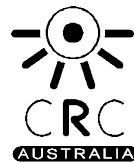


Factors limiting resilience and recovery of fished abalone populations

Craig Mundy, Karen Miller and Luisa Lyall



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2005/029 Factors limiting resilience and recovery of fished abalone populations.

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OBJECTIVES:

1. To determine the efficacy of translocation of mature abalone for stock rebuilding
2. To identify key ecological processes that limit stock recovery.
3. To quantify the scale of “spillover” from translocated populations.
4. Cost-benefit analysis of rehabilitated habitat

OUTCOMES ACHIEVED TO DATE

A brief summary of the key results are;

- 1) Approximately 2000 reproductively mature abalone were successfully translocated to each of three sites in a depleted region of the Tasmanian Eastern Zone fishery. The success of the translocation provides clear demonstration that translocation of abalone can be achieved easily, and at a relatively low cost.
- 2) Intensive monitoring of abalone at paired Translocation and Control sites over 24 months demonstrated that Translocation of mature abalone as a tool for rebuilding local populations is a feasible and relatively low cost activity, with high levels of survival easily achieved with appropriate handling and transport of abalone.
- 3) Surveys of abalone density and movement at the three Translocation sites revealed the translocated abalone responded differently at each site, with increased and earlier emigration of the translocated abalone at the sites and areas with low habitat complexity.
- 4) While the release of 2000 abalone into a small area at an initial density of eight abalone/m² resulted in a clear increase in the density of abalone, natural events such as localised recruitment, immigration and storm induced movement and mortality had an impact of a similar magnitude on local abalone density.
- 5) Formation and maintenance of abalone aggregations was weakly associated with topographic features such as crevices and boulder complexes, although the number of abalone in aggregations was variable through time. Aggregations did not always persist at specific locations, demonstrating that aggregations and aggregation behaviour in abalone is spatially and temporally dynamic, and importantly, was not related to local abalone density.
- 6) Larval recruitment to collectors was found to be highly variable among sites,

and through time. Larval recruitment within sites was also highly variable, although it could not be determined whether this variability was related to adult density or fine scale topographical variation

- 7) Investigation of connectivity at different spatial scales using population genetic tools strongly demonstrated that recruitment is highly localised, with very high levels of self-recruitment to sites or populations (scales of 100's of metres).
- 8) Techniques to tag abalone larvae using chemical based markers remain elusive. While Nile Red proved to be a successful stain for the lecithrophic abalone larvae, this marker was not retained at a useful intensity after metamorphosis.

A brief summary of the key conclusions are;

- 1) Despite the ease and success of a translocation event, emigration of translocated abalone away from the release site to surrounding reef habitat is inevitable, and at some sites this may negate the objective of creating an effective spawning population.
- 2) The time frame required to determine whether Translocation of adult abalone to create an 'instant' spawning population is at least seven years, and well beyond the typical funding period of research projects. Further surveys of the experimental sites in this project will be required to determine the longer term effect of creating localised spawning populations in depleted abalone habitat.
- 3) The clear demonstration of very high levels of self-recruitment by population genetic techniques has significant implications for any blacklip abalone stock rebuilding program. In the context of translocation, the spatial scale of benefit of remedial activity will be extremely limited regardless of whether that involves translocation of wild adults, release of juveniles, or release of larvae. We therefore suggest that stock rebuilding using such interventionist methods where the objective is to rebuild depleted stocks over geographic spatial scales is not viable as an industry funded commercial venture.
- 4) Translocation of wild abalone can only be used if there is an adequate source of mature abalone that are surplus to the requirements of the fishery. For this reason, it is expected that the circumstances where Translocation of wild abalone for the purposes of stock rebuilding can be undertaken will rarely occur.

Recovery of depleted abalone populations to a productive level will be dependent on recruitment success occurring over multiple years. High levels of exploitation or, natural mortality events, can reduce the reproductive capacity of populations to levels where local populations are no longer self-sustaining. Translocation of mature adult abalone to create standalone spawning populations have been proposed by managers, industry and researchers in a range of abalone fisheries around the world, although this concept has never been fully tested. In this study we examined translocation of wild

abalone to depleted abalone reefs in North-East Tasmania as a tool to enhance the rate of recovery of reefs that have failed to recover naturally over several decades.

Several experiments were conducted during this study, including a wild abalone translocation, a genetic study on connectivity, monitoring of larval recruitment at Treatment and Control sites, and a larval tagging study.

The translocation experiment involved moving around 2000 wild abalone to each of three Treatment sites at an initial abalone density of 8/m². Surveys of the three Translocation sites and three neighboring Control sites were conducted over a 24 month period, clearly showed an initial effect of the translocation with significant shifts in density. Density of larger abalone gradually declined over the subsequent 24 months as a result of natural processes, notably emigration at two of the Treatment sites, and mortality from a single severe storm event at the other. While the study time-frame was too short to determine whether the translocated wild abalone did indeed function as a viable spawning population, we were able to determine that translocation of wild abalone can be done with little cost, and with high initial survival.

The population genetic study confirmed results from previous genetic and field studies conducted in Tasmania, that dispersal of abalone larvae is limited, with most populations largely reliant on self-recruitment. This result has very clear implications for the scale of benefit that might be achieved from translocation of wild abalone, with the benefits largely restricted to the natal site (i.e. site of release). Thus to rebuild populations over a large geographic scale, a large number of release sites will be required. This finding applies equally to stock enhancement using hatchery raised larval or juvenile abalone.

In the context of management of wild abalone fisheries, maintenance of commercially viable densities of abalone on exploited reefs will also be dependent on the local reproductive biomass. Thus appropriate Minimum Legal Sizes (MLS) to preserve sufficient reproductive biomass, matched with appropriate Total Allowable Catch (TAC) is fundamental to ensuring that our abalone fisheries are resilient to ongoing exploitation.

Recruitment of abalone larvae to artificial collectors was highly variable, with the majority of recruitment to collectors occurring at just a single site. At the time when

spawning must of occurred, the site at which recruitment was observed was also the site with the highest density of abalone. This result is consistent with the concept of a threshold density, or Allee Effect, above which successful recruitment can occur. However, it was not possible to determine whether the limiting factor was suitable habitat or supply of larvae. Processing of larval collector samples is very slow, and requires high levels of human resources. We suggest some tactical research be undertaken in order to improve the efficiency of sample processing to make future recruitment studies feasible.

Experiments to identify suitable methods for chemical tagging of larvae were partially successful. Nile Red was the most successful tag, and acts by creating a fluorescent mark on lipid reserves of the egg or developing embryo. Nile Red had little impact on normal development of embryos, or settlement. However, the intensity of the fluorescent tag diminished rapidly at metamorphosis, and was therefore unlikely to be useful for the intended purpose, which was to distinguish larvae released from known points from larvae that may have arrived from elsewhere.

KEYWORDS: blacklip abalone, *Halitois rubra*, connectivity, gene flow, aggregation, translocation, stock rebuilding, movement.

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1. BACKGROUND

The blacklip abalone fishery is the largest and most valuable fishery in Tasmania. Following the collapse of major abalone fisheries elsewhere (South Africa, Japan, Mexico, California and British Columbia), Tasmania now supports the largest wild abalone fishery in the world and supplies 25% of the world's wild abalone catch. The Tasmanian abalone industry is worth approximately \$88m (ABARE 2003).

Between 2001 and 2004 the performance of the Tasmanian eastern zone fishery declined rapidly, necessitating a 32% reduction in the Total Allowable Commercial Catch (TACC) within this zone. TACC was reduced from 1120 to 770 tonnes and represents a loss of approximately \$10.5million per annum (equivalent to the entire NSW abalone fishery). The Tasmanian Abalone Management Plan (the plan) has clear expectations for an ecologically sustainable fishery. The plan identifies several objectives relevant to maintaining a sustainable fishery, as well as management actions to address declining fishery performance (catch and catch/effort). The relevant management actions available are;

1. Adjust the Total Allowable Catch for subsequent years;
2. Introduce area-specific management, such as catch limits, under the overall TACC;
3. Change the size limits, including the possibility of introducing maximum size limits;
4. Introduce seasonal closures; and
5. Introduce area closures.

In response to the declining eastern zone Fishery (falling catch, falling catch/effort), actions 1, 2, 3, and 4 have been implemented between 2002 and 2004. Fishery performance responded positively in most areas, and as of 2009 fishery performance in most of the eastern zone had stabilised and increased (Figure 1). However, a few areas that failed to recover following excessive exploitation in the 1980's again failed to recovery during the period of low TACC's and increased minimum legal size limits

established from 2002. The lack of response to these management actions by abalone populations in these areas is of great concern to all stakeholders, and it has become clear that we need to better understand the mechanisms that prevent stock recovery, as well as develop tools for rebuilding depleted stocks, as an essential component of the revision of management actions to ensure that the goal of ecological sustainability of the Tasmanian abalone fishery is achieved.

1.1 Consultation with stakeholders

Following the rationalisation of the Tasmanian abalone fishery in the mid 1980s, two productive areas in the eastern zone failed to recover (Blocks 28 and 30). The performance of Block 30 in particular was of concern to the industry, and there was strong interest in options for rebuilding abalone populations in Block 30 to previous levels of productivity. Critically, stakeholders were worried that the absence of recovery in Block 30 may be expressed in other parts of the fishery, even if management actions such as quota reduction were implemented. This concern has driven industry's request for a better understanding of the dynamics of stock recovery and the key factors that enable depleted stocks to rebuild.

Translocation and reseedling as a way to rebuild depleted stocks has been raised on several occasions over the past decade by the Tasmanian Abalone Industry during Abalone Fishery Advisory Committee (AbFAC) and Abalone Research Advisory Group (AbRAG). In 2003/2004 and 2004/2005, industry prioritised research into the dynamics of stock recovery, particularly in Block 30 (North East Tasmania). The research reported here addresses the industry need to understand the dynamics of stock recovery by exploring the utility of adult translocations as a mechanism to rebuild depleted abalone populations. It focuses specifically on Block 30, where abalone populations have declined dramatically, with catches now 4% of the long-term average catch. The first ever area closure in the Tasmanian Abalone Fishery (Action 5 above) was implemented for Block 30 in 2006. This is a drastic management action, and by the time there is support for such a management 'lever', ecologically it may be too late for recovery, or recovery time may take decades rather than years.

1.2 Ecological background:

Sustainable abalone fisheries depend on recruitment being equal to, or greater than, harvest. If the rebuilding of populations is to occur, then recruitment to the exploited

population must exceed the amount being harvested. Recruitment to the exploited population is a function of three important ecological processes and these will underpin the resilience and rebuilding of populations;

1) Reproductive success – the ability to produce viable larvae. Successful reproduction in subtidal broadcast spawners is a function of spawning synchrony, density, and aggregation (Levitan and Sewell 1998). In depleted populations, the density of mature abalone may be too low, with individuals scattered too far apart for effective fertilisation to occur (reproductive failure), and subsequently there will be insufficient larvae produced to sustain the population (Babcock and Keesing 1999; Tegner 2000; Dowling et al. 2004). It is often assumed that mature abalone in a depleted population re-aggregate after fishing (c.f. Gorfine et al. 1998; Officer et al. 2001), however, whether this does occur or whether aggregations form by emergence and movement of new recruits is not clear. If it is the latter, and there is reproductive and recruitment failure, then the population will collapse and be very slow to recover without assistance. Understanding movement and clustering of abalone to form successful spawning aggregations is the first challenge in determining how to rebuild depleted abalone populations.

