NORTHERN ABALONE, *Haliotis kamtschatkana*, JUVENILE SAMPLING TECHNIQUES: A SUMMARY OF METHODS TESTED IN THE BROKEN GROUP ISLANDS, BARKLEY SOUND, BRITISH COLUMBIA

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ABSTRACT

Curtis, L.F., Swan, K.D., and Lessard, J. 2021. Northern Abalone, *Haliotis kamtschatkana*, juvenile sampling techniques: A summary of methods tested in the Broken Group Islands, Barkley Sound, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 3420: x +53p.

The Northern Abalone (*Haliotis kamtschatkana*) is Canada's only abalone species and is currently listed as Endangered under the Species at Risk Act. Despite the closure of the abalone fishery in British Columbia (BC) in 1990, the species remains at risk of extinction. In 2002, Fisheries and Oceans Canada (DFO) and Parks Canada embarked on a multi-year study to test aggregation of adult abalone as a population rebuilding method. Abalone are slow growing and methods to sample juveniles efficiently were needed to detect the effects of these aggregations in a relatively short time span, to study population dynamics and to reduce the potential cost of this field experiment. However, juvenile abalone are difficult to sample, in part due to their cryptic nature. Consequently, DFO conducted a series of experiments to evaluate different methods for sampling juvenile abalone. These included two passive sampling methods (post-larval collectors and artificial habitats) and five active sampling methods (rock removal, Venturi suction, magnified searches, cryptic searches, and day/night surveys). Due to logistical constraints, not all methods were tested concurrently or in the same habitat. Here we review the efficacy,challenges, and limitations of each sampling technique. Most methods did not provide aconsistent and/or efficient means of sampling post-larval or juvenile abalone. The inclusion ofcryptic searches in abalone surveys is likely the best means to improve the detection of juvenilesin coastal BC. However, all of the methods we tested have limitations which need to beaddressed before they may be implemented in a widespread fashion.

RÉSUMÉ

Curtis, L.F., Swan, K.D., and Lessard, J. 2021. Northern Abalone, *Haliotis kamtschatkana*, juvenile sampling techniques: A summary of methods tested in the Broken Group Islands, Barkley Sound, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 3420: x +53p.

L'ormeau nordique (*Haliotis kamtschatkana*) est la seule espèce d'haliotide au Canada et est actuellement inscrit sur la liste des espèces en voie de disparition en vertu de la Loi sur les espèces en péril. Malgré la fermeture de la pêche à l'ormeau en Colombie-Britannique en 1990, l'espèce demeure menacée d'extinction. En 2002, Pêches et Océans Canada (MPO) et Parcs Canada ont entrepris une étude pour tester l'agrégation des ormeaux adultes comme méthode de reconstitution de la population. Les ormeaux ont une croissance lente et des méthodes d'échantillonnage efficace pour les juvéniles sont nécessaires pour détecter les effets de ces agrégations dans un laps de temps relativement court, pour étudier la dynamique des populations et pour réduire le coût potentiel de cette expérience. Cependant, les ormeaux juvéniles sont difficiles à échantillonner, en partie en raison de leur nature cryptique. Par conséquent, le MPO a mené une série d'expériences pour évaluer différentes méthodes d'échantillonnage des ormeaux juvéniles. Celles-ci comprenaient deux méthodes d'échantillonnage passives (collecteurs de post-larves et habitats artificiels) et cinq méthodes d'échantillonnage actif (enlèvement de roches, succion Venturi, échantillonnage avec une loupe, recherches cryptiques et échantillonnage jour/nuit). En raison de contraintes logistiques, toutes les méthodes n'ont pas été testées simultanément ou dans le même habitat. Nous passons ici en revue l'efficacité, les défis et les limites de chaque technique d'échantillonnage. La plupart des méthodes ne fournissaient pas de moyen consistant et/ou efficace d'échantillonner les ormeaux post-larvaires ou juvéniles. L'inclusion de recherches cryptiques dans les relevés d'ormeaux est probablement le meilleur moyen d'améliorer la détection des juvéniles sur la côte de la Colombie-Britannique. Cependant, toutes les méthodes que nous avons testées ont des limites qui doivent être étudiées avant de pouvoir être mises en œuvre de manière généralisée.

GENERAL INTRODUCTION

Abalone (Family: Haliotidae) are marine gastropods with a worldwide distribution (Geiger 1999). Several species of abalone have supported fishing and aquaculture industries for decades, but wild populations are threatened by fishing pressure, illegal harvest, and disease in many parts of their range (Crosson et al. 2014). The Northern Abalone (*Haliotis kamtschatkana*) is Canada's only abalone species, and is patchily distributed in rocky, nearshore areas from northern Alaska to Baja California, Mexico. The species has been a traditional food for indigenous communities, a popular recreational shellfish, and the target of a commercial fishery (Sloan and Breen 1988). Despite harvest restrictions, abalone abundance in British Columbia (BC) declined by more than 75% between 1978 and 1989-90 (DFO 2008); consequently, all abalone fisheries in BC (indigenous, recreational, and commercial) were closed in 1990.

Thirty years after the fishery closure(s), Northern Abalone densities at regularly surveyed locations in BC have been slow to recover - particularly for mature abalone (Obradovich et al. 2021). Northern Abalone (hereafter, "abalone", unless the species is unclear) are currently listed as Endangered under the Species at Risk Act (Government of Canada 2011). Although climate change and sea otter predation may play a role, low recruitment levels and illegal harvest are considered to be the most significant threats to abalone recovery (COSEWIC 2009; Obradovich et al. 2021).

The size and distribution of abalone populations required for effective reproduction and recruitment are unknown. Current knowledge of abalone reproduction suggests there needs to be a sufficient density of mature abalone to ensure fertilization of eggs and production of larvae (Babcock and Keesing 1999). Abalone spawn synchronously, with groups of males and females aggregated in shallow waters, broadcasting their gametes into the water column (Breen and Adkins 1980). Studies on abalone (Clavier 1992; McShane 1995a, b; Shepherd and Partington 1995; Babcock and Keesing 1999) and sea urchins (Levitan et al. 1992) have emphasized reduced fertilization success can be attributed to the dilution of gametes at low adult spawner density (Levitan and Sewell 1998). The size of adult females may also play a role in successful reproduction. In Northern Abalone, fecundity is linearly correlated to weight, meaning that larger abalone can release more eggs and could produce more offspring (Campbell et al. 2003). A relationship between mean abalone size, abalone abundance, and habitat type has also been described (Breen and Adkins 1979; Lessard and Campbell 2007). In general, in suitable habitats, abalone abundance increases with wave exposure while mean size decreases. Abalone that inhabit exposed areas tend to be small and found in relatively high abundances; these are often referred to as 'surf' abalone.

Maintaining the harvest closure is a key approach to meet abalone recovery objectives in BC. The Northern Abalone recovery team also recognized that additional recovery activities could be necessary, and recommended rebuilding experiments to bolster local populations (DFO 2007). "Rebuilding" activities could take the form of translocating hatchery-raised Northern Abalone to

the wild, moving individuals from highly wave exposed to sheltered habitats, or aggregating adult abalone to increase their local density. In Barkley Sound, transplanting Northern Abalone from exposed to more sheltered habitat led to enhanced individual growth rates (Emmett and Jamieson 1988). However, at the time of this study, aggregating abalone was not evaluated as a method to enhance reproductive output in BC. Prior to attempting large rebuilding efforts, feasibility studies were required to identify rebuilding method(s) which address the problem of poor adult recruitment, and to fill knowledge gaps on Northern Abalone biology and ecology (DFO 2007; DFO 2012).

In 2002, a five year (plus one year extension) collaborative, multifaceted study was initiated in the Broken Group Islands, Barkley Sound (Vancouver Island, BC; Figure 1), to determine if abalone recovery could be assisted by translocation and aggregation of adult abalone. It was hypothesized that translocating and aggregating adult abalone from exposed areas to more sheltered habitats could increase their local density and promote growth, therefore increasing their reproductive potential. Field studies are expensive and time-consuming; detecting a population-level impact following aggregation would take years, given that traditional survey methods are biased towards the detection of mature abalone (see Study 4, herein). Abalone grow slowly, reaching approximately 20 mm shell length (SL) in one year and sexual maturity (>50 mm SL) in 3-5 years (Quayle 1971), at which point they are emergent and more readily detected by surveyors (Zhang et al. 2007). Hence, reliance on traditional survey methods would require 5-7 years to assess the effectiveness of aggregation as a rebuilding tool.

An understanding of recruitment processes is important in developing a recovery strategy for Northern Abalone. To date, little information is available on the early life stages of abalone (but see COSEWIC 2009). Small juveniles (<50 mm SL), which represent the first 1-5 years of the benthic stage, are often cryptic and difficult for survey divers to find. Prior to 2002, the standard survey techniques used to measure adult abalone abundance were ineffective for measuring juvenile abalone density, because they did not entail searching under rocks, in small cracks, or under turf algae. Based on this known inefficacy for enumerating juveniles, alternate methods of sampling juveniles were needed.

This report describes the different methods that were tested to detect settlement and recruitment of Northern Abalone in the early 2000s. Recruitment can broadly be defined as “the addition of new individuals to populations or to successive life-cycle stages within populations” (Caley et al. 1996). Within fisheries science, the term “recruitment” most often applies to the number of individuals entering an exploited population (usually involving a minimum size requirement; McShane 1995b). For the purposes of this report, we use the more general interpretation and apply it to the life stage specified within each study. Other components of the collaborative research, namely the effects of aggregations and translocations on growth, density, and movement, will be reported elsewhere (J. Lessard, Pacific Biological Station, DFO, Nanaimo, BC). Unfortunately, due to the length of time that has passed since these experiments were

conducted, some data and methodological details have been lost. These gaps are identified and discussed where appropriate.

DETECTING JUVENILE RECRUITMENT

Effective management and conservation of endangered species is dependent on reliable population estimates (Yoccoz et al. 2001; Katzner et al. 2011), yet such estimates are challenging to obtain for species with low detection rates. Imperfect detection can hinder our understanding of species-habitat relationships (Monk 2014), population abundances, and range expansions or contractions, among other key conservation parameters. The rate at which we can reliably detect and record the presence or abundance of a given species may also vary with the age or life stage of individuals present. For example, in marine organisms with planktonic larvae, most populations are demographically open and recruitment may depend less on local reproduction than on the emigration of larvae from elsewhere (Caley et al. 1996). Newly settled juveniles can also be particularly difficult to locate and count. In turn, it is challenging to generate reliable estimates of juvenile recruitment for these species.

Juvenile recruitment reflects both adult fecundity and the survival of young (McConnell et al. 2018), and influences population structure and abundance in the short- and long-term (Gaillard et al. 2008). Consequently, variation in juvenile recruitment has a significant impact on projections of population growth (or loss). Without reliable estimates of recruitment rate and the factors that influence them, management decisions may be impaired.

The small size and cryptic habitat use of juvenile abalone make it difficult to locate them and estimate their abundances. Additionally, abalone grow slowly and undergo ontogenetic shifts in their habitat use – larvae are pelagic (aged 7 – 10 days; Olsen 1984 in Sloan and Breen 1988), while newly settled, post-larval juveniles are typically found on rocky substrates encrusted with coralline algae (Roberts 2003; Obradovich et al. 2021). As juveniles grow larger (~20 – 70 mm SL), they are thought to seek out shallower waters with foliose macroalgae, where they hide in crevices and the underside of rocks, particularly during the day (Sloan and Breen 1988; Cripps and Campbell 1998; Obradovich et al. 2021). Adult abalone (>70 mm SL) are most often emergent (i.e. not hidden in crevices of under rocks; Sloan and Breen 1988; Study 4 herein), but this may vary with predation risk, such as the presence of sea otters. These general age-related differences in habitat use and behaviour correspond with a sampling bias towards mature abalone – emergent individuals are more likely to be detected by visual survey than are individuals which utilize cryptic habitats (i.e. a majority of juveniles). Consequently, different sampling methods must be employed to collect reliable data on juvenile abalone.

Between 2002 and 2006, Fisheries and Oceans Canada (DFO) and Parks Canada tested a variety of methods for locating and enumerating post-larval and juvenile abalone. These pilot studies were relatively short term (ranging from 1 month to 5 years in duration) and designed to assess

the feasibility and relative efficacy of different sampling methods. The studies varied in location, variables recorded, and analyses performed, but are presented here as a group to allow qualitative comparisons of the advantages and disadvantages of each method tested. The pilot studies described here are in four sections: 1) Evaluating collectors as a method to estimate post-larval recruitment, 2) Effort-based comparison of techniques to sample juvenile abalone 3) Evaluating the utility of artificial habitats to estimate juvenile densities, and 4) Multi-year SCUBA surveys for juveniles in cryptic habitat.

STUDY AREA DESCRIPTION

All of the pilot studies took place in Barkley Sound, in and around the Broken Group Islands unit of Pacific Rim National Park Reserve (Figure 1). The study sites were chosen based on the results of a 1991 survey (DFO, unpublished data) and timed-swims. Sites with subjectively high abalone densities (in 1991) were included in the present study. The exact locations cannot be disclosed because poaching is the main threat to Northern Abalone (DFO 2012). Consequently, we refer to the various study islands by an abbreviated reference name and unique number. Because the juvenile/post-larval sampling studies described here were conducted within the context of the larger abalone aggregation study, some study sites are referred to by their aggregation study monikers (Prod1, Prod2, Prod3, Prod4 and Surf1). Each of these is a different island. The artificial habitat study (Study 3, herein) involved two islands not included in the aggregation study, so these are termed Condo1, Condo2. The post-larval collector study (Study 1, herein) included 20 sampling sites throughout the Broken Group Islands. Some of these sites were included in the other studies, and two are discussed in greater detail (Collector1 and Collector2) but most are not necessary to name in this report.

