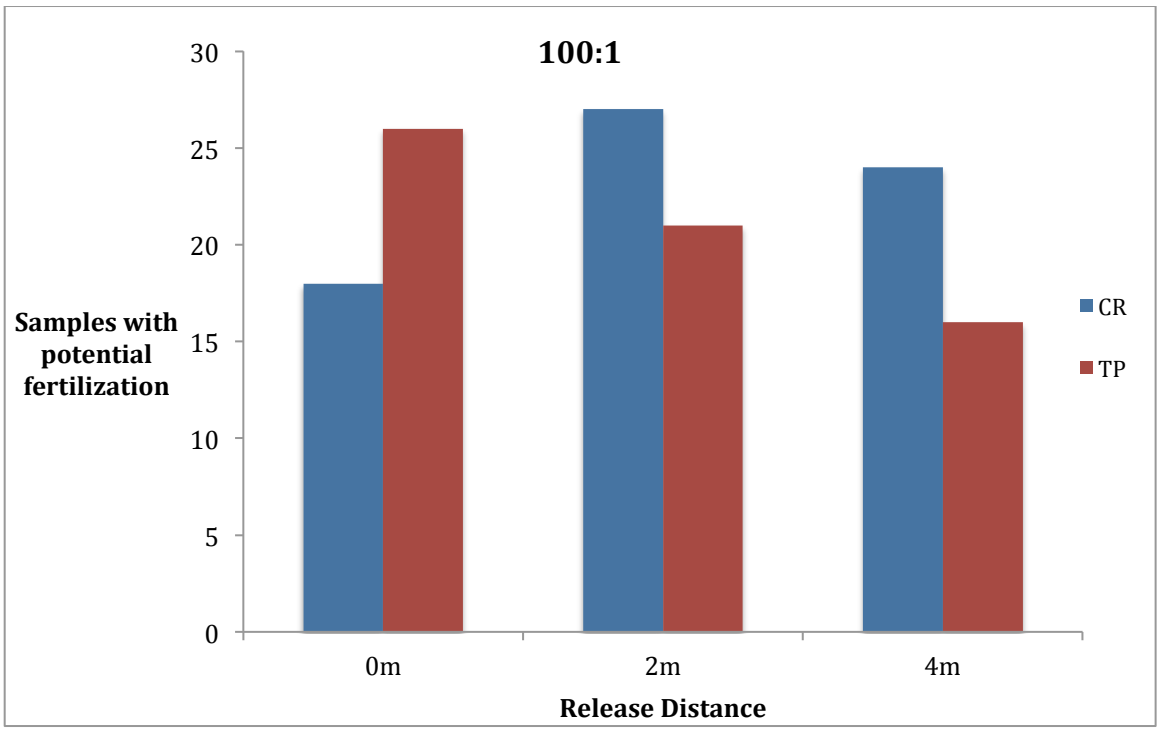

Figure 1.28 Total samples with potential fertilization based on the 100:1 sperm to egg criteria defining fertilization potential, showing patterns in release distance (P = 0.6220) and habitat type (P = 0.5528).

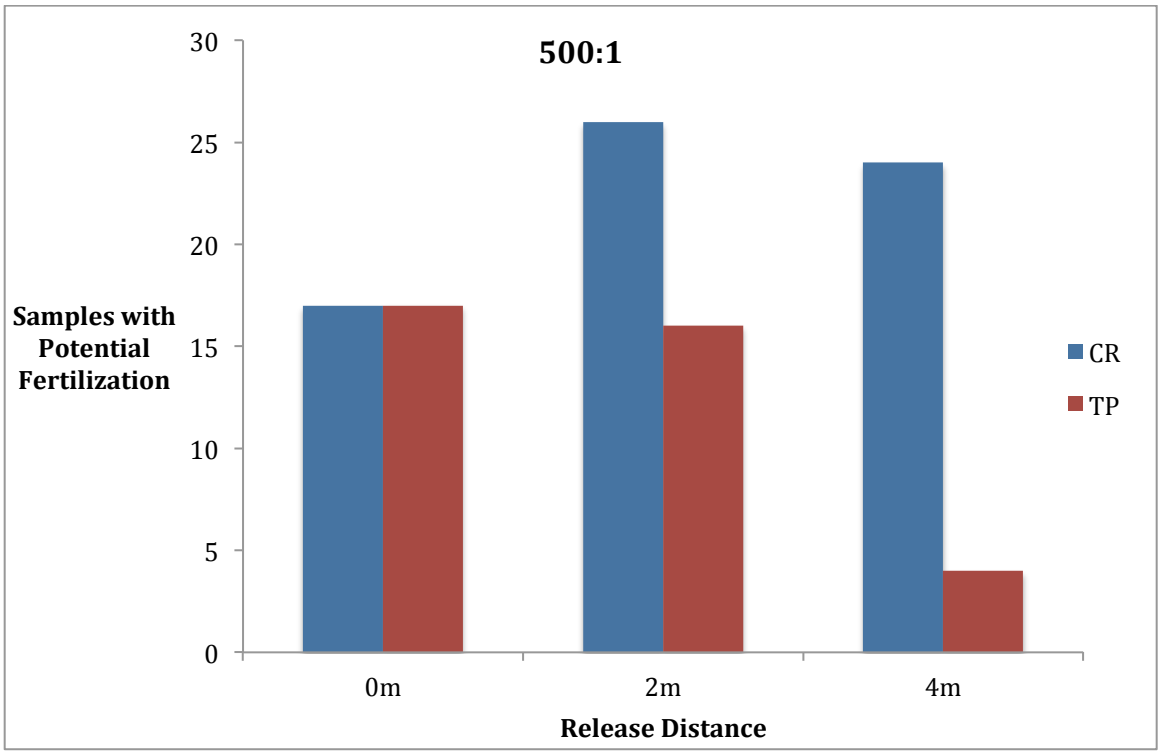

Figure 1.29 Total samples with potential fertilization based on the 500:1 sperm to egg criteria defining fertilization potential, showing patterns in release distance (P = 0.5729) and habitat type (0.1123).

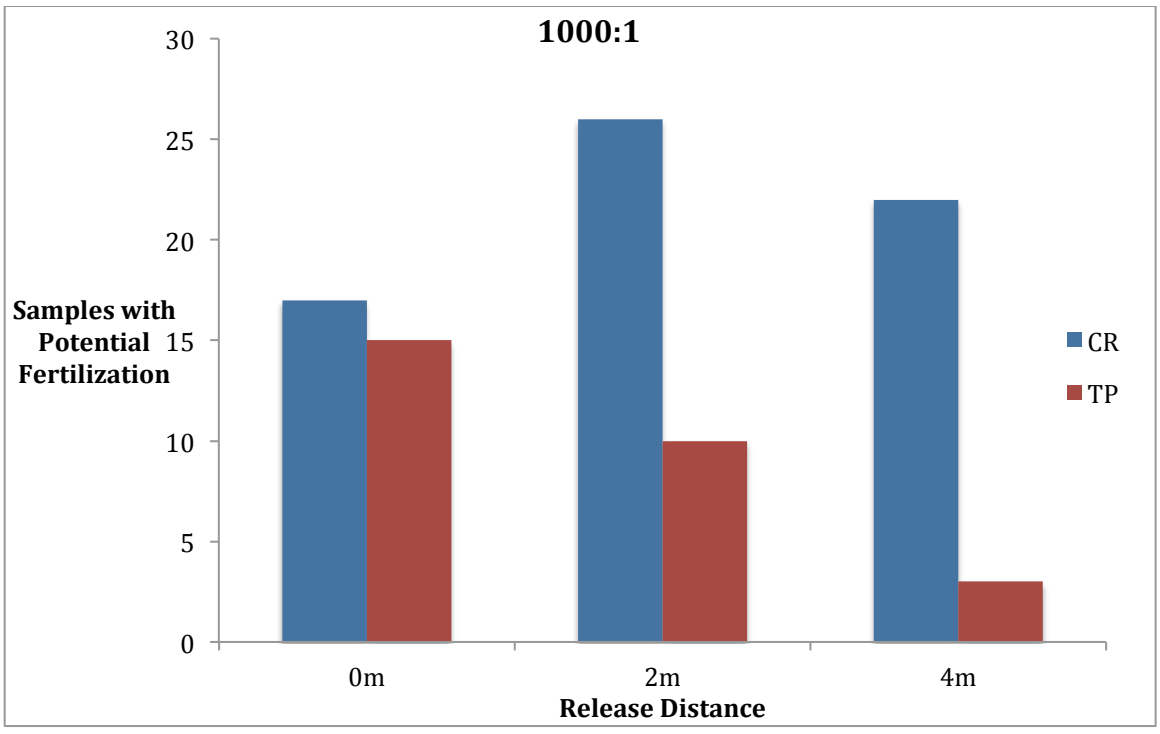

Figure 1.30 Total samples with potential fertilization based on the 1000:1 sperm to egg criteria defining fertilization potential, showing patterns in release distance (P = 0.454) and habitat type (P = 0.0578).

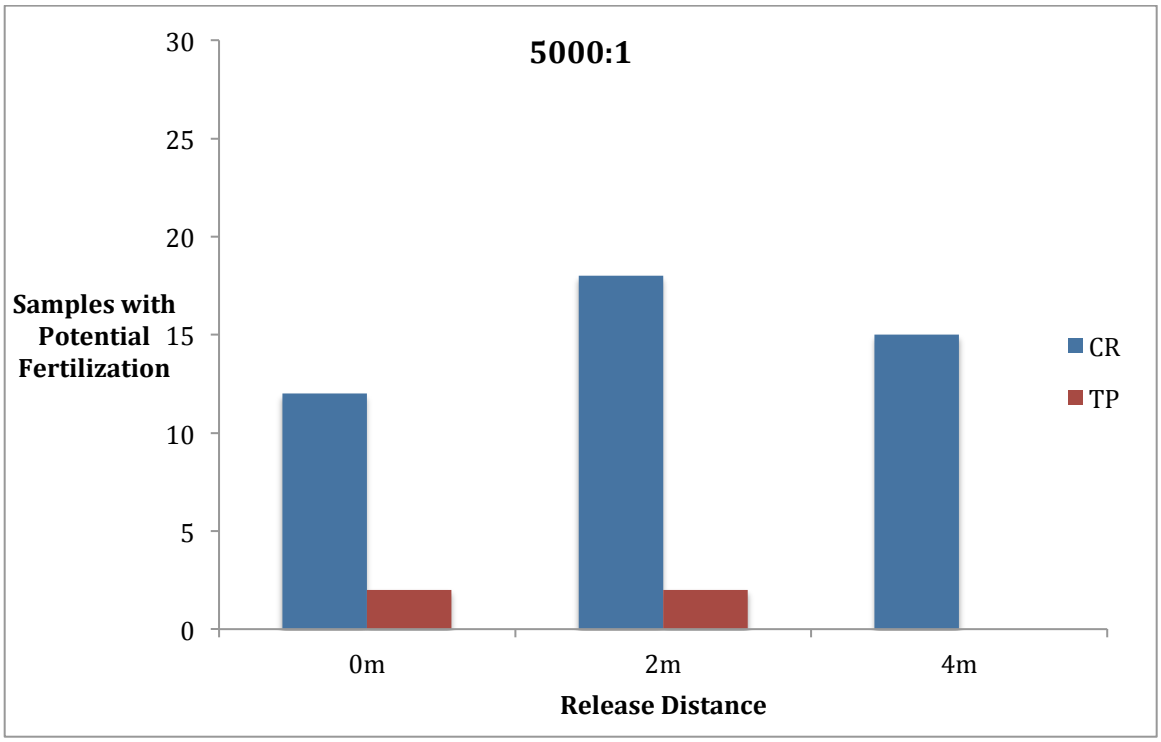

Figure 1.31 Total samples with potential fertilization based on the 5000:1 sperm to egg criteria defining fertilization potential, showing patterns in release distance (P = 0.8683) and habitat type (P = 0.0244).

Chapter 2

Maximum spawning distance determined from field experiments sheds light on population level dynamics exhibited in black abalone populations on San Nicolas Island, California

Abstract

Distances between individual spawning abalone may limit the recovery of abalone species in areas that have experienced depletion. The problem may be acute for the endangered black abalone (*Haliotis cracherodii* Leach, 1814), which occupies exposed rocky intertidal habitats along the coasts of California, USA, and northern Baja California, Mexico. US populations of black abalone are listed as endangered due primarily to disease effects and a history of excessive harvest. Annual surveys conducted in nine permanent sites around the periphery of San Nicolas Island (SNI) since 2005 have included black abalone abundance, size, and nearest neighbor information. I hypothesize that the differences in abalone densities among sites around SNI, following massive die-offs due to disease, may be responsible for the observed disparities in recovery rates. The purpose of this study is to analyze patterns in population growth and nearest neighbor distances to determine if the Allee effect, as mediated by patterns of distance among individuals, is causing the disparity in recovery between sites. Information on abalone abundance and nearest neighbor distances were used to calculate the amount of abalone

per square meter, annual growth rates, determine patterns in aggregations, and find correlations between population growth, patches of five or more abalone within 2 x 4 m^2 area, and nearest neighbor distances. The proportion of black abalone located within 0.1 m of each other increased by 3.7% annually between 2005 and 2012; and the amount of black abalone in contact increased by 9% annually since 2005. Additionally, the increase in absolute population growth and number of patches at each site annually, as well as the correlation between declining nearest neighbor distance and population growth are consistent with the Allee effect, potentially explaining the disparity in recovery rates among study sites.

Introduction

Intertidal broadcast spawners face several reproductive difficulties, mainly involving the mixing properties of water. The turbulent nature of intertidal water acts to simultaneously disperse and dilute gametes. Although spawning individuals may not need to be in contact to reproduce, if spawners are located too far apart, their gametes may not mix in sufficient concentrations to result in egg fertilization. Black abalone (*Haliotis cracherodii* Leach, 1814), are affected by this property as their populations struggle to recover from the effects of a debilitating disease, known as withering syndrome (WS) along the coasts of southern California.