2) Settlement success – supply or arrival of larvae to suitable habitats and successful settlement. The current paradigm of larval dispersal is that the dispersal distance of abalone larvae is on scales of 100s of metres ((Prince et al. 1987)), although this could vary across habitats (McShane et al. 1988; Shepherd et al. 1992; Temby et al. 2007). A consequence of short-distance dispersal is that recovery of local populations will be dependent on larvae produced by the local population (self-recruitment), or nearby populations. The presence of conspecific abalone also appears to aid settlement in many abalone species (Bryan and Qian 1998), and depleted populations may offer few settlement cues to the larvae that do arrive. Identifying the source of larval supply and suitable conditions for settlement is the second challenge in the rebuilding process.

3) Recruitment to the exploitable population – ability of newly settled larvae and juveniles to survive and enter the fishery. Adult abalone are rumoured to provide a 'habitat maintenance function' by maintaining areas of crustose coralline algae (CCA, or 'pink rock'), which may contribute to the survival of successful settlers and juveniles, although this has never been examined experimentally and thus remains an untested

hypothesis. Monitoring juvenile abundance as an indicator of recovery and the habitat maintenance function of abalone is the third challenge for understanding the process of rebuilding populations.

The research undertaken within this project directly addressed aspects of all three of these ecological processes in order to better understand both the failure of eastern zone abalone stocks to recover, as well as the feasibility of stock enhancement approaches to assist the recovery of depleted abalone populations.

1.3 Assisted recovery of exploited abalone populations:

Recovery of depleted populations may occur naturally over an extended period, although in several instances, populations never recover (e.g. no recovery in the Northern Abalone Fishery, British Columbia after 15 years of full closure; Californian white abalone now listed as an endangered species). Due to the lack of recovery in several heavily exploited abalone stocks around the world, stock enhancement by reseedling of larvae and/or juveniles has been trialled. However, release of either larvae or juvenile abalone is prohibitively expensive (Schiel 1992; Heasman et al. 2004) and there is no evidence that this form of stock enhancement is effective (Rogers-Bennett and Pearse 1998; Masuda and Tsukamoto 1998). An alternative approach to larval and juvenile reseedling is to translocate mature abalone into depleted abalone populations (Bell et al. 2005; Campbell et al. 2000). Tegner (1992, 2000) reported high numbers of juvenile green abalone (*Haliotis fulgens*) three years after translocation of mature abalone into depleted areas in California, whereas adjacent control areas showed no increase in juvenile abundance. Thus of all the possible enhancement procedures so far tested, translocation of adults appears to be the most successful, cost-efficient and practical. Furthermore, translocation experiments represent an ideal experimental tool with which to test aspects of the three ecological processes which are critical to successful recruitment.

1.4 Links with previous/concurrent research:

FRDC 2003/050 - Linking habitat mapping with fisheries assessment in key commercial fishing grounds. The area we chose for closure and translocation (Sub-block 30c) was one of two focus areas in the FRDC funded habitat mapping project conducted by TAFI. The research completed in project 2003/050 provided us with a detailed bathymetric and habitat map for the entire target study area, and in conjunction

with advice from commercial abalone divers, ensured our choice of study sites was optimal.

FRDC 2004/013 - Towards integrated multi-species management of Australia's SE reef fisheries: A Tasmanian example. Element 2 of 2004/013 addressed the association between resilience of a reef to abalone fishing and the nature of the understory/overstory kelp community. The study area we used was also assessed as part of FRDC 2004/013 and the resulting data were incorporated as part of the interpretation of our results.

FRDC 2001/074 Linking fishery-dependent and fishery-independent assessments of Abalone fisheries. Fishery independent survey techniques developed through 2001/074 were used to monitor abalone density over the duration of our study.

2. NEED

The socio-economic cost of complete closures in large areas of a fishery would be devastating for any fishing industry, creating considerable tension among researchers, managers and stakeholders. Importantly, although area closures are considered as the final option for stock rebuilding, complete fishery closures have not been successful as a management action to rebuild the Californian and British Columbian abalone fisheries. Therefore there is a need to identify key factors that are critical to ensuring sustainable management of fisheries and to develop tools to promote rapid and long term recovery, as better alternatives to full closure.

Stock enhancement has been the subject of a North American symposium on rebuilding abalone stocks, and the focus of two major FRDC funded research programs (1994/005; 2001/033). As outlined above, restocking of populations by releasing larvae and/or abalone seed (juveniles) was ineffective in Australia, Japan and North America, and is also prohibitively expensive. On the other hand, translocation of mature individuals may provide substantial benefits over the release of larvae and juveniles, and if successful, would be considerably more cost-effective (Tegner 2000; Campbell et al 2000). Translocation of mature abalone into depleted populations would also allow us to fast-track the recovery process.

Translocation of mature abalone may well be a useful tool for rebuilding depleted abalone populations, but there is also a clear need to understand the scale of influence of a translocation exercise. For example, if we were looking to facilitate recovery along a 50km length of coastline, would translocation of animals to one location in the middle serve to rebuild the entire area, or would we have to translocate animals to five locations spaced 10km apart, or 50 locations at 1km apart? The scale of influence of a translocation is therefore a crucial component of assessing translocation as a management tool. Additionally, using translocation as an experimental tool will enable a greater understanding of the key ecological processes limiting stock recovery and will enable management strategies to be implemented that either remove impediments to recovery, or trigger stock recovery.

Knowledge of recovery processes is required if the abalone fishery is to be managed sustainably, and is to continue to provide an important economic resource to Australia's rural coastal populations. Knowledge of ecological processes such as those underpinning stock recovery are clearly identified in the Australian National Research Priorities - An Environmentally Sustainable Australia - Sustainable use of Australia's biodiversity.

By understanding the key processes of reproductive success, recruitment and early survival, the proposed research addresses the Australian Marine Science and Technology Plan Program 1 – Understanding the Marine Ecosystem, by contributing to Objective 6 - Understand the biological processes in Australia's oceans and Objective 7 - To understand the dynamics of Australia's marine habitats and ecosystems. This research also addresses Program 6 – Using and caring for the Marine Environment, specifically Objective 6 - To improve the productivity and sustainability of the wild harvest fisheries, and to improve understanding of the relationships between fished stocks and the ecosystems that support them.

This project also addresses three of the high priority tasks in the current Tasmanian Abalone Strategic Research Plan (2005-2009): Recruitment, Stock Recovery and Stock Enhancement. The dynamics of stock recovery was identified as the highest priority research issue in 2004 by the Abalone Research Advisory Group.

3. THE TRANSLOCATION

The abalone fishery in statistical Block 30, North East Tasmania has had a classic boom-and-bust history. The area, more commonly known as the Bay of Fires, was a ‘Mecca’ for abalone divers in the late 1970’s and early 1980’s – beautiful environment, abundant abalone, and better weather than areas to the south and west. Up to 15 divers were resident in St Helens during the period of peak exploitation. This chapter provides a background to the fishery and the impetus for the translocation project, including site choice and site description.

3.1 History of Block 30 and impetus for stock rebuilding

Available catch records start in 1974, although the abalone fishery in Block 30 probably commenced in the early 1960’s. Effort data prior to 1985 are unreliable, and catch information prior to 1992 is also likely to be an under estimate of actual catch. However, it is apparent that the fishery was well established by 1974. Up to 28 divers fished annually in Block 30 through the 1970’s (Figure 1a), with total annual catches between 50 tonnes and 100 tonnes (Figure 1b). From 1980 to 1984 the annual catch exceeded 100 tonnes annually, and coincides with a marked increase in the number of divers active in Block 30. The fishery peaked in 1983, with 49 divers removing 299 tonnes. Following this spike in catch, the fishery and the number of active divers declined steadily to a low but stable catch of approximately 10 tonnes by 1992, although the number of active divers continued to decline through to 2005 (Figure 1a), when the fishery in Block 30 was closed.

The decline and subsequent low yields from the reefs in Block 30 was an issue of concern for many years. Assisted recovery through larval/juvenile reseeded or translocation of adult abalone was posed as a mechanism to try to restore the fishery in Block 30 to its earlier productive state. The only other option, closing the fishery, did not seem to be encouraged as a pre-cursor to active intervention.

3.2 Local knowledge: where did all those abalone come from?

Prior to a field based site selection process, habitat maps were obtained for the Block 30 region (Figure 2) to identify target areas. Discussions were held with several of the abalone divers that were active in this region during the peak of the fishery, and in the early phase of decline, to facilitate site selection. Older divers typically identified The

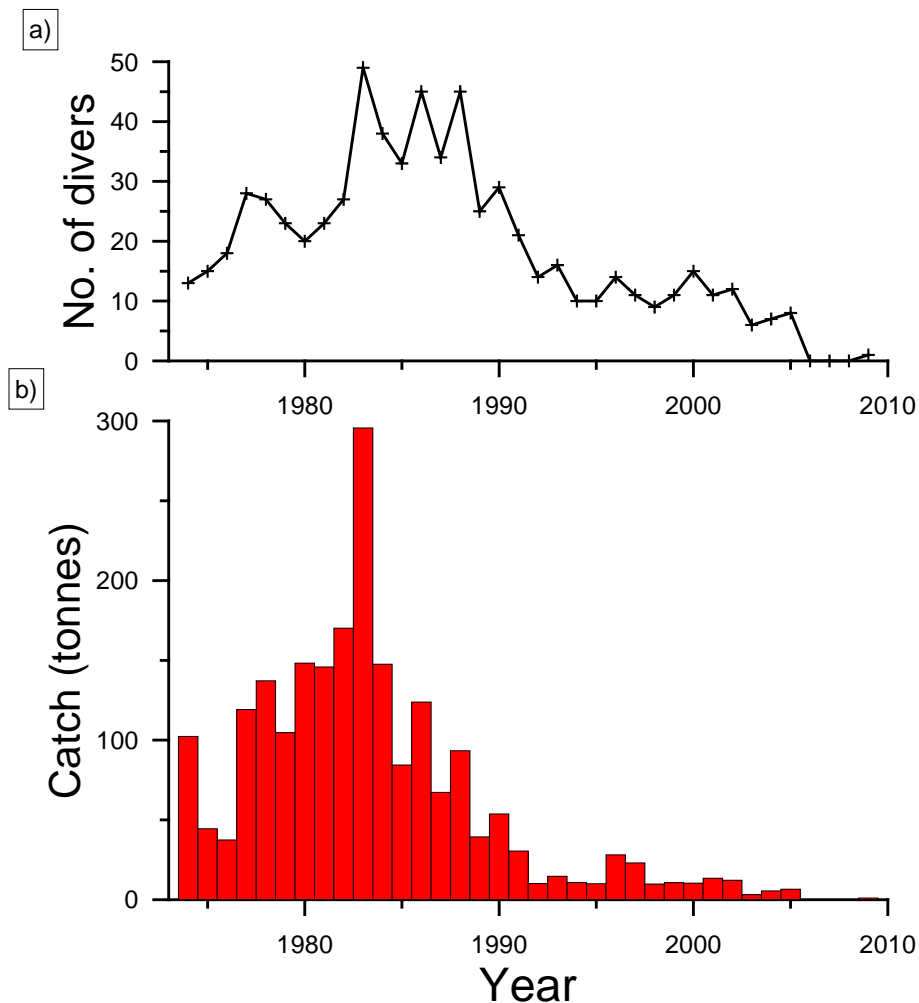


Figure 1. Catch history in Block 30 from 1974 to 2009. a) Number of divers with recorded catches in Block 30; b) Catch landed in each year.