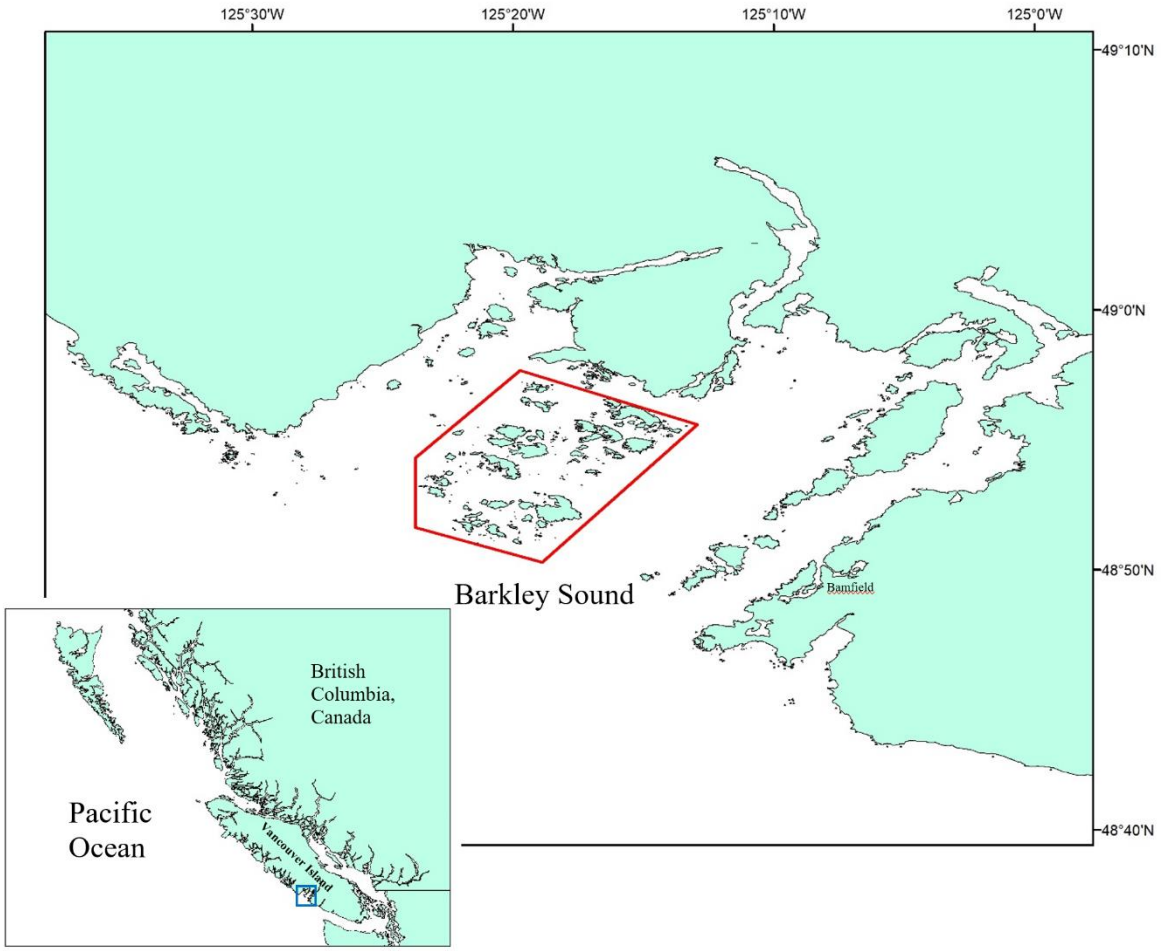

Figure 1. Map of Barkley Sound showing the general area of the study. Blue box (inset) shows the extent of the map. Red outline delineates the Broken Group Islands unit of the Pacific Rim National Park Reserve.

STUDY 1: EVALUATING COLLECTORS AS A METHOD TO ESTIMATE POST-LARVAL RECRUITMENT

INTRODUCTION

At the most general level, recruitment refers to the addition of new individuals to a population. For abalone and other broadcast spawners in the open ocean, recruitment is often measured at one of two points: 1) at the settlement phase, when pelagic larvae settle onto the benthic substrate (Caley et al. 1996), or 2) the point at which juveniles reach a particular minimum size (McShane 1995b; Obradovich et al. 2021). The earliest recruits (i.e. at the settlement stage) are termed "post-larval" juveniles and are typically < 400 μm in length, which makes monitoring and identifying them in the field particularly challenging. Consequently, little is known about this life stage of Northern Abalone.

Artificial collectors have been used to sample post-larval individuals belonging to a variety of species, with moderate success (e.g., Nash et al. 1995; Keesing et al. 1995; Rodda et al. 1997; Rossetto et al. 2013). These stationary, pre-conditioned surfaces are deployed in locations where larval abalone are expected to settle. The collectors are then monitored over time to track settlement rates and to estimate the relative abundance and density of post-larval juveniles.

In June 2002, we deployed 20 artificial larval collectors in the Broken Group Islands in Barkley Sound. Collectors were sampled and redeployed five times over the course of three months, for a total of six sampling periods. Morphometric data were collected from all suspected post-larval abalone juveniles and species identifications of unknown individuals were confirmed by DNA analysis. Reference abalone were obtained from the Bamfield Huu-ay-aht Community Abalone Project, from which DNA and post-larval shell lengths were sampled. The primary goals of this study were to refine the identification and aging of post-larval abalone and to evaluate the utility of collectors as a method to sample them. The ability to reliably sample post-larval juveniles could provide key early insight into the success (or lack thereof) of aggregation as a tool to restore abalone populations.

METHODS

Collector Design

The collectors used were based on modifications of those described in Nash et al. (1995) and Keesing et al. (1995). The collecting plates consisted of a set of four sheets of clear corrugated polycarbonate (25 x 30 cm) that were separated by spacers (2.5 cm). These plates were bolted to a flat PVC base (32 x 37 cm). The collecting plates were held off the bottom by attaching the flat base to a 60 cm tall PVC pipe (7 cm diameter) that was affixed to a round supporting base

(Figure 2). The supporting base of the entire unit was bolted to concrete anchors on the seafloor. The PVC pipe and supporting base plate remained at the site throughout the study. We do not have an estimate of the time it took to build the collectors.

Figure 2. A collector station, prior to deployment for collection of newly settled post-larval Northern Abalone in the Broken Group Islands, Barkley Sound, BC. The stand is approximately 60 cm in height.

Collector Deployment

Twenty larval collectors were deployed among 17 sites in the Broken Group Islands, Barkley Sound by one dive team from June 10-12, 2002 (Figure 2). On arriving at a selected site, reconnaissance dives were conducted to look for a suitable placement of the collector. The main criteria in choosing a location for a collector were that the substrate was flat enough for the collector to stand upright, that the depth was between 2.4 to 5.8 meters below chart datum, and that shore landmarks could be used to easily locate the collector. In order to help find the

collector in low visibility conditions, a lead line was attached to the collector and run along the bottom toward a landmark on shore.

Prior to deployment in the field, the collector plates were assembled and conditioned in a tank with flow-through filtered seawater (25 µm) in order to allow diatoms and bacteria to coat the plates (Keesing et al. 1995). The conditioning time varied and was not standardized throughout the study, but ~65% of the sets were conditioned for at least 11 days (range: 0 – 41 days; median: 14 days). Three sets of collector plates were deployed without conditioning. The collector plates were first deployed on June 10-12, 2002. They were collected and replaced with new plates approximately every two weeks from June 10 to September 18, 2002. Due to the distance between collector stations and other logistical concerns (e.g. time required to process the plates), plates were sampled over the course of one to three days (i.e. sites were not all sampled on the same date). The replacement plates were transported to the field sites in plastic totes filled with filtered seawater (25 µm). Once at the site, SCUBA divers located the collector, removed the soaked collector plate assembly and installed replacement plates from the totes. The soaked plates were brought back to the surface in the same tote and transported to the laboratory for processing. The total field-time required for retrieving and replacing the collector stations from 20 study sites was not recorded, unfortunately, but we estimate it took at least 17 days, and involved at least 3 field personnel each day.

Collector plate processing and sample sorting

The soaked collector plates were disassembled and immersed in an anaesthetic bath for 10 min (1% v/v ethanol in 25 µm filtered seawater; Prince and Ford 1985). The plates were then gently brushed and rinsed with 25 µm filtered seawater to dislodge small organisms. The rinsate was first filtered through a 2 mm sieve, to remove large debris, and then through a 120 µm sieve, to collect all individuals that were at least 120 µm in size. The 120 µm fraction was rinsed into a bottle containing 95% non-denatured ethanol and brought back to the lab for processing.

Once at the laboratory, the samples were filtered again using a 100 µm sieve to remove any remnant tiny particles or organisms. The samples were then re-suspended in filtered seawater (25 µm) and split using a Folsom Plankton Splitter. Sub-sampling was necessary due to time constraints. Half of the sample was saved, while the other half was split again until the amount of material remaining could be easily sorted in a standard plastic petri dish (85 mm diameter). The split ratio was recorded and the sub-sample was searched for post-larval abalone and other small gastropods using a stereomicroscope. The split ratio was not consistent or based on any established methodology to estimate densities like that of United States EPA (2003). A quality control assessment of how evenly the Folsom Plankton Splitter separated the samples was not performed; therefore the proportion of small-benthic invertebrates in our samples may not be representative of the total sample.

A total of 56, 25, 1 and 38 samples were split by 1/8, 1/4, 3/8 and 1/2, respectively, and then sorted. Suspected abalone and other small gastropods were transferred individually into tubes and preserved using 95% non-denatured ethanol for later identification, measurement, photography and DNA analysis (PCR). Unfortunately, the time spent processing and sorting collector plate samples was either not recorded or was lost over the 19 years that passed between the experiment and the present report. We estimate it took a full day for one technician to process and sort just one sample.

Densities of gastropods from each collector at each collection point (e.g., mid-July or late-July) were calculated based on the surface area for all four collecting plates (0.4 m^2). Total sample densities were calculated first by multiplying the number of animals found by the sample split volume ratio to get an estimate of the total number of animals in a sample. For example, if a quarter of the sample was sorted the split volume ratio would be 0.25. The total number of animals estimated was then divided by the estimated surface area of the collector plates (0.4 m^2). Given the filtration methods used prior to processing the samples, all collected individuals were larger than 100 um, but no greater than 2 mm in size. Hence, we refer to them as "small gastropods".

Morphological identification of post-settlement abalone

At the time of this study (2002) and to the best of our knowledge, identification keys or information about any distinctive morphology present in newly settled Northern Abalone were not available. Specifically, we did not find much information to assist in the morphological identification of abalone in samples containing other small sized gastropods (<500 µm). To aid with the identification of abalone in our samples, a reference collection of newly settled post-larval abalone was created using individuals raised in a hatchery (Bamfield Huu-ay-aht Community Abalone Project). Abalone were preserved in ethanol every two days, up to 21 days post-settlement (n = 10 per collection, except Day 1 (n = 24) and Day 5 (n = 5)). These specimens were photographed using a digital camera, and shell length was measured with calipers and a video camera connected to a stereomicroscope.

The relationship between shell length and the number of days post-settlement for hatchery-raised abalone was analyzed through multiple logarithmic regressions with 1000 fits and 1000 iterations, to explore the best fitting model. The assumptions of normality and constant variance were assessed through the Shapiro-Wilk and Levene's test, respectively (SigmaPlot version 13.0, Systat Software Inc., San Jose, California, USA).

DNA identification of post-settlement abalone

Given the lack of taxonomic keys and information on newly settled abalone, we suspected it would be difficult to identify newly settled abalone. To verify some of our visual identifications, genetic analyses were performed for a sub-sample of individuals suspected of being abalone (n = 10). Adult abalone tissue, as well as five hatchery reared post-larval abalone, (ranging from one to seventeen days post-settlement) were used as positive controls. Tissue from adult Red Abalone (*H. rufescens*) served as a negative control; it was tested with the other samples to ensure that a positive identification was accurate to the species level. DNA extraction and identification followed the method outlined by Withler et al. (2001).

RESULTS

The sub-samples we processed contained a total of 1440 whole gastropods and 402 gastropod shells over six sampling periods. Fifty-one of these individuals were initially suspected of being abalone, but only 7 were confirmed to be abalone after close morphological and DNA analyses were completed.

Morphological identification of post-settlement abalone

Based on our observations of hatchery-raised abalone, shell growth becomes visible between 1 and 3 days post-settlement (mean shell length ± 1 SE = 381 ± 6 µm, N=127), but does not become distinctive until two to three days post-settlement (Figure 3). Prior to this stage of growth, distinctive abalone-like shells may not be present or may be too small to differentiate abalone from other gastropods. These reference abalone helped us to identify six Northern Abalone by morphological features alone (these were not confirmed by DNA analysis, however). Although it was not recorded at the time of the study, we suspect the distinctive feature is a larger opercular opening with a fringing and ridged band of shell (Figure 3, Day 3 onwards; Rogers-Bennett et al. 2016). This band grows over time as the animal becomes dorso-ventrally flattened.

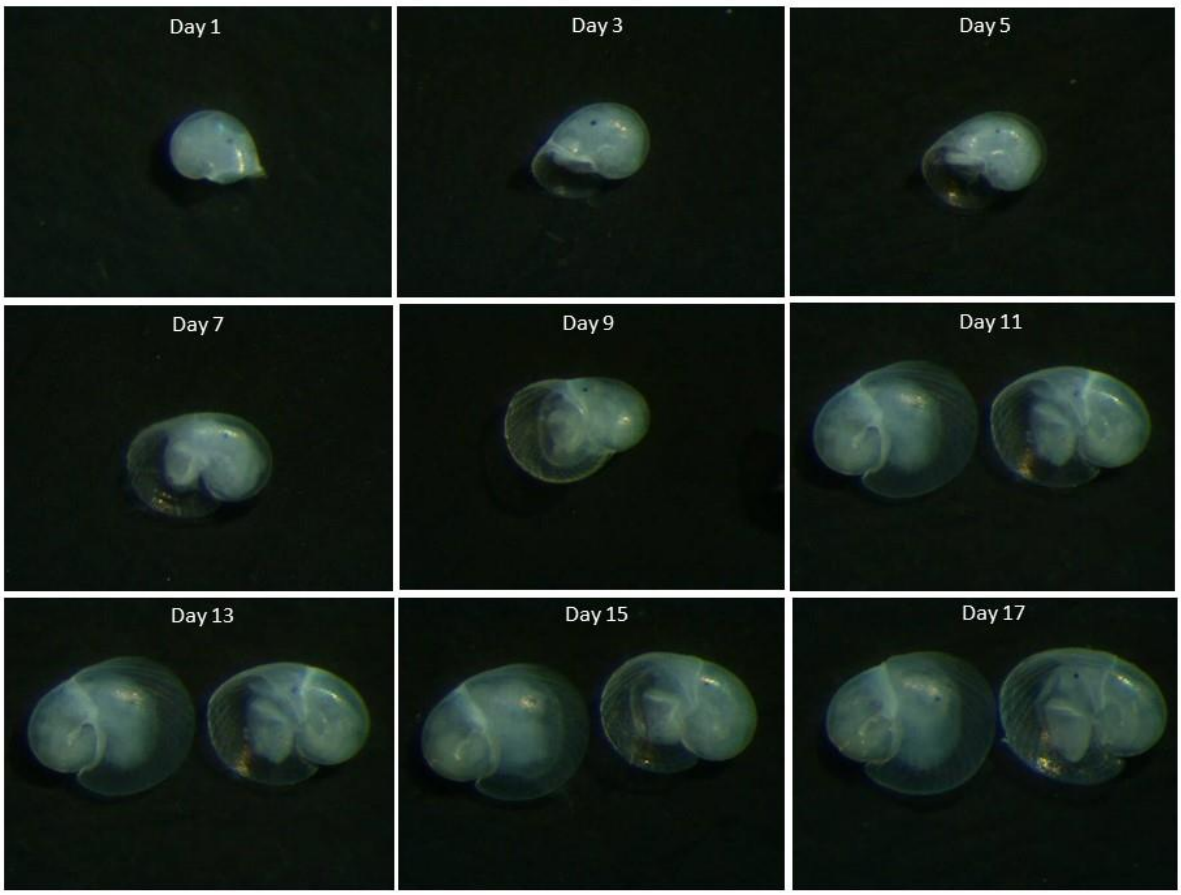

Figure 3. Photo reference collection of newly settled, hatchery reared abalone. Abalone were provided by the Bamfield Huu-ay-aht Community Abalone Project, preserved in ethanol and photographed by digital camera and measured with calipers (unfortunately no ruler was included in photos for scale).

