RESTORING ENDANGERED NORTHERN ABALONE (*HALIOTIS KAMTSCHATKANA*) POPULATIONS IN BRITISH COLUMBIA, CANADA, USING HATCHERY-RAISED INDIVIDUALS

by

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ABSTRACT

This study investigated the effectiveness of using hatchery-raised northern abalone (*Haliotis kamtschatkana*) to supplement wild populations in Barkley Sound, BC. Densities of abalone were assessed at sites outplanted by the Bamfield Huu-ay-aht Community Abalone Project, and all fell at or below the suspected Allee threshold. The potential for improving outplanting success was then examined by releasing both larval and juvenile hatchery-reared individuals at different treatment densities and tracking their survival over time using cohort analyses and mark-recapture methods, respectively. Predators represented the major source of mortality for outplanted abalone and congregated at outplant sites. Tagging, handling, and temperature stress did not result directly in mortality. Juvenile abalone were particularly vulnerable in the first 24 hours after outplanting, experiencing 64 % mortality during that period. The behaviours of hatchery-raised abalone differed from those of wild individuals. I recommend outplanting 50,000 larvae/m^2 or groups of 100 juveniles in predator exclosure cages.

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ACKNOWLEDGEMENTS

Für Oma
Waltraud Margarete Clara Hansen (*geb*. Welke)
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Chapter 1: General introduction

  The majority (55%) of recorded marine extinctions occurring across the globe are attributable to exploitation (Pauly et al. 1998; Rose and Kulka 1999; Dulvy et al. 2003). There is evidence that commercial fishing practices reduce community biomass by 80 % within 15 years of the start of exploitation and have decimated 90 % of marine predatory fish (Myers and Worm 2003). Concurrently, there has been a shift in fishing pressure from targeting predators dominating food chains to concentrating on fish and invertebrates occupying lower trophic levels (Pauly et al. 1998). Such invertebrates, particularly broadcast spawners, were once considered resilient to over-exploitation due to their widespread distributions and high fecundities (Hobday et al. 2001). Yet there is mounting evidence that stocks of broadcast-spawning invertebrates are collapsing as a result of overfishing (Hobday et al. 2001; Dulvy et al. 2003).

  Global catches of abalone, a highly fecund broadcast spawning invertebrate, have declined drastically since the inception of commercial abalone fisheries. Indeed, the peak in global abalone fisheries production occurred in 1968, at 27,600 tonnes (Prince and Guzman del Proo 1993; Prince 2005). This figure had declined to 15,000 tonnes by the late 1980s, to approximately 10 000 tonnes in 2000 (Prince 2005), and is still declining (Prince and Guzman del Proo 1993; Prince 2005). While these gross numbers recall widespread trends in fisheries production and are suggestive of unsustainable fishing practices, the records of individual abalone fisheries are far more telling. There has been a litany of collapses of fisheries for specific abalone species, characterized by boom and bust landings (Wallace 1999; Campbell 2000).

  The recognition that abalone populations are declining through fishing has often been delayed because of phenomena known as serial depletion and hyperstability of catches. Given that abalone distributions are highly aggregated (Shepherd and Brown 1993; McShane 1996), abalone can be progressively depleted in space and time until an entire region is depleted because divers can target one aggregation before moving on to the next (Jamieson 1993; Karpov et al. 2000; Hobday et al. 2001; Morales-Bojorques et al. 2008). The mechanism behind serial depletion is also partially responsible for a

discordance between catch-per-unit-effort (CPUE) and abundance of abalone (Prince and Guzman del Proo 1993; Campbell 2000; Dowling et al. 2004). In an abalone fishery, CPUE will be stable as long as divers can find new aggregations, areas or abalone species to exploit (Karpov et al. 2000; Hobday et al. 2001). Because they remain stable even as the stock is being depleted, abalone catch rates are often termed 'hyperstable' (McShane 1996; Prince 2005). These are two of the principal reasons for which abalone fisheries have so often been overfished (Karpov et al. 2000; Hobday et al. 2001; Prince 2005; Prince et al. 2008). Of approximately 75 species of abalone in existence, only 25 % are still commercially fished (Prince 2005).

The northern abalone (*Haliotis kamtschatkana*) is the only abalone species that occurs in Canada, where it is distributed along the British Columbia coastline. Historically, *H. kamtschatkana* supported First Nations, recreational and commercial fisheries in B.C. The peak in commercial landings of this species occurred in 1977 and prompted further study of the biology of the species, which had hitherto been largely ignored (Sloan and Breen 1988; Jamieson 1993; Campbell 2000). In fact, the first stock assessment was conducted after abalone landings had already begun to decline (Sloan and Breen 1988). Surveys indicate that declining landings of *H. kamtschatkana* in B.C. were mirrored by a 75% reduction in the abundance of this species (Campbell 2000). This has manifested itself in a decrease in the number of sites occupied by northern abalone, as well as the density of abalone at these sites (Campbell 2000). Eventually, concern about declining abalone populations led to a complete closure of the B.C. abalone fishery in 1990 (Jamieson 1993; Campbell 2000). Nevertheless, abalone stocks continued to decline, and *H. kamtschatkana* was listed as threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1999 and uplisted to endangered in 2009 (Sloan 2004; Zhang et al. 2007; COSEWIC 2009). The plight of *H. kamtschatkana* in B.C. demonstrates that when overfishing has taken place, abalone populations may not be able to recover on their own.

A major contributor to this continued decline is suspected to be a strong Allee effect (DFO 2004; Bouma 2007), resulting in a sharp drop in the reproductive success of

populations when the density decreases below a threshold level (Courchamp et al. 2008). In the marine environment, probability of successful fertilization among broadcast spawners is dependent on a variety of factors including: number, density and distribution of spawning individuals, synchrony of gamete release, currents and turbulence which alter the dilution of gametes, and gamete properties (Levitan and Sewell 1998; Courchamp et al. 2008). When abalone populations are reduced in number or density, the probability of successful fertilization is greatly reduced, and spawning synchrony may be impaired (Shepherd and Brown 1993; Babcock and Keesing 1999). Below a threshold density, known as the Allee threshold, spawning events will no longer sustain population growth. Species whose populations have been driven below an Allee threshold will likely require human intervention (or a fortuitous removal of the factors driving mortality) for continued persistence. According to Courchamp et al. (1999) artificial enhancement of such populations should be possible.

Enhancement methods that rely upon the natural recruitment of abalone are slow (Dixon et al. 2006). Two such enhancement methods are predator removal and translocation of wild adult abalone (Dixon et al. 2006). If abalone population densities are already so reduced that a fertilization efficiency Allee effect is acting on them, then predator removal will be insufficient to bolster the population. Translocating wild adult abalone from low density into high density populations may increase fertilization efficiency and subsequent local recruitment (Dixon et al. 2006). However, this involves depleting certain areas of abalone, and may render high density populations especially vulnerable to poaching (Dixon et al. 2006). Another method for enhancing wild populations involves outplanting hatchery-reared abalone into the wild. While this is theoretically a viable option, it has experienced extremely variable success in past experiments using other abalone species and remains largely unstudied with respect to northern abalone (Schiel and Welden 1987; McCormick et al. 1994; Dixon et al. 2006; Griffiths and Gosselin 2008).

The overarching goals of this thesis are to assess whether outplanting hatchery-raised individuals is an effective means of rebuilding wild northern abalone populations, and to determine means by which outplanting success can be improved.

Northern abalone have been previously outplanted in British Columbia by the Bamfield Huu-ay-aht Community Abalone Project (BHCAP). The success of these efforts, however, was not monitored. That organization outplanted nearly 4 million larval and 150 000 juvenile abalone in the vicinity of the Bamfield Marine Sciences Centre since 2003. The first goal of this Master's thesis was to assess whether these outplanting events had been successful in order to establish a baseline from which methods (and ideally success) can be improved. The impact of these outplanting efforts on northern abalone populations was assessed in this study and is discussed in Chapter 2.

Studies delving into outplanting other abalone species have generated variable results, with estimates of mortality as high as 100 % after 7 days and as low as 10 % after one year. These studies have demonstrated considerable potential to enhance the success of outplanting attempts by altering the life history stage, density, size and condition of abalone upon outplanting (McCormick et al. 1994; Olla et al. 1998; Sweijd et al. 1998; Dixon et al. 2006). Abalone are long-lived broadcast spawning gastropods that develop from pelagic larvae into benthic juveniles which reach sexual maturity only after several years of growth (Cox 1962). *H. kamtschatkana,* for example, becomes sexually mature at shell lengths of 50 to 70 mm, which represents more than three years of growth (Sloan and Breen 1988; Campbell et al. 1992; Hakewich et al. 2007; Zhang et al. 2007). There are accordingly several stages of development at which northern abalone could be outplanted. Abalone experience highest mortality during the larval and post-larval phase of their life-history (Moss and Tong 1992). As abalone grow, their vulnerability to predators declines (Sloan and Breen 1988; Schiel 1993; Griffiths and Gosselin 2008). Thus, it is generally believed that the larger the abalone, the greater its chances of survival upon outplanting (Schiel 1989; Roberts et al. 2007). However, it is expensive to raise abalone in a hatchery environment (Tong, Moss and Illingworth 1987; Schiel 1993; Roberts et al. 2007), and so the greater the age of the abalone, the greater the cost of

production (McCormick et al. 1994; Sweijd et al. 1998). For these reasons, there is a trade-off in the choice of seed size, namely, whether one should outplant many small abalone or fewer large abalone. The optimal life history stage, density, size and condition of northern abalone for maximizing outplanting success are addressed in Chapter 3.

In order to improve the success of outplanting efforts, it is necessary to understand the failures of current techniques. In general, attempts to outplant other abalone species have had limited success, which is attributable to the high mortality of outplanted individuals (Schiel and Welden 1987; McCormick et al. 1994; Dixon et al. 2006), particularly immediately following outplanting (McCormick et al. 1994; Dixon et al. 2006). Chapter 4 examines the principal causes of mortality of outplanted northern abalone, and whether site selection influences mortality rates. This chapter also includes an analysis of the congregative abilities of important northern abalone predators, which influence both mortality through predation and the importance of site selection.

In many cases, mortality rates of outplanted abalone greatly exceed those of their wild counterparts (Schiel 1993; Rogers-Bennett and Pearse 1998; Sweijd et al. 1998; Dixon et al. 2006). This is thought to be partially attributable to behavioural discrepancies between wild and hatchery-raised abalone (Schiel and Welden 1987). Notably, certain abalone behaviours, such as escape responses, nocturnal activity, and use of cryptic habitats may be learned through exposure to predators (Schiel and Welden 1987). Thus hatchery-reared abalone that have never been exposed to predators might not express these behaviours. In Chapter 5, I examine the behavioural differences that exist between wild and hatchery-raised northern abalone. The potential of exposing hatchery-raised abalone to predators to alter their behaviours and improve outplanting success is also addressed in this chapter.

In summary, the failure of *H. kamtschatkana* populations to recover on their own over the 20 years since the fishery closure suggests that restocking may be the only option for rebuilding wild populations above the threshold density. The purpose of this M.Sc. research is to develop an effective method for increasing abalone densities above the critical threshold by outplanting hatchery-reared abalone. This is addressed in three

ways: examining the success of past outplanting attempts (Chapter 2), identifying optimal characteristics for outplanting (Chapter 3), and identifying outplanting limitations (Chapter 4 and 5). Recommendations drawn from my entire thesis are summarized in Chapter 6.

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Chapter 2: Assessing past outplanting efforts by the Bamfield Huu-ay-aht Community Abalone Project in Barkley Sound

INTRODUCTION

Overfishing, in combination with ineffective management, has driven fisheries into a crisis across the globe (Pauly et al. 1998; Rose and Kulka 1999). In some cases entire fisheries have been closed, with no subsequent recovery of the exploited species. A Canadian example involves the northern abalone, *Haliotis kamtschatkana*. In response to dwindling stocks, northern abalone in British Columbia were declared off-limits to all harvesting in 1990 (DFO 2004). Nevertheless, abalone stocks continued to decline, and *H. kamtschatkana* was listed as threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1999 (DFO 2004). At the latest COSEWIC assessment in April 2009, northern abalone were re-classified as endangered (COSEWIC 2009).

A major contributor to the continued decline of this species is thought to be the Allee effect, in which a population's reproductive success drops sharply when its density decreases below a threshold level (DFO 2004; Bouma 2007). Although there is currently no estimate of a threshold density for northern abalone in the literature, threshold densities for greenlip (*H. laevigata*) and blacklip (*H. rubra*) abalone in Australia range from 0.15 to 0.3 individuals per m^2 (Shepherd and Brown 1993; Shepherd and Partington 1995; Babcock and Keesing 1999). It is important to note that the densities of abalone participating in spawning can be lower than the densities of reproductive adults, as they are dependent on sex ratios and on spawning synchronicity within a population (Babcock and Keesing 1999).

Given the similarities in reproductive biology (fecundity, sex ratios, and spawning synchronicity) between northern abalone and *H. laevigata* and *H. rubra*, the Allee threshold for northern abalone may be comparable to that of *H. laevigata* and *H. rubra*. For example, the two above-mentioned species of Australian abalone reach sexual maturity at approximately 3 years of age, as does the northern abalone, although the shell length at sexual maturity is greater in the Australian than the Canadian species (75-120

mm vs. 50-70 mm; Shepherd and Laws 1974; Sloan and Breen 1988; Campbell et al. 1992; Zhang et al. 2007). While fecundity does increase with shell length in abalone, estimates of maximum fecundity of *H. kamtschatkana* approximate those of other abalone species (Campbell et al. 1992; Campbell et al. 2003). The sex ratios of *H. kamtschatkana, H. rubra* and *H. laevigata* are all relatively close to a 1:1 ratio, with some variation between sites (Shepherd and Laws 1974; Breen and Adkins 1982; Sloan and Breen 1988; Litaay and DeSilva 2001). Finally, the synchronicity of spawning in these species is not well described, but is thought to be poor in *H. rubra* and to range from 50-100 % synchronicity in *H. laevigata* and *H. kamtschatkana* (Shepherd and Laws 1974; Babcock and Keesing 1999). Another consideration is that reproductive abalone do actively aggregate for spawning and thus increase densities and fertilization success to some extent. Northern abalone, being a species with relatively low rates of dispersal in comparison with other abalone species (Sloan and Breen 1988; Hansen and Lessard in prep.), might be expected to be most limited in forming spawning aggregations. As a result, we have chosen to adopt the more conservative value of 0.3 abalone/m^2 as a target minimal density or Allee threshold for *H. kamtschatkana* for the purposes of this study.

The failure of northern abalone to recover in the 20 years since the fishery closure suggests that human intervention is necessary. Indeed, either the closure of the fishery has not significantly reduced abalone mortality due to ongoing poaching, or wild populations have fallen below the threshold density, in which case restocking is one of the few remaining options for rebuilding populations. Yet previous outplanting experiments using hatchery-reared abalone of other species have had limited success due to high mortality (Schiel 1993; McCormick et al. 1994; Rogers-Bennett and Pearse 1998; Dixon et al. 2006).

The Canadian National Recovery Strategy for the northern abalone lists as a long-term goal that the number and density of wild northern abalone be increased to self-sustainable levels in each biogeographic zone of British Columbia (DFO 2004). Thus the primary measure of success of outplanting attempts will be whether adult densities at outplanted sites are higher than the Allee threshold. A secondary measure of success

involves determining whether there is evidence that outplanted abalone survived; in the current thesis this will be examined by cohort analyses, but genetic techniques are also being used in a partner project (Read 2010).

The Bamfield Huu-ay-aht Community Abalone Project (BHCAP) began outplanting northern abalone in the vicinity of the Bamfield Marine Sciences Centre in 2003 as part of their licensing agreement with the Department of Fisheries and Oceans. The BHCAP outplanting history is summarized in Table 1. The BHCAP outplanted abalone as larvae, as 2-3 month old juveniles and as 1 year old juveniles. Ten-day-old larvae were transported in syringes and squirted into crevices in rocks by SCUBA divers. Juveniles were grown out on cement blocks, which were packed into plastic crates for outplanting. This community-based non-profit organization was unable to verify the level of success of its outplanting efforts due to insufficient funds. Consequently, the present study addressed three objectives to identify the success of these past outplanting attempts, namely to (1) determine if densities of reproductive northern abalone at outplanted sites exceed densities at a control site, and whether those outplanting efforts increased abalone densities above the presumed Allee threshold of 0.3 abalone/m^2, (2) determine the growth rates of hatchery abalone in the wild, and (3) assess whether size frequencies of abalone at outplanted sites provide evidence of hatchery abalone presence.

Table 1. The Bamfield Huu-ay-aht Community Abalone Project northern abalone outplanting history. All locations are within Barkley Sound, B.C. Shorthand for outplanting locations are SB for Scott's Bay, GT for Goby Town and HI for Helby Island. The two life stages outplanted are larvae (L) and juveniles (J). Expected average sizes at the time of surveys in 2008 and 2009 are based on the von Bertalanffy growth model with parameters $L_\infty = 122.6$ and $k = 0.158$ based on wild abalone from Ellis Islet (EI), our control site or $L_\infty = 114.2$ and $k = 0.351$ based on wild abalone from Bauke Island (BI). These parameters were determined by Breen (1986).

Date	Location	Life stage	Number	EI Expected size		BI Expected size	
				2008	2009	2008	2009
09/2003	SB	L	64,000	64.0	72.5	92.0	98.6
09/2003	SB	J (1yr)	2000	72.5	79.9	98.6	103.2
06/2004	SB	L	500,000	57.4	67.0	86.2	94.5
09/2004	SB	L	1,330,000	53.9	64.0	82.7	92.0
03/2005	SB	J (2-3 mo)	72,000	50.2	60.7	78.7	89.2
11/2005	GT	L	1,000,000	42.2	53.9	69.5	82.7
11/2005	HI	L	1,000,000	42.2	53.9	69.5	82.7
03/2006	GT	J (2-3 mo)	75,000	37.8	50.2	63.8	78.7

METHODS

Field surveys

All outplanting sites used by BHCAP were located within Barkley Sound, near the town of Bamfield in British Columbia, Canada (Figure 1). Three locations were chosen by BHCAP for outplanting: the south end of Helby Island, Scott's Bay and a site outside Grappler Inlet known as Goby Town. In 2008 we selected one additional site (Ellis Islet) that had never received outplants and would therefore serve as a control, based on wave exposure and habitat characteristics that were similar to the outplanting sites.
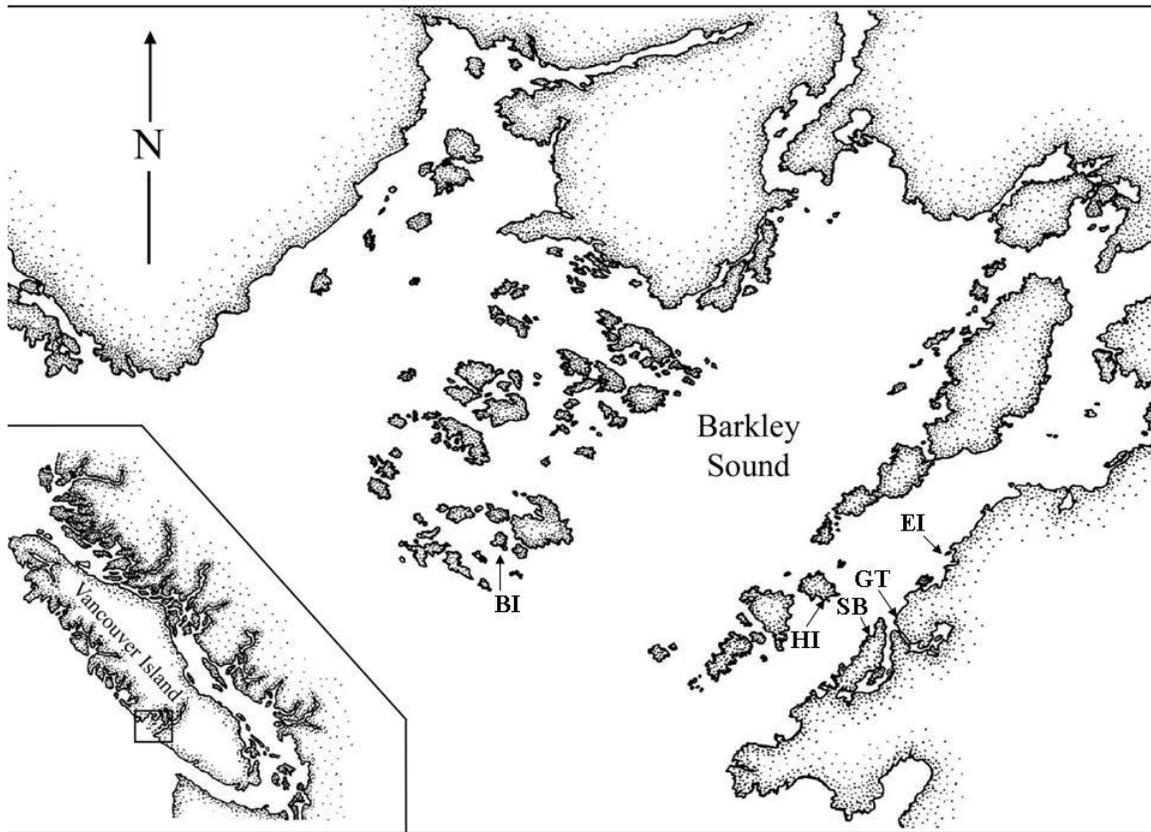
Figure 1. Map of past outplanting and control sites in Barkley Sound, located on the west coast of Vancouver Island (inset). Past outplanting sites and the control site are indicated by arrows, where HI is Helby Island, SB is Scott's Bay, GT is Goby Town and EI is Ellis Islet (the control). BI is Bauke Island, which was neither a control nor an outplanted site, but is a site for which northern abalone growth parameters are available (Breen 1986). Adapted from Gosselin and Chia (1995).

A stratified random sampling design, used by the Pacific branch of the Department of Fisheries and Oceans (Lessard et al. 2007), was modified slightly for the present study to determine densities of abalone at the control and experimental sites in 2008. This method, known as the plot survey, is illustrated in Figure 2. The plot survey consists of two reference lines, each 30 meters in length, laid at depths of 2.5 and 7.5 m

below chart datum. These depths represent the middle of the two major depth strata inhabited by northern abalone (Tomascik and Holmes 2003; Lessard and Campbell 2007; Zhang et al. 2007). A random number generator was used to determine the points along the reference lines at which the sampling transects began. Ten transects radiated perpendicularly from the shallow reference line, while eight transects were conducted along the deep reference line. Transects radiated alternately up and down from the reference line to reduce the likelihood of chasing abalone out of later transects. Each transect consisted of six 1 m^2 quadrats, unless the transect ventured into unsuitable habitat such as sand, in which case the transect was terminated prematurely. Every fourth quadrat was searched cryptically: all rocks and boulders within the quadrat that could be moved were turned over and closely examined for hidden abalone. All other quadrats were inspected only for exposed (emergent) abalone. An important feature of the plot survey is its use of multiple quadrats within the sampling unit of a transect; this minimizes the occurrence of zeros which are overemphasized in many abalone surveys due to the patchy nature of abalone distributions (Shepherd and Brown 1993; McShane 1996). All sea urchins were removed because their spine canopies often conceal abalone (J. Lessard, pers. comm.). Abalone found within the quadrat boundaries were measured with vernier calipers, and their shell length and position (whether cryptic or emergent) were recorded.
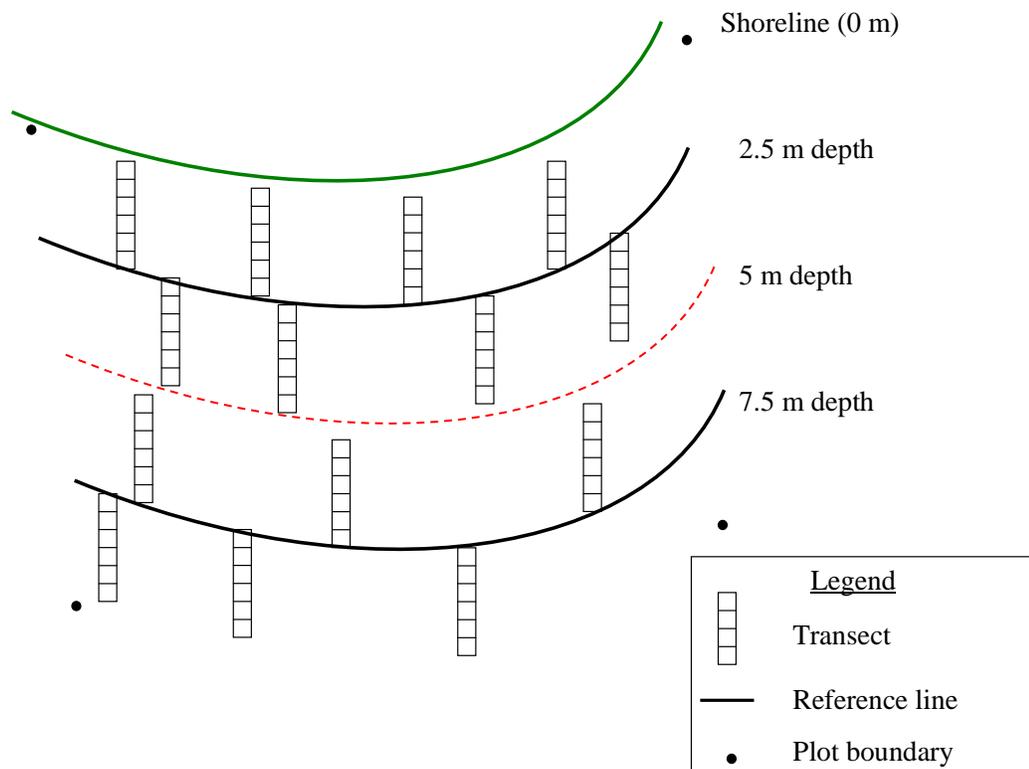
Figure 2. Plot survey design: lead lines measuring 30 m in length are laid at specific depths and serve as a reference line from which transects consisting of six 1 m^2 quadrats are extended.

The plot surveys were used to estimate abalone densities at all four sites. Because the plot survey is designed to assess densities rather than to maximize sightings of abalone within an area, size frequencies determined from plot survey data were insufficient for cohort analyses; size frequency data collected in conjunction with a partner project were used instead. This partner project was conducted under the supervision of Kaitlyn Read of the University of Guelph. Each outplanted site was methodically searched in 60 minute swims in 2008 and 2009 and all encountered abalone were placed in mesh collection bags and brought to the surface. There, tentacle samples

were taken for genetic analysis (partner project) and abalone were measured (this project) before being returned to their home sites.

Northern Abalone Densities

To determine overall abalone densities, the number of abalone observed in a transect was divided by the number of component quadrats. Given the 1 m^2 area of each quadrat, this calculation provided a density estimate in abalone per m^2. Densities were calculated separately for the shallow and deep strata, as well as for both strata combined. Moreover, because the density of reproductive abalone is especially relevant in determining population self-sufficiency, densities were also calculated separately for reproductive and immature abalone. I designed two equations to determine the density of reproductive abalone (Equation 1) and immature abalone (Equation 2).

Equation 1 $\quad \dfrac{(0.50)N_{a50-69} + N_{a>70}}{N_{quadrats}} =$ density of reproductive abalone

Equation 2 $\quad \dfrac{N_{a<50} + (0.50)N_{a50-69}}{N_{quadrats}} =$ density of immature abalone

In these two equations, N_{a50-69} is the number of abalone measuring between 50 and 69 mm in shell length (SL) and $N_{a>70}$ and $N_{a<50}$ are the number of abalone with SL longer than 70 mm and shorter than 50 mm, respectively. $N_{quadrats}$ is simply the number of quadrats. The SL for these calculations were chosen based on size at maturity information: some northern abalone mature at 50 mm SL, but 70 mm SL is the size at which 100 % of individuals are mature (Campbell et al. 1992; Zhang et al. 2007).

A three-way factorial repeated measures ANOVA was used to determine what factors influenced abalone densities. The response variable was density of abalone, and the three predictors were: abalone maturity (fixed; 2 categories), depth stratum (fixed; 2 depth categories), and site (fixed; 3 sites). Since both maturity stages (immature and reproductive) were recorded in the same quadrats, this variable was included in the ANOVA as a repeated measure. One-sample t-tests were subsequently used to determine whether the densities of abalone differed significantly from the Allee threshold of 0.3

abalone/m^2 at Ellis Islet and Scott's Bay. This was done for both the shallow (n=8) and deep (n=6) strata. Finally, the densities of abalone at Ellis Islet and Scott's Bay were compared using an independent samples t-test (n=16).

The increase in density resulting from outplanting was determined from the densities of hatchery-raised abalone identified by pedigree analyses at the three outplanted sites (Read 2010). These densities were calculated as the number of abalone identified as hatchery-raised divided by the area surveyed.

Northern Abalone Growth

The expected average SL of outplanted abalone at the time of the surveys in 2008 and 2009 were calculated using the von Bertalanffy equation (Rogers-Bennett et al. 2007; Bolker 2008; Zhang et al. 2009). The values for asymptotic average maximum length (L$_\infty$=122.6 and L$_\infty$=114.2) and the growth rate coefficient (k=0.158 and k=0.351) were derived from studies of wild abalone at Ellis Islet and Bauke Island, respectively (Breen 1986), both of which are in Barkley Sound (Figure 1). The von Bertalanffy equation was also rearranged (Equation 3) such that the age of abalone with a known shell length (L) could be estimated.

Equation 3 $Age = \dfrac{\ln(1 - L/L_\infty)}{k}$

Information on the year and life stage at which abalone were outplanted for each site allowed us to estimate the growth rates of the abalone that were found to originate from the hatchery. For example, if a 20 mm abalone was found at a given site in June 2008, and outplanting at this site only occurred in June 2006 and 2007 and involved only larvae, then the slowest possible growth rate of the abalone would be 10 mm/year (if outplanted in 2006), while the fastest possible growth rate would be 20 mm/year (if outplanted in 2007). Both the slowest and fastest possible growth rates are presented. A two-way repeated measures ANOVA was used to determine whether the growth rates of abalone differed between sites. The response variable was growth rate, and the predictor variables were site (fixed; 3 sites) and time since outplanting (fixed; 2 times). Time since outplanting is treated as a repeated measure in this ANOVA because both categories are

calculated from the same data (i.e. the earliest outplanting date yields an estimate of slow growth while the latest outplanting date yields an estimate of fast growth). A post-hoc Tukey's HSD test was used to compare growth rates among sites.

Northern Abalone Cohorts

All cohort analyses were performed in R, version 2.10.1, using the mixdist package. The parameters of interest in cohort analyses are μ, σ and π. Mu is the estimated mean of a component distribution (i.e. the mean SL of a cohort), while σ is the standard deviation of the component distribution. Finally, estimates of π show the relative importance of each cohort. In general, when working with abalone populations we expect variance to increase for older cohorts because differential growth of abalone will result in a broader range of sizes over time, and because the growth rate of northern abalone is inversely related to size (Emmett and Jamieson 1988). Older cohorts should accordingly become indistinguishable and appear as one broad flat peak, while young cohorts (particularly those representing new recruits) are the most likely to be clearly delimited (i.e. have small σ values). When the component distributions have equal proportions, the area under each curve will be equal; in such cases those cohorts with lower σ values will have higher peaks. When cohorts have equal importance, $\pi = \dfrac{1}{\#cohorts}$. Deviations from these standard π values indicate that some cohorts are proportionally more important than others.

Each of the cohort models referred to herein estimates all abalone generations existing at a particular site in a given year, based on the size frequencies of abalone. The fit between modeled size frequency distributions and actual frequencies of measured abalone was tested using a χ^2 analysis. The choice of the best cohort model was subjective, and was based on three criteria: (1) a small χ^2 value, which indicates that the model fits the data, (2) the model is able to estimate most or all parameters (i.e. it does not fail to compute any estimates), and (3) later cohorts do not have extremely small σ values (i.e. σ not smaller than 0.1, for 3rd year and older cohorts), as these would indicate that the model is not representative of a natural population.

RESULTS

Northern Abalone Densities

Overall there were significant differences in abalone densities ($F_{3,9}$=4.980, p=0.026) but these were not consistent for different abalone age groups at different depths. Indeed, there was a significant interaction between abalone maturity and depth (ANOVA: $F_{1,9}$=5.222, p=0.048), indicating that abalone at different life history stages are not evenly distributed across depth strata. Indeed, reproductive abalone were found in higher densities in the shallow zone (0-5 m below chart datum) while immature abalone were more prevalent in the deep zone (5-10 m below chart datum) (Figure 3).
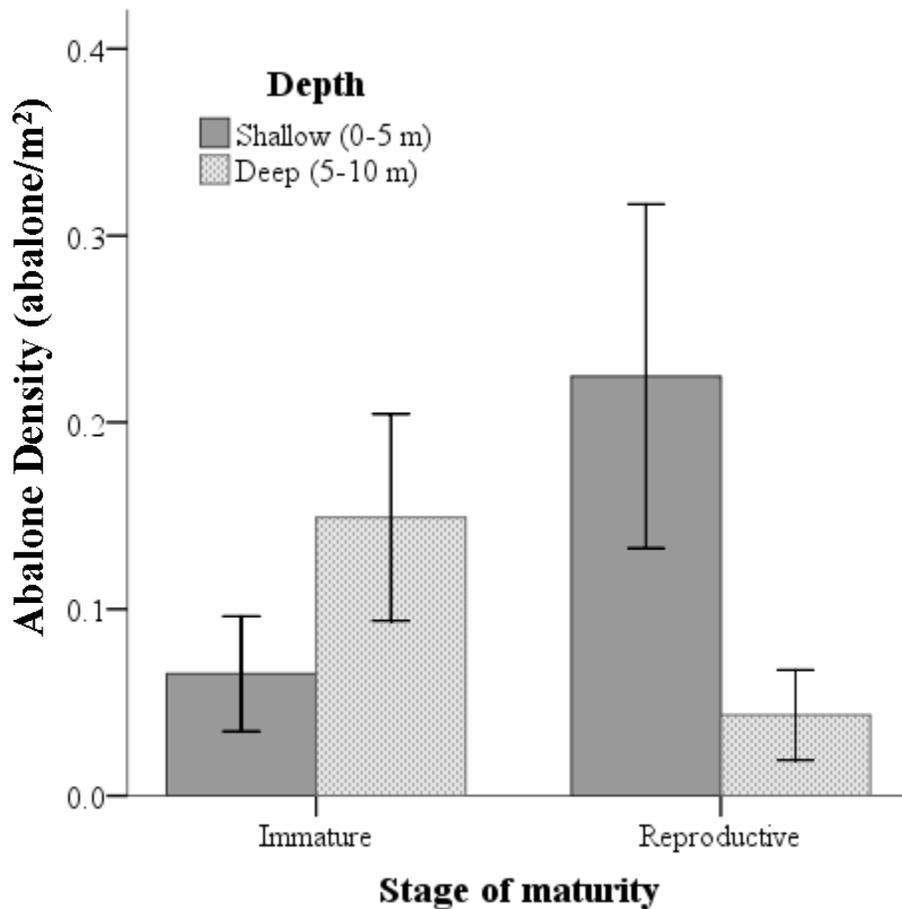
Figure 3. Mean density of immature and reproductive abalone (number of abalone/m²) in the shallow and deep strata. Data are pooled from all surveyed sites. Error bars represent ± 1 standard error (n=3).

The mean densities of reproductive abalone in the shallow stratum at both Ellis Islet and Scott's Bay (0.360 ± 0.174 and 0.343 ± 0.196, respectively) were slightly, but not significantly, higher than the proposed Allee threshold of 0.30 individuals/m^2 (One-sample t-test: $t_7=0.310$, $p=0.766$; $t_7=0.221$, $p=0.831$; Figure 4a). Reproductive abalone densities in the deep stratum were significantly lower than the Allee threshold at the Ellis Islet control site (One sample t-test: $t_5=2.920$, $p=0.033$) and Scott's Bay (One sample t-test: $t_5=10.950$, $p<0.001$) (Figure 4b). Adult abalone were absent from the surveyed plots at the Helby Island and Goby Town sites. Densities of reproductive abalone were not significantly different between Scott's Bay and Ellis Islet (Independent samples t-test: $t_{14}=0.480$, $p=0.639$).

A. Shallow

B. Deep
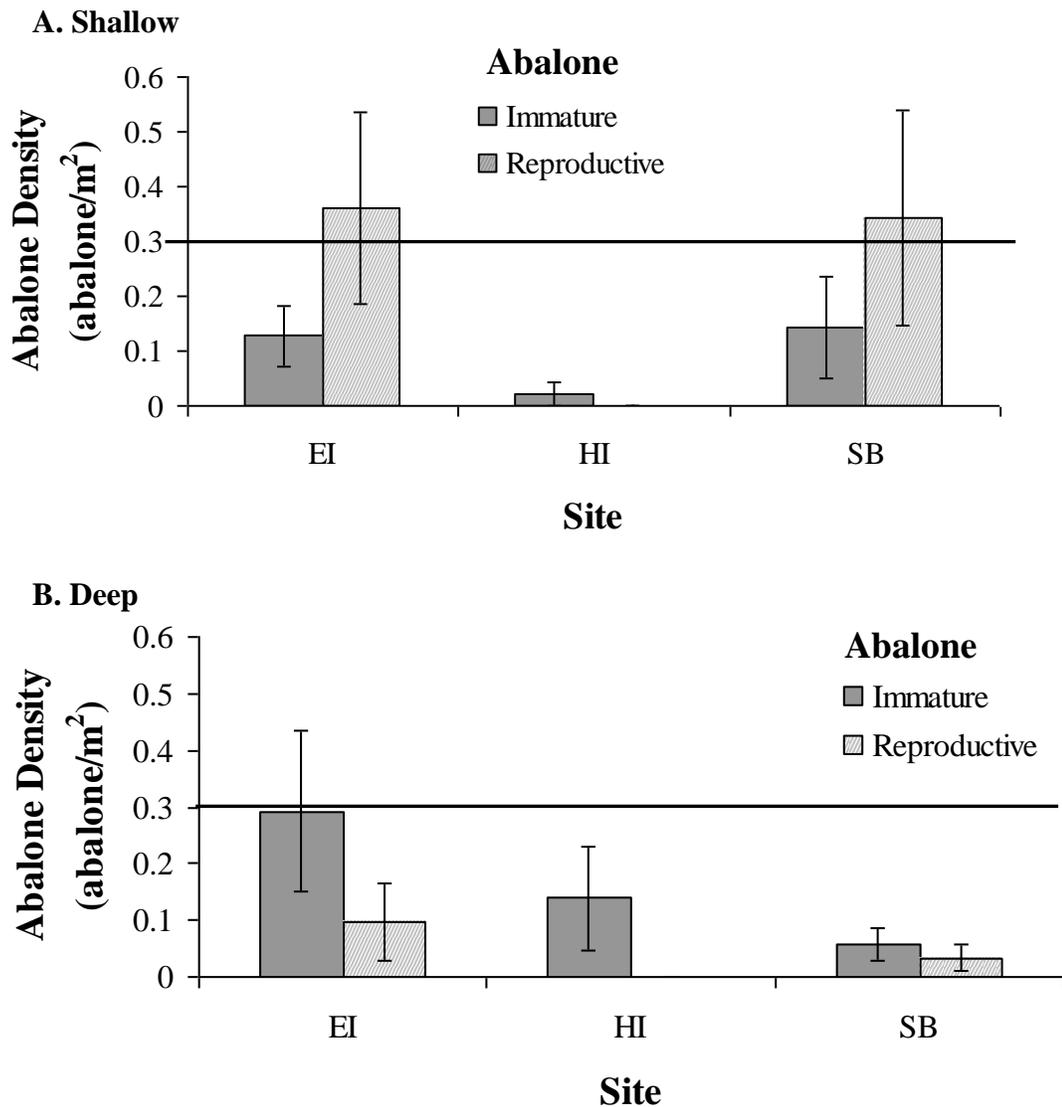
Figure 4. Mean density of immature and reproductive abalone (number of abalone/m^2) in (a) the shallow stratum, and (b) the deep stratum, at the three study sites near Bamfield, BC. Goby Town is not shown as no abalone were found. EI is Ellis Islet, HI is Helby Island, and SB is Scott's Bay. Error bars represent ± 1 standard error (n=8 for the shallow stratum and n=6 for the deep stratum). The horizontal line represents the proposed Allee threshold (Babcock and Keesing 1999).