Gardens, Sloop Rock, and Binalong Bay area as the best fishing grounds, but this does not necessarily imply most of the catch came from these areas. The largest reef system in Block 30 is in the north (Block 30C), extending from Ansons Bay approximately 8km south to Pebbly Beach. The Ansons Bay reef system was still considered moderately productive through the late 1980s. However, whether the catch was spread evenly through the four main reef systems, or concentrated in the south could not be

determined, as most divers had different recollections of where the catch predominantly came from.

The single common thread of information from the discussions with older divers was that the environment had changed significantly over the past 30 years. According to the divers, all of the shallow inshore reef from St Helens north past Eddystone Point and Georges Rocks was banded by a heavy curtain of string kelp (*Macrocystis pyrifera*). Among many tactics employed was the practice of divers swimming into the kelp beds along the reef, ascending and swimming back to the boat along the surface with their catch. The timing of the disappearance of the major kelp beds parallels the decline in the abalone fishery. At about the same time, several divers encountered large numbers of freshly shucked shells in the Ansons Bay area. There was thought to be an additional group of up to 5 divers based in St Helens harvesting illegally through the mid to late 1980's. Combined with intense summer recreational fishing, it is perhaps unlikely that exceptional levels of commercial exploitation is the only factor involved in the collapse of commercial blacklip abalone populations in Block 30.

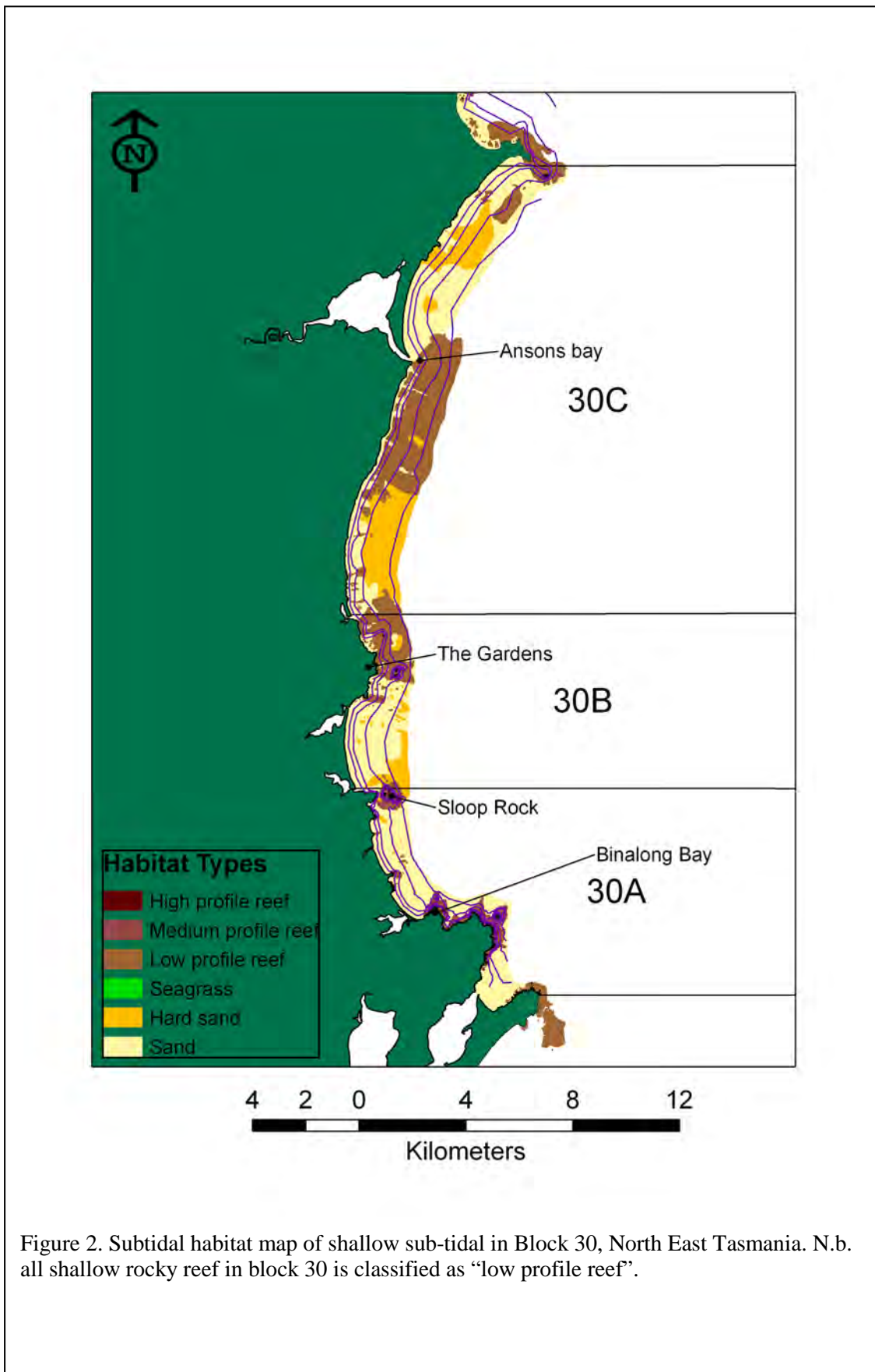


Figure 2. Subtidal habitat map of shallow sub-tidal in Block 30, North East Tasmania. N.b. all shallow rocky reef in block 30 is classified as “low profile reef”.

3.3 Procedure and logic on site selection

3.3.1 Site selection process

Following from discussions with divers, review of habitat maps, and consideration of the risk of interference from recreational fishing, the project was limited to areas in the more remote parts of Block 30 (30B and 30C). Extensive rapid surveys within Block 30 were done using a 10 minute count of all abalone and a qualitative assessment of habitat, to determine suitable locations for the experimental sites. Key factors that were necessary for the sites to be considered suitable were the presence of abalone habitat, evidence of reduced abalone density, and relatively discrete boundaries (i.e. site area consisting of reef mostly bounded by sand) to facilitate the containment of translocated abalone within the experimental sites.

One location in Block 30B (The Gardens) and two locations in Block 30C (Fancy Reefs and Pebbly Beach) were identified as suitable study areas. Each location was separated from the others by at least 5km (Figure 3), and each had different aspects. The aspect and exposure allowed the possibility of access to at least one pair of sites depending on swell or wind direction. At each of these locations, two sites separated by ~500m were established (a translocation site and a control site which represented no management action).

Each site was approximately 60m long x 30m wide, with the long axis aligned north-south and parallel to the shore. To facilitate easy relocation of each site, a centreline was established along the long axis and a steel pin cemented in place at each end. Sub-surface buoys were attached to the pins and GPS coordinates recorded for each pin. A system with a removable centreline was preferred, as there was a risk of the line coming loose during high swell events and posing a risk to boating. The pins were relocated and the removable centreline re-established at each visit. For the duration of each visit the centreline also functioned as baseline for abalone transect counts, and for establishing a grid for aggregation surveys, and kelp community assessment.

The centreline position at all sites was chosen to ensure the maximum depth across each site was <9m, to maximise the available dive time under the remote area restrictions within University of Tasmania Boating and Diving policy. Average depths at each site was approximately 6m, but ranged from 4m on shallow rock outcrops to 9m on the seaward edge of most sites.

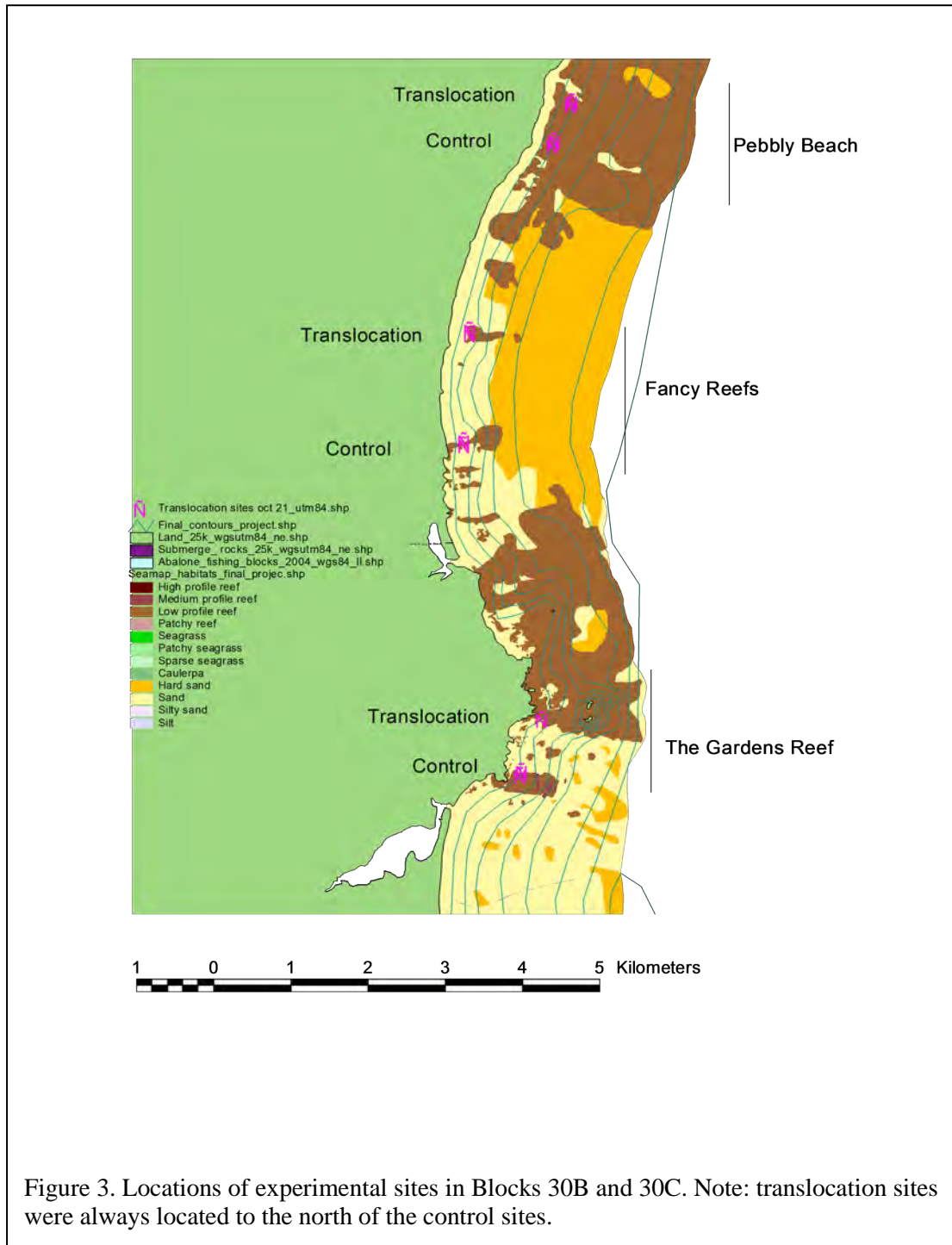


Figure 3. Locations of experimental sites in Blocks 30B and 30C. Note: translocation sites were always located to the north of the control sites.