The mean abalone shell length (± 1 SE) of hatchery reared individuals increased from 293 (± 3.7) µm on Day 1 to 544 (± 4.1) µm by Day 21 (N = 127; Figure 4). The relationship between shell length and the number of days post-settlement was best explained ($R^2 = 0.91$) by the logarithmic model in Equation 1 (Table 1). All other logarithmic models tested either explained less of the variance, or the data did not meet the model assumptions.

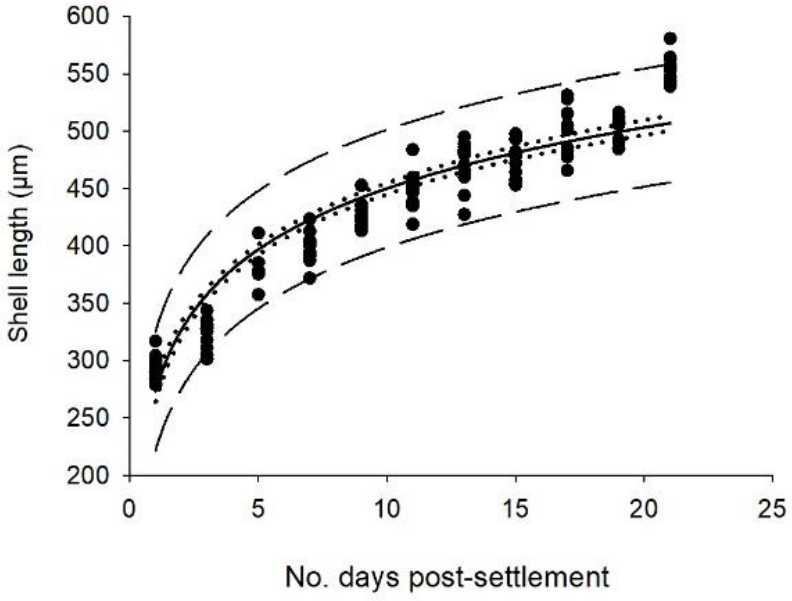

Figure 4. The relationship between abalone shell length and number of days after settlement. The round solid, black dots represent the raw data, the solid line is the estimated relationship given in Equation 1, the dotted lines are 95% confidence bounds of the line and the long dashed lines are the 95% prediction bounds of the relationship.

Equation 1:

$$f = if(x > 0, (\ 273.6 \pm 4.2) + (76.7 \pm 2.1) * \ln(abs(x)),0)$$

Table 1. The analysis of variance results of the relationship between the number of day post-settlement and abalone shell length (1000 fits and 1000 iterations).

Effect	DF	MS	F	P
Regression	2	838102.8	1258.4	<0.0001
Residual	125	666.0		
Total	126	7312.3		

DNA identification of post-settlement abalone

We successfully identified post-larval abalone to the species level through genetic analysis. The genetic material from individuals smaller than 500 µm was sufficient to confirm specimens that were too difficult to identify using morphology alone. All five positive controls (hatchery-raised and adult origin) confirmed that genetic analysis could identify post-larval abalone. The negative control, the Red Abalone, further indicated that this analysis was specific for Northern Abalone (Table 6). Only one of the ten unknown gastropods we chose for DNA identification was positively identified as a Northern Abalone (Figure 5; No. 7). This particular individual was difficult to distinguish morphologically from other small gastropods (Figure 6; No. 7).

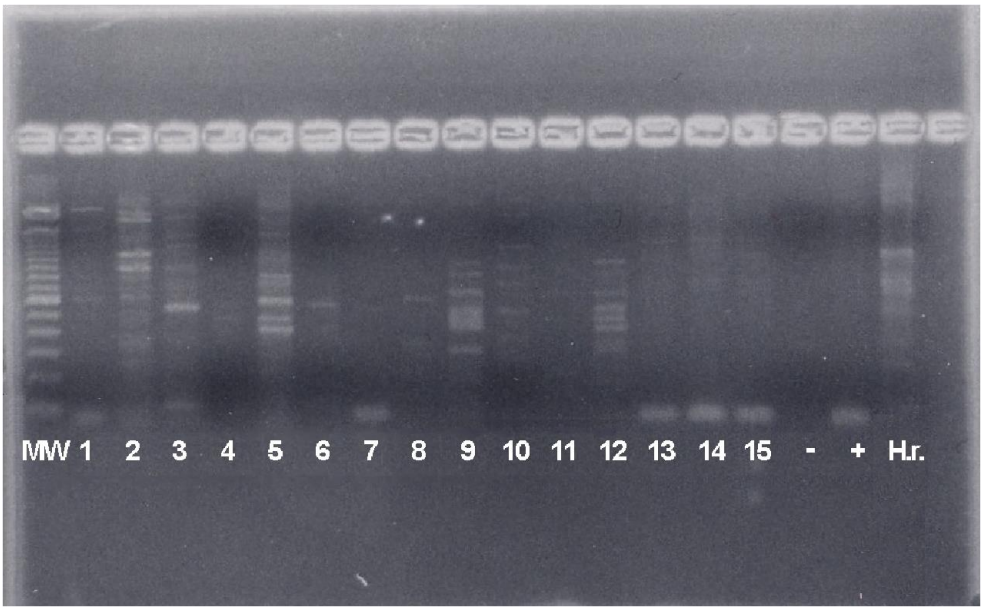

Figure 5. The electrophoresis gel (PCR) of DNA fragments from Northern Abalone and gastropods suspected, but not visually confirmed as Northern Abalone. The DNA fragments from Lane 7 are from the only non-confirmed gastropod to test positively as a Northern Abalone. Lanes 1, 13, 14, 15 are post-settlement Northern Abalone from the Bamfield Huu-ay-aht Community Abalone Project hatchery. + is a positive control of adult Northern Abalone tissue, MW are the molecular weight standards, - is a negative control, and H.r. is a tissue sample from Red Abalone, a closely related species which shows that the test is specific for Northern Abalone. Note that each positively identified abalone contains the same band as the "+" which is the genetic material from an adult Northern Abalone.

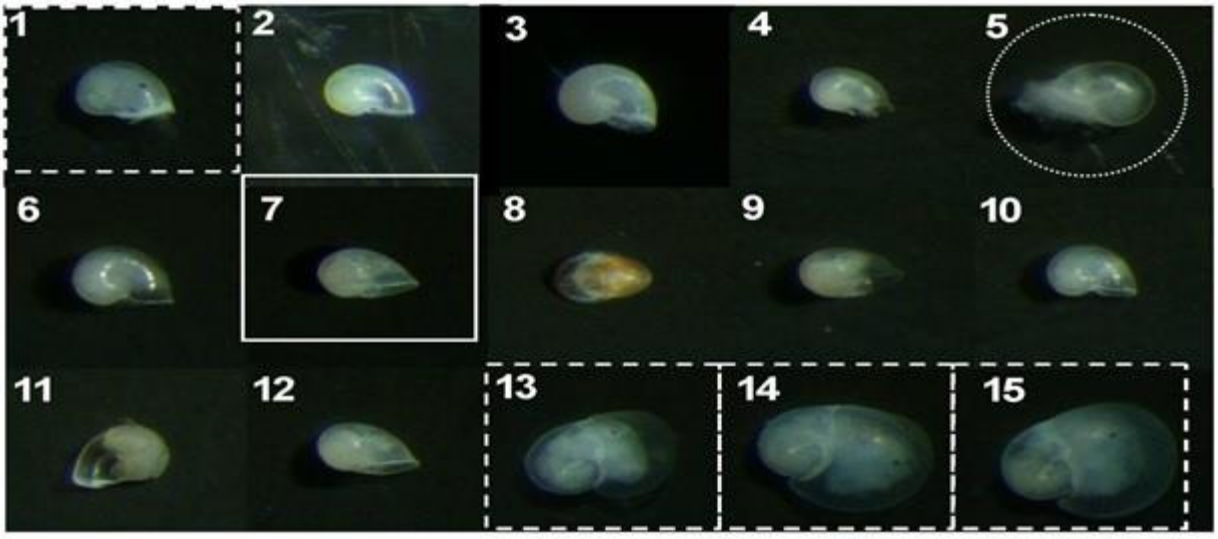

Figure 6. Gastropods from collector plates deployed in the Broken Group Islands, Barkley Sound, BC. Each was genetically tested to confirm species identification, all specimens in a square were positively identified as Northern Abalone through genetic analysis. Specimens 1, 13, 14, and 15 were abalone from the Bamfield Huu-ay-aht Community Abalone Project hatchery (long-dashed boxes). Specimen 7 (solid white box) was the only specimen from collector samples genetically identified as a Northern Abalone. Specimen 5 was not preserved properly (dashed circle), and all others were unknown gastropods morphologically suspected of being abalone, but were not genetically identified as abalone from collector plate origins.

Abalone counts

We identified seven post-larval abalone within three separate samples, based on their morphology (n = 6) and genetics (n = 1). These samples came from three different study sites (Collector1, Collector2, and Prod3). Six of the seven abalone settled on the collectors during the first deployment (mid-June through mid-July), while the seventh settled on a collector in early to late September. In addition, three empty abalone shells were found during the mid-June to mid-July deployment. Based on estimates derived from Equation 1, we determined that all specimens found were 3-4 days post-settlement (Table 2).

Table 2. The maximum shell length and estimated age of abalone (7 live abalone and 3 empty shells) found using larval collectors deployed in the Broken Group Islands, Barkley Sound, BC (*age is the estimated number of days after settlement derived from Equation 1). Some shell length measurements were lost since the study was conducted – these are identified as "unknown".

Specimen	Site	Deployment	Shell length (µm)	Age (days)*
Whole	Collector2	June 10 – July 10	400	3
	Prod3	June 11 – July 11	unknown	
	Prod3	June 11 – July 11	460	4
	Prod3	June 11 – July 11	400	3
	Prod3	June 11 – July 11	440	3.5
	Prod3	June 11 – July 11	480	4
	Collector1	Sept. 5 – Sept. 18	unknown	
Shell	Collector2	June 10 – July 10	400	3
	Prod3	June 11 – July 11	400	3
	Prod3	June 11 – July 11	440	3.5

Gastropod densities

The mean density (± SE) of small gastropods (≤ 2 mm SL) collected in mid-July (524 ± 120 gastropods · m^{-2}) was approximately ten times greater than those collected in early and late-August, and 27 and 55 times greater than those found in late-July and early September, respectively (all sites and samples pooled; Figure 7). There was significant variation between sample sites in the mid-July sample, whereas subsequent sampling periods showed lower spatial variation in gastropod density (Figure 7). In early September, small gastropods were at their lowest density of the study period - most sites had fewer than 9.5 ± 2.3 small gastropods · m^{-2} (Figure 7). However, by late September, densities had increased again and we collected an average of 125.0 ± 21.0 small gastropods · m^{-2} – the second highest density over the course of the study (Figure 7).

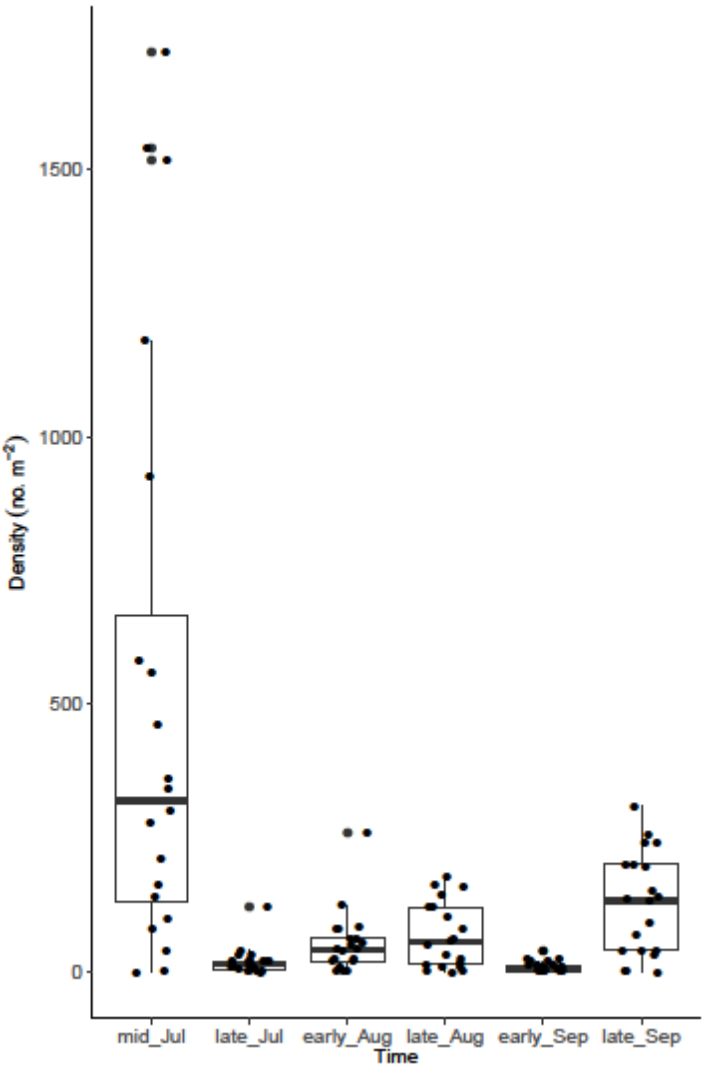

Figure 7. A boxplot of small gastropod densities (< 2 mm SL) found on collectors deployed in the Broken Group Islands, Barkley Sound, BC, from mid-July through late September, 2002. Within the box is the median (line) and the data within it represents the interquartile range. Data outside the box whiskers represent outliers. Data points (solid black circles) represent the density of each collector within each time period (e. g. mid July).