Genetic analyses revealed that a total of 41 abalone of 149 surveyed in 2008 and 2009 were determined to be of hatchery origin (Read 2010); of these, 2 were found at Goby Town, 23 at Helby Island, and 16 at Scott's Bay. The number of hatchery-raised abalone found as a function of the area searched corresponds to increases in density of only 0.010, 0.115 and 0.080 abalone/m^2 at Goby Town, Helby Island and Scott's Bay, respectively. Most of these hatchery-raised abalone, however, were still juveniles; when considering only reproductive adults the increases in density were only 0.003, 0.008, and 0.033 abalone/m^2.

Northern Abalone Growth

The site at which a hatchery abalone was outplanted significantly influenced its growth (ANOVA: $F_{2,4}=44.495$, $p=0.002$) with abalone at Helby Island having faster growth than abalone at either Scott's Bay or Goby Town (Tukey's HSD tests: $p<0.001$, and $p=0.032$, respectively; Figure 5). There was no difference in growth rates of abalone between Scott's Bay and Goby Town (Tukey's HSD test: $p=0.260$).
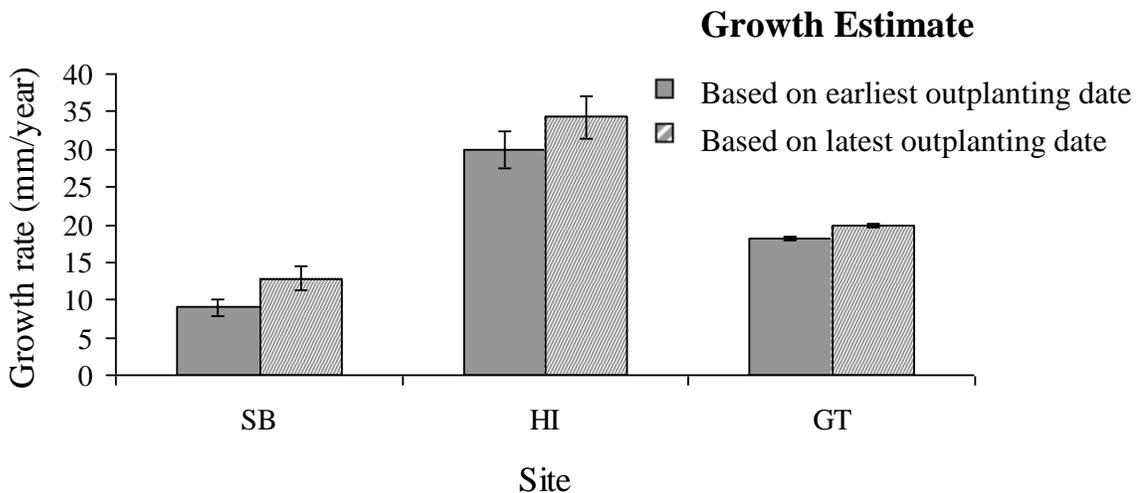
Figure 5. Mean growth rate of hatchery-raised abalone in the wild. The slowest growth was calculated from the earliest outplanting date at a site and fastest growth from the latest outplanting date at a site. Sites are GT for Goby Town, HI for Helby Island, and SB for Scott's Bay. Error bars are ± 1 SE, n=2 at GT, n=23 at HI and n=16 at SB. Read (2010) used pedigree analyses to identify the hatchery abalone represented herein.

Northern Abalone Cohorts

Estimates of μ, σ and π and their associated standard errors from the cohort models that best fit the size frequencies of abalone in this study are presented in Table 2.

Table 2. Cohort analyses results. Number refers to the cohorts found at a site (from smallest to largest), π is a measure of the dominance of the cohort, μ is the mean SL of the cohort in cm, σ is the standard deviation of the component distribution, and SEs are the standard errors of these estimates. Degrees of freedom, Chi square values and p-values refer to the cohort models themselves.

Year	Site	#	π	SE	μ	SE	σ	SE	χ^2	df	p
2008	SB	1	0.073	0.055	1.125	0.482	0.473	0.586	17.331	19	0.567
		2	0.167	0.080	2.971	0.301	0.515	0.276			
		3	0.426	0.120	5.837	0.364	1.024	0.454			
		4	0.205	0.156	8.627	0.318	0.457	0.442			
		5	0.129	0.106	9.946	0.398	0.403	0.248			
2008	HI	1	0.140	0.090	0.957	0.065	0.081	0.090	6.669	12	0.879
		2	0.165	0.096	2.288	0.492	0.609	0.454			
		3	0.088	0.063	4.973	0.204	0.039	0.295			
		4	0.306	0.177	7.492	0.905	1.269	0.814			
		5	0.300	0.157	9.565	0.197	0.444	0.196			
2008	EI	1	0.278	0.075	1.052	0.072	0.217	0.064	9.049	17	0.939
		2	0.216	0.072	4.962	0.227	0.545	0.197			
		3	0.098	0.066	6.557	0.091	0.112	0.091			
		4	0.281	0.098	8.777	0.363	0.801	0.350			
		5	0.128	0.072	10.567	0.167	0.256	0.127			
2009	SB	1	0.516	0.076	2.717	0.226	0.950	0.195	16.336	29	0.972
		2	0.196	0.070	6.072	0.129	0.286	0.123			
		3	0.202	0.079	7.629	0.221	0.486	0.288			
		4	0.086	0.054	9.510	0.390	0.395	0.345			
2009	HI	1	0.385	0.083	3.141	0.232	0.796	0.190	14.029	20	0.829
		2	0.536	0.108	8.485	0.445	1.459	0.360			
		3	0.079	0.078	10.691	0.209	0.205	0.208			

The size frequencies of abalone at Scott's Bay, Helby Island and Ellis Islet in 2008 were best described by five-cohort models (Table 2, Figure 6). In other words, there appear to have been five cohorts of abalone coexisting at each of these sites in 2008. At Scott's Bay, the mean sizes of these five cohorts are 1.125, 2.971, 5.837, 8.627, and 9.946 cm SL. Note that the cohort with a mean of 5.837 cm is represented by a peak that is both broad and tall (Figure 6a) and has a much larger π value than the other cohorts, indicating that this cohort is proportionally more abundant than the others. The cohort with the second highest proportional importance is cohort number 4 (mean SL = 8.627 cm). If we assume that growth of outplanted abalone follows a similar pattern to that of wild abalone at Ellis Islet then all of the Scott's Bay outplants would have SL encompassed by the most abundant cohort at Scott's Bay, that with a mean size of 5.837 cm. Alternatively, if outplanted abalone grew more rapidly, such as wild abalone at Bauke Island, they would then fall under the cohort with mean size 8.627 cm.
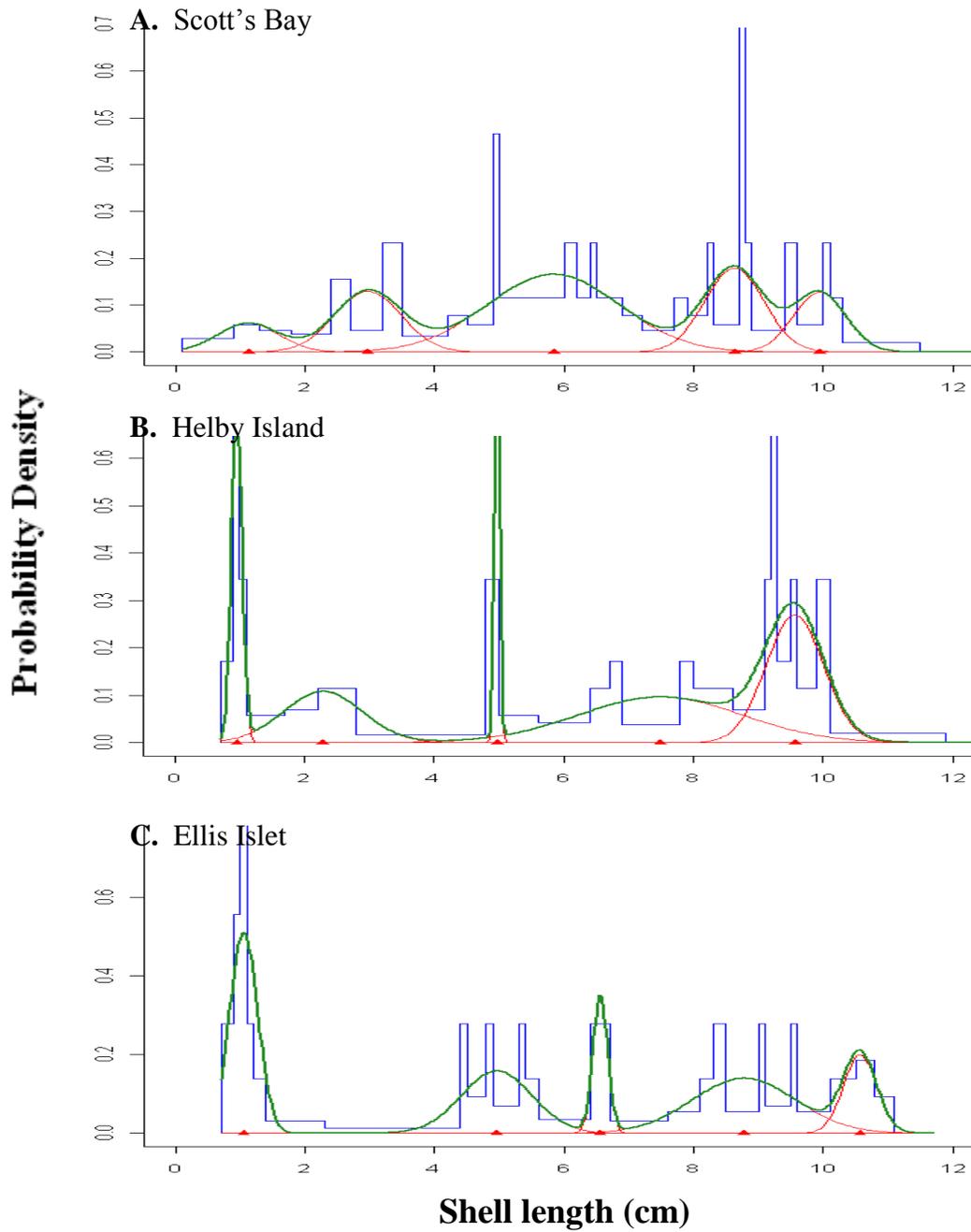
Figure 6. Cohort analyses of the size frequencies of abalone at three sites in 2008: (a) Scott's Bay, (b) Helby Island, and (c) Ellis Islet. The blue line is the actual frequency distribution, the green line shows all modeled cohorts while the red lines show individual cohorts and red triangles indicate the mean shell lengths of each cohort.

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For size frequencies of abalone at Helby Island in 2008 (Figure 6b), the five-cohort model had the drawback of a very small σ value for an intermediate cohort (0.039 for the cohort with mean size 4.973 cm SL). At Helby Island, the last two cohorts were those with the largest π values. The expected sizes of outplanted abalone based on growth at Ellis Islet do not correspond to any peaks at Helby Island, whereas expected sizes based on growth at Bauke Island encompass the strongest cohort at Helby Island.

A cohort of new recruits was the only cohort occurring across all sites in 2008, with the exception of Goby Town where no abalone of any size were observed. This young cohort had a mean SL of 1.125 cm at Scott's Bay, 0.957 cm at Helby Island, and 1.052 cm at Ellis Islet. Von Bertalanffy parameters estimated by Breen (1986) suggest that 1 year old wild northern abalone have a mean size of 1.79 ± 1.43 cm SL (mean $\pm$ 1 SE), thus this new cohort likely corresponds to abalone that settled within the past year. Another distinct cohort of mean size 2.288 and 2.971 cm SL was present at Helby Island and Scott's Bay, respectively, yet this cohort is completely absent at Ellis Islet. Helby Island and Ellis Islet share a larger cohort of mean size 4.973 and 4.962 cm SL.

The size frequencies of abalone at Scott's Bay one year later (in 2009) are described well by a model with four cohorts of mean size 2.717, 6.072, 7.629 and 9.510 cm SL (Figure 7a). This size frequency distribution is atypical. Indeed, we see a decline in the relative importance of cohorts with increasing size at Scott's Bay in 2009, whereas natural abalone populations are characterized by an accumulation of older individuals and thus relatively large importance among the larger size classes. The two strongest cohorts were those with mean SL 2.717 and 7.629 cm, with the former being by far the strongest (Table 2). The cohort with mean SL of 7.629 cm encompasses the expected sizes of all abalone outplanted at Scott's Bay. If growth rates were similar to those at Ellis Islet, then outplanted abalone would fall under the left tail of the cohort, whereas growth rates similar to those at Bauke Island would put outplanted abalone under the right-tail of the distribution.

A. Scott's Bay

B. Helby Island

Probability density

Shell length (cm)
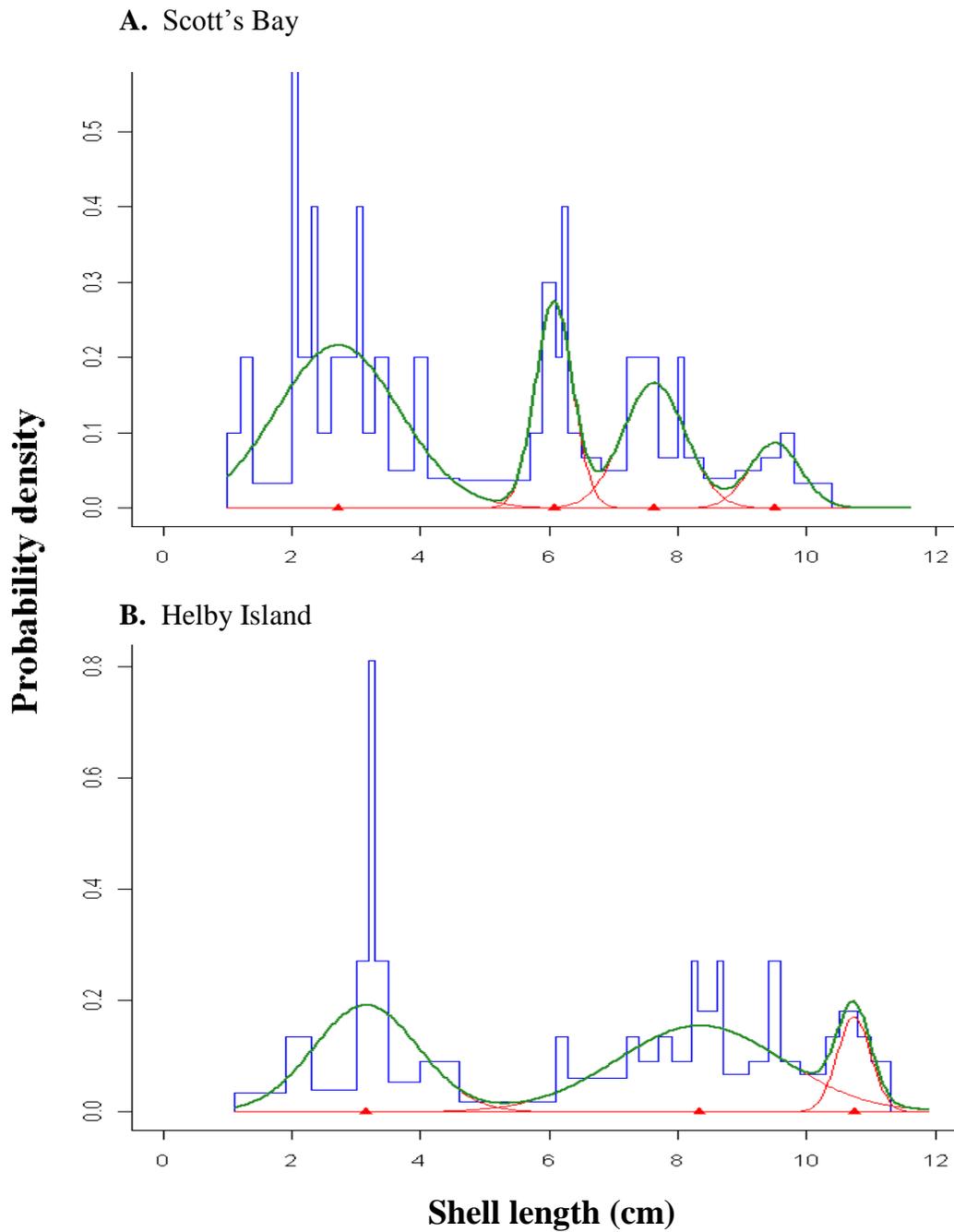
Figure 7. Cohort analyses of the size frequencies of abalone found in 2009 at (a) Scott's Bay, and (b) Helby Island. The blue line is the actual frequency distribution, the green line shows all modeled cohorts while the red lines show individual cohorts and red triangles indicate the mean SL of each cohort.

A three-cohort model was selected as the best model to describe the size frequencies of abalone at Helby Island in 2009. The three cohorts consist of abalone with the following mean sizes: 3.141, 8.485, and 10.691 cm SL (Figure 7b). As in the case of Scott's Bay in 2009, the oldest cohort was found to be relatively small and narrow. The cohort with a mean SL of 8.485 cm was proportionally the most important cohort at Helby Island in 2009. The range in SL of this cohort encompass the expected SL of abalone outplanted at Helby Island, if growth mimics that at Bauke Island, but not if it is any slower.

There are no obvious matching cohorts at Scott's Bay and Helby Island in 2009. The youngest cohorts detected at these sites in 2009 could correspond to new recruits if growth was rapid (mean SL were 2.717 and 3.141 at Scott's Bay and Helby Island, respectively). However, the youngest cohorts at these sites in 2008 had considerably smaller SL (1.125 cm at Scott's Bay and 0.957 cm at Helby Island), suggesting that the youngest cohorts seen in 2009 were survivors from previous years, not new recruits. This is suggestive of a recruitment failure in 2008.

DISCUSSION

Northern Abalone Densities

Given that the greatest density of adult abalone was found at the control site and no adults were found at two of the three outplanting sites during our plot surveys in 2008, one might speculate that the outplanting was detrimental to the health of wild abalone populations. However, several other facts make it clear that this is not the case. For example, the densities at Ellis Islet were not significantly different from those at Scott's Bay. In 2008, densities of northern abalone were highest at Ellis Islet and Scott's Bay, intermediate at Helby Island and extremely low at Goby Town. In hindsight, it is likely that Ellis Islet was suitable as a control for Scott's Bay but not Helby Island or Goby Town where the algal community structure and ratio of boulders to cobble were different (pers. obs.). Indeed, I suspect that the low densities of abalone found at the latter two sites are related to habitat characteristics. It would have been most advantageous to have one

control site for every outplanted site; however, no such control sites were known and time and resource constraints prevented an extensive search for additional control sites.

The fact that abalone were almost non-existent at Goby Town may be related to poor habitat. Indeed, when surveys were conducted in 2008 and 2009, Goby Town was almost entirely overgrown with *Agarum fimbriatum* (pers. obs.). *Agarum fimbriatum* is an understory kelp known for having some of the highest phenolic content of all brown kelps in the Pacific Northwest (Durante and Chia 1991). Phenolic content of kelps has a strong inverse relationship with palatability to grazers such as abalone (Durante and Chia 1991; Duggins and Eckman 1997), and thus the overbearing presence of this kelp at Goby Town may be an indicator of less than ideal abalone habitat.

The habitat at Helby Island did not show any indications of being inappropriate for abalone but did consist of more large stacked boulders than were present at either Ellis Islet or Scott's Bay. The stacking of such boulders creates plenty of cryptic space in which even large abalone can seek refuge. As few of these boulders could be overturned by divers during the plot survey, it is possible that our density estimates were artificially reduced by our inability to survey as much of the cryptic space as at other sites. The idea that reproductive abalone are present at Helby Island but were cryptic at the time of the plot survey in 2008 is supported by the fact that adult abalone were found there at other times. In fact, 23 adult abalone were observed near the plot survey site at Helby Island in timed swims in 2008, and 37 were found within and around the plot survey site in 2009. Thus this study appears to be one of many in which the refuge seeking behaviour of abalone species can reduce the accuracy of density estimations (McShane 1994; Maliao et al. 2004; DeFreitas 2005; DFO 2007; Leaf et al. 2007). The possibility of using chemical cues to "chase" abalone out of cryptic habitat should be considered for studies where accurate density or population size information is required. Northern abalone react strongly to *Pycnopodia helianthoides* saponins and conspecific stress mucus (Bullock 1953; Feder 1963; Montgomery 1967; Mackie 1970; Chapter 5). It is possible that extracts from either of these substances could be injected deep into crevices to encourage abalone to emerge. Abalone forced to emerge in this way would however be moving very

rapidly and would accordingly still be difficult to survey. This could also prove to be very destructive, as abalone moving rapidly and releasing stress mucus are vulnerable to predators (Knudsen 1960; Feder 1963). Moreover, it is unknown whether all size classes would respond to the *P. helianthoides* saponins equally or emerge at the same rate.

Certain habitat features such as kelp community structure appear to be a better predictor of abalone density than whether the site has been subject to outplanting. However, this statement is currently based only on personal observations and few field sites. The relationship between habitat features and abalone densities as well as outplanting success accordingly requires further examination.

Densities of reproductive abalone were not significantly higher than the presumed Allee threshold of 0.3 individuals/m^2 at any of the three outplanting sites. Thus, we must conclude that past outplantings of *H. kamtschatkana* in Barkley Sound were not successful in raising densities above the threshold density of 0.3 abalone per m^2. Densities of reproductive abalone were not, however, significantly lower than the threshold density at the control site, nor at Scott's Bay, an outplanted site.

Note that outplanting did increase abalone densities (hatchery-reared abalone were identified by pedigree analyses), but not by a large amount, and not above the Allee threshold. One possible cause for the failure of outplanting to substantially raise abalone densities may have been the practice of choosing sites based on their proximity to the abalone rearing facilities rather than on habitat features. Two recent studies should facilitate the use of habitat features to guide choices for future northern abalone outplanting sites (see Tomascik and Holmes 2003, and Lessard and Campbell 2007).

This study provides further support for northern abalone distribution being defined by two depth strata (Tomascik and Holmes 2003; Lessard and Campbell 2007; Zhang et al. 2007). I recommend that when the densities of reproductive or immature northern abalone are being assessed in future studies, the two depth categories – 0 to 5 m and 5 to 10 m – be considered separately.

Northern Abalone Growth

The choice of sites for outplanting is made even more important by the fact that site influenced the growth rates of outplanted abalone in this study, which varied from 9.00 ± 1.09 mm/year to 34.3 ± 2.79 mm/year. In fact, the mean growth rate of abalone at Helby Island exceeded the growth predicted by the von Bertalanffy model based on data from Breen (1986), whereas the mean growth rates of abalone at Scott's Bay and Goby Town were lower and more closely approximated rates of growth in the hatchery. It is likely that growth rates varied between sites because of differing habitat quality, including features such as the algal community structure.

The von Bertalanffy equation, based on consistent asymptotic growth throughout juvenile and adult life, performs poorly when describing the growth of young abalone (Rogers-Bennett et al. 2007). The von Bertalanffy equation with parameters based on Ellis Islet and Bauke Island abalone predicts sizes of 1.79 and 3.38 cm after one year of growth in the wild. The growth of abalone in the first year of life is likely overestimated by the von Bertalanffy equation (Rogers-Bennett et al. 2007), a fact that is supported by the observation that northern abalone grow approximately 10 mm per annum in the hatchery environment (J. Richards pers. comm.). That said, neither of these predictions (hatchery or wild growth) are expected to conform exactly to the growth of hatchery abalone in the wild. Nevertheless, these predictions give us a range within which we expect true growth rates to fall. These predicted growth rates can be combined with observations of abalone cohorts to draw important conclusions regarding recruitment.

Northern Abalone Cohorts

If outplanting by BHCAP had been successful, one should find unusually large cohorts corresponding to the expected size of outplanted abalone. Strength of a cohort could be manifested as a relatively high value of π, or, in the case of equal π values, as a narrower peak (smaller σ value) than might otherwise be expected. Past outplantings by BHCAP did not produce large pulses of recruits that could be easily distinguished from natural fluctuations in recruitment. This observation is supported by the pedigree analysis

performed by Read (2010), as the 41 hatchery abalone identified at the three outplanted sites contribute only 0.010, 0.115, and 0.080 abalone per m^2 to the overall densities at Goby Town, Helby Island and Scott's Bay. These increases are so small that they are obscured by natural variability.

There is, however, evidence that outplanted hatchery-reared abalone contributed to the strength of certain cohorts. Notably, the two cohorts that are proportionally most important in Scott's Bay in 2008 have a range of SL that encompass the expected SL of all abalone outplanted at Scott's Bay. It is possible that outplanted abalone are responsible for the relative strength of these cohorts. Yet the SL of the two strongest cohorts at Helby Island exceed the expected SL of outplanted abalone. Interestingly, there is a discrepancy between the expected sizes of outplanted abalone at Helby Island and the actual sizes of those identified by pedigree analysis. Indeed, the growth of abalone outplanted at Helby Island was extremely rapid, and the actual sizes of abalone outplanted at Helby Island do correspond to the strongest cohorts. Given the rapid growth of abalone observed among recovered hatchery abalone at Helby Island, it is also likely that outplanted abalone were responsible for boosting the strongest cohort there. The second strongest cohort of abalone in Scott's Bay in 2009 consists of abalone with a range of SL that include the expected size of abalone outplanted as larvae in 2004 and juveniles in 2005. Thus these strong cohorts all lend support to the conclusion that cohorts with large π values may have been slightly enhanced by outplanting. One exception is the strongest cohort at Scott's Bay in 2009 (mean size 2.717 with π equal to 0.516), which has a SL that does not correspond to the expected sizes of any outplanted abalone. It is accordingly evident that the fluctuations in cohort strength observed throughout this study can also be generated through natural events, and the cohort analyses only provide an indication – not conclusive evidence – that outplanted abalone are contributing to the strength of cohorts at surveyed sites.

The smallest cohort (~ 1 cm SL) observed at Scott's Bay, Helby Island and Ellis Islet in 2008, represents abalone that were spawned within the year prior to surveys. The second cohort (2-3 cm SL) that is present at Scott's Bay and Helby Island, but not Ellis

Islet, may be the result of an earlier spawning event in the same year as the first cohort were spawned, or a spawning in the previous year. In either case, these two cohorts provide evidence of successful wild spawning events, since no larval or juvenile abalone were outplanted between the summer of 2006 and the surveys of 2008. Furthermore, they provide evidence of at least one recruitment failure occurring at Ellis Islet within the same time frame.

Recruitment failures are thought to have been characteristic of northern abalone populations even before they began to decline in earnest, and to have contributed to the decline (McShane 1995; Bouma et al. 2006). This is reflected in the fact that Ellis Islet was the site with the highest surveyed abalone densities in this study, and yet was still subject to a recruitment failure at a time when other sites received new recruits. Scott's Bay and Helby Island also appear to have experienced recruitment failures in 2008, as indicated by a lack of young abalone in 2009. Until recently, it was commonly held that abalone recruits were not necessarily produced by the site at which they settled, but emerging evidence indicates that abalone larvae are highly philopatric, recruiting back to the same patches of habitat occupied by their parents (Prince et al. 1987, 1988; McShane 1992, 1995; Davis et al. 1996). Metapopulation theory may best describe the connectivity of abalone populations, with the majority of recruitment coming from local sources, but occasional recruits from outside populations providing some genetic exchange (Shepherd and Brown 1993). An increase in the occurrence of recruitment failures is often an indication that populations are close to or below the Allee threshold (Shepherd and Baker 1998; Frank and Brickman 2000).

The population structure of northern abalone at Scott's Bay in 2009 is typical of a heavily fished population. Fishing has been banned since 1990, and the likelihood that large abalone are consistently being picked off by poachers is low for several reasons. Firstly, Scott's Bay is in very close proximity to both a Coast Guard outpost and the Bamfield Marine Sciences Centre, and is accordingly protected by surveillance. It is, moreover, a site that is overlooked by a few houses and is passed by a high volume of boat traffic, and would accordingly be an unlikely site for poachers to target. Thus the

atypical population structure suggests that old abalone are simply dying off in Scott's Bay and are not being replenished through recruitment, and is consistent with an Allee Effect.

Conclusion

Outplanting, as it was conducted in the past, was not successful in causing a substantial increase in densities of the northern abalone. Outplanting does appear to have increased densities of reproductive adults, but only by only 0.003 to 0.033 individuals/m^2, leaving the population densities below the presumed Allee threshold. Furthermore, cohort analyses suggest that very few of the abalone outplanted by BHCAP survived until 2008 and 2009 when outplanted sites were reassessed. Nevertheless, the survival of some outplanted northern abalone is promising, particularly since we believe that outplanting methods, and hence success, can be significantly improved, and this will form the focus of the remaining chapters of my thesis.

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Chapter 3: Optimizing outplanting strategies: Density and Life Stage Manipulations

INTRODUCTION

Studies on the viability and methods of abalone stock enhancement by release of hatchery-raised individuals have become relatively common in the last two decades (McCormick et al. 1994; Dixon et al. 2006). Nevertheless, results remain extremely variable and no solution has been identified that functions consistently to ease the plight of wild abalone populations. Estimates of mortality of outplanted abalone range from only 10% after one year up to 100% after only 7 days (McCormick et al. 1994). Many factors have been identified as influencing the survival of abalone outplants; these include site selection, density at outplanting, the life history stage, size and condition of the abalone, as well as the stress suffered prior to outplanting (McCormick et al. 1994; Olla et al. 1998; Sweijd et al. 1998; Dixon et al. 2006). The optimal choice of site, outplanting density and size vary according to the species of abalone being outplanted, as does the relative importance of these factors.

One of the most important considerations for outplanting involves the choice of age group, from larvae to late-stage juveniles (McCormick et al. 1994). Generally, it is thought that the further along an abalone is in its life history (from larva to adult), the greater its chances of survival upon outplanting (Schiel 1989; Roberts et al. 2007). However, it is expensive to raise abalone in the hatchery environment (Tong, Moss and Illingworth 1987; Schiel 1993; Roberts et al. 2007), and so the greater the age of the abalone, the greater the cost of production and likely the fewer individuals that can be outplanted (McCormick et al. 1994; Sweijd et al. 1998). For these reasons, there is a trade-off in the choice of seed size: namely, should one outplant many small abalone or few large abalone?

Abalone development proceeds from fertilization to a pelagic lecithotrophic phase involving a trochophore and then a veliger. This pelagic phase lasts 7-10 days (Strathmann 1987), during which the veliger develops traits necessary for recognizing settlement cues and beginning benthic life, thus becoming metamorphically competent. Once it is competent, the veliger settles and metamorphoses into a benthic juvenile that

has the adult form but lacks reproductive organs (Mottet 1978; Garland et al. 1985; Strathmann 1987). Over a period of several years, the juvenile grows, shifts from a cryptic to an emergent lifestyle and becomes a reproductive adult (Sloan and Breen 1988). There are specific advantages and disadvantages for outplanting of abalone which are inherent to each stage of this ontogeny.

One consideration for the outplanting of larvae is that density-dependent mortality has been recognized in the recruits of a number of abalone species; so there is likely an upper limit to the number of larvae that should be outplanted (McShane 1991; McShane and Naylor 1995). If density-dependent mortality is an important factor for *H. kamtschatkana* larvae, it may be advantageous to outplant low densities of larvae. Alternatively, the larval and early post-larval phase of an abalone's life history are the most vulnerable (Moss and Tong 1992), and high densities at outplanting may achieve predator dilution and therefore be more successful. The predator dilution effect describes the fact that for a given number of predator attacks, as the number of prey in an aggregation increases, each prey individual's likelihood of being a victim decreases (Foster and Treherne 1981; Levitan and Petersen 1995; Petersen and Levitan 2001). Indeed, even before settling on the substrate, abalone are at risk of predation from a variety of predators. They can be removed from the water column by filter feeders such as cnidarians, coelenterates, bivalves, barnacles, bryozoans and ascidians (Bingham and Walters 1989). Not only are hard substrate communities often dominated by such filter-feeders (Bingham and Walters 1989), but the abalone's preferred settlement substrate, crustose coralline algae (Moss and Tong 1992), hosts a number of infaunal deposit-feeding predators that can target newly settled larvae (Naylor and McShane 1997). These infaunal predators include polychaetes and turbellarians (Naylor and McShane 1997). Naylor and McShane (1997) even went so far as to conclude that larval abalone preferences for corallines might be greatly limiting their success, although Thorson (1950) identified predation as the most important source of mortality for planktonic invertebrate larvae in general.

The survival of juvenile hatchery-reared abalone in the wild is positively related to size in a number of species (Tegner and Butler 1985; McCormick et al. 1994; Roberts et al. 2007). This relationship appears to level out at a different size for different species, such that each has a unique optimal size for outplanting (McCormick et al. 1994). For example, survival increases with size to 50 mm in *H. discus hannai*, to 70 mm in *H. rufescens*, and to at least 25 mm in *H. iris* (Saito 1984; Tegner and Butler 1985; Roberts et al. 2007). The size of northern abalone that maximizes survival upon outplanting is currently unknown, although Griffiths and Gosselin (2008) found that the risk of predation decreases with increasing shell length, particularly at 12-13 mm shell length.

This study was designed primarily to assess whether survival of outplanted larval or juvenile abalone is influenced by outplanting density, and how these two life stages compare in terms of outplanting success. My initial hypothesis was that low densities of larval outplants would be advantageous in that they would reduce competition at the post-larval stage. Similarly, if juvenile abalone dispersal after outplanting is negligible and the densities in which they are outplanted persist, high density treatments are expected to be at a disadvantage through competition for refuges and food. The influence of body size of juvenile abalone outplants on post-outplanting survival will also be addressed. Each section of this study will be divided into two principal components to facilitate comparison between the two life stages. Larval outplanting, for which the overall goal is to assess the value of larval outplanting for increasing recruitment into wild northern abalone populations, will be addressed first, and will be followed by juvenile outplanting. The objectives for larval outplanting were to determine (1) whether larval outplanting increases densities of post-larval recruits, and (2) whether the density of larvae at outplanting influences recruitment success. The juvenile outplanting objectives were to determine if post-outplanting survivorship is influenced by: (1) outplanting density, or (2) abalone size.

METHODS

Larval Outplanting

The abundance of cobble and boulders with a high percentage cover of crustose coralline algae was used to select 12 locations (1 m^2) for outplanting within a single site in Barkley Sound, BC, Canada (Figure 8), based on the known preference of abalone larvae for settlement on such substrates (Moss and Tong 1992). The 12 locations were subsequently randomly assigned one of three treatment densities. The number of larvae to be used in this experiment was limited by what could be produced by the Bamfield Huu-ay-aht Community Abalone Project (BHCAP) hatchery in a single spawning event. Given this limitation, the three treatment densities consisted of: (1) 0 larvae/m^2, (2) 50,000 larvae/m^2, and (3) 100,000 larvae/m^2. To prevent the transportation of unsettled larvae away from outplanting locations by water movement, outplanted larvae were confined within tents. Eight nitex tents (Figure 9) were affixed to the substrate at the locations identified to receive treatments 2 and 3. The 200 μm mesh size of the larval tents was chosen to be slightly smaller than metamorphically-competent northern abalone larvae, which are 270 – 300 μm in diameter (Strathmann 1987; Page 1997*a*, 1997*b*; L. Page pers. comm.). Nitex tents were not placed at the control locations. Ideally I would have had two types of control where no abalone were outplanted, one with a nitex tent and one without, but this was not feasible given the cost and time involved in constructing the tents. All 1 m^2 treatment and control locations were permanently marked with Z-spar Splash Zone Epoxy ® and 4 cm diameter eyebolts attached to bedrock or boulders at the corners of the plots.
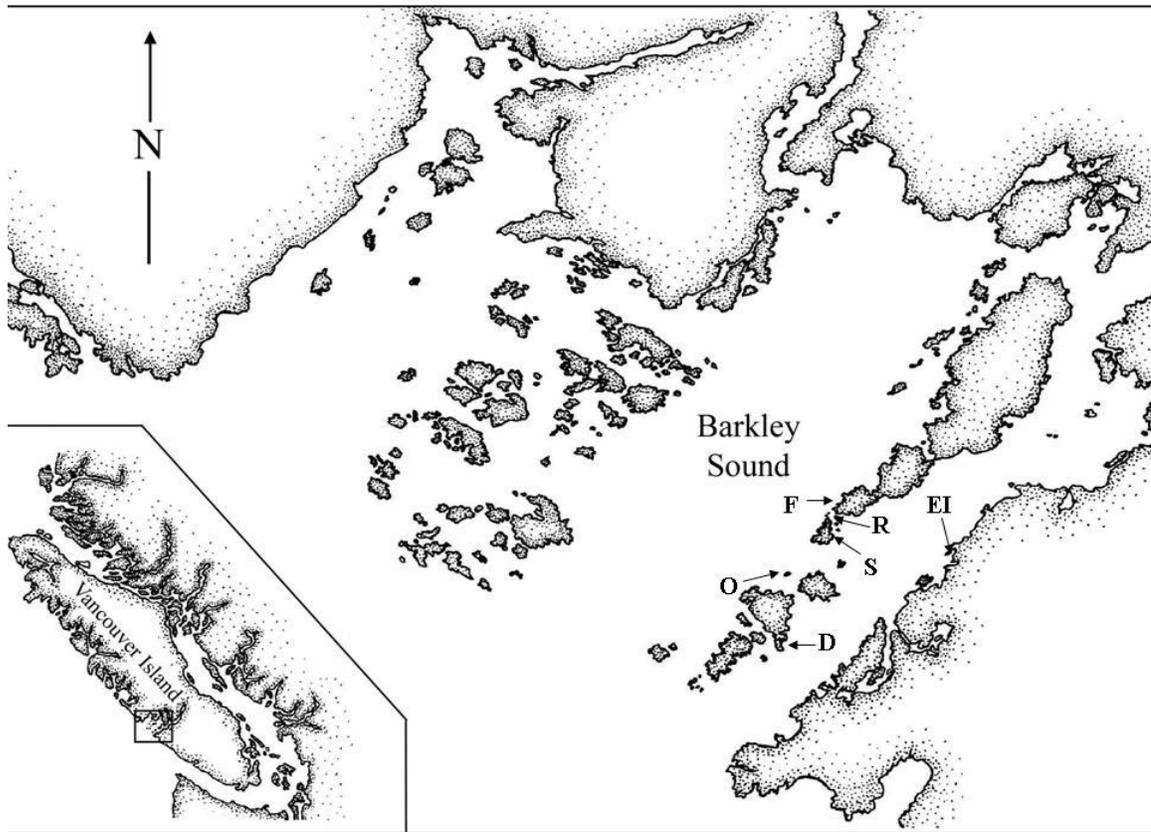
Figure 8. Map of larval and juvenile study sites in Barkley Sound, located on the west coast of Vancouver Island (inset). Outplanting sites are indicated by arrows, where S (Sanford Island) served as a larval and juvenile outplanting site, and O (Ohiat), F (Fleming Island), R (Ross Islets) and D (Diana Island) were strictly juvenile outplanting sites. EI (Ellis Islet) is a site for which wild northern abalone growth parameters are available. Adapted from Gosselin and Chia (1995).
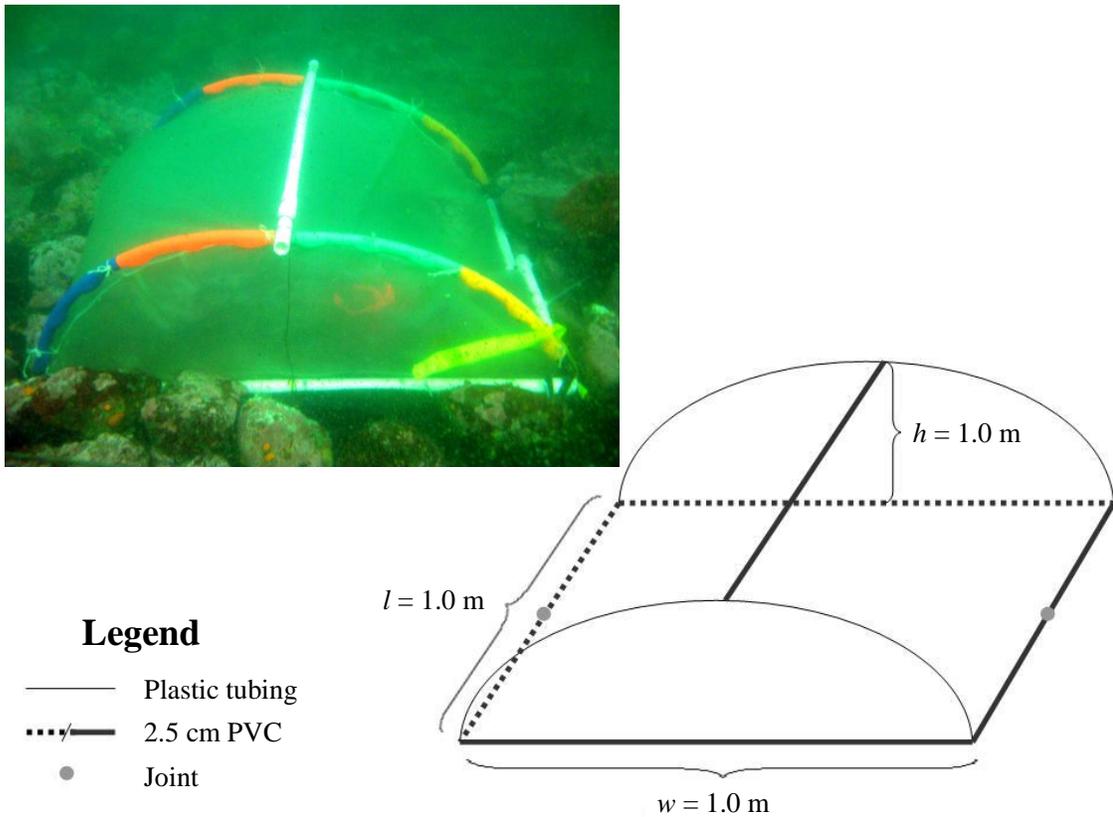
Figure 9. Nitex tent for larval abalone outplanting. The coloured inset shows a nitex tent deployed in the field. The frame consists of 2.5 cm PVC and plastic tubing. The 200 µm nitex is sewn into the same shape and has the same dimensions as the frame, with seams three times reinforced (sewn, serged, folded over and sewn again). Strings that were sewn into the seams are used to tie the nitex to the plastic tubing, while the bottom square of the nitex is hot glued in place on the PVC.