3.3.2 Pre-Translocation density surveys

Prior to the translocation event, density and aggregation surveys were conducted at all 6 sites, and all resident abalone at the translocation sites were tagged. The density of abalone at each of the sites was determined using a modified belt transect procedure. At each site, abalone were counted and measured along 10 replicate 15m x 1m transects, with position along the centreline and the side (East/West) randomised. As expected,

density of resident abalone was low at each of the sites and ranged from an average of 0.007 abalone/m² at the Fancy Reef control site to 0.38 abalone/m² at The Gardens control site (Figure 4).

The average size of abalone at each of the sites was below the legal size limit of 136mm, although some legal sized animals were recorded at most sites (Table 1). The abalone densities recorded in this study contrast strongly with data collected recently from commercially fished sites in Block 31A to the north (0.83 to 1.29 abalone/m²) and from Storm Bay in the south (0.25 to 1.7 abalone/m²). The results from the initial density surveys on our target reefs emphasise the reduced state of the abalone stocks within Block 30 prior to the translocation.

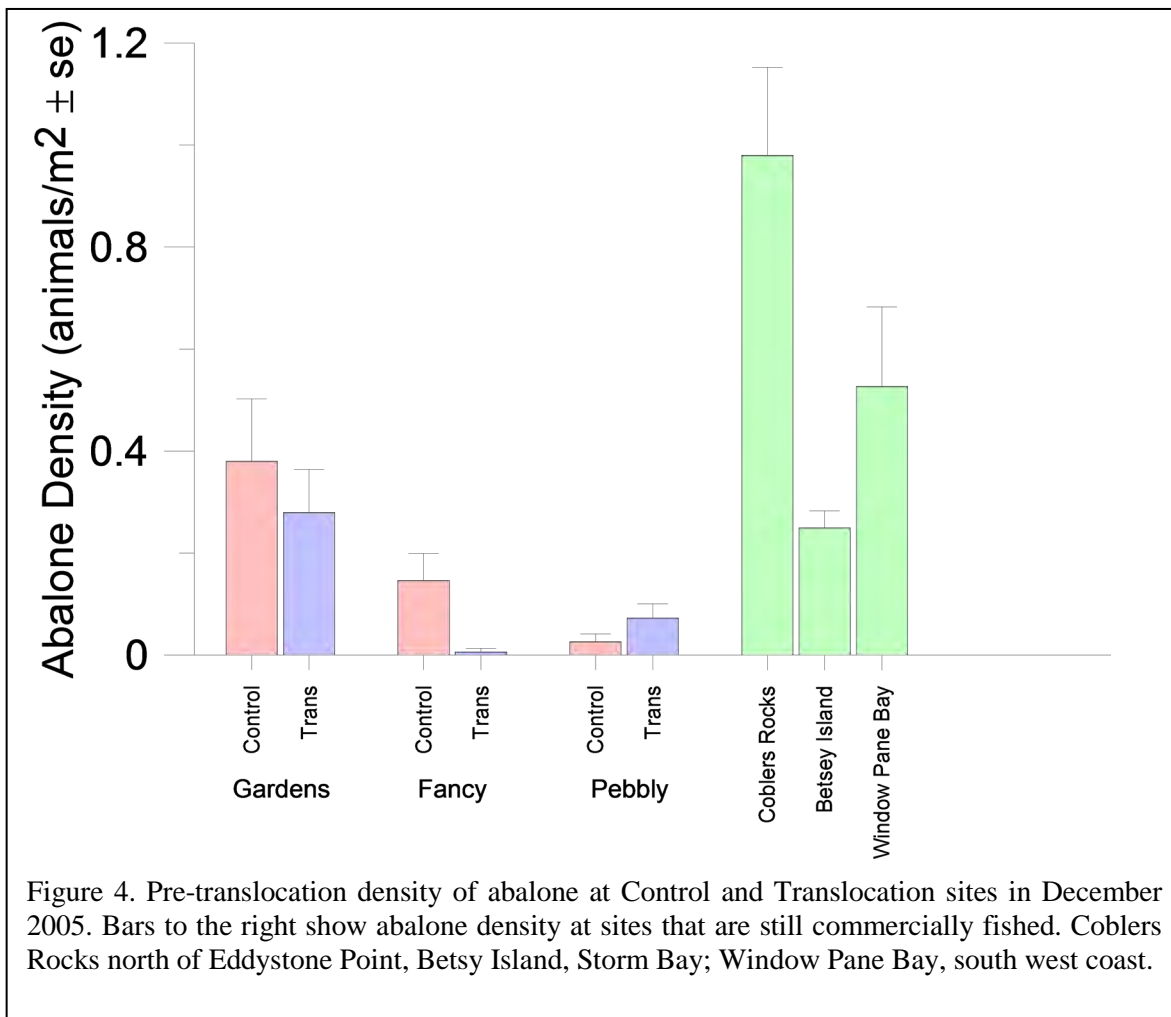


Table 1. Summary of the size of “resident” abalone recorded at each site (60m x 30m) from density surveys carried out prior to the translocation. Survey methods were 10 replicate 15m x 1m belt transects.

Location	Treatment	Average size (mm)	Minimum size (mm)	Maximum size (mm)	Total no. abalone
The Gardens	Control	110.7	60	158	57
	Treatment	120.0	47	150	42
Fancy Reefs	Control	133.4	100	176	22
	Treatment	118	118	118	1
Pebbly Beach	Control	115.3	100	142	4
	Treatment	86.1	30	152	11

3.3.3 Pre-Translocation aggregation surveys

Systematic surveys to determine natural aggregation structure of resident abalone were also conducted at all sites prior to the translocation. These surveys were done by dividing the site into four quadrants (NE, NW, SE, and SW). Each quadrant was divided into 6 bands 5m in width, by attaching one end of a 15m weighted rope (lead coil) to the centreline and laying the weighted rope in an East or West direction (depending on the quadrant) using compass bearings. Each band was then searched systematically, and all abalone observed marked with fluorescent surveyor’s crayon to prevent multiple counts. These aggregation surveys recorded the number and size of abalone clusters, and the number of legal sized abalone in each aggregation. Data on the habitat (vertical/horizontal crevice, boulder junction, open rock etc) where the abalone were located were also recorded.

Abalone larger than approximately 80mm were tagged in situ using plastic sheep ear tags attached with a plastic rivet inserted through the proximal respiratory opening. Abalone less than 80mm shell length were tagged using Floy disc tags attached using 2-part underwater epoxy (Z-Spar Splash Zone). Tagging of resident abalone at each site was also done during these initial aggregation surveys. At the Gardens Control site, weather conditions restricted sampling to a brief aggregation survey with abalone recorded as either above or below legal size ($\geq 136\text{mm}$), with insufficient time available to measure and tag residents.

3.3.4 Characterisation of kelp community

The kelp community at all three locations was similar, although rock type and physical structure differed substantially among locations. To describe the kelp community, and in order to detect potential future changes associated with recovery of abalone populations, it was necessary to conduct a baseline survey of benthic community

structure at each site. This was done in association with the initial abalone density surveys, enabling coupling of abalone density data with benthic community structure. On each of the 10 replicate transects conducted for abalone density, canopy cover was estimated using a line intercept method, where the canopy at each 1m increment along the transect was recorded.

The baseline surveys revealed that the dominant canopy-forming species on experimental reefs was *Phyllospora comosa* (Figure 1Figure 5), reaching > 50 % cover on all reefs (Table 2). *Phyllospora comosa* was particularly dominant on the Fancy Reefs, where it comprised > 80 % cover. *Ecklonia radiata* also represented a significant component of the canopy on some reefs, while cover of *Durvillea potatorum* was generally low (<10 % cover).

The dominant components of the understory community in experimental sites included encrusting algae, foliose algae and encrusting invertebrates. The abundance of these broad groups varied among locations, although the magnitude of the differences depended on the particular component concerned. For example, the abundance of encrusting algae was broadly similar between sites (ranging between 18-32 %), while encrusting invertebrates varied substantially (e.g. 22 % at The Gardens translocation site, to 61 % at Fancy Reef translocation site).

Table 2. Cover of dominant canopy species on experimental and control reefs.

Location	Treatment	Canopy cover (+/- SE)			
		<i>Phyllospora comosa</i>	<i>Ecklonia radiata</i>	Mixed <i>Phyllospora/Ecklonia</i>	<i>Durvillea potatorum</i>
The Gardens	Control	73.3(4.6)	8.6 (2.4)	1.3 (1.3)	14.0 (6.4)
	Treatment	53.3 (7.2)	35.3 (5.2)	1.3 (1.3)	0.7 (0.7)
Fancy Reefs	Control	83.3 (5.7)	0.7 (0.7)	0	8.7 (5.9)
	Treatment	90 (6.0)	0.7 (0.7)	0	8.7 (6.0)
Pebbly Beach	Control	58.7 (6.4)	16.7 (4.2)	3.3 (2.3)	18.7 (9.5)
	Treatment	60.3 (3.7)	16.3 (4.4)	16.0 (4.5)	2.7 (1.8)



Figure 5. Typical *Phyllospora comosa* dominated canopy at approximately 6m depth. The top of the canopy was typically 1.5m to 2m above the substrate, but varied among locations. The central base line is visible in the upper right corner of the photograph

3.4 The Translocation

3.4.1 Source populations

Potential source populations of abalone for the translocation were identified by Tasmanian Abalone Council Executive members Greg Woodham and Nigel Wallace. The target areas on the East Coast were at Seymour Point (immediately south of the

study sites in Block 30), and several inshore populations north of Eddystone Point. Dives at seven sites identified by Nigel Wallace resulted in the identification of four locations north of Eddystone Point from which we could confidently source 3000-4000 adult abalone (Figure 6) with little impact on the resident populations. The Eddystone

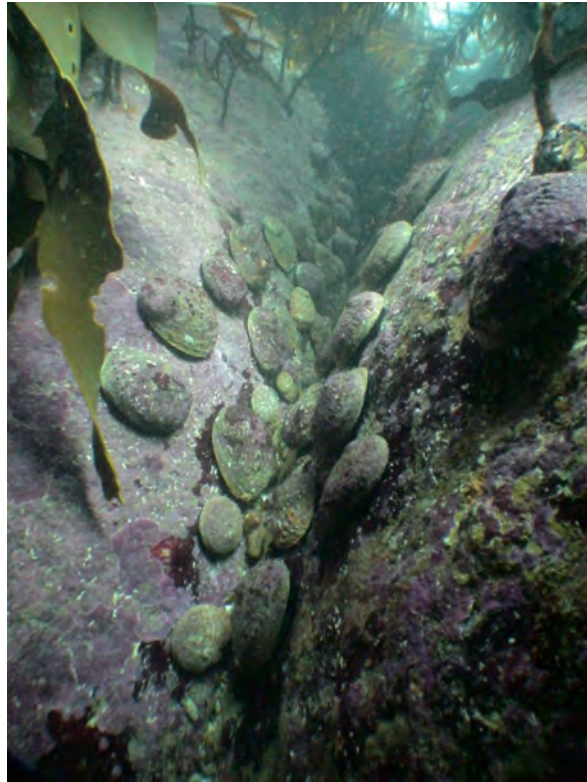


Figure 6. Aggregations at Purdon Point, one of the sites used as a source population for the translocation. Purdon Point is approximately 10km to the north of the Pebbly Beach site.