DISCUSSION

Our test of collector plates to detect post-larval juvenile abalone in the Broken Group Islands produced mixed results. While the collectors were straightforward to install and sample in the field, only a tiny proportion of the gastropods we collected were identified as Northern Abalone (0.004%). The densities of larval gastropods appeared to vary with time, but we identified significant limitations to our methods which warrant caution in interpreting these results. Our

efforts to identify post-larval abalone both visually and by DNA sampling were more informative.

Morphological identification of newly settled abalone remains a significant challenge. From hatchery-reared individuals, we observed that distinctive abalone-like shell morphology does not appear until ~3 days post-settlement. At this age, the shells are roughly 350 μm in length. While potentially identifiable as abalone, these individuals are so small that identifying them requires considerable expertise. Our wild, collector plate samples confirmed as much – 10 of the 1440 gastropods we found were too small and/or morphologically ambiguous to be visually identified by trained observers. It is also important to note that the growth model we present may not perfectly represent post-settlement growth in the field. Hatchery-based studies demonstrate that diet, light, and temperature influence growth rates (Rodda et al. 1997; Day et al. 2004; Moss 1999; Takami et al. 2006).

Genetic analysis proved to be a reliable method for identifying post-larval Northern Abalone among a group of morphologically similar gastropods. Among our ten ambiguous individuals, we identified one Northern Abalone, indicating that quantitative PCR is useful for distinguishing larval Northern Abalone from other molluscan species, in spite of very small tissue samples. The technique could be applied to other sampling methods in the future, such as eDNA collection from sea water samples, thereby decreasing the costs associated with microscopy work for enumeration (Vadopalas et al. 2006; Quinteiro et al. 2011). Until a reliable method for identifying the smallest of Northern Abalone via morphometry is developed (e.g. as for Red Abalone; Rogers-Bennett et al. 2016), DNA identification will remain a valuable tool for studying post-larval juveniles of this species.

Our relatively low success with collector plates may reflect a low density of spawners (and thus post-larval juveniles) in the sampled areas of the Broken Group Islands. However, it is likely that a combination of study limitations reduced the efficacy of the collectors. Firstly, given that 9 of the 10 abalone (6 whole and 3 shells) were collected during the first sampling period (mid-June to mid-July), it is possible that we caught the tail end of a peak settlement period. Perhaps deploying the collector stations earlier in the year (April or May) would have resulted in higher numbers of post-larval abalone. Secondly, our collectors may not have been placed in ideal locations, despite our best efforts to do so. Larval dispersal distance and settlement locations may be influenced by local hydrodynamics, pelagic larval duration, pre-settlement larval mortality, and larval behaviour, among other things (see Miyake et al. 2017 for review). Lastly, the biota which settled on each collector plate over the study period were sub-sampled until the remaining material could be sorted in a single petri dish. Sub-sampling was necessary due to time constraints; however this may have reduced the probability of detecting Northern Abalone if they were present, given that the species is relatively rare and spawning is quite variable in time and space. At the time of this study, there was insufficient personnel availability to sort the remaining material. Should this method be explored for future uses, the temporal effort required to isolate and identify individuals in the lab should be considered. At the very least, a set number of

randomly selected samples could be processed in their entirety *before* splitting them, to determine if sub-sampling would accurately reflect the densities of abalone on the plates. Standard operating procedures for rare species also exist and can provide guidelines on how many splits to perform in order to accurately estimate the density of rare species (U.S. EPA 2003). Alternately, one could split a small number of samples and then process *all* of the component subsamples to quantify the variability between them. This would clarify whether sample splitting is justified in this context. Modifications to improve the speed of processing could also be explored (e.g., fluorescence *in situ* hybridization and cell sorting, FISH-CS; Becker et al. 2012; and tray sorting; Curtis et al. 2021).

It should also be noted that a Folsom splitter is most commonly used for splitting samples of pre-settlement zooplankton, which can be relatively evenly distributed throughout the sample. Animals which are larger will sink to the bottom. Thus our sub-samples obtained via Folsom splitter may not capture a representative number of individuals – adding uncertainty to density estimates derived from these sub-samples alone. For that reason, we did not calculate or report estimated abalone densities from the post-larval collectors (rather, we pooled all gastropods and presented their estimates and variation).

It is also possible that insufficient conditioning and a lack of encrusting algae on the collector plates reduced their efficacy. Abalone larvae rely on external chemical cues to induce settlement, and these may include signals from mucous trails of abalone (Seki and Kan-no 1981 in Moss and Tong 1992), encrusting algae (Morse and Morse 1984; Daume et al. 1999), and/or various bacteria and microalgae (Moss and Tong 1992). The conditioning period we established was presumably long enough for some bacteria and microalgae to establish on the artificial surface; however, neither coralline algae nor extensive mats of diatoms or bio-films were observed on our settlement plates (J. Lessard, pers. obs.). Given the frequently observed association between post-larval Northern Abalone and encrusting algae in particular (Roberts 2003; Obradovich et al. 2021), it may be that our collector plates were not attractive to larval abalone, relative to surrounding natural areas. Encrusting algae releases a GABA-like molecule that serves as a settlement cue for abalone and other gastropods (Roberts 2001). Future studies should test the role of encrusting algae as a settlement cue for Northern Abalone, and its potential use in collector plate-based sampling. In the absence of such tests, ensuring the presence of algae on each collector plate, combined with long, consistent conditioning periods, could improve overall success.

Future studies involving larval collector plates may improve upon our sampling design by having replicate collectors at each site, rather than spreading them widely among different islands. Increasing the sampling effort at a reduced number of sampling sites would increase statistical power. Collectors should also be installed earlier in the spring, and the plates collected at consistent two week intervals, to improve temporal resolution over the sampling period.

In spite of the aforementioned limitations of the methods we tested, the apparent seasonality of gastropod settlement that we observed (Figure 7) reflected similar patterns for gastropods around the globe (McShane and Smith 1991; Keesing et al. 1995). While most studies suggest recruitment occurs throughout much of the year, peak settlement and recruitment occurs from late-Spring to early Fall for most abalone species (McShane and Smith 1991; Keesing et al. 1995). Similarly, high inter-site variability in the density of post-settlement and newly recruited abalone is common among species elsewhere in the world (McShane and Smith 1991; Keesing et al. 1995; Rodda et al. 1997). This inter-site variability was also observed in our dataset.

The results of this study indicated considerable effort would be necessary to sample post-larval abalone via collectors. The effort and resources required to assemble, deploy, and retrieve the collectors in the field (~1 month), and to enumerate filtered samples in the laboratory (several months), were deemed too great to be of use for the aggregation experiment or other rebuilding studies in BC.

STUDY 2: EFFORT-BASED COMPARISON OF TECHNIQUES TO SAMPLE JUVENILE ABALONE

INTRODUCTION

Surveys of abalone populations rely on the ability of trained divers to locate individuals under variable conditions. The task is made difficult for juvenile abalone (1 – 10 mm shell length; SL) as they are often cryptic (Lessard et al. 2007; Zhang et al. 2007; Figure 14 herein) and spend the majority of their time in crevices, under articulated coralline algae, and under rocks. It can be time consuming, labour-intensive, and/or infeasible to search these microhabitats for cryptic juveniles (Sloan and Breen 1988; Takami and Kawamura 2018). Consequently, visual surveys are often biased towards mature, emergent abalone (i.e., abalone that can be readily seen at the surface of rocks and vegetation; Cripps and Campbell 1998; Obradovich et al. 2021).

Given that conventional survey methods are biased towards detection of larger abalone, alternative methods of surveying for juvenile abalone are required. Modified techniques for sampling cryptic juveniles within their natural habitat include: the use of Venturi-suction samplers and magnifying glasses (McShane and Smith 1988), the collection of substrate material and treatment with an anaesthetic (Prince and Ford 1985; McShane and Smith 1988; Sasaki and Shepherd 2001; Hart et al. 2020) and night-time surveys (Mortimor et al. 2003; see also Study 3, herein).

In July 2003, we tested three survey techniques for sampling juvenile abalone, modelled after the work of McShane and Smith (1998). Of primary interest was the temporal effort required for each technique, relative to the effectiveness. The objective of this pilot study was to identify an effective sampling technique for juvenile abalone, which could be utilized in later abalone aggregation experiments.

METHODS

Three methods of sampling juvenile abalone (1 – 10 mm SL) were tested from July 22 to July 24, 2003 in the Broken Group Islands, BC (see Figure 1 for map of study area). These SCUBA-based methods were: (1) Venturi suction sampling (henceforth Venturi), (2) rock removal, and (3) searching with a magnifying glass (hereafter termed magnified searches). Methods were tested in a variety of substrate types, including cobble (8 – 30 cm), boulders (>30 cm), bedrock with crevices (rock with cracks and crevices) and smooth bedrock (flat, smooth surfaces). Rock removal could only be assessed in habitats with moveable rocks (1 substrate category; Table 3). Samples were taken from 0.04 m^2 quadrats placed haphazardly within the four desired substrate types (Table 3). One technique was tested per quadrat.

Table 3. The number of quadrats (area = 0.04 m^2) sampled for juvenile Northern Abalone (1 – 10 mm shell length) in the Broken Group Islands, Barkley Sound, BC, using various techniques on different substrate types.

Technique	**Microhabitat**				**Quadrats Sampled**	**Total Area (m^2)**
	Cobble	Boulders	Creviced bedrock	Smooth bedrock		
Venturi	10	10	10	10	40	1.6
Rock removal	10	-	-	-	10	0.4
Magnified search	10	10	10	15	45	1.8

The Venturi technique employed a gas powered suction sampler attached to an anchored boat (Figure 8; McShane and Smith 1988). Standard household vacuum cleaner attachments were interchanged depending on the type of substrate within the quadrat (e.g., a brush attachment was used for smooth surfaces while a narrow nozzle attachment was used for crevices). A stiff brush was used to loosen any organisms during Venturi sampling. The filter bags (800 µm) were attached to the sampler, removed after each quadrat was sampled, and placed into resealable plastic bags. At the surface, each filter bag was rinsed with seawater and the filtrate was preserved in 90% ethanol for later sorting.

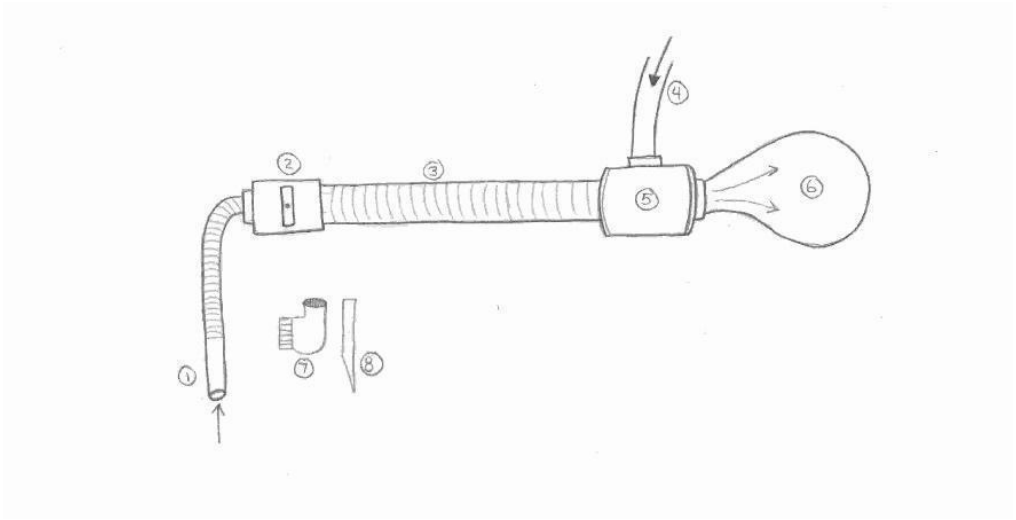

Figure 8. Modified Venturi suction sampler used to collect Northern Abalone from various substrate types. (1) Standard Vacuum Hose; (2) Shut off Valve; (3) 8cm suction hose reduced to 4cm by using the vacuum hose (1) and valve (2); (4) 8m surface water supply hose from 3hp

pump on boat; (5) Venturi device; (6) 0.8 mm mesh size filter bag; (7) Brush attachment; and (8) Crevice attachment. Illustration by Pauline Ridings.

The rock removal technique involved the removal and collection of all cobble within a quadrat (McShane and Smith 1988), and could therefore only sample cobble habitats. Divers removed the cobbles and placed them in large resealable plastic bags that were brought to the surface for processing. Once at the surface, the cobbles were removed from the bags and placed into anaesthetic baths (50% v/v ethanol) for 5-10 minutes. Each rock was then gently brushed with a soft brush and rinsed with filtered seawater to remove epifauna. The water from each bath and from within each resealable bag was filtered through an 800µm sieve and the filtrate was preserved in 90% ethanol for later sorting.

Magnified searches involved divers systematically searching each quadrat for abalone with the use of a magnifying glass and a light to find juvenile abalone. Unfortunately, the magnification level was not recorded by the observers.

Field data collection and sample sorting

The following data were collected by divers within each quadrat for each of the juvenile enumeration techniques tested: depth, substrate type, and time required to sample each quadrat (minutes per quadrat).

The filtered and preserved Venturi and rock removal samples were filtered (800 µm), sorted and specimens were identified using a stereomicroscope. When abalone were found they were measured to the nearest millimeter using calipers and photography. The presence of other invertebrates (e.g. limpets, chitons, urchins, and snails) was noted but not enumerated. The time to sort through samples (minutes per sample) was also recorded to compare sorting effort for the Venturi (all substrates) and rock removal (cobble only) techniques.

