Investigations into Mortality in Juvenile *Haliotis kamtschatkana* (northern abalone) and Factors That Affect Outplanting

by

Allison Muriel Griffiths
B.Sc., Simon Fraser University, 2003

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

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Abstract

The predation pressures on juvenile *Haliotis kamtschatkana,* northern abalone, in Barkley Sound, British Columbia, were investigated. Thirty-seven potential predators were tested in the laboratory to determine if they would consume juvenile abalone 1- 25 mm shell length (SL). Six of these potential predators consumed > 10 % of the abalone offered to them and were considered major predators. Natural mortality for juvenile *H. kamtschatkana* was then estimated by outplanting calcein marked and bee tagged hatchery-reared abalone at field sites. Calcein concentrations between 20 – 40 mg/L produced clear fluorescent marks for 3- 5 mm SL abalone when exposed to a double calcein marking procedure and abalone ≥ 15 mm SL immersed in calcein for 72 h showed the most distinct marks. Recoveries of outplanted abalone were highest (24%) for 15.1- 20 mm juvenile abalone. I recommend outplanting juvenile abalone larger than 12 mm to increase chances of survivorship in the wild.

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Dedication

To my family and friends who have supported me and encouraged in my endeavours and to the abalone whose lives had to be sacrificed for this project.

Chapter 1: General Introduction

Abalone live in marine environments world wide, and are found in the intertidal to depths exceeding 30 m (Shepherd and Breen 1992; Campbell 2000). *Haliotis kamtschatkana* (northern or pinto abalone) (Jonas 1845) is the only abalone species in British Columbia and is found from Alaska to California (Emmett and Jamieson 1988; Sloan and Breen 1988; Campbell 2000). *H. kamtschatkana* are slow growing and reach maturity after 3-8 years (Quayle 1971; Sloan and Breen 1988; Campbell 2000). Early spring is a peak time for this species to reproduce, although they are know to spawn throughout the year (Quayle 1971; Sloan and Breen 1988). *H. kamtschatkana* reproduce by broadcast spawning in which gametes of both sexes are released into the water column (Bevelander 1988; Sloan and Breen 1988). Non-feeding larvae settle and metamorphose 11-12 days post-fertilization (Page 1997). After this time the young abalone use a structure called a radula, a chitinous ribbon covered with multiple rows of hard re-curved teeth (Buchsbaum et al. 1976; Bevelander 1988), to graze on benthic diatoms and possibly coralline algae (Sloan and Breen 1988). As juvenile *H. kamtschatkana* grow they change their diet from grazing algae to primarily trapping drift kelp (Sloan and Breen 1988). Smaller *H. kamtschatkana* (< 70 mm) tend to remain in cryptic locations such as under rocks whereas, mature (> 100 mm) are commonly observed in exposed habitats (Campbell 2000).

Due to stock depletion and possible collapse of *H. kamtschatkana* populations, all commercial and sport fishing was closed in B.C. in December 1990 (Campbell 2000). To date, populations of *H. kamtschatkana* have not recovered, and therefore in April of 1999, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) listed *H. kamtschatkana* as threatened (Campbell 2000; Lessard et al 2004). The lack of recovery is thought to be due to slow growth, late age of maturity, and reproduction through broadcast spawning, the effectiveness of the latter being density dependent (Campbell 2000). Disease, predation (Watson 2000) and poaching of adult abalone may also have contributed to the collapse of abalone populations (Campbell 2000). Causes of natural mortality for abalone include predation by crab, seastars, and sea otters, competition for food and space with sea urchins, environmental factors such as high

temperature and low salinity, parasites, and diseases (Sloan and Breen 1988; Shepherd and Breen 1992; Bower 2000). Little is known about the biology of juvenile *H. kamtschatkana* (Sloan and Breen 1988) and factors influencing survival of juveniles. This thesis describes a series of field and laboratory experiments aimed at understanding juvenile *H. kamtschatkana* biology and more specifically mortality.

In chapter 2, potential predators of juvenile *H. kamtschatkana* were collected from the field, and laboratory trials were carried out to determine which species prey upon juvenile *H. kamtschatkana*. Six predators were identified that consumed > 10% of the abalone offered to them. Using four of the six predators, investigations were carried out to determine how abalone survival changes with ontogeny. Abalone smaller than 12 mm SL were found to be most vulnerable to the predator species.

Natural mortality of juvenile *H. kamtschatkana* was investigated in chapter 3 by releasing hatchery-reared *H. kamtschatkana* into the field and estimating their natural mortality through a mark-recapture procedure. This experiment was successful at determining the sizes of juvenile abalone that are most vulnerable to mortality.

In chapter 4, the fluorescent dye calcein was used to mark juvenile *H. kamtschatkana* to determine the optimal concentrations and duration of exposure of the calcein. Calcein is useful to mark juvenile *H. kamtschatkana* smaller than 5 mm that can not be tagged using other identification methods typically used for larger organisms. The effect of abalone size on the calcein mark intensity was also investigated.

Chapter 2: Potential Predators and Ontogeny of Vulnerability to Predation of Juvenile *Haliotis kamtschatkana*

ABSTRACT

Benthic invertebrates are subject to high mortality during the early juvenile stage. Juvenile mortality due to factors such as predation and environmental stressors can be as high as 90 % in some species. Vulnerability to mortality factors often decreases as the size of the individual increases. I identified the major predators of juvenile *Haliotis kamtschatkana* (northern abalone) and examined predation on juvenile *H. kamtschatkana* in Barkley Sound, British Columbia, to determine which sizes of abalone are most vulnerable to predation. Thirty-seven potential predator species were tested to determine if they would consume juvenile *H. kamtschatkana* 1-25 mm shell length (SL). Six of these species consumed >10 % of the juvenile abalone offered to them. These six predators are: *Scyra acutifrons* (sharp-nosed crabs), *Cancer productus* (red rock crabs), *Lophopanopeus bellus* (black-clawed crabs), *Pycnopodia helianthoides* (sunflower stars), *Amphissa columbiana* (wrinkled Amphissa) and *Rhacochilus vacca* (pile perch). *Lophopanopeus bellus* was found in highest abundance during field surveys with one of three field sites having 1.61 individuals m^{-2} for 12.1- 15 mm crab. However, *L. bellus* was the only predator of those tested that did not successfully prey upon abalone greater than 19 mm SL. Juvenile *H. kamtschatkana* that reach 12 mm were found to be less vulnerable to predation than ≤ 10 mm SL juvenile abalone, thus, size is likely to be a major determinant of juvenile abalone survival.

INTRODUCTION

Juvenile mortality among benthic marine invertebrates such as ascidians, bivalves, barnacles, gastropods, bryozoans, and echinoderms has been reported to be > 90% in 20 of 30 studies of age-specific mortality reviewed by Gosselin and Qian (1997). During the initial days to weeks of juvenile life, survivorship decreases exponentially and mortality can exceed 30% on the first day (Gosselin and Qian 1997). Early juvenile mortality can influence the size and distribution of populations as well as community structure (Sih et al. 1985; Gosselin and Qian 1997; Moran 1999; Osman and Whitlatch 2004). For instance, potential recruitment bottlenecks within a population can occur if

predation on early juveniles decreases the number of available recruits for further life stages (Smith and Herrnkind 1992). Juvenile mortality is also a determinant of age at maturity (Gosselin and Qian 1997) and is important in determining how many individuals in a population are likely to survive to reproduce.

Predation, competition, physiological stress, biological and physical disturbances and delayed metamorphosis all contribute to mortality in early juvenile invertebrates (Spight 1976; Rumrill 1990; Gosselin and Qian 1997; Hunt and Scheibling 1997; Moran 1999). Of these factors predation is the best documented mortality factor (Hunt and Scheibling 1997). Decapod crustaceans are often major predators of early juveniles because of their high abundances, broad vertical distribution (eg. intertidal and subtidal), requirement to obtain large quantities of food (Juanes 1992; Gosselin and Qian 1997) and high mobility and ability to crush protective structures (Seed and Hughes 1995; Gosselin and Qian 1997; Yamada and Boulding 1998; Schenk and Wainwright 2001). Grazers such as limpets and sea urchins can accidentally ingest early post-settled juveniles but vulnerability to grazers decreases with body size and with substrate complexity (Hunt and Scheibling 1997).

In newly metamorphosed juveniles, protective structures such as shells, carapaces or teguments are generally not yet completely formed, resulting in increased vulnerability during the first 1 to 2 d of life (Gosselin and Qian 1997; Osman and Whitlatch 2004). As early juveniles grow they become less vulnerable to physical and biological constraints such as predation and competition for space (Rumrill 1990; Gosselin and Qian 1997; Osman and Whitlatch 2004). Predators can be less efficient at capturing prey if their prey reach a size large enough to escape the handling capacities of the predator (Paine 1976; Peterson 1982; Boulding 1984; Palmer 1990). Palmer (1990) defined critical size as "the size above which a given type of prey cannot be consumed by an individual predator of a given size." Prey are vulnerable for a longer period of time if their critical size for a particular predator is large (Palmer 1990). In species that can eventually reach a size refuge, selection should favour rapid early growth (Paine 1976; Rumrill 1990). Many species reach a refuge from predators where predators of a smaller size can not prey upon them. After that size, however, some prey species become susceptible to larger predator species (Hunt and Scheibling 1997; Osman and Whitlatch 2004).

Predators known to prey upon adult *H. kamtschatkana* include: *Enhyra lutris* (sea otter) (Linnaeus 1758), crabs such as *Cancer productus* (red rock crab) (Randall 1839), *Octopus dolfleini* (Giant Pacific octopus) (Wulker 1910), the seastar, *Pycnopodia helanthanthoides* (Brandt 1835) (Emmett and Jamieson 1988; Sloan and Breen 1988; Campbell 2000; Watson 2000), and fish such as *Anarrhichthys ocellatus* (wolfeels) (Ayres 1855), and *Scorpaenichthys marmoratus* (cabezon) (Ayres 1854) (Emmett and Jamieson 1988). However, little is known about the predators of the early juvenile stage (first 3 years) of *H. kamtschatkana* (Sloan and Breen 1988; Campbell 2000). Suggested sources of natural mortality for juvenile *H. kamtschatkana* include: environmental factors (extreme temperature, low salinity, and storm disturbance), starvation, competition, parasitism, disease and predation (Sloan and Breen 1988; Campbell 2000). The importance of each of these potential early juvenile mortality factors, however, is currently unknown. In other species of abalone, predators known to feed upon small juvenile abalone include gastropods (Shepherd and Breen 1992), polychaete worms (Naylor and McShane 1997), anemones (Shepherd 1998), wrasses (Shepherd et al. 2000), crabs, seastars and lobsters (Schiel and Welden 1987).

The objectives of this study were to determine (1) which animal species will prey on early juvenile *H. kamtschatkana*, (2) the abundance and size-structure of predator populations in the field, and (3) if juvenile *H. kamtschatkana* reach a size at which they gain refuge from early juvenile predators.

METHODS

All field work was carried out in Barkley Sound located on the West Coast of Vancouver Island, British Columbia, Canada. This area has pristine waters rich in sea life and receives large amounts of nutrients from the Pacific Ocean. All laboratory work was carried out at the Bamfield Marine Sciences Centre (BMSC) (Figure 1).

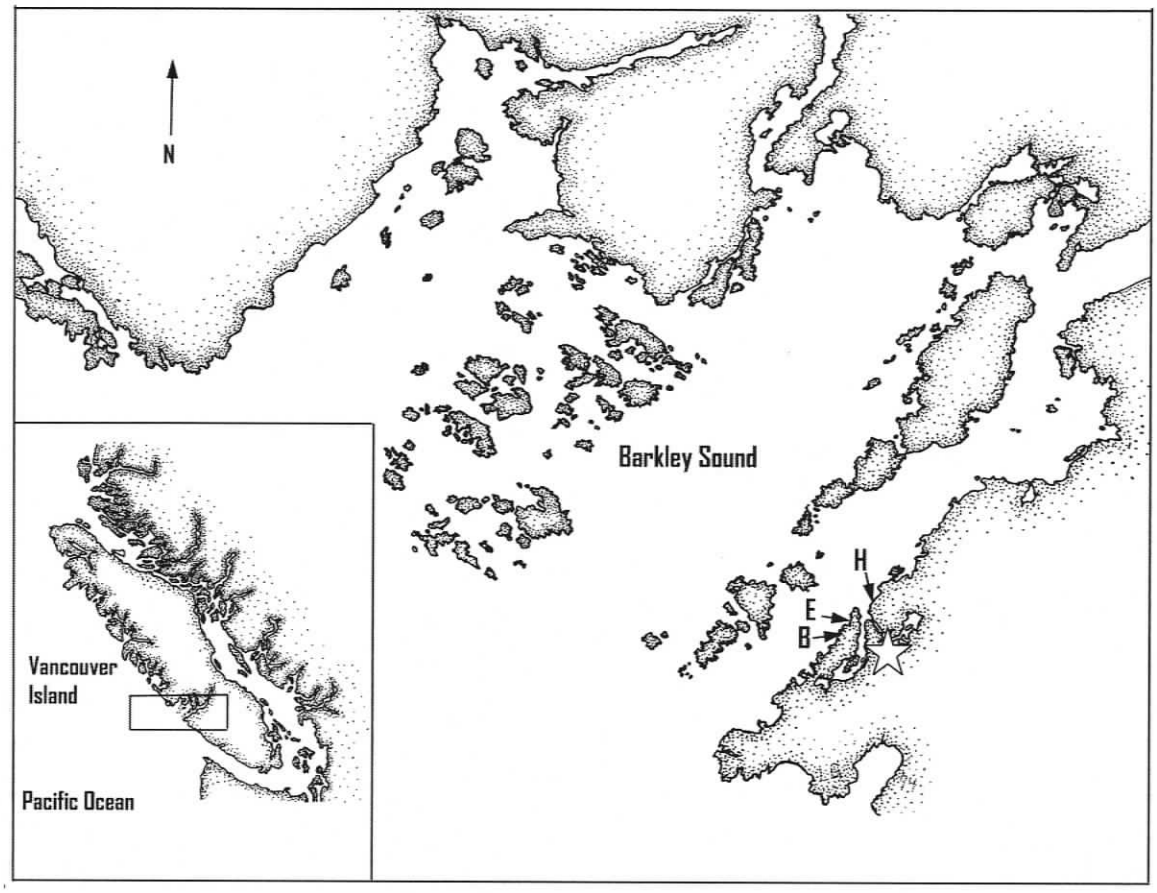

Figure 1: Map of Barkley Sound, British Columbia, Canada.
Bamfield Marine Sciences Centre is represented by the star symbol. The three field sites, Brady's Rock, Eagle Bay and Bamfield Harbour entrance are represented in the figure by B, E, and H respectively.

Abalone used in this study were obtained from the Bamfield Huu-Ay-Aht Community Abalone Project (BHCAP) hatchery located in Bamfield B.C. The selection of abalone size classes used in these experiments was based on past research with small juvenile abalone (Shepherd and Turner 1985; Schiel and Welden 1987; Schiel 1993; Rogers-Bennett and Pearse 1998; Seki and Taniguchi 2000) but was also constrained by availability from the BHCAP hatchery at the time of experimentation. Field work was carried out between 22 May and 15 June 2005 at Eagle Bay (48°50.252′ N, 125°08.670′ W), Brady's Rock (48°49.940′ N, 125°09.021′ W) and Bamfield Harbour entrance (48°50.561′ N, 125°07.995′ W) (Figure 1).

Potential Predators

To determine which species might prey on juvenile abalone, 37 species of potential predators were collected from Eagle Bay and Brady's Rock and brought to the laboratory. Table 1 summarizes the methods used to collect each potential predator species as well as the collection dates. Crabs, snails, sea urchins, and worms were hand picked by SCUBA divers. Fish were captured underwater in dip nets by SCUBA divers, in beach seines, or using hook and line fishing. All SCUBA diving collections occurred at depths of 5-15 m. Predators that were rare and smaller sizes of some other species were difficult to find to test with juvenile *H. kamtschatkana*. A few fish species such as *Enophrys bison* (buffalo sculpin) (Girard 1854), *Scorpaenichthys marmoratus* (cabezon), *Ophiodon elongates* (lingcod) (Girard 1854), and *Anarrhichthys ocellatus* (wolf-eel) were difficult to capture by SCUBA diving, beach seining or fishing and were not obtained for use in the laboratory trials.

Table 1: Collection dates and methods of potential predators.
"X" indicates the method used and "n" represents the sample size for each predator.

Potential Predator	n	Dates Collected	Collection Methods		
			SCUBA Diving	Beach Seine	Hook and Line
Gastropods					
Amphissa columbiana	6	20 May 04- 3 July 04, 31 May 05- 01 June 05	X		
Ceratostoma foliatum	4	11 May 04- 3 July 04	X		
Fish					
Artedius fenestralis	1	11 May 04	X		
Asemichthys taylori	2	22 June 04	X		
Coryphopterus nicholsi	3	22 June 04	X		
Cymatogaster aggregata	5	22 July 04- 2 Oct 04		X	
Embiotoca lateralis	3	2 Oct 04		X	
Hexagrammos decagrammus	2	28 Nov 04			X
Hexagrammos stelleri	2	2 Oct 04, 30 May 05		X	
Leptocottus armatus	2	2 Oct 04		X	
Micrometras frenatus	6	22 July 04- 2 Oct 04			
Oligocottus maculosus	4	22 July 04		X	
Rhacochilus vacca	5	2 Oct 04		X	
Sebastes melanops	1	18 Nov 04			X
Scorpaenichthys marmoratus	1	30 May 05		X	
Seastars					
Crossaster papposus	2	14 July 04- 20 July 04	X		
Dermasterias imbricata	5	12 May 04- 28 June 04	X		
Evasterias troschelii	4	11 May 04- 28 June 04	X		
Orthasterias koehleri	6	12 May 04- 14 July 04	X		
Pycnopodia helianthoides	4	11 May 04- 28 June 04 31 May 05- 01 June 05	X		
Solaster dawsoni	4	12 May 04- 1 July 04	X		
Solaster stimpsoni	2	12 May 04- 1 July 04	X		
Worms					
Halosydna brevisetosa	4	20 May 04- 28 June 04	X		
Pseudostylochus ostreophagus	1	20 May 04- 28 June 04	X		

Potential Predator	n	Dates Collected	Collection Methods		
			SCUBA Diving	Beach Seine	Line and Hook
Crabs					
Cancer productus	3	11 May 04- 22 July 04	X		
Lophopanopeus bellus	9	11 May 04- 14 July 04 31 May 05- 01 June 05	X		
Mimulus foliatus	1	22 June 04	X		
Pachycheles rudis	4	11 May 04- 3 July 04	X		
Paguristes turgidus	1	24 June 04	X		
Pagurus caurinus	1	24 June 04	X		
Pagurus hemphilli	5	28 June 04	X		
Pelia tumida	1	22 June 04- 24 June 04	X		
Petrolisthes eriomerus	2	11 May 04- 3 July 04	X		
Scyra acutifrons	6	11 May 04- 24 June 04 31 May 05- 01 June 05	X		
Sea urchins					
Strongylocentrotus droebachiensis	3	20 Nov 04, 2 July 05	X		
Strongylocentrotus franciscanus	13	20 Nov 04, 2 July 05	X		
Strongylocentrotus purpuratus	3	20 Nov 04, 2 July 05	X		

Predator Size

Crab size was determined by measuring the widest part of the carapace. Hermit crabs were weighed with their shells because crabs could not be removed from the shells without causing damage. Using shell weight is not the ideal measurement for hermit crab size, however, shell weight can be used as a relative comparison of hermit crab size. Seastar size was determined by measuring the diameter of the central disk. Fish size was determined by measuring the distance from snout to caudal fork. Snails were measured from the apex of their shell to the most anterior tip of the aperture. Worms were measured from the anterior tip of the body to the posterior point. Sea urchin size was determined by measuring the test diameter. Potential predators were identified using the following identification keys: Hart (1973), Lamb and Edgell (1986), Jensen (1995), Kozloff (1996), Harbo (1999) and Lamb and Hanby (2005).