The metamorphic competence of abalone larvae increases with larval age until the end of larval life expectancy (Moss and Tong 1992; Roberts 2001). For example, *Haliotis iris* is competent at 9 days, but was held for 13 days by Tong et al. (1987) prior to outplanting, to ensure larvae would settle readily. Cultures of 10-day old competent larvae were concentrated to densities of 1000 larvae/mL (by removing water through a nitex screen) and were exposed to gamma-amino-n-butyric acid (GABA) for 30 minutes immediately prior to outplanting, to stimulate settlement and metamorphosis (Morse et al.

1979; Richards 2010). At the field site, approximately 10 minutes before outplanting, the GABA-treated larval abalone were sucked into 50 mL syringes for a density of 50,000 abalone per syringe. The syringes were then slowly emptied into the appropriate nitex tents, 1 syringe for the 50,000 treatment and 2 syringes for the 100,000 treatment. Outplanting of larvae occurred on June 10th, 2009.

      The nitex tents were removed 48 hours following the outplanting event, by which time all competent larvae were expected to have settled; larvae in the hatchery that were generated in the same spawning event had all settled by this time. Immediately prior to the removal of nitex tents, 1 L samples of the seawater in the tents and at the four control locations were taken with a suction sampler (Figure 10), and returned to the laboratory. Nitex tents were removed and returned to the surface, where the inner surfaces were rinsed and the contents concentrated into 50 mL tubes. Both the suction and tent samples were briefly rinsed with freshwater, and preserved in 4 % formalin for 12 hours. The samples were then rinsed with and stored in 70 % ethanol. These samples were later examined in the laboratory using a dissecting scope to assess the numbers of unsettled larvae and whether any larvae had settled on the nitex tents. This was done to confirm our assumption that the majority of abalone had settled out of the water column after 48 hours and to ensure that abalone were settling on the substrate and not on the tents themselves.
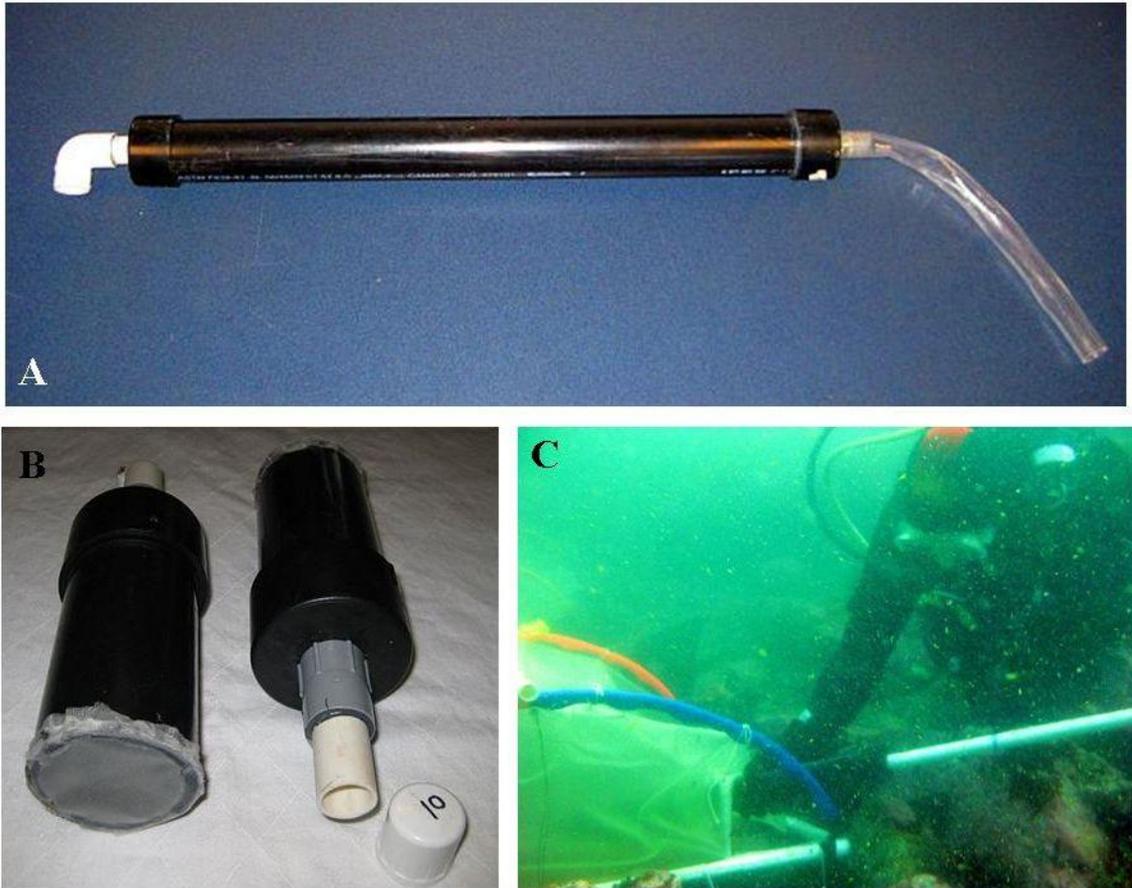
Figure 10. Suction sampler (A) and collection tubes (B). A diver is collecting a sample of the water inside a larval tent using the suction sampler in (C). We constructed this modified version of the Yabbie Pump (Limestone Media, Austin, TX), using: 5 cm diameter PVC and 2 caps, 2 cm diameter PVC, cap and elbow, a 4 cm diameter washer and 5 cm PVC test plug. Collection tube design was based on Fisher (2007), with 200 µm nitex used at the base of each tube.

On July 7th-8th, 2010, 13 months following the outplanting of larvae, we surveyed the $1m^2$ permanent plots on which larvae had been outplanted. All abalone observed were counted and measured with vernier calipers. A cohort analysis was used to determine the proportions of juveniles originating from hatchery outplanting as opposed to natural

reproduction. For a description of the assumptions and methods behind cohort analyses, see Chapter 2. The substrate preferences of new recruits are analyzed in Appendix A.

The effect of initial larval density treatment on the density of abalone recruits after 13 months was assessed via a one-way factorial ANOVA. Tukey's HSD test was conducted as a post-hoc test to identify where the differences in group means existed (Quinn and Keough 2002). Only abalone measuring less than 3.2 cm shell length were included in the analyses (ANOVA and Tukey's HSD test), as this was thought to represent the maximum shell size that could be achieved by our outplanted larval abalone by the time of the surveys. This size was chosen based on expected average growth in the first 13 months (1.8 ± 1.4 cm) as determined by a von Bertalanffy growth equation. The parameter estimates for the von Bertalanffy growth equation were derived by Breen (1986) based on wild abalone from Ellis Islet, a site relatively near our larval outplanting site (Figure 8).

As I was primarily interested in whether outplanting could raise densities of reproductive adults, the densities of new recruits were extrapolated to adult densities using survival rates published in the literature. Notably, an annual survival rate estimate for 1-3 year old abalone is 86.1 % (Olsen 1984 cited in Sloan and Breen 1988), while that for 3-5 year olds is 81.9 % (Breen 1980; Fournier and Breen 1983; Breen 1986; Sloan and Breen 1988).

Juvenile Outplanting

All abalone to be used in this study were given a unique tag so that the survival of hatchery reared individuals could be monitored after their release into the wild. Tagging involved drying the abalone shell with paper towels and measuring the abalone to the nearest millimeter using vernier calipers, molding a small dot of Z-Spar Splash Zone Epoxy ® onto the shell near the spire, and pressing a numbered bee tag into the epoxy (Figure 11). Recorded information for each abalone included the colour and number of the tag and the animal's shell size, which was determined by measuring the shell length from the posterior end at the level of the spire along the longest axis. Abalone shell

colouration and shell damage were also recorded, and are analysed in Appendix B. A total of 1875 tagged abalone ranging in size from 2.3 to 4.8 cm were used in this study. Tagged abalone were replaced into flow-through hatchery tanks with water flow and aeration at maximum strength so that any chemicals leaching from the z-spar would be diluted. The following week, tagged abalone were packed into 15-20 cm diameter PVC tubes containing fresh *Nereocystis leutkeana* blades. Vexxar mesh was used to cover the ends of the PVC tubes to prevent abalone from escaping these 'outplanting modules'.

Figure 11. Methods for individually tagging hatchery-raised abalone. A three-person production line (A) dries the abalone shell, (B) applies z-spar just above the spire, presses a coloured bee tag into the z-spar, measures shell length, shown in (C) and records all information.

The effect of juvenile outplanting density on survival was determined using five density treatments: (1) no abalone, (2) 25 abalone, (3) 50 abalone, (4) 100 abalone, and (5) 200 abalone outplanted in a 1m^2 area. The number of abalone per tube never exceeded 75. This was done to eliminate possible density effects prior to outplanting, since Schiel (1993) has demonstrated density-dependent mortality in juvenile abalone during transportation. Tubes packed with abalone were returned to hatchery tanks for 24 hours to allow the abalone to properly attach themselves to the PVC and to recover from handling prior to outplanting. Then one tube was used for each of treatments 1-3, 2 tubes for treatment 4, and 3 tubes for treatment 5.

Previous work by Lessard and Campbell (2007) examined optimal northern abalone habitat and identified site features which were indicators of good habitat. I worked in conjunction with Joanne Lessard of the Department of Fisheries and Oceans to select five sites with appropriate habitat for this study. Criteria for appropriate habitat included a combination of cobble and boulder habitat, presence of crustose coralline and articulated coralline algae, nearby *N. leutkeana* or *Macrocystis pyrifera* beds, presence of wild abalone, and at least a 75 m stretch of such habitat within 0 to 10 m depth. Five outplanting locations, each separated by 15 m, were identified at each site and were randomly assigned one of the five treatments (i.e. to receive 0, 25, 50, 100 or 200 juvenile abalone), such that each site had a full complement of all five density treatments. Thus we established a randomized block design with site as the blocking factor. We affixed 2.5 cm diameter eye-bolts to the substrate to mark the centre of each treatment location. The surface of the rock was scraped clean at the sites to be marked, and the eye-bolts were attached using Z-Spar Splash Zone Epoxy ®.

The PVC tubes containing tagged abalone were then outplanted at the five sites (Figure 8) on the 7th, 11th, 14th and 16th of August 2008. At each treatment location, the vexxar mesh ends were removed from the PVC tubes, which were then anchored in place immediately next to the eyebolt by stacking rocks on top of them. This allowed the abalone to leave the PVC in favour of surrounding natural habitat.

One day and three days following outplanting, circular swath surveys (Figure 12) centered on the outplant locations were conducted to locate and identify surviving hatchery abalone. The PVC outplanting modules were also checked for any hatchery-raised abalone remaining inside. In addition, a second circular swath survey, this time of 3 m radius, was conducted at each location 14 days after outplanting. This second survey consisted of overturning every rock and searching all crevices within the designated area so that both emergent and cryptic abalone would be observed. To determine whether any outplanted abalone were still alive and present near the outplant location after one year, an additional 5 m radius cryptic survey was conducted at each outplant location between July 20th and 30th, 2009.
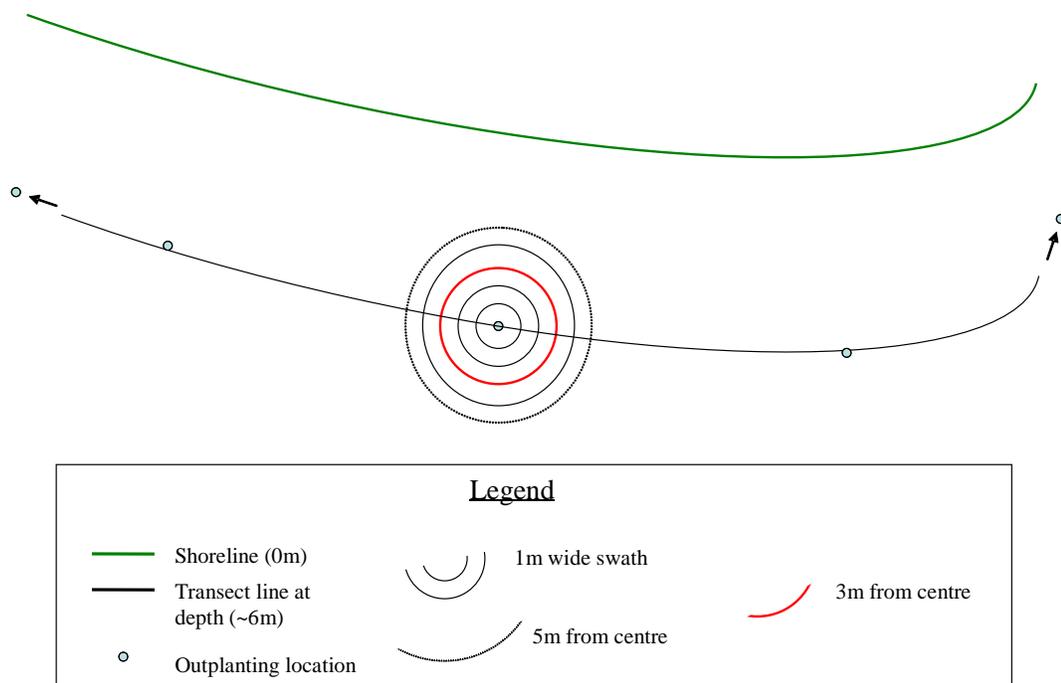
Figure 12. Circular swath surveys. Only one site is shown, and only one circular swath survey is depicted at that site. Pre-outplanting, 1-day and 3-day post-outplanting surveys were conducted to a 5 m distance, whereas the 14-day post-outplanting surveys were conducted to a 3 m distance. Each concentric ring represents a 1 m swath searched by divers.

A Jolly-Seber analysis was performed on the recovery data for abalone within a 3 m radius of outplanting sites (Krebs 1999). Abalone found at a distance of 3 to 5 m from the outplanting locations were used to estimate dispersal. The Jolly-Seber method provides estimates of apparent survival (ϕ; alive and stayed) and recapture probability (p), but does not separate apparent survival into true survival (S; alive but stayed or left) and site fidelity (F; probability of remaining in the area). In other words, the Jolly-Seber method does not distinguish between abalone which are dead and those which have left the sampling area. This method therefore provided minimum estimates of survival. The Jolly-Seber method requires a minimum of three different surveys, on different dates to estimate survival over the first interval.

The Barker model, which incorporates live-recapture, live-resight and recovery of dead individuals, was used in program MARK 6.0 to estimate maximum survival (S) for all three time intervals. The best model, as determined by Akaike's Information Criterion (AIC), was used to derive parameter estimates except when the change in AIC score between models was less than 2 (Burnham and Anderson 2004). In the latter case, all models with ΔAIC of less than 2 were used to generate model averaged parameter estimates (Burnham and Anderson 2004). Finally, it is important to note that we had to employ restricted models (with all parameters not of direct interest set to 0.5), in order to obtain acceptable bootstrap Goodness of Fit test results (S. Bonner pers. comm.). Only parameters S, p and F are of interest from the Barker models and will be presented in this study; these parameters and model shorthand are described in Table 3. Although only S is directly relevant to the determination of outplant survivorship, p and F can provide insight into the behaviour of abalone after outplanting, as well as the success of our survey methods. Site was included as a grouping factor in all general models. Because of adverse weather conditions, the three surveys were not completed at all sites; only those sites with complete data (Diana Island, Ross Islets and Sanford Island) were used with Barker models. Finally, shell recoveries are reported.

Table 3. Barker model parameter descriptions and shorthand. Only survival (S), recapture (p), and site fidelity (F) are presented in this paper. The subscripts can be used in conjunction with any parameter to describe whether it was found to be site or time dependent.

Shorthand	Parameter	Description
S_i	Survival	The probability that an abalone alive at i is still alive at $i+1$
p_i	Recapture	The probability that a live abalone at risk of capture at i is actually captured at i
F_i	Site fidelity	The probability that a live abalone at risk of capture at i is still at risk of capture at $i+1$
Subscript (t)		Any parameter with this subscript is time-dependent
Subscript (g)		Any parameter with this subscript is site-dependent
Subscript (.)		Any parameter with this subscript is neither time nor site-dependent.

The Jolly-Seber method estimates mortality based on abalone that are never seen again after any given date and tempers this estimate with information on abalone that are not observed on certain occasions, but are seen later. The Barker model further takes into account observations of deceased abalone. Since the recovery of shells is expected to represent only a fraction of all deceased abalone (Schiel and Welden 1987), and the Barker method attempts to estimate a large number of parameters using only a few unique encounter histories, I considered the Jolly-Seber estimates of survival to be more accurate. Emmett and Jamieson (1988) set a precedent of using two methods to provide a range of estimates within which true survival is expected to occur. I present results from both methods, with the caveat that when Jolly-Seber estimates of mortality are available, they are more reliable than the Barker estimates.

The extent of abalone dispersal after outplanting was assessed by examining the mean percent of abalone resighted at different distances from outplanting locations over time. The percent of abalone resighted, $\frac{\#\,resighted}{\#\,outplanted}*100$, was transformed as follows to achieve homogeneous variances: $\frac{(Percent\ resighted - mean)^2}{mean}$, where mean is the mean percent resighted across treatments. A two-way repeated measures ANOVA was

used for the dispersal analysis, with the transformed percent resighted as the response variable, and time and distance from outplanting location as the predictor variables. The unit of replication was site. Neither site, outplanting density treatment, nor any associated interaction terms, were significant and these factors and interactions were accordingly removed from the ANOVA model, thus n = 25 (5 sites x 5 treatments).

Recoveries of outplanted abalone one-year following outplanting were used to calculate the mean growth of outplanted hatchery-raised abalone. The densities of abalone recovered after one year were calculated and extrapolated by two and four years, using the afore-mentioned published survival rates (see larval outplanting methods), to determine the increases in density of reproductive abalone occasioned by outplanting.

A two-way repeated measures ANOVA with abalone survivorship as the response variable and time and treatment as the predictor variables was used to assess the relative merit of outplanting different densities of juveniles. Site was the unit of replication (5 sites). Post hoc Tukey's HSD t-tests were performed to identify between which treatments differences in abalone survival existed.

Finally, estimates of individual survival (whether an abalone was seen alive on a particular survey or anytime thereafter) were used to assess whether abalone size at outplanting influenced survival to each of the three time periods via logistic regressions. In these regressions, the binary response variable was survival, while the predictor variable was site (categorical). The unit of replication was site and each site had 375 outplanted abalone. In addition, a one-way factorial ANOVA was used to determine whether the deceased abalone that were recovered differed in size by survey date, and Tukey's HSD tests were conducted as a post-hoc test to identify among which dates the discrepancies existed.

RESULTS
Larval Outplanting

No larval or post-larval abalone were present in the suction samples from the control, 50,000 or 100,000 density treatments. A number of larval abalone were identified

in the samples collected from the inside surface of the tent screen, with more being found in samples from the 100,000 density treatment than the 50,000 density treatment (Table 4). The larval abalone identified within the tent samples were largely characterized by the veliger shell (Figure 13a), although one individual was found with the beginnings of a juvenile shell (Figure 13b).

Table 4. Larval abalone presence in nitex tent samples. Tents were present over the 50,000 and 100,000 larval outplanting density treatments only (not the control). The average given includes ± 1 standard error.

Replicate	50,000 Treatment	100,000 Treatment
1	0	3
2	1	3
3	0	4
4	3	3
Average	1.00 ± 0.71	3.25 ± 0.25
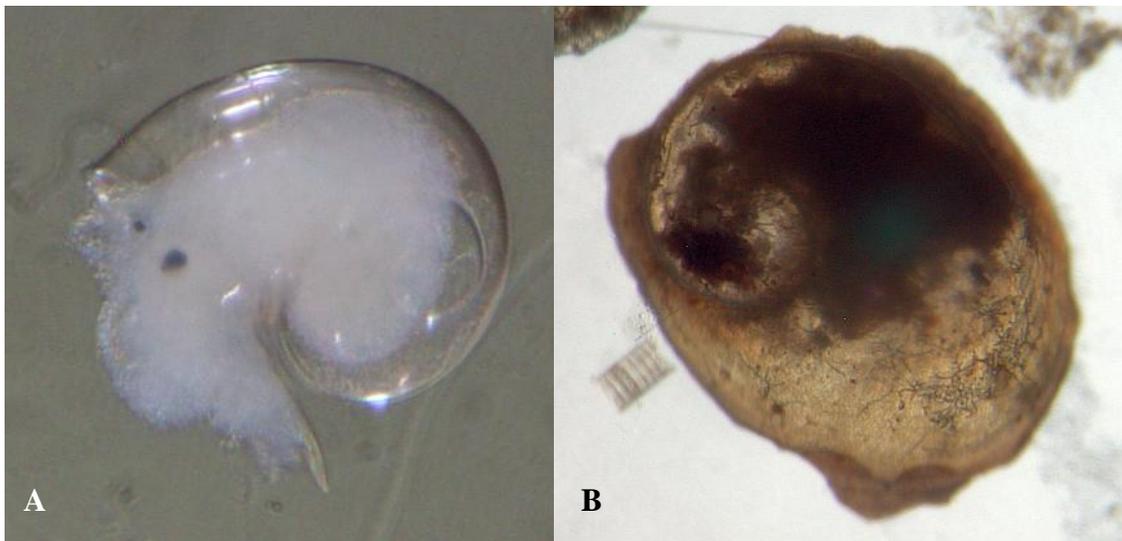
Figure 13. Examples of northern abalone identified in the tent samples. The individual in **A** still has a shell typical of the veliger stage, whereas that in **B** is beginning to take on the flattened juvenile morphology. The photographs were taken through a dissecting scope at magnifications of 180 x and 112 x, respectively.

The effect of larval outplanting treatments on the subsequent densities of recruits was marginally significant (ANOVA: $F_{2,11}=3.500$, $p=0.067$). Tukey's HSD test demonstrated that this resulted from greater densities of recruits in the 50,000 treatment than in the control (Tukey's HSD test: $p=0.066$). The increase in density within 50,000 plots represents a 260 % increase in the number of new recruits relative to control plots (Figure 14).

Taking into account the densities of recruits 13 months after outplanting, published mortality rates, growth rates and information on size at maturity, each 50,000 treatment application is expected to raise densities of reproductive adults by 0.487 individuals/m^2 after 3 years. After 5 years all outplanted larvae are expected to have reached sexual maturity, thus the density of reproductive individuals is expected to increase by 0.721 individuals/m^2 relative to the control.
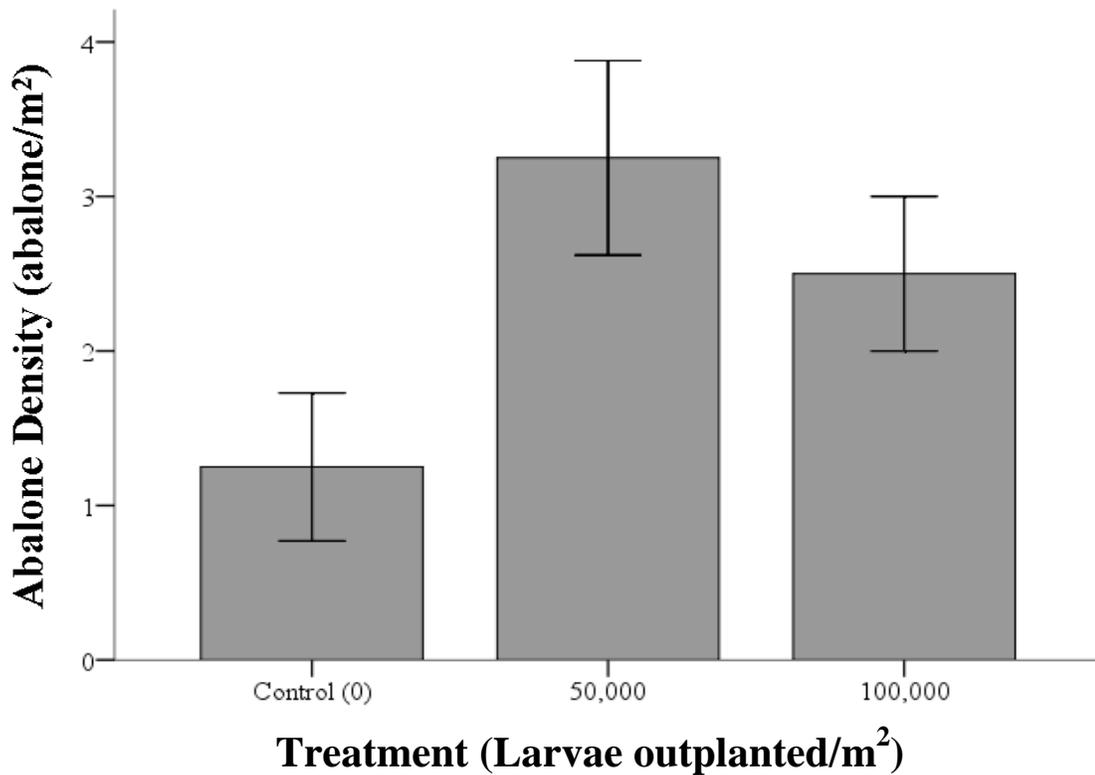
Figure 14. Mean densities (number of abalone/m^2) of new recruits (<3.2 cm shell length) in the three larval outplanting treatments (0, 50,000, and 100,000 larval abalone outplanted/m^2) 13 months after outplanting. Error bars are ± 1 SE (n=4).

The cohort analyses confirmed the existence of a cohort of new recruits that was present across all treatments (Figure 15). This cohort had mean sizes of 1.651 ± 0.077 cm, 1.678 ± 0.307 cm, and 1.578 ± 0.354 cm, in the control, 50,000, and 100,000 larvae/m^2 treatments, respectively. The relative prominence of this cohort was greatest in the 50,000 and 100,000 treatments ($\pi = 0.614 \pm 0.134$, $\pi = 0.630 \pm 0.172$, respectively) and considerably lower in the control treatment ($\pi = 0.152 \pm 0.082$). Similarly, of the 27 new recruits identified during surveys, 55.56 % were found in the 50,000 treatment plots, 33.33 % in the 100,000 treatment plots and only 11.11 % in the control plots. Thus all results suggest that the 50,000 treatment density had the greatest positive influence on densities of new recruits. Interestingly, the cohort analyses also identified a group of new recruits with mean shell length 0.575 ± 0.082 cm within the 50,000 treatment plots, that was absent in all others and may be a false cohort, given that this group of small individuals was only identified in the 50,000 treatment plots which were randomly interdespersed among all other plots. Thus this 'cohort' likely consists of slow growing individuals belonging to the larger outplanted cohort.
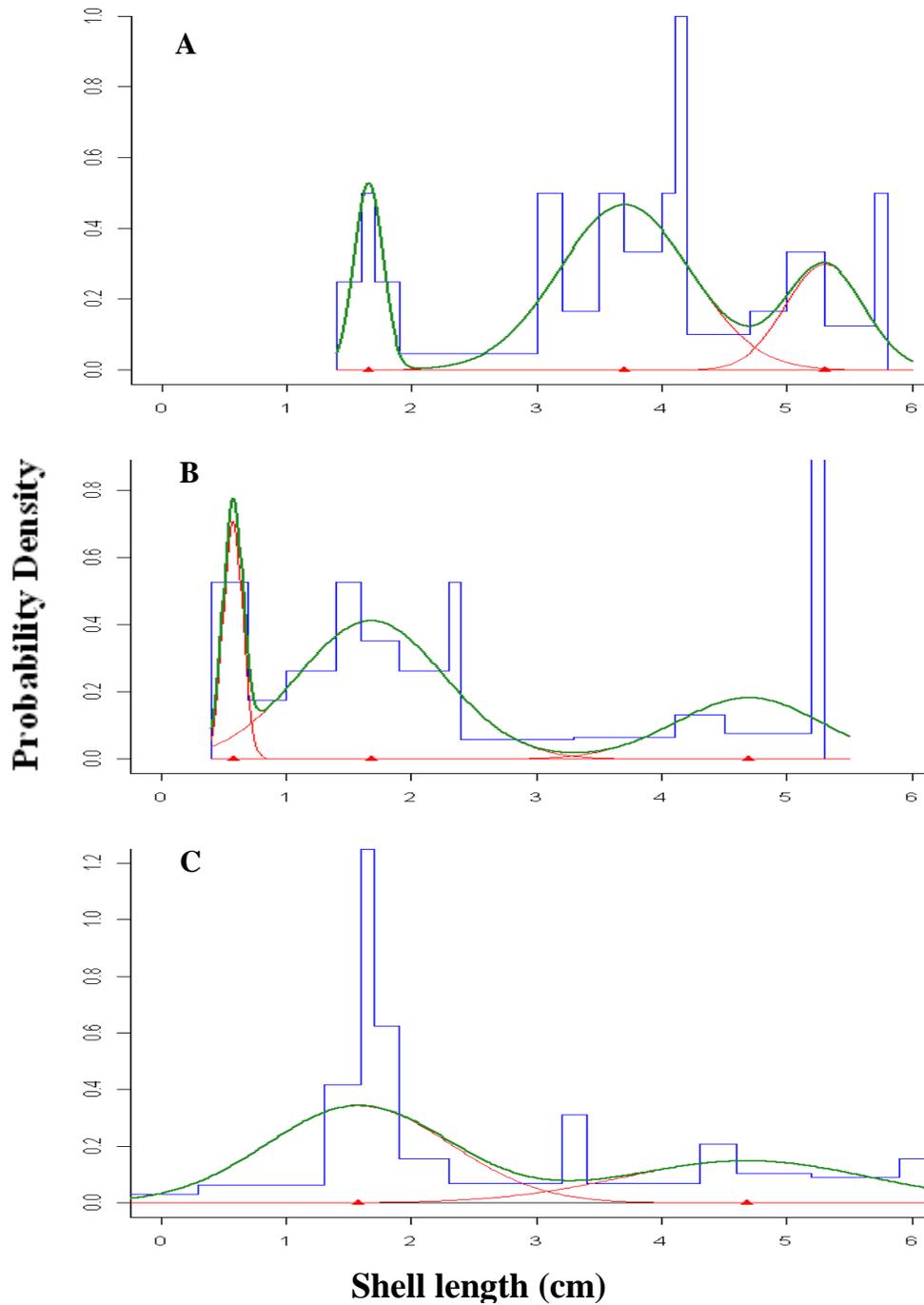
Figure 15. Cohort analyses of abalone found in the (a) control, (b) 50,000 larvae/m^2, and (c) 100,000 larvae/m^2 treatment permanent plots. The blue line is the actual frequency distribution, the green line shows all modeled cohorts while the red lines show individual cohorts and red triangles indicate the mean shell lengths of each cohort.

Juvenile Outplanting
<u>Survival and density effects of outplanted juveniles</u>

Survivorship of outplanted juvenile abalone, irrespective of density, site or any individual characteristics, was highly dependent upon the method of determination. The Jolly-Seber analysis revealed that only 36.45 % of the outplanted abalone were still alive and present within a 3m radius of the outplanting locations after 24 hours. The Jolly-Seber method uses sightings of previously unaccounted for individuals to establish 95% confidence intervals, calculated in this case to be 33.04 % to 39.77 %. The narrow range of our confidence intervals indicates that our surveys had high recapture probability (Krebs 1999). Numbers of abalone found at a distance of 3 to 5 m from the outplant locations demonstrate that only 0.03% of all tagged abalone dispersed more than 3 m within 24 hours (Figure 16). This low dispersal rate, in combination with the high recapture probability of our survey methods provides support for the use of abalone presence as a measure of minimum survival in this experiment. Mean survival of outplanted juvenile abalone after two weeks, as determined by resightings, was 7.93 ± 0.02 %. The Barker model – which incorporates live recovery, resighting, and dead recovery information – yields a higher estimate of mean survival after 24 hours (46.13 ± 4.12) and after two weeks (37.81 ± 1.70 %). The Barker method also found a moderate recapture probability (55.46 ± 17.08 %) over the two week period. Recall that the discrepancy in survival estimates arises from the way in which they are calculated, and that the lower Jolly-Seber estimates are expected to be more accurate.

A total of 92 dead abalone shells (only 5 % of all outplants) were recovered at all five sites over the two weeks that these sites were surveyed. Of these, 49 were recovered one day following outplanting, 38 were recovered three days after outplanting and 30 were recovered after 14 days at large. All collected shells were found free of flesh and approximately 35 % were intact, the remained being broken.

Abalone moved away from the outplant sites over time, as is evidenced by a significant interaction between time and distance with the transformed percent of abalone

recovered at different distances from the outplanting locations as a response variable

(ANOVA: $F_{1,24}$=7.659, p=0.011; figure 16).
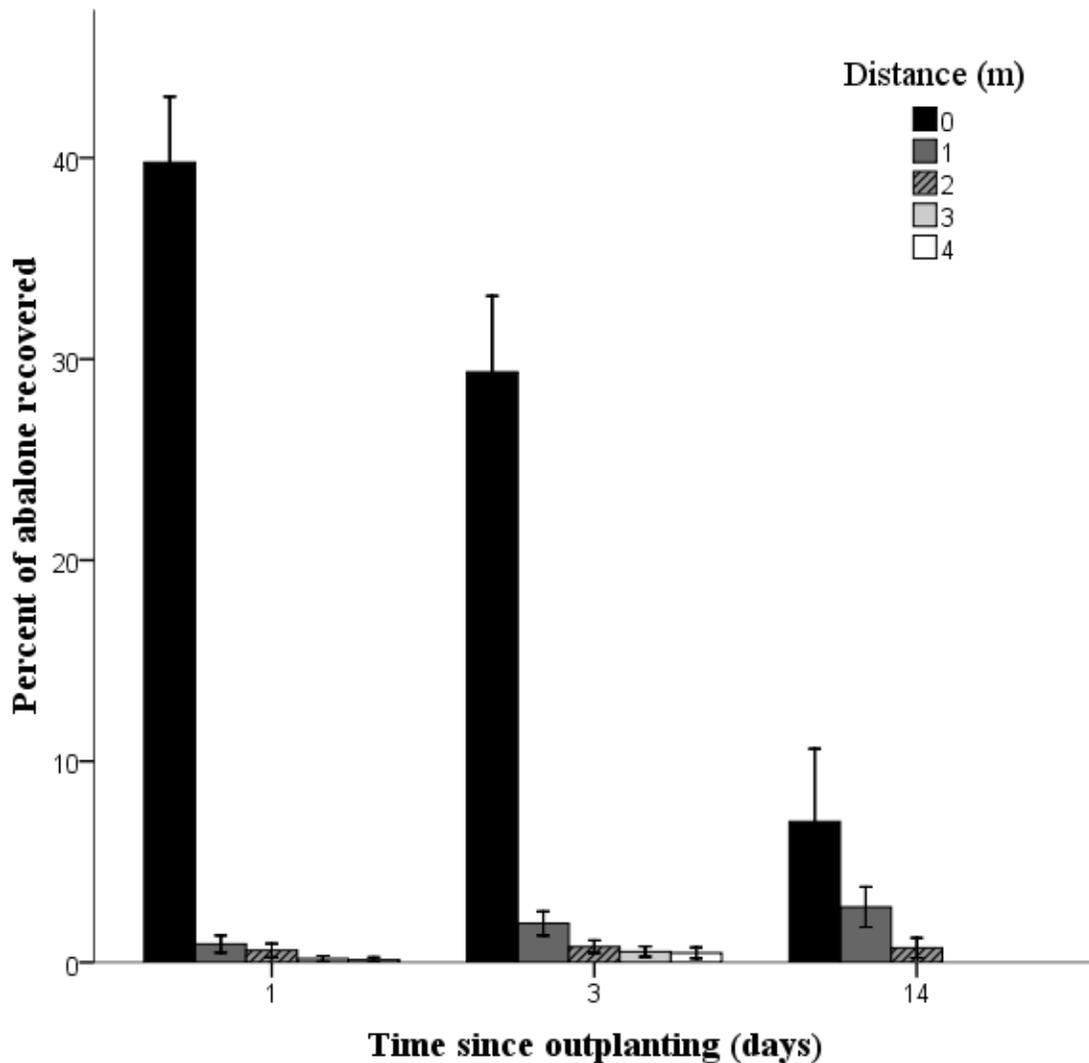
Figure 16. Mean percent of juvenile abalone recovered at different distances from the outplanting locations, as an indication of dispersal. Percent of abalone recovered is calculated as the number recovered / the number outplanted * 100. Error bars represent ± 1 standard error (n=25). The survey on day 14 only covered the first three distance categories.

During surveys conducted in 2009, approximately 12 months after outplanting, only 13 of the original 1875 outplanted abalone were found (Table 5). Some abalone that had lost their tags were still identifiable as originating from the hatchery due to distinct changes in shell colour and in some cases scarring where the tag had been attached to the shell (Figure 17). For those abalone that had retained their tags, mean growth over the year was 1.63 cm ± 0.15, which corresponds to a 146 ± 5 % increase in shell length. This growth exceeds expectations. Indeed, according to the von Bertalanffy growth equation with parameters $L_\infty = 122.6$ and $k = 0.158$ (Breen 1986), it would take wild abalone of equivalent size approximately 16 months to achieve this level of growth.

Table 5. Recovery of hatchery-raised abalone after one year in the wild. When recovered abalone could be individually identified, their exact growth was calculated. When recovered abalone were not individually identifiable, their growth was calculated from the size of the largest abalone outplanted (minimum growth) and from the mean size of outplanted abalone (growth from mean). The acronym NL (given in abalone ID) indicates that one or both digits on the tag were not legible.