Data analyses

We ran two ANOVAs to examine the differences in temporal effort for each enumeration technique. First we used a two-way ANOVA to test the influence of substrate (boulder, cobble, creviced bedrock, and smooth bedrock) and technique (Venturi suction and magnified search) on SCUBA sampling time (i.e. under water temporal effort). Rock removal could not be analysed relative to the other SCUBA sampling methods, because it was only used in one substrate category (cobble only). We then ran a one-way ANOVA to test the influence of substrate on Venturi sampling sorting time (i.e. above water temporal effort). The assumptions of normality and homogeneity of variance were assessed through quantile plots, predicted versus residual

plots and Levene's tests (JMP, version 14, SAS). Data were square-root transformed and one extreme outlier was removed from the Venturi sample processing analysis in order to meet the model assumptions.

RESULTS

Abalone enumeration

A total of four abalone were detected in our survey areas (Table 4) - two were found using the rock removal technique (cobble substrate), one abalone (shell) was found by Venturi sampling (on bedrock with crevices) and one was observed using the magnified search technique (on smooth bedrock). Individuals ranged in size from 3 to 6.5 mm shell length (Figure 9).

Table 4. Number of abalone collected by each sampling technique and substrate. "-" indicates the technique was not used on a given substrate.

Substrate	Rock Removal	Magnified Search	Venturi Suction	Total
Boulder	-	0	0	0
Cobble	2	0	0	2
Bedrock w crevices	-	0	1	1
Smooth bedrock	-	1	0	1
TOTAL	2	1	1	4

Limpets and chitons were found in most of the quadrats sampled by rock removal and Venturi techniques. Unidentified abalone-like gastropods of 1 mm or less and juvenile urchins were also collected by both the rock removal and Venturi methods (Figure 10).

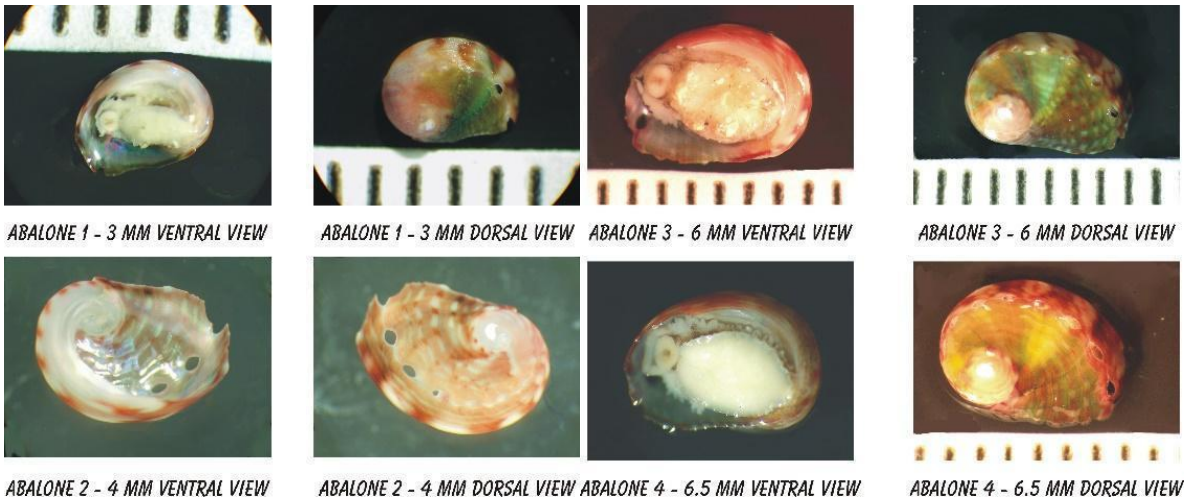

Figure 9. The ventral and dorsal views of all abalone found using three collection/enumeration techniques, Venturi suction sampling, rock removal and magnified search, in the Broken Group Islands, Barkley Sound, BC (note: the ruler spacing is 1 mm when shown).

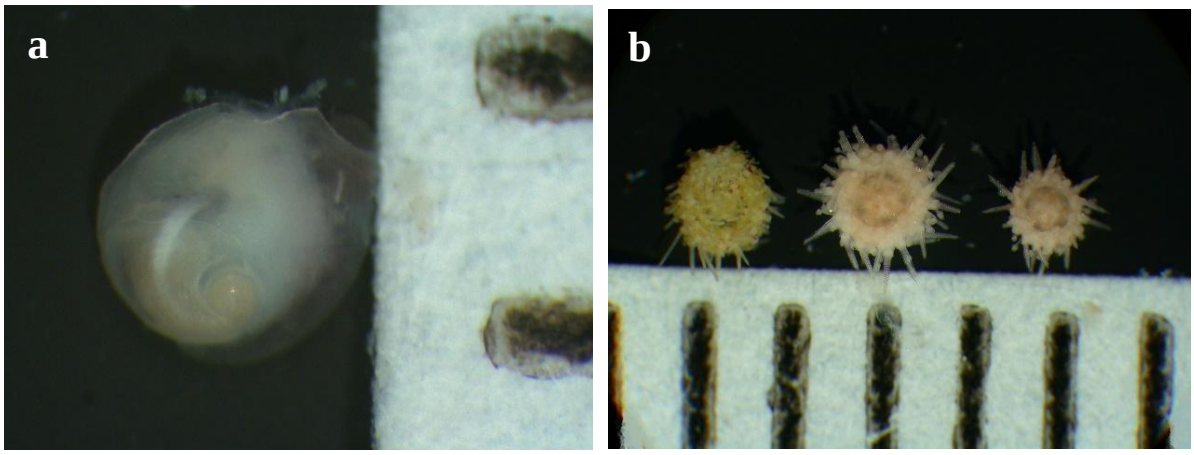

Figure 10. (a) An unidentified gastropod, similar in shape and size to very small juvenile abalone, and (b) urchins collected using the Venturi suction sampling technique in the Broken Group Islands, Barkley Sound, BC. (Note: the ruler spacing is 1 mm when shown).

Juvenile enumeration technique – analyses of effort

Sampling technique and substrate type significantly affected the time required to collect or enumerate abalone underwater (Table 5, Figure 11a). There was also a significant interaction between technique and substrate type. The magnified SCUBA search required significantly more time than all other combinations of substrate and technique, except when searching smooth bedrock. Venturi suction sampling on smooth bedrock required the least amount of effort when compared to all other combinations of substrate and technique. Magnified searches of smooth bedrock also required significantly more effort than Venturi suction sampling of boulders.

The effort required to process Venturi samples was significantly influenced by the substrate type (Table 6, Figure 11b). Venturi samples collected on cobble substrate required more processing effort than all other substrates.

Although statistical comparisons of the rock removal effort were not possible, qualitative comparisons suggest potential differences in effort. The rock removal effort underwater was lower than the magnified search on cobble substrate and the lab processing effort was likely equivalent to that of samples collected by Venturi suction sampling on cobble (Figure 11b).

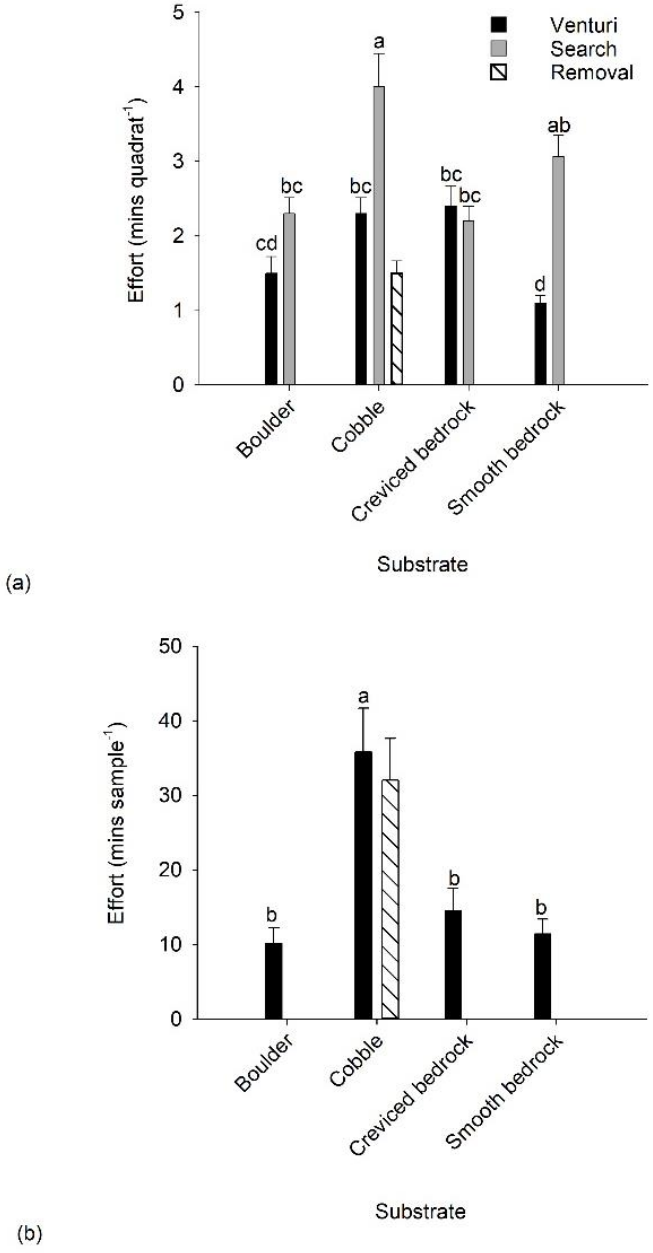

Figure 11. The (a) underwater and (b) laboratory processing time (mean + 1 SE) to enumerate juvenile Northern Abalone on different substrates through Venturi suction sampling (Venturi), magnified SCUBA search (Search) and, rock removal (Removal) in the Broken Group Islands, Barkley Sound, BC (Bars not connected by the same letter are significantly different; the legend is within (a). Rock removal is shown here, but was not statistically analyzed).

Table 5. Two-way ANOVA results for the effects of technique and substrate on the underwater effort to collect or enumerate juvenile Northern Abalone (SCUBA; minutes per quadrat) in the Broken Group Islands, Barkley Sound, BC.

Effect	DF	SS	F	*p*
Technique	1	2.36	40.0	<0.0001
Substrate	3	1.99	11.2	<0.0001
Technique*Substrate	3	1.40	7.95	<0.0004

Table 6. One-way ANOVA results for the effect of substrate type on the effort (minutes per sample) to process samples collected through Venturi suction sampling in the Broken Group Islands, Barkley Sound, BC.

Effect	DF	SS	F	*p*
Substrate	3	66.9	14.46	<0.0001
Error	45	69.42	1.54	

DISCUSSION

The juvenile abalone sampling techniques we tested resulted in too few abalone to make conclusions about their relative effectiveness. While the quadrats used were small enough to facilitate fine-scale searches via magnifying glass, rock removal, and Venturi suction, they were not large enough to capture any potential variation in the effectiveness of these techniques. We located four juvenile abalone after searching a combined area of 3.8 m^2 (i.e., 1.05 abalone per square metre searched), which suggests that juvenile abalone were indeed present at detectable levels at our study sites. Increased sampling or larger quadrats may have resulted in higher numbers of abalone, with sufficient variation to compare each of the sampling techniques.

Ideally each sampling technique would be tested in the exact same quadrat so that the number of abalone present was directly comparable. However, the collection-based, destructive methods for two techniques (rock removal and Venturi suction) rendered this impossible. Thus we cannot say for certain that any differences in number of abalone detected reflect the effectiveness of the sampling techniques (and not just natural variation within sampling areas). Regardless, with only four abalone detected (two via rock removal, one via Venturi sampling, and one via magnified search), we can not make conclusions about overall effectiveness. Future tests of these techniques could involve magnified searches followed by one collection-based method (rock collection or Venturi suction) in the same quadrat.

Despite the few abalone observed, we collected useful data on the temporal effort and logistical challenges associated with each sampling technique. All three techniques required the use of SCUBA, but two (Venturi suction and rock removal) required additional effort in the form of processing samples through microscopy. Our ANOVA analyses revealed that the type of substrate affected both the underwater temporal effort and the laboratory processing effort. Cobble substrates were the most time consuming to sample via magnified search. Similarly, Venturi suction samples obtained from cobble substrates were the most time consuming to process in the laboratory (compared to Venturi samples from boulders, creviced bedrock, and/or smooth bedrock; Figure 11). Rock removal could only be performed for cobble substrates; the laboratory processing time for these samples was similar to that of Venturi suction. Rock removal also required the least under water sampling time compared to magnified search and Venturi suction, but the logistical constraints of this technique limit its utility across varied substrates.

In contrast to rock removal, the Venturi method was suitable for rapid sampling on a variety of substrates. Despite yielding just one juvenile abalone shell, we collected numerous small chitons, limpets, and urchins using the Venturi technique, which suggests that the strength of suction would be sufficient to remove juvenile abalone. We therefore suspect that the Venturi technique would be useful in areas with a higher density of abalone. The Venturi sampler can reach into crevices and under rocks to remove abalone that may not otherwise have been detected by magnified or other visual search, but our study did not attempt to test the limits of the sampler. Some abalone could have been present within our quadrats but not reachable (particularly on bedrock with crevices). Additionally, like rock removal, the Venturi technique is destructive to the habitat and to the abalone themselves, which is not ideal for sampling species at risk. Should either of these methods be considered in the future, in situ sorting and release of the sampled individuals could be considered (rather than preserving them in ethanol for later laboratory processing). However, the survival of the returned individuals is uncertain, as they could be injured, highly stressed, and/or more vulnerable to predation.

Magnified searches were non-destructive and required the least temporal effort, given that laboratory processing was not necessary. However, divers found it difficult to use the handheld magnifying glass while maintaining a steady position in the quadrat. The three-dimensional nature of the substrate, combined with the small size of the magnifying glass made it challenging to survey the entire quadrat effectively. Additionally, the potential for inter-observer variation in abalone detection (due to varying levels of experience) can be substantial (J. Lessard, pers. obs.), so should be considered if this method is implemented elsewhere.