Each collected animal was placed in a plastic cage with four rectangular windows covered with 600 μm mesh. Three sizes of containers were used depending on the size of the potential predator being tested: 16 × 16 × 3 cm, 34 × 22 × 17 cm, and 33 × 33 × 8 cm. Fish species were tested either in one of the two larger sized containers or in a seawater tank (117 × 119 × 36 cm). These potential predators were held in these cages with flowing seawater without food for 72 h after collection to acclimate to their new surroundings prior to feeding trials. Each cage then received three abalone < 5 mm, two abalone 5-10 mm, two abalone 10-15 mm, and one abalone > 15 mm SL. The number of live abalone were then counted after 24, 48 and 72 h. In addition, images were taken of evidence of abalone remnants after a predator attack to help identify mortality sources for dead abalone collected in the field. Potential predators were classified as major predators if they consumed more than 10% of the abalone offered to them or as occasional predators if they consumed 1-10% of juvenile abalone offered.

Abundance of Predators in the Field

Field surveys were carried out in the summer of 2005 to determine the population abundance and size structure of the species identified in 2004 as major predators of juvenile *H. kamtschatkana.* The six species surveyed included: *Scyra acutifrons* (sharp-nosed crabs) (Dana 1851), *Cancer productus* (red rock crabs), *Lophopanopeus bellus* (black-clawed crabs) (Stimpson 1860), *Pycnopodia helianthoides* (sunflower stars),

Amphissa columbiana (wrinkled Amphissa) (Dall 1871) and *Rhacochilus vacca* (pile perch). Two 60 minute surveys were carried out at each site. During the surveys, a 15 m transect line was set at depths between 7-10 m. Sampling took place within a 1 m^2 quadrat placed at 2 m intervals along the transect line. Rocks were overturned to search for predators. Predators found were measured using plastic calipers with accuracy to 0.1 mm. Once a predator was measured and recorded, it was placed approximately 0.5 m outside the sampling quadrat to avoid sampling the individuals more than once. Two SCUBA divers each surveyed half of each quadrat with both divers measuring predators and a third diver recorded predator sizes. A total of 13 quadrats were sampled at each site: five quadrats were sampled at each site during the first sampling period 22 May - 2 June and eight quadrats were sampled at each site on the second sampling period 4 June - 22 June.

Size Vulnerability

Abalone Size Feeding Trials

Specimens of the five major predators were collected from Eagle Bay and Brady's Rock from 15 May – 1 July 2005 to use in laboratory trials to determine the size range of juvenile abalone that predator species could consume. *Rhacochilus vacca* (pile perch) were not used in laboratory trials because they were not found at the field sites surveyed. The predator size ranges tested were: *S. acutifrons* (8-32 mm carapace width (CW)), *L. bellus* (8-26 mm CW), *C. productus* (65-101 mm CW), *P. helianthoides* (13-53 mm central disk diameter (CD)), *A. columbiana* (10-16 mm shell length (SL)). *Cancer productus* were collected from an eel grass bed approximately 1 km away from Eagle Bay by snorkel collection because too few were found at the two study sites. These animals were brought back to the laboratory, placed in separate cages and were held without food for 72 h to acclimate prior to experimentation. Abalone size classes used were: 10-12 mm, 15-19 mm, 21-24 mm, and 25-28 mm SL. After the 72 h acclimation period, three abalone from the smallest size class were added to each cage. After 48 h the number of consumed abalone was recorded. Previous predator trials had revealed that 24 h was too short a trial period for predators to consume juvenile abalone, and therefore the number of consumed abalone was recorded after 48 h. If a predator ate at least one

abalone from a size class, the size class was considered to be below the size refuge and the predator was then offered the next larger abalone size class. If the predator did not consume any abalone, the predator was again offered three abalone from the same size class. If this second time the predator still did not succeed in consuming an abalone of that size class, feeding trials for that individual were considered complete and the largest size class that predator consumed was recorded. After each trial, abalone shells and fragments were removed from the containers. The method by which the predators consumed the abalone was also noted. Incomplete predation (i.e. cracked abalone shells) were noted but were not recorded as successful predation. When crabs moulted during trials, the trials were ceased and a new individual was tested.

Crab Claw Morphologies

Photographs were taken of the three crab species *S. acutifrons*, *C. productus,* and *L. bellus* to compare morphology and potential strength of the claws to relate differences between the three species in their ability to consume juvenile *H. kamtschatkana*.

Predation Susceptibility Index

A predation susceptibility index, as defined by Gosselin (1997), was calculated to determine how susceptibility to predators in the field changes as juvenile abalone grow. The index value for a given abalone size at a field site represents the density at that site of juvenile abalone predators that would feed on an abalone of this size. To determine the index value, first the size-range of *L. bellus* capable of killing juvenile abalone of this given size was defined from the above abalone size feeding trials. Second, field densities (# of individuals m^{-2}) of *L. bellus* within this size range were determined using size-frequency data obtained from the field surveys. The two steps were then repeated for *S. acutifrons*, *C. productus* and *P. helianthoides*. The resulting densities (# of individuals m^{-2}) for all four predators were totalled resulting in the index value for that given abalone size. The predation susceptibility index is thus an estimate of the potential predation pressure on juvenile *H. kamtschatkana* for a given site.

Statistical Analyses

To determine if there was a difference in size frequency distributions for each predator within the three field sites surveyed, two-sample Kolmogorov-Smirnov tests were carried out using the statistical analysis software SYSTAT. To determine if field

densities of *Lophopanopeus bellus* and *Scyra acutifrons* were different a t-test was carried out using the statistical software Sigma Stat. To determine the relationship between predator size and the maximum abalone size the given predator could consume, linear regressions were carried out using the statistical software SPSS. Only predators that consumed abalone were included in the regressions.

RESULTS

Potential Predators

Thirty-seven potential predator species were tested to determine if they would eat juvenile *H. kamtschatkana*. Of these, 14 species consumed at least one juvenile abalone in the trials (Table 2). The six species of potential predators that consumed more than 10% of the juvenile abalone offered were: the crabs *Cancer productus*, *Lophopanopeus bellus,* and *Scyra acutifrons,* the seastar *Pycnopodia helianthoides*, the pile perch *Rhacochilus vacca* (Girard 1855), and the snail *Amphissa columbiana. Cancer productus* consumed an average of 67% of juvenile abalone making this species the top potential predator tested. *Cancer productus* left only fragments of juvenile abalone shells behind or sometimes no traces, and *L. bellus* and *S. acutifrons* left abalone shell fragments behind or chipped shells near the whorl (Figure 2A). *Pycnopodia helianthoides* consumed all the abalone tissue and left behind clean intact shells. *Amphissa columbiana* produced 1-2 drill holes approximately 1 mm in diameter in the soft tissues of the abalone they attacked and did not consume the entire abalone (Figure 2B).

Eight predators species consumed 1-10% of the abalone offered: *Embiotica lateralis* (striped perch) (Agassiz 1854), *Evasterias troscheli* (mottled star) (Stimpsoni 1862), *Solaster dawsoni* (morning star) (Verrill 1880), *Dermasterias imbricata* (leather star) (Grube 1857), *Orthasterias koehleri* (painted star) (deLoriol 1897), *Cymatogaster aggregata* (shiner perch) (Gibbons 1854), and *Micrometras frenatus* (kelp perch) (Gill 1862). Abalone that had been preyed upon by *E. lateralis*, *C. aggregata* and *M. frenatus* had damaged shells or were crushed into many fragments (Figure 2C-D). *Rhacochilus vacca*, *C. aggregata*, and *M. frenatus* only consumed juvenile abalone smaller than 10 mm SL.

Table 2: Potential predators of juvenile *Haliotis kamtschatkana*

AP anterior to posterior, CW carapace width, CD central disk diameter, SF standard fork, TD test diameter, SL shell length, n= number of predators. Predator classification: MJ = major predator (≥ 10% of abalone consumed), OC = occasional predator (≤ 10% of abalone consumed), N = not a predator.

Potential Predator Species	Predator Size Range Tested	n	Abalone Sizes Offered (mm SL)	Ave. % Of Abalone Consumed	Predator Status
Controls	No Predator	63	1-25	1.9± 0.1	
Annelida					
Polychaeta					
Halosydna brevisetosa	22-40 mm AP	4	1-20	0± 0	N
Platyhelminthes					
Turbellaria					
Pseudostylochus ostreophagus	28 mm AP	1	1-20	0	N
Mollusca					
Gastropoda					
Prosobranchia					
Neogastropoda					
Muricidae					
Ceratostoma foliatum	6.14-11.6 g	4	1-20	0± 0	N
Columbellidae					
Amphissa columbiana	11-16 mm SL	6	1-25	15.0± 0.1	MJ
Arthropoda					
Crustacea					
Decapoda					
Cancridae					
Cancer productus	58-95 mm CW	3	1-25	67.0± 0.4	MJ
Majidae					
Scyra acutifrons	16-30 mm CW	6	1-25	22.0± 0.4	MJ
Mimulus foliatus	22 mm CW	1	1-25	0	N
Paguridae					
Pagurus hemphilli	1.75-10.37g	5	1-25	0± 0	N
Pagurus caurinus	0.995 g	1	1-15	0	N
Paguristes turgidus	Not recorded	1	1-20	0	N
Pisidae					
Pelia tumida	16 mm CW	1	1-20	43.0	MJ
Porcellanidae					
Pachycheles rudis	10-17 mm CW	4	1-25	0± 0	N
Petrolisthes eriomerus	10 mm CW	2	1-15	0± 0	N
Xanthidae					
Lophopanopeus bellus	10-25 mm CW	9	1-25	35.0± 0.3	MJ

Potential Predator Species	Predator Size Range Tested	n	Abalone Sizes Offered (mm SL)	Ave. % Of Abalone Consumed	Predator Status
Echinodemata					
Asteroidea					
Forcipulatida					
Asteriidae					
Evasterias troschelii	11-24 mm CD	4	1-20	8.0± 0.1	OC
Orthasterias koehleri	5-35 mm CD	6	1-25	4.0± 0.1	OC
Pycnopodia helianthoides	6-31 mm CD	4	1-20	32.0± 0.4	MJ
Spinulosida					
Solasteridae					
Crossaster papposus	47-55 mm CD	2	1-25	0± 0	N
Solaster dawsoni	21-60 mm CD	4	1-25	5.0± 0.1	OC
Solaster stimpsoni	43-55 mm CD	2	1-25	0± 0	N
Valvatida					
Asteropseidae					
Dermasterias imbricata	36-50 mm CD	5	1-25	5.0± 0.1	OC
Echinoidea					
Echinoida					
Strongylocentrotidae					
Strongylocentrotus droebachiensis	63-55 mm TD	3	9-16	0± 0	N
Strongylocentrotus franciscanus	85-125 mm TD	13	6-25	0± 0	N
Strongylocentrotus purpuratus	60-65 mm TD	3	7-16	0± 0	N
Chordata					
Osteichthyes					
Teleostei					
Perciformes					
Embiotocidae					
Rhacochilus vacca	65-82 mm SF	5	1-25	17.0± 0.2	MJ
Embiotoca lateralis	70-100 mm SF	3	1-25	8.0± 0.2	OC
Cymatogaster aggregata	45-95 mm SF	5	1-25	2.0± 0.1	OC
Micrometras frenatus	65-90 mm SF	6	1-25	2.0± 0.1	OC
Gobiidae					
Coryphopterus nicholsi	5-65 mm SF	3	1-5	0± 0	N
Scorpaeniformes					
Cottidae					
Artedius fenestralis	85 mm SF	1	1-25	0	N
Asemichthys taylori	50-65 mm SF	2	1-5	0± 0	N
Leptocottus armatus	150-190 mm SF	2	1-25	0± 0	N
Oligocottus maculosus	42-50 mm SF	4	1-5	0± 0	N
Hexagrammidae					
Hexagrammos decagrammus	200-250 mm SF	2	7-21	0± 0	N
Hexagrammos stelleri	70-115 mm SF	2	1-25	0± 0	N
Scorpaenidae					
Scorpaenichthys marmoratus	90 mm SF	1	1-25	0	N
Sebastes melanops	200 mm SF	1	7-25	0	N

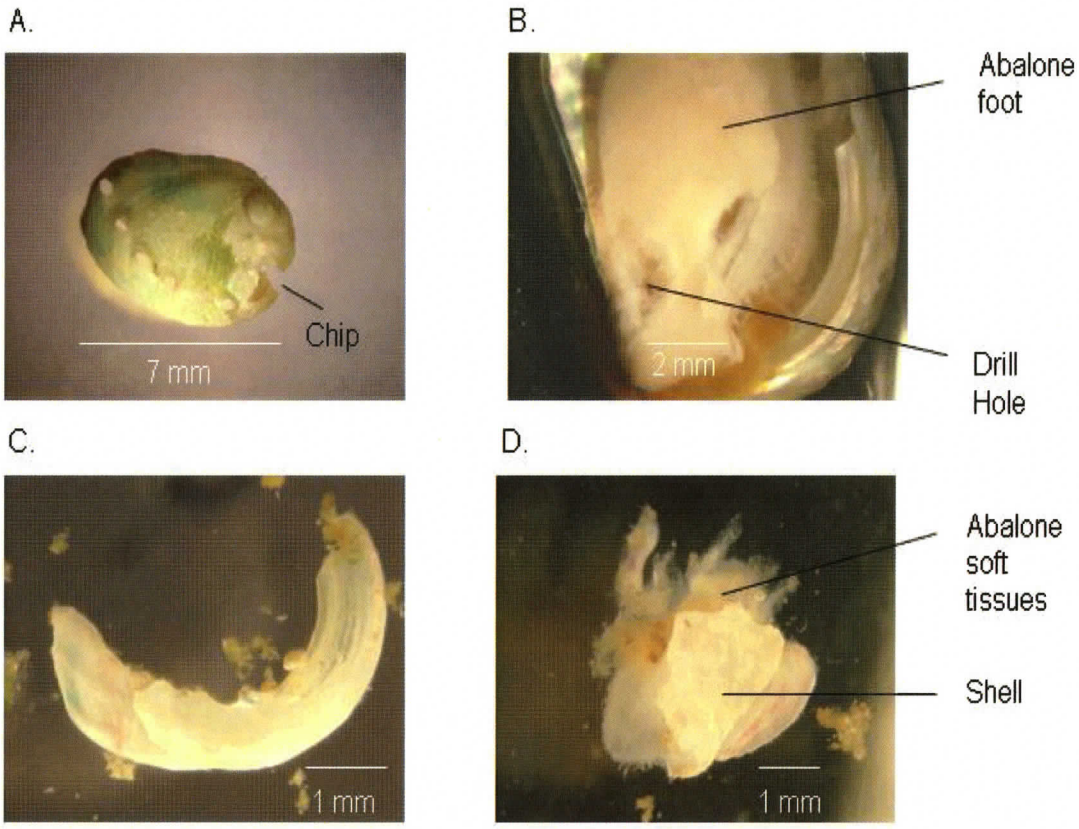

Figure 2: Evidence of juvenile *H. kamtschatkana* predation.
(A) Shell break by a *Scyra acutifrons* with the chipped shell on the bottom right corner. (B) Drill holes in the foot of the abalone that had been preyed upon by *Amphissa columbiana*. (C) Evidence of predation by *Rhacochilus vacca*. The margin of the abalone shell is present. (D) the second piece of shell fragments remaining from the abalone attacked by *Rhacochilus vacca*. Note: the soft tissue of the abalone has been pulled apart.

Predation Pressure on Early Juvenile *H. kamtschatkana*

Field Surveys of Predator Abundances

Of the six predator species surveyed in the field, *Amphissa columbiana* were found at the highest density. Brady's Rock had the highest density of 1.76 individuals m^{-2} for the 12.1- 15 mm SL size followed by Eagle Bay with 1.31 individuals m^{-2} for the 9.1- 12 mm SL size and 0.615 individuals m^{-2} for the 9.1- 12 mm SL size at Bamfield Harbour entrance (Figure 3A). There was no difference in size frequency distribution of any of the major predators among the three field sites (Table 3).

Eagle Bay had the highest abundance of *L. bellus* with 1.61 individuals m^{-2} for the 12.1- 15 mm CW size, followed by Brady's Rock with a maximum abundance of 1.15 individuals m^{-2} for the 9.1-12 mm CW size. Bamfield Harbour entrance had a maximum abundance of 1.07 individuals m^{-2} for the 12.1- 15 mm CW size (Figure 3B). *Scyra acutifrons* were found in the highest abundance at Bamfield Harbour entrance with a maximum density of 1.30 individuals m^{-2} for the 5.1- 10 mm CW size. At Brady's Rock the greatest abundance of *S. acutifrons* was 0.69 individuals m^{-2} for the 5.1- 10 mm CW size and 0.38 individuals m^{-2} for the 10.1- 15 mm CW size at Eagle Bay. These subtidal crabs were difficult to remove from crevices to measure and might have been under-estimated because of their cryptic behaviour. The most common size range of *S. acutifrons* found within all three sites was 5.1- 15 mm CW (Figure 3C). When all field site densities were combined, *L. bellus* were found at higher densities (mean = 0.399 individuals m^{-2}, standard deviation = 0.440) compared to the second most abundant *S. acutifrons* (mean = 0.231 individuals m^{-2}, standard deviation = 0.341). However, the difference between the mean densities were not statistically significant (t-test, $p = 0.179$, $\alpha = 0.05$, $n = 27$).

Densities of *P. helianthoides* and *C. productus* were very low at all three field sites. The highest abundance of *P. helianthoides* was 0.15 individuals m^{-2} for the 0-15 mm CD size at Eagle Bay and Brady's Rock, 30.1- 45 mm CD at Eagle Bay and 45.1- 60 mm CD at Bamfield Harbour (Figure 3D). Only two *C. productus* (25 mm CW and 75 mm CW) were found at Brady's Rock, buried in sand.