Point sites were particularly attractive as they were only 10km to 15km from the northern most translocation site, minimising any risk of disease transfer, and of introducing rare genotypes that may be more common in distant populations (e.g. West Coast).

3.4.2 Collection, Transport and Release

Over a period of ten days in May 2006, 6587 adult abalone were collected from Seymour Point (2000) and Eddystone Point (4587), and relocated to the three Translocation sites in Block 30 (Fig 3). Approximately 4000 abalone were collected by two commercial divers (Nigel Wallace and Greg Woodham), with the remainder collected by the TAFI research team.

Abalone collected from the Eddystone Point area by the TAFI Abalone Team were held in open mesh bags below the *RV Wobbecong* until brought on board for measurement and tagging. These abalone were collected, tagged and relocated to the translocation sites in three small batches, to minimise desiccation stress. Abalone collected by the two commercial divers were transferred to *FV Tacoma* (Figure 7) and either tagged immediately and placed into live wells, or placed directly into live wells for tagging the next day. This enabled us to minimise the period of time abalone were exposed to desiccation stress. While out of water, abalone were layered in bins with Hessian sacks, and kept moist using a deck hose, or buckets. All animals were tagged (using standard sheep ear swivel tags) and measured to the nearest millimetre with an electronic measuring board (ScieElex). A different colour tag was used for abalone destined to each of the translocation sites (Gardens –Blue, Fancy Reef – Green, Pebbly Beach- Yellow). Abalone were held in the *FV Tacoma* live-wells for 1-3 days prior to release. This procedure placed minimal stress on the abalone so as to ensure maximum survival rates following tagging.



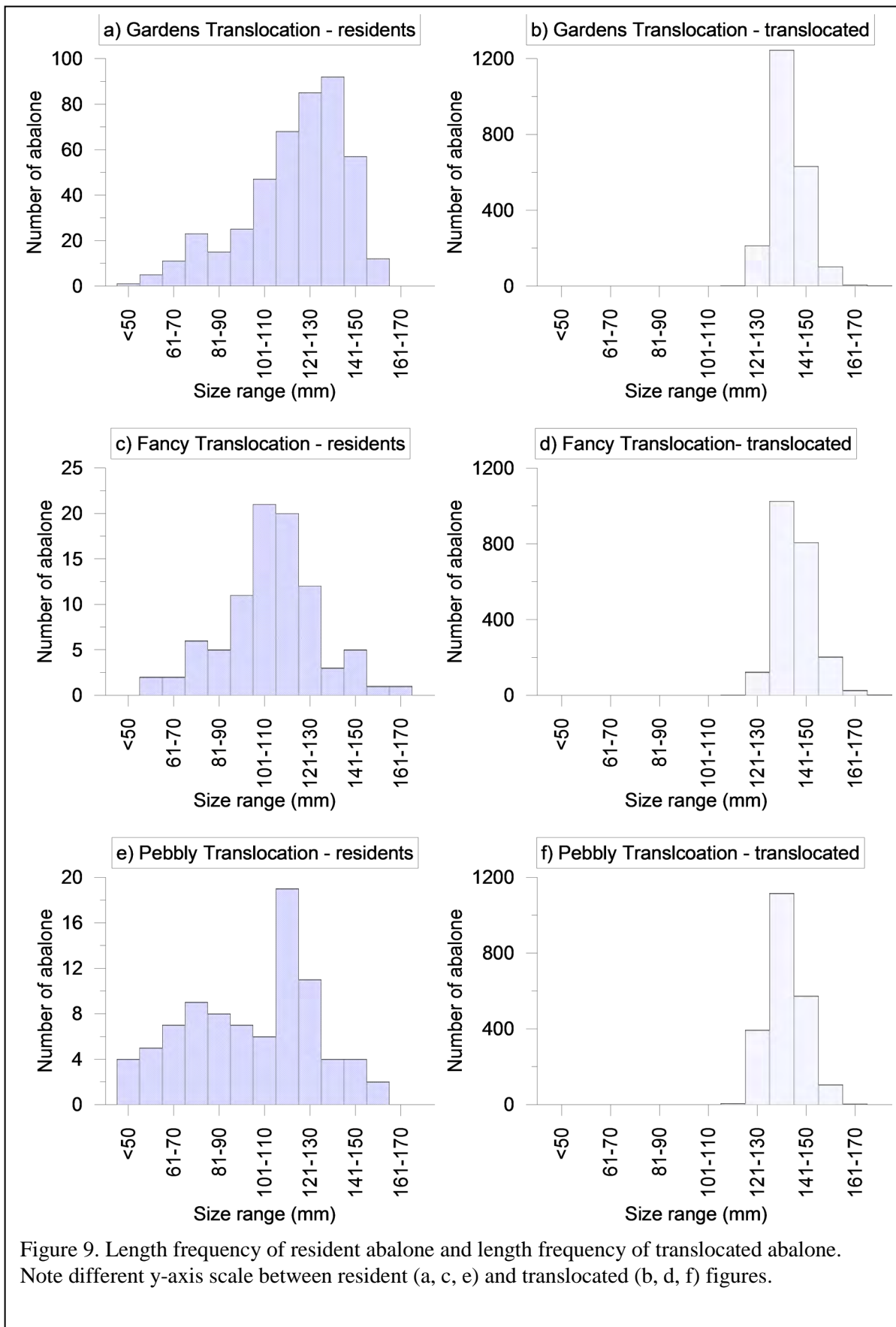
Figure 7. *FV Tacoma* used as a mother ship to hold and transport abalone collected for the translocation.

The release component involved placing abalone from the live wells into bins layered with Hessian sacks and then transferring animals to the study site by dinghy. The bins of tagged abalone were then placed intact underwater, and each abalone hand-placed onto suitable exposed rocky substrate by divers. The tagged abalone were held gently against the rock until they were securely attached (i.e. could not be removed by hand). While this was very time consuming, this manual approach served to minimise stress, maximise attachment, and to ensure translocated abalone were initially safe from predation (Figure 8). All abalone released were placed in an approximately 45m x10m strip in the centre of each of the three 60m x 30m Translocation sites.



Figure 8. Tagged abalone at Fancy Reefs the day after the translocation, May 2006. The older animal in the foreground with tag#720 is a large animal from north of Eddystone point. Note the different shell fouling and shell shape to the remainder of the abalone in this picture.

In total, 2199, 2189 and 2199 animals were translocated to each of the translocation sites at Pebbly Beach, Fancy Reef and The Gardens respectively. Equal proportions of abalone from Seymour Point (1/3) and Eddystone Point (2/3) were released at each site. Abalone collected for the translocation represented reproductively mature animals (Figure 8) and ranged in size from 119-175mm, with most measuring between 126-140mm (Figure 9). In comparison to the resident animals at each site (generally 90-120mm), the translocated abalone are larger and hence likely to have greater reproductive output to facilitate recovery at each of the sites (Figure 9). The density achieved at each of the three Translocation sites in theory would be in excess of that considered by some as the minimum for population maintenance (Shepherd and Brown 1992).



4. SURVIVAL AND PERSISTENCE OF TRANSLOCATED ADULT BLACKLIP ABALONE

4.1 Introduction

The introduction of a large number of abalone within several adult size classes to a depleted population is expected to effect a change both in population density and the population size structure. The rate of initial survival and the rate of emigration of translocated abalone will influence both the magnitude of the effect of the translocation on the resident population, and the time frame over which this effect persists. Detailed studies of the population effects associated with translocation of adult abalone have not been described previously. The magnitude of any measured effect of a translocation also needs to be considered relative to natural events that may occur during the time frame of the study.

Several studies suggest that abalone tend to aggregate into patches of high density. If such behavioural processes operate in blacklip abalone then we would expect to see population density increase dramatically associated with the translocation, and for density to decline only slowly, largely in response to natural mortality with relatively little decrease in density attributable to emigration.

Positive or negative feedback effects may also be associated with a sudden increase in population density. Negative feedback may occur where the pre-translocation population density is close to carrying capacity for that site, and the addition of a large number of mature abalone leads to a population size that exceeds the carrying capacity of that site. Positive feedback might occur in several forms. Abalone outside of the core study area may be attracted by the higher density of abalone within the core area, and migrate into the region of higher density created by the translocation. The presence of a high(er) density of larger, adult abalone may also contribute to greater survival of juvenile abalone.

Increased survival of juvenile size classes is often proposed as a positive benefit of the presence of larger abalone. This could occur through beneficial grazing and maintenance of area of non-geniculate coralline algae, which would serve to improve or increase the habitat available for very small abalone (< 20mm shell length). As juvenile

blacklip abalone tend to shelter in crevices prior to emergence, typically behind larger abalone, the presence of larger abalone may serve to decrease mortality of the small cryptic size classes. Both of these mechanisms are a poorly understood component of density-dependent effects. The former mechanism (effect on small juveniles) will not be detectable within the time-frame of this study. The latter mechanism (effects on larger juveniles) may apply in this study, although survival rates of these size classes cannot be measured due to the difficulty of locating, tagging, and re-locating juvenile blacklip abalone in cryptic habitat.

The objective of this chapter is to quantify the short term (two years) effect of the translocation on population density and size structure. It cannot consider the longer term effects associated with any potential increase in population density or change in population structure associated with increased biological recruitment.

4.2 Methods

Summary of density surveys and census events are presented in three contexts. Firstly, the initial retention and survival of abalone immediately after the translocation event to determine whether high levels of mortality and/or emigration occur rapidly after release. The second set of analyses relate to the survival of abalone over a longer period (~ 24 months) and the impact of the translocation on population density. The third section focuses on the persistence of the translocated abalone through time, in the context of population size structure. The latter two approaches are required due to the poor tag retention in abalone sourced from Eddystone Point and the limited capacity to conduct formal mark-recapture based survival analyses. Furthermore the observation that the pattern of size structure varied through time suggested the population size structure of residents was relatively dynamic at some sites, and contributed to the changes observed in density.

4.2.1 Post-translocation survey for mortality and density estimates

To obtain an initial assessment of the success of the translocation, transect based density surveys were undertaken in June 2006, two weeks after the release of abalone at the translocation sites to provide a comparison with pre-translocation estimates of density. For further comparison, an additional transect survey was conducted in May 2008 two years after the translocation. The purpose of the initial post-translocation survey was to determine if there was any large scale mortality associated with the

translocation of abalone, and, to gain an initial estimate of density at both translocation and control sites. At each of the six sites (3 x translocation, 3 x control) abalone density was counted in 10 replicate 15m x 1m belt transects, using the modified transect procedure described above (see section 3.3.2). Two surveys were also done between the initial post-translocation survey and the final survey. However because many sites were not sampled during these intermediate surveys due to weather restrictions the final data set was unbalanced and could not be used for rigorous statistical analysis. The results presented here therefore are based only on the initial and final survey periods.

A factorial ANOVA was used to test for change in density associated with the Translocation of 2000 abalone into the three translocation sites, above any natural change that might have occurred. The three factors were Time (Before, 2 weeks after, 2 years after), Treatment (Control/Translocation) and Location (Gardens, Fancy Reef, Pebbly Beach), with 10 replicates at each factor combination. Time and Treatment were fixed, and Location was random. Of principal interest is a significant Time x Treatment interaction term, with density in the Translocation treatment higher than the Control Treatment after the translocation of abalone. As $\log(x + 1)$ transformation improved the scatter of residuals vs predicted, the Log transformed data were used in this analysis.