VanBlaricom began annual surveys of black abalone populations at nine permanent sites around the periphery of San Nicolas Island (SNI) in 1981 (Figure 2.1) to document

patterns in abalone abundance in response to the translocation of sea otters from the mainland California coast. Although sea otter presence has had no apparent effect on black abalone abundance to date, VanBlaricom's data documented the appearance and consequences of withering syndrome as population counts around SNI began to plummet. Total numbers at all study sites combined declined from over 25,000 in 1992 to less than 200 by 2001 (VanBlaricom et al., 2012a). Since 2001, a slight recovery has been seen around the island. Total population counts have increased to over 1,000 individuals (VanBlaricom et al., 2012a), with inconsistent recovery among the different sites.

Prior to the appearance of WS, black abalone on SNI were abundant, often attached to shells of conspecifics (VanBlaricom, personal communication). After the onset of the disease, population counts dropped, and abalone densities likely became a limiting factor affecting population recovery and growth. The sharp declines and lack of subsequent recovery led to the listing of black abalone as "endangered" under the US Endangered Species Act of 1973 as amended (16 US Code §§ 1531-1543 *et seq*.) on January 14, 2009 (74 FR 1937).

Withering syndrome is caused by a rickettsiales-like prokaryotic pathogen (Friedman et al., 2000). The pathogen inhibits the absorption of materials from the gut lumen by disrupting the production of digestive enzymes (Friedman et al., 2000). Infected abalone lose soft tissue mass, especially in the pedal muscle, resulting in increased risk of predation and dislodgement from their substrata by breaking surf. The disease was first documented on Santa Cruz Island in 1985 (Tissot, 1991, 1995; Haaker et al., 1992), and

quickly spread to the mainland of California and other Channel Islands (Douros, 1987; Lafferty and Kuris, 1993; Richards and Davis, 1993; VanBlaricom et al., 1993).

In 2005, VanBlaricom began including collection of data on nearest neighbor distances between individual black abalone as a standard sampling protocol in annual surveys at SNI (VanBlaricom et al., 2012a). Analyses of the nearest neighbor data showed an increase in clustered distribution patterns as the populations recover (VanBlaricom et al., 2012b). Since 2005, there has been a marked increase in the number of observed abalone with nearest neighbor distances less than 10 cm (from 60% in 2005 to over 80% in 2012), with more than 90% of the individuals within 1 m of another black abalone (VanBlaricom et al., 2012a). VanBlaricom hypothesized that the recent trend in decreasing nearest neighbor distances will lead to an increase in the probability of successful fertilization when black abalone spawn (VanBlaricom et al., 2012a).

Abalone are dioecious, broadcast spawners. Fertilization success is likely directly correlated to the spatial proximity and temporal synchronicity of spawning male and female abalone (Blaud 2013, MS thesis, Chapter 1). This appears to be an example of the Allee effect, analogous to depensation, which describes the positive correlation between population density and recruitment. Among intertidal broadcast spawners, fertilization success decreases as a result of low population density (Levitan, 1991; Yund, 1995), increased wave action (Pennington, 1985; Denny and Shibata, 1989), and increased distance between spawners (Pennington, 1985; Yund, 1990; Levitan et al., 1992).

Simulated spawning experiments were conducted in two intertidal habitat types on SNI (see Chapter 1). Surrogate eggs and sperm were released at increasing distances apart that followed a linear trajectory, and water samples collected at different distance and time intervals were used to measure surrogate gamete concentration and determine if fertilization was possible based on a range of criteria using the ratios of surrogate sperm to surrogate eggs. Results from these experiments indicate that fertilization is highly influenced by habitat type, with more particles retained within the crevice habitat. In the crevice habitat, fertilization is possible when spawning abalone are located 4 m apart, even by the strictest surrogate sperm-to-egg ratio criterion of 5000:1.

I hypothesize that the differences in abalone densities between sites around SNI may help explain the observed disparities in population recovery rates between 2005 and 2012. Black abalone density, nearest neighbor distance, and population counts were measured and compared to determine patterns in recovery rates among study sites around SNI. The objective of this study was to analyze patterns in population growth, numbers of dense patches of abalone, and nearest neighbor distances to determine if the Allee effect, as mediated by patterns of distance among individuals, is causing the disparity in recovery among sites.

Methods

Nearest neighbor data were obtained during black abalone population surveys at the nine SNI study sites, indicated in Figure 2.1. Information on long-term survey protocols is

provided in detail by VanBlaricom (1993) and is briefly summarized here. Each site was surveyed once every two years from 1981 through 1997 on average, and has been surveyed annually since 2001 with a sampling hiatus from 1998 through 2000. Collection of the nearest neighbor data was added to the survey protocol beginning in 2005. At each study site, a group of permanent transects was demarcated in known black abalone habitat by stainless steel eyebolts permanently embedded in holes drilled in the rock surface, and secured therein with marine epoxy compound ("Splash Zone," Kop-Coat Inc., Marine Group East, Rockaway, New Jersey). During surveys, a transect tape was attached to the eyebolts for a given transect, to define the sampling space. Data on black abalone number, individual size, distance to nearest neighbor, microhabitat use, and feeding activity were collected in quadrats of 1 m^2 area spaced in contiguous fashion along each side of the transect. Thus, for example, surveys along a 20 m long transect involved obtaining data from a 2 x 20 m array of contiguous quadrats of 1 m^2, for a total sampled transect area of 40 m^2. A total of 44 permanent transects were distributed among the nine study sites with a mean length of 24 m, and a total survey area of 2,054 m^2. For each abalone encountered in sampled space, the distance to the closest adjacent abalone was measured in cm with a measuring tape. Distance took precedence over location within the sampled space in selection of a nearest neighbor. Thus, nearest neighboring individuals were not necessarily part of the sampled space, and were selected solely on the basis of distance from the subject abalone that were within the sampled space. From 2005 through 2012 a total of 5,495 nearest neighbor measurements were obtained for black abalones in permanent intertidal study sites at SNI.

Total counts per survey area were used to calculate the overall density of abalone in each individual site and track population growth around SNI. The nearest neighbor distances were compared to the density of abalone to examine the relationship between these two variables over time. Clustering patterns were further investigated by measuring the proportion of black abalone within 2 m, 1 m, 0.1 m, and 0 m (in contact) of nearest neighbors over time. Simple linear regressions were computed for the change over time in the size of each of the bins used for nearest neighbor data. Trendlines with equations provide visual indices of temporal trends in the binned nearest neighbor data.

For purposes of linking my findings from Chapter 1 to available population data for black abalone at SNI, I hypothesized that an area with five sexually mature black abalone in a habitat surface area of 2 x 4 m^2 is the minimum number and proximity of animals to successfully fertilize and produce larvae at spawning. The choice of habitat area and configuration (2 x 4 m^2), with associated assumptions is based on results described in Chapter 1 and justified below. Gender determination in black abalone is not possible without removing animals from the substratum, which risks mortal injury to the abalone and was therefore deemed unacceptable. I chose 5 individuals as the minimum number of contiguous animals for reproductive success based on the assumptions that there is no gender bias in the spatial distribution of black abalone, and that gender ratio in black abalone is 50% of each sex. From these assumptions it follows that an unbiased sample of five black abalone will have a 0.9375 probability of including at least one individual of each gender, based on simple equivalent binomial probabilities. I used black abalone population data collected by VanBlaricom at his nine study sites at SNI during annual

surveys from 2001 through 2012. As noted above, the data are recorded by individual quadrats (area 1 m^2) in contiguous arrays two quadrats wide by L quadrats in length, where L is transect length in meters (Figure 2.2).

For the accumulated data for each transect, I established an initial plot of 2 x 4 m^2 at the transect origin, then advanced the plot boundaries successively in 1 m increments, determining abalone counts in each plot so defined. The process continued until the leading edge of the advancing plot reached the end of each subject transect. For a transect of length of L m, this process resulted in identifying abalone counts in L-3 overlapping plots of 2 x 4 m^2 each. Although successively-assessed plots overlapped, I tracked the identity of each qualifying abalone patch to ensure that no patch was counted more than once. Once patches were identified, the included animals were cross-checked with a separate data set obtained by VanBlaricom during the indicated population surveys, containing shell lengths for nearly all abalone enumerated. Animals with shell lengths of 5 cm or more were categorized as reproductive adults, based on information from C.S. Friedman (personal communication). A given patch was retained as a qualifying entry in the set of patches identified as having reproductive potential only if five or more abalone, each with shell lengths of at least 5 cm, were present within the 2 x 4 m^2 plot indicated in the population data set. By determining abalone numbers in 2 x 4 m^2 subsets of the data recorded for quadrats at each transect in each sample year, and cross-checking qualifying abalone patches to ensure the presence of at least five reproductively mature animals, I determined the number of patches by site and year in the permanent plots at SNI meeting the criteria for reproductive capacity as defined

above. The method includes an error rate of 6.25% for identifying qualifying individual patches containing five abalone, such that a few non-qualifying patches are included with qualifiers. However, the method has conservative attributes as well, as it excludes patches of abalone with less than five individuals even if both males and females are present. In addition, the calculated error rate of 6.25% progressively declines as the number of abalone in identified patches increases above five individuals.

Given the finding that maximum distance over which two adult black abalone can be separated and still engage in successful fertilization is approximately 4 m (Chapter 1), I have assumed that two mature black abalone of opposite gender within a habitat surface area of 2 x 4 m^2 would be capable of fertilization. This assumption includes several components and reflects several constraints, but it also affords substantial advantages in terms of application of the results from Chapter 1 to the actual spatial distribution of black abalone in intertidal habitats at SNI, the primary goal of this chapter. First, the selection of the 2 x 4 m^2 plot area is consistent with the key finding of the simulated spawning experiments, as virtually all possible distances between pairs of black abalone within a 2 x 4 m^2 plot are ≤ 4 m, and the few possible exceptions are likely to be rare and involve distances only slightly greater than 4 m. Second, the 2 x 4 m^2 configuration generally matches the linear geomorphology and unidirectional flow pattern that characterized study sites for simulated spawning experiments as described in chapter 1. Third, use of the 2 x 4 m^2 plot configuration is highly compatible with black abalone population survey data collected by VanBlaricom at SNI since 1981, as described in chapter 1, with all surveys involving linear arrays of quadrats of 1 square meter in area,

placed in contiguous fashion along a permanent transect line, such that data are collected by quadrat in a 2 x L m^2 array where L is transect length. The principal constraint of the assumption is that black abalone may spawn under circumstances other than linear, predominantly unidirectional flow at relatively low tidal levels. Based on the available data from SNI, I suggest that the design used in my study and the associated assumptions are robust for intertidal black abalone habitats at low tide along SNI. Most abalone aggregations at the island are in fact linear in configuration, reflecting the linear nature of preferred crevice habitats typical at the SNI. There are exceptions to this configuration, and the extent to which the SNI pattern may be generalized to other geographic locations in the range of black abalone is open to question. The degree to which black abalone spawn successfully at higher tidal levels, during which flow patterns arguably would differ substantially from those at low tide, is unknown, with the consequence that the validity of generalizing my findings across temporally fluctuating tidal levels, even at SNI, remains an open question. Based on limited observation during snorkel and SCUBA dives at high tide at SNI (VanBlaricom, personal communication; such observations are safely manageable only during periods of unusual calm), it appears that the spatial distribution of black abalone at SNI is independent of tidally-driven temporal fluctuations in sea surface level.

Results

A summary of the relationship between abalone counts and average nearest neighbor distance is displayed graphically for the entire island, as well as each study site (Figures 2.3 to 2.12; note that the vertical scale for mean nearest neighbor distances varies widely

among the indicated figures). Overall, abalone counts increased at each site, with the majority of black abalone found in crevice habitat (with the exception of Site 6, which has limited crevice habitat available). The average nearest neighbor distance displayed a general decreasing trend, except for several study sites that showed a spike in mean nearest neighbor distance in 2007.

In general, the proportion of black abalone with nearest neighbor distances within 2 m, 1 m, 0.1 m, and 0 m (in contact) has increased since 2005 (Figure 2.13). Trendlines with associated regression equations show the level of increase in clustering patterns (Figure 2.13), where the greatest increase was in abalone within 0.1m (mean annual increase of 3.7%) and abalone within contact (mean annual increase of 9%). Cluster proportions are highly variable among individual study sites (Figures 2.14 to 2.22), showing inconsistent population growth and anomalies in grouping patterns, with an overall trend decreasing nearest neighbor distances.

The absolute population growth was calculated by the total change in abalone counts, which is compared to the patch number among study sites for each study site (Figures 2.23 to 2.31). There is a positive correlation between the annual change in population size and the number of patches documented at each site (Table 2.1).

Discussion

Initial settlements of abalone larvae are likely triggered by chemical cues, such as γ-aminobutyric acid (aka "GABA") and potassium chloride (Yu et al., 2010). Other cues for settlement are based on presence of crustose red algae (Morse and Morse, 1984), and presence of diatoms, which are an important food source for young, post-settlement individuals (Takami, 1997). As abalone increase in size, and their diet changes from crustose algae to macroalgae, they move out of the more protected, highly cryptic habitat underneath rocks, and possibly in other microhabitats as yet unidentified, and gather in their preferred crevice habitat (Prince et al., 1987). While the occurrence of several individuals within a particular crevice could occur randomly, the results of this study indicate that abalone are grouping together, in particular within crevices, at a rate that exceeds expectations if abalone were uniformly distributed. This result suggests that a trigger, cue, or behavioral strategy may be directing their clustered distribution.

Other intertidal invertebrates follow cues for grouping. The Galacian snail, *Littorina saxatilis*, aggregates based on season (breeding versus nonbreeding season) (Erlandsson and Kosylev, 1995), using mucus trails left by conspecifics to differentiate between male and female paths (Erlandsson et al., 1999). The intertidal snails *Echinolittorina trochoides* and *E. radiata* also follow mucus trails to converge in crevices (Stafford et al., 2008). The less mobile blue mussel, *Mytilus edulis* is driven by species-specific chemical cues to settle near large groups (De Vooys, 2003; Liu et al., 2011). The benefits for aggregating patterns among black abalone may include protection from desiccation

during low tide (Stafford et al., 2008), protection from direct impacts of breaking surf and wave-born projectiles in the intertidal (Shanks and Wright, 1986), predation by sea otters (Lowry and Pearse, 1973; Cooper et al., 1977; Fanshawe et al., 2003), and increased fertilization due to decreased distance between spawners (Pennington, 1985; Yund, 1990; Levitan et al., 1992).