Rhacochilus vacca (pile perch) were not present at any of the field sites and thus are not shown in Figure 3. *Rhacochilus vacca* were not used in size vulnerability experiments because *R. vacca* were not present at the field sites.

Size Vulnerability

Abalone Size Feeding Trials

Lophopanopeus bellus (< 20 mm CW) tested were able to eat abalone 10 -12 mm SL. The larger 26 mm CW *L. bellus* was able to eat abalone 10 -19 mm SL in this experiment. *L. bellus* size explained approximately 67% of the variation in the size of abalone preyed upon ($R^2 = 0.674$, $p = 0.045$) (Figure 4).

Scyra acutifrons < 22 mm CW were unable to eat abalone larger than 10-12 mm SL (Figure 4). However, one 25 mm CW *S. acutifrons* ate abalone in the size class 15-19 mm SL and a 28 mm CW *S. acutifrons* was able to consume a 24-28 mm abalone. Abalone that were not consumed were often found with their respiratory pores cracked open. There was a weak relationship between predator size and prey size for *S. acutifrons* and abalone ($R^2 = 0.499$, $p = 0.076$). Small *S. acutifrons* (< 22 mm CW) would leave behind pieces of abalone soft tissue in the shells of the dead abalone.

All *Cancer productus* used in this experiment consumed all sizes of abalone offered (Figure 4). Abalone eaten by *C. productus* had no soft tissues left in them and the abalone shells were either completely crushed, crushed in half, or were broken at the shell whorl.

A strong relationship exists between predator size and largest prey size for *P. helianthoides* and abalone ($R^2 = 0.888$, $p = 0.005$) (Figure 4). It is likely that *P. helianthoides* > 55 mm could consume abalone larger than 28 mm SL because the four largest *P. helianthoides* tested could consume the 28 mm SL abalone. Consequently, the two largest *P. helianthoides* tested were not included in the regression because the maximum abalone size that these *P. helianthoides* could consume was larger than the largest abalone offered, and therefore the maximum abalone size these seastars could consume was not known. Abalone eaten by *P. helianthoides* had no soft tissues left and their shell insides were shiny and clean.

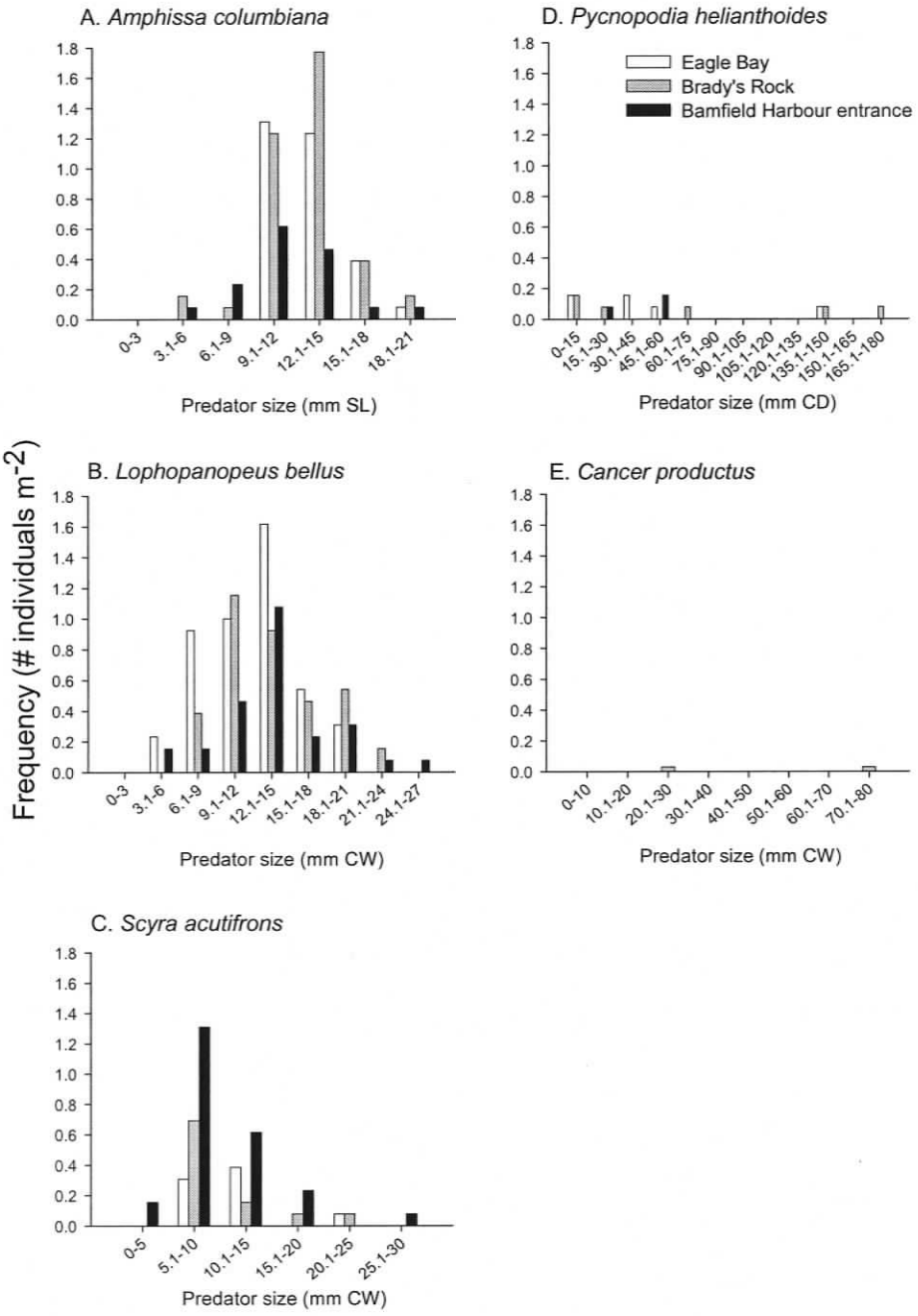

Figure 3: Size frequency distributions of juvenile *H. kamtschatkana* predators. (A) *Amphissa columbiana*. (B) *Lophopanopeus bellus*. (C) *Scyra acutifrons*. (D) *Pycnopodia helianthoides*. (E) *Cancer productus*. Note: CW = carapace width, SL = shell length, CD = central disk diameter.

Table 3: Comparison of predator abundances among the three field sites using two-sample Kolmogorov-Smirnov tests

	Z	*p*	n
Amphissa columbiana			
Eagle Bay vs Brady's Rock	0.286	0.919	7
Eagle Bay vs Bamfield Harbour entrance	0.286	0.919	7
Brady's Rock vs Bamfield Harbour entrance	0.286	0.919	7
Lophopanopeus bellus			
Eagle Bay vs Brady's Rock	0.111	1.000	9
Eagle Bay vs Bamfield Harbour entrance	0.333	0.662	9
Brady's Rock vs Bamfield Harbour entrance	0.333	0.662	9
Scyra acutifrons			
Eagle Bay vs Brady's Rock	0.167	1.000	6
Eagle Bay vs Bamfield Harbour entrance	0.333	0.778	6
Brady's Rock vs Bamfield Harbour entrance	0.333	0.778	6
Pycnopodia helianthoides			
Eagle Bay vs Brady's Rock	0.083	1.000	12
Eagle Bay vs Bamfield Harbour entrance	0.167	0.985	12
Brady's Rock vs Bamfield Harbour entrance	0.250	0.769	12

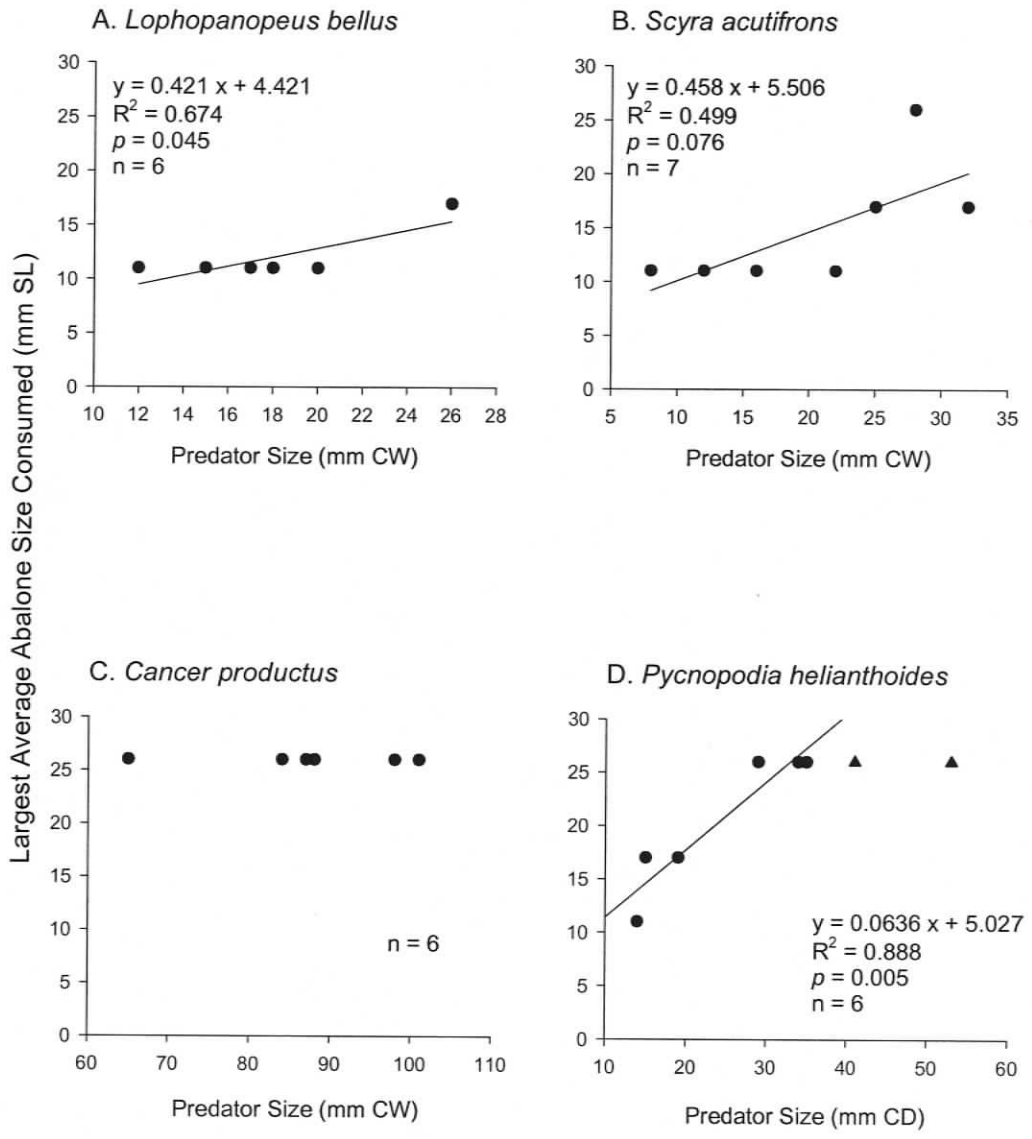

Figure 4: Predator size and the largest average juvenile *H. kamtschatkana* size consumed. Note: CW = carapace width, CD = central disk diameter. The triangle symbols in (D) represent values that were not used to calculate the regression (see text).

Of 20 *A. columbiana* tested in the potential predator trials, none ate any abalone. This was an unexpected finding given that the six *A. columbiana* used in the initial potential predation experiment consumed on average 15% of the 2-13 mm SL juvenile abalone offered to them.

Predation Susceptibility Index

The predation susceptibility index value for a given abalone size at a field site represents the total density (individuals m^{-2}) of juvenile abalone predators (*Lophopanopeus bellus, Scyra acutifrons, Cancer productus and Pycnopodia helianthoides*) at that site that would feed on an abalone of that size. The predation susceptibility index decreased rapidly with increasing abalone size, levelling off at an index value of ≤ 1 at abalone sizes ≥ 12 mm SL at Eagle Bay, and 13 mm SL at Brady's Rock and Bamfield Harbour entrance (Figure 5A). Eagle Bay had the highest predation susceptibility index for juvenile abalone < 5 mm SL, followed by Brady's Rock and Bamfield Harbour entrance. The predation susceptibility index calculations were then repeated without data from *L. bellus*, the most abundant predator in the field at all three sites, to investigate the impact *L. bellus* had on the combined PSI from all four predators (Figure 5B). *Lophopanopeus bellus* is the most important contributor at these sites to the pattern of ontogenetic change in susceptibility. Without *L. bellus* the predation susceptibility index was considerably lower at all sites. Bamfield Harbour entrance had the highest predation susceptibility index value of 2.69 followed by Brady's Rock and Eagle Bay with index values of 1.54 and 1.23 respectively. The predation susceptibility index without *L. bellus* levelled off at an index value ≤ 1 at an abalone size of $\approx$10 mm SL at Eagle Bay, $\approx$11 mm SL at Brady's Rock and $\approx$12 mm SL at Bamfield Harbour entrance.

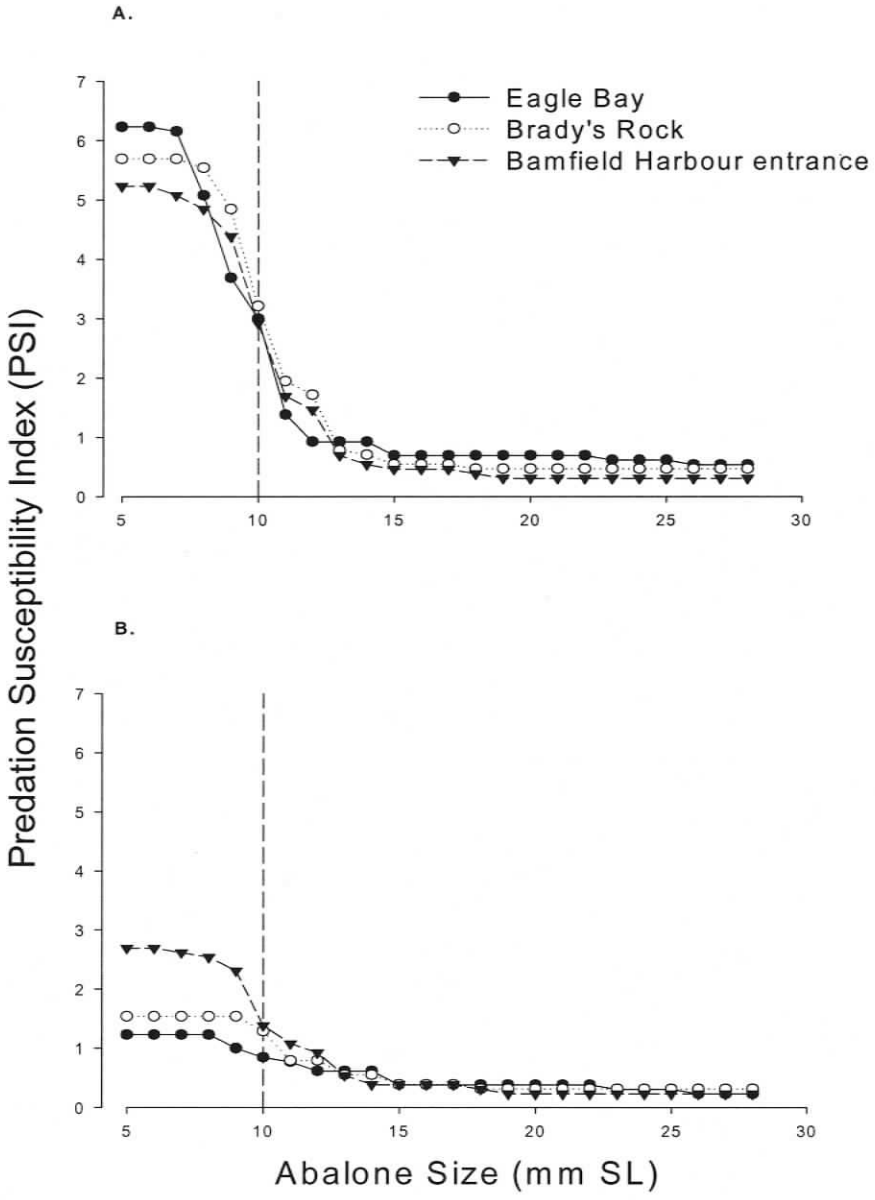

Figure 5: Predation susceptibility index (PSI) for juvenile *Haliotis kamtschatkana*. The predation susceptibility index consists of the total density (# of individuals m^{-2}) of all juvenile abalone predators (*Lophopanopeus bellus*, *Scyra acutifrons*, *Cancer productus* and *Pycnopodia helianthoides*). (A) represents the ontogenetic change in the PSI including all four juvenile abalone predator species, and (B) represents the ontogenetic change in the PSI excluding *L. bellus*. Index values were calculated for every 1 mm shell length size increment. The dashed line at 10 mm represents the smallest size of abalone that was tested in the laboratory with the four predators; PSI values for abalone < 10 mm SL are based on extrapolations of the regression analyses between 10 and 28 mm.

Crab Claw Morphologies

The morphologies of the principle claws of the three crab predators (*C. productus*, *S. acutifrons* and *L. bellus*) are distinct, with considerable differences in size. *Cancer productus* have claws that are powerful and are larger than those of *L. bellus* or *S. acutifrons* for crab of similar size (Figure 6A). *Scyra acutifrons* have a narrower claw than *C. productus* (Figure 6B). *Lophopanopeus bellus* have wider claws than *S. acutifrons* but they are not as wide as the claws of *C. productus* (Figure 6C). Molariform teeth can be seen on *C. productus* and *S. acutifrons*, but are smaller on *L. bellus*. It is also apparent that the teeth on *S. acutifrons* are smaller than those of *C. productus*.