Abalone ID	Site	Treatment density	2008 size (cm)	2009 size (cm)	Growth	Minimum growth (cm)	Growth from mean (cm)
Y88	D	100	3.75	5.42	1.67	–	–
no tag	D	unknown	–	5.51	–	1.07	2.08
Y7	F	100	3.23	5.08	1.85	–	–
no tag	F	unknown	–	5.61	–	0.79	2.04
no tag	F	unknown	–	5.76	–	0.94	2.19
no tag	F	unknown	–	6.03	–	1.21	2.46
W1NL	F	unknown	–	4.94	–	0.12	1.37
R44	R	100	4.15	5.23	1.08	–	–
RNL	R	100	–	5.49	–	1.05	2.09
Y30	S	200	3.79	5.74	1.95	–	–
Y34	S	200	3.33	4.95	1.62	–	–
no tag	S	unknown	–	5.45	–	0.93	1.96
no tag	S	unknown	–	4.92	–	0.4	1.43
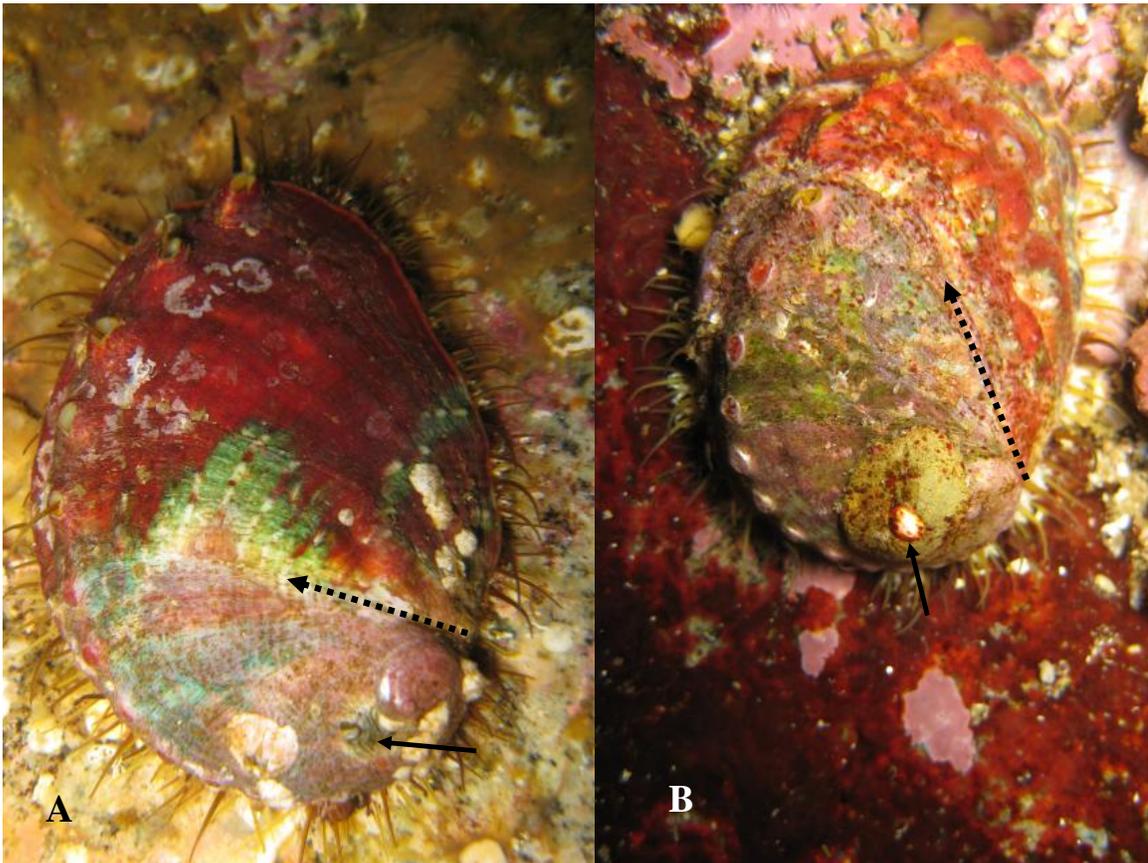
Figure 17. Two different hatchery abalone (A and B) resighted in the wild one year after release. Dashed arrows indicate the point at which the old "hatchery shell" ends and new growth, once in the wild, commenced. Solid arrows indicate the mark where the tag was lost (in A) and the present but abraded bee tag (in B).

Abalone that could be identified as coming from the 100 density treatment corresponded to an increase in density of 0.010 ± 0.005 abalone/m^2 one year after outplanting (Figure 18). The different treatment densities have different projected impacts on abalone densities, with the greatest increase in abalone densities being associated with an outplanting density of 100 juveniles. Applications of 100 juveniles are expected to increase densities of abalone at outplanting sites by 0.009 ± 0.004

individuals/m^2 two years after outplanting and 0.006 ± 0.003 individuals/m^2 four years after outplanting. Given that the smallest outplanted juvenile is expected to reach 50 mm shell length after two years and 70 mm shell length after four years, the increases in reproductive densities after these time periods should be 0.005 ± 0.004 individuals/m^2 and 0.006 ± 0.003 individuals/m^2, respectively.
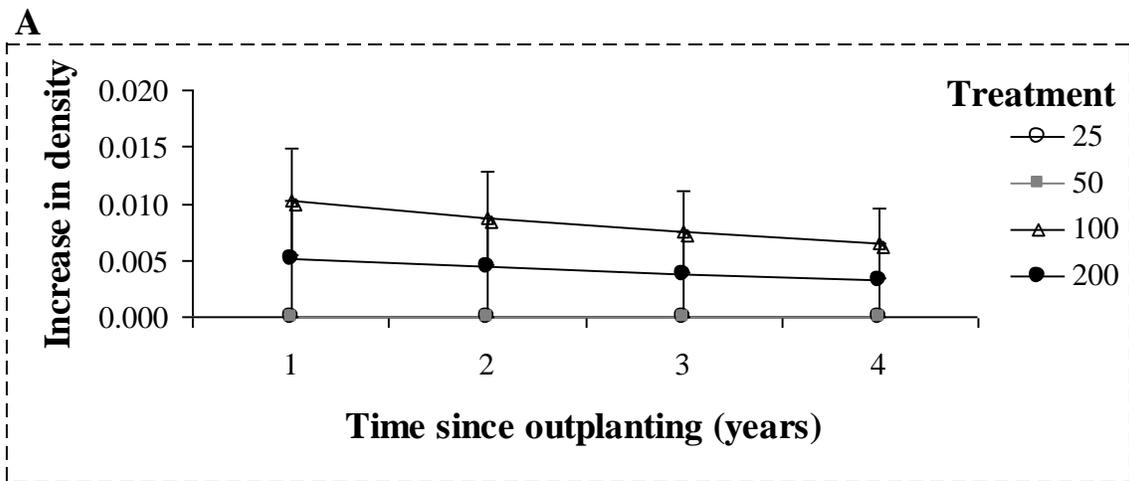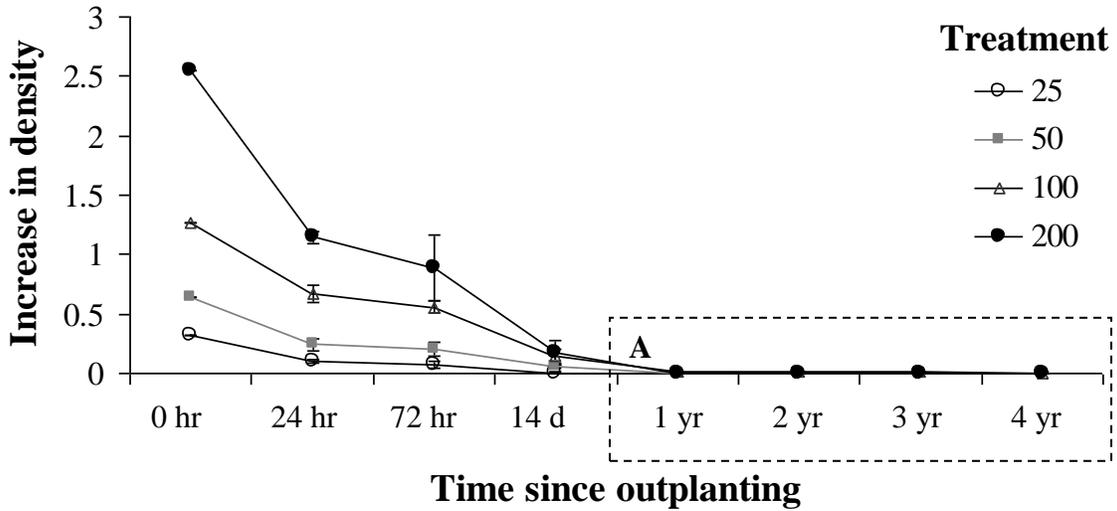
Figure 18. Projected increases in abalone density over time, produced by outplanting different densities of hatchery-raised juvenile abalone. The inset A is magnified. Error bars are $\pm$ 1 SE.

Survival estimates, based upon whether an abalone was seen on a survey or any survey thereafter, provided evidence of a difference in survivorship between treatments (ANOVA: $F_{3,12}=16.735$, $p<0.001$). This resulted from lower survival estimates in the 25 treatment density than the 100 treatment density (Tukey's HSD test: $p=0.006$; Figure 19).
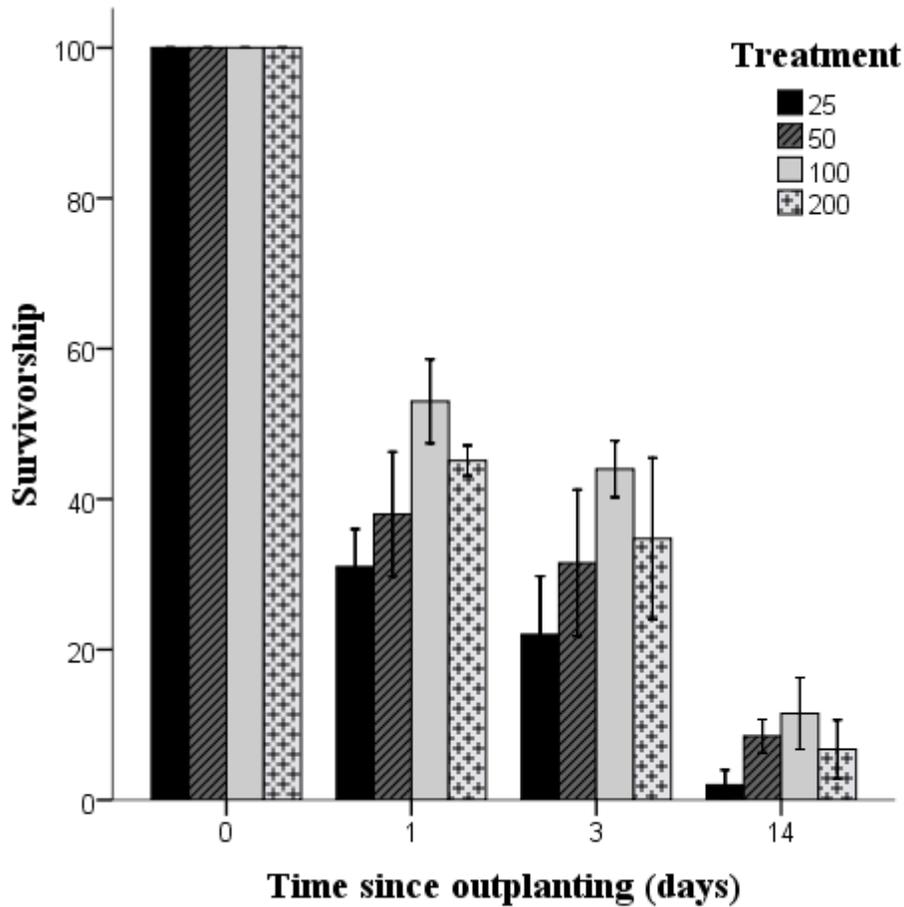
Figure 19. Mean survivorship of abalone outplanted at different treatment densities at outplanting and to the three survey times. Survivorship is the number seen on a particular survey or any survey thereafter divided by the number outplanted *100. Error bars represent ± 1 SE (n=5).

The best Barker model (smallest AIC value) describing survival of abalone outplanted in groups of 25 individuals was $\{S_{(t)} \, p_{(g)} \, r_{(0.5)} \, R_{(0.5)} \, R'_{(0.5)} \, F_{(t)} \, F'_{(0.5)}\}$, which demonstrates time-dependence in survival and site fidelity, and site dependence in recapture probability. The best models for the 50 and 100 treatment densities were as follows: $\{S_{(g*t)} \, p_{(t)} \, r_{(0.5)} \, R_{(0.5)} \, R'_{(0.5)} \, F_{(t)} \, F'_{(0.5)}\}$ and $\{S_{(g*t)} \, p_{(.)} \, r_{(0.5)} \, R_{(0.5)} \, R'_{(0.5)} \, F_{(g*t)} \, F'_{(0.5)}\}$. The 200 treatment density generated two models with low AIC scores that differed by less than two AIC values. Both models were accordingly used to generate model-averaged parameter estimates (Table 6). Estimates from the Barker models indicate highest survivorship of abalone in the 100 treatment density, followed by the 200, 50, and 25 treatment densities, in that order. Estimates of mean cumulative survival over the fourteen days in these treatments are: 41.5 ± 7.1 %, 39.8 ± 3.0 %, 29.8 ± 10.8 % and 22.6 ± 2.3 %, respectively. Recapture probabilities of abalone were consistently higher in the 100 treatment density. In general, site fidelity was moderate in the interval immediately following outplanting, highest between day 1 and day 3 surveys and lowest between the day 3 and day 14 surveys. There were no apparent trends in site fidelity between treatments. Correspondingly, the standardized percent of abalone recovered at different distances from the outplanting locations over time did not differ significantly among treatment densities (ANOVA: $F_{3,24}=1.325$, $p=0.289$; recall that this parameter was removed from the ANOVA model, see page 54).

Table 6. The three best models, according to AICc values, describing encounter histories for juvenile abalone outplanted in groups of 25, 50, 100 and 200 individuals. AICc is the corrected Akaike Information Criterion, Δ AICc is the change in AICc between models, AICc W gives the relative weights of the models (used to calculate model averaged parameters), and No. Par. is the number of parameters estimated by the model. Parameters and shorthand are described in table 3.

25 Treatment

Model	AICc	Δ AICc	AICc W	No. Par.
{S(t) p(g) r(0.5) R(0.5) R'(0.5) F(t) F'(0.5)}	426.441	0.000	0.951	10
{S(t) p(g*t) r(0.5) R(0.5) R'(0.5) F(t) F'(0.5)}	433.850	7.409	0.025	15
{S(g*t) p(g) r(0.5) R(0.5) R'(0.5) F(t) F'(0.5)}	436.227	9.786	0.008	17

50 Treatment

Model	AICc	Δ AICc	AICc W	No. Par.
{S(g*t) p(t) r(0.5) R(0.5) R'(0.5) F(t) F'(0.5)}	824.816	0.000	0.799	18
{S(g*t) p(.) r(0.5) R(0.5) R'(0.5) F(t) F'(0.5)}	829.151	4.336	0.091	16
{S(g*t) p(g) r(0.5) R(0.5) R'(0.5) F(t) F'(0.5)}	829.159	4.343	0.091	18

100 Treatment

Model	AICc	Δ AICc	AICc W	No. Par.
{S(g*t) p(.) r(0.5) R(0.5) R'(0.5) F(g*t) F'(0.5)}	1811.225	0.000	0.599	22
{S(g*t) p(t) r(0.5) R(0.5) R'(0.5) F(g*t) F'(0.5)}	1813.369	2.144	0.205	24
{S(g*t) p(g) r(0.5) R(0.5) R'(0.5) F(g*t) F'(0.5)}	1814.045	2.821	0.146	24

200 Treatment

Model	AICc	Δ AICc	AICc W	No. Par.
{S(g*t) p(g*t) r(0.5) R(0.5) R'(0.5) F(t) F'(0.5)}	3375.401	0.000	0.583	24
{S(g*t) p(g*t) r(0.5) R(0.5) R'(0.5) F(g*t) F'(0.5)}	3376.076	0.675	0.416	29
{S(g*t) p(g*t) r(0.5) R(0.5) R'(0.5) F(.) F'(0.5)}	3389.420	14.019	0.001	22

Of the thirteen hatchery-reared abalone resighted in 2009, six were still individually identifiable (Table 5). All six of these abalone had been outplanted in the 100 or 200 treatment densities in 2008.

Influence of shell length on juvenile outplant survival

The likelihood of surviving one day past outplanting decreased with increasing size of abalone, as evidenced by a regression with survival as the binary response and size as the predictor (Logistic regression: $F_{1,374}=45.477$, $p<0.001$; Figure 20). Size had the same significant effect on survival to 3 days post-outplanting (Logistic regression: $F_{1,374}=10.203$, $p=0.002$). Size was not significant to survival after 14-days, likely because so few individuals were resighted alive at this time (Logistic regression: $F_{1,374}=0.223$, $p=0.637$). Thus smaller abalone initially have greater survivorship over the range of shell lengths tested.
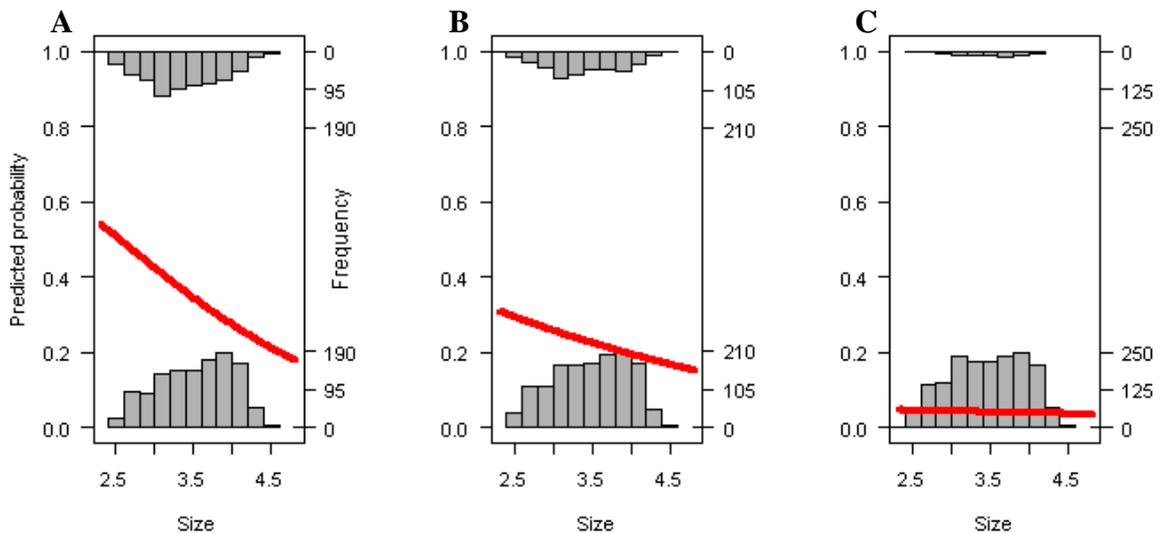
Figure 20. Logistic regressions showing the probability of surviving (a) one day, (b) three days, and (c) 14 days post-outplanting for different sizes of juvenile hatchery-raised northern abalone. The left y-axis, labeled predicted probability, indicates the probability of survival for a given size of juvenile abalone outplant. The right y-axis, labeled frequency, represents the number of abalone of each size that survived (top histogram) or died (bottom histogram). Abalone are considered to be deceased if they are never seen again. As there is missing data (surveys were not completed at Fleming on day 1 or day 14, nor at Ohiat on day 3), the sample size at each survey time (a, b and c) is 1500, not1875.

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When we consider the sizes of the recovered deceased abalone, it becomes apparent that the average size of individuals dying immediately after outplanting was larger than in the time after the three-day survey (Figure 21). In fact, the sizes of recovered dead abalone shells differed significantly by survey times (ANOVA: $F_{2,78}=3.501$, $p=0.035$), with larger abalone being found dead on the 3-day survey than on the 14-day survey (Tukey's HSD test: $p=0.038$).
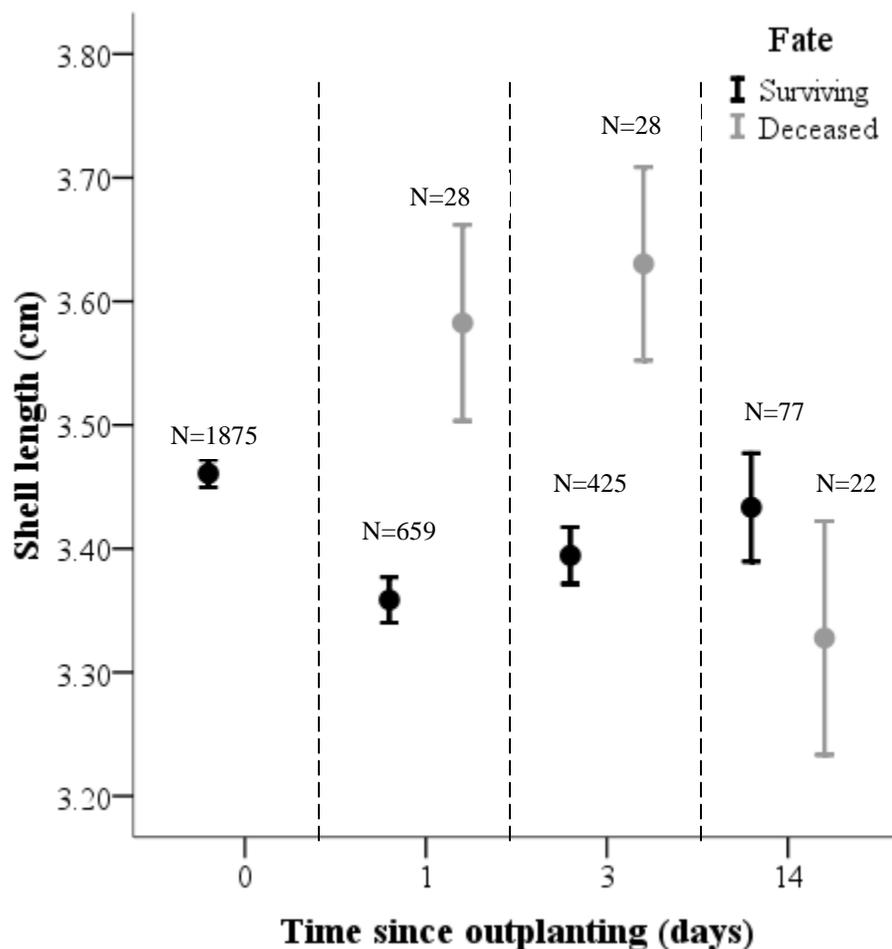
Figure 21. Mean sizes ± 1 SE of resighted abalone (surviving) and recovered abalone shells (deceased) over time. Sample sizes are given above the error bars.

DISCUSSION

The major findings of this chapter are: (1) that larval outplanting (of 50,000 individuals/m^2) can increase densities of new recruits; (2) that larval outplanting is more effective than juvenile outplanting; (3) that intermediate densities of juveniles (100/m^2) upon outplanting result in the highest increases in abalone densities; and (4) that large abalone are most vulnerable immediately after outplanting.

Larval Outplanting

Thirteen months after outplanting, densities of new recruits were higher at locations outplanted with 50,000 larvae than at control locations, demonstrating that larval outplanting can successfully bolster the densities of northern abalone recruits. This finding is further supported by the cohort analyses in which new recruits had greater prominence within outplanted treatments than within the control treatment. The findings in this study are consistent with density effects among new northern abalone recruits outplanted as larvae. The 100,000 treatment had intermediate densities of new recruits, albeit not significantly different from the other treatments, and is therefore consistent with negative density dependence at high densities. The lack of significance may be due to the small sample size (n=4) in this experiment. I accordingly recommend further experimentation to determine the influence of larval outplant density upon recruitment success. Given that the low density (50,000 larvae/m^2) treatment resulted in increased densities of new recruits, this is currently the method I recommend for future outplanting attempts.

Finally, extrapolation of our experimental results suggests that each application of 50,000 larval abalone will result in a localized increase of 0.487 reproductive abalone per m^2 after three years or 0.721 reproductive abalone per m^2 after 5 years, not considering spill-over into adjacent areas. There are two assumptions inherent to this extrapolation: (1) hatchery-raised abalone that remained within outplanted plots after one year will not disperse after that time, and (2) the survival of hatchery-raised abalone larvae outplanted in the wild will approximate that of wild-spawned individuals. The first assumption is unlikely to be met because abalone migrate from deep to shallow waters as they age

(Sloan and Breen 1988). As a result, further experimentation is required to compare actual increases in densities of reproductive adults produced by larval outplanting to these predictions. The second assumption is likely to be met by abalone released as larvae that have survived over a year in the wild.

The nitex tents designed for this study appeared to perform well. After 48 hours deployed at approximately 10 m depth at a wave exposed site, the tents were still in good condition. The fact that outplanting effects could be determined in the permanent plots one year after outplanting also indicates that the tents performed their intended function of confining larval settlement within chosen plots. In past larval outplanting, larval abalone were released from syringes into crevices (pers. comm. Richards), and could have been swept away from appropriate settlement substrates by strong currents or surge. In fact, the transport of larvae into unsuitable habitats is one of the major sources of larval mortality identified by Thorson (1950), and counteracting it through the use of larval tents can greatly influence outplanting success. An experiment in which larval abalone are outplanted within and outside of tents should be considered in a future study, as it would better address the suitability of tents.

One predicted disadvantage to nitex tents – that larval abalone would settle on them and be killed when the tents were removed – was not an important factor. When 50,000 abalone were outplanted into a tent, on average only one abalone settled on the tent, and only 3 abalone settled on the tents in each of the 100, 000 treatments. One recommended modification is that a smaller mesh size be placed on the base of the sample collection containers used in conjunction with the suction sampler. It is possible that larval abalone were forced through the 200 µm mesh at the high pressures generated by the suction sampler when expelling samples. For this reason, it is possible that some unsettled larval abalone were in fact present in the water within the tents 48 hours after outplanting but were not detected. Nevertheless, the number of unsettled outplanted larvae is likely to have been minimal, given that all larval abalone retained in the hatchery from the same spawning event had settled during the same time frame. BHCAP's method of outplanting larval abalone in syringes was adopted in this study for

its practicality and ease; however it would be wise to conduct a small-scale experiment examining whether the high concentrations and pressures experienced by larvae outplanted in this way are detrimental.

In summary, this study provides evidence that larval outplanting can successfully bolster cohorts of new recruits and accordingly raise their densities. This result is particularly encouraging because most larval seeding experiments fail due to high rates of larval mortality (Tong et al. 1987; McCormick et al. 1994; Preece et al. 1997; Schiel 1997; Dixon et al. 2006).

Juvenile Outplanting

<u>Survival and density effects of outplanted juveniles</u>

Survival of outplanted abalone is notoriously difficult to determine accurately due to the highly patchy nature of their aggregations and because they often occupy cryptic habitat which is inaccessible to survey divers (Breen 1992, Shepherd and Breen 1992, Schiel 1993, McCormick et al. 1994, Sweijd et al. 1998). Although determining mortality by collecting shells seems like a straightforward solution, it does not provide a good measure of mortality (Tegner and Butler 1985; Scott 1997, Sweijd et al. 1998, Dixon et al. 2006, Griffiths 2006, Griffiths and Gosselin 2008). Many crab predators crush the shell when consuming abalone (Tegner and Butler 1985; Emmet and Jamieson 1988; Griffiths 2006; Griffiths and Gosselin 2008). In fact, in a laboratory experiment examining the responses of red abalone to predators, Schiel and Welden (1987) discovered that only 68 to 88 % of shell material could be recovered from the tanks following crab trials, whereas all shell material was recovered in seastar trials. Although seastar, fish and octopus predators leave the shell relatively intact, they often remove them from the outplanting site (McCormick et al. 1994). Those shells that do remain intact and within the study area risk being swept away by swell and currents (Rogers-Bennett and Pearse 1998). It follows that recovery of shell material in an in situ experiment would be even lower than the 68-88 % reported in the laboratory. As a result, estimating survival from resighting events in mark-recapture experiments remains the

preferred method (McCormick et al. 1994). Nevertheless, as illustrated in this study, there is still considerable variation in survival estimates, resulting partially from the choice of mark-recapture model. Notably, the Jolly-Seber method indicated that mortality of outplants was extremely high, with estimates of 64 % in 24 hours. When resightings are taken as a proxy for survival, mortality is estimated at 92 % after two weeks. In contrast, the Barker method indicated that mortality was 56 % after 24 hours and that cumulative mortality over 2 weeks was only 62 %. Thus this study illustrates that mortality rates over the first 24 hours after outplanting are extremely high, regardless what method of survival determination is used.

Each replicate outplant of juveniles is expected to increase densities of reproductive adults by only 0.005 individuals/m^2 after 2 years. As the juveniles continue to grow and reach maturity, this is expected to become an increase in density of 0.006 reproductive individuals/m^2 after four years. This extrapolation involves two key assumptions: (1) the hatchery-raised abalone observed at the time of the 1-year survey do not disperse out of the area in future years, and (2) mortality rates of hatchery-raised juveniles more than one year after outplanting approximate those of wild abalone of the same age. We believe these assumptions will be met because adult northern abalone exhibit low dispersal rates (Hansen and Lessard in prep.), and because the mortality of hatchery-raised abalone is only expected to be high in the period immediately following outplanting (Schiel 1993; Sweijd et al. 1998).

Both the Jolly-Seber and Barker methods clearly demonstrate that the lowest density outplant treatment has the worst performance (highest mortality), and that high densities of 100 and 200 abalone/m^2 generate the highest survival rates, with 100 abalone/m^2 being the optimal density. I propose that this is due to failure of predator dilution at low density outplant locations. Our highest density outplant locations were not most successful, possibly due to excessive concentrations of abalone stress mucus, which is attractive to predators, or competition for refuge space. Both of these explanations require further experimentation.

Outplanted abalone slowly dispersed away from their outplant location over time, with less than 1 % of abalone dispersing more than 1 m in 24 hours despite the fact that stress can prompt abalone to disperse (Emmet and Jamieson 1988; Kojima 1995; Werner et al. 1995; Sweijd et al. 1998). Site fidelity values, however, suggest that the majority of dispersal in this experiment occurred more than three days after outplanting. Nevertheless, the low dispersal values are consistent with recent findings that northern abalone are relatively stationary in comparison to other abalone species (Poore 1972; Shepherd 1986; Schiel 1993; Rogers-Bennet and Pearse 1998; Hansen and Lessard, in prep.). Dispersal did not differ significantly by treatment density, although I suspect that methods with greater accuracy in determining average dispersal will show lowest movement of abalone in lower density treatments, as abalone outplanted in higher densities attempt to avoid competition for refuges and food.

Influence of abalone shell length on juvenile outplant survival

To my knowledge, this is the first study to show that large abalone experience the greatest mortality immediately after outplanting. This may relate to the fact that the time scale of this study is considerably shorter than most others (McCormick et al. 1994), providing greater resolution of the events that occur immediately after outplanting. It is thought that when abalone are first outplanted, disorientation and stress make them particularly vulnerable to predators (Emmet and Jamieson 1988; Schiel 1993; McCormick et al. 1994; Sweijd et al. 1998). I propose that during this period predators focus their efforts upon the largest abalone in accordance with optimal foraging theory (Lendrem 1986). Once outplanted abalone recover from the stress of outplanting and adhere properly to the substrate, large abalone experience a size refuge from predators, at which point the predators return to feeding upon smaller abalone. This study thus provides evidence of a critical period in outplant mortality which occurs immediately after outplanting.

Apparent survival of the outplanted abalone in this study actually decreased with increasing size between 2.3 and 4.8 cm SL in the first two surveys, and was independent

of size after two weeks. There are two possible explanations for this trend: larger abalone are more difficult to detect immediately after outplanting, perhaps because they disperse more quickly, or larger abalone experience greater initial mortality. The former is unlikely because recapture probabilities were highest in the first survey and site fidelity values were also relatively high during this period. Moreover, the sizes of recovered shells (i.e. dead abalone) provide support for the second explanation. Indeed, it appears that the largest individuals died off in the first three days. This result was unexpected for two reasons: not only does vulnerability of abalone to certain predators tend to drop off with increasing size (Sloan and Breen 1988; Griffiths and Gosselin 2008), but in other outplanting experiments, survivorship has been shown to increase with size of abalone (Saito 1984; Schiel 1993; Dixon et al. 2006; Roberts et al. 2007). It is possible that the optimal size for survival of outplanted juvenile northern abalone is closer to the size at which they first experience a reduced vulnerability to predators (12-13 mm in Griffiths and Gosselin 2008). Use of abalone covering a greater range of shell lengths might reveal that survival increases with size up to a particular shell length, which was missed because we only outplanted abalone measuring 23 to 48 mm. Other studies have also found that increasing the shell length of outplants above the optimal size does not increase survivorship (McCormick et al. 1994).

Thirteen outplanted abalone were resighted after one year, during which time their average growth (1.63 cm) exceeded the expected average growth of wild abalone of equivalent size. This suggests that hatchery-raised abalone, once established, can perform well in the wild. Moreover, the resighted abalone were approximately 5 cm in length or larger in 2009, which is the size at which northern abalone begin to reach sexual maturity (Lessard et al. 2007). It is accordingly possible that some of these outplanted abalone contributed to spawning events in 2009. This in turn suggests that even those juvenile abalone outplanting attempts with extremely high mortality have the potential to contribute spawners to wild spawning events.

Conclusion

There were indications of density-dependent effects on post-outplanting survival for both larval and juvenile outplants in this study. While treatments of 50,000 larvae significantly increased densities of recruits after one year, treatments of 100,000 larvae did not. On the other hand, higher densities of juvenile abalone performed better, with groups of 100 outplanted individuals having higher survival than groups of 25 individuals.

The larval outplanting treatments used in this study are expected to raise densities of reproductive adults by 0.487 to 0.721 individuals/m^2 after three and five years, whereas juvenile outplanting is expected to increase densities by only 0.005 to 0.006 reproductive individuals/m^2 over two and four years, respectively (albeit over a larger area). Even when considering the area over which densities are expected to increase, the increase in reproductive adults produced by outplanting 50,000 larvae exceeds that produced by outplanting 100 juveniles. Although these predictions do not take into account the dispersal which abalone might undertake over a course of four to five years, nor inter-annual variation in survivorship, they provide support for adopting larval outplanting over juvenile outplanting, particularly given the lower costs incurred in larval production (Richards 2010). In comparison, past outplanting efforts by BHCAP raised densities of reproductive abalone by 0.003 to 0.033 individuals per m^2 at three sites (Chapter 2). Hence by altering outplanting methods we were able to improve outplanting success.

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Chapter 4: Identifying the limitations of outplanting: Hatchery abalone mortality

INTRODUCTION

The shallow subtidal zone along exposed rocky coastlines can prove inhospitable to even the most tenacious clingers among benthic organisms, as they risk being dislodged and swept out to sea, or scoured and crushed by the movement of sand and rocks in extreme wave action (Sainsbury 1982; Schiel 1992; Trussel et al. 1993; Alfaro and Carpenter 1999). Even abalone, which are famous for their large and muscular foot among a family of creatures named for this feature – *gastropod* is latin for stomach foot – are absent from shallow water at extremely exposed sites (Cox 1962; Sloan and Breen 1988). Yet northern abalone can be found at exposed sites where swell and turbulence are sufficient to limit their ability to capture and feed upon drift kelp (Sloan and Breen 1988; Zhang et al. 2007). Abalone at such sites become stunted in growth relative to those occupying more sheltered sites (Campbell et al. 2003; Zhang et al. 2007). Why would abalone occupy sites where their ability to feed is compromised? The answer relates to the avoidance of an even bigger threat, namely predation.

Northern abalone are preyed upon by a myriad of predators, from crabs (*Cancer productus, C. magister, Scyra acutifrons,* and *Lophopanopeus bellus*) and seastars (*Pycnopodia helianthoides*) to fishes (*Anarrhichthys ocellatus,* and *Scorpaenichthys marmoratus*), octopuses (*Enteroctopus dofleini*), and even sea otters (*Enhydra lutris*) (Emmett and Jamieson 1988; Griffiths 2006; Griffiths and Gosselin 2008; Watson 2000), and are a preferred prey item of many of these predators (Tegner and Butler 1985). According to Hines and Pearse (1982), "the persistence of a preferred prey population under intense predation pressure may depend on the existence of a physical refuge". Exposed sites may represent such a refuge for northern abalone. In fact, while the unique mechanical properties of gastropod mucus (Denny 1980, 1984) in combination with the muscular foot permit abalone to maintain a purchase on the substrate in challenging conditions, many of their predators are discouraged by the same conditions (Menge 1978; Denny 1988).

Given the predator avoidance strategies of wild abalone, it is perhaps unsurprising that abalone raised in the absence of predators in calm hatchery tanks experience high rates of mortality when released into their natural habitat (Rogers-Bennett and Pearse 1998; Dixon et al. 2006). Indeed, past studies have hypothesized that abalone raised in hatchery environments are naïve to predators (Schiel and Welden 1987; Tegner and Butler 1989; McCormick et al. 1994; Shepherd et al. 2000). Not only would outplanted abalone suddenly be facing new threats, but the disorientation inherent to such a transfer of surroundings could further interfere with their ability to recognize and respond to threats (Olla et al. 1998). Chapter 3 of this thesis revealed that 64 % of hatchery-raised northern abalone died within 24 hours of being outplanted. In comparison, wild individuals of the same age are expected to experience a daily mortality of less than 0.1 % (Sloan and Breen 1988). This result reflects those of a number of studies in which other abalone species were outplanted: namely, that recently outplanted abalone experience unusually high mortality, be it through stress, predation or other causes (Schiel 1993; Sweijd et al. 1998).

Predation is a major source of mortality for other species of abalone when outplanted (Tegner and Butler 1985; Schiel and Welden 1987). *Pycnopodia helianthoides*, a voracious generalist predator, hones in on weakened and stressed northern abalone and has been observed targeting abalone which have recently been transplanted (Emmett and Jamieson 1988). Furthermore, scavengers that are usually only capable of preying on weak abalone have been found to congregate at sites where abalone are outplanted (Kiyomoto 2007), as have predators (Tegner and Butler 1985). It is currently unknown whether the high mortality of outplanted northern abalone is caused by predators, or whether predators simply consume abalone that are already dying or dead. It is also not known whether predators congregate on northern abalone outplant locations.

To improve the success of outplanting attempts, it is necessary to identify and counteract the major sources of outplant mortality. This study examines (1) whether predators congregate at northern abalone outplant locations, (2) whether the survivorship

of outplanted abalone at a site is related to the abundance of predators at the site, (3) whether predators are an important source of outplant mortality, (4) whether handling, tagging and outplanting stress cause mortality, and (5) whether predator exclosures can (a) improve outplant survival or (b) alter abalone behaviours post outplanting.