Previous modeling results for *H. laevigata* indicated that abalone spawning in the subtidal must be within 1.6 m of a conspecific of opposite sex to successfully reproduce (Babcock and Keesing, 1999). Similar studies conducted with sea urchins (Levitan, 1991; Levitan et al., 1992; Lundquist and Botsford, 2011), fluted giant clams (Neo et al., 2013), ascidians (Yund, 1995; Crean and Marshall, 2008), and blue mussels (Stewart et al., 2012) indicate the required spawning proximity differs greatly among species, but is a significant factor limiting reproductive success when densities are low. A computer simulated spawning model, developed by Zhang (2008) indicated a positive correlation between population density and fertilization success in spawning abalone, however the abalone species, habitat type (intertidal versus subtidal), or other such factors that may affect fertilization success were not specified.

Each of the study sites at SNI has exhibited an overall increase in population growth since 2005, with decreasing nearest neighbor distances, supported by the comparison of absolute annual population growth at individual sites with the number of patches representing substantial reproductive potential (five abalone within a 2 x 4 m^2 plot as defined above; hereinafter "patches") since 2002. The apparent correlation between

nearest neighbor distance and number of patches with population growth, respectively, indicates there may be a critical threshold in abundance and density that must be reached before sustainable population growth may occur on a local scale. Babcock and Keesing (1999) identified a critical threshold after recruitment failures occurred when densities fell below 0.3 abalone/m^2 for *H. laevigata*. Similarly, Neuman et al. (2010) estimated a minimum threshold of 0.34 abalone/m^2 for recruitment and population growth based on three long-term studies of black abalone abundance in California.

Studies of dispersal patterns have important implications for black abalone recruitment. Abalone larvae remain pelagic for 5 to 14 days before settling (Ault 1985), and at SNI may be dependent on currents near the island for dispersal. In a study at SNI 1,989 drift cards were recovered (Chambers et al., 2005). Of those recovered, 74% were found within 2 km of the release site, 25% were recovered between 2 to 10 km of the release site, and less than 1% were recovered from other islands or the mainland (Chambers et al., 2005). This study suggests that the majority of recruitment at SNI may result from spawning adults located within sites or at relatively nearby sites at SNI. A genetic study of newly emergent black abalone along SNI and other California Islands suggested a strong relationship among abalone on these islands further illustrating local recruitment (Chambers et al., 2006). Other genetic studies investigating mitochondrial cytochrome oxidase subunit one DNA sequences, microsatellites, and amplified fragment length polymorphisms of black abalone populations similarly indicated there is limited larval dispersal, and recruitment is likely local (Gruenthal and Burton, 2008), and that strong population structure exists between black abalone sites along the California mainland

(Hamm and Burton, 2000). A more detailed examination of population genetics, including parentage assignment tests between populations, would highlight dispersal patterns in greater resolution on a finer scale. Knowledge of dispersal patterns would indicate if there are source-sink relationships among study sites, where spawners from one or more locations are supplying the recruits that appear at other locations.

The Allee effect describes the positive correlation of population density and recruitment, and may explain the disparities in recovery rates among the study sites. This correlation was displayed by the relationship between the absolute population growth and numbers of patches on SNI, as well as the increase in counts corresponding to the decrease in nearest neighbor distances. By utilizing the maximum distance between spawning abalone that allows potential fertilization in simulated spawning experiments, I was able to estimate numbers of abalone patches with reproductive potential over the period from 2001 through 2012. The majority of increases in population counts at each site corresponded to the larger numbers of patches. It is also possible that the proposed link between variation in patch count and variation in absolute numbers is coincidental rather than causal. That is, changes in patch number may simply be a direct and simple response to changes in absolute numbers of abalone, with both metrics of population growth reflecting some other process, such as some combination of increased recruitment and increased survival that is independent of patch count. Data presently available do not permit exclusion of this hypothesis. Using live gametes in the simulated spawning experiments to confirm the experimental design and additional simulated spawning

experiments using the surrogate gametes could further support the experiments from Chapter 1.

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Tables

Table 2.1 Linear regression equations and R^2 values for absolution population growth and number of patches between 2002 and 2012.

	Linear Regression		R^2	
Site	Number of Patches	Absolute Growth	Number of Patches	Absolute Growth
1	y = 1.0545x - 4.8364	y = 0.0699x - 0.1212	0.19152	0.14985
2	y = 0.0385x - 0.1667	y = 0.6455x - 2.4273	0.23077	0.099
3	y = 0.4032x - 1.3636	y = 0.5x + 6.1364	0.63781	0.00227
4	y = 0.0699x - 0.2879	y = 0.2909x - 0.6727	0.41958	0.07596
5	y = 0.3287x - 0.303	y = -0.6x + 9.2909	0.65271	0.01543
6	y = 0.0699x - 0.2879	y = 0.7091x - 2.7818	0.41958	0.29166
7	y = 0.6993x - 0.2121	y = 6.7x - 21.991	0.75464	0.19109
8	y = 0.8112x + 5.8939	y = 14.364x - 25.455	0.81353	0.44877
9	y = 0.1713x - 0.3636	y = 0.7091x - 1.9636	0.67161	0.19895

Figures

Figure 2.1 Map of San Nicolas Island, located approximately 120 km WSW of Los Angeles, California. Numbers refer to the nine sites sampled by VanBlaricom (Figure courtesy of D. Witting).

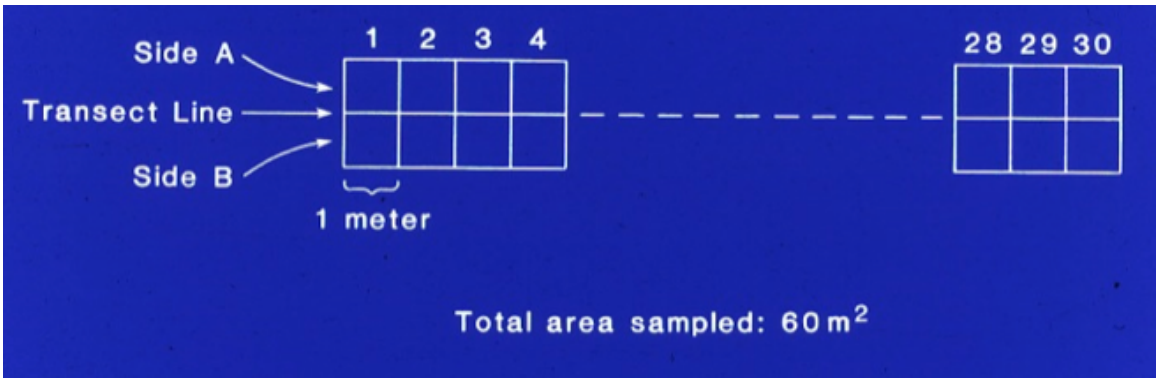

Figure 2.2 Conceptual survey design (VanBlaricom, 1993; VanBlaricom et al., 2012a).

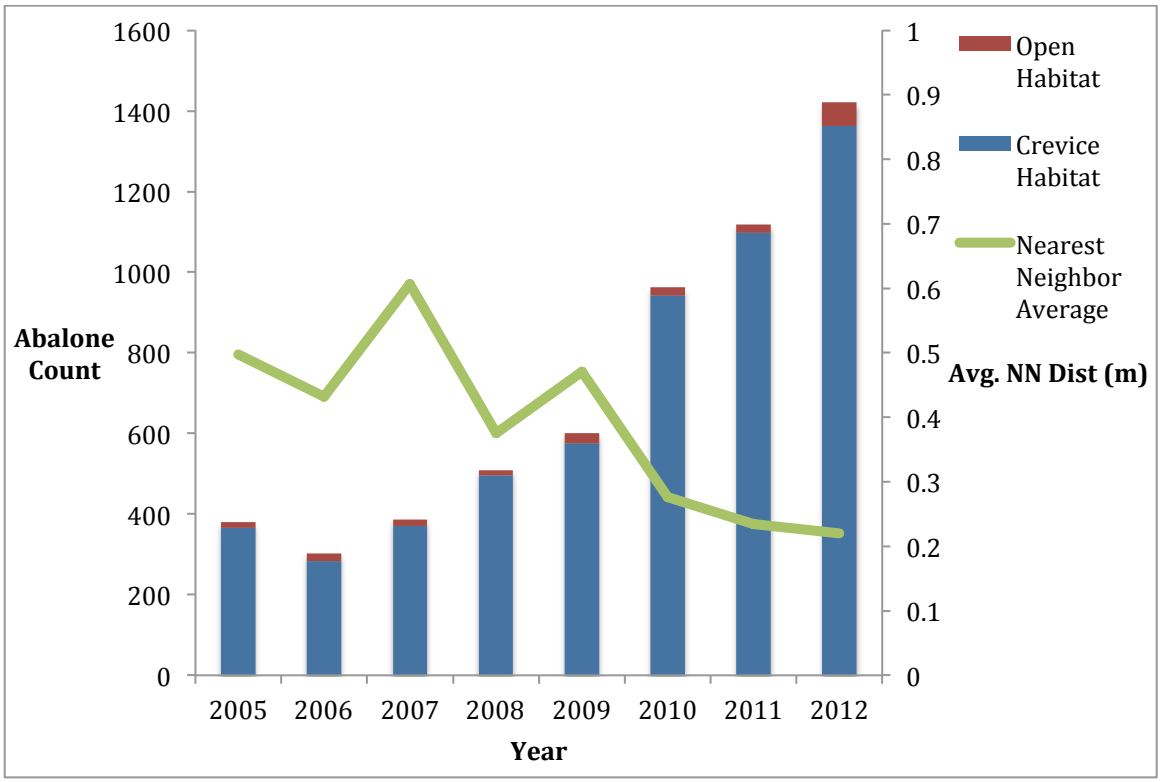

Figure 2.3 Summary of SNI abalone counts between 2005 and 2012, with the amount of abalone in the crevice (blue) and open (red) habitats indicated on the bar plot, and the trend in nearest neighbor (NN) distances (green) in a line transect.

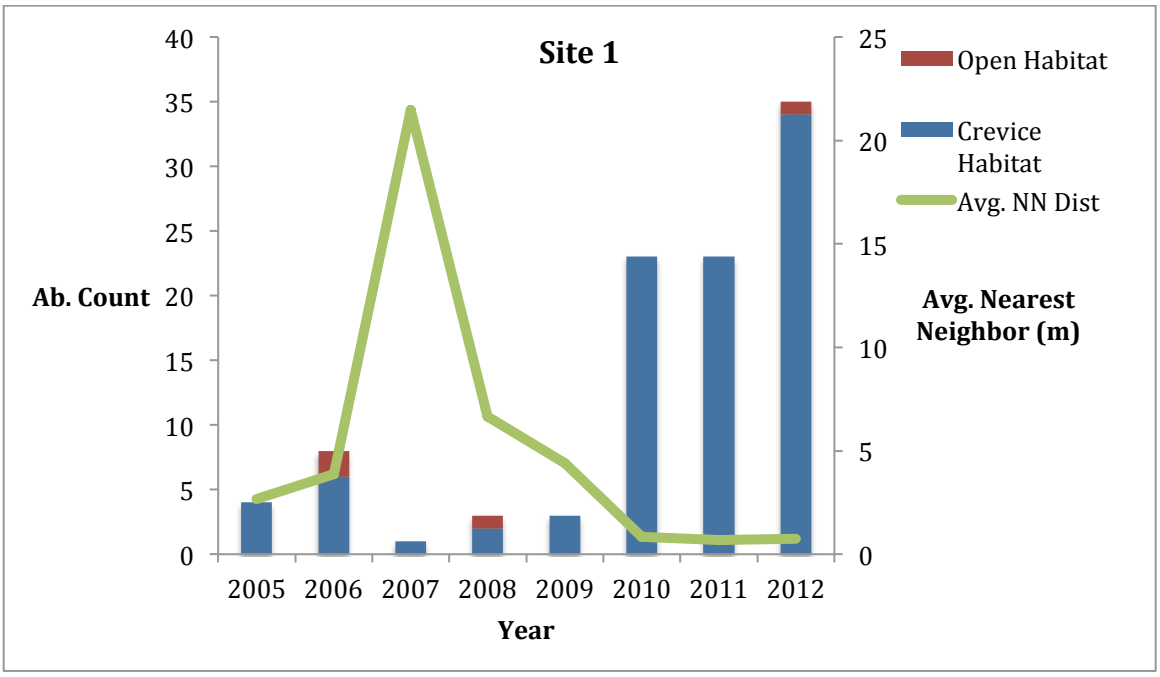

Figure 2.4 Summary of SNI abalone counts at Site 1 between 2005 and 2012, with the amount of abalone in the crevice (blue) and open (red) habitats indicated on the bar plot, and the trend in nearest neighbor (NN) distances (green) in a line transect.