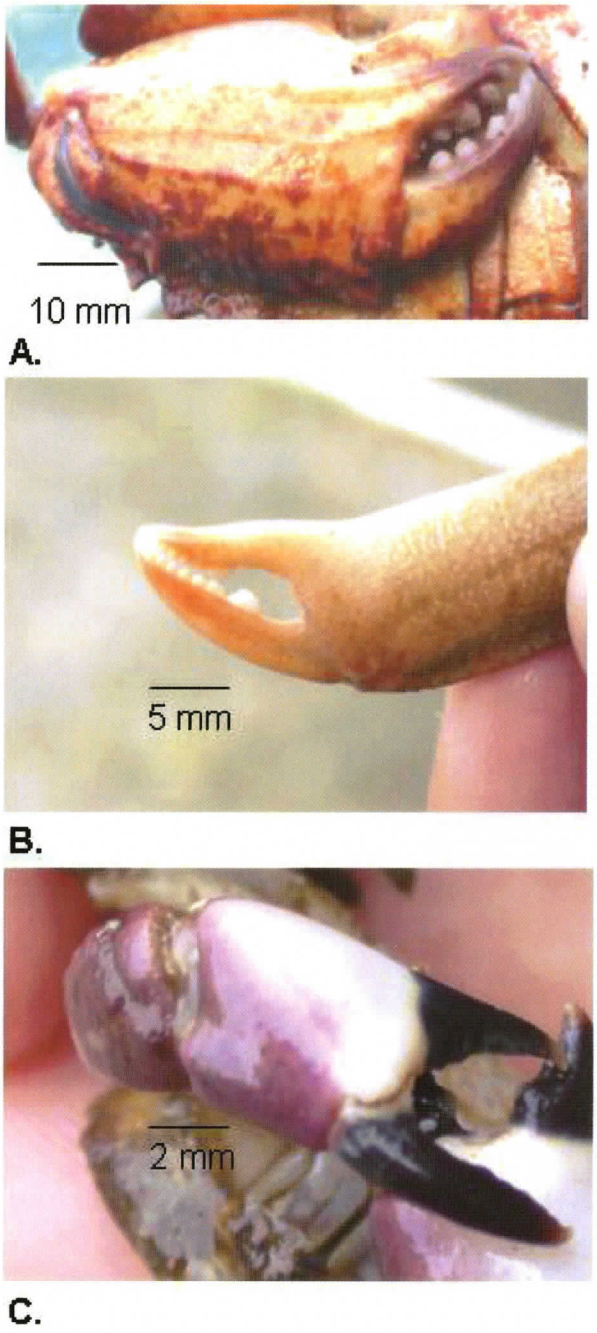

Figure 6: Claws from crab predators.
(A) A 84 mm CW *Cancer productus*. (B) A 22 mm CW *Scyra acutifrons*. (C) A 17 mm CW *Lophopanopeus bellus*.

DISCUSSION

Potential Predators

Of the 37 species of potential predators tested, my data indicate 14 may actually feed on juvenile *Haliotis kamtschatkana* in the field. Of these, six are likely to be main threats to the juvenile abalone. Feeding laboratory experiments revealed that *Lophopanopeus bellus, Pycnopodia helianthoides, Cancer productus, Scyra acutifrons,* and *Amphissa columbiana* are major predators of juvenile *H. kamtschatkana*. Campbell (2000), and Emmett and Jamieson (1988) both stated that *C. productus*, and *P.helianthoides* are predators of adult *H. kamtschatkana* and thus juvenile and adult *H. kamtschatkana* share these predators. *Amphissa columbiana* were found to be major predators when tested in first laboratory trials determining the potential predators of juvenile abalone and were the only gastropod in my study to prey upon the juvenile *H. kamtschatkana*. However, when tested in size vulnerability experiments, *A. columbiana* did not consume abalone. Perhaps when *A. columbiana* consumed abalone in the first laboratory experiment, the abalone were either stressed, or unhealthy. These gastropods will actively scavenge any carcass releasing body fluids (Lamb and Hanby 2005). Neogastropods in the genera *Nassarius, Buccinum, Kelletia, and Pleuroploca* have been reported to prey on abalone species such as *H. rubra* (Leach 1815), and *H. scalaris* (Leach 1814) in Australia (Shepherd 1973). Shepherd and Breen (1992) reported that weakened, stressed or aged abalone comprise the majority of reported cases of all gastropod predation where the gastropod inserts their proboscis into the abalone soft tissues. *Amphissa columbiana* are abundant in the field but probably do not pose a major threat to healthy juvenile *H. kamtschatkana*.

The fish species *Enophrys bison*, *Scorpaenichthys marmoratus*, *Ophiodon elongates*, and *Anarrhichthys ocellatus* were not tested in the laboratory trials as mentioned earlier. Four of the thirteen fish species that were tested to determine the potential predators of juvenile *H. kamtschatkana* consumed at least one juvenile abalone, and one fish species, *Rhacochilus vacca,* consumed several abalone and was classified as a major predator. Thus, it is unlikely that the fish species that were not tested would be major predators of the juvenile abalone.

When interpreting the results of the predation trials, it is important to remember that these are laboratory results where predator enclosures are used. Predator dispersal is hindered by the enclosures (Sih et al 1985) and some of these potential predators may not have preyed upon abalone in the laboratory because they were stressed.

Frequency of Predators in the Field

Lophopanopeus bellus were found in the highest abundance at Eagle Bay. These cryptic crabs are found under rocks, gravel, sand, or mud (Kozloff 1996; Harbo 1999). They are mobile predators that can move rapidly and prey upon juvenile *H. kamtschatkana* in the field (personal observations). Often when rocks are overturned, *L. bellus* were seen quickly taking shelter in the sand.

Scyra acutifrons are commonly found between and underneath rocks (Jensen 1995; Harbo 1999). In my field surveys, *S. acutifrons* were moderately abundant. Eighty-five percent of the *S. acutifrons* found in the field were smaller than 20 mm CW. *Cancer productus* were found in very low densities at the three study sites.

Pycnopodia helianthoides were occasionally observed in the field surveys, ranging in size from 6-175 mm CD. *Pycnopodia helianthoides* 15 mm or less did not consume abalone larger than 19 mm SL in my feeding trials. Sea urchins and bivalves are thought to be preferred prey of *P. helianthoides*, although they are generalist feeders and will feed on other prey species (Shivji et al 1983). In the wild, *P. helianthoides* arms can be used to move adult abalone off rocks by inducing the abalone escape response (Emmett and Jamieson 1988; Sloan and Breen 1988) and is also effective with juvenile *H. kamtschatkana* (personal observations). Thus, *P. helianthoides* appear to pose a potential threat to juvenile abalone in both the laboratory and the wild.

The importance of habitat refugia is seen in situations where prey do not have a size refugium (Peterson 1982). This is true for juvenile *H. kamtschatkana* in which there exist specific size refugia for some predators, but other predators can consume all sizes of juvenile abalone tested in the laboratory experiments. Therefore, habitat refugia are critical for the survival of juvenile abalone.

Size Vulnerability

Abalone size feeding trials

Juvenile *H. kamtschatkana* do not appear to have a size refugia from either *C. productus* or *P. helianthoides*. However, both of these predators were found at low densities in the field. The largest *L. bellus* tested (26 mm CW) was able to eat 15-19 mm SL abalone but *L. bellus* of that size were very rare at my field sites. Juvenile *H. kamtschatkana* reaching a size larger than 12 mm SL are therefore less vulnerable to predation by *L. bellus*. *Scyra acutifrons* smaller than 20 mm CW could only eat juvenile *H. kamtschatkana* up to 10 mm SL. *Scyra acutifrons* larger than 20 mm CW are able to consume abalone larger than 10 mm SL, however, these crabs are rare in the field and therefore do not appear to pose a major threat to juvenile abalone survival.

Predation Susceptibility Index (PSI)

Lophopanopeus bellus were the most abundant predators in the field and therefore have a substantial impact on the predator susceptibility index values. When *L. bellus* are included in the PSI calculations, juvenile abalone ≥12 mm SL are at a considerably lower risk of predation than smaller sized abalone. However when *L. bellus* are excluded from the PSI calculations, an abalone size of ≥ 10 mm SL is at a lower risk of predation than ≤ 10 mm. If juvenile abalone can reach a size of 10 mm they may be less susceptible to predation risks by *C. productus, S. acutifrons,* and *P. helanthoides* at the three study sites used in this study. However, juvenile abalone might not be less susceptible from *L. bellus* until they are ≥ 12 mm.

The PSI demonstrates that, for juvenile abalone, size appears to be a critical factor in determining susceptibility to predation. A slight 2 mm difference in shell length in juvenile abalone may be the determining factor between survival and mortality. Selection should favour rapid early growth for juvenile abalone if the size refugia from *L.bellus* is present in the wild once the juvenile abalone reach ≥ 12 mm (Paine 1976). *L. bellus* typically live under rocks buried in the sand (personal observations). Juvenile abalone ≤ 10 mm SL would be less susceptible to predation by *L. bellus* if they avoided taking refuge under rocks until they are ≥ 12 mm. However, if these ≤ 10 mm SL juvenile abalone do not take refuge under rocks, they are vulnerable to predators such as

C. productus, S. acutifrons, and *P. helanthoides* that are predominantly found on the surface of rocks and sand (personal observations). Thus, it is likely that juvenile *H. kamtschatkana* ≥12 mm SL are less restricted to specific habitats than juvenile *H. kamtschatkana* ≤ 10 mm SL.

Bamfield Harbour entrance had a slightly lower PSI (all predators combined) than the two other sites, suggesting it might be a safer location for juvenile abalone ≥ 12 mm SL. This site would be better suited for outplanting juvenile abalone then at a site such as Eagle Bay which has a higher PSI. The differences in PSI values between field sites may help to explain why adult abalone populations are higher at certain field sites and lower at other sites. If juvenile abalone are more susceptible at one field site, they have a lower chance of reaching adult size. Field sites with no or few *L. bellus* should be used.

Crab Claw Morphology

Crabs that prey on hard-shelled animals can be categorized into two functional groups: generalists and specialists (Yamada and Boulding 1998). Generalists usually have omnivorous diets and weak claws with small fine denticles compared to the specialists that have large powerful claws with wide blunt molars (Yamada and Boulding 1998; Seed and Hughes, 1995). Generalists have lower mechanical advantage of the claw's lever functioning compared to specialists (Yamada and Boulding 1998; Schenk and Wainwright 2001; Seed and Hughes 1995). *Cancer productus* and *L. bellus* are specialists (Yamada and Boulding 1998). The molariform teeth of *C. productus*, structures on crab claws that are used to aid in the stabilization of prey, are large indicating that perhaps this species is at a mechanical advantage to crab species without large molariform teeth (Bertness and Cunningham 1981). *Cancer productus* eat clams (Boulding 1984), snails (Emmett and Jamieson 1988; Yamada and Boulding 1998; Campbell 2000), mussels, barnacles and smaller crabs (Jensen 1995) and *L. bellus* are omnivorous, feeding on small molluscs (Yamada and Boulding 1998), algae and barnacles (Jensen 1995).

Scyra acutifrons feed primarily on detritus and sessile invertebrates (Jensen 1995). These crabs are in the family Majidae that includes species such as *Libinia*

emarginata (spider crab), and have long claws with minimal dentition (Schenk and Wainwright 2001). *Libinia emarginata* feed mainly (up to 75%) on plants but is known to consume molluscs (Schenk and Wainwright 2001). Also *L. emarginata* does not have dimorphic claws (Schenk and Wainwright 2001) commonly found in crabs that feed on hard-shelled molluscs, with one claw used as the crusher that is larger and more powerful and the other smaller, less robust claw used for shearing (Seed and Hughes 1995; Schenk and Wainwright 2001). Thus, it is likely that *S. acutifrons* are generalist feeders.

Boulding (1984) found that as *Cancer productus* size increased (carapace width) both chela gape and chela strength increased. This is consistent with my findings that larger crabs could consume larger abalone. It is surprising though that *L. bellus* did not consume abalone larger than 17 mm SL but similar sized *S. acutifrons* consumed abalone as large as 27 mm SL. *Lophopanopeus bellus* claws are built for crushing hard-shelled prey (Yamada and Boulding 1998) and *S. acutifrons* feed primarily on detritus and sessile invertebrates (Jensen 1995). However, a review of 41 studies of decapod crustacean predation on molluscan prey by Juanes (1992) revealed that small sized prey were preferred by a variety of predator sizes even though the predators could consume all prey size ranges offered to them. Shepherd and Breen (1992) also found that crabs prefer smaller prey that they can easily handle. The larger shells are more energetically expensive to break and increase the chance of claw damage to the predator (Boulding 1984; Juanes 1992; Seed and Hughes 1995).

The limited shell crushing ability of some crabs can sometimes be compensated by specialized shell opening behaviour (Seed and Hughes 1995; Schenk and Wainwright 2001). Bertness and Cunningham (1981), discuss the difference between crab predators that crush and those that peel the shells of their prey. When the prey's shell is relatively small compared to the predator's claw, crushing is used, whereas when the prey's shell is larger the predator can peel the shell. All of the juvenile *H. kamtschatkana* that were killed by *C. productus* were crushed or broken at the whorl suggesting they have the potential to consume *H. kamtschatkana* larger than 24-28 mm SL. The four *L. bellus* that ate the 15-19 mm abalone showed evidence of shell peeling along the respiratory pores of abalone shells. Similarly, two of four *S. acutifrons* peeled abalone shells (10-12 mm SL)

around the respiratory pores and abalone size classes above 10-12 mm were not consumed.

Releasing hatchery-reared abalone

Applying the results from the predation trials to restocking efforts for *H. kamtschatkana* populations in the wild through release of hatchery-reared abalone, I would suggest outplanting juvenile *H. kamtschatkana* ≥ 12 mm SL to decrease the vulnerability to predation for the abalone. It is difficult to remove predators such as cryptic *L. bellus* from a field site before releasing abalone, and it is easier to release larger *H. kamtschatkana*. Larger, more visible predators such as *C. productus* and *P. helianthoides* could be removed from the release sites and relocated to an alternative location to increase the chances of survival of the juvenile *H. kamtschatkana*. A field site such as Bamfield Harbour entrance that has a lower predation susceptibility index should be used as a location to release the juvenile *H. kamtschatkana*.

Chapter 3: Estimating Natural Mortality in Juvenile *Haliotis kamtschatkana* Using Mark- Recapture

ABSTRACT

The first days to months for early juvenile invertebrates are critical periods of life when vulnerability to mortality is high. Predation, competition, and environmental stresses are among the factors that contribute to early juvenile mortality. The primary goals of this study were to quantify early juvenile mortality in *H. kamtschatkana* and to document ontogenetic changes in mortality rates for juveniles up to a size of 25 mm SL. This was accomplished using a mark-recapture outplanting study using bee tags and calcein marks. To quantify juvenile mortality as accurately as possible, four additional sets of experiments were carried out to (1) determine the efficiency of the search method at locating juvenile *H. kamtschatkana in situ*, (2) estimate the dispersal rate of juvenile *H. kamtschatkana* to determine if individuals might have crawled out of the study area during the mark and recapture study, (3) compare recovery rates of juvenile abalone by divers during daytime and nighttime surveys in the field and (4) determine if the number of empty shells of dead abalone found in the field can be used as an estimate of mortality. Hatchery-reared juvenile *H. kamtschatkana* (1- 25 mm shell length (SL)) were released (outplanted) at two field sites in Barkley Sound, British Columbia. The initial health of abalone < 3 mm SL appears to have been poor and these abalone can experience up to 24 % mortality due to prior health. Predation had an impact on mortality in all abalone size classes tested and appears to be responsible for > 60 % of the mortality observed in field treatments. As juvenile abalone increase in shell size they become less vulnerable to mortality from prior health, handling and predation. From the present study, it can be suggested that juvenile abalone larger than 3.1 mm SL are > 50 % less vulnerable to mortality than the < 3 mm SL abalone and are a reasonable size to release by outplanting to increase wild abalone populations.

INTRODUCTION

Populations of marine invertebrates are affected by physical and biological factors that vary spatially and temporally. One of the most important factors that structure

populations, but is poorly understood, is natural mortality (Sih et al 1985; Shepherd and Breen 1992). Natural mortality is defined as the frequency of death due to non-anthropogenic causes (Rumrill 1990). Natural mortality rates are poorly understood because of variations in physical and biological factors that affect populations from location to location and from year to year (Shepherd and Breen 1992).

For newly metamorphosed marine invertebrates, mortality caused by predation, competition, delay of metamorphosis, physiological stress and abiotic disturbances is high (Gosselin and Qian 1997; Hunt and Scheibling 1997; Moran 1999). In addition to their high vulnerability, early juvenile benthic invertebrates are often ecologically distinct from later juvenile and adult stages because they may have different predators, be more vulnerable to abiotic factors, have different food requirements, different distribution in habitat, different behaviour and different body coloration (Gosselin 1997).

The early juvenile stage of *Haliotis kamtschatkana* (northern abalone) has not been well studied (Sloan and Breen 1988; Campbell 2000). However, juvenile *H. kamtschatkana* are thought to have mortality rates that are higher than adults (Sloan and Breen 1988). For juvenile *H. kamtschatkana* some natural mortality is thought to be caused by predation, competition and environmental factors (Sloan and Breen 1988; Campbell 2000). Juvenile *H. kamtschatkana* are highly cryptic which may reflect their vulnerability to predation (Sloan and Breen 1988). However, there are no documented cases of predation on juvenile *H. kamtschatkana* (Sloan and Breen 1988).

A common and effective method for estimating natural juvenile mortality in motile invertebrates such as abalone is through mark-recapture experiments (Quayle 1971; Shepherd and Breen 1992; Shepherd 1998). For motile animals, mark-recapture is often the only monitoring method available for short-term studies during which animal dispersal is limited (Shepherd and Godoy 1989; Levin 1990; Gosselin and Qian 1997). Various methods of marking and tagging individuals have been used for both juvenile and adult abalone, including bee tags (Shepherd et al. 2000), coded wire tags (Seki and Taniguchi 2000), spaghetti tags (Quayle 1971; Breen and Adkins 1980; Emmett and Jamieson 1988), hatchery coloration (Rogers-Bennett and Pearse 1998), yellow plastic cement (Schiel and Welden 1987) and more recently acoustic transmitters (Holmes and Tomacik 2004).

In the first few months after metamorphosis, mortality in the hatchery is high for juvenile abalone and as the juvenile abalone grow their mortality rates decrease considerably (Dr. Dawn Renfrew, personal communication). It is not clear, however, when this change in mortality rates occurs. The primary goals of this study were therefore to quantify early juvenile mortality in *H. kamtschatkana* in the field and to document ontogenetic changes in mortality rates for juveniles up to a size of 25 mm shell length (SL) which corresponds to approximately the first 24 months of juvenile life in this species (Dr. Dawn Renfrew, personal communication). This was accomplished using a mark-recapture outplanting study. To quantify juvenile mortality as accurately as possible, four additional sets of experiments were carried out to (1) determine the efficiency of the search method for locating juvenile *H. kamtschatkana in situ*, (2) estimate the dispersal rate of juvenile *H. kamtschatkana* to determine if individuals might have crawled out of the study area during the mark and recapture study, (3) compare recovery rates of juvenile abalone by divers during daytime and nighttime surveys in the field, and (4) determine if the number of empty shells of dead abalone found in the field can be used as an estimate of juvenile mortality.

METHODS

To quantify early juvenile mortality in *H. kamtschatkana* and to document ontogenetic changes in mortality rates for juveniles up to a size of 25 mm SL four field experiments were carried out. Experiment one involved determining the efficiency of the search method for locating juvenile abalone on site to ensure that all abalone present were being located. Experiment two involved estimating dispersal rates of tagged hatchery-reared juvenile *H. kamtschatkana* to determine if abalone were able to crawl out of the study area during the mark-recapture study and therefore not be located. Experiment three compared recovery rates of marked juveniles in the field between daytime and nighttime surveys. Finally, experiment four determined if the empty shells found in a mark-recapture study are good estimates of mortality. The abalone sizes, numbers and marking methods used for each experiment are listed in Table 4.