METHODS

Predator congregation and the relationship between predator density and outplant survival

All outplanting sites in this experiment were located within Barkley Sound on the west coast of Vancouver Island, BC, Canada. This experiment was designed to determine if abalone predators congregate on outplanting sites, and whether the degree of predator congregation is dependent on abalone density. This involved outplanting juvenile abalone at five densities, 0, 25, 50, 100 and 200 individuals per m^2, and then monitoring predator densities near each outplanting location. The 5 treatment densities were spaced 15 m apart and replicated at five sites (Figure 22); outplanting was done in 2008 following the outplanting methodology described in Chapter 3. Prior to outplanting, the abundance of predators at each treatment location within each site was assessed using circular swath surveys of 5 m radius (Figure 23). For the circular swath surveys, a team of divers attached one end of a measuring tape to an eyebolt that had been permanently fastened to the center of each outplant location. The divers then searched each 1 m swath, swimming in alternating directions until a circle of 5 m radius had been covered. The circular swath surveys were repeated 1 and 3 days post-outplanting to reassess predator densities and monitor abalone survival. Fourteen days following outplanting, a 3 m radius cryptic survey (see Chapter 3 for details) was conducted to reassess abalone survival. Adverse weather conditions prevented the completion of the 1-day and 14-day surveys at Fleming Island and the 3-day survey at Ohiat Islet. The choice of predators to monitor was based on the identification, by Griffiths and Gosselin (2008), of important predators of juvenile northern abalone. Every sighting of one of the following northern abalone predators was recorded: *Cancer magister*, *C. productus*, *Lophopanopeus bellus*, *Scyra acutifrons*, and *Pycnopodia helianthoides*.
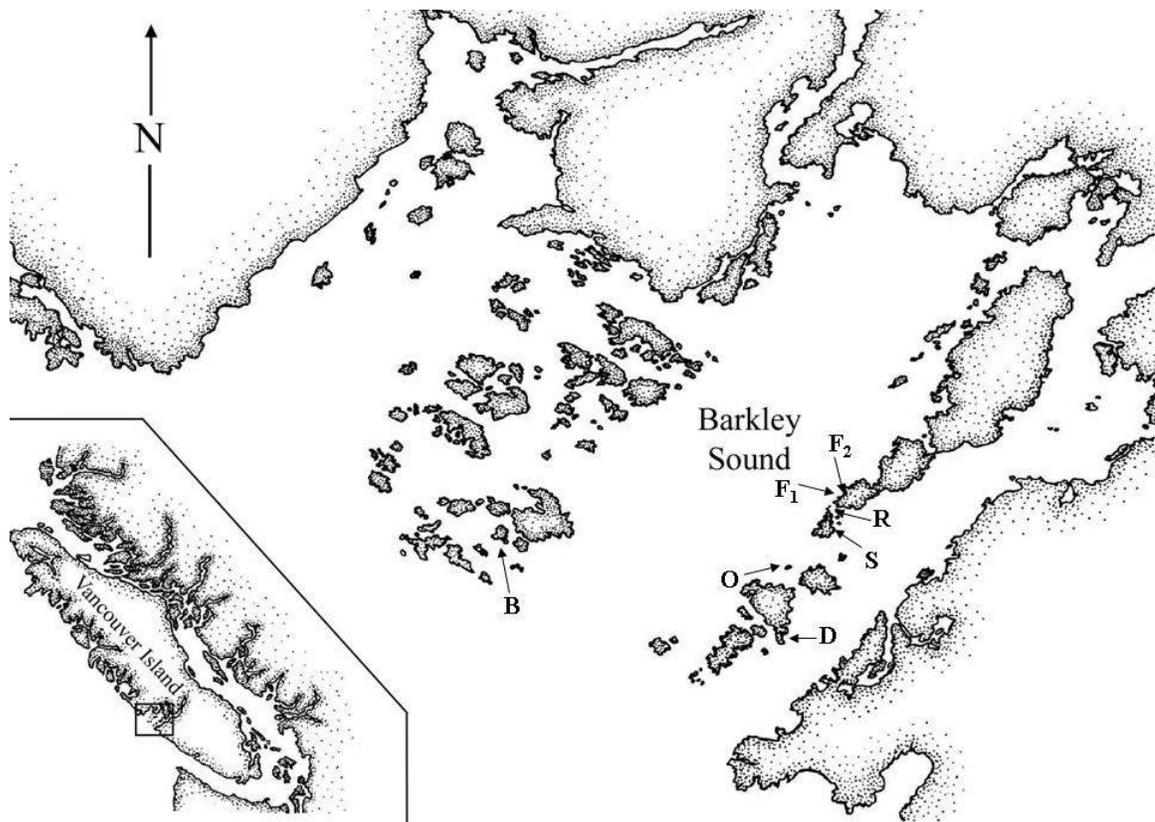
Figure 22. Map of study sites in Barkley Sound, located on the west coast of Vancouver Island (inset). Outplanting sites are indicated by arrows, where S (Sanford Island), O (Ohiat Islet), F_1 (Fleming Island), R (Ross Islets) and D (Diana Island) were those sites used for determining the influence of outplanting on predator densities. F_2 (Fleming Island) is the site at which juvenile abalone were outplanted in the predator exclosure part of the experiment. B (Bauke Island) is a site for which morphometric measurements of wild abalone are available. Adapted from Gosselin and Chia (1995).
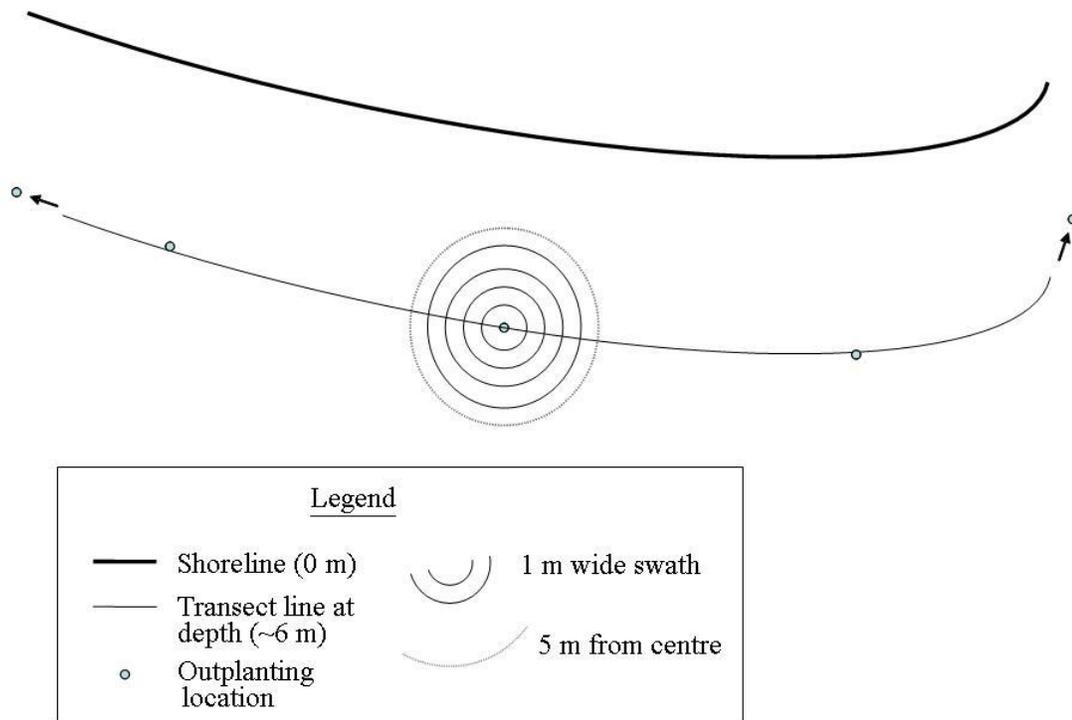
Figure 23. Circular swath survey methodology. Only one site is shown, and only one circular swath survey is depicted at that site. Each concentric ring represents a 1 m swath searched by divers.

Densities of four of the five above-mentioned abalone predators (*P. helianthoides, C. productus, C. magister*, and *L. bellus*) were calculated for each concentric 1 m swath surrounding the treatment locations, as well as the overall density within each 5 m radius circle. The presence of *Scyra acutifrons* was recorded during surveys but was omitted from analyses because some divers misidentified individuals of this species. To determine if predators congregated on outplant locations, a four-way repeated measures ANOVA was performed for each of the four predator species. The response variable was the density of a given predator. The repeated measure was time, with one survey conducted prior to outplanting, and two conducted post-outplanting (day 1 and day 3

surveys; the day 14 survey was omitted from this analysis because the cryptic habitat was also searched on this survey. The other three predictor variables were distance from outplanting location (fixed; 5 distances), abalone outplanting density treatment (fixed; 4 treatments), and site (random; 5 sites on the pre-outplanting survey, and 4 sites on the day 1 and day 3 surveys). As such, the total degrees of freedom for these analyses were 324, with 96 degrees of freedom for the error term.

General linear models were used to assess whether predator densities influenced abalone survivorship. In these analyses, abalone survivorship to one-day post outplanting was the response variable, while the two predictor variables were: (1) the density of *P. helianthoides* prior to outplanting, and (2) the density of *C. productus* prior to outplanting.

To compare the 5 sites in terms of predator abundance, densities calculated for the 5 m circles were averaged across all five treatment locations within a site. When densities differed significantly between sites, the densities are reported, as are the cumulative survival rates of outplanted abalone over a two-week period. The numbers of hatchery-raised abalone resighted in the wild after one year is also summarized by site.

The shells of deceased hatchery abalone were collected when encountered during the surveys, and suspected mode of death (induced by a crab or a seastar) was identified based on the occurrence and type of shell damage, as per Emmett and Jamieson (1988). The relative importance of *P. helianthoides* as opposed to *Cancer spp.* predation upon outplanted abalone was compared between sites using a two-way repeated measures ANOVA. The response variable was the proportion of mortalities attributable to *Cancer spp.* (i.e. the number of mortalities caused by *Cancer spp.* divided by the total number of mortalities), while the predictor variables were site and time. Although there were 5 sites and 3 survey times included in the analysis, the error and total degrees of freedom for this analysis were 5 and 11, respectively, due to the three surveys which could not be completed.

Identifying and counteracting sources of outplant mortality

The importance of predators, predator exclosures, and stress caused by handling, tagging and outplanting to abalone survivorship were examined in this second experiment using 560 hatchery-raised abalone ranging in size from 4.23 to 6.46 cm shell length (SL). Individual abalone were measured to the nearest 0.01 cm with vernier calipers, weighed to the nearest 0.01 g on a digital scale, and individually labeled with bee tags (Queen Marking Kits from The Bee Works, ON, Canada) attached with superglue. An analysis of the body tissue condition index of these abalone is presented in Appendix D. A second size class of 0.1 to 2.0 cm SL abalone was also used, but this part of the experiment resulted in recovery rates that did not accurately reflect survival and is not further discussed in this chapter (Appendix E).

Abalone were returned to the hatchery tanks after tagging and were fed *ad libitum* with the kelp *Nereocystis leutkeana* for one week prior to outplanting. In preparation for outplanting, abalone were packed into half-sections of PVC tubes (30 cm length x 15 – 20 cm diameter), which were then paired up with the matching PVC half-section and held together with elastic bands to form a tube containing the abalone. A few blades of *N. leutkeana* were placed in the tubes and then the ends were sealed with Vexar mesh. Each treatment replicate consisted of one tube containing 20 abalone. Tubes containing abalone were held in hatchery tanks for 24 hours before outplanting to allow the abalone to properly attach themselves to the PVC.

PVC outplanting modules were used to outplant abalone because they appear to minimize the stress experienced by abalone during the outplanting process, in that they allow an abalone to adhere undisturbed to the same surface before, during and after outplanting, until dispersal. This method did however necessitate that 35 large PVC outplanting modules be carried by two divers. To make this possible we devised a method using a lift bag and rope (see Appendix F for details).

A site with more than 70 m of abalone habitat at 0-12 m depth along Fleming Island (Figure 22) was selected to receive outplants in this experiment. Lead lines were placed at 9 m depth along the 70 m stretch of habitat and affixed to cement blocks at

either end. The lead lines were further weighed down by rolling large cobble and small boulders on top. Seven replicate outplanting locations separated by 10 m intervals along this line were identified and marked with zip-ties. At each location, one suspended and one grounded cage were attached to the lead line using herring clips (Figure 24).
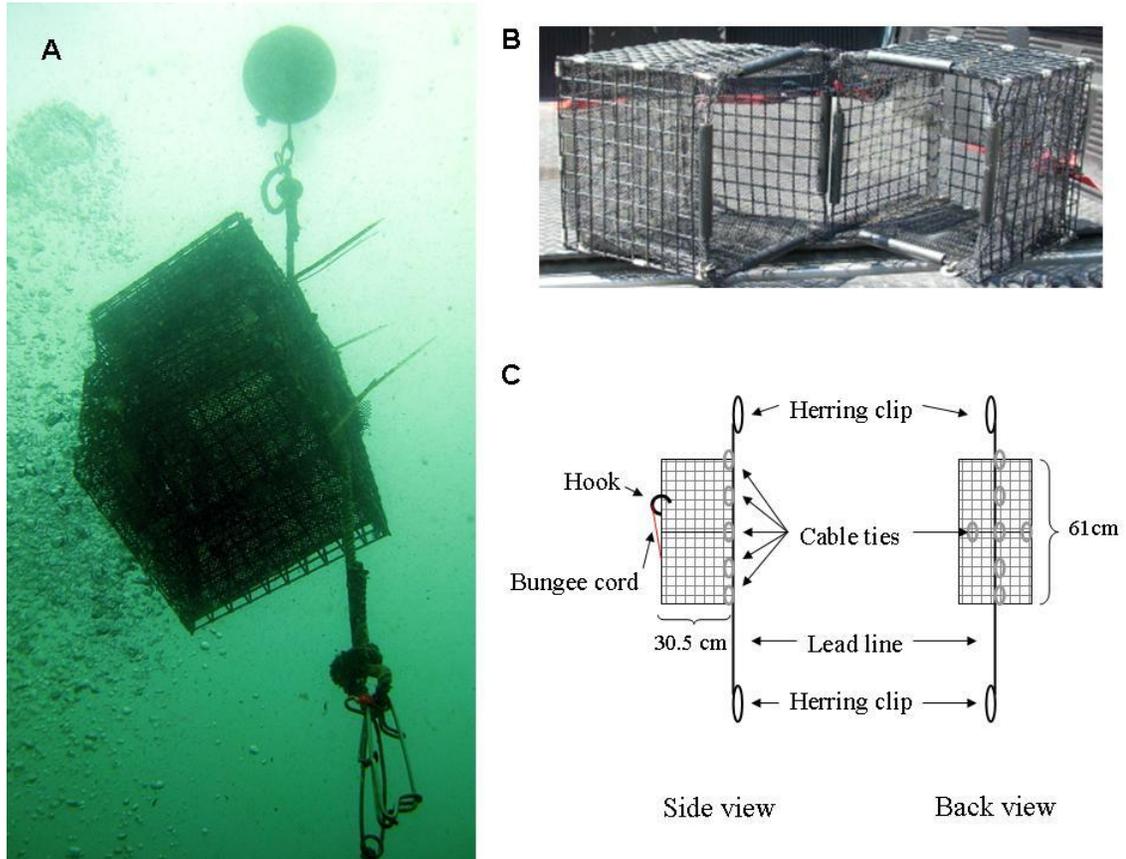
Figure 24. Predator exclosures for juvenile abalone outplanting. The coloured inset (A) shows a suspended predator exclosure cage deployed in the field. The predator exclosure cages consisted of two wire cages measuring 30.5 x 30.5 x 30.5 cm, which were attached by cable ties on one edge (B). The mesh size of the wires is ~ 2 x 2 cm, but the cages were also lined with vexxar of 0.5 x 0.5 cm mesh size. The edge opposite to that hinged with cable ties received a bungee cord and hook so that it could be opened and closed when the abalone within needed to be surveyed. Lead lines, 1.5 m in length, with herring clips at either end were cable tied to the back side of the cage and served to attach the cages to other lead lines and buoys (C).

This experiment involved the following six treatments: (1) tagged abalone outplanted into suspended predator exclusion cages, (2) tagged abalone outplanted into grounded predator exclusion cages, (3) tagged abalone outplanted directly onto the substrate (no cage), at the base of the predator exclusion cages, (4) tagged abalone handled in the same way as outplanted abalone and brought to the field, on the dive, and returned to laboratory tanks, (5) untagged abalone brought to the field, as well as along on the dive during the outplanting work, and returned to laboratory tanks, and (6) untagged abalone that were not handled and were left in laboratory tanks for the duration of the experiment. One week after outplanting, all abalone in treatments 1 and 2 were released from their respective cages by placing the PVC tubes on the substrate at the base of the cages and weighing the tubes down with rocks, thus allowing the abalone within to disperse. Treatments 4, 5, and 6 served as controls for handling and tagging.

Circular swath surveys of outplanted abalone (5 m in diameter centered on each outplant location) were conducted 1, 3, 7, 10 and 14 days after outplanting. Abalone in the predator exclusion cages were also examined concurrently with the circular swath surveys on days 1, 3 and 7 to determine the number of surviving and deceased abalone in these treatments. The survivorship of control abalone in the laboratory (treatments 4, 5 and 6) was monitored up to day 7.

Eight iButton® temperature loggers (Maxim Integrated Products, Inc., CA, USA), set to record at 1 min intervals, were randomly distributed among the replicates of treatments 4 and 5 to measure the temperatures experienced by abalone during outplanting. The same temperature loggers were used for two days prior to outplanting to determine the baseline temperature in hatchery tanks.

Outplanting success was measured as survivorship of outplanted abalone over time. As in Chapter 3, the Jolly-Seber method was used to estimate survivorship of abalone in all treatments outplanted into the field (treatments 1, 2 and 3). Resighting information was transcribed into vectors such that the Barker model in program MARK 6.0 could be employed to estimate site fidelity and recapture probability for the three outplanted treatments. Since abalone from treatments 4, 5 and 6 were maintained in

separate laboratory tanks, mark-recapture modeling was not necessary to determine survivorship in these treatments. The survivorship of abalone was compared between treatments using a randomized complete block two-way ANOVA. In this ANOVA, the bivariate response variable was survivorship on day 7, while the predictor was outplanting treatment (fixed; 6 treatment levels) and the blocking factor was location (random; 7 blocks). Tukey's HSD post-hoc test was used to compare survivorship among individual treatments. In this first analysis, the survivorship being compared between treatments was that of abalone 7 days after outplanting, regardless of time spent on the substrate. A second comparison was made between treatments considering the length of time that abalone had been free upon the substrate rather than the time since outplanting. Thus the 10-day survivorship of abalone outplanted in cages and then released after seven days (treatments 1 and 2) was compared to the 3-day survivorship of abalone outplanted directly onto the substrate. Similarly, the 14-day survivorship in treatments 1 and 2 were compared to the 7-day survivorship in treatment 3. This analysis is a repeated measures, randomized complete block three-way ANOVA, in which time is the repeated measure (fixed; 2 times), location is the blocking factor (random; 7 blocks), and treatment is the predictor (fixed; 3 treatment levels). Once again, Tukey's HSD test was used to compare survivorship among individual treatments.

As I was primarily interested in determining whether outplanting can raise densities of reproductive adults in the field, the densities of surviving abalone were extrapolated to adult densities using survival rates published in the literature for wild abalone. Notably, the annual survival rate for 3-5 year olds is 81.9 % (Breen 1980; Fournier and Breen 1983; Breen 1986; Sloan and Breen 1988).

Temperature stress was calculated as the differential, in degree minutes (DM), between hatchery and field conditions following:

Equation 1 $DM = \sum (To_1 - Th) + ... + (To_n - Th)$; where To_{1-n} is the temperature in the outplanting module measured at 1 min intervals from the time the abalone were removed from the hatchery tanks on outplanting day (To_1) to the time during the

outplanting dive when temperatures settle back to the base temperature (To_n). The temperature of the seawater in hatchery tanks (*Th*) serves as the base temperature.

RESULTS

Predator congregation and the relationship between predator density and outplant survival

Predator congregation at outplanting locations

Of the four predators monitored, *Cancer magister* showed no evidence of congregation, *Lophopanopeus bellus* showed some indication of congregation, and both *Pycnopodia helianthoides* and *C. productus* showed strong evidence of congregation at the outplanting sites. The distribution of *C. magister* was sparse at all study sites (<0.003 individuals/m^2) and there were no detectable differences in its densities over time (ANOVA: $F_{2, 96}$=0.481, p=0.620), at different distances from the outplant locations (ANOVA: $F_{4, 96}$=0.894, p=0.471), or between outplanting treatments (ANOVA: $F_{4, 96}$=0.581, p=0.677).

Although the densities of *L. bellus* appeared to increase over time, particularly at the center of locations in which abalone had been outplanted (Figure 25), this trend was not statistically significant (ANOVA: $F_{32, 96}$=1.125, p=0.323). No *L. bellus* individuals were observed at control locations during any of the three surveys. The lack of statistical significance in the distributions of this predator are thought to be due to the highly patchy nature of its distribution, which result in large variances in its densities.
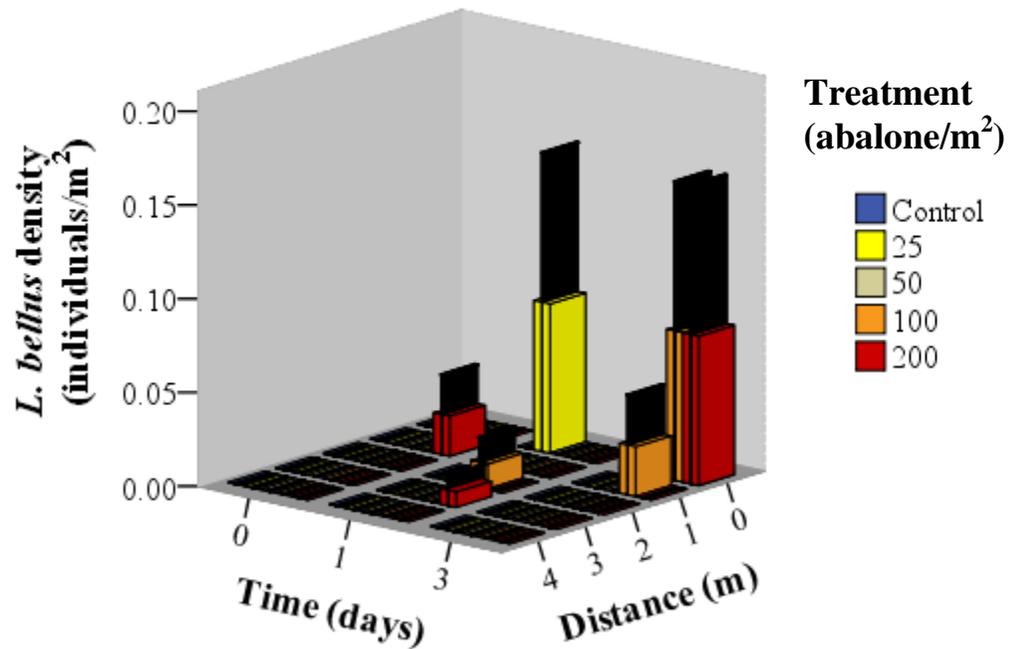
Figure 25. Changes in *L. bellus* densities over time at different abalone outplanting densities. Times are: prior to outplanting (0), 1-day (1), and 3-days (3) post-outplanting. The treatments are the different abalone outplanting densities (0, 25, 50, 100 or 200 abalone/m^2). Error bars are $\pm$ 1 SE (n=5, 4 and 4 for the three survey times, respectively).

As for *P. helianthoides*, two significant interactions influenced its distribution: a three-way interaction between treatment, distance and site (ANOVA: $F_{64, 96}$=2.794, $p<0.001$), and a three-way interaction between time, distance and site (ANOVA: $F_{24, 96}$=1.813, $p=0.023$). The latter interaction reflects the fact that *P. helianthoides* moved in towards the center of outplant locations over time at Fleming Island (Figure 26a).

Densities of *P. helianthoides* were significantly higher in the 1 m radius area at the center of outplant locations, than at greater distances from the centre (Tukey HSD test: *p*<0.001; Figure 26b).

A

B
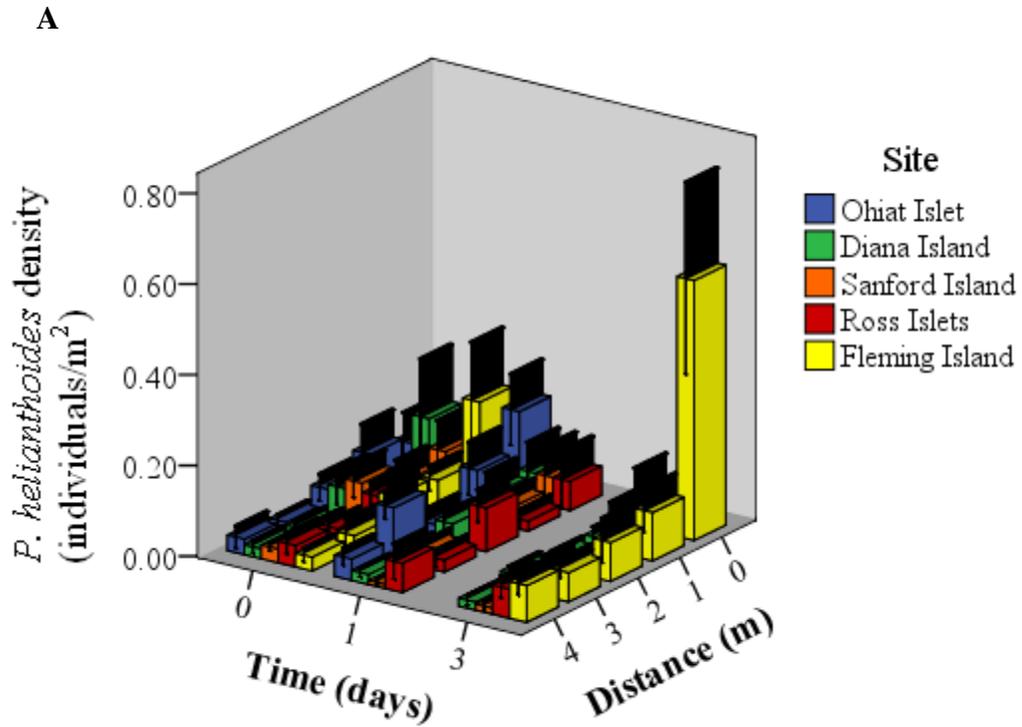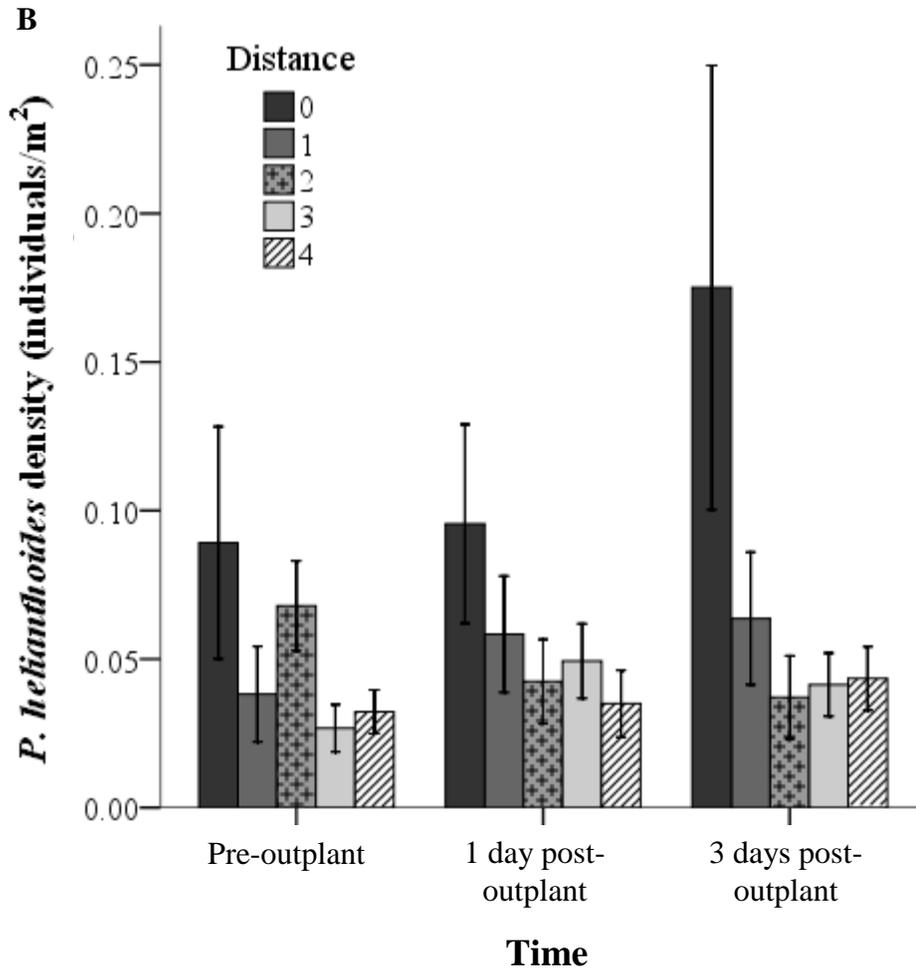
Figure 26. Mean densities of sunflower stars (*P. helianthoides*) over time (a) at different distances from the outplant locations at five outplanted sites (n=5), and (b) at the different distances only (n=25, 20, and 20 for the three surveys, respectively). Error bars are ± 1 SE.

Densities of *C. productus* were significantly influenced by an interaction between the time of the survey and the distance from the center of outplanting locations (ANOVA: $F_{8,96}=3.425$, $p=0.002$); densities of *C. productus* increased over time at the center of outplant locations (Figure 27a). Treatment locations were not fully independent of one another in terms of densities of *C. productus*. Indeed, densities of this predator increased at the outplanting locations immediately after hatchery abalone were released, with an associated decrease in density at control locations (Figure 27b), suggesting that the red

rock crabs moved between outplant locations. This suggests *C. productus* can distinguish locations that differ in abalone density and can relocate accordingly.
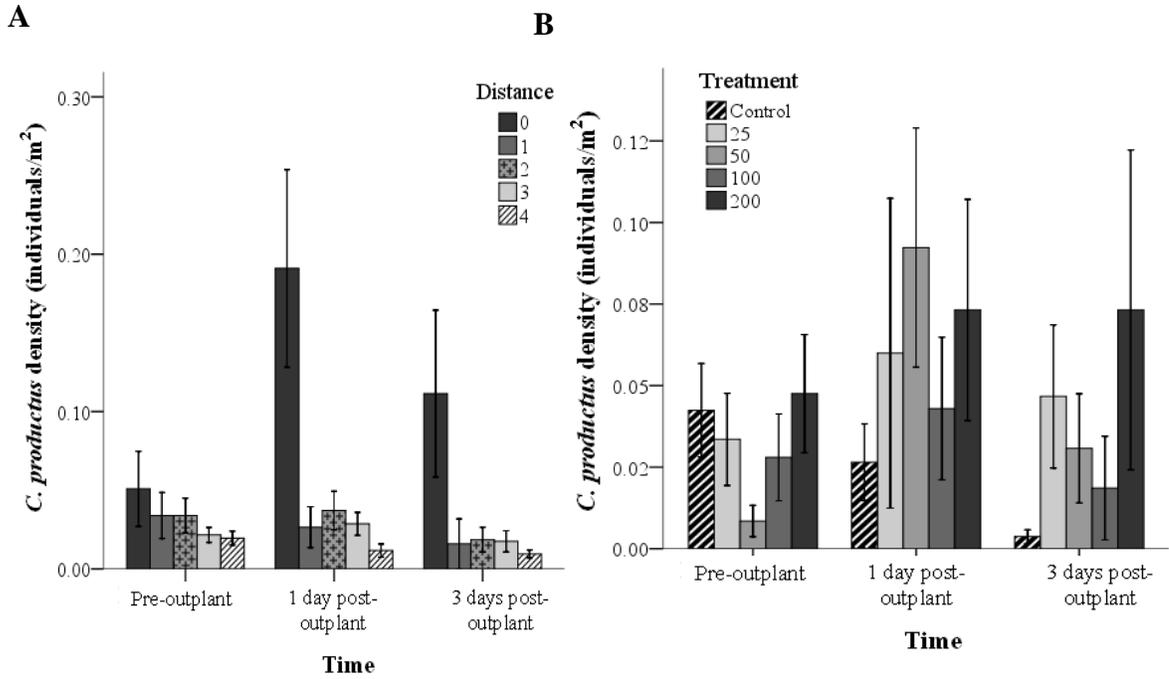
Figure 27. Mean densities of red rock crabs (*C. productus*) over time (a) at different distances from the outplant locations, and (b) at the different abalone outplant density treatments. Error bars are ± 1 SE (n=25, 20, and 20 for the three survey times, respectively).

Influence of predator density on outplant survival

Abalone survivorship was related to the densities, in the 5 m diameter area, of *C. productus* (GLM: $F_{1, 6}=11.784$, *p*=0.007) and *P. helianthoides* (GLM: $F_{1,6}=7.729$, *p*=0.021), but not to the densities of *L. bellus* (GLM: $F_{1,9}=0.006$, *p*=0.941). In fact, the densities of *C. productus* and *P. helianthoides*, and an interaction between the densities of these two predators, explained approximately 60 % of the variation in abalone survivorship to one-day post-outplanting (GLM: $R^2=0.596$, *p*=0.002; Figure 28).
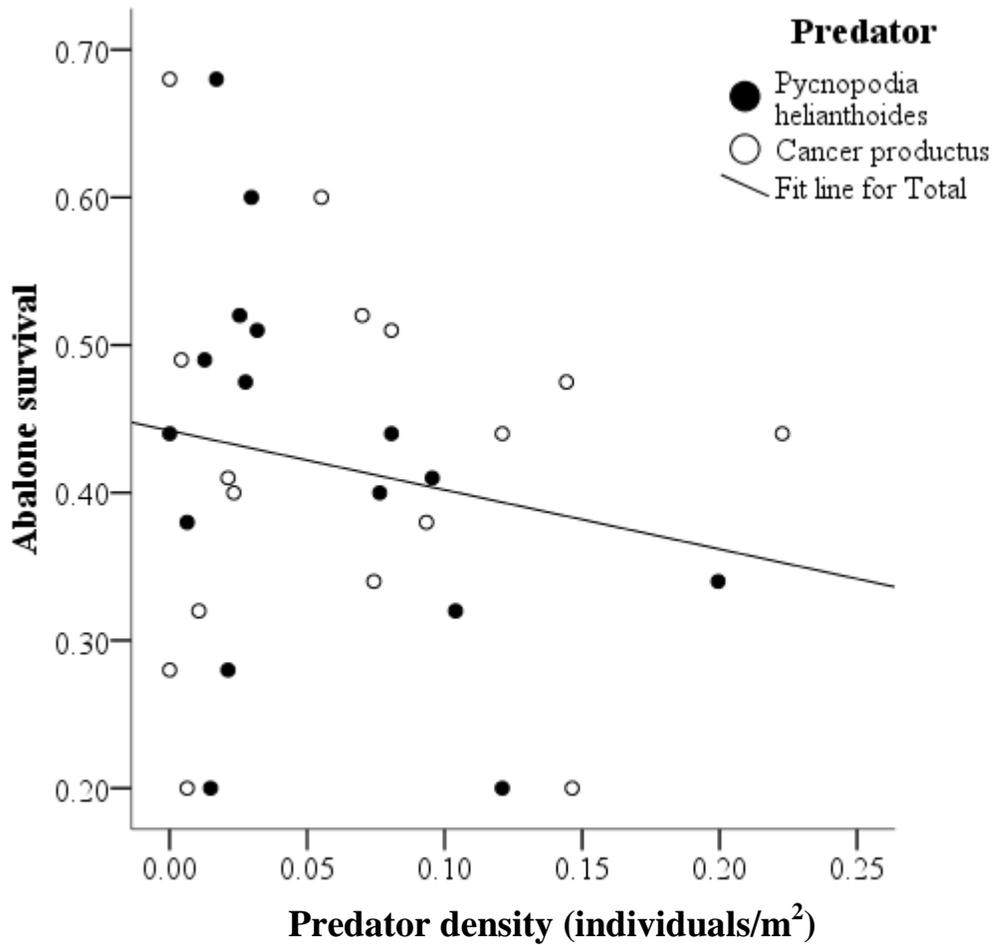
Figure 28. Abalone survival probability at all survey times as a function of the densities of *P. helianthoides* and *C. productus*.

Only the densities of *C. productus* differed between sites before outplanting, as determined by a one-way ANOVA ($F_{1,123}=3.96$, $p=0.049$). *Cancer productus* densities (averaged across sites) were highest at Ross and Ohiat, with 0.087 individuals/m^2, followed by densities at Diana with 0.077 individuals/m^2. Densities of *C. productus* were relatively low at both Sanford and Fleming, at 0.033 and 0.025 individuals/m^2, respectively.

After two weeks, abalone survivorship (averaged across treatments) was highest at Sanford (14.67 %) and lowest at Ohiat (2.67 %). Ross had a two week survivorship of 9.87 %, while survivorship at Diana was 4.53 %. Fleming was only surveyed 3 days after outplanting and therefore no two week survivorship estimate is available. However, at the time of the 3-day survey, survivorship was highest at Fleming (46.63 %) and second highest at Sanford (31.00 %).

During surveys conducted in July 2009, one year after outplanting, thirteen of the 1875 outplanted abalone were found alive, with the majority (five) found at Fleming Island. Four were located at Sanford Island, and two were found at each of the Diana Island and Ross Islets outplanting sites. No outplanted abalone were resighted at Ohiat Islet in 2009.

Of the 92 abalone shells recovered during surveys in the first two weeks following outplanting, there was considerable variation in proportions consumed by crabs versus seastars at different sites (Table 7). In fact, site was a significant predictor of the proportion of mortalities attributable to *Cancer* spp. (ANOVA: $F_{1,5}=16.057$, $p=0.010$), explaining almost 60 % of the variation therein ($R^2=0.578$, $p=0.002$). Interestingly, the sites with high *C. productus* densities are also those with low survivorship of abalone, low resighting of abalone after one year and large proportions of deaths attributable to crab predators.

Table 7. Number of outplanted abalone whose deaths are attributable to either *P. helianthoides* (PH) or *C. productus* (CP) at the five sites over time, based on recovered shells.

Time	1-day		3-days		14-days		Overall	
Site	PH	CP	PH	CP	PH	CP	PH	CP
Ohiat	1	8	na	na	0	2	1	10
Diana	0	27	0	10	1	4	1	41
Sanford	5	4	6	4	14	4	25	16
Ross	1	0	4	4	3	6	8	6
Fleming	na	na	6	4	na	na	6	4

Identifying and counteracting sources of outplant mortality

All abalone outplanted into cages, whether grounded or suspended, survived the first 24 hours, whereas 16.6 ± 5.3 % (mean $\pm$ SE) of abalone outplanted directly onto the substrate died within 24 hours. The first dead abalone in cages were observed on the 7-day survey. The survivorship of abalone to 7-days post-outplanting was significantly influenced by the outplanting treatment (ANOVA: $F_{5,30}=34.097$, $p<0.001$). In fact, the survivorship of abalone initially outplanted directly onto the substrate was significantly lower than that of abalone in any other treatment (Tukey's HSD test: $p<0.001$; Figure 29). This demonstrates that predator exclosures improved abalone survivorship while abalone were within the cages. During the first week, survivorship of abalone released directly onto the substrate (treatment 3) was estimated at 57.9 ± 2.5 % by the Jolly-Seber method, compared to $97.1 - 100.0$ % in the other treatments. The survivorship of abalone in suspended (treatment 1) and grounded (treatment 2) cages was significantly lower than that of untagged abalone brought into the field and returned to the laboratory (treatment 5) (Tukey's HSD test: $p=0.022$ and $p=0.007$, respectively), or untagged abalone maintained in the laboratory (treatment 6) (Tukey's HSD test: $p=0.003$ and $p=0.001$, respectively), but no different from tagged abalone brought into the field and returned to the laboratory (treatment 4) (Tukey's HSD test: $p=0.244$ and $p=0.113$, respectively). Nevertheless, abalone within cages had extremely high survivorship throughout their period of internment (>97 %). There were no significant differences in abalone survivorship among any of the control treatments, nor between the two types of cage treatments.
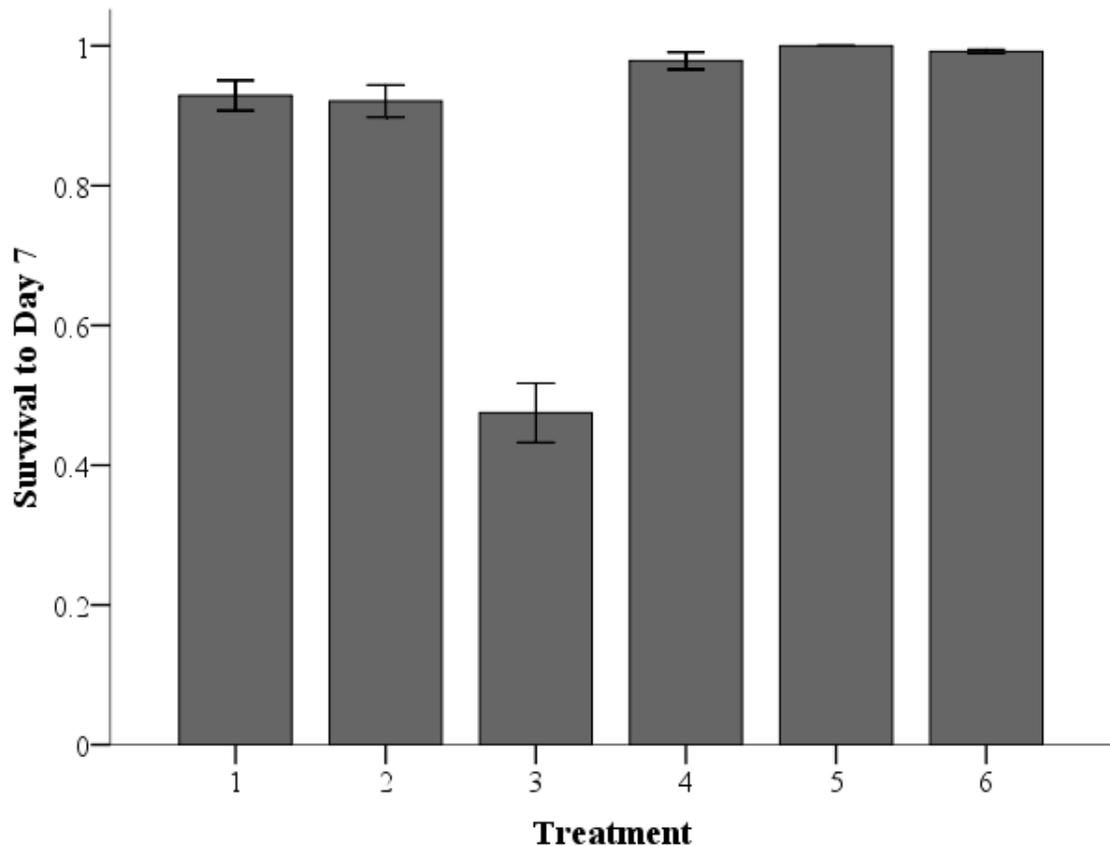
Figure 29. Survival of treatment and control abalone over time. Treatment abalone were outplanted in (1) suspended cages, (2) grounded cages, and (3) outside of cages. Treatment 4 abalone are those that were tagged, brought to the field and returned, Treatment 5 abalone were not tagged, but were brought to the field and returned, and Treatment 6 abalone were not tagged or handled, but left undisturbed in the hatchery. Treatments 1, 2 and 3 were monitored for 14 days, whereas Treatments 4, 5, and 6 were only monitored for 7 days. Error bars are $\pm$ 1 SE (n=7).