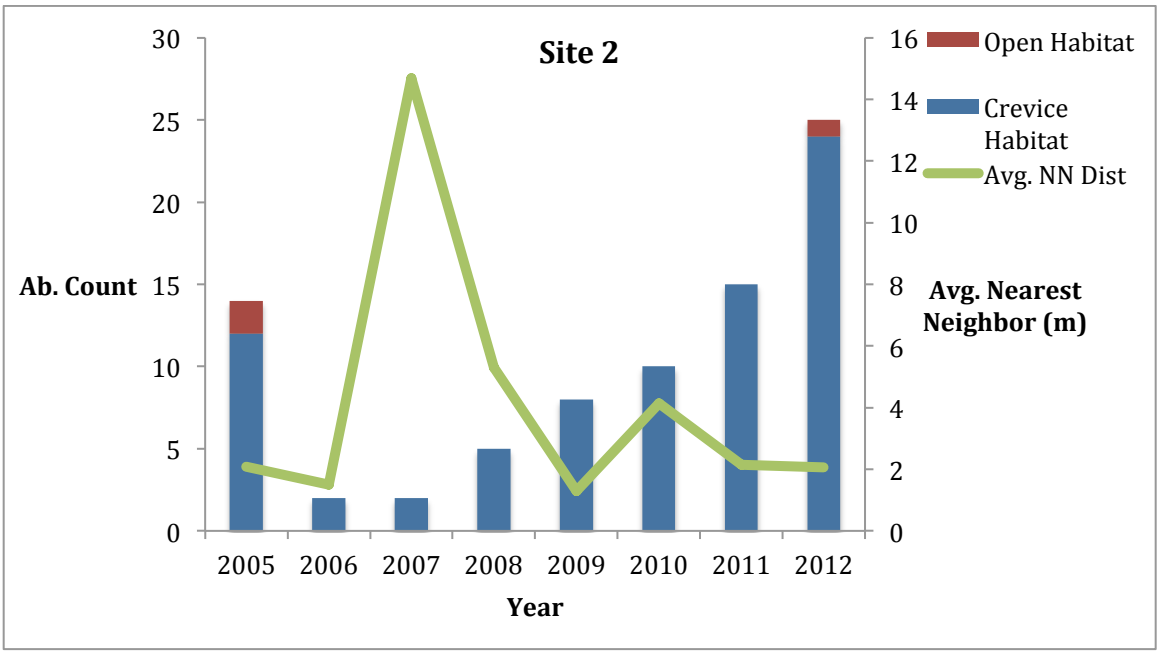

Figure 2.5 Summary of SNI abalone counts at Site 2 between 2005 and 2012, with the amount of abalone in the crevice (blue) and open (red) habitats indicated on the bar plot, and the trend in nearest neighbor (NN) distances (green) in a line transect.

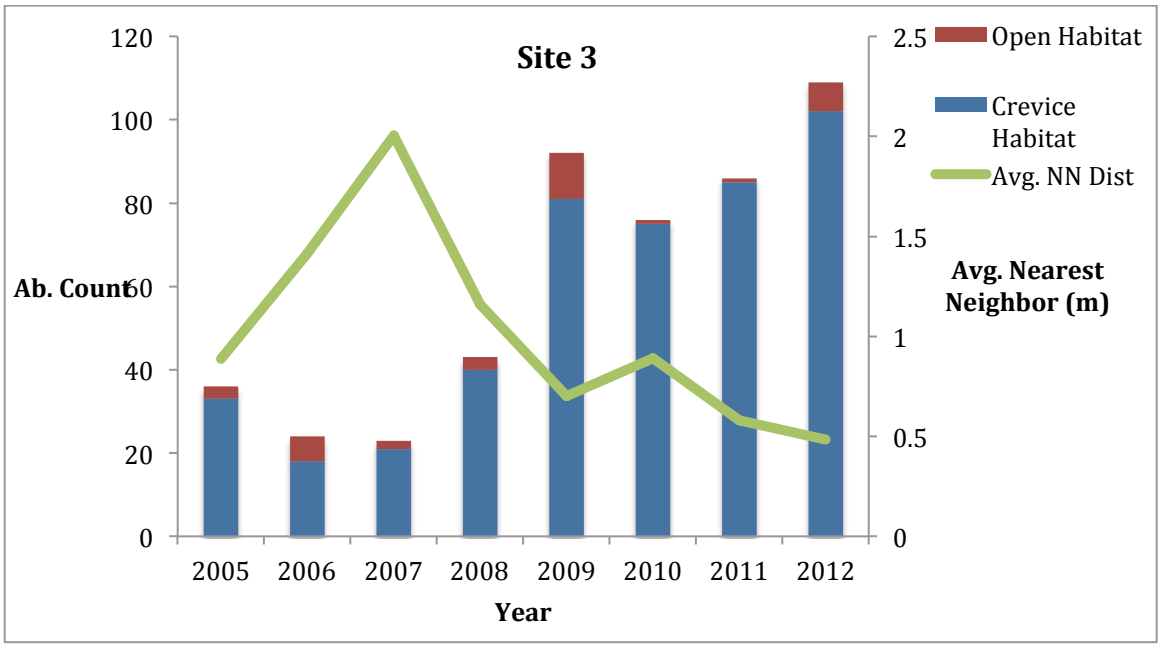

Figure 2.6 Summary of SNI abalone counts at Site 3 between 2005 and 2012, with the amount of abalone in the crevice (blue) and open (red) habitats indicated on the bar plot, and the trend in nearest neighbor (NN) distances (green) in a line transect.

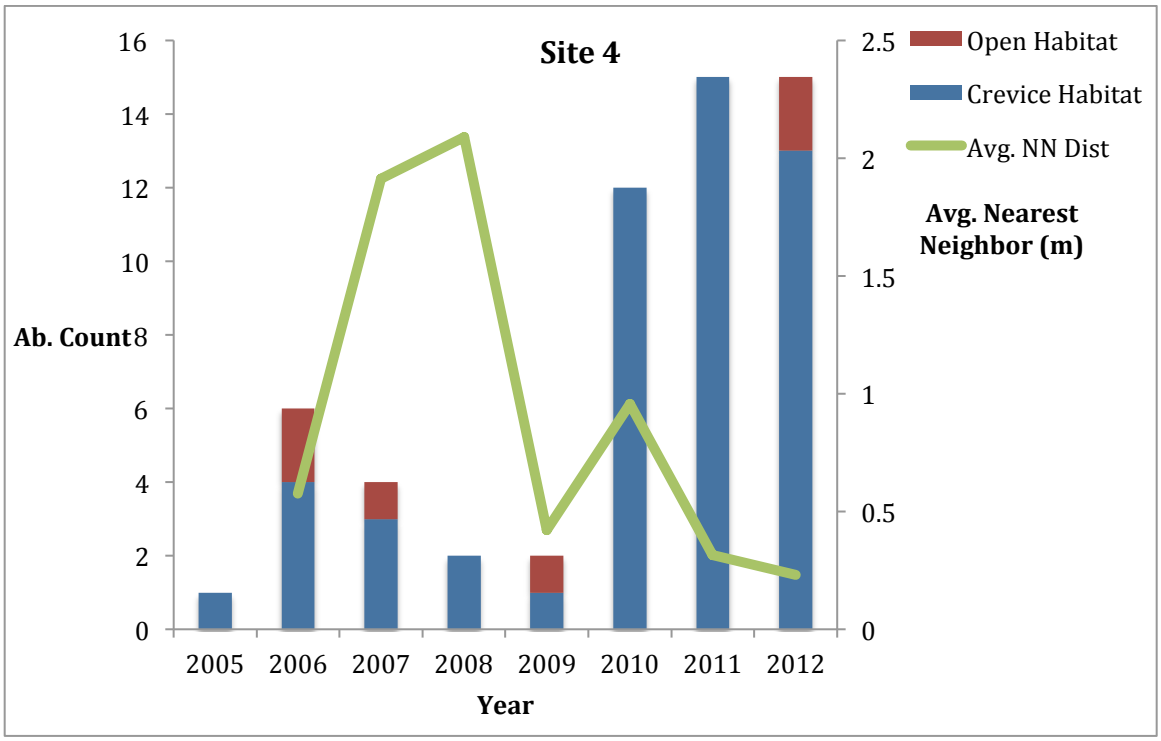

Figure 2.7 Summary of SNI abalone counts at Site 4 between 2005 and 2012, with the amount of abalone in the crevice (blue) and open (red) habitats indicated on the bar plot, and the trend in nearest neighbor (NN) distances (green) in a line transect.

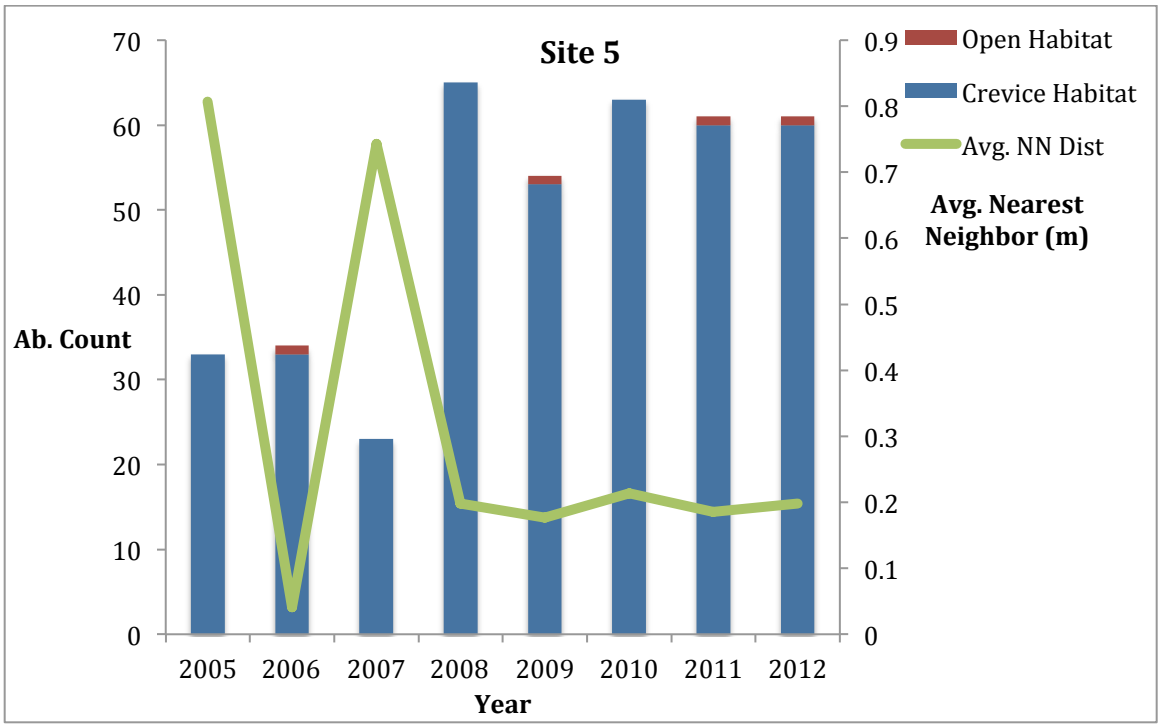

Figure 2.8 Summary of SNI abalone counts at Site 5 between 2005 and 2012, with the amount of abalone in the crevice (blue) and open (red) habitats indicated on the bar plot, and the trend in nearest neighbor (NN) distances (green) in a line transect.

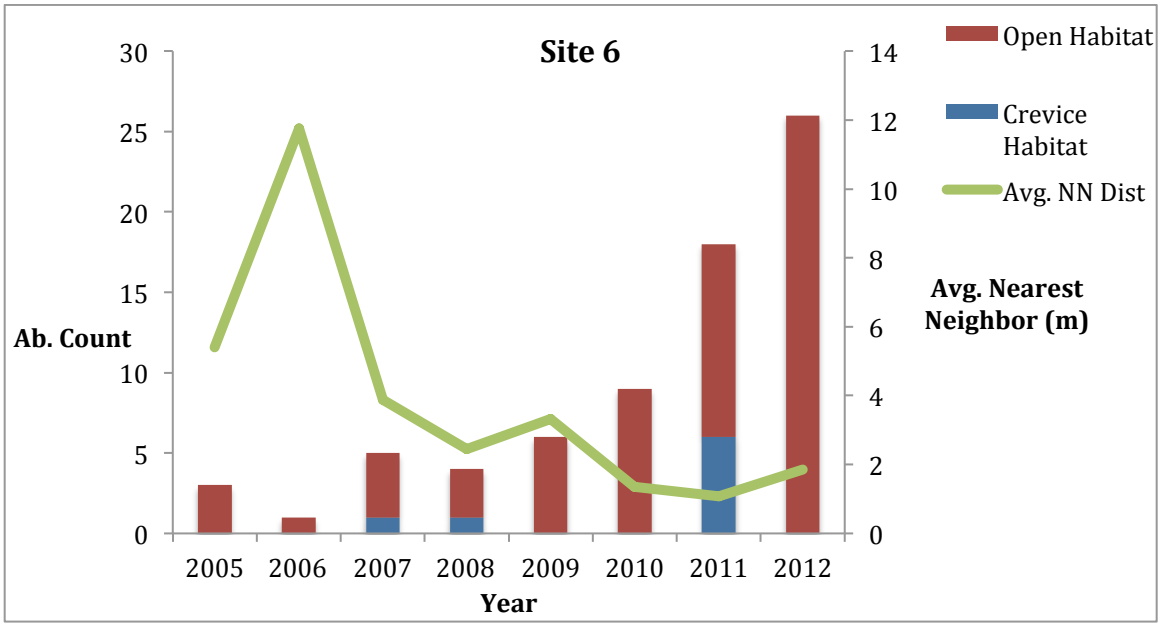

Figure 2.9 Summary of SNI abalone counts at Site 6 between 2005 and 2012, with the amount of abalone in the crevice (blue) and open (red) habitats indicated on the bar plot, and the trend in nearest neighbor (NN) distances (green) in a line transect.