Table 4: Abalone sizes, and marking methods used in each experiment described herin. Within each of these experiments unequal numbers of each abalone size class were used based on abalone availability from the Bamfield Huu-Aht Community Abalone Hatchery. Note: The 20.1-25 mm SL abalone were not used in experiment two.

Abalone SL Size Class (mm)	Marking Method Used	Treatments		
		# of abalone outplanted in each quadrat	# of abalone in each field control	# of abalone in each laboratory control
1-3	Calcein	20	20	20
3.1-5	Calcein	20	20	20
5.1-10	Blue bee tags	10	10	10
10.1-15	Yellow bee tags	10	10	10
15.1-20	Green bee tags	5	5	5
20.1-25	Orange bee tags	5	5	5

Preparation of Abalone

All juvenile *H. kamtschatkana* used in this study were obtained from the Bamfield Huu-Ay-Aht Community Abalone Project (BHCAP) hatchery located at the Bamfield Marine Sciences Centre (BMSC) in Bamfield, B.C. (Figure 7). Wild *H. kamtschatkana* brood stock were spawned in the hatchery and larvae were then settled onto plastic plates (39 × 32 cm). Newly settled abalone were reared in 1100 L tanks with approximately 50,000 individuals in each tank. Seawater used for the hatchery system was drawn from Bamfield Inlet at a depth of 30 m and was then filtered through a 10 μm mesh filtration system. The selection of abalone size classes used in these experiments was based on past research with small juvenile abalone carried out by Shepherd and Turner (1985), Schiel and Welden (1987), Schiel (1993) and Rogers-Bennett and Pearse (1998), but was also constrained by availability from the BHCAP hatchery at the time of experimentation.

Marking and tagging

Calcein was used to mark 1-5 mm SL juvenile *H. kamtschatkana* to increase the probability of relocating them in the field after release. Calcein solutions of 40.8 mg/L were prepared by mixing seawater and calcein powder together (Product #C0875, SIGMA). The abalone on plastic settlement plates (39 × 32 cm), were placed in a sea table (160 × 65 × 7 cm) immersed in the calcein solution. Abalone were fed diatoms and were immersed in the calcein solution for 48 h (c.f. chapter 4 for complete details on the calcein marking procedure).

Bee tags, purchased through Bee Works (Ontario, Canada), were used to tag juvenile abalone ranging from 5.1-25 mm SL. Bee tags are circular plastic disks, 1 mm diameter and are pre-numbered and colored. To apply bee tags, abalone were removed from seawater, placed on a damp cloth, and their shells blotted dry with a towel. One drop of superglue was placed closest to the spire of the shell and a bee tag was placed on top of the glue. The glue was then left to dry for at least one minute before abalone were returned to seawater. Different tag colours were used for each of three abalone size classes to help distinguish between size classes in the field.

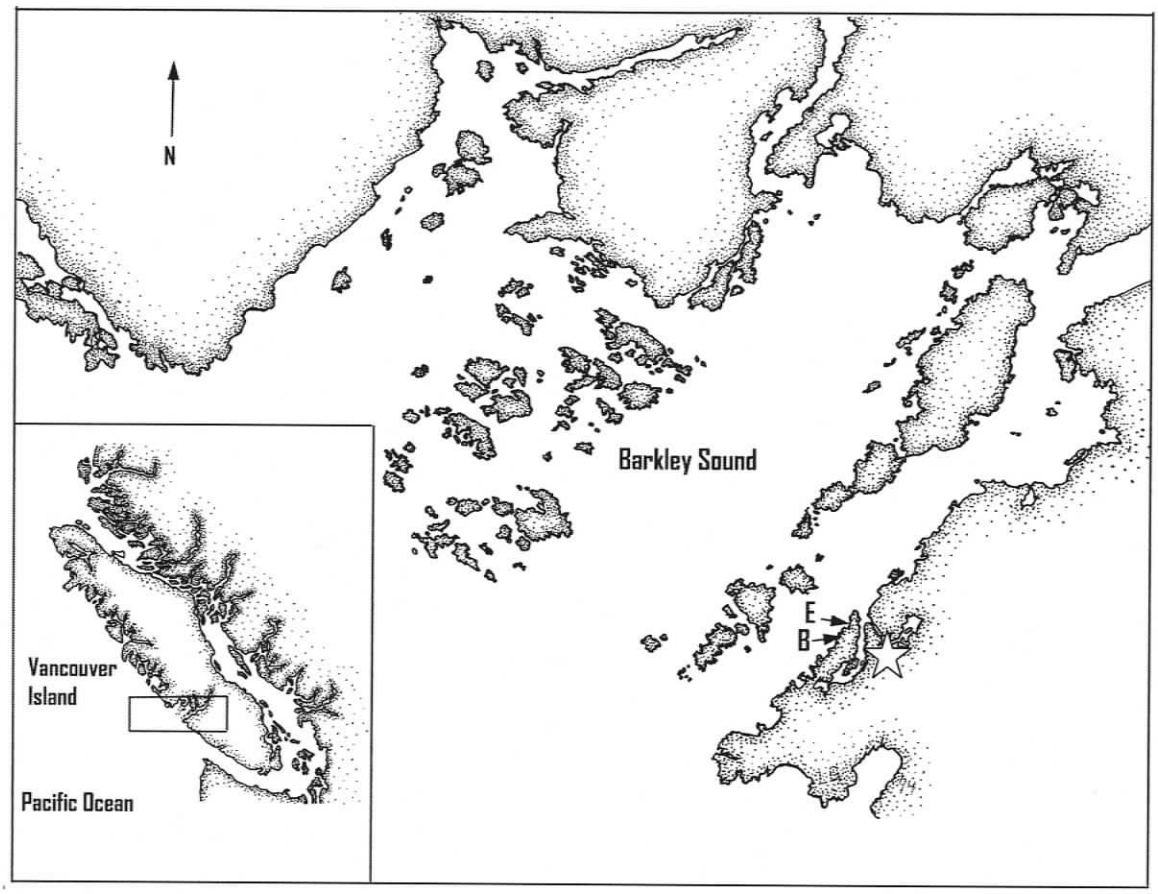

Figure 7: Map of Barkley Sound, British Columbia, Canada.
Laboratory facilities located at the Bamfield Marine Sciences Centre (star symbol). The two field sites: Brady's Rock, and Eagle Bay are identified in the figure by B, and E respectively.

Field Site Selection

The two field sites used for outplanting juvenile *H. kamtschatkana* were Eagle Bay (48°50.252′ N, 125°08.670′ W) and Brady's Rock (48°49.940′ N, 125°09.021′ W) (Figure 7). The criteria for selecting field sites were that they should be located within 30 minutes travel by boat from BMSC, have an abundance of kelp growing at the site for food for the abalone, and have wild abalone present in the area as well as complex boulder substrates on which juvenile *H. kamtschatkana* could take shelter. These sites were chosen by investigating previous data on *H. kamtschatkana* abundance in Barkley Sound (Mortimor et al. 2003) and by conducting three survey dives at Eagle Bay and Brady's Rock between March 2004 and May 2004 to search for adult abalone.

Johnson (2001) commented on the presence of adult *H. kamtschatkana* in the Eagle Bay and Brady's Rock area and estimated their population densities at 2.6- 3.8 abalone m^{-2}. Mortimor et al. (2003) carried out daytime and nighttime surveys in Eagle Bay and found 0.4 abalone m^{-2} in the daytime and 0.7 abalone m^{-2} at nighttime, ranging in size from 8-110 mm SL. *Macrocystis integrifolia* (giant kelp) (Bory 1826), and *Nereocystis luetkeana* (bull kelp) (Postels and Ruprecht 1840), and suitable substrate (i.e. boulders 15-30 cm diameter, cobble 1-5 cm diameter, and coralline algae) were present at both field sites. Finally, Eagle Bay and Brady's Rock sites also had moderate wave exposure and were close to the BMSC facilities, and were therefore likely to be accessible even in poor weather.

Preparation of abalone and outplanting

Twelve hours before the outplantings 1-5 mm SL abalone were placed in a sealed plastic bag (20 × 20 cm) whereas 5.1-25 mm abalone were placed in a separate plastic bag (40 × 30 cm). Each bag also had a piece of PVC pipe (20 cm diameter) that had been cut in half and placed in the bags for shelter and a piece of *M. integrifolia* for food (Figure 8A). Juvenile *H. kamtschatkana* were transported in the early morning to the outplanting site by boat in a seawater-filled plastic ice chest (59 × 30 × 30 cm). The time taken to transport the juvenile abalone from their laboratory holding tanks to the field outplant sites at depths of 5-7 m was approximately 35 minutes. Abalone were brought down to the benthos by divers and 5.1-25 mm SL juvenile abalone were individually taken out of the plastic bags and placed foot down on rocks in the middle of the quadrats.

The bags containing 1-5 mm SL abalone were turned inside out and abalone were settled in the centre of the quadrat.

Searches for Outplanted Juvenile *H. kamtschatkana*

For each location where juvenile abalone were outplanted, an area of 3 × 3 m was searched, centered on the location where the abalone were outplanted.

Field Controls

Field controls were used to quantify non-predatory mortality of outplanted *H. kamtschatkana*. All abalone in the field controls had the same calcein mark or bee tags as the outplanted abalone. Each outplanting location had two matching field control cages, one with 1-5 mm SL abalone and one with 5.1-25 mm SL abalone. These cages were attached directly beside the open outplanting locations (Figure 8 B). The cage used for 1-5 mm SL abalone was 13 × 13 × 8 cm and the cage used to hold 5.1-25 mm SL abalone was 20.5 × 20.5 × 10 cm. Each of the two control cages had two sides covered by 600 µm Nitex mesh to allow for water flow. Each time a survey was conducted to find outplanted abalone, the field control cages were also checked for survivorship. Field controls were monitored until day 7 after the outplanting; they were not monitored on day 30 of the survey because control cages were lost due to extreme weather conditions.

Laboratory controls

Two sets of laboratory controls were used to control for mortality unrelated to field conditions (eg. due to handling or abalone health). One set of controls consisted of calcein marked or bee-tagged abalone in cages. The other laboratory control consisted of non-tagged abalone in cages. Both tagged and untagged abalone cages were placed in a single seawater table (160 × 65 × 22 cm) with flowing filtered seawater. Filter bags with a mesh size of 1 µm were cleaned every 24 h to prevent clogging. The number and sizes of abalone used as tagged and marked laboratory controls are listed in Table 4. Benthic diatoms, obtained from the BHCAP abalone hatchery, were fed to the 1-5 mm abalone once every 3 d by pouring the diatom-seawater mixture into the abalone containers as is normally done in the hatchery for juveniles this size. The 5.1-25 mm SL abalone were fed 2 × 2 cm pieces of *M. integrifolia* every second day. Laboratory controls were checked for surviving abalone within 2 h after each survey of field outplants.

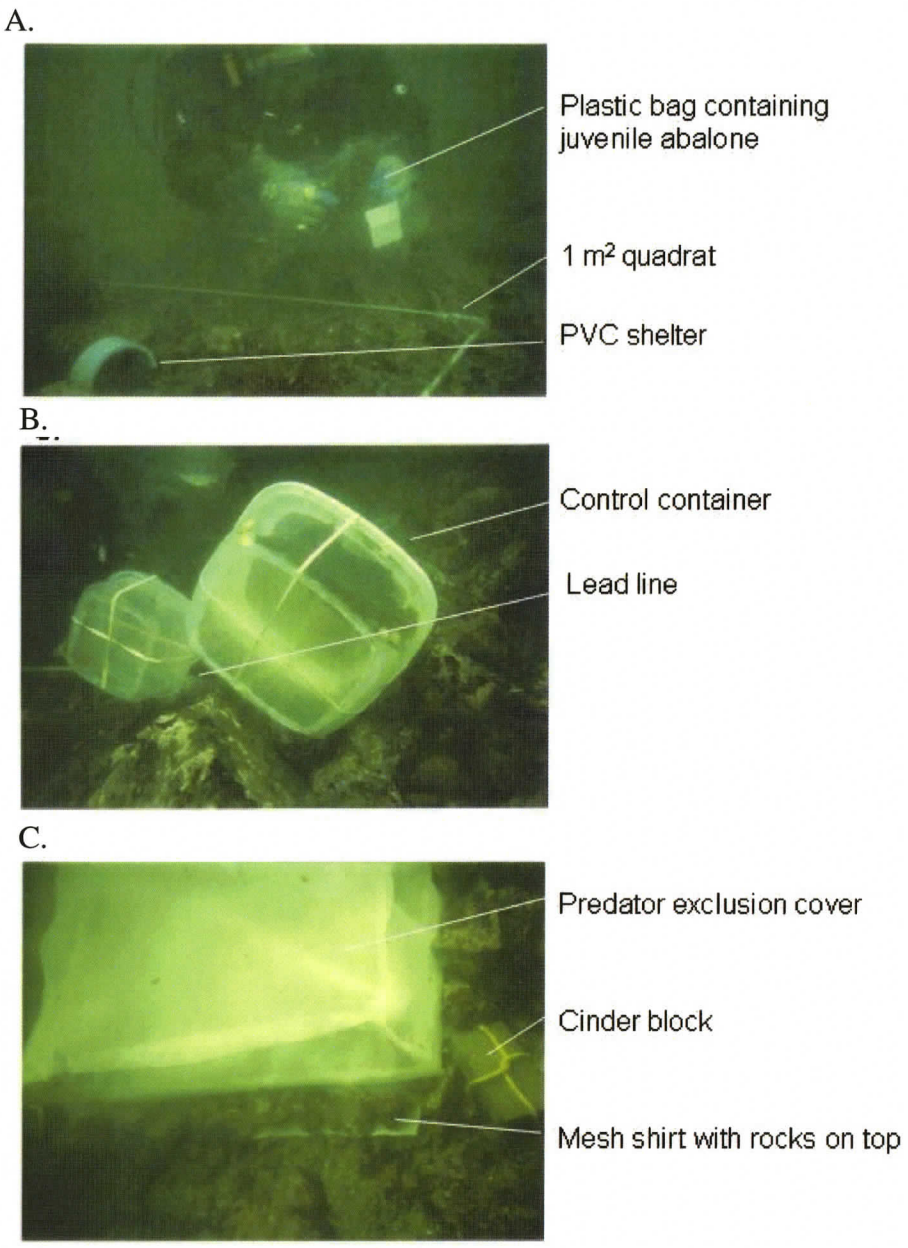

Figure 8: Underwater photographs of outplanting juvenile *Haliotis kamtschatkana*. (A) Outplanting of juvenile *Haliotis kamtschatkana* in plastic bags. (B) Field control container attached to a lead line. Container on the left containing 1-5 mm SL abalone and the container on the right containing 5.1-25 mm abalone. (C) View from above of the predator exclusion cover (experiment 2) with "skirt" and a cinder block anchoring the corner.

Experimental Methods

Experiment 1: Efficiency of search method at locating juvenile *H. kamtschatkana*

An experiment was carried out at the Brady's Rock field site on 20 September 2004 to determine if the method of turning over rocks and visually inspecting the quadrats *in situ* for outplanted abalone was an efficient search method. Three 1 m^2 quadrats were laid out at 1 m intervals along a 10 m lead transect line that was placed parallel to shore. Abalone were outplanted in each quadrat according to the method described earlier. The numbers and sizes of abalone outplanted are found in Table 4.

Forty-eight hours after outplanting, a survey was carried out *in situ* to recover outplanted abalone. The recovered calcein marked abalone were measured using plastic calipers accurate to 1 mm and the numbers on the bee tagged abalone that were recovered were recorded onto a slate. All rocks and shell fragments large enough to pick up with gloved hands (≈ 3 mm or larger), in each location were then gently brought up to the surface in mesh collection bags and carefully searched again for abalone in daylight on the deck of the dive boat.

Experiment 2: Dispersal of Juvenile *H. kamtschatkana*

This experiment was carried out on 5 August 2004 at Brady's Rock to determine how far juvenile *H. kamtschatkana* travel in a period of up to 4 d. At the field site a 10 m lead line was laid out and permanently attached to the bottom parallel to the shore to mark the site. One plastic 1 m^2 quadrat was then laid down beside the lead line and the area within the quadrat was prepared by removing all sea urchins, sea cucumbers, sculpins, gobies, crabs and any other potential predators of abalone. A 1 m^2 predator exclusion cover, made from a 1 m^2 PVC quadrat with plastic screen (2.5 cm mesh size), cable tied together at the corners, was then placed over the prepared 1 m^2 area. Abalone were then brought down to the outplanting site. The number and sizes of abalone outplanted were as listed in Table 4. The exclusion cover was opened at a 90 ° angle and the juvenile abalone were placed into the quadrat. A ruler was used to measure the position of the abalone and shelters relative to the centre of the quadrat. The predator exclusion cover was then closed. To prevent predators from getting under the extremities of the exclusion cover a 10 cm "skirt" of extra screen was added around the edge of the 1 m^2 quadrat. Small cinder blocks were then attached with cable ties to each of the four

corners of the quadrat to anchor the cover down and rocks were placed on top of the mesh skirt (Figure 8C). On days one and four after the outplanting, the predator exclusion cover was removed and each 1 m^2 outplant location was searched for tagged and marked juvenile abalone. Additionally, 1 m outside the 1 m^2 boundary was searched for a total search area of 3 × 3 m. The position of each abalone was measured relative to the centre of the outplant location and control cages were checked for live abalone.

Experiment 3: Mark and recapture experiment

Experiment 3.1: Juvenile *H. kamtschatkana* Mortality in the Field

To quantify and compare relative mortality rates between 1-25 mm SL *H. kamtschatkana* in the field, juvenile abalone were outplanted at Eagle Bay on 18 August 2004 and at Brady's Rock on 19 August 2004. The numbers, sizes and methods of marking and tagging used for the juvenile *H. kamtschatkana* outplanting were as listed in Table 4. Outplantings occurred at depths of 7 m at Brady's Rock and 5 m at Eagle Bay. At each of the two sites, a 10 m lead line was attached to the bottom parallel to shore as a site marker for the duration of the study. Markers were attached to the lead line at 1 m intervals to mark the location where each set of abalone would be outplanted. One m^2 PVC quadrats were placed on top of the markers to the right of the lead line and used as a reference to ensure that the abalone were outplanted within the centre of the quadrat. Five replicate outplanting locations were used at each of the two field sites.

Surveys to search for outplanted abalone were carried out 2 d, 7 d and 30 d after the outplantings. On average it took 65 minutes per site to check all controls and treatment quadrats for outplanted abalone. Field and laboratory controls were only monitored up to day seven. On each survey, 1 x 1 m^2 plastic PVC frames were placed with their left corner each centered on the outplanting location of the five marked locations along the lead line. Abalone that were visible without disturbing the rock substrate were surveyed first, after which rocks and debris in each quadrat were gently overturned to search for cryptic abalone, shells, and tags within the 3 × 3 m search area. When abalone were found, their size, bee tag number and color was recorded. Dead abalone were brought back to the laboratory to be closely inspected for possible cause of death.