There was no difference in survivorship between outplanted treatments when considering time since release onto the substrate rather than time since outplanting (ANOVA: $F_{2,12}=3.546$, $p=0.062$). Finally, at 14-days post-outplanting, cumulative survivorship had dropped to 48.4 ± 2.5 %, 45.7 ± 2.5 %, and 33.8 ± 2.4 % in treatments 1, 2, and 3, respectively.

Estimates of recapture probabilities for abalone outplanted directly onto the substrate ranged from 34.0 ± 8.2 % to 55.1 ± 14.4 % over the two weeks following outplanting. There were no differences in recapture probabilities or site fidelity over time (Table 8).

Table 8. Instantaneous survival rates (S), recapture probabilities (p) and site fidelity (F) of hatchery-reared abalone outplanted into the wild in three treatments over time. The treatments are: (T 1) in suspended cages for one week, (T 2) in grounded cages for one week, and (T 3) onto the substrate. Survival estimates were calculated using the Jolly-Seber method, while recapture probabilities and site fidelity were estimated using Barker models.

	Time since outplanting (days)									
	1		3		7		10		14	
Survival	S	SE	S	SE	S	SE	S	SE	S	SE
T 1	1.000	0.000	1.000	0.000	0.971	0.021	0.714	0.064	0.698	0.094
T 2	1.000	0.000	1.000	0.000	0.971	0.010	0.738	0.140	0.637	0.121
T 3	0.834	0.053	0.842	0.057	0.825	0.071	0.904	0.078	0.646	0.143
Recapture probability	p	SE	p	SE	p	SE	p	SE	p	SE
T 1	1.000	0.000	1.000	0.000	1.000	0.000	0.325	0.044	0.235	0.041
T 2	1.000	0.000	1.000	0.000	1.000	0.000	0.335	0.043	0.219	0.040
T 3	0.551	0.144	0.454	0.084	0.399	0.079	0.340	0.082	0.375	0.098
Site fidelity	F	SE	F	SE	F	SE	F	SE	F	SE
T 1	1.000	0.000	1.000	0.000	1.000	0.000	0.991	0.005	0.991	0.005
T 2	1.000	0.000	1.000	0.000	1.000	0.000	0.991	0.005	0.991	0.005
T 3	0.819	0.148	0.924	0.046	0.938	0.030	0.964	0.034	0.958	0.062

Initially, these outplantings raised densities of abalone by 1.4 individuals/m^2 over the 5 m radius area surveyed. The projected increase in density over time is illustrated in Figure 30, although this only accounts for abalone mortality, not dispersal. The von Bertalanffy growth equation with parameters L∞ = 122.6 and k = 0.158 (based on abalone from Ellis Islet; Breen 1986), indicates that the smallest abalone outplanted in this study, measuring 4.23 cm, would reach sexual maturity at the latest after 3 years of

growth (i.e. at 7.00 cm). Three years after outplanting, the increases in density resulting from outplanting 60 abalone at a given location ranged from 0.303 to 0.434 individuals/m^2 depending on the outplanting treatment.
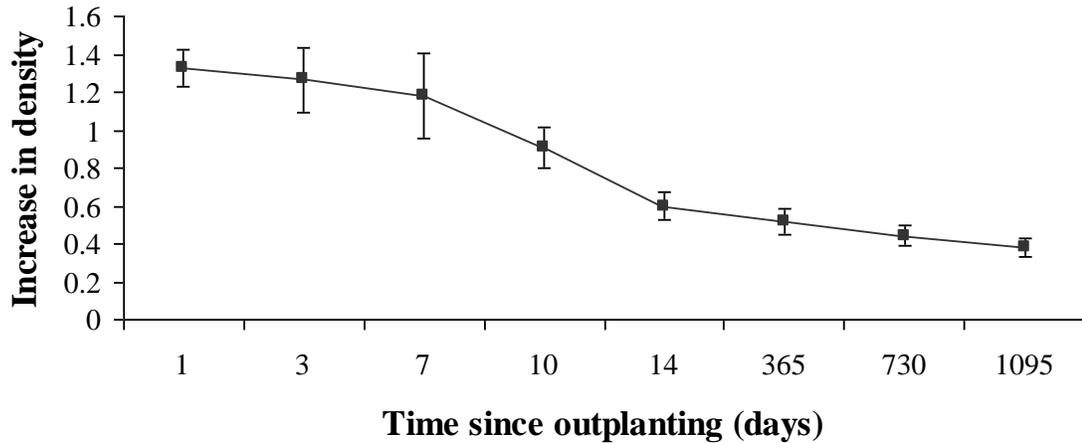
Figure 30. Predicted increase in density over time resulting from outplanting abalone. Density is the number of abalone per m^2 and is averaged across the three outplanting treatments. Error bars are $\pm$ 1 SE.

The average seawater temperature within hatchery tanks was 10.89 $\pm$ 0.00ºC over the first seven days of the experiment. The mean value of degree minutes (DM, a measure of temperature stress) reached during outplanting was 161.23 $\pm$ 60.73ºC. The minimum DM recorded in any outplanting module was 2.19ºC, whereas the maximum was 550.14ºC. This great discrepancy occurred because the replicates were outplanted one after another (see Appendix F), and it was necessary for divers to return to the surface once to switch tanks and take a surface interval during outplanting; thus abalone in modules outplanted at the end of the second dive reached much higher DM values than those outplanted at the start of the first dive. Abalone in treatments 4 and 5 were brought to the field and returned to hatchery tanks to control for the effects of temperature stress. Recall that the survivorship of these abalone was not significantly different from the

survivorship of abalone left undisturbed in hatchery tanks, suggesting that temperature stress did not directly result in any mortality.

DISCUSSION

Predator congregation and the relationship between predator density and outplant survival

This study provides the first evidence of predators congregating at northern abalone outplant locations. Predators demonstrating the greatest congregating response to outplanted abalone were the crab *Cancer productus* and the seastar *Pycnopodia helianthoides*. Densities of these two abalone predators at the center of outplant locations increased by as much as 300% and 200%, respectively, over a period of only 1 day. However, not all predators responded in this way; densities of the crabs *Lophopanopeus bellus* and *C. magister* did not change significantly in response to abalone outplanting.

It was hypothesized that predators would respond differently to different densities of outplanted abalone, honing in on high densities in an aggregative response (Shepherd et al. 2000; Gascoigne and Lipcius 2004). An interaction between time, distance and treatment would have been indicative of such an aggregative response, but was not detected for any of the predators surveyed herein. Thus this study suggests that high densities of outplanted abalone are not more attractive to predators.

Outplanting locations were placed only 15 m apart, due to limited availability of appropriate habitat at each site. Large predators were capable of moving between outplanting locations (though not from one site to another) over the course of the experiment, and the outplanting locations were accordingly not fully independent with regards to the densities of the motile predators such as *C. productus* and *P. helianthoides*. Notably, *P. helianthoides* is known to use distance chemoreception to track down injured prey, and can move up to 28 m/hr (Brewer and Konar 2005). The caveat that one outplant location may therefore be influencing results at another has important implications for the interpretation of results. Interestingly, an increase in density of *C. productus* at outplanting locations was associated with a decrease in density at control locations. This provides strong support for the hypothesis that these predators distinguish locations with

low abalone densities from locations with higher abalone densities, and will accordingly relocate from low to high abalone density locations, presumably to prey on the abalone.

Due to the lack of independence among treatment locations, comparisons of predator densities among sites are thought to be more telling for the motile predators, *C. productus* and *P. helianthoides*. The two experimental sites with the highest outplant survivorship (Sanford and Fleming Islands) were also those with the lowest *C. productus* densities. Moreover, the proportion of abalone lost to crabs, based on recovered shells, was much higher at Diana Island and Ohiat Islet, the two sites with the highest abalone mortality and *C. productus* densities. All of this suggests that low *C. productus* densities at a site may be a key indicator of optimal abalone outplant survival. Moreover, *C. productus* densities at a site prior to outplanting were found to be a significant predictor of abalone survivorship, as were the densities of *P. helianthoides*. This indicates that predator densities should be considered when choosing sites for outplanting. Moreover, the mobility of predators and the associated temporal variation in their densities should be considered when attempting to choose sites for outplanting based on low predator densities.

As in several other studies of abalone outplanting, survivorship differed significantly among sites (Schiel 1993; Roberts et al. 2007), reinforcing the importance of selecting sites with suitable characteristics. After 3 days, survivorship was highest at Fleming Island, whereas after 14 days it was highest at Sanford Island. Although the former site was not surveyed on day 14 due to adverse weather conditions, it is possible that it was a better site than Sanford Island. Sanford and Fleming Islands were also the two sites with the greatest recovery of hatchery abalone one year following outplanting. Given that a team of experts had initially worked together to identify sites with appropriate juvenile abalone habitat, all of which are situated within Barkley Sound, the fact that they nonetheless show considerable variability in outplant survivorship indicates that we still have much to learn about habitat features that influence the survival of juvenile abalone.

Identifying and counteracting sources of outplant mortality

This study revealed that predators are the primary cause of mortality of newly outplanted juvenile *Haliotis kamtschatkana*. Indeed, during the first seven days of the experiment almost all of the abalone outplanted into cages survived, whereas only 57% of abalone outplanted directly onto the open substrate survived. This finding is consistent with studies of other abalone species that have suggested predators to be the main cause of mortality of outplanted abalone (Tegner and Butler 1985; Schiel and Welden 1987; McCormick et al. 1994; Scott 1997 cited in Sweijd et al. 1998; Rogers-Bennett and Pearse 1998; Dixon et al. 2006). Both the suspended and grounded predator exclosure cages used in this study were successful in excluding all predators, as indicated by the low mortality within, and the fact that no predators were ever observed within the cages (See Appendix G for discussion of cages). Abalone mortality is generally highest immediately after outplanting (Schiel 1993; Sweijd et al. 1998; Chapter 3), and predator exclosure cages were devised as a means of protecting abalone during this period of high vulnerability. Interestingly, this particular study presents an exception in that mortality of abalone on the substrate was considerably lower during the first 24 hours than in previous experiments (16.6 ± 5.3 % as compared to 63.5 ± 1.7 % in a previous experiment, see Chapter 3).

Handling stress was not an important cause of mortality. Although it is widely held that stress induced from being handled, tagged and introduced to a new environment is responsible for substantial abalone mortality upon outplanting (Schiel 1993; McCormick et al. 1994; Sweijd et al. 1998; Shepherd et al. 2000; Tegner 2000; Dixon et al. 2007; Kiyomoto 2007), no stress-related mortality was detected in this study even though the treatments in this experiment were specifically designed to reveal such effects. Notably, the lack of difference in survival rates between untagged abalone kept in the laboratory and untagged abalone brought to the field and then returned to the laboratory indicates that handling during the outplanting process did not directly cause abalone mortality. Similarly, increases in temperature during outplanting were expected to negatively impact abalone, but the lack of difference in survivorship between control

treatments suggests that temperature stress during the outplanting process does not cause mortality. These findings may have resulted from our adoption of methods designed to minimize stress, such as the use of outplanting tubes that did not require the dislodgement of abalone at the time of outplanting. There was a significant difference in survivorship between tagged control abalone and untagged control abalone, suggesting that tagging may cause some mortality. The difference, however, was very small, with less than 3 % difference in survivorship between treatments. Although the stress experienced by abalone during the tagging, handling and outplanting procedures appears to cause very little mortality on its own, it is nevertheless possible that it makes them more vulnerable to predators, as suggested by Olla et al. (1998).

Conclusion

The key findings of this chapter are that: (1) predators are the main source of abalone outplant mortality, (2) *C. productus* and *P. helianthoides* rapidly congregate at outplant locations, and (3) high *C. productus* and *P. helianthoides* densities are associated with low outplant survivorship.

The methods adopted in this study successfully minimized the effects of tagging, handling and outplanting stress on abalone survivorship, and can accordingly be recommended for future outplanting attempts. Similarly, the cages used herein were successful in excluding predators, but a one week acclimation period within such cages does not appear to improve the survival of abalone after their release.

Given the ability of some *H. kamtschatkana* predators to congregate at outplant locations and the apparent relationship between *C. productus* density, *P. helianthoides* density and outplant survivorship, it is important to consider predator densities when selecting sites for outplanting in addition to features such as the presence of cobble, boulder and bedrock substrates and appropriate kelp communities.

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Chapter 5: Identifying the limitations of outplanting: Hatchery abalone behaviour

INTRODUCTION

The mortality of recently outplanted abalone is considerably higher than that of their wild counterparts (Schiel 1993; Rogers-Bennett and Pearse 1998; Sweijd et al. 1998; Dixon et al. 2006; Chapter 3), and predation is one of the major sources of mortality for outplanted abalone (Tegner and Butler 1985; Schiel and Welden 1987; Chapter 4). When organisms are raised in the absence of predators, as they are in hatcheries, learned predator recognition and avoidance behaviours may not be adequately expressed. Hatchery conditions can accordingly result in a lack of proper behaviour or the development of new behaviours which make organisms more vulnerable to predators than their wild counterparts (McCormick et al. 1994). Behavioural differences have been cited in a number of studies as the cause of increased vulnerability of outplanted abalone to predators in comparison with wild abalone (Schiel and Welden 1987; Tegner and Butler 1989; but see Tegner and Butler 1985). In 1987, Schiel and Welden determined that hatchery-raised red abalone (*Haliotis rufescens*) were slower to seek refuge when placed on artificial reefs, and therefore experienced higher predation than wild red abalone. An abalone's reflex reaction to disturbance involves firmly attaching itself to the substrate and drawing its shell down, but this behaviour is often delayed in hatchery-raised individuals (Tegner and Butler 1985; Schiel and Welden 1987). To some extent, the failure of hatchery-raised individuals to recognize and respond to threats after outplanting may be exacerbated by the shock involved in being transplanted into a new environment (Olla et al. 1998). Hatchery-raised red abalone are able to acclimatize, minimize their response times and even learn to respond more appropriately to predators (Schiel and Welden 1987). However, predators and scavengers that feed upon weak organisms may target outplant locations (Tegner and Butler 1985; Kiyomoto 2007; Chapter 4), and predation of outplanted abalone is often intense during the first hours and days after being outplanted to the field (Emmett and Jamieson 1988; Chapter 3). Thus if abalone lacking a refuge-seeking behaviour are directly outplanted to the field they may

not have sufficient time to learn predator avoidance behaviours before they are besieged by predators.

Abalone have an abundance of organs designed to detect threats. Notably, abalone have cephalic tentacles, eyes, statocysts, and a more elaborate epipodium than any other mollusc (Cox 1962). The latter is covered in epipodial tentacles that are both light and touch sensitive (Cox 1962). Abalone also have osphradia; these chemosensory organs, located in the mantle cavity, test the water that constantly filters over the gills, allowing the detection of upstream predators (Kohn 1961; Cox 1962; Szal 1971). The two principal responses of abalone to predators are often described as clamping and flight responses (Clark 1958). Clamping responses, which involve the cessation of movement and the clamping of the abalone's shell to the substrate, are often elicited by mechanical stimulation, including contact with crabs (Bullock 1953; Clark 1958; Knudsen 1960; Kohn 1961; Feder 1963; Montgomery 1967). In contrast, flight responses are often triggered by chemical cues such as asteroid saponins, and can be extremely vigorous and complex (Bullock 1953; Clark 1958; Kohn 1961; Feder 1963; Montgomery 1967).

Abalone behaviours can accordingly range from the simple extension, waving and retraction of their cephalic and epipodial tentacles, to the protraction, retraction, twisting, and clamping of the shell, and even include several gaits of movement, from slow crawling to galloping (Bullock 1953; Clark 1958; Feder 1963; Montgomery 1967; Voltzow 1986). Moreoever, an abalone that has the misfortune of being dislodged and landing on its shell can rapidly right itself (Minchin 1975; Sloan and Breen 1988; pers. obs.).

To reduce early mortality of outplanted northern abalone, it is necessary to understand whether behavioural anomalies are responsible for the increased vulnerability of these abalone and, if so, how the problem might be alleviated. The objectives of this study are therefore to (1) assess whether hatchery-raised northern abalone respond differently to shadows, movement, mechanical stimulation or contact with *Pycnopodia helianthoides* than wild northern abalone, (2) determine whether exposing hatchery-raised northern abalone to predator cues prior to outplanting leads to a (a) change in

behaviour, or (b) increase in their survivorship after outplanting, and finally (3) determine whether the abalone's potential to learn responses to predators changes with age.

METHODS

All experiments were conducted in the vicinity of Bamfield, on the west coast of Vancouver Island, BC, Canada, with laboratory experiments being conducted at the Bamfield Marine Sciences Centre.

Comparing wild and hatchery-raised abalone behaviours

The behaviours of abalone in response to three cues were recorded in both the laboratory and natural environment. Laboratory observations were conducted only on hatchery-raised northern abalone of two size classes: early-stage hatchery juveniles measuring 0.94 to 2.16 cm shell length (SL) (1 year old), and late-stage hatchery juveniles measuring 4.41 – 5.93 cm SL (4 years old). Abalone were placed in clear Sterilite® containers (30 cm x 15 cm x 10 cm), one individual per container. The sides of the containers had been cut out and replaced with mesh to allow for water flow, and the containers were held in a seawater table in which the water was maintained at a depth of 5 cm. Abalone were allowed to acclimatize to their container for 60 minutes prior to the start of the experiment. Treatment cues were first applied to the individual that was the furthest downstream; once the response of this individual was recorded, it was removed from the water table and the next individual, one cage upstream, was exposed to the cue. In a pilot experiment, it was found that upstream abalone never reacted to downstream cues; this setup therefore allowed us to increase sample size over a limited period of time. The three treatments, applied to haphazardly selected abalone, consisted of: (a) removal of the container's lid, no other cue applied (control; n=36 small juveniles and 32 large juveniles), (b) lid removed, then touching the abalone with a probe for 30 seconds (n=36 small juveniles and 45 large juveniles), and (c) lid removed, then touching the abalone with *Pycnopodia helianthoides* for 30 seconds (n=42 small juveniles and 36 large juveniles). Treatments *b* and *c* were always applied to the posterior end of the abalone (Figure 31). The timing and type of reactions of abalone during the three minutes

following application of the cue were then recorded. Each occurrence of the following reactions was recorded (see Figure 31 for details of abalone anatomy): extension of the cephalic tentacles, extension of the epipodium and associated tentacles, mushrooming, clamping of the shell, twisting of the shell, release of a viscous fluid, and rate of movement (slow or fast). For a description of the behaviours observed see Table 9. At the conclusion of each trial, the abalone's shell length was measured to the nearest 0.01 cm using vernier calipers.
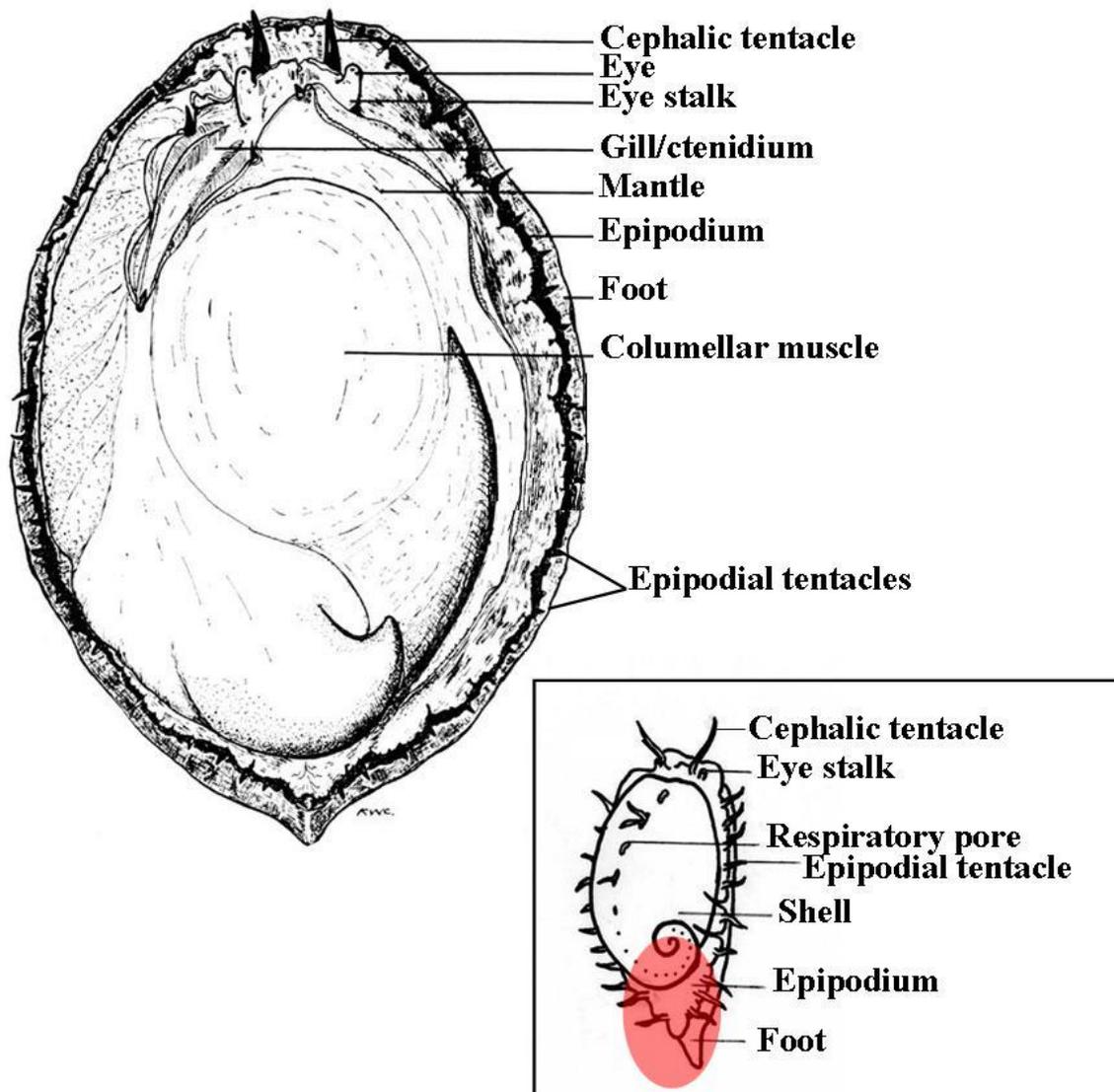
Figure 31. Gross anatomy of an abalone. Adapted from Cox (1962) and Montgomery (1967). The inset shows the anatomy of an abalone with shell intact, whereas the principal figure gives a dorsal view of an abalone with the shell removed. The shaded circle in the inset shows the posterior region which was stimulated with mechanical and chemosensory cues.

Table 9. Description of northern abalone behaviours

Behaviour	Description	Illustration
Epipodium extended	The epipodium and associated tentacles are extended beyond the margin of the shell	Figure 32*a*
Cephalic tentacles extended	Cephalic tentacles are extended and wavering	na
Mushrooming	The foot contracts into a columnar shape, raising the shell	Figure 32*b*
Slow movement	The abalone moves slowly, the entire margin of the foot appears to remain in contact with the substrate	na
Fast movement	The abalone moves quickly, the entire margin of the foot appears to remain in contact with the substrate	na
Galloping	The abalone moves rapidly, waves of muscle contraction are apparent along the margin of the foot, and the abalone appears to be rocking	na
Clamping	The epipodium and cephalic tentacles are retracted and the shell is pulled down to the substrate	na
Twisting	The shell is lifted from the foot and twisted back and forth almost 180 degrees	Figure 32*c*
Fluid	The abalone expels a viscous fluid from the respiratory pores	na
"Tail" over shell	The posterior end of the foot is raised onto the shell and swept around the shell's perimeter	Figure 32*d*
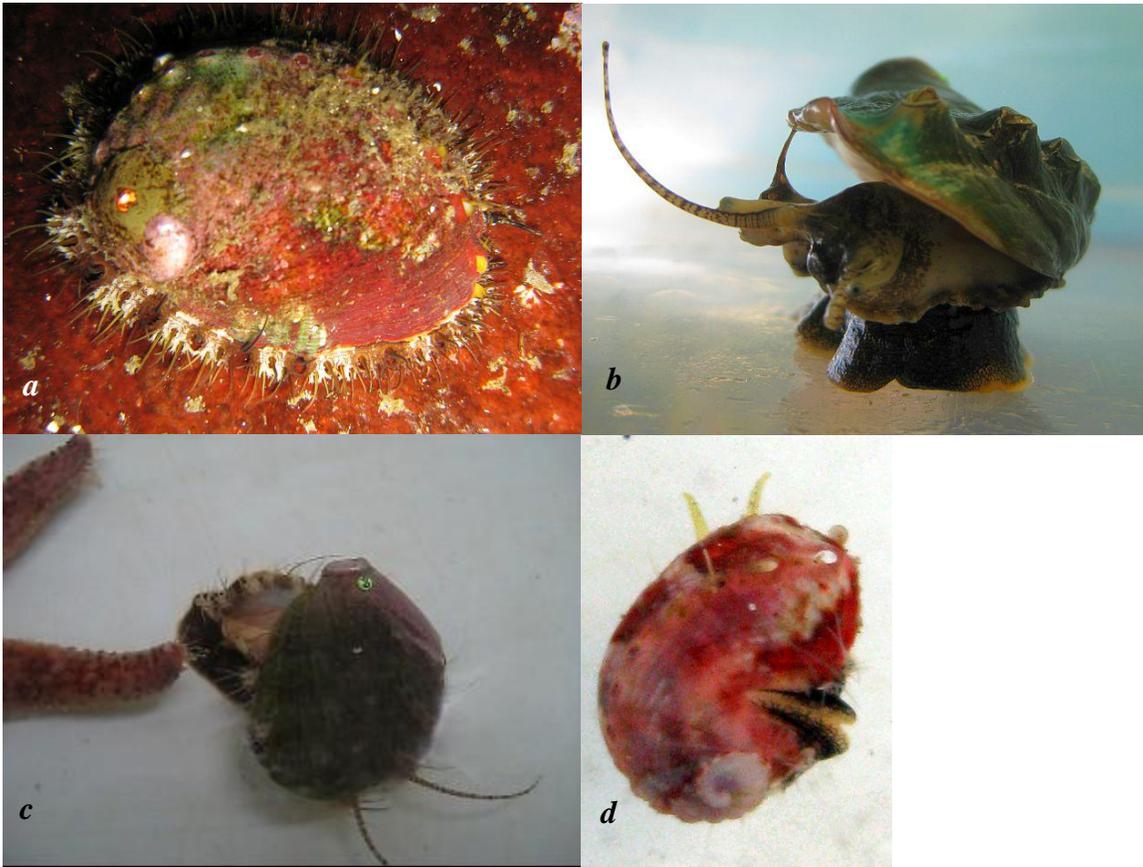
Figure 32. Northern abalone behaviours. *(a)* Extension of epipodium and associated tentacles, *(b)* mushrooming, *(c)* twisting, and *(d)* "tail" over shell.

A second experiment, to compare the responses of wild and outplanted (hatchery-raised) abalone, was carried out in the natural environment. The site used for the predator exclusion experiment, described in Chapter 4, was searched for abalone on 5 occasions over a period of two weeks, approximately 1 month after abalone had been outplanted for that experiment. Every abalone that was found was haphazardly assigned one of the three treatments, and the treatment was applied to the individual *in situ*. In this experiment we therefore recorded observations for a range of sizes of abalone and for both wild and hatchery-raised individuals. One diver searched for abalone while a second followed,

prepared to apply a cue. The three treatments were: (a) displacing rocks and hovering near the abalone (n=36 wild abalone and 27 outplanted abalone), (b) touching the abalone with a probe for 30 seconds (n=15 wild abalone and 20 outplanted abalone), and (c) touching the abalone with *P. helianthoides* for 30 seconds (n=12 wild abalone and 12 outplanted abalone). In this experiment, we also recorded the time and type of reaction over a 3 minute period following application of the treatment. Treatment *a* served as a control for the effects of the contact cues of treatments b and c. In addition, since some abalone were found emergent on rocks while others were found by turning rocks over, the control also revealed the effect of habitat disturbance without directly contacting the abalone. After each trial, the abalone was measured with vernier calipers and its origin (i.e. whether hatchery-raised or wild) was recorded. Hatchery-raised individuals could be distinguished from wild individuals by their shell colouration, and in many cases, by the retention of tags from the predator exclusion experiment.

The reactions observed in the laboratory experiment and in the field experiment were later graded on a numerical scale ranging from 0 (no reaction) to 8 (strongest reaction; see Table 10). Reaction times were taken into account in this scale because they have been shown to be good measures of the intensity of responses of marine invertebrates to predators (Legault and Himmelman 1993). A three-way ANCOVA, with reaction grade as the response variable, was used to elucidate the factors influencing abalone behaviour. The predictor variables in this analysis are cue treatment (fixed; 3 treatment groups), type of abalone (fixed; 4 types of abalone), and position (fixed; 2 positions), and the covariate is abalone shell length. I also noted every case in which an abalone responded to a cue by raising the posterior end of its foot over the shell and sweeping it back and forth, as this was an unexpected reaction.

Table 10. Scale for grading northern abalone reactions	
Assigned value	Behaviours
0	No reaction
1	Epipodial tentacles extended
2	Cephalic tentacles extended
3	Slow movement after 30 seconds, or slow and short movement
4	Movement within the first 30 seconds, or delayed but rapid movement (not galloping)
5	Galloping or twisting after 60 seconds, or immediate clamping
6	Galloping or twisting between 30-60 seconds after the cue
7	Galloping or twisting between 10-30 seconds after the cue
8	Galloping or twisting within 10 seconds of the cue

Influence of predator cues on hatchery-raised abalone behaviour and survival

This experiment, delving into the ability of hatchery-raised northern abalone to learn predator avoidance behaviours, was carried out in outdoor seawater tanks at the Bamfield Marine Sciences Centre. Tanks were located outdoors under an awning and were selected for equivalent levels of ambient light in all tanks. Sixteen 62.5 L clear Sterilite® containers were distributed among six tanks and served as aquaria. Rocks of variable size with crustose coralline algal growth were collected from abalone habitat at Sanford Island (Figure 33) and stacked within each aquarium so as to include plastic and rock substrates and provide cryptic and exposed habitat. Twenty-five hatchery-raised abalone, with a mean size of 5.30 ± 0.02 cm SL, were placed in each aquarium. Flow to the tanks was set at a moderate rate, tanks were covered with black mesh to reduce light levels and abalone were given 24 hours to acclimatize to their new surroundings. After this acclimatization period, the aquaria were randomly assigned one of eight treatments. The experiment involved cues from two predators, *P. helianthoides* and *Cancer*

productus, and four treatments per predator species: (i) control, (ii) scent of unfed predators, (iii) scent of predators feeding on abalone, and (iv) physical presence of predators near, but not in contact with, the abalone. All treatments involved rerouting the seawater source through a cue container and then into the aquarium containing abalone (Figure 34). In the case of odour treatments (ii and iii), the appropriate predator was placed in the cue container with or without an accompanying abalone. The cue container was empty in treatments i and iv. In treatment iv, a predator was placed in a mesh bag within the aquarium itself.
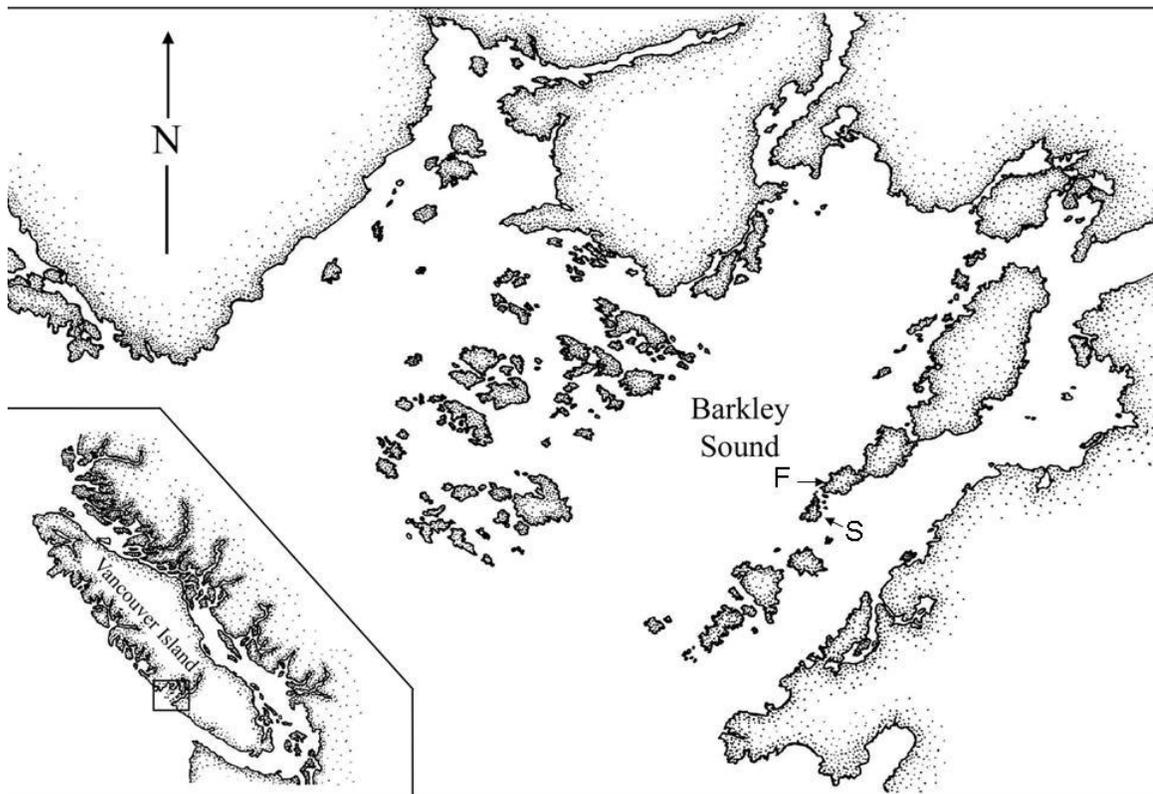
Figure 33. Map of study sites in Barkley Sound, located on the west coast of Vancouver Island (inset). Rocks were collected from Sanford Island (S) to construct reefs in laboratory aquaria. Behavioural abalone were outplanted at a site on Fleming Island (F). Adapted from Gosselin and Chia (1995).
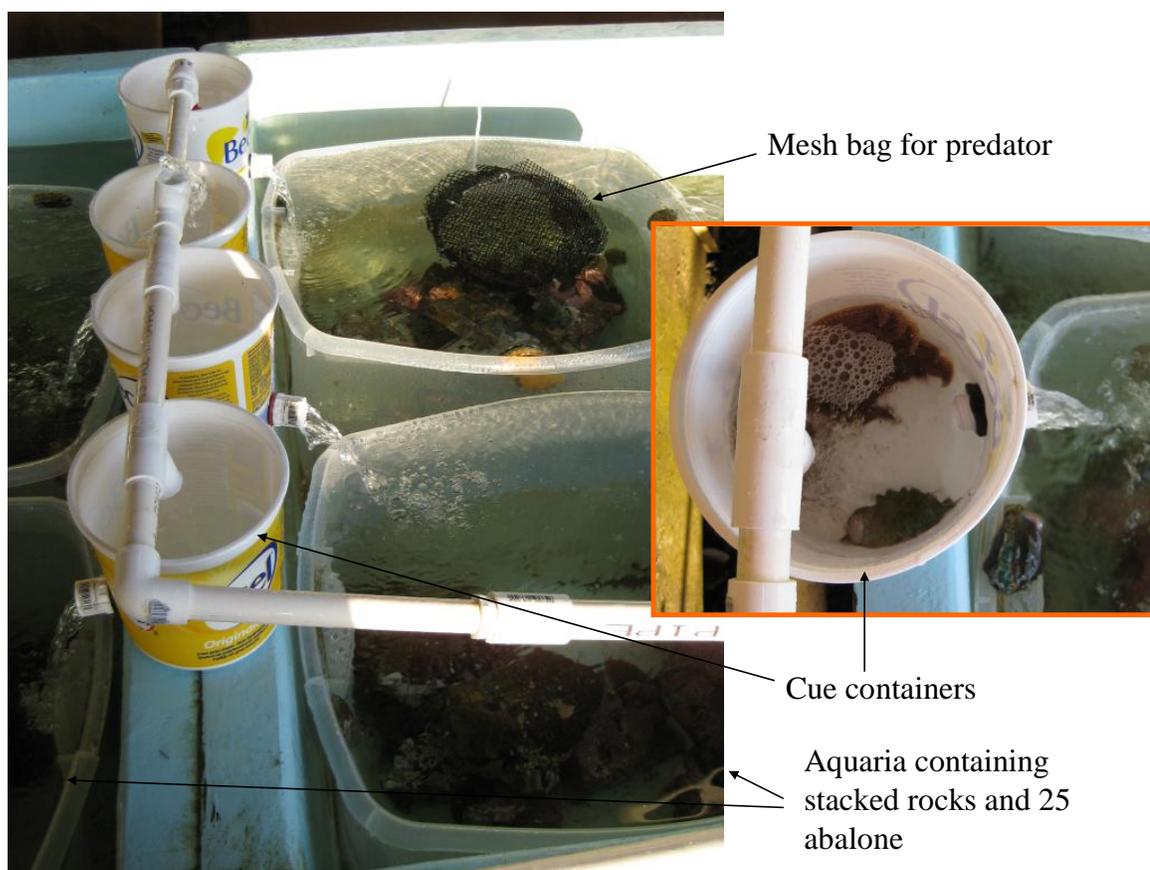
Figure 34. Water flow during cue application. Water is directed into the cue containers, from which it flows into the appropriate Sterilite aquarium. Here you see two aquaria sitting in two larger outdoor tanks. The inset shows a cue container with *P. helianthoides* and a northern abalone individual (the *P. helianthoides* feeding cue).

I recorded the positions (cryptic or emergent), substrate (rock or plastic) and behaviour of abalone immediately prior to the start of each trial and then again after applying a cue for one hour. The cues were then removed and the tanks were covered and left undisturbed for 24 hours before the process was repeated with the same cues being applied again to the same groups of abalone. These trials were conducted daily over a period of six days to determine if abalone progressively change their responses with repeated exposure to the cues. Abalone were fed at night on three occasions: during the

initial 24-hour acclimatization period, on the third day of the experiment after trials were completed, and on day 6 after this first phase of the experiment was completed. Four separate three-way factorial ANOVAs were used to determine if behaviour changed during the course of this experiment. Notably, the response variables in these four analyses were: (1) proportion of abalone occupying cryptic space, (2) proportion of abalone occupying rock substrates, (3) proportion of abalone on the edge of the tank (i.e. attempting to escape), and (4) proportion of abalone rearing. The predictor variables were the same in all four analyses, and are as follows: cue treatment (fixed; 7 treatment groups), time (fixed; 2 times), and day of experiment (fixed; 6 days). In the first ANOVA, with proportion cryptic as the response variable, all of the interaction terms were found to be non-significant and were therefore removed. Tukey's HSD tests were used as a post-hoc test to identify the nature of the differences identified in the ANOVA.

On day 7, after the abalone had been exposed to treatment cues for six consecutive days, the abalone were outplanted at Fleming Island (Figure 33). At this site, two locations at 10 m depth each received seven sets of 25 abalone (one set from each cue treatment) from the laboratory experiment. The survival of these abalone was monitored via circular-swath surveys 1, 3, and 14 days after outplanting. Circular swath survey methods are described in detail in Chapter 3. The Jolly-Seber method was used to calculate the survival rate of outplanted abalone over the first interval (from outplanting until the one-day survey), and the Barker model was used to estimate recapture probabilities and site fidelity of abalone at all survey times (see Chapter 3 for detailed methods).

Ontogenetic changes in behaviour and learning

To assess whether predator-avoidance behaviours change with age, the above predator cue trials were repeated with younger hatchery-raised juveniles. These had a mean shell length of 1.22 ± 0.03 cm and were approximately 1 year old. Early stage juvenile abalone feed upon diatom films, crustose coralline algae and other benthic microalgae (Sloan and Breen 1988; Tutschulte and Connell 1988; Wood and Buxton

1996; Day and Branch 2002), and were therefore not fed *N. leutkeana* during the experimental period. However, tanks were prepared three days in advance of the experiment so that a diatom film was available to abalone upon transfer into these tanks.