4.2.2 Recapture of translocated abalone

Recaptures of tagged resident and translocated abalone occurred during the density surveys (Section 4.2.1 and the aggregation surveys (Section 3.3.3). During the density surveys, the colour, tag type and tag number of tags on abalone were recorded, along with an estimate of size. The position within the site was not recorded. During aggregation surveys, details of the tag were recorded along with the spatial position within the site, and the habitat class that best described the surface where the abalone was found (e.g. crevice, ledge, boulder junction etc). To avoid handling-induced movement or mortality, abalone were not removed (ie. chipped from the rock) during either rapid density or aggregation surveys. Tags were cleaned of excess crustose coralline algae if required to read the tag number.

4.2.3 Effects of translocation on abalone population size structure

Aggregation surveys and tagging of residents were used to characterise the population structure (size structure, distribution, aggregation patterns) of each site prior to the translocation of abalone to Treatment sites. Pre-translocation aggregation surveys were

completed at all sites, with the total number of abalone and the number of legal sized abalone ($\geq 136\text{mm SL}$) in each patch recorded (see Section 3.3.3 for details on pre-translocation aggregation survey methods). This provided an initial break down of the proportion of the population in the core study area that comprised mature adults.

As weather conditions deteriorated in early summer 2005/2006, the tagging program for resident abalone, where shell length of individual abalone is recorded, was only completed at the Treatment site at each location. Consequently, data of the exact length for all abalone at a site was available only from the Translocation (Treatment) sites and not for the control site at each location.

The size structure of populations post-translocation was obtained during aggregation surveys (see section 5.2.1 for details on aggregation survey methods), conducted at four sampling periods after the translocation (Oct 2006, June/July 2007, Jan 2008, Aug 2008). During the June/July 2007 survey a severe storm halted survey work, resulting in some sites or sections of sites being completed after weather conditions eased. Pebbly Beach Control and Translocation sites, 75% of Gardens Translocation and Fancy Control sites were surveyed prior to the storm, whereas Gardens Control, Fancy Reef Translocation, and the unsurveyed components of the Gardens Translocation and Fancy Control sites were surveyed after the storm. Insufficient time and resources were available to complete a full re-survey of all six sites after the storm.

Weather conditions also prevented sampling at several sites in January 2008 (Fancy Reef Control, Fancy Reef Translocation, Gardens Control) and in August 2008 (Pebbly Beach Control). During all post-translocation surveys, shell length was measured wherever possible without removing or disturbing the abalone, or where the position of the abalone prevented direct measurement the shell length was estimated (to the nearest 10mm) with the assistance of 10mm intervals marked on an abalone iron, and/or a steel ruler attached to a polypropylene slate.

Abundance of sub-legal and legal abalone prior to the translocation are summarised for all six sites. Length-frequency histograms of abalone shell length were constructed (10mm size class bins) for the three Treatment sites prior to the translocation, and all successful surveys after the translocation.

4.3 Results

4.3.1 Changes in abalone density associated with the translocation

Two weeks after the translocation, abalone density at all three translocation sites had increased, with densities ranging from 0.44/m² at Pebbly Beach Translocation to 0.65/m² at The Gardens Translocation (Figure 10). Subsequently, density declined at all three translocation sites, but this was also apparent at two of the three control sites (Figure 10). However the results were complex, with significant variation among locations, and a significant interaction, as expected, between the Time and Treatment factors (Table 3).

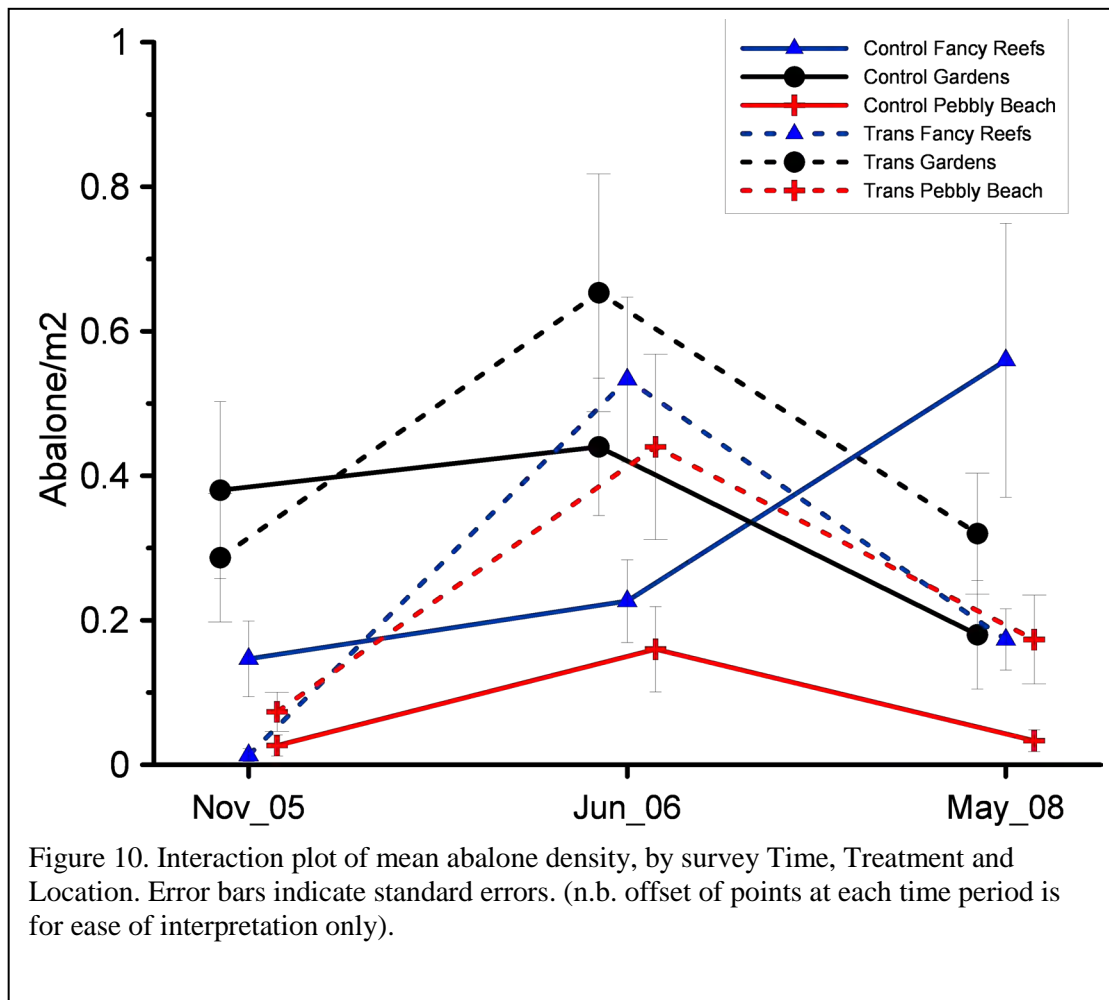
Table 3. Factorial ANOVA of the effect of the translocation through time on abalone density at Control and Treatment sites across three locations. Time (before, 2 weeks after, 2 years after) and Treatment (Control/Translocation) are Fixed effects, and Location (Gardens, Fancy Reef, Pebbly Beach) is a Random effect.

Source	df	Mean Square	F-ratio	Prob	Denom MS
Time	2	11.438	6.839	0.051	MS _{TmLcn}
Treatment	1	1.838	0.828	0.46	MS _{TrLcn}
Tm*Trt	2	2.398	2.057	0.24	MS _{TmTrLcn}
Location	2	7.888	12.688	0.0001	MS _E
Tm*Lcn	4	1.672	2.691	0.033	MS _E
Trt*Lcn	2	2.229	3.587	0.03	MS _E
Tm*Trt*Lcn	4	1.168	1.872	0.118	MS _E
Error	162	0.628			

Given the significant interaction, the full model was broken down and an analysis of the Location and Treatment effects were conducted at each of the three time periods. Examination of Main Effects revealed no significant Treatment effect at Time 1 (pre-translocation), but a significant Location effect (Location: d.f_{2,54}, MS=10.42, F=11.5, p= 0.001) reflecting the substantial differences in abalone density among locations prior to the translocation (Figure 10). At the second survey, six months after the translocation, Treatment sites had a significantly higher density of abalone (Treatment: d.f_{1,2}, MS=10.33, F=20.05, p= 0.046), while there was also significant variation among Locations (Location: d.f_{2,54}, MS=4.33, F=3.65, p= 0.033). At the third survey, two years after the translocation there was a significant main effect of Location, and a significant Treatment x Location interaction (Treatment X Location: d.f_{2,54},

MS=5.48, F=4.18, p= 0.02), reflecting the variable decrease in density across the Treatment sites at each location, and the marked increase in density at the Fancy Reef control site (Figure 10).

Density at all Sites increased between Nov 2005 and June 2006 (after the translocation), but with the increase at the Translocation sites substantially larger than at the Control sites (Figure 10). Theoretically, the release rate at each of the translocation sites should have resulted in a minimum density of 1.22 abalone per m² (based on site dimensions of 1800m², and 2200 abalone released) above the existing density at the time of the translocation. The observed immediate post-translocation density however was < 1/ m² at all three treatment sites



4.3.2 *Recapture of tagged translocated abalone*

High tag-loss was experienced at all translocation sites at the first full aggregation/recapture survey conducted six months after the translocation event. Tag loss was primarily associated with the abalone sourced from Eddystone Point (66% of all translocated abalone) where the shells were more degraded and fragile than shells of abalone sourced from the south. The loss of tags was immediately evident at the commencement of the six month survey, and therefore an attempt was made on this initial re-capture survey to categorise abalone sighted as either 1) untagged residents, 2) tagged resident or tagged translocated abalone, and 3) lost-tag abalone, in order to gain an understanding of the extent of tag loss. This was feasible as part of the process of attaching tags to the shell using a plastic rivet required some widening of the respiratory pore. A uniform circular hole larger than the adjacent respiratory pores was clearly evident on abalone that had been tagged and where the rivet had subsequently pulled through the shell. However, at the second post-translocation survey, classifying abalone as either 1) untagged resident or 3) lost-tag abalone was sufficiently difficult, that the practice was discontinued.

A primary consequence of the high tag loss was that insufficient recaptures were obtained to accurately determine survival and emigration parameters at the translocation sites. Instead, time-series of abalone density and population size-structure are used here to follow the effect of translocation on the depleted populations in this study (see result sections 4.3.1 and 4.3.3).



Figure 11. *Coscinasterias muricata* found preying on translocated abalone at Pebbly Beach Translocation site.

Evidence of mortality in the form of the presence of dead shells was low at all sites throughout the study, with typically less than 10 shells found at each site, on each survey. Higher than normal densities of dead shells were observed in two locations a) along the seaward sand edge, but not the leeward sand edge, of the Gardens Translocation site and, b) at the Pebbly Beach translocation site. The cause of mortality at the Gardens Translocation site sand edge was unknown, whereas at Pebbly Beach, several individuals of the predatory sea star *Coscinasterias muricata* were associated with an area of local high density on a rock rib parallel to the centre of the site. Predatory events by *C. muricata* (Figure 11) were observed on several occasions within several meters of dead shells at the bottom of the rock rib, below the abalone aggregations.