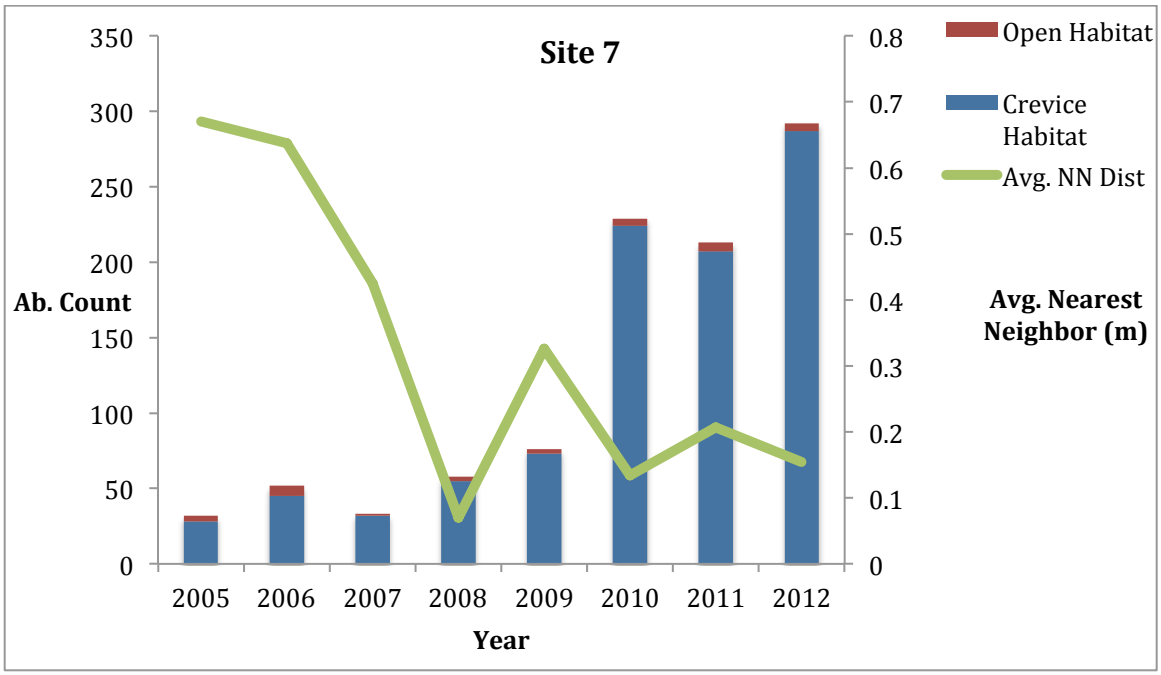

Figure 2.10 Summary of SNI abalone counts at Site 7 between 2005 and 2012, with the amount of abalone in the crevice (blue) and open (red) habitats indicated on the bar plot, and the trend in nearest neighbor (NN) distances (green) in a line transect.

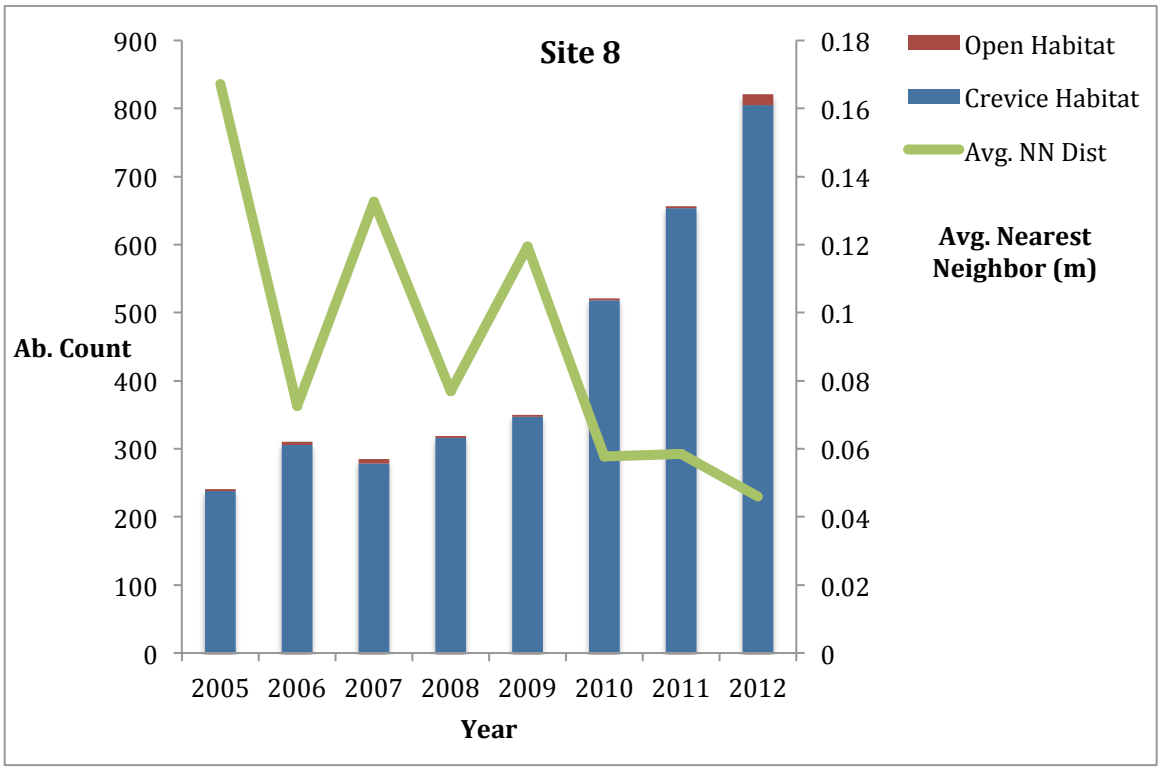

Figure 2.11 Summary of SNI abalone counts at Site 8 between 2005 and 2012, with the amount of abalone in the crevice (blue) and open (red) habitats indicated on the bar plot, and the trend in nearest neighbor (NN) distances (green) in a line transect.

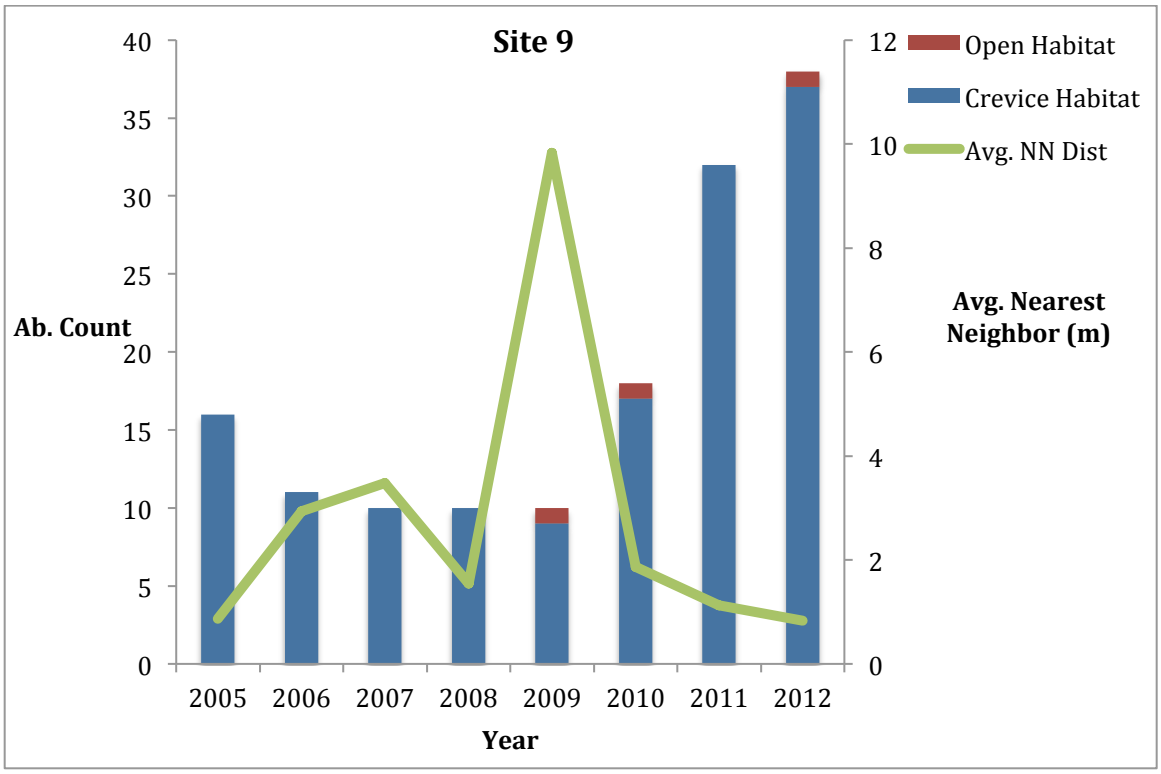

Figure 2.12 Summary of SNI abalone counts at Site 9 between 2005 and 2012, with the amount of abalone in the crevice (blue) and open (red) habitats indicated on the bar plot, and the trend in nearest neighbor (NN) distances (green) in a line transect.

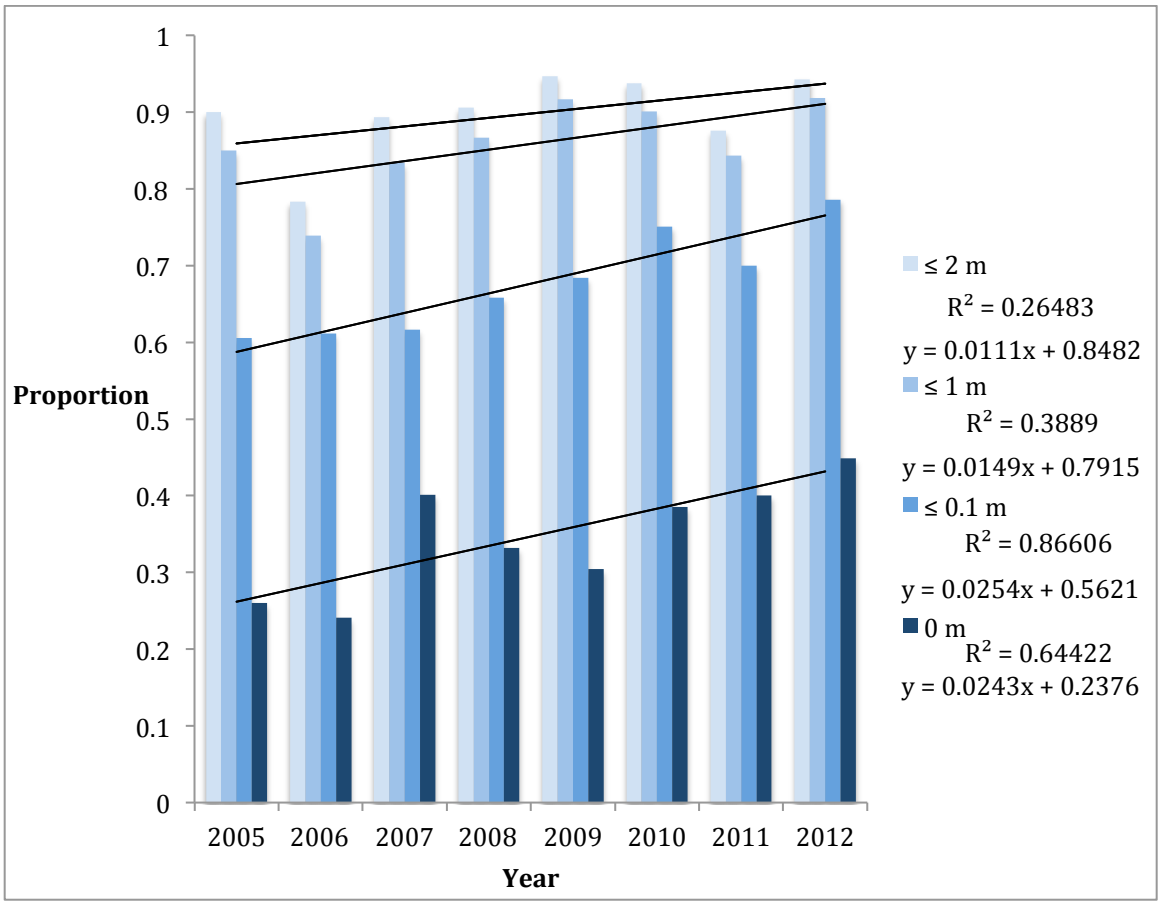

Figure 2.13 Variations in nearest neighbor proportions over time, based on data for all SNI study sites combined. The proportion of total abalone counts on SNI within 2m, 1m, 0.1m, and 0m (in physical contact) is shown for annual surveys from 2005 to 2012. Trendlines with the equations are shown to include the slope of the trendline, indicating the overall increase in aggregations.

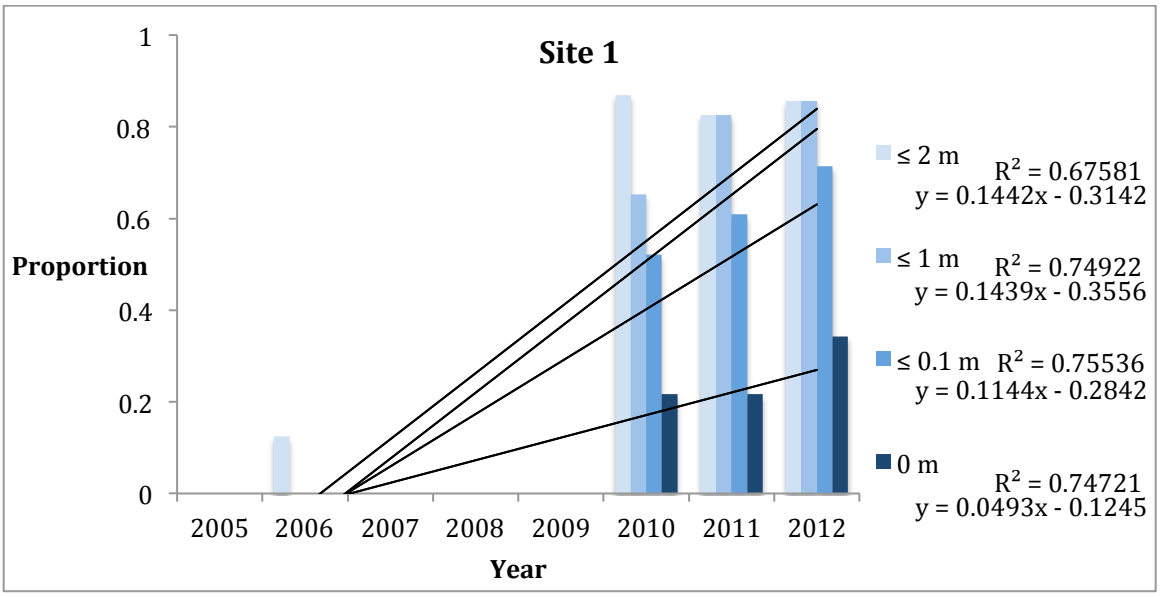

Figure 2.14 Variation in nearest neighbor proportions at Site 1 over time. Trendlines in plots match with indicated simple linear regression equations associated with each bin of proportion data.