Fewer rocks were made available to the small juvenile abalone as their small size and shell colour made them harder to locate than the larger juveniles, as such there was less surface area that had to be searched for these cryptic individuals. Even so, enough rock was provided that all individuals could occupy any of the habitat types if they so chose. The tanks were randomly assigned to each of the 7 treatments (2 predator species x 3 cues per predator and 1 control), although this time the two predators used were *P. helianthoides* and the crab *Lophopanopeus bellus*. This small crab was chosen in place of *C. productus* because it is a more important predator of small juvenile northern abalone (Griffiths 2006). Fifteen abalone were used per treatment. A three-way factorial ANOVA was used to assess the behaviours of small juvenile abalone for each of the two response variables, which were: (1) the proportion of small juveniles occupying cryptic spaces, and (2) the proportion of juveniles occupying rock substrates (rather than the walls of the plastic containers). The predictor variables were the same for both ANOVAs and consisted of: the cue treatment (fixed; 7 treatment groups), time (fixed; 2 times), and day of experiment (fixed; 6 days). None of the interaction terms in these ANOVAs were significant, thus the ANOVAs were carried out a second time without the interaction terms. Tukey's HSD tests were used as a post-hoc multiple comparisons test. The survival of small juveniles was not assessed post-outplanting due to the very low recapture probabilities associated with field surveys of abalone belonging to this size group (see Appendix E).

Independent samples t-tests (n=380) were used to compare the predator avoidance strategies of large abalone with that of small juvenile abalone. The two response variables analyzed were: (1) proportion occupying cryptic space, and (2) proportion occupying rock substrates. Although the experiments with large and small abalone were run at different times and the two abalone size classes were exposed to a different crab predator, I believe the comparison is nevertheless valid because all other

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factors and aspects of the methods were the same in both experiments, and conditions at the times of the two experiments were similar.

RESULTS

Comparing wild and hatchery-raised abalone behaviours

The reactions of abalone, graded from 0 to 8 (no reaction to strong reaction), were found to be significantly influenced by an interaction between the type of abalone and the cue being applied (ANCOVA: $F_{6,17}=3.974$, $p=0.011$). This interaction resulted because small juvenile hatchery abalone responded more strongly to the control and being prodded with an unscented probe than large hatchery abalone in the lab or the field (Figure 35). Similarly, wild abalone responded more strongly to these two cues than large hatchery-raised abalone (Figure 35). Interestingly, there was no difference in reaction grade between hatchery juveniles and wild individuals, nor between the two groups of large hatchery-raised abalone, be they in the lab or the field (Figure 35). Reactions were strongest in response to *Pycnopodia helianthoides,* moderate in response to the control (removal of lid or movement of rocks) and weakest in response to being prodded with an unscented probe (Figure 35). Emergent hatchery-raised abalone in the wild did not respond to nearby disturbances or to being prodded, while their cryptic counterparts did (Figure 35). However, this interaction was not detected statistically (ANCOVA: $F_{1,17}=0.878$, $p=0.372$).
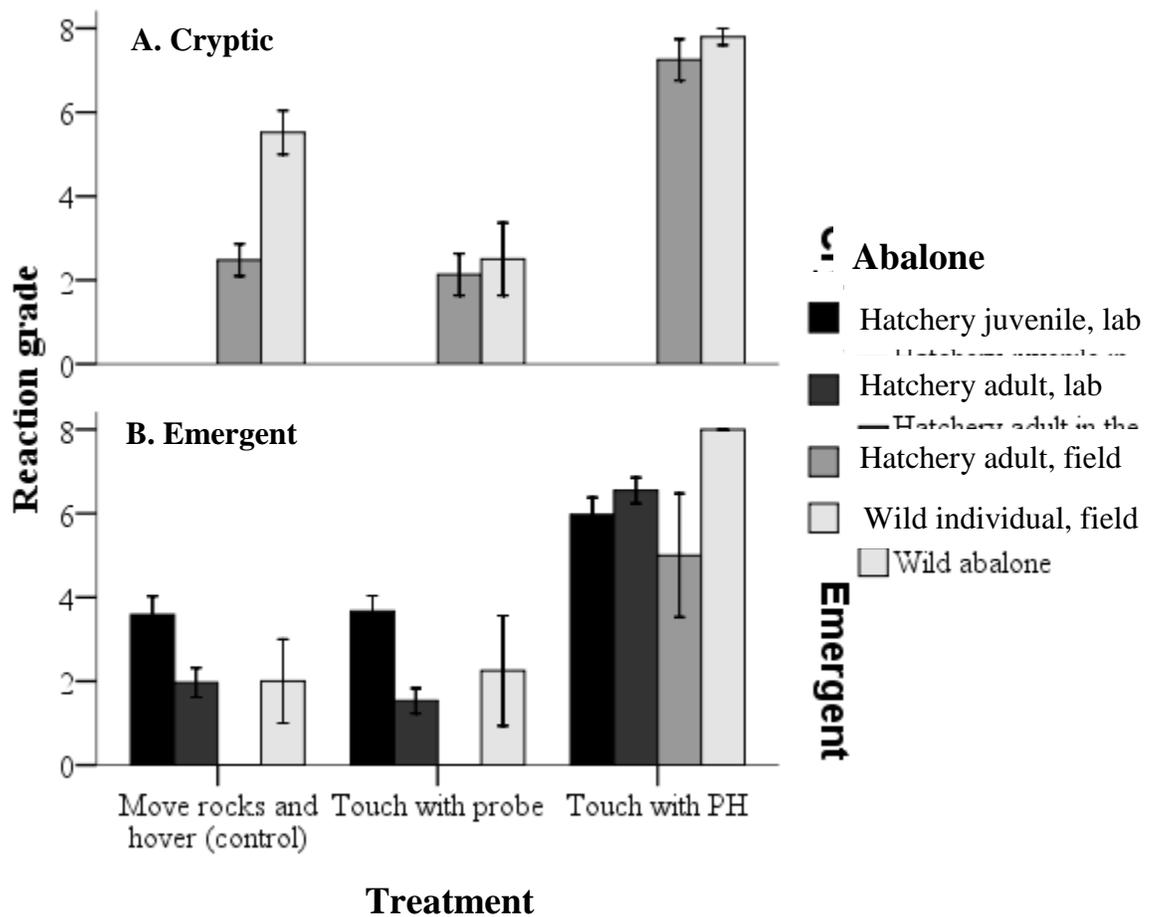
Figure 35. Reactions of different groups of northern abalone in response to three cues when their initial position is either (a) cryptic or (b) emergent. PH is shorthand for *P. helianthoides*. There are no laboratory observations for cryptic abalone. Error bars are ± 1 SE.

In some cases, hatchery-raised abalone responded to stimulation with *P. helianthoides* by raising the posterior end of their foot and sweeping it over the surface of their shell. This behaviour was only ever observed in hatchery-raised individuals in the laboratory environment. Four individuals from the small juvenile size class (n=42), representing 9.5 % of this group, responded in this way, as did four individuals from the adult size class, equivalent to 11.1 % (n=36).

Influence of predator cues on hatchery-raised abalone behaviour and survival

The proportion of large juvenile abalone occupying cryptic spaces among the rocks in the aquaria (34.8 ± 1.3 %) was significantly influenced by the cue treatment applied ($F_{6,95}=9.483$, $p<0.001$) and the day of the trial (ANOVA: $F_{5,95}=8.812$, $p<0.001$), but not the time of the observation, be it before or during cue application ($F_{1,95}=0.141$, $p=0.708$). In fact, the proportion of cryptic abalone was highest in the *P. helianthoides* presence treatment and lowest in the *P. helianthoides* scent feeding and control treatments (Figure 36). In addition, across all treatments, the proportion of cryptic individuals changed during the course of the experiment, initially increasing up to day 3, then decreasing to slightly lower values (Figure 37).
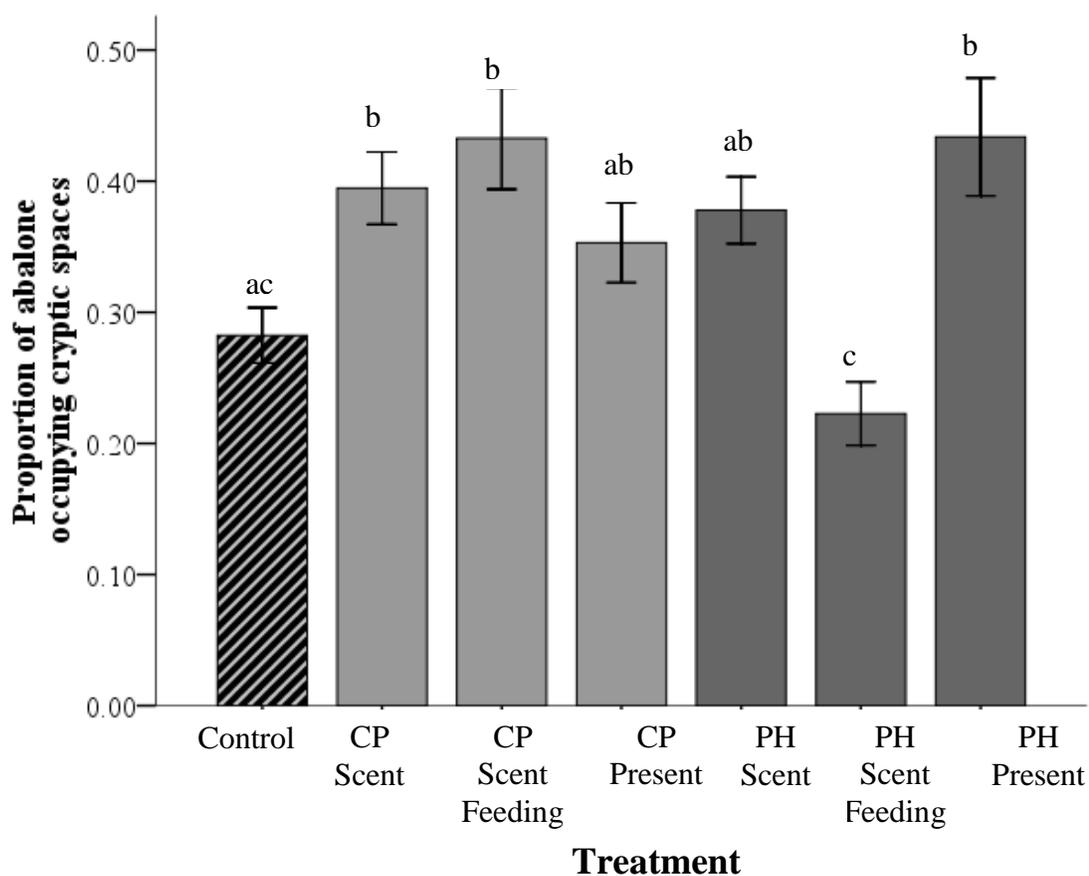
Figure 36. Mean proportion of abalone occupying cryptic positions in each of the seven treatment groups. The treatments are: control, no predator cue; scent, with scent of the predator only; scent feeding, with scent of the predator feeding upon abalone, and present, indicating a predator was present within the tank. The two predators are *C. productus* (CP) and *P. helianthoides* (PH). Error bars are ± 1 SE (pooling across day and time, thus n=48 for the control and 24 for all other treatments). Letters above error bars indicate significant differences between treatments, as determined by Tukey's HSD test.
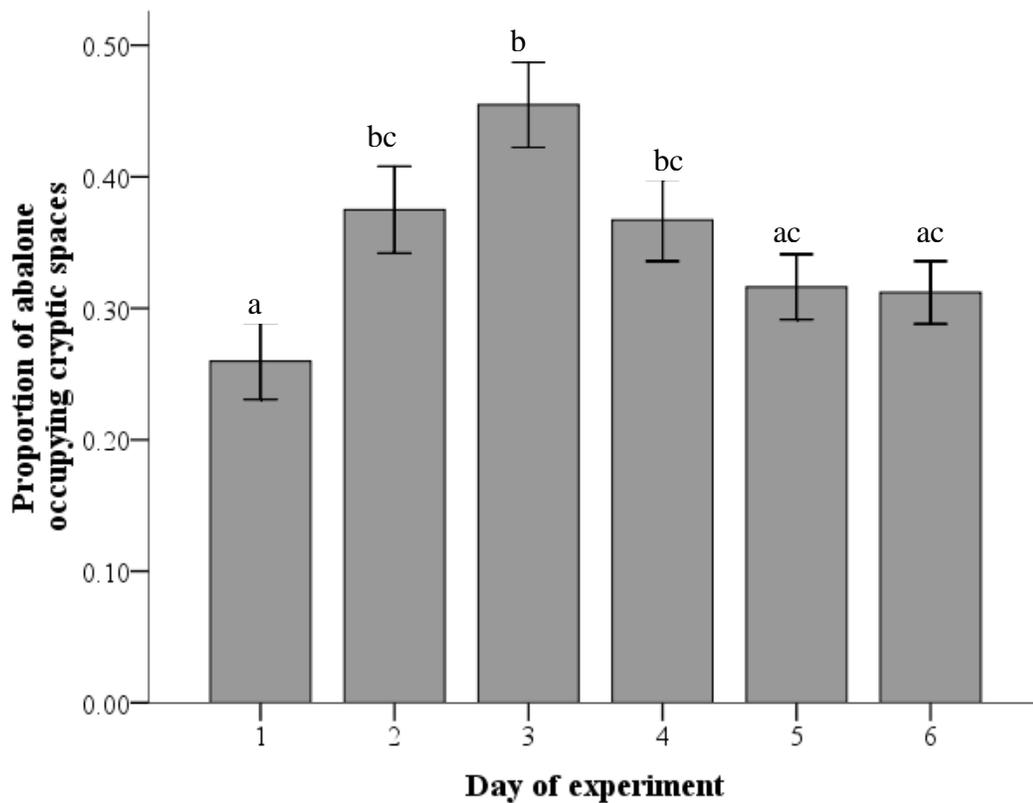
Figure 37. Mean proportion of abalone occupying cryptic positions over the course of the experiment. Error bars are ± 1 SE (pooling across treatments and time, thus n= 32). Letters above error bars indicate significant differences between the days of the experiment, as determined by Tukey's HSD test.

As for the proportion of abalone attached to rocks (5.2 ± 0.1 %) and plastic substrates (94.8 ± 0.1 %), there was a significant three-way interaction between the day of the experiment, the time of the observation and the cue treatment ($F_{30,95}$=5.451, p<0.001). Overall, however, abalone appeared to prefer plastic substrates, since the percentage found on rock was always lower than 15 % (Figure 38), and the surface area available on each substrate was approximately equivalent.
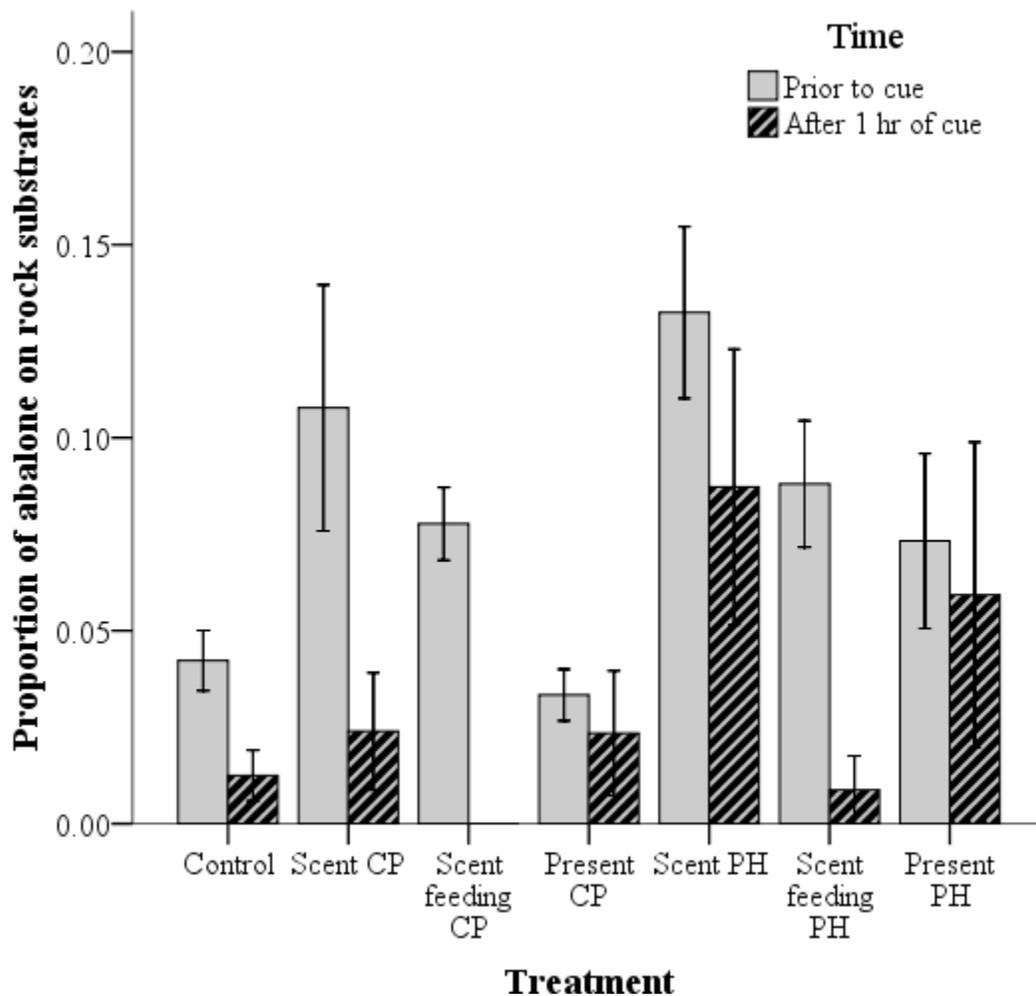
Figure 38. Mean proportion of abalone occupying rock substrates in each of the seven treatment groups both before and during cue application. The treatments are: control, no predator cue; scent, with scent of the predator only; scent feeding, with scent of the predator feeding upon abalone, and present, indicating a predator was present within the tank. The two predators are *C. productus* (CP) and *P. helianthoides* (PH). Error bars are ± 1 SE (pooling across day, thus n=24 for the control and 12 for all other treatments).

The proportion of abalone found on the edge of the tank (i.e. attempting to crawl out of the water and out of the tank) was significantly influenced by an interaction between the day of the experiment, the time of the observation and the cue treatment

(ANOVA: $F_{30,95}=3.218$, $p<0.001$). This interaction simply reflects the fact that abalone responded to the *P. helianthoides* scent feeding cue by attempting to climb out of the water (Figure 39), especially on the first day of the experiment.
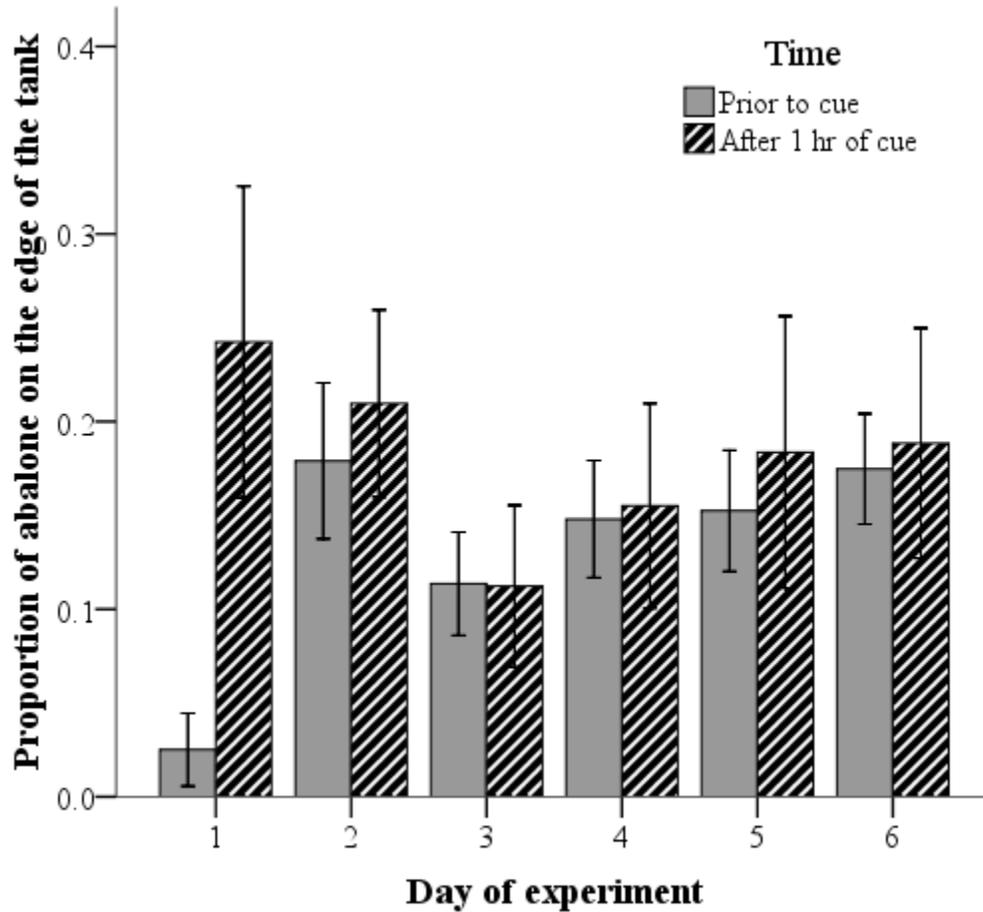
Figure 39. Mean proportion of abalone on the edge of the tank (i.e. attempting to escape) over the course of the experiment, both before and during cue application. Error bars are ± 1 SE (n=16).

Finally, the proportion of abalone rearing (i.e. adopting a feeding posture) was significantly influenced by an interaction between the day of the experiment, the time of the observation and the treatment (ANOVA: $F_{30,95}=2.798$, $p<0.001$). This interaction

reflects the fact that abalone only reared after cue application on the first day of the experiment in the *P. helianthoides* scent treatment. The only other incidences of rearing occurred prior to cue application on the fifth and sixth days of the experiment (Figure 40). Note that the highest incidence of rearing involved only 3 % of all abalone.
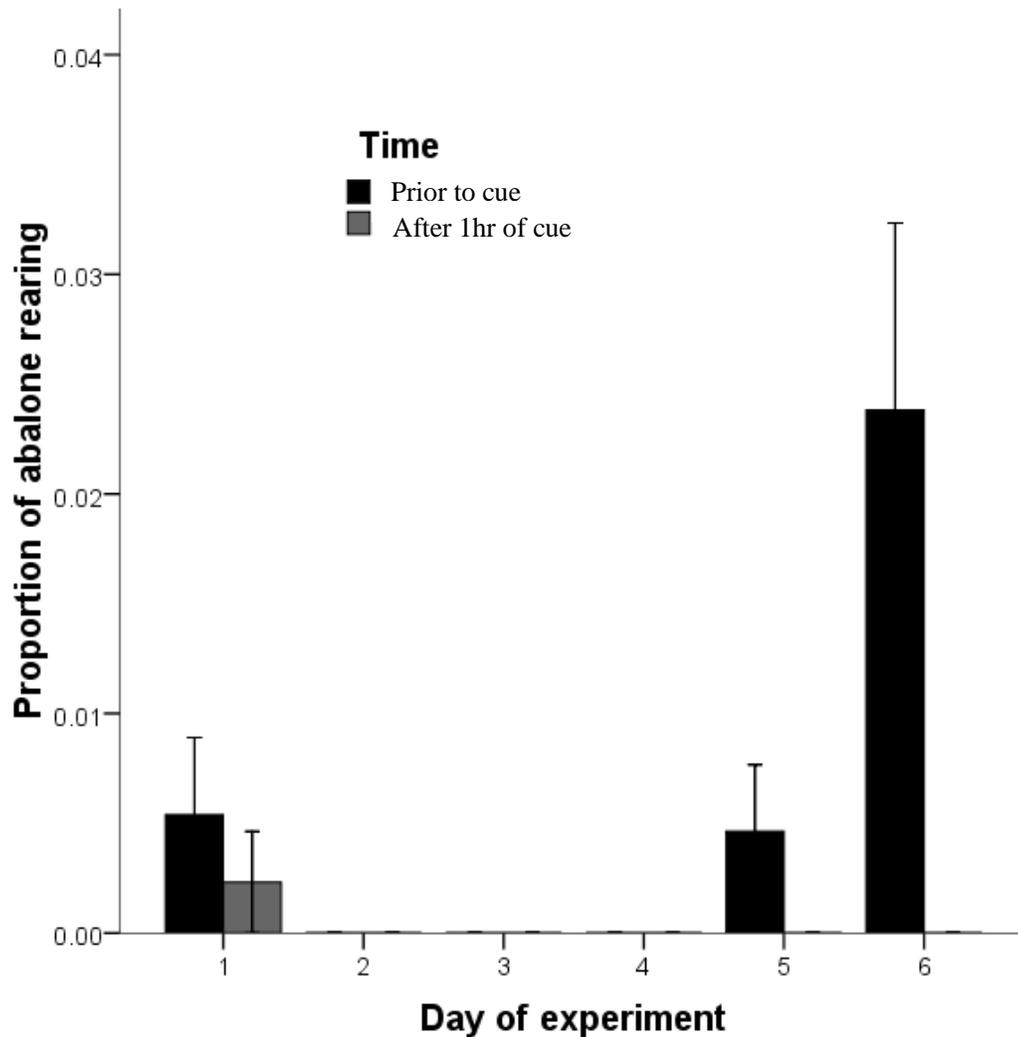
Figure 40. Mean proportion of abalone rearing over the course of the experiment, both before and during cue application. Error bars are ± 1 SE (n=16).

Estimated survival over the first 24 hours after outplanting appeared to be lowest for abalone exposed to *C. productus* scent feeding treatment and highest for those exposed to *C. productus* presence. However, due to large error terms in estimating survival, there are no significant differences in the survival of abalone between treatments (Table 11).

Table 11. Jolly-Seber estimates of abalone survival over the first 24 hours since outplanting, for abalone exposed to different predator cues in the laboratory. *S* is the estimated survival, L SE is the lower standard error and U SE is the upper standard error.

Predator	Treatment	*S*	L SE	U SE
	Control	0.765	0.067	0.128
C. productus	Scent	0.665	0.040	0.094
C. productus	Scent feeding	0.451	0.004	0.246
C. productus	Present	0.906	0.059	0.047
P. helianthoides	Scent	0.812	0.067	0.052
P. helianthoides	Scent feeding	0.785	0.016	0.108
P. helianthoides	Present	0.700	0.018	0.150

The variability associated with site fidelity and recapture probability estimates was extremely large, and no differences exist between treatments for these parameters (Table 12).

Table 12. Recapture probabilities and site fidelities of outplanted abalone exposed to different predator cues. Recapture probabilities are denoted p, site fidelity is F, and standard errors are SE.

		Time (days)					
		1		3		14	
Predator	Treatment	p	SE	p	SE	p	SE
	Control	0.866	0.356	0.719	0.347	0.444	0.394
C. productus	Scent	0.903	0.193	0.829	0.203	0.705	0.296
C. productus	Scent feeding	0.661	0.226	0.523	0.295	0.515	0.303
C. productus	Present	1.000	0.000	1.000	0.000	1.000	0.000
P. helianthoides	Scent	0.973	0.155	0.930	0.162	0.838	0.251
P. helianthoides	Scent feeding	0.870	0.220	0.735	0.366	0.697	0.413
P. helianthoides	Present	0.767	0.216	0.767	0.216	0.767	0.216
		F	SE	F	SE	F	SE
	Control	0.806	0.331	0.764	0.281	0.686	0.250
C. productus	Scent	0.693	0.191	0.615	0.137	0.503	0.157
C. productus	Scent feeding	0.868	0.280	0.724	0.277	0.770	0.267
C. productus	Present	0.684	0.075	0.387	0.096	0.237	0.115
P. helianthoides	Scent	0.665	0.086	0.665	0.086	0.665	0.086
P. helianthoides	Scent feeding	0.834	0.165	0.492	0.371	0.565	0.337
P. helianthoides	Present	0.650	0.138	0.650	0.138	0.650	0.138

Ontogenetic changes in behaviour and learning

The proportion of small juvenile abalone found in cryptic positions on the artificial reefs was significantly influenced by treatment (ANOVA: $F_{6,95}=2.781$, $p=0.016$). Notably, there were fewer abalone occupying cryptic space in the *P. helianthoides* scent treatment than either the control (Tukey's HSD test: $p=0.017$) or *P. helianthoides* scent feeding treatment (Tukey's HSD test: $p=0.017$; Figure 41). On average, 31.7 ± 0.0 % were found in cryptic positions (68.3 ± 0.0 % were exposed).
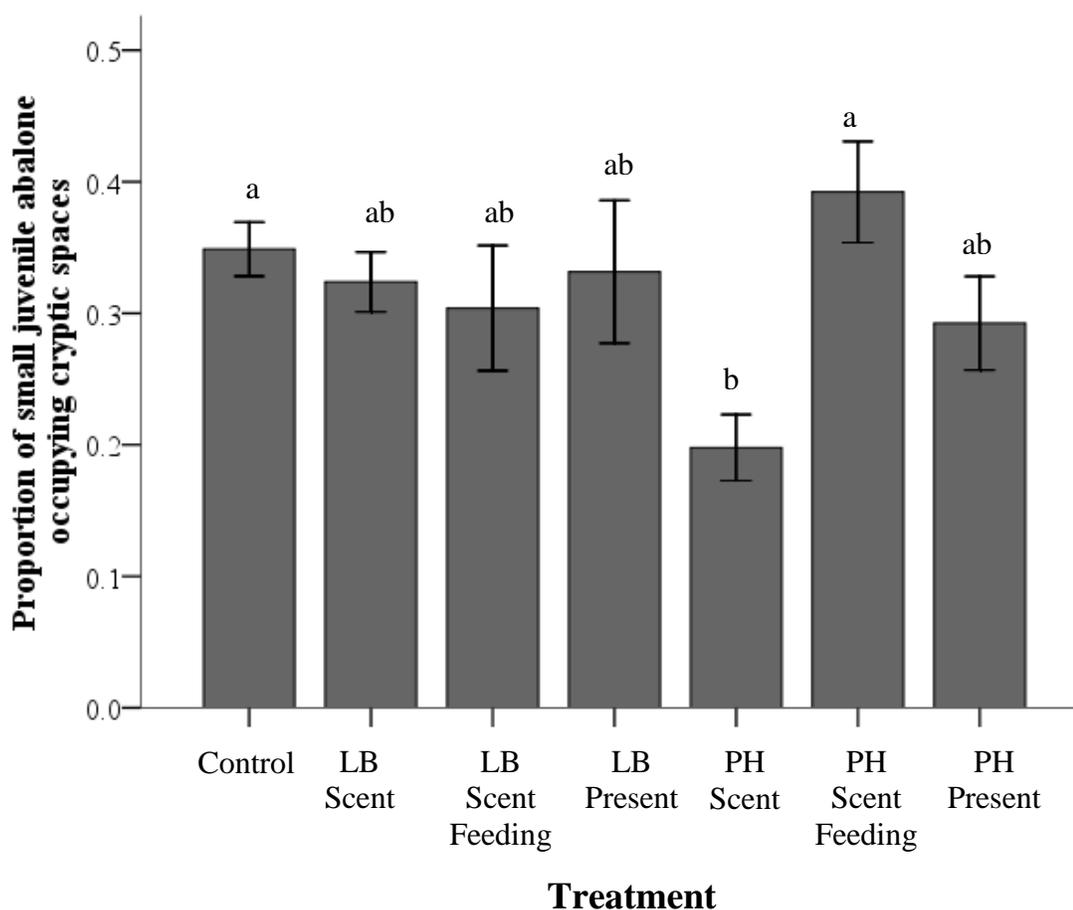
Figure 41. Mean proportion of small juvenile abalone occupying cryptic spaces in the seven different predator treatments. The treatments are: control, no predator cue; scent, with scent of the predator only; scent feeding, with scent of the predator feeding upon abalone, and present, indicating a predator was present within the tank. The two predators are *L. bellus* (LB) and *P. helianthoides* (PH). Error bars are ± 1 SE (n=24 for the control and 12 for all other treatments).Letters above error bars indicate significant differences between the days of the experiment, as determined by Tukey's HSD test.

Small juveniles preferred rock substrates to the plastic alternative, with 77.7 ± 0.0 % being attached to rocks over the course of the experiment. The proportion of small juveniles found on rock substrates was significantly influenced by the cue treatment (ANOVA: $F_{6,95}=9.620$, $p<0.001$). Indeed, there were fewer individuals to be found on rock in the *L. bellus* scent treatment than in any other treatment (Tukey's HSD test: $p<0.001$). There was no time dependence to these responses (over the 6 days of the

experiment), and they were equivalent both before and during cue application. If substrate preferences differed between treatments but not times, it follows that this preference was not learned as a result of the experiment, but was innate and expressed only in response to certain cues. This in turn suggests that early-stage juveniles did not learn during this experiment

The reactions of early and late stage juveniles to predator cues in the laboratory experiment showed both similarities and discrepancies. The proportion occupying cryptic space did not differ between the two age groups, with 31.7 ± 1.3 % of small juveniles and 34.8 ± 1.3 % of large juveniles occupying cryptic spaces (Independent samples t-test: $t_{378}=1.387$, $p=0.166$). However, rearing and moving to the edge of the tank were reactions that were only ever expressed by late-stage juveniles. Abalone age also influenced substrate choice. Significantly fewer large juvenile abalone occupied rock substrates than their small counterparts (5.15 ± 0.06 % as compared to 77.69 ± 0.94 %, respectively; Independent samples t-test: $t_{378}=48.907$, $p<0.001$).

DISCUSSION

Comparing wild and hatchery-raised abalone behaviours

The behaviours of hatchery-raised northern abalone differed from those of wild abalone both before and after the former were outplanted, but not in response to all three cues tested. In fact, contrary to our expectations, the majority of hatchery-raised abalone responded to stimulation with *P. helianthoides* in the same manner as, and with equal intensity to, wild abalone. This finding is not consistent with the hypothesis that abalone raised in the absence of predators are entirely naïve. Nevertheless, the response of young juveniles from the hatchery to nearby movement appeared somewhat subdued relative to wild abalone (not significantly so), and the reactions of older hatchery abalone were significantly subdued compared to wild individuals. Those discrepancies appear to have resulted from habituation in the hatchery environment, not from the absence of learning. This suggests that abalone become accustomed to nearby movement over time in the hatchery, possibly because hatchery personnel moved along the tanks daily to remove

any deceased individuals and bi-weekly to feed the abalone. One might therefore expect that hatchery abalone perceive nearby movement as benign or even associate it with the arrival of food. The displacement of nearby rocks in the natural environment might normally foreshadow the arrival of *C. productus*, given that these predators can shift rocks to gain access to cryptic abalone (Schiel and Welden 1987). Similarly, shadows could warn abalone of the approach of any motile predator. Thus the habituation of hatchery-raised abalone to nearby movement or shadows is expected to be detrimental to their survival after outplanting.

The category of abalone that responded most strongly to stimulation with an unscented probe was that of small hatchery juveniles. Large hatchery abalone demonstrated significantly less reaction in response to this cue. The responses of wild abalone to an unscented probe spanned a range of reaction grades, yet reaction grades in response to this cue were not related to the shell lengths of wild abalone. That being said, the smallest wild abalone stimulated with an unscented probe was 3.93 cm long, considerably larger than the hatchery juveniles which responded strongly to this cue (1.22 ± 0.03 cm). Given that juvenile abalone are particularly vulnerable to small crab predators such as *S. acutifrons* and *L. bellus* until these abalone reach a size of 1.2 to 1.3 cm shell length (Griffiths and Gosselin 2008), it is possible that we failed to detect size-dependence among wild abalone because all individuals tested were above the 1.3 cm SL size refuge. The strong reactions to mechanical stimulation at small sizes are likely an adaptive response to crabs which are efficient predators of small abalone. There is a precedent for this assumption in the literature, as predator avoidance behaviours have been proven to be most developed when risk of predation is high (Sih 1987; Legault and Himmelman 1993). Moreover, Montgomery (1967) has also shown that predator avoidance reactions of abalone are stronger among small individuals. Hence, as abalone grow and their vulnerability to certain predators decreases, they modify their escape and avoidance behaviours. Our study provides evidence of an ontogenetic shift in behaviour which occurs even among abalone held in the hatchery environment, and which is consistent with an ontogenetic shift in susceptibility to predators.

Emergent hatchery-raised abalone failed to react to mechanical stimulation altogether. The reactions of cryptic hatchery abalone, cryptic wild abalone and emergent wild abalone were approximately equivalent. A failure to exhibit a flight response when stimulated with an unscented probe is not unprecedented, even among wild individuals. Notably, Montgomery (1967) found that wild adult *H. assimilis* and *H. rufescens* responded to this stimulus simply by retracting their tentacles and clamping down. We observed both clamping and flight responses among wild *H. kamtschatkana* that were prodded. The failure to react to which we refer is not only a failure to flee, but also a failure to clamp. Hence emergent hatchery abalone in this study were aberrant both relative to their cryptic hatchery counterparts, their cryptic and emergent wild counterparts, as well as relative to other abalone species (Clark 1958; Montgomery 1967). It is possible that whatever mechanism prompts an abalone to adopt an emergent lifestyle is associated with a failure to recognize tactile stimuli as threatening in hatchery-raised abalone. An alternative explanation is that some abalone learn and adopt behaviours such as crypsis upon outplanting, while other abalone do not learn any appropriate responses and therefore remain exposed. Whatever the cause of the emergent hatchery abalone's nonchalance, it is a cause for concern. In fact, incidents of predation upon abalone are not solely dependent upon the abundance of abalone relative to other prey items, but are also moderated by the relative intensity of their escape responses (Hines and Pearse 1982; Legault and Himmelman 1993). In other words, we can expect that emergent hatchery abalone, with their reduced responses to tactile stimulation, will be a more vulnerable prey item for generalist predators.

Cryptic and emergent abalone also differed in their response to nearby movement. In fact, emergent hatchery-raised abalone did not respond to nearby movement or shadows, whereas the majority of their cryptic counterparts did. To some extent, this behavioural discrepancy was also found in wild abalone, with cryptic individuals responding more strongly to nearby movement than emergent ones. The difference in response may simply reflect the fact that the cue itself was somewhat different for cryptic and emergent abalone. Notably, a cryptic abalone would be found when rocks above it

were removed or the rock it was on was turned over, whereas emergent abalone were immediately visible. Thus, a cryptic abalone would experience an increase in light as it was exposed, in addition to the shadows and rock movement that constituted the cue. This explanation is supported by the fact that both hatchery-raised abalone and wild abalone responded more strongly to the control cue when their initial position was cryptic.

Approximately 10 percent of hatchery-raised northern abalone responded to *P. helianthoides* by raising the posterior portion of the foot over the shell and sweeping it around the perimeter of the shell. This behaviour is postulated to be a maintenance behaviour, used to clear settling debris off of the shell (pers. comm. J. Lessard 2010; pers. comm. J. Richards 2010; pers. obs.). To my knowledge, this is the first report of abalone demonstrating such a behaviour in response to a predator. The only similar report is of *H. rufescens* raising the mantle and extending the epipodium over the shell in response to stimulation with *P. helianthoides* tube feet (Montgomery 1967). The latter serves as an effective escape behaviour because it can prevent *P. helianthoides* from obtaining a purchase on the potential victim's shell (Margolin 1964). However, the posterior end of an abalone's foot will not achieve this purpose because it covers only a small portion of the shell at a time. The sweeping behaviour was never observed in wild abalone or in hatchery-raised abalone once they had been outplanted into the wild for a month. It is possible that abalone exhibiting this behaviour in response to *P. helianthoides* did not recognize the latter as a threat, attempting to sweep away what they perceived to be a benign object on the shell. Such abalone would accordingly have rapidly fallen victim to predatory sea stars once outplanted, which accounts for the absence of this behaviour in the wild. The sweeping behaviour in response to *P. helianthoides* constitutes the principle evidence of naivety observed among hatchery-raised northern abalone in this study.

Influence of predator cues on hatchery-raised abalone behaviour and survival

Behavioural crypsis, or the occupation of crevices and cryptic spaces, is believed to be a predator avoidance adaptation in abalone (Sloan and Breen 1988). As abalone

grow and their vulnerability to predators declines, they generally shift to an emergent lifestyle (Sloan and Breen 1988). In fact, during observations of wild abalone in the field, Lessard et al. (2007) found that the proportion of cryptic northern abalone dropped from 100 % at less than 2.0 cm to 50 % at approximately 4.5 cm SL, values consistent with our finding that, less than 50 % of hatchery-raised abalone measuring 4.41-5.93 cm SL occupied cryptic positions. Exposure to predator cues nevertheless exerted some influence on this behaviour. In my laboratory experiment, the *Cancer productus* scent and scent feeding treatments and the *Pycnopodia helianthoides* present treatment raised the cryptic proportion relative to the control. Furthermore, the proportion of juvenile abalone located in a cryptic position changed over time, peaking on the third day of the experiment; this progressive change in behaviour suggests these abalone learned over the course of the experiment. This finding also suggests that the vulnerability of hatchery-reared abalone to predators might be reduced by exposing them to the odours of feeding *C. productus* or placing them in proximity to *P. helianthoides* for two days and then outplanting them on the third day.