At the six month post-translocation aggregation survey, the total population size was greater at both Control and Translocation sites (Table 4), with a much greater increase in abundance at the Treatment sites. At the Treatment sites (translocation), increases in abalone abundance were between 343% to 994%, whereas at all three Control sites, the increase in abundance was less than 100% (Table 4). The results from these detailed surveys where a complete census was undertaken of the core study area, match with the results obtained from the density surveys where the site was sub-sampled using 15m x 1m belt transects.



Figure 12. Aggregation at Pebbly Beach Translocation site targeted by *Coscinasterias muricata*.

The number of recaptures of translocated abalone at Fancy Reefs and Pebbly Beach sites were substantially lower than at the Gardens site (Table 5), and this appears to be a consequence of differential emigration rather than differential mortality. Movement at the Gardens Translocation site was more restricted due to a longer sand boundary separating this reef from reefs to the south and east, than at the other two translocation sites. At the Fancy Reef site, a casual search conducted inshore and seaward of the core 60m x 30m study area revealed many tagged abalone (~ 20) associated with a complex

boulder habitat with mixed *Durvillea/Phyllospora* observed 10m – 30m to the seaward of the site. At the Pebbly Beach Translocation site, a rocky rib running east of the middle of the site in a north-south plane appears to be an attractive feature to the translocated abalone, and may have contributed to the higher retention rates at this site.

Table 4. Total number of abalone found at each site within the 60m x 30m core study area before and 6 months after translocation of mature abalone. Counts are from systematic swims providing a complete census of abalone at each site.

	Translocation			Control		
	Gardens	Fancy Reefs	Pebbly Beach	Gardens	Fancy Reefs	Pebbly Beach
Before	504	89	83	469	217	59
6 months	1727	536	825	827	330	75
% increase	343%	602%	994%	57%	66%	79%
# translocated	2199	2189	2199			

Table 5. Number of translocated abalone (tag present or identified as lost tag) recorded in the core 60m x 30m study area 6 months after the translocation.

	Gardens	Fancy Reefs	Pebbly Beach
Translocated Abalone with Tag	723	160	296
Translocated Abalone without Tag	390	236	353
Total	1113	396	649
Percent recapture	50.6%	18%	29.5%

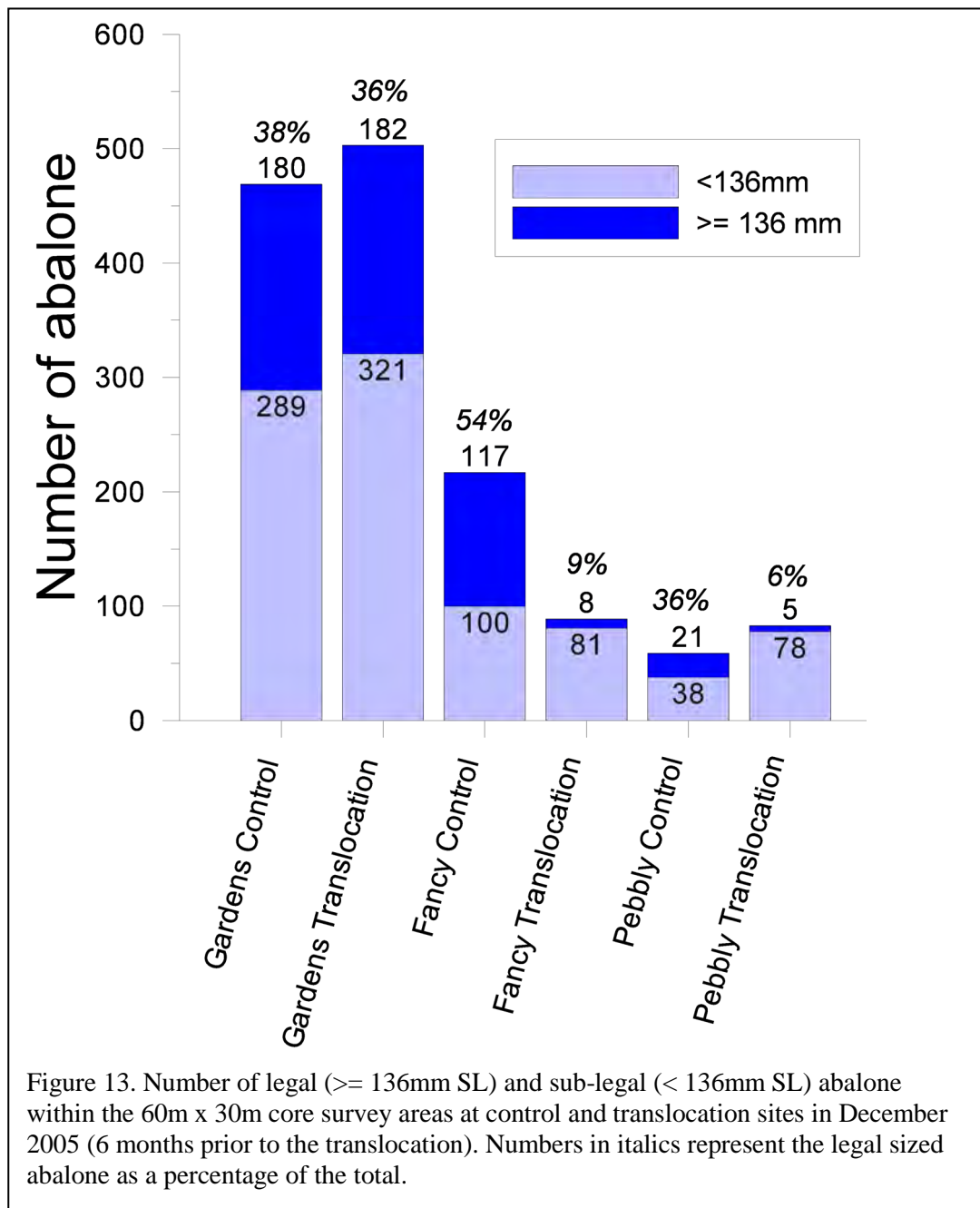
Some mortality of abalone was associated with a severe easterly storm that affected the north-east coast of Tasmania in June 2007. A drop in abalone numbers was observed during aggregation surveys, and other visits to the sites following this storm (see Figure 14 - Figure 16). In particular, the Gardens Reefs appeared to be affected to a larger extent than the other locations. The Gardens sites are more protected than those at Fancy Reefs and Pebbly Beach, as the Gardens sites are relatively sheltered from the southerly swells by the headland at St Helens, and from the northerly and north-easterly swells by the rocky reefs that make up most of the Gardens Reef structure. Large numbers of dead shells littered crevices on the north-west (leeward) side of the reef in the weeks following the storm. While some of these were older shells that were uncovered by significant shifting of sand adjacent to the reef, many were from tagged translocated abalone.

4.3.3 Population size-structure as a measure of persistence of translocated abalone

Prior to the translocation, the proportion of the population represented by legal-sized, reproductively mature abalone varied across the six sites, but did not appear to be

linked to total abundance. At two of the Treatment sites, Fancy Reef Translocation and Pebbly Beach Translocation, legal sized abalone represented less than 10% of the total abalone observed (Figure 13). Legal sized abalone represented at least one third of the total abalone at the other four sites. The greatest proportion of legal to sub-legal sized abalone was recorded at the Fancy Reef Control (Figure 13), where more than half of the population consisted of legal sized abalone. At the Treatment (Translocation) sites prior to the translocation, the modal size class at the two northern locations (Fancy Reefs and Pebbly Beach) was less than 120mm, whereas at the Gardens the modal size class was larger at 130mm to 140mm (Figure 14, Figure 15, Figure 16)

Six months after the translocation, the evidence of an impact of the addition of ~2200 mature abalone on the population size structure varied at the Treatment sites across the three locations. At the Gardens and Pebbly Beach locations, the translocated abalone clearly dominated the population (Figure 14, Figure 16). At the Fancy Reef location, the effect of the translocation on population size structure was still apparent, but greatly reduced compared to the Gardens and Pebbly Beach sites (Figure 15).



The surveys scheduled for one year after the translocation were interrupted by a long period of severe winter weather, including a major storm event in June 2007 (see 5.2.1 for details). The Pebbly Beach sites were surveyed prior to the June storm, with the Translocation site still showing a clear signal of the addition of adult abalone 12 months earlier, and the Control site showing no change in size structure or abundance (Figure 16). At the two southerly locations, including those largely surveyed prior to the major storm there was clearly an effect of the storms on the population size

structure (Figure 14 and Figure 15). While the modal size classes remained the same as observed at the six month survey, the overall abundance was considerably lower, particularly the two sites that were surveyed entirely in July 2007 (Gardens Control and Fancy Reef Translocation).

By August 2008, 27 months after the translocation, the size structure had changed markedly at all three treatment (translocation) sites, though the presence of the larger size classes introduced via the translocation was still apparent at the Gardens and the Pebbly Beach Treatment sites (Figure 14, Figure 16). At the Fancy Reef translocation site, the effect of the translocation on the size structure was negligible. The effect of the June 2000 storm appeared to be most severe at the Gardens and Pebbly Beach locations (Figure 14, Figure 15, Figure 16).

The change in population size structure at the three Control sites was equally variable over the two year study period. Notably the larger size classes at the Gardens Control had contracted, although the modal size class remained the same as the observed for the survey in October 2006. At the Fancy Reef Control site, a clear increase in the juvenile size classes was evident between October 2006 and June 2007, and very large increase between June 2007 and August 2008. The population at Pebbly Beach Control remained depleted and virtually unchanged between October 2006 and January 2008.