Experiment 3.2: Daytime and Nighttime surveys

As other investigators have reported higher abundances of *H. kamtschatkana* at nighttime, possibly because they spend daytime hours hidden from visual predators in cryptic locations (Shepherd 1973; Watson 2000; Mortimor et al. 2003), I also carried out one survey for outplanted abalone at nighttime. At Eagle Bay, the nighttime survey was carried out on 23 August 2004, seven days after the juvenile *H. kamtschatkana* abalone outplanting, and at Brady's Rock on 25 August 2004, eight days after outplanting due to weather conditions inhibiting diving at nighttime on the seventh day. Nighttime surveys began after twilight.

Experiment 4: Empty Shells as a Measurement of Mortality

To determine if the collection of empty juvenile abalone shells in the field is a good estimate of mortality an experiment was carried out at the Brady's Rock field site on 20 September 2004. Empty, intact juvenile *H. kamtschatkana* shells were obtained from the BHCAP hatchery and placed in the centre of a 1 m^2 quadrat at a depth of 7 m. The number and sizes of juvenile *H. kamtschatkana* shells used was as listed in Table 4. Abalone shells were placed in the quadrat and 48 h later the shells remaining in the 3 × 3 m area were retrieved and the shell numbers and sizes were recorded.

Statistical Analyses

To test whether marking juvenile *H. kamtschatkana* with calcein or bee tags had an effect on their survival, the survival of laboratory control abalone after seven days that had been marked or tagged were compared to the laboratory control abalone that had not been marked or tagged. A Cox Time Dependent Regression was carried out using the statistical analysis software SPSS to determine if there was an effect of the marking and tagging on juvenile abalone survival. The Cox Time Dependent Regression was also used to determine if the field controls with tagged abalone from both sites differed in survival compared to the tagged abalone in the laboratory controls. To determine if there was a difference between the number of abalone recovered on daytime and nighttime surveys, paired T-tests were carried out using SPSS.

RESULTS

Experiment 1: Efficiency of search method at locating juvenile *H. kamtschatkana*

The numbers of juvenile *H. kamtschatkana* observed on location at the quadrat sites was exactly the same as the number found at the surface on the deck of the dive boat (Figure 9). From these results no correction is needed for observed abalone numbers in the field. It should be noted that bringing the rocks to the surface for inspection resulted in 31% of the 1-5 mm abalone being crushed, whereas no abalone were found crushed during the initial survey on the bottom. Approximately 50% of the larger 5.1-25 mm abalone had chips out of their shells and were slightly damaged when brought to the surface.

Experiment 2: Dispersal of Juvenile *H. kamtschatkana*

Average displacement distances of each juvenile abalone size class after day one and day four under the predation exclusion cover are shown in Table 5. Overall, as the time since abalone release increased, the displacement distances increased except for the 1-3 mm SL abalone. The 15.1-20 mm SL abalone travelled, on average, the furthest out of all the size classes used in this experiment. The furthest displacement a single 10.1 -15 mm abalone travelled after day four was 154 cm and the second furthest displacement by a single 10.1 -15 mm abalone travelled was 120 cm. The furthest displacement a single 15.1 – 20 mm abalone travelled after day four was 120 cm. All abalone < 10 mm SL are believed to have stayed within the search area; however, some abalone > 10.1 mm SL may have crawled beyond the area searched. No mortality corrections are needed for the abalone < 10 mm SL but the mortality estimates for abalone > 10.1 mm SL may be higher than what was calculated.

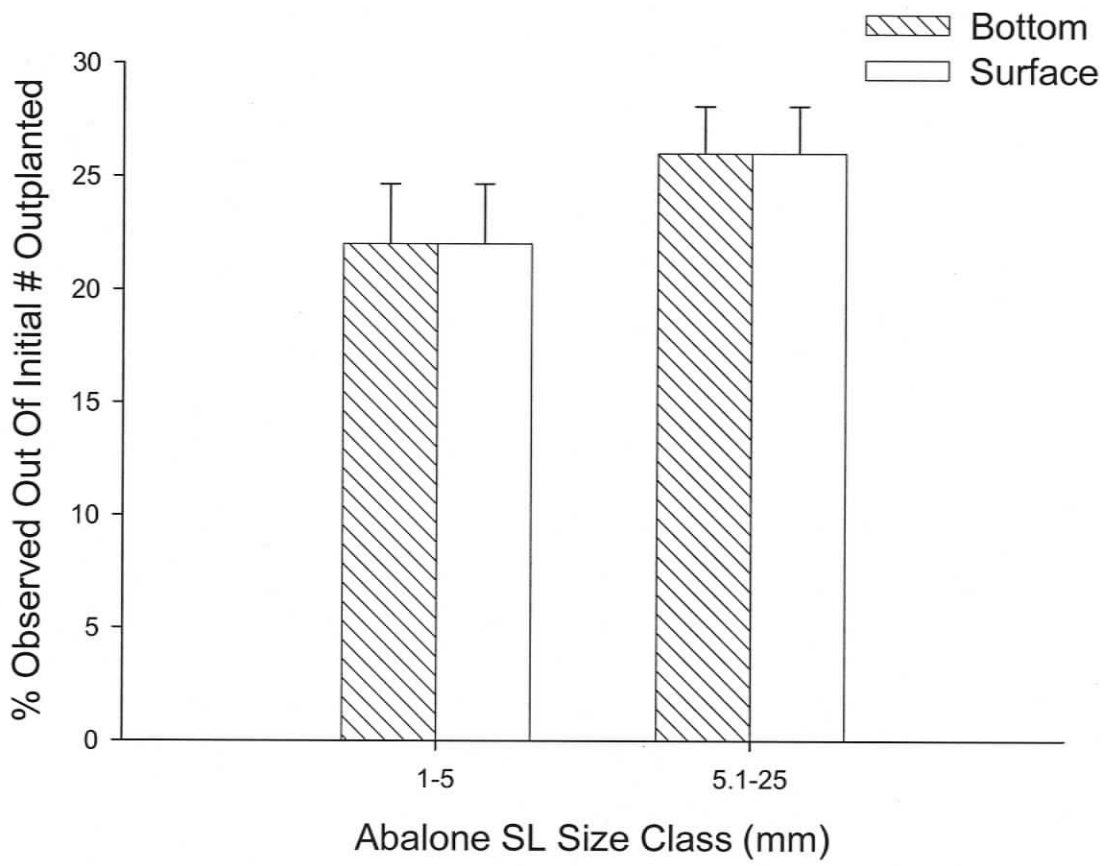

Figure 9: Percent of juvenile *H. kamtschatkana* observed out of the initial number outplanted on the bottom of the ocean floor and on the surface.
Error bars represent standard deviation.

Table 5: Average displacement for Juvenile Abalone after day 1 and day 4 in the Field. Error terms are standard error.

Abalone size class (mm)	Average displacement (cm)	
	After day 1	After day 4
1.0-3.0	1.8 ± 0.3	1.0 ± 0.0
3.1-5.0	1.9 ± 0.2	14.7 ± 10.7
5.1-10.0	2.8 ± 1.0	31.3 ± 15.3
10.1-15.0	3.3 ± 1.5	8.0 ± 3.4
15.1-20.0	6.3 ± 1.2	51.7 ± 32.8

Experiment 3: Mark and Recapture

Experiment 3.1: Juvenile *H. kamtschatkana* Mortality in the Field

Based on the results of experiments one and two, no correction in mortality estimates was needed for the ability of divers to located juvenile abalone on the benthos, or due to dispersal of juvenile abalone < 10.1 mm SL. As well, calcein marking did not have a significant effect on abalone survival (Hazard Ratio (Quinn and Keough 2002) HR = 0.896, n = 400, $p = 0.412$), nor did bee tagging (HR = 0.907, n = 300, $p = 0.907$) based on comparison of marked and tagged abalone in laboratory controls with unmarked and untagged abalone in laboratory controls. Abalone mortality in Eagle Bay field controls did not differ from mortality of abalone in the tagged laboratory controls (HR = 1.003, n = 300, $p = 0.959$) nor did Brady's Rock (HR = 1.021, n = 300, $p = 0.606$). Thus no correction for mortality estimates needs to be made due to marking and tagging techniques.

In the field treatments, the day two surveys recovered more abalone than the day seven surveys at both field sites. The greatest number of abalone not recovered in the field treatment was in the 1- 3 mm and 3.1 -5 mm abalone. As abalone size increased, recoveries were higher (Figure 10). On the day 30 survey, only one 24 mm SL tagged abalone was found, attached to the underside of a boulder.

Mortality in the field controls was as high as 60 % after day seven for the 1- 3 mm juvenile abalone and even in the marked abalone laboratory control mortality was 50 %. Natural mortality in the benign laboratory controls was as high as 25 % for the smallest juvenile abalone sizes of 1-3 mm SL. Mortality decreased as abalone size class increased but was variable (Figure 10).

Brady's Rock and Eagle Bay field treatment mortality data (% of abalone not recovered), field control data (% mortality) and laboratory mortality data (% mortality) were combined because the results were similar and used to estimate the percent of mortality assumed to be caused by predation, handling and prior health conditions. The methods used to calculate these values are listed in Table 6. Estimates for the percent of mortality assumed to be caused by predation, handling and prior health conditions are shown in Figure 11. Day two results are shown because dispersal of abalone was lower after day two than day seven thus, the day two data are a better estimate of mortality.

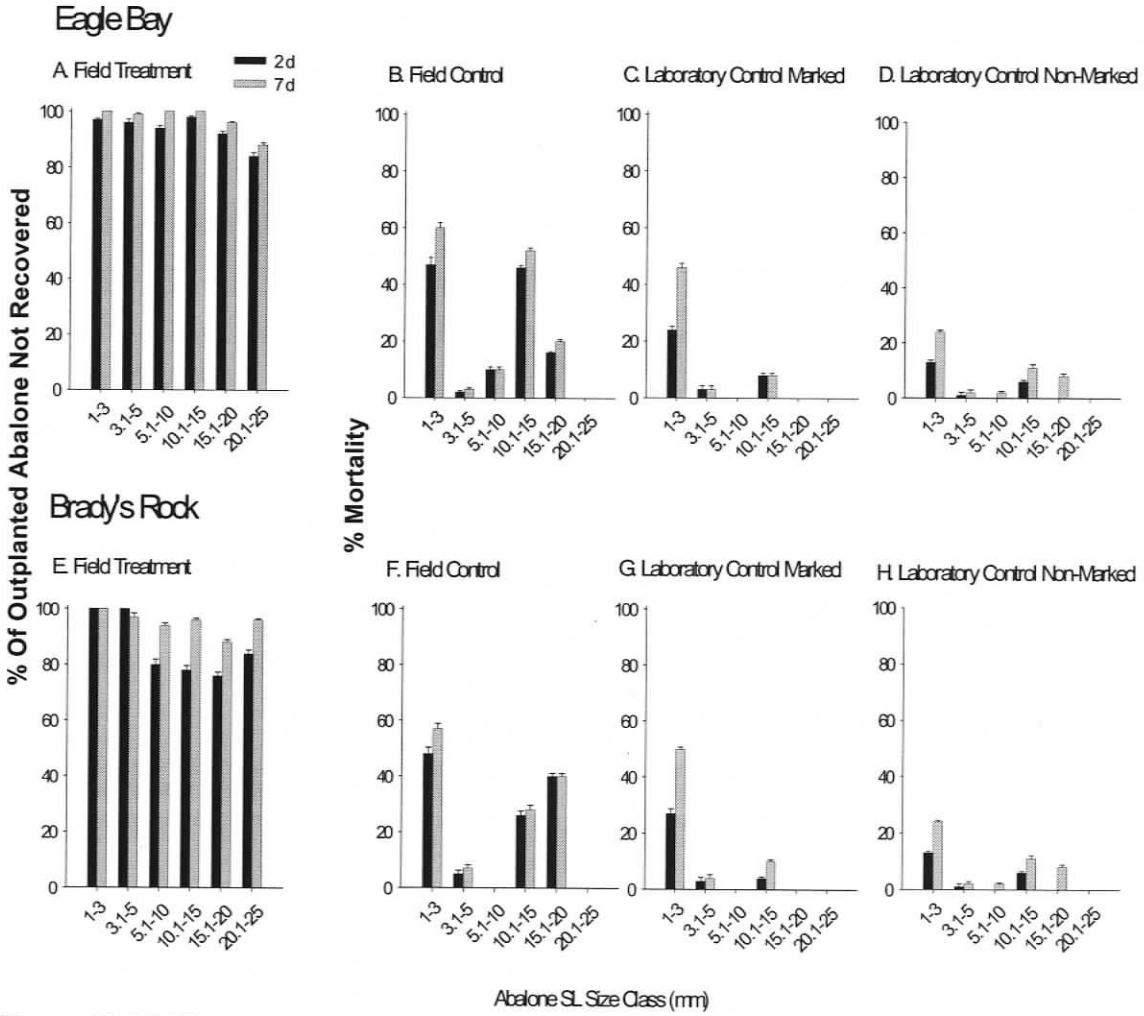

Figure 10: Field treatment and laboratory control results for abalone outplantings after day two and day seven.

(A) Eagle Bay field treatment % of outplanted abalone not recovered (B) % mortality in the Eagle Bay field control (C) % mortality in the marked laboratory control (D) % mortality in the non-marked laboratory control (E) Brady's Rock field treatment percent of outplanted abalone not recovered (F) % mortality in the Brady's Rock field control (G) % mortality in the marked laboratory control (H) % mortality in the non-marked laboratory control. Error bars represent standard deviation.

Table 6: Factors responsible for juvenile abalone mortality and the corresponding data source.

Factors Responsible For Mortality	Source of Data
Predation	% Abalone not recovered in field treatments – field control mortality
Handling	Field control mortality – mortality of marked abalone in laboratory controls
Prior Health Conditions	Mortality in non-marked laboratory control abalone
Marking and Tagging	Mortality in laboratory control marked abalone – mortality in laboratory control non-marked abalone

The initial health of the 1- 3 mm SL abalone was estimated to have been responsible for contributing 30 % of the total mortality (Figure 11). The 10.1-15 mm abalone size class also had high mortality assumed to be caused by prior health conditions. Handling did not contribute to mortality for the largest abalone size class of 20.1-25 mm SL but did for the remaining size classes. Handling is assumed to have contributed to 30 % of the total mortality experienced by the 10.1-15 mm SL abalone. This high mortality due to handling for the 10.1-15 mm SL abalone is thought to be due to an employee handling the juvenile abalone without care when cleaning tanks in the hatchery prior to these experiments. Predation is thought to have contributed to mortality in all abalone size classes and as mentioned previously abalone dispersal in the 10.1 – 25 mm abalone may have contributed to the lower recoveries of outplanted abalone.

Experiment 3.2: Daytime and Nighttime Surveys

The proportion of juvenile *H. kamtschatkana* found during the nighttime survey at both sites were not significantly different from daytime surveys (paired T-test Eagle Bay, $t = -0.848$, $n = 6$, $p = 0.435$, Brady's Rock, $t = 1.464$, $n = 6$, $p = 0.203$) (Figure 12A,B). From these data, there appears to have been no difference between daytime and nighttime surveys in our ability to locate juvenile abalone, and thus no corrections to the mortality estimates need to be made using this data.

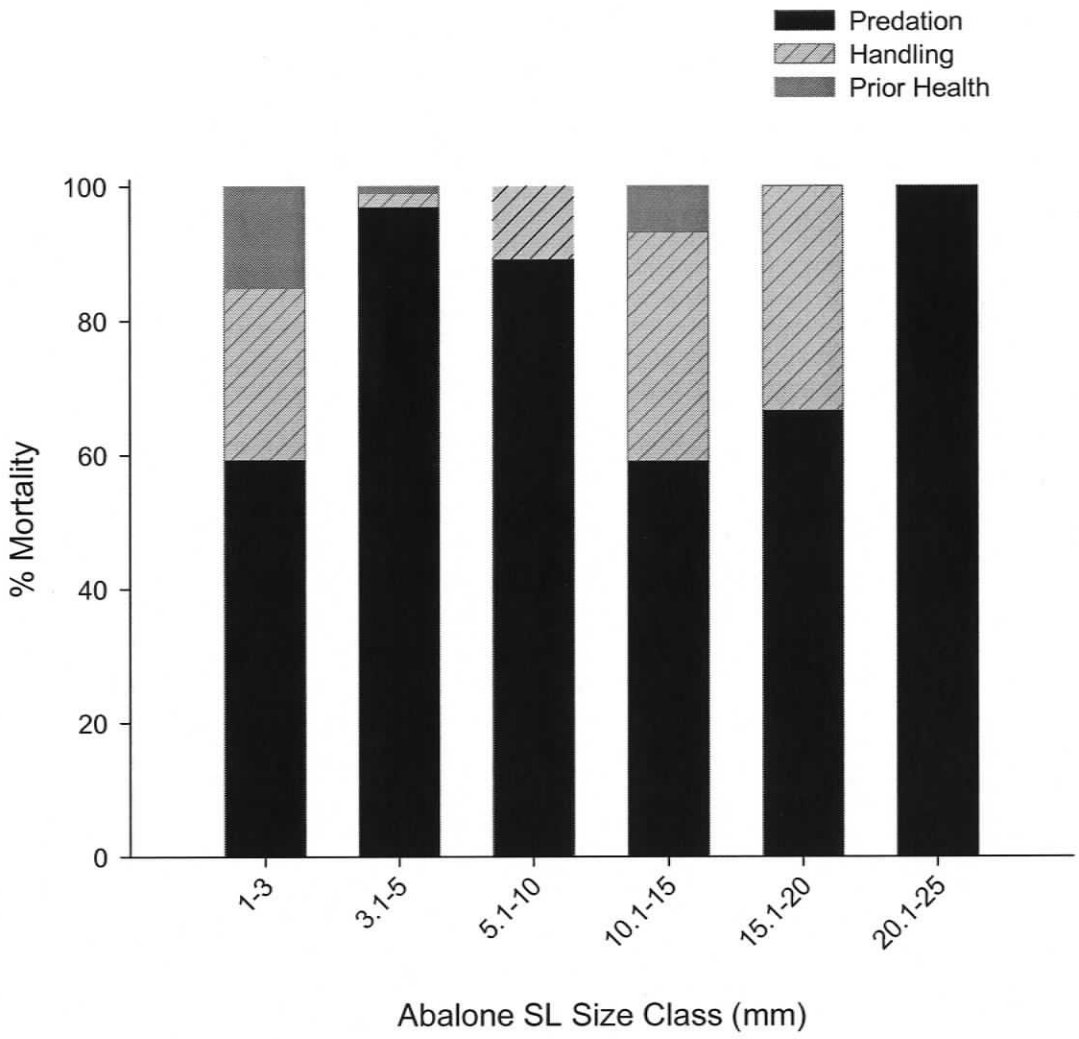

Figure 11: Percent of juvenile abalone mortality assumed to be due to predation, handling and prior health conditions with both Eagle Bay and Brady's Rock data combined after day two.