One of the basic defences of an abalone is its ability to attach itself firmly to the substrate (Cox 1962; Mottet 1978). At the micrometer scale, an abalone's foot consists of setae terminating in cylindrical fibrils, which enable abalone to adhere to surfaces of varying roughness (Lin et al. 2009). In effect, the fibrils allow the foot to conform to the microtopography of the substrate, sealing the interface and strengthening the attachment through capillary and van der Waals forces (Lin et al. 2009). One would accordingly expect that an abalone can attach firmly to both smooth plastic and rough rock substrates. Nevertheless, it was expected that hatchery-raised abalone would demonstrate a preference for plastic substrates over rock substrates, not because of attachment abilities but because these abalone would not have been previously exposed to rock substrates. This expectation was confirmed, with an average of only 5 % of the large juvenile hatchery-raised abalone being found on rock substrates throughout this experiment. When the abalone were disturbed, for example by removing tank covers and applying cues, the proportion of individuals on rock dropped even further, most notably in the *C.*

productus scent feeding treatment. This demonstrates a preference for plastic substrates in hatchery abalone, particularly when stressed, with the sole exception of abalone exposed to *P. helianthoides* scent favouring rock substrates on day 3. This preference is not expected to negatively affect hatchery-raised abalone, although it could slow their dispersal from plastic outplanting modules into the surrounding natural environment. It would be interesting to test whether wild abalone also prefer plastic substrates. While surveying outplanting sites in the predator exclosure experiment (Chapter 4), I often observed wild abalone in the PVC outplanting modules, but it is unclear whether they were attracted by their conspecifics or to the PVC itself.

Pycnopodia helianthoides is recognized as one of the most active and voracious predators of subtidal gastropods in the northeast Pacific (Brewer and Konar 2005). These asteroids are also highly mobile, running down their prey, which they can detect using well-developed chemosensory abilities (Brewer and Konar 2005). Given the formidable abilities of this predator, most of its prey species react to its approach by fleeing and displaying "mild hysteria" (Haderlie 1947, cited in Bullock 1953). In this study, large juvenile *H. kamtschatkana* responded to *P. helianthoides* with that same hysteria, galloping rapidly upwards, twisting the shell, and even climbing out of the water. The abalone's reaction to *P. helianthoides* in the absence of tactile stimuli reflects the abalone's ability to detect saponins released by the predator (Mackie 1970; Legault and Himmelman 1993), a chemosensory ability which forewarns them of the predator's presence. Interestingly, the distressed reactions of abalone were particularly pronounced when the *P. helianthoides* cue involved an abalone being fed upon. Notably, the proportion of abalone on the edge of the tank (attempting to crawl out of the water) was greatest when the *P. helianthoides* feeding treatment was being applied (54.6 ± 5.7 %) and was most extreme upon the abalone's first exposure to this cue. Since the above fleeing response of abalone to the *P. helianthoides* feeding treatment was strongest on the first day of the experiment, it appears that this response is innate, and was not learned as a result of the predator cue trials.

The abalone individuals placed in the cue container with *P. helianthoides* to provide the *P. helianthoides* scent feeding cue were often observed releasing a viscous mucous from their respiratory pores. The release of mucous by stressed abalone has been noted by Montgomery (1967). The mucous seems to serve as a warning for other abalone, which react very strongly to it (pers. obs; pers. comm. Lessard 2010). Other organisms with predators common to those of the northern abalone have also been observed fleeing when presented with this mucous (pers. comm. Lessard 2010).

In the natural environment, a number of abalone species, including *H. kamtschatkana*, are nocturnal foragers (Wood and Buxton 1996; Allen et al. 2006). This is particularly true for juveniles, which occupy cryptic habitat during the day and must move out into the open to feed at night (Wood and Buxton 1996). Such nocturnal feeding is thought to be a predator avoidance strategy against diurnal predators (Wood and Buxton 1996). Many abalone species feed on drift kelp, which they capture by rearing onto the posterior portion of their foot and grasping with the anterior end of the foot as it drifts by (Momma and Sato 1969; Tutschulte and Connell 1988; Wood and Buxton 1996; Day and Branch 2002; Lafferty et al. 2004; Allen et al. 2006). Northern abalone are no exception (Sloan and Breen 1988). Hatchery-raised northern abalone can be observed rearing when *N. leutkeana* is placed in their tanks (pers. obs.). Hatchery personnel feed abalone during the daytime and cast shadows when placing *N. leutkeana* in the hatchery tanks. Shadows alone may cause hatchery abalone to rear (pers. obs.). Similarly, Allen et al. (2006) found that *H. iris* given algae in the hatchery would adopt feeding postures both during the day and night. Providing abalone with kelp during the daytime likely increases their vulnerability twofold: firstly by eliminating their diurnal avoidance of predators, and secondly by giving them a positive association with shadows, which in the wild would often foretell the arrival of predators.

On the first day of the laboratory experiment, a small proportion of abalone reared both prior to and during cue application in the *P. helianthoides* scent treatment. All further instances of rearing occurred prior to, not during, cue application. This was true for both control and treatment abalone, indicating that some aspect of the experimental

procedure other than treatment was sufficient to break the abalone's tendency to associate shadows with food. Over the course of the experiment, abalone were only fed at night. It is possible that night-time feeding, and day-time shadows in the absence of food (i.e. when tank covers were removed and abalone were observed during the experiment) may have been responsible for breaking the hatchery-raised abalone's dangerous habit of rearing in response to shadows.

Exposing hatchery-reared abalone to predator cues for six days prior to outplanting had no detectable influence on their subsequent survival and behaviours. This is likely due to the small number of abalone outplanted per treatment and the use of only two replicates. As such, I recommend that this part of the experiment be repeated with more replicates and more abalone used per treatment.

Ontogenetic changes in behaviour and learning

There was no evidence of learning among early-stage hatchery-raised abalone over the course of this experiment, whereas late-stage abalone altered their behaviours over the course of the experiment, suggesting that they were learning from their exposure to predator cues. There were also discrepancies in the distributions and behaviours of different age groups of northern abalone presented with predator cues. The average percentage of small hatchery-raised juveniles occupying cryptic space in experiments (32 %) was considerably lower than the ~ 90 % observed for wild northern abalone of the same size (Lessard et al. 2007), but was similar to the percentage of large hatchery-raised juveniles occupying cryptic space (35 %). This suggests that the hatchery environment promotes the adoption of an emergent lifestyle early on, which may greatly increase the vulnerability of outplanted abalone to predators. This hatchery-induced behavioural anomaly is a cause for concern since the persistence of a prey population under intense predation pressure is believed to be dependent upon physical refuges from predators, such as crevices and the undersides of rocks that are inaccessible to the predators of small abalone (Hines and Pearse 1982).

The two size classes of juvenile abalone differed in their substrate preferences. Substantially more small juveniles positioned themselves on rock surfaces than large juveniles, suggesting that development in the hatchery environment may lead to a preference for plastic substrates.

Another notable difference between the two groups of abalone is that, unlike their larger counterparts, small juveniles were never observed rearing over the course of this experiment. This probably reflects the different feeding habits of young juveniles, which do not capture drift kelp but rather graze upon diatom films, crustose coralline algae and some attached microalgae (Sloan and Breen 1988; Tutschulte and Connell 1988; Wood and Buxton 1996; Day and Branch 2002). Although large abalone often crawled upwards and attempted to escape the aquaria when predator cues were being applied, particularly those involving *P. helianthoides*, this was not observed in the small size class. The climbing response, however, has been noted among other gastropods exposed to seastars (Feder 1963). The present study provides an indication that the climbing response is not expressed uniformly across all ages of abalone, being absent in early-stage juveniles and developing later in an abalone's ontogeny. The development of this response later in life may be associated with the shift from deep habitats to shallow habitats as abalone age (Sloan and Breen 1988). A late-stage juvenile abalone that has begun its migration to shallower waters may be able to confuse potential predators cueing in on its scent by climbing out of the water. On the other hand, an early-stage juvenile located in deep water has no chance of climbing out of the water.

The relatively short-term exposure to predator cues applied in this study had little influence on the behaviours of early-stage juveniles, and what changes were observed are not considered beneficial. Notably, the movement of abalone to plastic substrates in the *L. bellus* scent trials will not benefit abalone upon release into natural habitats. The fact that both early and late-stage juveniles responded to crab predators by moving onto plastic substrates contradicts the idea that abalone can attach equally well to plastic and rock (Lin et al. 2009), and suggests that they respond to crabs by attempting to get a good grip on the substrate. Knudsen (1960) observed crabs trying unsuccessfully to pry black

abalone (*H. cracherodii*) off of rocks, and suggested that the crabs were more successful at tearing pieces of flesh from an unsuspecting abalone's foot as it passed. Hence, withdrawing the foot and clamping the shell down to the substrate may be a more effective defence against crabs than seeking cryptic habitat, given that large crabs can dismantle this habitat. The attachment strength of abalone on different substrates requires further investigation as it could elucidate whether the preference of hatchery-raised individuals for plastic substrates arises from habituation in the hatchery environment or from an effective defensive strategy.

Conclusion

The present study revealed that the behaviours of hatchery-raised northern abalone differed from those of wild abalone in response to shadows, nearby movement, and tactile stimulation, but not in response to contact with *P. helianthoides*. Behavioural differences appear to result largely from habituation in the hatchery environment rather than naivety. This study revealed that the behaviour of juvenile northern abalone can be altered by exposing them to predator cues. Notably, the substrate preferences and preferences for cryptic surfaces of both early and late-stage juvenile abalone were modified through predator exposure trials. Moreover, late-stage juveniles appeared to learn not to rear in response to shadows. However, such treatments do not appear to improve survival after outplanting

Four behaviours of hatchery-reared abalone that are likely to prove disadvantageous to outplanting efforts are: reduced reactions or even rearing in response to shadows and movement, foraging during the daytime, early adoption of an emergent life-style and occasionally responding to *P. helianthoides* by sweeping the posterior portion of the foot over the shell.

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Chapter 6: General Conclusion

To develop an effective method for increasing abalone densities above the suspected Allee threshold of 0.3 reproductive adults/m^2 by outplanting hatchery-reared abalone I (1) examined the success of past outplanting attempts, (2) identified optimal characteristics for outplanting, and (3) identified outplanting limitations. Of primary importance in this study is the finding that past efforts to outplant northern abalone have had limited success in increasing population densities, but that outplanting methods and success can be improved.

Past outplanting attempts raised densities of reproductive adults by 0.003 to 0.033 individuals/m^2, but did not raise densities above the critical threshold. Moreover, there was evidence of recruitment failures occurring at all surveyed sites in Barkley Sound, BC, Canada.

Outplanting larval northern abalone at densities of 50,000 individuals/m^2 increased the densities of new recruits after one year. There were no density effects detected among larval outplants, whereas outplanted juvenile northern abalone are subject to density effects, with groups of 100 individuals/m^2 having the greatest survival after outplanting. Furthermore, juveniles experienced a critical period in mortality immediately after outplanting, as approximately 64 % died within the first 24 hours. Predators represent the major source of outplant mortality, whereas tagging, handling and outplanting stress have only a negligible effect on outplant survival. Neither the exposure of juveniles to predator cues prior to outplanting nor the use of predator exlosure cages upon outplanting influenced the subsequent survival of juvenile hatchery-reared abalone. The behaviours of hatchery-raised abalone differed from those of wild abalone. Notably, hatchery-raised abalone were habituated to shadows, movement, and unscented physical contact in the hatchery environment. However, hatchery-raised abalone are not naïve of predators.

The major finding, in terms of improving outplanting success by identifying optimal characteristics is that larval outplanting has greater potential than juvenile outplanting. Indeed, each application of 50,000 larvae is expected to increase densities of

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reproductive abalone by 0.721 individuals/m^2 after 5 years, whereas juvenile outplants are expected to increase densities by only 0.006 individuals/m^2.

DEVELOPMENTS IN METHODOLOGY

Over the course of this study, we devised several novel survey and outplanting methods which proved to be quite useful. Circular swath surveys allowed us to determine the dispersal, survival and densities of abalone concurrently with the congregation of predators (see Chapters 3 and 4). The larval tents described in Chapter 3 appeared to confine larval settlement, for indeed recruits from outplanting events were located within outplanted plots one year after larval outplanting. These larval tents withstood currents and surge at a 12 m depth for 48 hours and very few larvae settled on the structures themselves. Finally, both the suspended and grounded predator exclosure cages were successful in excluding abalone predators, without any detectable negative effects on abalone held within.

CONCLUSIONS AND FUTURE DIRECTIONS

Several conclusions arrived at through this study bear implications for future restoration work with the northern abalone. Most importantly, we have shown that the success of outplanting attempts can be improved through research into optimal life history stages and outplanting densities. Contrary to our expectations, larval outplanting appears to be the most promising means of raising northern abalone densities with hatchery-reared individuals. Abalone raised in the hatchery for any length of time are extremely vulnerable to predators upon release. If juvenile abalone are to be outplanted, sites should be chosen for low *C. productus* densities. Feeding hatchery-raised abalone at night in the absence of shadows may also raise outplanting success.

Although we currently recommend outplanting larvae at densities of 50,000 individuals per m^2, further research should be conducted to refine the optimal density for larval outplanting. Such a study should also examine the spill-over of abalone into areas surrounding tents, and ideally follow the cohorts over several years to determine whether our extrapolated estimates of increased adult densities are accurate. Furthermore, the

extent of inter-annual variability in outplanting success could be determined concurrently with such a study. It would also be propitious to confirm the density at which northern abalone experience an Allee threshold.

Continued research aimed at identifying an optimal strategy for outplanting is critical in light of recent findings which indicate that global warming may negatively affect northern abalone populations. Notably, a recent study indicates that larval Haliotids in Australia are unable to form shells in acidic waters, and as a consequence fail to settle and metamorphose into juveniles (M. Byrne pers. comm.). As such, one might expect mortality during the larval phase to increase with increasing ocean acidification. Not only would this negatively impact the reproductive success of natural populations, but it would also impact abalone being outplanted as larvae.

As the only abalone species found in Canada and the first marine invertebrate to be listed as endangered by the Committee on the Status of Endangered Wildlife in Canada, *H. kamtschatkana* is a flagship species. In this thesis I have demonstrated that hatchery-raised individuals can be used to supplement wild populations, and that both the methods and the success of northern abalone outplanting efforts can be improved. Given the representative status of this species and the positive results that are attainable, as demonstrated by the experiments herein, it is imperative that efforts be made to restore northern abalone populations in Canada and thus establish a precedent for protecting the marine environment and its resources.

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Introduction to Appendices

Over the course of this study a number of side projects and observations were inconclusive, not easily explicable, or not directly relevant to the original research aims. Yet these observations still bear implications for future northern abalone outplanting work and have accordingly been included as appendices.

While determining the effectiveness of larval outplanting in Chapter 3, I also assessed the substrate preferences of newly recruited northern abalone. This work is not directly relevant to the question of larval outplanting, but is nonetheless of interest. It is therefore presented in Appendix A.

One hypothesis for the high mortality of hatchery-raised abalone relative to their wild counterparts is that the aberrant shell colouration of individuals originating in the hatchery reduces crypsis and increases vulnerability to visual predators. This hypothesis is partially discounted in Appendix B, wherein I examine the influence of abalone shell colour and damage to post outplanting survival. In fact, pale and damaged abalone had higher survival than abalone with wild shell colouration or undamaged abalone. Although this result appears to contradict the hypothesis, it is currently inexplicable and thus I have relegated this portion of the Chapter 3 experiment to the appendices.

When considering possible explanations for the high mortality of outplanted northern abalone, I thought hatchery-raised abalone might be in worse physical condition than wild individuals, and accordingly be more vulnerable. In Appendix C, I compare the condition of hatchery-raised and wild abalone and examine the influence of condition on post outplanting survival. I also report the condition indices and weight to length relationships of these two groups of abalone, as these may serve as a basis of comparison in future studies.

A small size class of abalone was originally included in the experiment described in Chapter 4. I include a description of the methods and difficulties associated with this size class in Appendix D, such that these same problems can be avoided in future studies. I have included a description of our methodology for deploying multiple outplanting modules in Appendix E, and considerations for for designing effective predator exclusion

cages in Appendix F, under a similar rationale; except in these cases, the methods were successful.

Appendix A: Substrate and positional preferences of newly recruited northern abalone

During the larval outplanting experiment described in Chapter 3, we addressed one additional objective. Namely, determining whether one-year-old abalone show any preference for particular substrates and/or positions on rocks.

METHODS

Thirteen months following outplanting, when the permanent plots were searched for newly recruited abalone (see Chapter 3), the positions and substrates of these abalone were recorded i.e. whether they were located on the upper or lower surface, or sides of rocks, and the type of substrate they were on. A three-way ANOVA as used to determine whether newly recruited abalone (those with a shell length < 3.2 cm) exhibited any substrate preferences. The response variable was the number of new recruits. The predictors were substrate type (fixed; 4 groups), treatment (fixed; 3 treatments), and replicate (random; 4 replicates). Moreover, a three-way ANOVA was used to assess whether the sizes of abalone differed by treatment, substrate, or position (fixed; 3 position categories).

RESULTS

New recruits were not randomly distributed across the different substrates, but rather exhibited clear preferences. Indeed, there was a significant interaction between substrate and treatment (ANOVA: $F_{6,18}=8.283$, $p<0.001$; Figure 42). Notably, new recruits in the 50,000 and 100,000 treatment plots demonstrated a preference for crustose coralline algae over bare rock, whereas no preferences were apparent for abalone in control plots, likely as a result of the small sample size of new recruits within these plots (n=5).
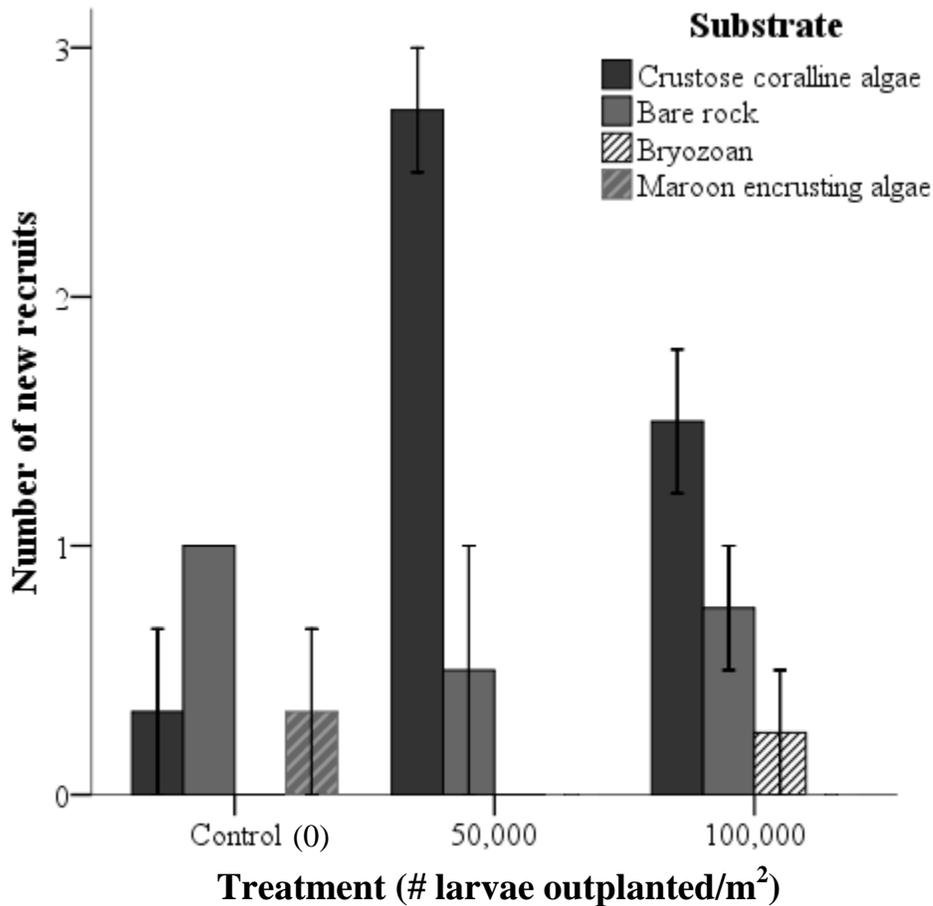
Figure 42. Mean number of new recruits (<3.2 cm) occupying different substrates in the three larval outplanting density treatments 13 months after outplanting.

The mean size of abalone was significantly influenced by an interaction between the substrate type and the outplanting treatment (ANOVA: $F_{6,33}$=2.519, p=0.041). In general, smaller abalone were found on crustose coralline algae than on bryozoans, with the exception of one small individual found on bryozoans in the 100,000 treatment. Abalone found on crustose coralline algae in the 50,000 treatment were also smaller than those found on bare rock.

There were no trends in the sizes of abalone occupying different positions (cryptic, emergent, or the sides) on rocks over the range of shell lengths observed in this study ($F_{2,33}=0.403$, $p=672$), nor in the percentage of new recruits occupying these positions.

DISCUSSION

Although early work by Crofts (1929) and Morse and Morse (1984) demonstrated that many Haliotids preferentially settle on crustose coralline algae, it is currently unknown at what age abalone begin to shift to other substrates. Crustose coralline algae were expected to be the preferred substrate for newly recruited northern abalone, since abalone feed upon the upper layer of this alga, ingesting both the algal cuticle and the bacteria living on it and thus gleaning a pink shell colour early in life (Garland et al. 1985; Sloan and Breen 1988). In keeping with this expectation, more new recruits occupied crustose coralline algae than any other substrate in the outplanted treatments. The second favoured substrate was bare rock, upon which certain abalone species are known to find diatoms and foraminifera for grazing (Crofts 1937; Cox 1962; Breen 1980). Strangely, there were no signs that new recruits in control plots preferred any one substrate type over another, although this may have been an artifact of the very low numbers of new recruits recovered within such plots.

There was a detectable shift in the choice of substrate with increasing shell length among newly recruited northern abalone, with the preference changing from crustose coralline algae to bare rock and maroon encrusting algae to bryozoans. This is complementary to previous evidence that abalone habitat use alters over time to facilitate a different diet at different life-stages as well as to avoid predators at particularly vulnerable stages (Sloan and Breen 1988), and suggests that the shift occurs when abalone are approximately one year of age. Juveniles eventually move from the surfaces of encrusting corallines or bare rock in deep water to shallow water where they feed upon fleshy algae (Sloan and Breen 1988). The shift in habitat use identified in other studies (see Sloan and Breen 1988) exists on a larger scale – abalone move from deep to shallow

waters. We now have evidence that juvenile abalone exhibit shifting preferences for substrates as they grow and before they begin migrating to shallower water.

Northern abalone also shift from exposed to cryptic and back to exposed surfaces over the course of their life history (Sloan and Breen 1988). Sloan and Breen (1988) did not find evidence for a relationship between crypsis and shell length among 10-70 mm northern abalone in British Columbia. However, more recently Lessard et al. (2007) confirmed that 50 % of northern abalone measuring approximately 50 mm shell length were occupying cryptic spaces. There was no clear preference for cryptic or exposed substrates among 0.2-30 mm abalone in this study. As such, the timing of a northern abalone's initial shift from exposed to cryptic habitats is still unknown.

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Appendix B: Influence of shell colour and damage on juvenile survival

INTRODUCTION

There is considerable evidence that hatchery-reared abalone, even large individuals, experience greater mortality in the field than their wild counterparts (Rogers-Bennett and Pearse 1998; for an exception see Tegner and Butler 1985, who compared large hatchery abalone to small wild individuals). This is thought to be largely due to the behavioural anomalies that exist among hatchery-raised individuals (Tegner and Butler 1985; Schiel and Welden 1987; Rogers-Bennett and Pearse 1998). However, the physical condition of hatchery-raised abalone or differences in phenotypes such as shell colouration could also be at fault.

The colouration of abalone shells is highly dependent upon the individual's diet (Ricketts et al. 1985), a relationship which may serve to fine-tune the camouflage of wild abalone (Fallu 1991; Liu et al. 2009). Abalone raised in a hatchery often have a different shell colour than wild abalone (Roberts et al. 2007, Heath and Moss 2009). In the case of the northern abalone, wild individuals tend to have a pink to dark red hue, whereas hatchery-raised individuals range in colour from pale green/blue to an almost wild shell colouration (pers. obs.). Notably, wild abalone incorporate algal pigment from crustose coralline algae into their shells, particularly during early life stages (Cox 1962; Sloan and Breen 1988), while young abalone in the hatchery forage upon diatom growth (Richards 2010). Some hatchery-raised northern abalone even have an orange shell (pers. obs.). Similarly, wild individuals of the Pacific abalone (*H. discus hannai*) are characterized by a dark-brown or green colouration, yet orange variants of this species have been observed in hatcheries (Liu et al. 2007, Liu et al. 2009). The discovery of a large number of orange Pacific abalone at a hatchery in China in 1992 prompted further research on abalone shell colour, and it has since been found that shell colour is genetically controlled but modified by diet (Liu et al. 2009).

Given that wild northern abalone are extremely camouflaged in their rocky subtidal habitat, particularly on pink and red encrusting growth such as crustose coralline algae, it is possible that the aberrant colouration of hatchery individuals increases their

vulnerability to visual predators upon outplanting. Given the limited success of past *Haliotis kamtschatkana* outplanting attempts (see Chapter 2), it is imperative that species-specific characteristics that optimize outplant survival be identified.

METHODS

The importance of shell colour and condition to abalone survival was determined as part of the juvenile outplanting experiment described in Chapter 3. When the colour and number of the abalone's bee tag was recorded, the colour and condition (e.g. shell damage) of the abalone were also noted. Abalone were assigned to one of three shell colour categories: normal (i.e. approximating the colouration of wild abalone), pale, or orange morphology. Features of shell condition that were recorded were limited to: fused aperture holes, notches in the shell, stunting of the shell, abnormal shell shape, and/or exposure of the nacreous layer or a hole in the shell. For ease of analysis, abalone with one or more of these features were simply considered as being damaged. Circular swath surveys were used to assess the survivorship of outplanted abalone over time (see chapter 3 for details). A repeated measures four-way ANOVA was used to assess whether shell colour or condition influence survival of hatchery-raised abalone after outplanting. Survival was the bivariate response variable, while the predictor variables were time (fixed; the repeated measure; 3 times), site (random; 5 sites), colour (fixed; 3 colour categories), and damage (fixed; 2 damage categories).

RESULTS

Although it was expected that abalone with a wild colouration would have higher survivorship than pale individuals and that undamaged abalone would have higher survival than damaged abalone, this was not the case. In fact, there was a significant interaction between shell colour and damage (ANOVA: $F_{2,16}=8.409$, $p=0.003$), which reflected the fact that pale and damaged hatchery abalone had higher survivorship than any other group.

DISCUSSION

Contrary to our expectations, hatchery abalone that were considered to be damaged did not experience higher mortality rates than their undamaged counterparts upon outplanting. This is thought to be due to the inclusion of stunted individuals in the damaged category. Stunted individuals may have redirected energy away from shell growth to somatic growth, resulting in a better condition index (weight/shell length) (McShane et al. 1988).

Growth in the hatchery environment is inversely related to stocking density (Mgaya and Mercer 1995, Capinpin et al. 1999, Huchette et al. 2003, Lloyd and Bates 2008). Interestingly, in a study of northern abalone at the hatchery that supplied abalone for our experiments, Lloyd and Bates (2008) found density-dependent growth that was not attributable to food availability. Indeed, abalone at the Bamfield Huu-ay-aht Community Abalone Project hatchery were fed *ad libitum* and reduced growth at high densities was most likely due to the stacking behaviour observed in abalone held at these densities.

Stacking is expected to reduce both the mobility and foraging behaviours of abalone. Notably, Lloyd and Bates (2008) found that abalone in stacks spent significantly less time feeding. Moreover, abalone will graze on diatoms which grow on all available surfaces, including other abalone shells. Scraping of the radula over shell material appears to cause minor damage to the outer prismatic $CaCO_3$ section of the shell, such that the iridescent nacreous layer becomes exposed (personal observation; Graham and Sarikaya 2000, Wang et al. 2003). When abalone are maintained at high densities, this destructive behaviour becomes more prevalent. Hence the shells of hatchery-raised abalone that become deformed and even damaged as a byproduct of high density stocking in hatcheries are likely also those that are stunted. In summary, an abnormal shell does not appear to be either disadvantageous or a signal of poor health in hatchery abalone, as abalone possessing a damaged shell did not experience greater mortality upon outplanting.

Although it was expected that hatchery-abalone with a wild shell colouration would have higher survivorship than their pale counterparts, this was not the case. Hatchery abalone with an orange shell colouration did not appear to be at a disadvantage either. One currently inexplicable result is that pale and damaged individuals appeared to outperform other groups.

Conclusion

While attempting to optimize outplanting strategies by using abalone at different stages in their life history and manipulating their densities, we were able to assess the importance of certain morphological and phenotypic features. Shell colouration was unimportant to the survival of hatchery-raised abalone after outplanting. Contrary to our expectations, individuals identified as damaged or having aberrant colouration were not found to be at a disadvantage upon outplanting.

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Appendix C: Influence of condition index on juvenile abalone survival

METHODS

Five hundred and eighty hatchery-raised abalone used in the predator exclusion study (Chapter 4) were measured to the nearest 0.01 cm shell length, and were weighed to the nearest 0.01 g. The condition index (CI) of abalone was calculated with the formula: $CI = \dfrac{weight(g)}{SL(cm)}$. I also calculated the condition index for wild abalone using weight and length data from Quayle (1971) for wild northern abalone at Bauke Island, a site in Barkley Sound on the west coast of Vancouver Island (n=914; Figure 22). The condition indices of hatchery-raised and wild abalone were compared using an Independent samples t-test. The morphometric relationship between northern abalone weight and shell length (W = a L^{b}; where *W* is weight, *L* is length, and *a* and *b* are constants) was calculated for both wild and hatchery-raised individuals using non-linear regressions. It was determined whether abalone origin influenced the relationship in a one-way ANCOVA with weight as the response variable, abalone origin (hatchery-raised or wild; 2 categories) as the predictor variable, and shell length as a covariate. Finally, the influences of condition index and size on the survival of the 420 outplanted abalone were assessed with two two-way ANCOVAs. The bivariate response variable was survival to 10-days post-outplanting, while the predictor variables were replicate (7 replicates) and treatment (3 treatment groups). The covariate was shell length in the first ANCOVA, and condition index in the second.

RESULTS

The average condition index of outplanted hatchery-raised abalone was 4.05 ± 0.03 g/cm SL, whereas that of wild abalone from Bauke Island was 11.89 ± 0.16 g/cm SL. The condition indices of these two groups of abalone were significantly different (Independent samples t-test: t=49.030, df=968, *p*<0.001). There was a relationship between the length and weight of hatchery-raised abalone (Figure 43). Indeed, the former variable explained 89 % of the variation in weight observed in hatchery-raised abalone,

although the regression was marginally non-significant (Non-linear Regression: R^2= 0.890, p=0.085). Given the relationship $W = aL^b$, the estimates of a and b for hatchery-raised juvenile abalone in this study are 0.179 ± 0.015 and 2.883 ± 0.050, respectively (Non-linear regression: $F_{1,419}$=3390.893, p<0.001). The relationship between the length and weight of wild abalone at Bauke Island was similar (Figure 43). In fact, the estimates of a and b for wild abalone at Bauke Island are 0.130 ± 0.011 and 2.988 ± 0.130 (Non-linear Regression: $F_{1,913}$=5955.232, p<0.001). Length explained 87 % of the variation in weight of wild abalone at Bauke Island (Non-linear regression: R^2=0.867, p<0.001). The relationship between weight and shell length differed significantly between the two groups of abalone (ANCOVA: $F_{1,1492}$=507.494, p<0.001). Neither shell length (ANCOVA: $F_{1,12}$=0.007, p=0.935) nor condition index ($F_{1,12}$=0.011, p=0.918) was a significant predictor of survival to ten days post-outplanting.
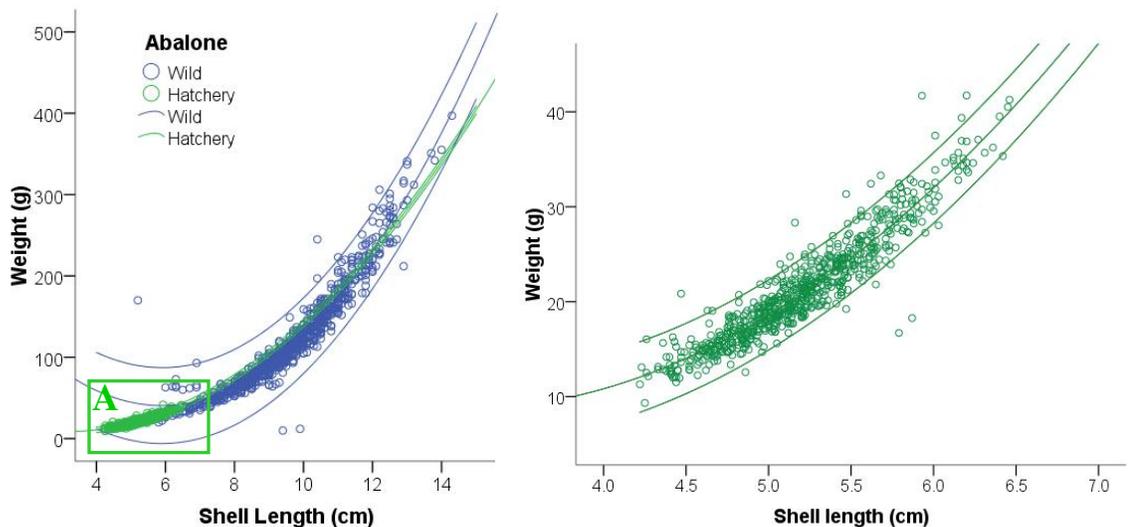
Figure 43. Relationship between the shell length (cm) and weight (g) of hatchery-raised abalone (outplanted in this study) and wild northern abalone (surveyed at Bauke Island by Quayle in 1971). The center curve represents the relationship between length and weight, while the outer curves show the 95 % confidence intervals of the relationship. Hatchery-raised abalone all had a shell length of less than 6.5 cm, and this portion of the graph is expanded in *A*.

DISCUSSION

Hatchery-raised abalone selected for outplanting exhibited strong relationships in their weight to length ratios, as did wild abalone. The relationship for hatchery-raised abalone can be expressed as $W = 0.179L^{2.883}$, while that of wild abalone is $W = 0.130L^{2.988}$. The morphometric relationship for wild abalone was calculated based on data collected by Quayle (1971) at Bauke Island, one of the Broken Group Islands, before northern abalone populations in British Columbia began to decline precipitously. We have chosen to compare hatchery-raised abalone to these wild abalone because we believe they represent a healthy and relatively natural population at a site near our own outplanting sites. The constant *b* (2.883 and 2.988 above) has also been used as a measure of the relative condition of individuals, and was found to approach 3 in other abalone species (McShane et al. 1988; Rogers-Bennett et al. 2007). Although the morphometric relationships overlap for the hatchery-raised and wild individuals, they are significantly different, and the average condition index of hatchery-raised abalone was significantly lower than that of wild abalone, suggesting the former were in worse condition. However, given that the condition index of hatchery-raised abalone was a poor predictor of survival after outplanting in this study, the low condition may be unimportant.

The survival of hatchery-raised abalone after outplanting was not related to shell length in this study. Shell length was, however, related to survival after outplanting in hatchery-reared abalone outplanted in 2008, with smaller individuals actually performing better (Chapter 3). Yet the two week survival of the large size group (4.2 to 6.5 cm) in this study, $66.0 \pm 1.9\ \%$, was significantly higher than that of the small size group (2.3 to 4.8 cm) in the previous study, $7.9 \pm 1.8\ \%$. The unpredictable nature of the relationship between size and survival over time suggests that interannual variation in conditions may be more important to outplant survival than size. The relationship between shell length and post-outplanting survival is complex, and survival cannot be said to increase with size over the 2.3 to 4.8 cm shell length range. Survival is known to increase with size to an optimal size in a number of other abalone species (Saito 1984; Tegner and Butler

1985; McCormick et al. 1994; Roberts et al. 2007). It is possible that this also occurs in northern abalone, but at a smaller size than tested herein. Indeed, Griffiths and Gosselin (2008) found that the vulnerability of northern abalone to predators declined considerably at 1.2-1.3 cm shell length, so it is possible that the optimal size for outplanting of this species exists between 1.2 and 2.3 cm.

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Appendix D: Small size class of abalone in predator exclusion cages

The predator exclusion experiment was actually conducted with two size classes of abalone. In addition to the 4.23 – 6.46 cm individuals mentioned in Chapter 4, we used a group of ten month old individuals ranging in size from 0.1 to 2.0 cm. The small abalone were not tagged for individual identification but were distinguishable from wild individuals based on shell colour. The outplanting modules for this size class consisted of halved 10 cm diameter PVC cut into 12 cm lengths and held together with elastic bands. Nitex mesh (210 µm) was used to prevent the escape of abalone from these modules. Unfortunately, the halved PVC which proved a great method for larger abalone was problematic for the small size class. The mesh size of predator exclosures was larger than many of the small abalone. The modules were therefore placed inside predator exclosures with nitex ends still in place. The rattling of these modules within predator exclosures in high swell caused the PVC halves to shift, and small individuals subsequently escaped both the modules and the predator exclosures. Moreover, when the modules were opened during the surveys, abalone occasionally fell out and could not be retrieved. Finally, the ability of divers to resight abalone measuring less than 0.3 cm was low, and subject to considerable observer bias. For these reasons, this part of the experiment was rejected.

Appendix E: Methodology for deploying multiple outplanting modules

The predator exclusion experiment described in Chapter 5 required that 35 large PVC outplanting modules (30 cm length x 15 – 20 cm diameter) be deployed into underwater predator exclusion cages. It was important to minimize the time between deploying each module so that replicates would be comparable. Moreover, although these modules were much too bulky for two divers to carry and swim between outplanting locations, the divers could not surface multiple times as this would generate health concerns. We devised a method to minimize both the time between module deployments and the number of times divers would have to surface.

Divers would locate the first set of cages and deploy a lift bag. A surface team manning a dive skiff would approach the lift bag and attach the first replicate of outplanting modules (treatments 1 through 5) by clipping them to the rope with herring clips. They would then remove the air from the lift bag and tug three times on the rope. The divers would in turn reel in the rope and attached modules, place the modules for treatments 1 and 2 in the appropriate cages, release abalone from the 3rd treatment module, and leave the modules for treatments 4 and 5 attached to the rope. The divers would swim to the next set of cages, re-inflate the lift bag, and allow it to rise back to the surface where the surface team would return the modules for treatments 4 and 5 to seawater containers and attach the next replicate of treatments to the rope, and so on.

Appendix F: Considerations for designing effective predator exclusion cages

The success of the predator exclosure cages is in strong contrast to several other cage prototypes used, which were easily infiltrated by predators. The small mesh size used within the final cages seems to have been key in excluding predators (pers. obs.). There are, however, disadvantages to the use of a small mesh size. Notably, small mesh sizes can disrupt flow (Miller and Gaylord 2007) and thus limit the availability of diatoms or drift kelp for the abalone's consumption. Cages with smaller mesh sizes are also expected to be overgrown more rapidly and require cleaning (pers. obs.). Thus the cages used in this study are only recommended for the short-term protection of outplanted abalone. For longer term studies, one should examine the effectiveness of a suspended cage with larger mesh size. The suspended cages did not have any detectable negative influence on abalone in this study, despite exposure to currents and some swell. One consideration is that the suspended cages attracted a number of fish, such as copper (*Sebastes caurinus*) and black rockfish (*S. melanops*), which are known to feed opportunistically on small *Haliotis kamtschatkana* individuals (DeFreitas 2005). Furthermore, the presence of the rockfish might attract predators capable of preying upon large abalone. The giant pacific octopus (*Enteroctopus dofleini*) for example, preys upon both rockfish and abalone (Hartwick et al. 1981; Robinson 1983; DeFreitas 2005). One immature *E. dofleini* individual did adopt an empty outplanting tube as a home base over the duration of this study.

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