All the juvenile sampling techniques we tested had significant limitations. However, by identifying both the limitations and benefits of these techniques, we hope to assist future researchers in their efforts to effectively detect, monitor, and protect abalone. In summary, the rock removal and Venturi methods were the fastest to implement under water, but required additional processing time at the surface and in the laboratory and were destructive to abalone

habitat. Although rock removal has been used successfully in Japan (Sasaki and Shepherd 2001), the logistical constraints of the technique limit its application to cobble substrates, which hinders its utility for sampling abalone populations across BC's varied coastline. The magnified search technique was the least invasive method and the fastest sampling method overall, but was the most challenging to implement under water and was subject to observer bias. Future studies that require sampling juvenile abalone would do well to consider the relative merits of these techniques for their particular objectives. Since the time of this study, Fisheries and Oceans Canada has implemented cryptic quadrat searches for abalone to better detect juveniles. These involve detailed visual searches, including turning cobbles and lifting articulated coralline algae to locate cryptic individuals. Cryptic search methods are described in Study 4 (herein).

STUDY 3: EVALUATING THE UTILITY OF ARTIFICIAL HABITATS TO ESTIMATE THE DENSITY OF JUVENILE ABALONE

INTRODUCTION

Among the variety of techniques available to sample juvenile abalone (e.g. Study 2, herein), the use of artificial habitats is likely the most widely adopted (e.g., Davis 1995; Defreitas 2003; Bouma et al. 2012; Kawana et al. 2019). Surrogate abalone habitats provide a standardized area to sample cryptic juveniles and are usually constructed out of cement blocks and modified fishing traps. While thorough searches of natural habitat for juvenile abalone are often destructive (e.g., Tegner et al. 1989), artificial habitats can be readily examined by divers with limited disruption to the surrounding area.

Davis (1995) demonstrated that both native and hatchery reared Red Abalone (*H. rufescens*) would utilize artificial habitats in the wild. At the time, however, it was unclear how closely the abundance of abalone in artificial habitats represented that of the surrounding natural habitat. Defreitas (2003) subsequently compared juvenile abalone abundance in artificial habitats to that of surrounding habitats in British Columbia and found juvenile abalone to be significantly more abundant in the artificial habitats. This method of sampling was subsequently adopted to monitor juvenile abalone in conjunction with rebuilding experiments conducted in Haida and Kitasoo First Nation Territories (Defreitas 2003).

Around the time of the aforementioned studies, Parks Canada biologists had detected juvenile abalone at significantly higher density during night dive surveys than during the day (Tomascik and Holmes 2003). Similar observations had previously been made for other species of Haliotids. For example, Shepherd (1973) recorded the movements of five species of abalone in Australia and noted that young individuals emerged from their crevice habitats to forage at night, presumably to avoid diurnal predators. Mortimor et al. (2003) also detected higher densities of Northern Abalone at night (1.13 abalone $\cdot$ m^{-2}) than in the day (0.50 abalone $\cdot$ m^{-2}) at a site in Barkley Sound near the Broken Group Islands. Consequently, we sought to determine how estimates of abalone densities derived from artificial habitats compared to those from day and night surveys of adjacent natural habitat.

Between 2003 and 2006, we installed artificial abalone habitats (hereafter termed “condos”) at multiple sites in the Broken Group Islands. Condos and their adjacent habitat were surveyed for abalone, and estimates of density for three age/size classes (young-of-the-year [≤ 25 mm shell length (SL)], immature [> 25 and < 70 mm SL], and mature [≥ 70 mm SL]), were generated. During the same period, Parks Canada conducted day and night transect surveys of habitats adjacent to our condos, to simultaneously test the hypothesis that juvenile Northern Abalone would be observed at higher densities (in their natural habitat) at night. We intended to use the day and night survey results to better understand how abalone in different age groups utilize the

condos and natural habitats, and to determine if condos are a feasible alternative to transect surveys.

Unfortunately, we encountered significant obstacles over the course of the study, which prevented a robust statistical analysis of our results. Here we describe our two separate attempts to deploy and survey condos and provide a qualitative assessment of our results.

METHODS

Condo design and deployment

Condos were modelled after Defreitas (2003) and consisted of modified commercial crab traps with 24 small concrete blocks stacked inside. The frame and stainless steel mesh of the commercial crab traps were retained, while the central "fishing" components were removed to make room for the concrete blocks. Additional design details can be found in Defreitas (2003).

In July 2003, five condos were placed at both Condo1 and Condo2 (a total of ten condos deployed). Both sites are north of the Broken Group Islands, outside the park boundary. The condos were installed approximately 6 m deep (chart datum) and 5 m apart from one another. This area is exposed to considerably more swell and wave action than sites inside the Broken Group Islands. Consequently, of the 10 condos deployed, only one could be sampled the following year, while five had completely disappeared and the rest were filled with shells and other debris.

A second attempt was made in 2005, when five condos were placed in known abalone habitat at each of sites Prod1, Prod2, and Prod3, within the Broken Group Islands (total of 15 condos deployed). These sites are less exposed to swell and have lower wave action than those selected in 2003. The condos were installed approximately 20 m apart and at 6 – 9 m depth, on substrates primarily composed of boulders. Excluding reconnaissance searches for sites, it took approximately half a day to set up five condos at each site, depending on ocean conditions.

Survey methods

The condos were surveyed by SCUBA divers in February and July 2006. A pair of divers sampled each condo by removing and examining each concrete mini-block for abalone. All live abalone and empty shells were removed and measured (maximum shell length) to the nearest millimeter using calipers. After all blocks were examined, they were repositioned back into each condo along with the abalone sampled.

During the same period in February 2006 (but not July), Parks Canada conducted day and night SCUBA surveys of natural habitat adjacent to the condos at two of our study sites (Prod1 and

Prod2), following their survey method (Tomascik & Holmes 2003). Experimental abalone aggregation studies were also conducted at these sites (Prod 1 and Prod 3; see General Introduction for additional context). At each site, two transects, 25 m long and approximately 30 m apart were sampled. One transect was limited to shallow habitat (1-5 m) and the other was in slightly deeper habitat (6-10 m). Along both transects, fifteen 1 m^2 quadrats (n = 15 per transect, N = 30 per site) were sampled. The depth of each transect and the positions of the quadrats along each transect, were randomly determined prior to each survey. Once reaching the selected depth, the starting point of each transect was selected haphazardly by swimming along the depth contour and dropping the quadrat after about a 1-2 minute swim. Once the starting point was determined, the divers proceeded to flip the quadrat along the transect until they reached the first predetermined, randomly selected position.

Quadrat sampling included divers carefully lifting up (but not removing) all large macrophytes from the quadrat area to facilitate the systematic search for both emergent and cryptic abalone (see Study 4, herein, for information on cryptic abalone). The maximum length of live abalone and empty abalone shells were measured and recorded to the nearest mm using calipers. Once sampling of the quadrat was completed, divers proceeded to flip the quadrat to the next randomly selected position along the virtual transect. This procedure was repeated until all fifteen quadrats were sampled, or until divers had to surface due to safety considerations.

For data analyses, abalone were binned into the following size classes: young-of-the-year (YOY; ≤ 25 mm SL), immature (> 25 and < 70 mm SL) and mature (≥ 70 mm SL). Calculations of abalone densities in condos were based on the 3.5 m^2 surface area of each condo (Defreitas 2003), while the densities in surrounding habitats were calculated at the quadrat level (per 1 m^2).

RESULTS

Our first attempt to assess abalone densities using condos was prevented by the loss and/or destruction of 9 of 10 condos we deployed in 2003, presumably due to high swells and wave action. The sole condo that remained in place did not contain any abalone when surveyed in 2004.

In 2006, we had variable results. A total of 35 live abalone and 5 empty shells were found in the condos: 16 live abalone and two empty shells at Prod1, zero live abalone but three empty shells at Prod2, and 19 live abalone and zero empty shells at Prod3. Prod3 had the highest density of abalone in condos with $1.09 \pm 0.66 \cdot m^{-2}$ (mean ± SE; 3.8/condo), while the density in condos at Prod1 was $0.91 \pm 1.28 \cdot m^{-2}$ (3.2/condo; Table 7). The mean shell length of abalone found at Prod3 and Prod1 were 30.1 mm and 19.8 mm respectively. The two sites had similar abalone size ranges (between 4 mm and 42 mm), although Prod1 also had a few individuals above 70 mm SL.

Transect surveys of the natural habitat surrounding condos (during both day and night) were only conducted at Prod1 and Prod2. As we only found abalone through day and night surveys as well as within condos at one site, it was not possible to statistically analyze the efficacy of condos relative to abalone densities within adjacent habitats at different times of day. Qualitative comparisons of our results suggest condos at Prod1 housed somewhat higher densities of YOY abalone than adjacent habitats in the day. However, the daytime density of YOY abalone in condos was markedly lower than that adjacent habitats at night (Table 7; Figure 12). There was greater variability in mature abalone densities in condos, but they had similar mean densities of mature abalone as the surrounding habitat at Prod1 and Prod2 in both day and night (Table 7). While the condos at Prod2 did not contain any abalone when sampled in February and July, YOY and mature abalone were detected in the area surrounding the condos in February.

Table 7. The mean density per m^2 (± 1 SE) of abalone within condos at Prod1, Prod2, and Prod3 in February and July 2006, and on day and night transect surveys of adjacent habitats at Prod1 and Prod2 in February 2006. Shaded cells highlight the density estimates derived from sampling conducted during the same time period (February 2006). No transect surveys of habitat adjacent to condos were conducted at Prod3. See *Survey methods* for details about size classes.

Site	**Size Class**	**Mean Condo Densities**		**Mean Transect Densities**	
		February	**July**	**Day**	**Night**
Prod1	Total	0.91 ±0.37	0.46 ±0.25	0.28 ±0.09	1.78 ±0.27
	Mature	0.17 ±0.17	0	0.10 ±0.04	0.17 ±0.05
	Immature	0.11 ±0.07	0.29 ±0.50	0.13 ±0.06	0.17 ±0.05
	YOY	0.63 ±0.23	0.17 ±0.07	0.05 ±0.02	1.45 ±0.23
Prod2	Total	0	0	0.35 ±0.10	1.28 ±0.29
	Mature	0	0	0.02 ±0.02	0
	Immature	0	0	0	0
	YOY	0	0	0.33 ±0.10	1.28 ±0.29
Prod3	Total	1.09 ±0.19	0.63 ±0.28		
	Mature	0	0		
	Immature	0.29 ±1.67	0.29 ±0.16		
	YOY	0.80 ±0.19	0.34 ±0.21		

The mean density ± 1 SE of YOY for both sites combined during the day was $0.19 \cdot m^{-2} \pm 0.05$, while the mean density of YOY found at night was $1.37 \cdot m^{-2} \pm 0.19$. At Prod1 and Prod2, for both shallow and deep transects, the number of emergent abalone observed was substantially higher during the night surveys (Table 8). No mature abalone were found in shallow waters at Prod2, or at night at either depths assessed.

Table 8. Summary of mean abalone densities (per m^2; ± SE) found during day and night dive surveys along shallow (1 – 5 m depth) and deep (6 – 10 m depth) transect lines at Prod1 and Prod2 in February 2006.

	Day		Night	
Site	**Shallow**	**Deep**	**Shallow**	**Deep**
Prod1	0.30 ± 0.13	0.27 ±0.15	2.03 ±0.42	1.53 ± 0.34
Prod2	0.60 ±0.18	0.1 ± 0.07	1.4 ±0.45	1.17 ±0.37

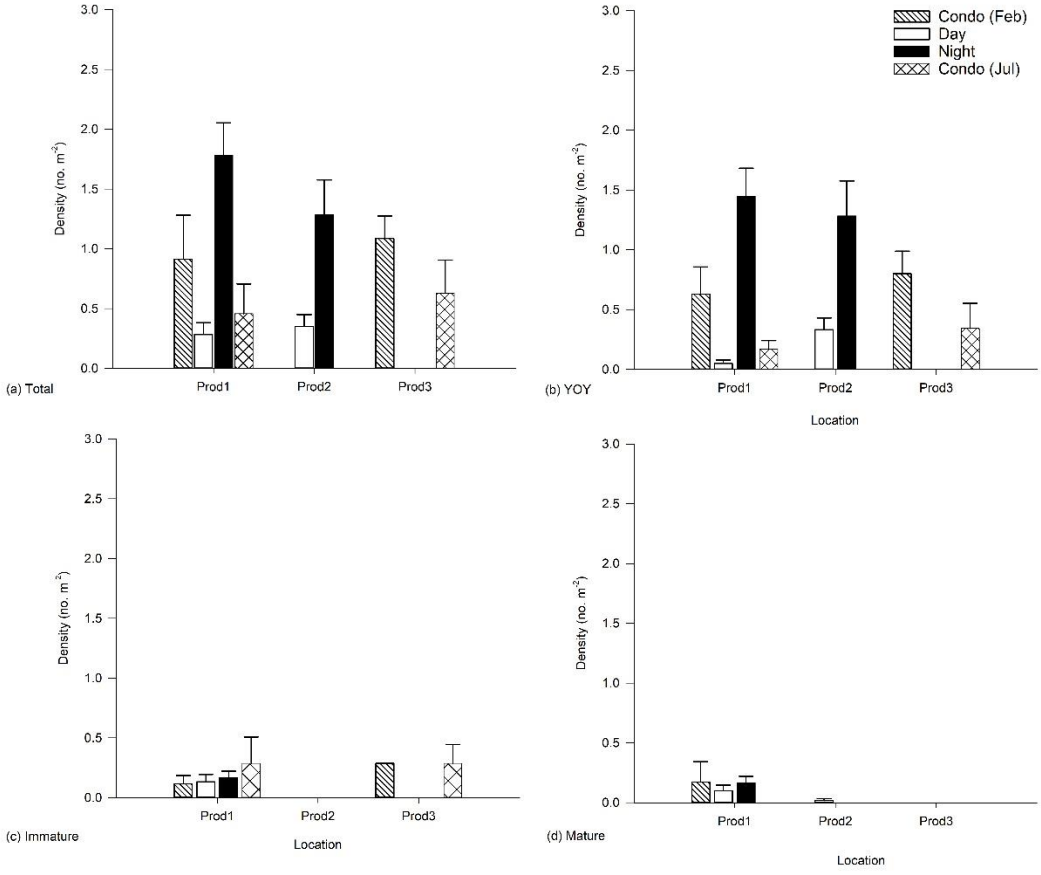

Figure 12. Mean density (± 1 SE) of (a) total, (b) young-of-the-year (YOY; ≤25), (c) immature (> 25 to < 70 mm SL), and (d) mature (> 70 mm SL) Northern Abalone found in February 2006 during day and night transect surveys, and within artificial habitats ("condos") in February and July 2006 at three sites in the Broken Group Islands, Barkley Sound, BC.