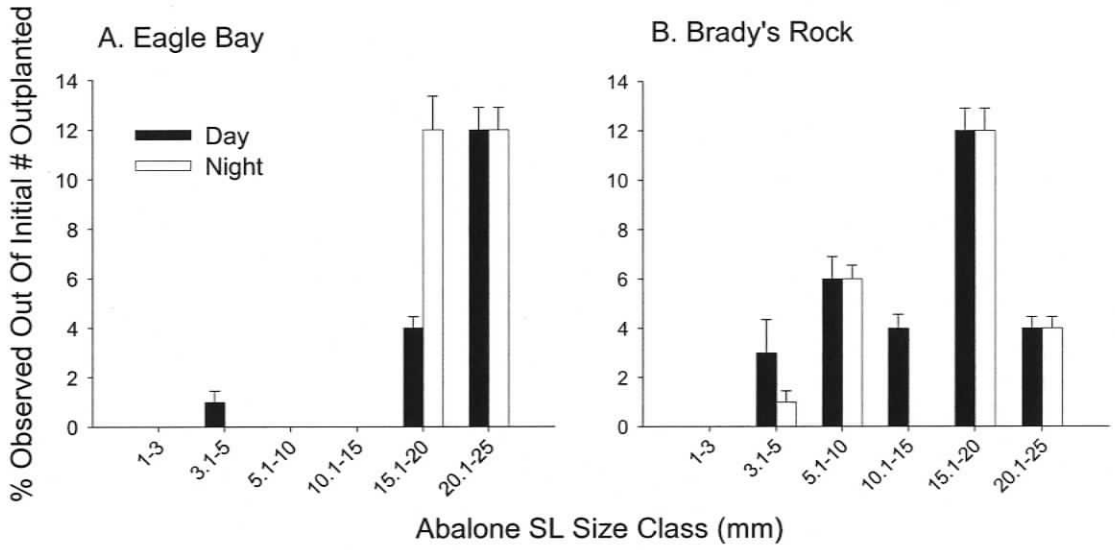

Figure 12: Comparison of daytime and nighttime survey results
Day seven at Eagle Bay (A) and on day eight at Brady's Rock (B). Values represent % observed on the given day relative to initial outplanted numbers. Error bars represent standard deviation.

Experiment 4: Empty Shells as an Estimate of Mortality

Thirty-five percent of the 1-5 mm SL abalone shells and 83% of the 5.1-25 mm shells were recovered. The recovered abalone shells had not dispersed more than 25 cm from their original placement. Complete recovery of all empty shells did not occur.

DISCUSSION

Is the underwater search method efficient at locating juvenile *H. kamtschatkana*?

The underwater diver survey method used for this mark-recapture experiment was efficient at finding juvenile *H. kamtschatkana.* Shepherd et al. (2000) suggested that bee tagging improves a diver's ability to see small organisms and the chances of finding them increases. I was also able to see the calcein marks and bee tags on the juvenile abalone underwater, which enhanced the recovery of the cryptic abalone. Thus based on experiment one, no correction was needed for the mortality estimates due to error in search method used.

The majority of abalone < 10 mm SL remained within the 3 × 3 m search area based on their limited dispersal observed on day four in experiment two and did not affected mortality estimates for day seven. However, some individuals from the 10.1 - 25 mm SL size classes may have escaped the search area after day seven. This statement is based on the two abalone that travelled the furthest distance in the dispersal experiment. The largest size class of 20.1-25 mm SL was not used in the dispersal experiment (experiment two), but it is likely that they could travel just as far as the 15.1-20 mm SL abalone. Therefore, the percent of juveniles not recovered at the field sites are most accurate for abalone < 10 mm SL. However, a higher number of 10.1- 25 mm abalone may have survived than was estimated by this study.

The distances travelled by juvenile *H. kamtschatkana* in the present study are less than in other studies. For example, Kojima (1995) observed movement as far as 4 to 6 m in abalone 8 to 18 mm SL the week following outplanting of *Haliotis discus discus*. He thus was unable to account for 46 to 56% of outplanted abalone in tide channels (McCormick et al 1994). In addition, adult *H. kamtschatkana* have been found to travel distances up to 50 m in 1-2 years (Lessard and Campbell 2004).

Abalone movement and mortality might be due to disturbance from divers carrying out the surveys (i.e. overturning boulders) (Shepherd and Godoy 1989).

However, disruption of the habitat is required to search for cryptic abalone which makes standardizing search areas difficult from site to site (Davis 1995).

Daytime surveys for juvenile abalone appear to be just as effective as nighttime surveys and it is easier to find small cryptic juvenile abalone in the daytime than at nighttime. Daytime surveys are easier to carry out because the full light is more favourable to search for small organisms. Nocturnal behaviour of adult and juvenile abalone has been observed by researchers such as Shepherd (1973), and with *H. kamtschatkana* (Mortimor et al. 2003). However, the highest number of *H. kamtschatkana* found by Mortimor et al. (2003) in nighttime surveys were mature abalone ($\geq$ 70 mm SL) suggesting that nighttime surveys possibly are most effective for searching for larger mature abalone.

The collection of shells is not a reliable method to estimate mortality in the field. However the recovery of shells may help identify causes of mortality. Recoveries of the small 1-5 mm SL abalone shells were low considering the short time span that they were left in the field in the shell dispersal experiment and demonstrated that shell loss is high especially in small abalone shells. These small abalone shells are fragile and can crush easily. Some of the shells that were not recovered may have been lost by burial or dissolution (Shepherd et al. 1992). There were higher recoveries of the larger 5-25 mm SL abalone shells. Breakdown of 15- 45 mm SL abalone shells is slower than for shells < 5 mm SL with an average half life of 48 d (Shepherd 1998) and could explain why abalone shells > 5 mm SL were recovered after day two.

How much mortality do juvenile *H. kamtschatkana* face?

As juvenile abalone grow, their vulnerability to natural mortality decreases. However, mortality caused by handling can affect all sizes of juvenile abalone. Predation is thought to be a major contributor to mortality for all sizes of juvenile abalone.

In benign laboratory conditions the smallest abalone size class used in this study (1-3 mm SL) faced mortality as high as 24 % after day two. These small juveniles are also affected by handling, which can contribute to 25 % of the total mortality. Predation can also contribute up to 60% of the total mortality for 1-3 mm SL abalone. Once the juvenile abalone are larger than 3.1 mm SL, they are less vulnerable to mortality in benign laboratory conditions. However, larger 10.1-20 mm SL abalone can face high

handling mortality (up to 30 % of the total mortality experienced). Predation appears to be responsible for all mortality observed in the 20.1-25 mm SL size class. In this larger abalone size class, dispersal was thought to have affected the field recovery estimates because individuals could have crawled out of the survey area.

In chapter 2 the predators of juvenile *H. kamtschatkana* were determined to be *Lophopanopeus bellus, Pycnopodia helianthoides, Cancer productus, Scyra acutifrons,* and *Amphissa columbiana*. These predators were found to exist at both Eagle Bay and Brady's Rock. All sizes of abalone outplanted in the present study can potentially be killed by at least one of the known predators. A predation susceptibility index was used in chapter 2 to determine how juvenile abalone vulnerability to predation changes as they increase in shell size. Abalone < 12 mm SL were found to be most susceptible to predation. Similarly, in the present chapter, smaller < 10 mm SL abalone were recovered in the field in the lowest numbers from outplanting experiments and were found to be susceptible to mortality caused by prior health, handling and predation. Therefore, abalone size is a critical factor that limits survival in juvenile *H. kamtschatkana.*

In addition to predation perhaps some juvenile abalone died of starvation because they could not adapt to finding food on their own. The hatchery-reared juvenile *H. kamtschatkana* outplanted in this study did not have to search much for food prior to their release into the wild. However, I observed approximately half the outplanted juvenile *H. kamtschatkana* attached firmly to the underside of rocks and the remaining abalone attached to the upper side of rocks suggesting that they are successfully adjusting to their new field environment. Concerns also remain about outplanted juvenile abalone that do not adjust to the new environment. Behavioural differences between hatchery-reared abalone and wild abalone are still a question of concern if more outplantings of hatchery-reared abalone are to occur. Schiel and Welden (1987) demonstrated that in the laboratory, hatchery abalone were slow at taking shelter compared to wild abalone when faced with predators; as a result, mortality due to predation was higher in hatchery abalone than in wild abalone. However, in my outplanting experiment, one abalone was found after day 30 in the field which demonstrated that this abalone was able to adjust and survive in the field and potentially other juvenile abalone survived but were not recovered after this time.

The optimal size of hatchery-reared abalone that should be released to enhance wild populations is under debate. Release of very small juveniles has the advantage of low production cost and ability to produce and release large numbers at a time (Schiel and Welden 1987; Rogers-Bennett and Pearse 1998; Seki and Taniguchi 2000; Tegner 2000). However, as previously stated, mortality is high in early juvenile benthic marine invertebrates and body size is thought to be a major determining factor of vulnerability (Gosselin 1997; Gosselin and Qian 1997; Hunt and Scheibling 1997; Moran 1999; Osman and Whitlatch 2004). Combining information on natural mortality in the field for juvenile abalone and predator vulnerability could allow us to find an optimal outplanting size for juvenile abalone. From the present study, I suggest outplanting juvenile abalone larger than 3.1 mm SL because they are > 50 % less vulnerable to mortality than abalone < 3 mm SL. The initial health of abalone < 3 mm SL appears to be have been poor and these abalone experienced up to 24 % mortality compared to juveniles > 3 mm SL that experience less than half of that mortality. As seen in the present study, larger abalone > 10 mm SL can be affected by mortality due to handling and thus it would be more cost effective to release smaller juveniles such as 3.1-5 mm SL that are less vulnerable to mortality than < 3 mm SL but do not take as much rearing time as the larger abalone.

Chapter 4: Calcein: A Non-invasive Method to Mark Juvenile *Haliotis kamtschatkana*

ABSTRACT

Early juvenile invertebrates can be difficult to monitor for studies of growth, movement and mortality. Marking these small juveniles with fluorescent dyes is a relatively harmless method that can enhance our ability to identify the individuals days to months later. No methods have been developed to mark small juvenile abalone; thus a calcein marking method was adapted to fluorescently mark juvenile *Haliotis kamtschatkana* (northern abalone) by determining a concentration and duration that produce that brightest and most clearly visible marks for juvenile abalone in the 3-5 mm SL size range and determining the relationship between abalone size and calcein mark intensity for 7- 28 mm SL juvenile abalone. Calcein concentrations of 10, 20, 40, 60 and 80 mg/L were used with exposure time of abalone to 24, 48, and 72 h. An additional double calcein exposure treatment involved abalone exposed to calcein for 72 h followed by a 48 h treatment of fresh seawater then an additional 72 h exposure to calcein. Calcein concentrations between 20 – 40 mg/L produced a distinct fluorescent band at the growing edge of juvenile abalone shells. The calcein band was particularly bright for small juvenile abalone (3-5 mm SL) in the double calcein exposure treatment. As abalone size increased, brighter and more clearly visible calcein mark were observed. Abalone that were 15 mm SL or larger immersed in calcein for 72 h had the brightest and most clearly visible marks.

INTRODUCTION

Tagging and marking of organisms are common procedures used to monitor growth (Breen and Adkins 1980), and carry out mark-recapture experiments to estimate natural mortality (Shepherd and Breen 1992). It is also often useful to mark cohorts of hatchery-reared organisms, such as molluscs, before they are released into the field in order to monitor their movement (Trevelyan and Chang 1987; Levin 1990). A tag or mark is needed that is long lasting and recognizable to scientists but not revealing to predators.

Fluorochromes are fluorescent dyes that can be used to mark organisms (Wilson and Beckman 1987; Day et al. 1995; Rowley and Mackinnon 1995). Tetracycline, oxytetracycline, xylenol orange, alizarin red and calcein are common fluorochomes that function by binding to calcium and are integrated into newly deposited shell or mineralizing bone (Day et al. 1995). Fluorescent marking is useful for smaller organisms, is an alternative method to tagging, and has been used to measure growth and survivorship (Wilson and Beckman 1987; Day et al. 1995; Rowley and Mackinnon 1995; Kaehler and McQuaid 1999). Otoliths of fish (Wilson and Beckman 1987), tests of sea urchins (Rowley 1990), mollusc shells (Levin 1990) and other calcified structures can be marked using fluorescent compounds. Fluorochromes do not affect the growth rate of individuals being marked (Rowley and Mackinnon 1995).

Calcein marking is considered a safe and effective procedure that for abalone does not require invasive procedures to view calcein marks as is necessary for observing calcium binding fluorochromes in other invertebrates (Day et al. 1995; Rowley and Mackinnon 1995). Calcein can be administered either by injecting the calcein solution into the soft tissues of the animal of interest or by immersing the animal in the calcein solution and as the animal feeds calcein will be imported into the mantle tissue before deposition onto the shell (Pirker and Schiel 1993; Day et al. 1995). The immersion method has been found to be less stressful to the animal than injection (Pirker and Schiel 1993; Day et al. 1995). The calcein marks are of highest intensity when the individual has been feeding prior to staining and thus the success of calcein is dependent on growth (Day et al. 1995). To view calcein marks, UV or blue light is required as the excitation wavelength maximum of calcein is 495 nm (Day et al. 1995; Rowley and Mackinnon 1995). Under these wavelengths calcein fluoresces bright green because the calcein emission wavelength maxima is 520 nm, within the green spectrum of 492-577 nm (Kaehler and McQuaid 1999). In the field, calcein marks are known to persist for 50 d in *Nucella ostrina* (Moran 2000) and in the calcium carbonate skeletons of marked animals such as brachiopods, the fluorescent marks can be retained for up to one year (Rowley and Mackinnon 1995). Day et al. (1995) found that of five calcein specific fluorochromes (oxytetracycline, tetracycline, calcein, alzarian red and xylenol orange) calcein was the most successful in marking *Haliotis rubra,* red abalone, and calcein

concentrations of 10-120 mg/L for 12-48 h yielded the best results for 70-100 mm shell length (SL) abalone. No methods have been used to mark small juvenile abalone < 5 mm SL.

Alternative identification methods such as spaghetti-type fish tags, and washers attached by epoxy are not suitable for abalone smaller than approximately 50 mm SL because the attachment methods used may interrupt growth (Shepherd and Breen 1992). Bee tags are round plastic disks (1 mm diameter) that come pre-numbered and are used to tag honey bees. Bee tags are too large for small abalone < 5 mm SL because the tag and glue are too large for the shell. In addition, respiratory pore holes on small abalone can easily get filled with glue when attaching bee tags to their shells. Calcein, however, can be used to mark large numbers of abalone at a time and does not require the direct handling of fragile juvenile abalone, and therefore might be effective for labelling early juvenile abalone. The objective of this study was therefore to develop a method of marking juvenile *H. kamtschatkana* with calcein by determining a concentration and duration that produce that brightest and most clearly visible marks for juvenile abalone in the 3-5 mm SL size range and determining the relationship between abalone size and calcein mark intensity for 7- 28 mm SL juvenile abalone.

METHODS

Laboratory work was carried out at the Bamfield Marine Sciences Centre (BMSC) located in Bamfield B.C. All juvenile *H. kamtschatkana* used in this study were obtained from the Bamfield Huu-Ay-Aht Community Abalone Project (BHCAP) hatchery located in Bamfield B.C.

Preparation of calcein solutions

Calcein solutions were prepared by dissolving calcein powder in seawater (Product #C0875, SIGMA). Abalone on 5 × 3 cm sections of plastic settlement plates from the hatchery were immersed in plastic containers (34 × 22 × 17 cm) containing the calcein solutions. The settlement plates were coated in diatoms which provided food for the abalone; the abalone were also provided with 1 cm^2 pieces of the kelp *Macrocystis integrifolia* every other day. The containers were maintained at 10-12°C by immersion in seawater trays provided with flowing seawater.

Calcein mark scoring

After the calcein exposure, abalone were removed from the calcein solution, placed in fresh seawater and examined for calcein marks. Calcein marks were then identified by shining a blue L.E.D. dive light on the abalone and selectively visualizing light emitted from excited calcein marks by viewing the animals through an amber filter film (both products were supplied by Northwest Marine Technologies Inc ®). Calcein marks were scored by intensity on the following scale: 0 = no mark, 1 = very faint, 2 = clear and visible, 3 = bright and easy to see, a scoring scheme similar to that used by Day et al. (1995). Fresh solutions of calcein were made immediately prior to each experiment.

Laboratory controls

One control container with 10 abalone from each size class and fresh seawater (no calcein) was placed in a seawater tray next to the treatment containers for each marking experiment. These controls were used to determine if the marking method affected abalone survival and differentiate between natural and calcein fluorescence in abalone shells. Day et al. (1995) noted that there is some natural fluorescence occurring in abalone shells, although, at a slightly different emission wavelength (590 nm) than calcein. Abalone in the controls were also fed diatoms and 1 cm^2 pieces of *M. integrifolia*. The holding containers were not provided with flowing water, but were continuously aerated using airstones. To determine the persistence of calcein marks up to 30 days, six additional abalone (10 mm SL marked with 50 mg/L calcein), were kept in a holding container with flowing seawater. Abalone were checked for the presence of calcein marks daily for 30 days.

Experiment 1: Marking 3-5 mm SL juvenile abalone

To determine the concentration of calcein and the duration of exposure that produce the brightest and most distinct marks in 3-5 mm SL abalone, 10 abalone of this size class were added to each of five different calcein concentration treatments: 10, 20, 40, 60 and 80 mg/L. Abalone < 1-3 mm SL were not available from the BHCAP hatchery at the time of experimentation. Individuals were then checked for calcein marks after 24, 48 and 72 h of exposure to calcein.

An additional set of treatments involved exposing abalone to two successive calcein treatments to determine if two separate calcein bands would be produced, as obtained by Rowley and Mackinnon (1995) with brachiopods. I did this by exposing abalone to calcein for 72 h followed by a 48 h exposure to fresh seawater, then an additional 72 h exposure to calcein. Ten 3-5 mm SL abalone were added to each of five different double calcein treatments: 10, 20, 40, 60 and 80 mg/L.

Experiment 2: Marking 7-28 mm SL juvenile abalone

To determine the effectiveness of calcein for marking juvenile *H. kamtschatkana* > 5 mm SL, 10 abalone from each of four larger size classes (7-10, 15-19, 21-24 and 25-28 mm SL) were added to a calcein solution of 50 mg/L for 24, 48 and 72 h. Calcein solutions of 50 mg/L were used because from the previous experiment with 3-5 mm SL abalone, the 20 mg/L and the 80 mg/L treatments produced the clearest calcein marks and thus 50 mg/L was chosen for the larger abalone because it was likely to be a concentration high enough to produce clear marks.