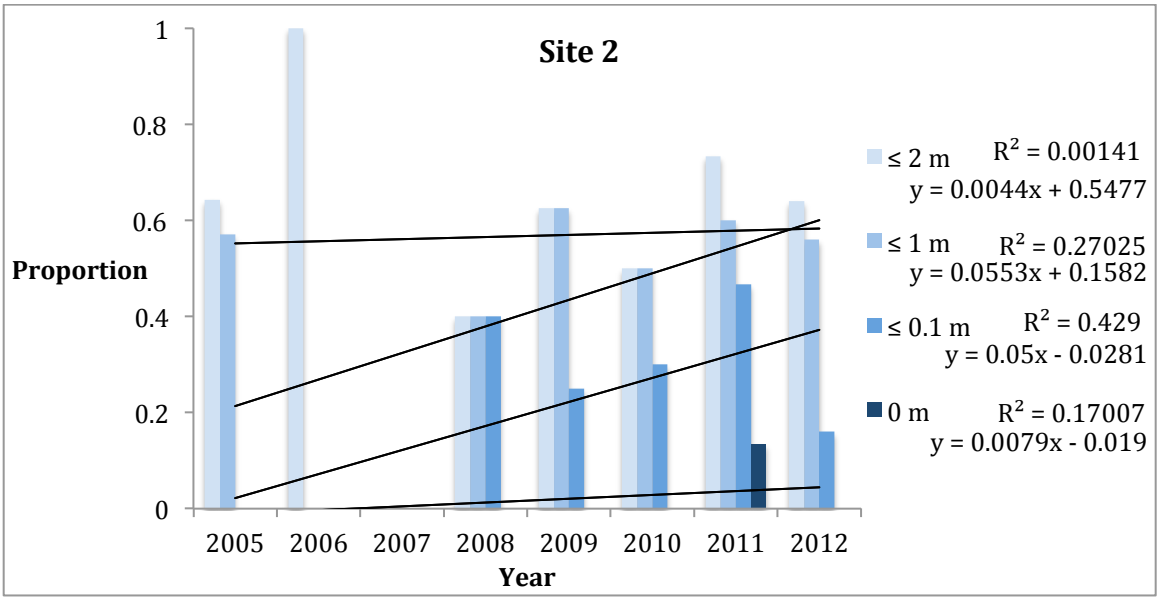

Figure 2.15 Variation in nearest neighbor proportions at Site 2 over time. Trendlines in plots match with indicated simple linear regression equations associated with each bin of proportion data.

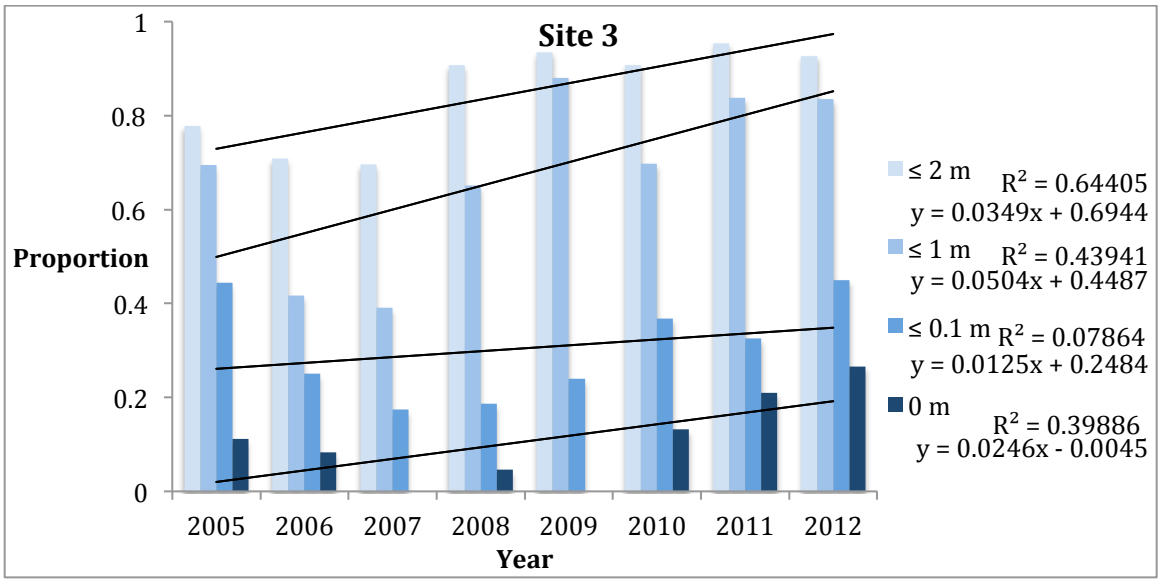

Figure 2.16 Variation in nearest neighbor proportions at Site 3 over time. Trendlines in plots match with indicated simple linear regression equations associated with each bin of proportion data.

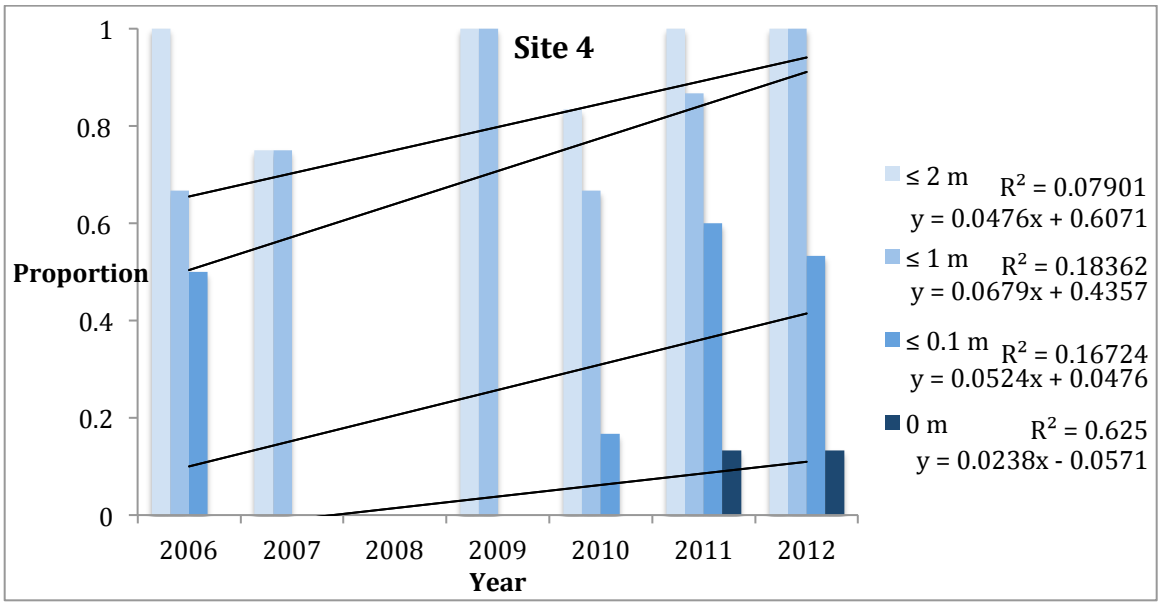

Figure 2.17 Variation in nearest neighbor proportions at Site 4 over time. Trendlines in plots match with indicated simple linear regression equations associated with each bin of proportion data.

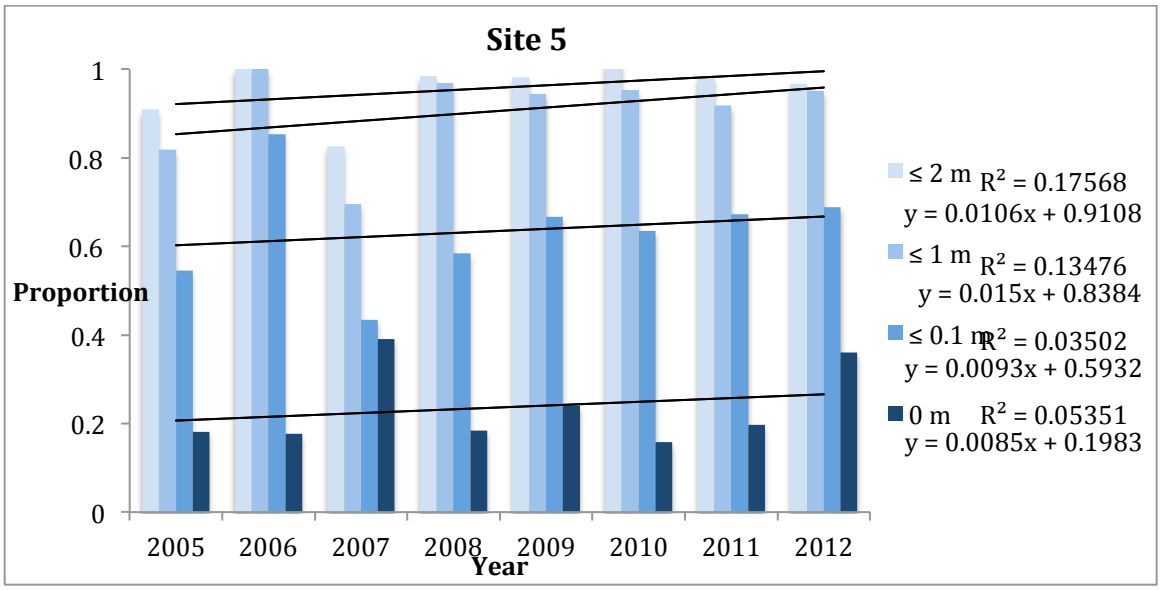

Figure 2.18 Variation in nearest neighbor proportions at Site 5 over time. Trendlines in plots match with indicated simple linear regression equations associated with each bin of proportion data.

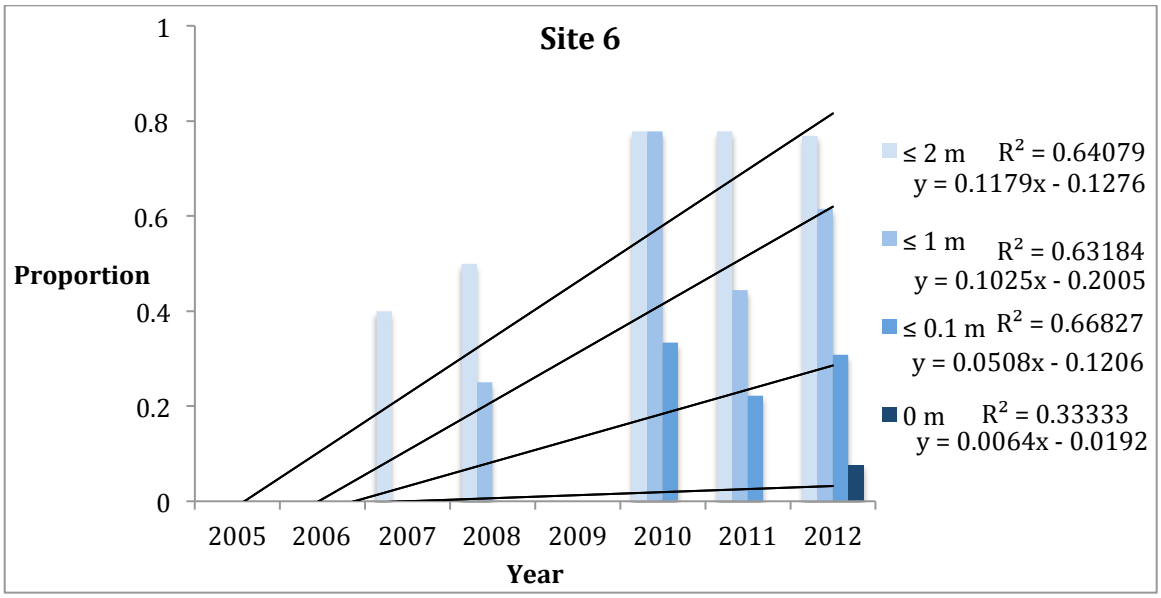

Figure 2.19 Variation in nearest neighbor proportions at Site 6 over time. Trendlines in plots match with indicated simple linear regression equations associated with each bin of proportion data.

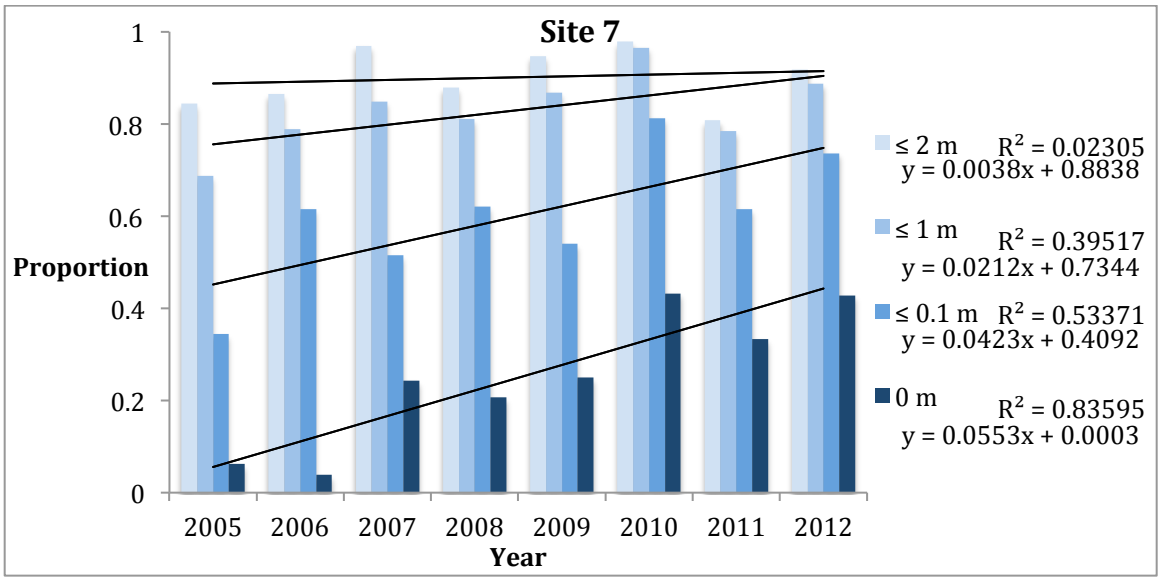

Figure 2.20 Variation in nearest neighbor proportions at Site 7 over time. Trendlines in plots match with indicated simple linear regression equations associated with each bin of proportion data.