DISCUSSION

Artificial habitats have shown promise for sampling Northern Abalone in other studies (e.g., Defreitas 2003) and for other species of Haliotids (e.g., Rogers-Bennett et al. 2004). However, we found limited evidence that condos provide estimates of abalone density consistent with those observed in adjacent natural habitats (particularly for juvenile abalone – our age group of greatest interest). The condos were utilized by abalone at both Prod1 and Prod3, but not at Prod2, despite detection of abalone in the area during transect surveys. While the condos were placed on similar substrates at all study sites (predominantly boulders), it may be that Prod2 had a higher availability of natural cryptic habitat and thus our condos were less enticing.

Night-time transect surveys yielded the highest abalone counts – particularly for juveniles. However, safety and logistical concerns may preclude the use of night diving on a regular basis. This is particularly true in remote areas where quick access to diving-specific medical care is not possible. In general, operations also become more difficult at night as divers encounter more safety risks, such as becoming ensnared in kelp, and boat tenders may find it more difficult to track divers and navigate shallow waters. Night SCUBA surveys also require specialized equipment and training/certification for divers. While night diving may be an effective method to find and survey juvenile abalone, it poses serious logistical and safety challenges and thus is not always feasible to implement.

The lack of mature abalone detected in general (at all three of our study sites) may have been influenced by a large storm, with up to 20 m wave heights, which occurred just prior to sampling (DFO 2019). Mature abalone may be disproportionately affected by storms, due to their larger body size, which increases their probability of dislodgement by mechanical drag forces (Denny et al. 1985).

While artificial habitats do provide a standardized area for sampling, the factors which influence their use by abalone are not well understood. Davis (1995) compared the abundance of native abalone in artificial habitats that had been pre-seeded by hatchery reared individuals to that of artificial habitats that were not pre-seeded. The author found seeded artificial habitats contained 2.4 times more native abalone, but this apparent selection for habitats containing conspecifics was only detected in the first year after seeding. Additionally, the released, hatchery-reared juveniles did not remain in the artificial habitats for the duration of the two year study – the majority had either died or moved out within six weeks (Davis 1995). The author noted that his study sites “represented a wide range of ecological conditions for abalone with regard to abundance of food (kelp), juvenile predators (sea stars), and competitors (urchins)”, but did not discuss the potential influence of this variability on the use of the artificial habitats by released abalone. The movement of juveniles out of the artificial habitats may suggest the adjacent natural habitat was preferable to them.

A few more recent studies have compared abalone density estimates derived from artificial habitats to those of nearby natural habitats. In California, at sites with high abalone abundance

and density, Rogers-Bennett et al. (2004) found similar numbers of abalone juveniles in artificial habitats as on adjacent natural rocky reefs. At sites with low abalone density, too few individuals utilized their artificial habitats to make comparisons between natural and artificial habitat use. The authors did not provide details about the substrates upon which artificial habitats were installed, but noted that all were placed on rocky reefs. In contrast, Defreitas (2003) documented *greater* mean densities of juvenile abalone in artificial habitats than in adjacent natural habitats. The author's study areas in Haida Gwaii, BC, were dominated by "boulders and cobble encrusted with red coralline algae", suggesting the surroundings did contain a certain amount of cryptic habitat, yet juveniles sheltered in the artificial habitats at higher densities. While Defreitas (2003) did conduct some cryptic searches of habitat in the natural areas (by lifting and searching underneath cobble), it is not clear if they also searched inside and under the vegetation present (particularly among the holdfasts of coralline algae), which also serves as important cryptic habitat for abalone (see Study 4, herein). The habitat complexity of areas in which artificial habitats are utilized, and the method of surveying the adjacent natural habitats, are important to consider when interpreting the accuracy of estimates of juvenile density derived from those artificial habitats. Indeed, an experiment in the Broughton Archipelago suggested abalone may be more inclined to occupy condos situated on bedrock than on boulder substrate (Lessard et al. 2007). Artificial habitats may therefore be more successful as a sampling tool in some habitats than others. Until these factors are better understood, it will be important to incorporate additional survey methods when first deploying condos in a new area. Artificial habitats do provide a standardized *area* for sampling, but to obtain standardized estimates of recruitment or density (e.g. for comparisons between different sites), many other habitat variables should be controlled for.

In spite of the challenges encountered, artificial habitats hold promise as a method to estimate recruitment of Northern Abalone. The condos were inexpensive, easy to install and survey, and provided a standardized sampling area that facilitated density estimation. However, our qualitative comparison of day/night transect surveys and condo sampling suggests that the density estimates obtained from condos may not always reflect that of the natural environment. A relationship between the densities of abalone in condos and those in surrounding habitats has yet to be quantified. Future studies that aim to use artificial habitats to compare abalone densities at different sites/locations should quantify or at least consider the relative availability of natural cryptic habitat at each site.

STUDY 4: MULTI-YEAR SCUBA SURVEYS FOR JUVENILES IN CRYPTIC HABITAT

INTRODUCTION

Despite their potential bias towards emergent adult abalone, visual transects and/or plot surveys remain the primary non-destructive method to monitor abalone populations around the world (excluding catch rates; McGarvey et al. 2008). In BC, long-term monitoring of abalone has been conducted via surveys of index-sites on a five-year rotation since 1978. These index site surveys are conducted in six regions of the province: the central coast, southeast coast of Haida Gwaii, west coast of Haida Gwaii, Queen Charlotte Strait, Georgia Basin, and the west coast of Vancouver Island (Obradovich et al. 2021). Most abalone index site surveys conducted between 1978 and 1994 included cryptic searches (Boutillier et al. 1985; Carolsfeld et al. 1988; Thomas et al. 1992; Hansen et al. 2020), which involve carefully lifting all moveable substrates and vegetation to locate abalone that are otherwise hidden from view. Cryptic searches take approximately three times longer to complete than standard searches of sample quadrats (an average of 3 minutes per 1 m^2 quadrat, as opposed to 1 minute per quadrat; J. Lessard, unpublished data); thus cryptic searches have not been implemented in all abalone surveys in BC. However, historical data from index site surveys suggest young-of-the-year abalone (YOY, <20 mm shell length) are almost always cryptic (Lessard et al. 2007; Zhang et al. 2007) Population estimates derived from surveys that do not include cryptic searches (e.g. index site surveys from 1995 to 2005, as well as 2009, 2011, and 2012) may therefore be biased towards mature, emergent abalone (Lessard et al. 2002; Curtis and Zhang 2018; Obradovich et al. 2021).

When abalone aggregation experiments commenced in the Broken Group Islands in 2002, cryptic searches were incorporated into transect and plot surveys designed to estimate abalone densities. The primary goal was to obtain accurate data on juvenile abalone to later inform stock-recruitment estimates in the short-term abalone aggregation experiment (see General Introduction). Additionally, morphometric data collected from all abalone (both cryptic and emergent), could refine our understanding of age- and size-related differences in their habitat use on the west coast of Vancouver Island. Here, we present the results of cryptic searches for abalone in the Broken Group Islands using a plot survey design. We assess whether cryptic searches can inform recruitment and abundance estimates for abalone in BC, by quantifying the relative abundance of individuals that go undetected in non-cryptic surveys. This report summarizes the results from plot surveys conducted between 2003 and 2007. Transect surveys were completed in 2002 and 2003. A comparison of results from the transect and plot surveys will be published in a separate technical report.

METHODS

In May 2003 and June 2004, 2005, 2006, and 2007, we surveyed abalone habitats at five different sites in the Broken Group Islands using a plot survey design (Figure 13; described in detail in DFO 2016). Two permanent reference lines, each 40 m long, were placed at 2.5 m and 7.5 m depth (chart datum) at each of Prod1, Prod2, Prod3, Prod4, and Surf1. The reference lines were located in the middle of two depth strata (0-5 m and 5-10 m). On either side of the reference lines were 4 to 5 one metre wide transects, along which 1 m^2 quadrats were placed for sampling. The start location of each transect was chosen randomly prior to the start of the survey and most transects were 8−9 m long. Every quadrat was surveyed using standard search methods to enumerate and measure "emergent abalone" (i.e., abalone that can be seen without overturning rocks; Obradovich et al. 2021), and every 10^{th} quadrat was also subject to a cryptic search in addition to the standard search methods. During these cryptic searches, divers turned over and inspected all moveable substrates (e.g. under cobble, rocks, small boulders, and mats of coralline algae) within each quadrat looking for hidden abalone. The first cryptic quadrat was chosen randomly, after which every 10^{th} quadrat was also searched for cryptic abalone.

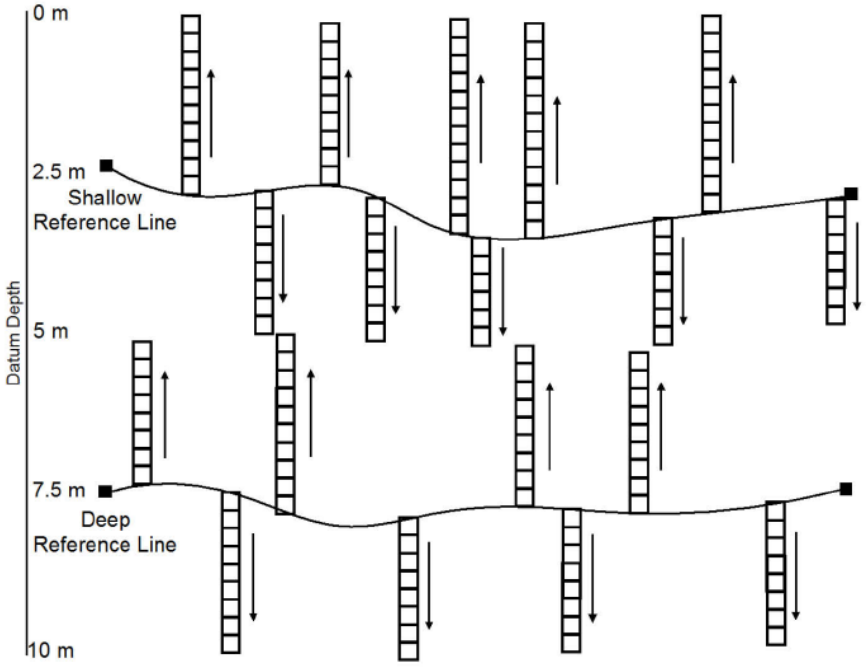

Figure 13. Schematic view of the plot survey design. Numbers on the left side are depths in meters (relative to chart datum). Arrows indicate the direction of survey of each quadrat. Reproduced from DFO (2016).

We recorded the shell length (SL in mm) of every abalone identified, and noted whether individuals were cryptic (hidden under rocks or other materials) or emergent (readily visible on the surface). In order to minimize habitat damage, algae were not removed. When a transect was completed, the divers moved to the next spot and repeated the procedure until all transects were completed within the depth strata.

Proportion and densities of cryptic abalone found across size classes

The relationship between shell length and cryptic proportion was investigated through multiple regressions with 1000 fits and 1000 iterations, to explore the best fitting model. We first pooled data from all cryptically searched quadrats across survey sites and years. Pooling was necessary to obtain sufficient data for a range of abalone sizes. Abalone measurements were then binned into groups at 5 mm intervals (i.e., 0 – 5 mm, 6 – 10 mm, 11 – 15 mm … 115 – 120 mm SL). The number of cryptic abalone were divided by the total number of abalone found in each size group and this was defined as the cryptic proportion. The assumptions of normality and homogenous variance for our regression analyses were assessed through the Shapiro-Wilk and Levene's test, respectively (SigmaPlot version 13.0, Systat Software Inc.). There was no evidence to reject the null hypothesis of homogenous variance ($p > 0.05$). After an extreme outlier was removed, normality subsequently improved (p increased from 0.0005 to 0.035), but this model assumption was not met. However, regression analysis is robust to violations of normality as long as other assumptions are not violated (Quinn and Keough 2002), which was not the case in this study.

The mean densities of cryptic and emergent abalone were also estimated for abalone in three age/size classes (young-of-the-year: ≤ 25 mm shell length (SL)); immature: > 25 to < 70 mm SL; mature: ≥ 70 mm SL) and plotted for each survey year and study site.

RESULTS

Cryptic proportion across all size classes

On average, over 90% of the YOY abalone we identified were cryptic. This was also reflected in the modelled relationship (Figure 14). A sigmoidal model (3-parameter) best explained the relationship between shell length and cryptic proportion (5 mm SL groupings; $R^2 = 0.93$; Table 9; Equation 2; Figure 14). The prediction bounds around the relationship suggest at minimum, 40 to 70 % of abalone smaller than 50 mm will be found in cryptic habitats, while a maximum of 25% of adults greater than 70 mm in size are cryptic (Figure 14).

Equation 2

$$f = (0.97 \pm 0.065)/(1 + \exp(-\left(x - \frac{57.4 \pm 3.2}{-12.5 \pm 2.6}\right))$$

Table 9. The analysis of variance results of the relationship between the abalone shell length (mm) and cryptic proportion of data collected during plot surveys in the Broken Group Islands, Barkley Sound, BC (1000 fits and 1000 iterations). Cryptic proportion is the proportion of individuals in each size class that were hidden during cryptic quadrat searches.