Statistical Procedures

Univariate ANOVAs were carried out for experiment 1, using the statistical software SPSS, to determine if calcein concentration, immersion time or abalone size affected calcein mark intensity. To determine if there were differences between concentration, calcein exposure time and abalone size, the Tukey HSD Post Hoc test was carried out using SPSS. Univariate ANOVAs were carried out for experiment 2, using the statistical software SPSS, to determine if calcein immersion time or abalone size affected calcein mark intensity.

RESULTS

To demonstrate the appearance of calcein marking and natural fluorescence, a photograph was taken of a marked 8 mm SL abalone using a Cannon A75 digital camera within an underwater housing filtered with a piece of amber filter film over the lens. Fluorescent green calcein marks were visible in new shell growth near the margins of the abalone shells as well as natural fluorescence (pink) (Figure 13). Natural and fluorochrome fluorescence differ in that calcein fluoresces green and appears near the new shell growth at the aperture, whereas natural fluorescence appears pink and is

observed primarily near the apical whorl of the shell. Bright and easy to see calcein marks were still visible in the present study on juvenile abalone (3-28 mm SL) after 30 d, similar to the results obtained by Moran (2000) who found calcein marks still present in *Nucella ostrina* 50 days after staining.

The combined mortality experienced by abalone in experiment one and two was 13 %. This mortality was due to abalone crawling up the sides of treatment containers and out above the waterline.

Experiment 1: Marking 3-5 mm SL juvenile abalone

Calcein concentration had a significant effect on calcein mark intensity (ANOVA, df = 4, n = 123, F = 7.371, $p < 0.001$). The calcein concentration that produced the brightest and most clearly visible marks for 3-5 mm SL abalone was 20 mg/L because this concentration required less calcein to obtain the same mark intensity observed at 60 mg/L and 80 mg/L (Table 7).

Overall, the double calcein exposure treatment produced the highest mark intensity scores (Figure 14). The 10 mg/L calcein solution treatment was the only treatment that did not show clear and visible calcein marks after the double calcein exposure treatment. Calcein exposure time had a significant effect on calcein mark intensity (ANOVA, df = 2, n = 123, F = 24.605, $p < 0.001$) (Table 7). After 24 h of calcein immersion, none of the concentration treatments produced detectable marks. Marks were visible however, after 48 h.

Experiment 2: Marking 7-28 mm SL juvenile abalone

Calcein mark intensity increased with abalone size. Abalone size had a significant effect on calcein mark intensity (ANOVA, df = 3, n = 76, F = 79.686, $p < 0.001$) (Table 7). Calcein exposure also significantly affected calcein mark intensity with the clearest calcein marks seen after 72 h (ANOVA, df = 1, n = 76, F = 64.655, $p < 0.001$). After 24 h calcein marks were not visible; marks were visible, however, after 48 h (Figure 15). The 25-28 mm SL abalone showed the clearest and brightest calcein marks after both 48 and 72 h calcein exposure.

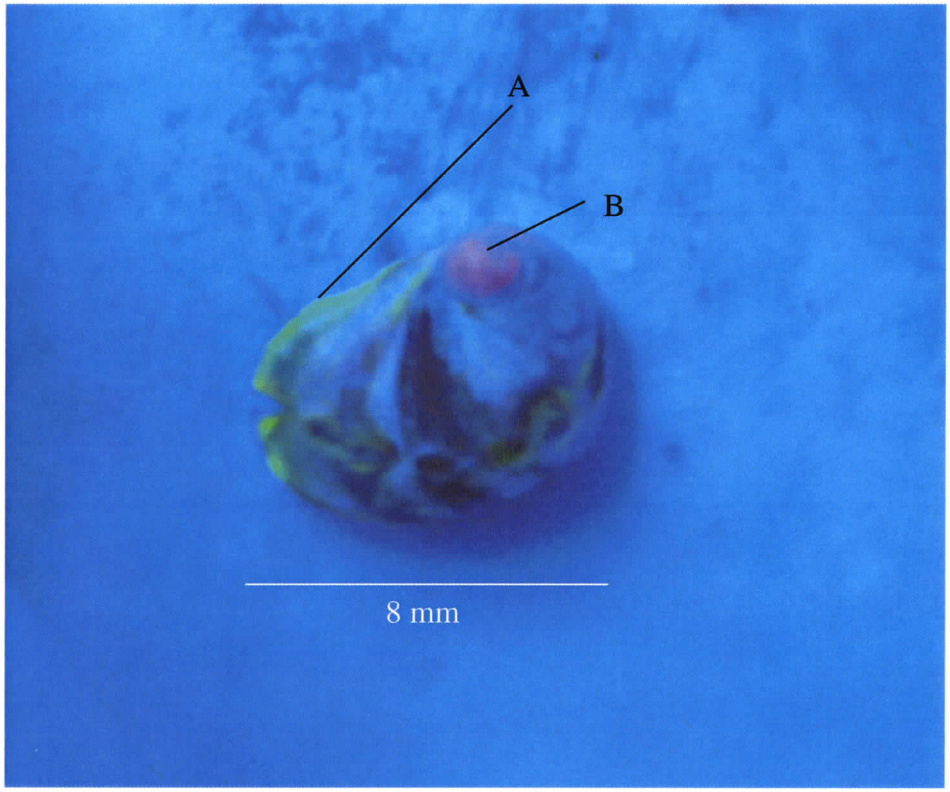

Figure 13: Calcein marked abalone
(A) A calcein mark (green) at the margin of the juvenile *H. kamtschatkana* shell. (B) Natural fluorescence in the abalone apical whorl (pink).

Table 7: ANOVA results of the effect of calcein concentration, immersion time and abalone size on calcein marking intensity.

	p-value
Experiment 1	
Calcein Concentration (mg/L)	
10 vs. 20	< 0.00
10 vs. 40	0.989
10 vs. 60	0.004
10 vs. 80	0.079
20 vs. 40	0.002
20 vs. 60	0.964
20 vs. 80	0.242
40 vs. 60	0.031
40 vs. 80	0.313
60 vs. 80	0.739
Calcein Immersion Time (h)	
48 vs. 72	0.002
48 vs. double calcein treatment	< 0.00
72 vs. double calcein treatment	< 0.00
Experiment 2	
Abalone SL Size Class (mm)	
7-10 vs. 15-19	< 0.00
7-10 vs. 21-24	< 0.00
7-10 vs. 25-28	< 0.00
15-19 vs. 21-24	< 0.00
15-19 vs. 25-28	< 0.00
21-24 vs. 25-28	0.005
Calcein Immersion Time (h)	
48 vs. 72	0.192

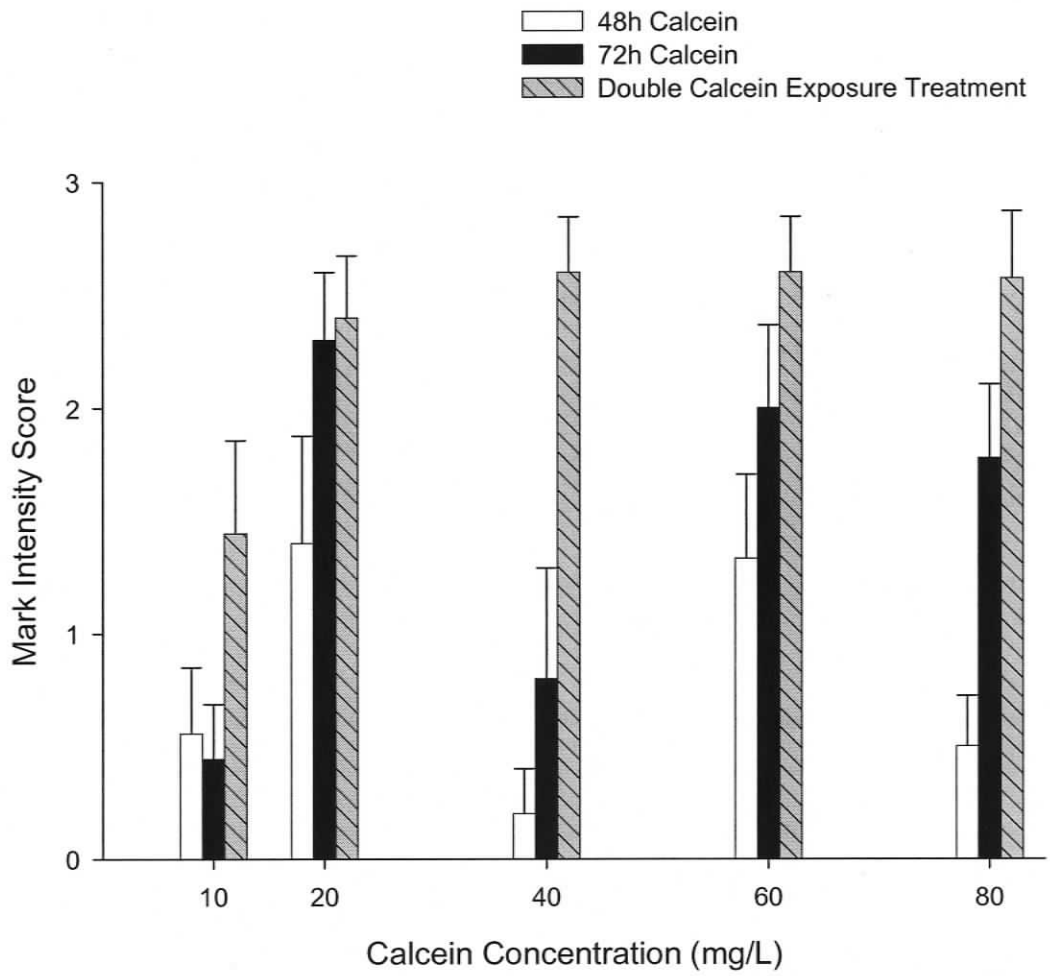

Figure 14: Experiment 1. Influence of calcein concentration on mark intensity for 3-5 mm SL juvenile *H. kamtschatkana* after 48 h, 72 h, and a double calcein exposure treatment.

Error bars represent standard error. Sample sizes at the end of the trials are as follows: 10 mg/L treatment n = 9 for all 3 treatments; 20 mg/L n = 10 for all 3 treatments; 40 mg/L n = 10 for 48 h and n = 5 for both the 72 h treatment and the double calcein exposure treatment; 60 mg/L n = 9 for the 48 h treatment, n = 6 for the 72 h treatment, and n = 5 for the double calcein exposure treatment; 80 mg/L n = 10 for the 48 h treatment, n = 9 for the 72 h treatment, and n = 7 for the double calcein exposure treatment. Mark intensity scores are as follows: 0 = no mark, 1 = very faint, 2 = clear and visible, 3 = bright and easy to see.

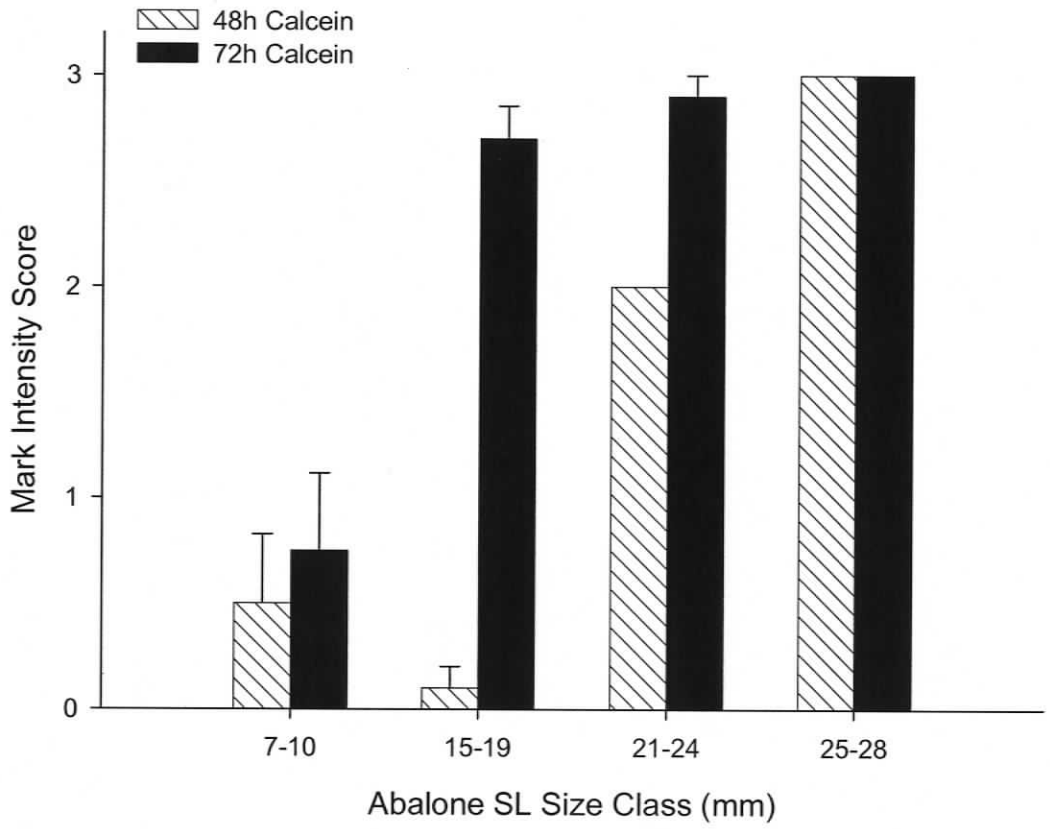

Figure 15: Experiment 2. Mark intensity scores for juvenile *H. kamtschatkana* treated in a calcein solution of 50 mg/L for 48 and 72 h.
Error bars represent standard error. Sample sizes at the end of the trials were 10 abalone for all durations and abalone size classes except the 7-10 mm SL where n = 8 for both exposures. Mark intensity scores are as follows: 0 = no mark, 1 = very faint, 2 = clear and visible, 3 = bright and easy to see.

DISCUSSION

Calcein concentration and duration of exposure to calcein affected the calcein mark intensity in 3-5 mm SL juvenile *H. kamtschatkana*. The double calcein exposure treatment produced the brightest, most visible marks among all calcein treatments. In this double calcein exposure treatment one calcein mark was produced, not two. Interestingly, in past research involving calcein marking of brachiopods two separate calcein marked regions were produced when the animals were placed in calcein for 24 h, then for 24 hours in sea water, and then again 24 h with calcein (Rowley and Mackinnon 1995). Perhaps this was because our abalone had a slower growth rate than the brachiopods, such that in the time between the first and second calcein exposures the abalone did not grow enough for distinct growth bands to be visible.

The 20 mg/L calcein treatment had calcein marks that were as good as or better than the 40-80 mg/L calcein treatments. Day et al. (1995) used calcein concentrations of 10-120 mg/L to mark 70-100 mm SL abalone which are similar to the results just mentioned for juvenile 3-5 mm SL abalone.

Although calcein was particularly effective when used to mark abalone > 15 mm SL, the 50 mg/L calcein solution did produce visible marks even in the 7-10 mm SL abalone. These smaller abalone were likely not growing as much as the larger abalone. As juvenile abalone grow, they change their diets from grazing on diatoms to trapping drift kelp such as *Macrocystis integrifolia* or *Nereocystis luetkeana* (Sloan and Breen 1988). After this diet change has occurred, perhaps the juvenile abalone grow more rapidly and thus the calcein marks are stronger in larger abalone. Day et al. (1995) used *Haliotis rubra* that were 70-100 mm SL and observed calcein marks after 12- 48 h using the same concentrations as used in this experiment. Their results suggest that larger abalone are growing at a higher rate than smaller abalone. All of the 25-28 mm abalone showed marks suggesting that they were all growing during the trial period.

The 7-28 mm SL abalone obtained the strongest calcein marks after the longest exposure time used in this experiment of 72 h. Leaving the abalone in the calcein solutions for an extra 24 h might have allowed for more feeding to occur and growth to

be enhanced, thus as time increased the strength of the calcein marks increased. Day et al. (1995) also found that as the duration of immersion increases from 12 to 48 h, the marks improve.

Applications

For juvenile *H. kamtschatkana* smaller than 5 mm SL, a calcein concentration of 20 mg/L and an immersion time of 48 h will produce clear and visible calcein bands. However, for bright and easy to see calcein marks, a double calcein exposure treatment is preferred. For juvenile *H. kamtschatkana* > 7 mm SL a calcein concentration of 50 mg/L would work well especially with abalone > 15 mm SL for a 72 h exposure to calcein.

Calcein marking is an effective procedure that can be applied to juvenile *H. kamtschatkana* for identification of hatchery-reared abalone in the field after release to monitor parameters such as growth and survival. This procedure is particularly important for juvenile abalone that are too small to be marked or tagged using other procedures. Abalone need to be feeding prior to and during the treatment periods to allow for optimal growth and thus retention of the calcein.

Chapter 5: General Conclusions

In chapter 2, fourteen predator species were found to consume 1-25 mm SL juvenile *Haliotis kamtschatkana* in the laboratory, of which six species are likely to pose an important threat to juvenile *H. kamtschatkana* in the wild. At the time of this study, *Lophopanopeus bellus* posed the greatest threat to the survival of juvenile *H. kamtschatkana* as they were found in the highest abundance in the field, consumed juvenile abalone as large as 19 mm SL, and are specialist predators that are known to feed on small molluscs (Yamada and Boulding 1998). Juvenile *H. kamtschatkana* were most vulnerable to predation in the field until they reached ≥ 12 mm SL.

A mark-recapture procedure was used in chapter 3 as a technique to estimate natural mortality in juvenile *H. kamtschatkana*. Predation had an impact on mortality in all abalone size classes tested and appears to be responsible for > 60 % of the mortality observed in field treatments. As juvenile abalone increase in shell size they become less vulnerable to mortality from prior health, handling and predation.

A successful method was developed in chapter 4 for marking small 3-5 mm SL juvenile *H. kamtschatkana* as an identification method using the fluorochrome calcein. Larger 7- 28 mm abalone can also be successfully marked using calcein for an additional identification method to tagging with bee tags, epoxy and other techniques. Calcein was therefore found to be an effective non-invasive technique to mark juvenile *H. kamtschatkana*.

The findings of this thesis can be applied to outplanting hatchery-reared juvenile *H. kamtschatkana* into the wild to increase wild abalone populations. Small juvenile *H. kamtschatkana* 1-3 mm SL are available in greater number from hatcheries but they face the highest natural mortality. *Haliotis kamtschatkana* larger than 3.1 mm SL *H. kamtschatkana* are less susceptible to natural mortality and would stand a higher chance of survival in the field than abalone < 3.1 mm SL. Until juvenile *H. kamtschatkana* reach 12 mm SL, they are most vulnerable to predation, which plays a major role in survival. Prior to outplanting juvenile abalone, suitable field sites should identified by determining the sites with the lowest predator abundances, and suitable substrate for shelter to increase success rates.

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