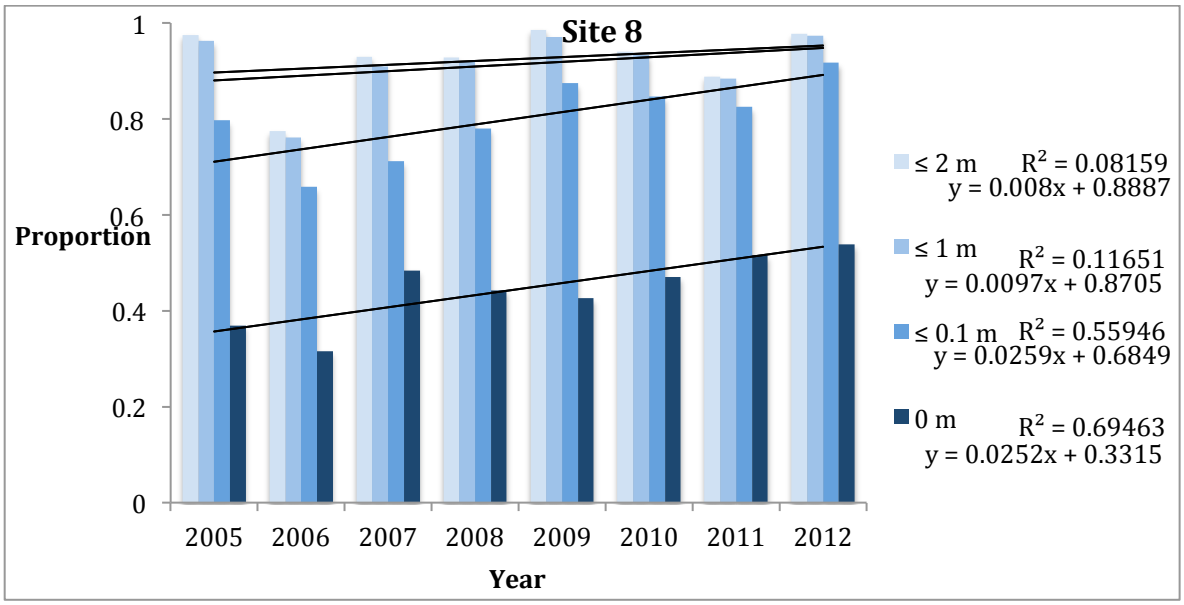

Figure 2.21 Variation in nearest neighbor proportions at Site 8 over time. Trendlines in plots match with indicated simple linear regression equations associated with each bin of proportion data.

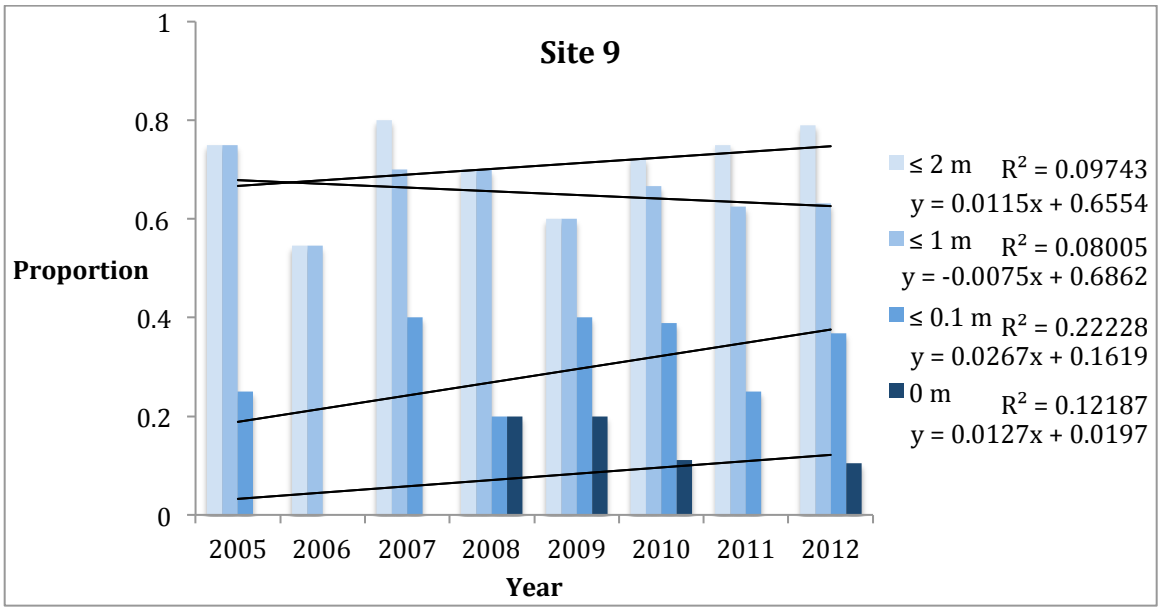

Figure 2.22 Variation in nearest neighbor proportions at Site 9 over time. Trendlines in plots match with indicated simple linear regression equations associated with each bin of proportion data.

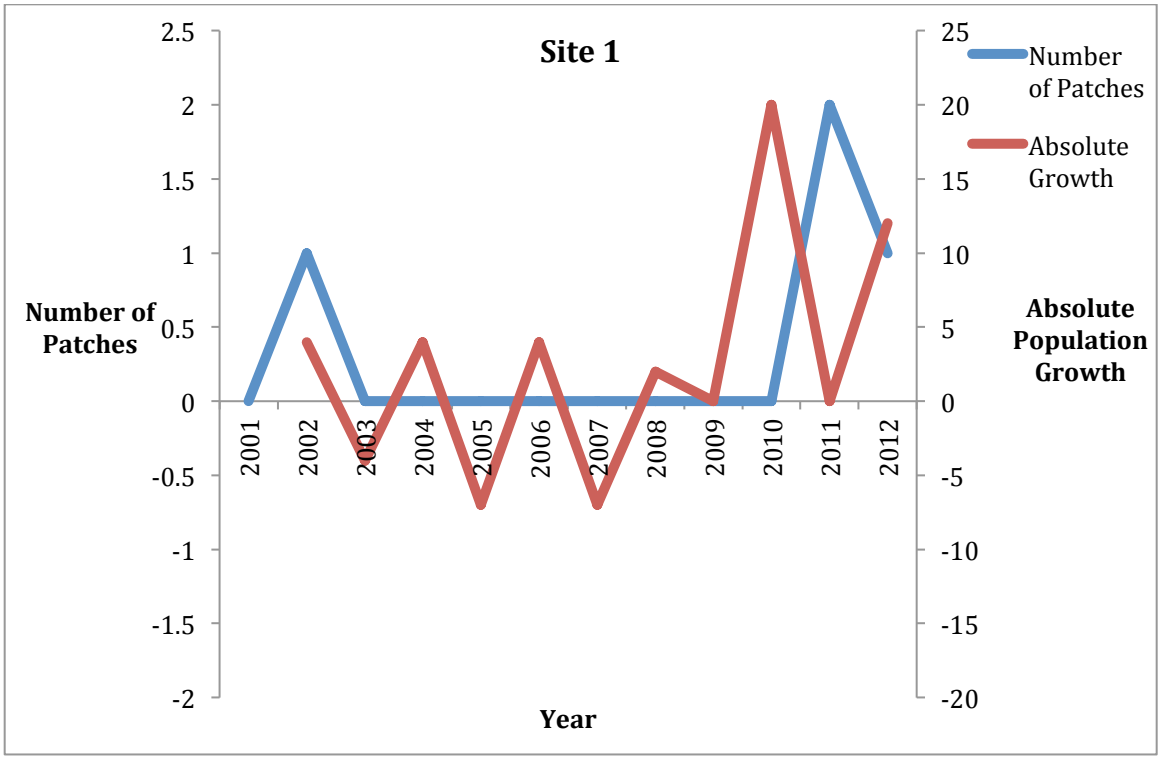

Figure 2.23 The comparison of absolute population growth (in numbers of abalone) and the number of patches, shown between 2001 and 2012 at Site 1.

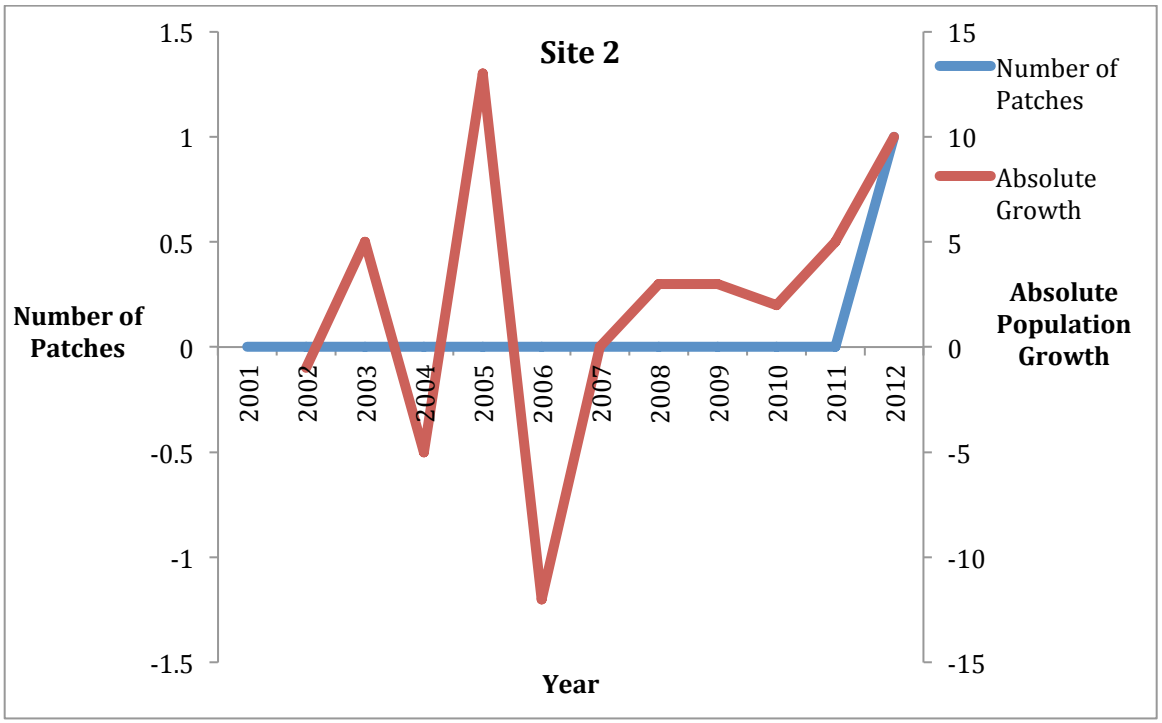

Figure 2.24 The comparison of the number of patches and the absolute population growth (in numbers of abalone), shown between 2001 and 2012 at Site 2.

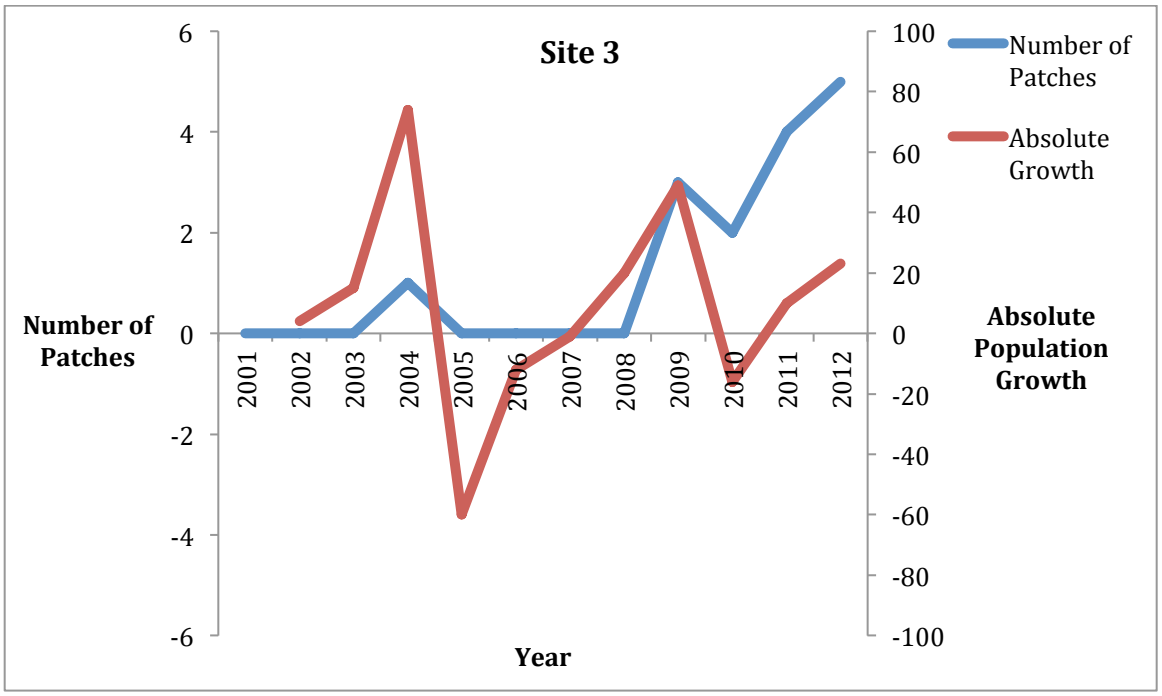

Figure 2.25 The comparison of the number of patches and the absolute population growth (in numbers of abalone), shown between 2001 and 2012 at Site 3.