Effect	DF	MS	F	p
Regression	2	2.97	130.0	<0.0001
Residual	19	0.22		
Total	21	3.19		

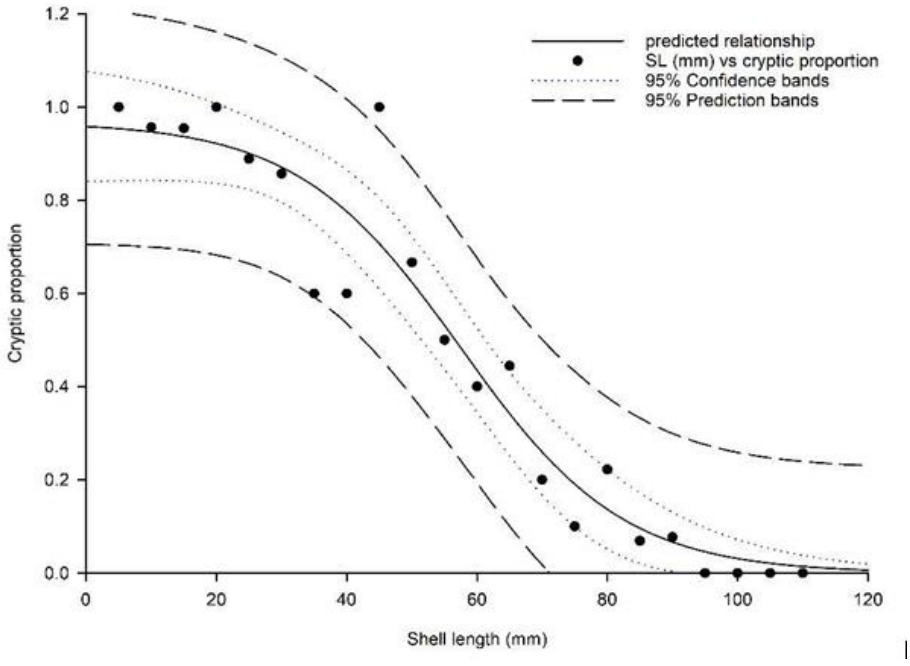

Figure 14. The relationship between abalone shell length (mm; in 5 mm groupings) and the cryptic proportion of abalone found in cryptically searched quadrats from the Broken Group Islands, Barkley Sound, BC, using a plot design.

The mean densities of each size class, across study sites and years, revealed similar patterns. Cryptic individuals were predominantly YOY abalone (Figure 15a), and density estimates of cryptic YOY abalone were higher than densities of emergent YOY abalone (Figure 15b). Density estimates of emergent and cryptic immature abalone were similar, while density estimates of mature emergent abalone were generally higher than those of mature cryptic abalone.

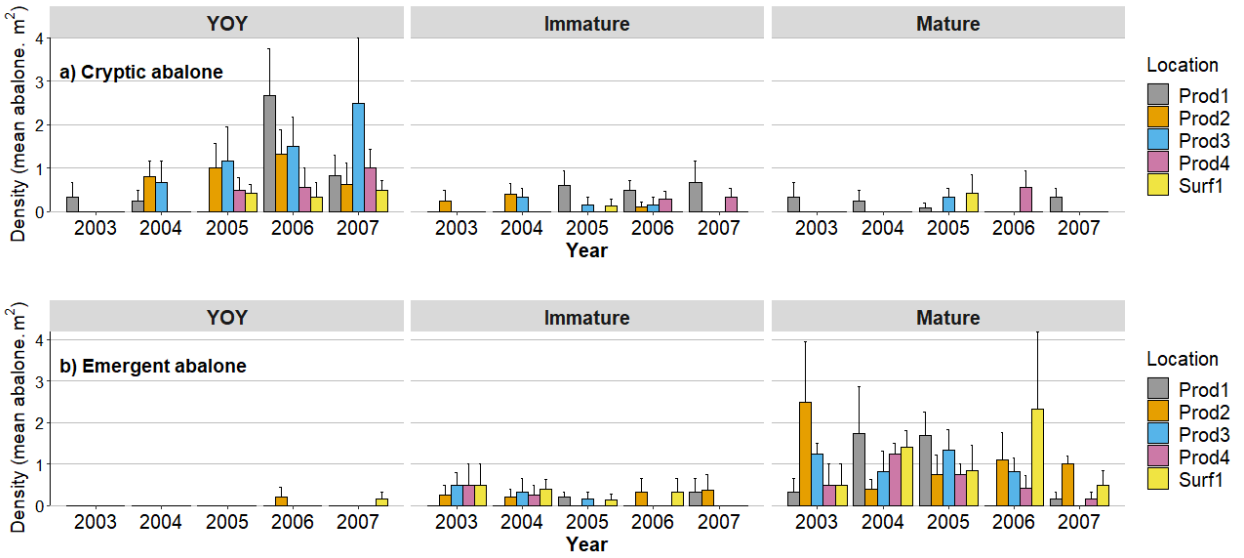

Figure 15. Mean density (+1 SE) of young-of-the year (YOY; ≤ 25 mm SL), immature (> 25 to < 70 mm SL), and mature (≥ 70 mm SL) abalone found in cryptically searched quadrats in 2003-2007. Panel (a) shows the densities of cryptic abalone belonging to each age group, while panel (b) shows the densities of emergent abalone belonging to each age group. Quadrats were sampled within a plot-survey design at various sites within the Broken Group Islands, Barkley Sound, BC.

DISCUSSION

Our five years of survey data suggest there is an ontogenetic shift in microhabitat use by abalone in the Broken Group Islands. Cryptic searches were far more likely to reveal small, young-of-the-year abalone than larger, older individuals. Mature abalone were predominantly emergent, while immature abalone tended to use both cryptic and emergent habitats to a similar extent. These size- and age-related differences in habitat use may reflect differences in predator avoidance behaviours and/or diet. Griffiths and Gosselin (2008) observed a rapid decrease in predation risk for Northern Abalone as their body size increased - particularly for abalone over 12-13 mm in size. Cryptic habitat use by the smallest juveniles may mitigate some predation risk

by limiting encounters with common predators such as small crabs (Griffiths and Gosselin 2008). An ontogenetic shift in habitat use has also been recorded in *Haliotis discus hannai* (Takami and Kawamura 2018). Young individuals (<20-30 mm) of this species tend to inhabit deeper crustose coralline algae-dominated habitats, whereas individuals > 40 mm are primarily found in kelp beds. Takami and Kawamura (2018) noted this shift in habitat use was likely associated with predator avoidance behaviours and changes in diet from benthic diatoms to macroalgae.

Our results suggest that inclusion of cryptically searched quadrats in abalone surveys would result in higher abundance and density estimates for young-of-the-year abalone. The implications for stock-recruitment modelling are significant. However, this method of sampling does have drawbacks. While visual surveys are relatively non-disruptive to the abalone and their habitat, cryptic searches require physical manipulation of the rocks and boulders that provide the cryptic habitat for small juveniles. If the rocks are not returned to their exact previous locations, resident abalone may be left vulnerable to predation. Even if the rocks are replaced as found, we do not know the impact of the brief disturbance to abalone or the other microfauna that utilize these cryptic habitats. However, given the small scale of this impact relative to seasonal storms which can also turn over large rocks and boulders (without replacing them), we find it unlikely that cryptic searches could have a population level impact on resident abalone.

An additional consideration for the inclusion of cryptic searches in plot or transect surveys is that many quadrats will not contain cryptic habitat (e.g. smooth bedrock with no algal growth), or they may contain cryptic habitat that is not searchable (e.g. deep cracks in bedrock or large boulders that cannot be lifted). These limitations may bias the analyses if records are not kept regarding which quadrats did not contain any searchable cryptic habitat. For example, if subsequent analyses of abalone density treated all quadrats as equal, the results might imply densities of cryptic abalone are much lower than they truly are.

While cryptic searches are time-consuming under water, they require no laboratory analysis (as opposed to rock collection and Venturi-suction sampling; see Study 2 herein), and likely result in higher juvenile abalone counts, depending on the substrate. Future research could explore the development of models to estimate the proportion of cryptic individuals in quadrats that contain cryptic habitat but are not cryptically searched. A better understanding of the role of substrate type and temporal variation in cryptic habitat use could inform abalone status assessments and population models in the future.

SUMMARY

The four studies presented here illustrate the wide variety of obstacles to obtaining reliable estimates of juvenile abalone abundance and density. Our pilot study using post-larval collectors to sample the smallest juveniles yielded very few abalone, but provided some information on settlement patterns, particularly for other gastropod species. While collectors offer a standardized sampling area that can facilitate comparisons of abalone settlement over time, we identified some issues with the study design, and find the time and expenses associated with this method make it less practical for large scale studies. We suggest that DNA-based approaches would provide a better method to detect and quantify early life stages (e.g. Vadopalas et al. 2006; Quinteiro et al. 2011; Becker et al., 2012; see review by Duarte et al., 2021).

Our comparison of three SCUBA-based sampling methods (Venturi suction, rock removal, and magnified search) was also hindered by low numbers of abalone. Our analyses of temporal effort indicated that Venturi suction and rock removal were the fastest to implement under water, but ultimately required more time to obtain results than magnified searches, due to substantial laboratory processing times. Venturi suction can be performed in a wide variety of substrates but is destructive, so it should be further tested and considered carefully before being implemented in the future.

Artificial habitats (i.e. condos) showed some promise for attracting juvenile abalone and facilitating estimates of abalone densities, but were not successful in all years and study areas. Strong winter storms destroyed condos that were deployed in areas exposed to high wave action, while other condos were simply not utilized by abalone (relative to their density in adjacent habitats), which may limit their value as a sampling method at some sites. At one study site where condos were used by abalone, densities in the condos were higher than those generated from day surveys, but lower than those that resulted from night surveys. We suspect that with additional research and concurrent use of other sampling techniques, the use of condos to sample juvenile abalone could be optimized. In particular, the availability of cryptic habitat at study sites should be considered when evaluating the density of abalone in condos relative to natural habitat.

The inclusion of cryptic searches in abalone surveys is likely the best means to improve juvenile detection. Our results confirmed that the smallest (i.e. youngest) abalone are most likely to use cryptic habitats, whereas mature abalone are most likely to be emergent. Cryptic searches can be readily incorporated into existing dive survey methods, but are more time consuming to complete and more disruptive than visual searches alone. To facilitate a qualitative comparison of cryptic searches with other juvenile sampling techniques presented in this report, we plotted the mean density of abalone detected at three sites where condo experiments, cryptic searches, and night/day surveys were conducted in the same year (2006; Figure 16).

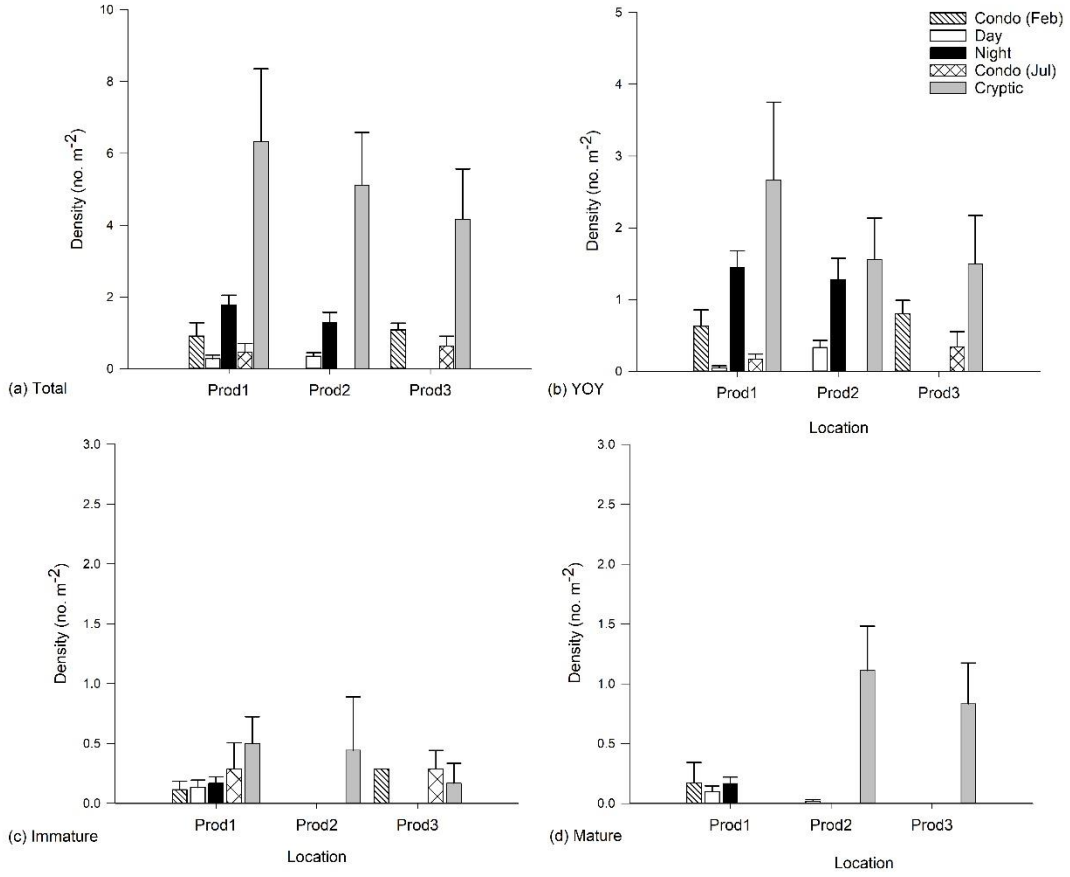

Figure 16. The mean density (+ 1 SE) of (a) total, (b) young-of-the-year (YOY; ≤ 25 mm SL), (c) immature (> 25 to < 70 mm SL), and (d) mature (≥ 70 mm SL) Northern Abalone, found during day- and night-time SCUBA surveys (February 2006), within condos (February and July 2006), and in cryptic quadrat searches (June 2006). Surveys were conducted at sites within the Broken Group Islands, Barkley Sound, BC. *The samples which generated these density estimates were not spatially or temporally consistent, but are shown here for general comparison. Note: the y-axes ranges are not the same on all panels.

In nearly all instances, plot surveys that incorporated cryptic searches provided the highest mean density estimates of YOY and mature abalone (one exception was Prod1 – fewer mature abalone were detected via cryptic searches than in condos or via night and day surveys). Only a controlled study designed to compare these methods can truly inform us which method yields the most representative abalone densities within a standardized area. The spatial area sampled by each of these techniques differs and the minimum survey area required to generate reliable density estimates for abalone is unknown. Abalone are known to aggregate (Shepherd and Partington 1995; Dowling et al. 2004) and are not evenly distributed on the seafloor, thus the

spatial extent and sample sizes of any juvenile sampling technique must be sufficient to capture this variation.

Each of the methods tested here therefore has important limitations and caveats that need to be considered before using them in future studies. No single method was clearly superior to the others, and the method(s) which would work best in future studies will depend on the specific objective of the study and the resources available.

By identifying methodological weaknesses and qualitatively comparing seven juvenile sampling methods trialed between 2002 and 2006, this report consolidates much of the current knowledge on juvenile abalone sampling in BC and represents an important step towards developing an effective method for sampling juvenile abalone. We suggest that the inclusion of cryptic searches in abalone surveys is likely the best means to improve the detection of abalone juveniles in coastal BC.

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