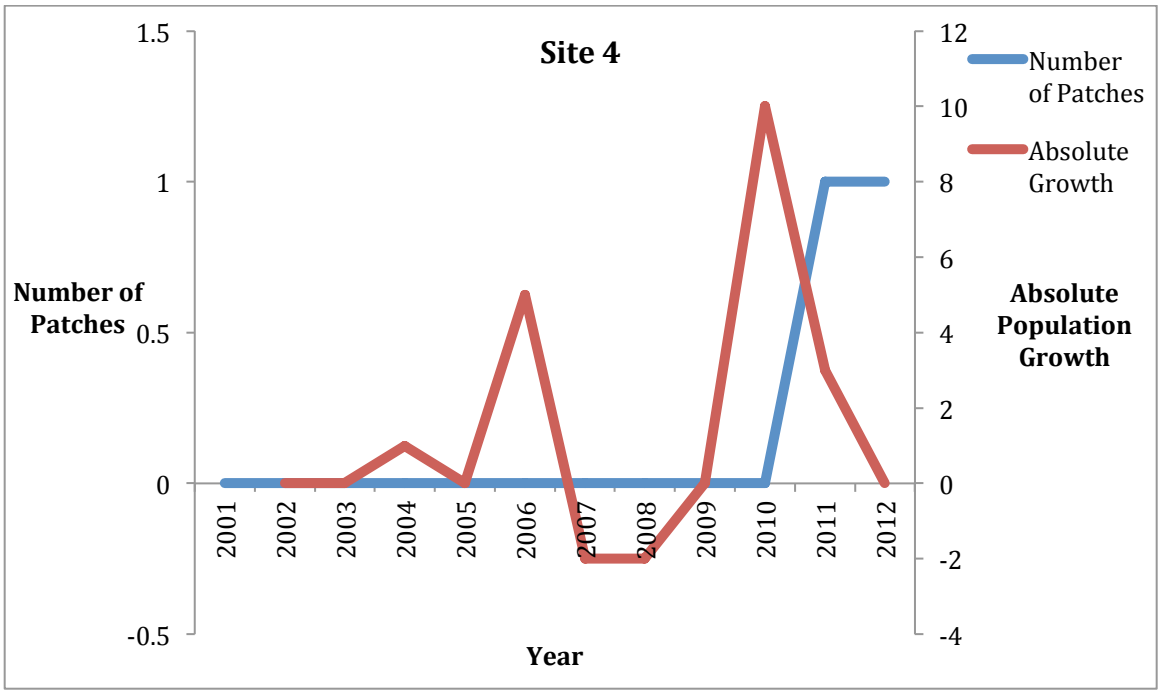

Figure 2.26 The comparison of the number of patches and the absolute population growth (in numbers of abalone), shown between 2001 and 2012 at Site 4.

Figure 2.27 The comparison of the number of patches and the absolute population growth (in numbers of abalone), shown between 2001 and 2012 at Site 5.

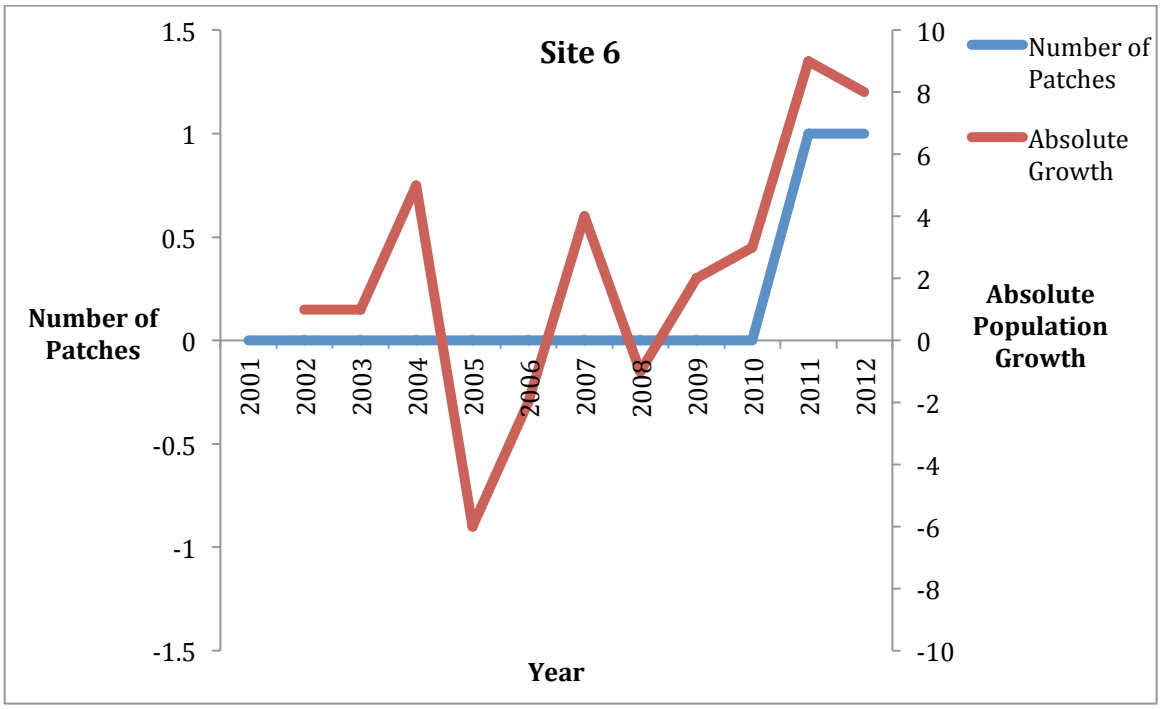

Figure 2.28 The comparison of the number of patches and the absolute population growth (in numbers of abalone), shown between 2001 and 2012 at Site 6.

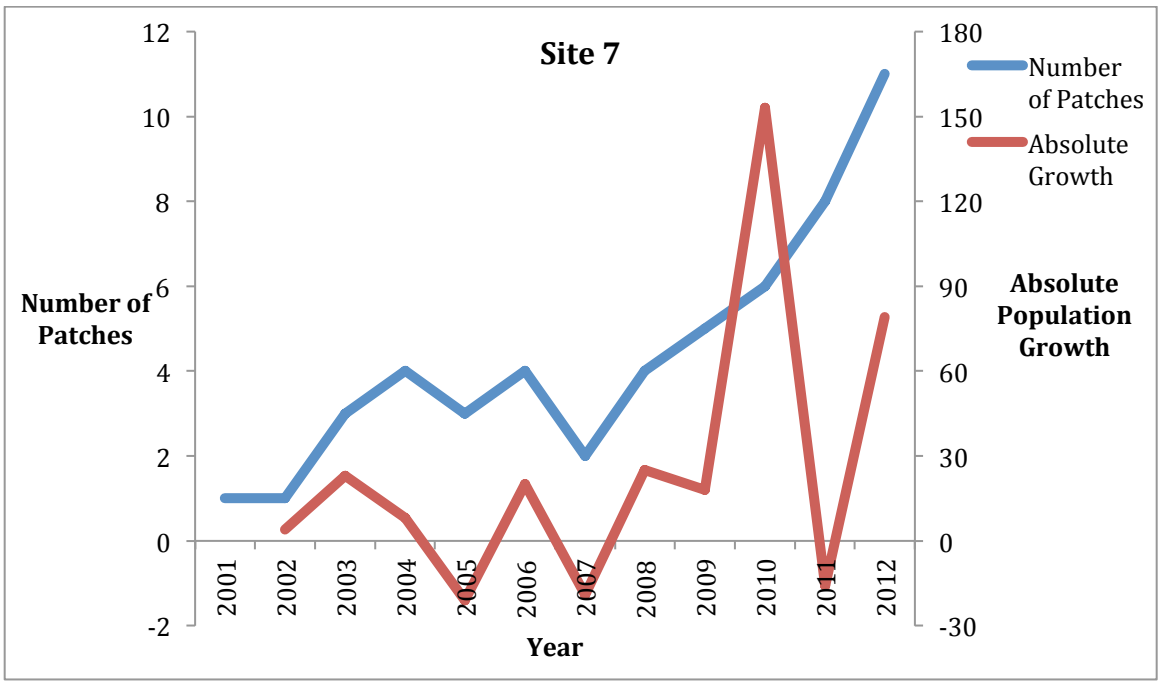

Figure 2.29 The comparison of the number of patches and the absolute population growth (in numbers of abalone), shown between 2001 and 2012 at Site 7.

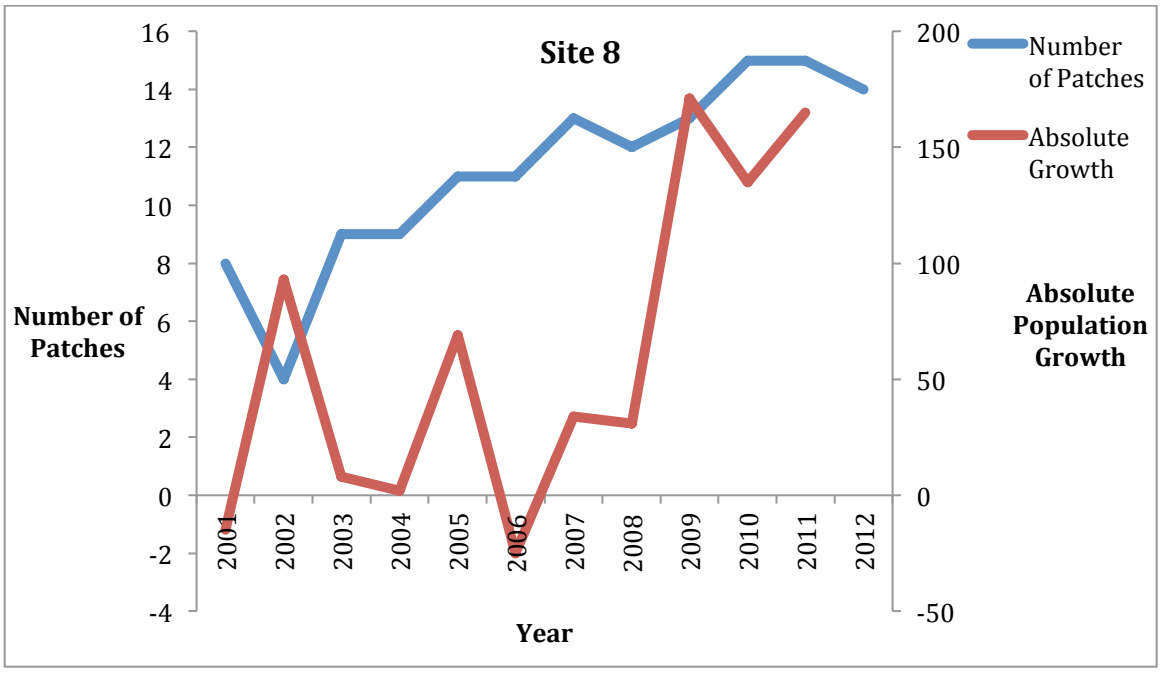

Figure 2.30 The comparison of the number of patches and the absolute population growth (in numbers of abalone), shown between 2001 and 2012 at Site 8.

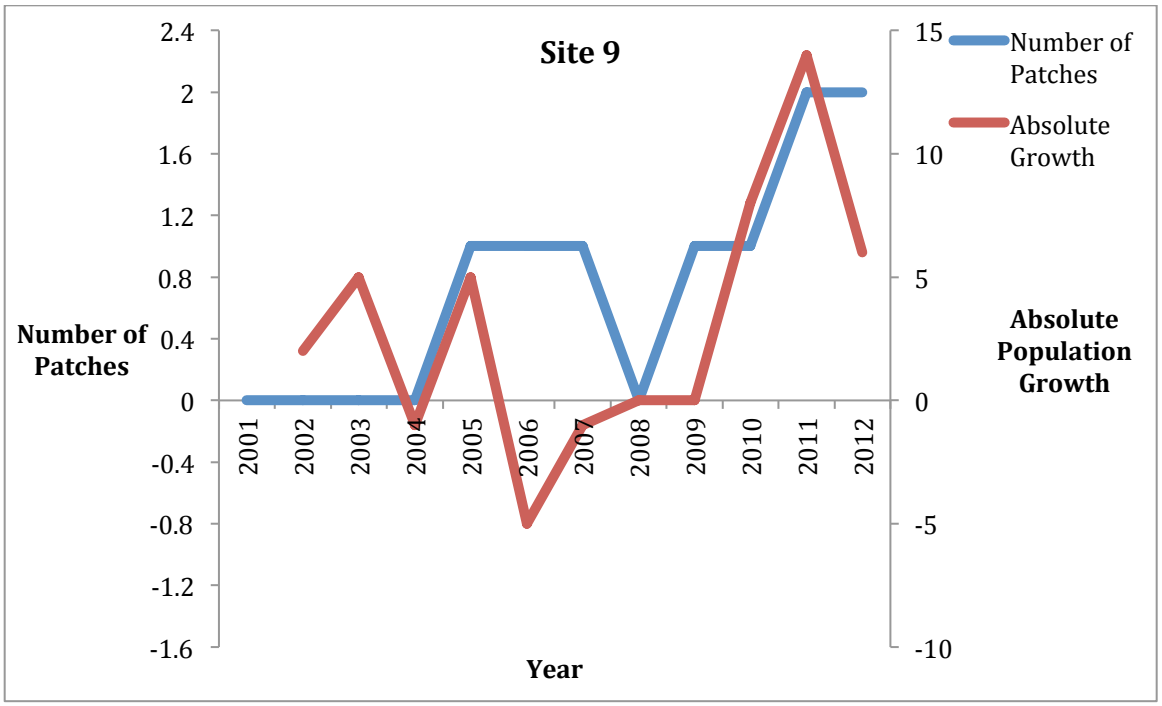

Figure 2.31 The comparison of the number of patches and the absolute population growth (in numbers of abalone), shown between 2001 and 2012 at Site 9.