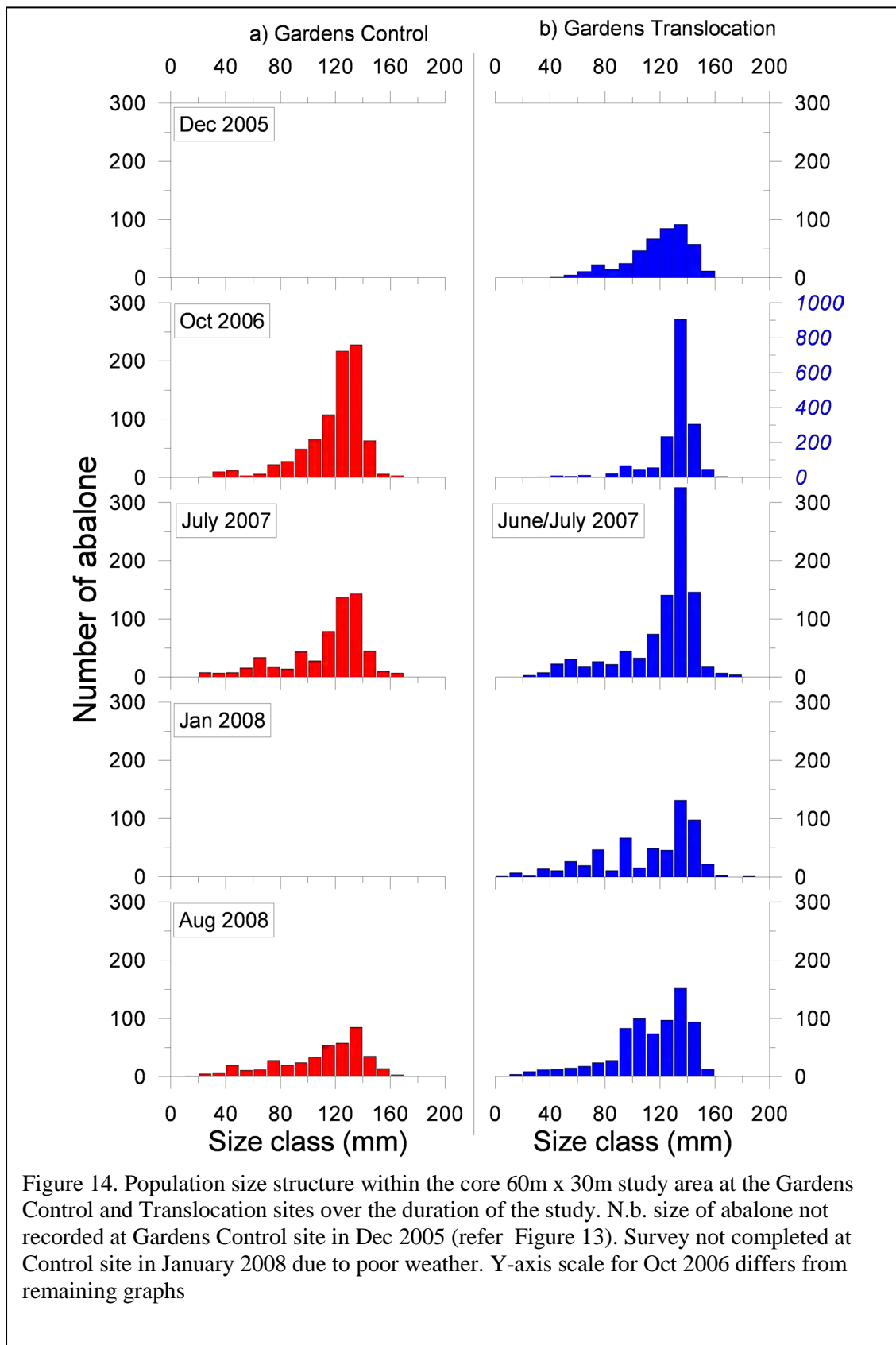
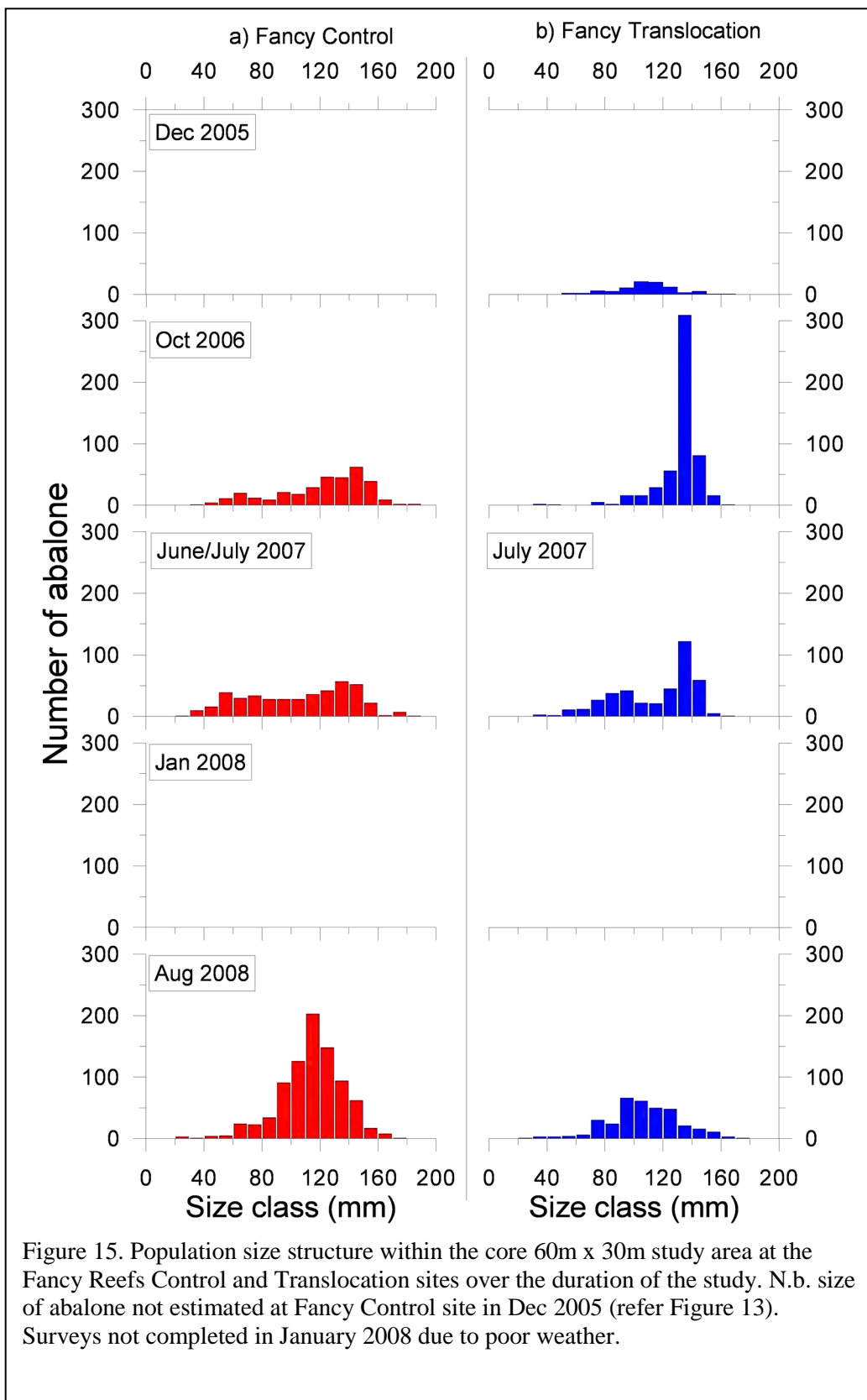
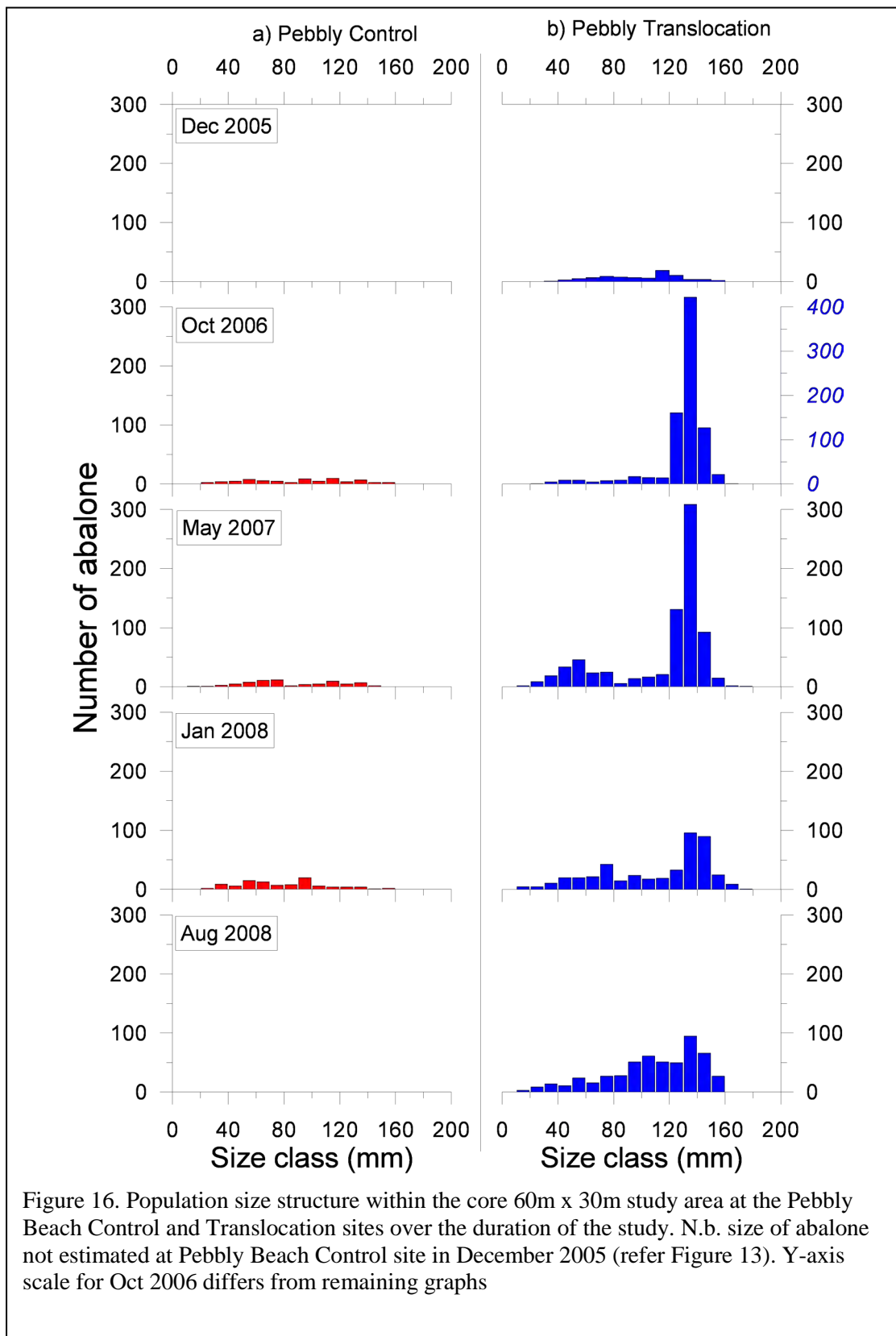


Figure 14. Population size structure within the core 60m x 30m study area at the Gardens Control and Translocation sites over the duration of the study. N.b. size of abalone not recorded at Gardens Control site in Dec 2005 (refer Figure 13). Survey not completed at Control site in January 2008 due to poor weather. Y-axis scale for Oct 2006 differs from remaining graphs





4.4 Discussion

Proposals to rebuild populations through translocation of mature, reproductive adults rely on two key assumptions; 1) the translocated abalone will survive, and 2) the translocated abalone will remain at the location for sufficient time to contribute effectively to the reproductive output of a population. The former is discussed in section 4.4.1. The latter assumption, contribution to reproductive output, however, is far more difficult to demonstrate and quantify, and is partially addressed in section 4.4.2. Both assumptions above also apply to projects that propose to use juvenile abalone, and are substantially more difficult to demonstrate for juvenile abalone given their small size and cryptic nature. Lastly, the impact of translocation on populations needs to be considered in the context of natural events and is discussed in section 4.4.3.

4.4.1 Persistence of translocated abalone within populations over 27 months

The results of this study clearly demonstrate that, with appropriate handling procedures, abalone will survive the translocation process with little overall mortality through the translocation event. Most abalone research programs will have reached an understanding that this will be the case, as the process of collecting, tagging and returning abalone to a reef for growth and mortality studies is not that dissimilar to the translocation process. However, the success or otherwise of a translocation is also dependent on the success of choosing an appropriate destination for the translocated abalone.

In this study, the exact location of the Fancy Reef Translocation site proved to be marginal for the translocated abalone. While the substrate and overstory canopy of the selected area was comparable to the general area, it appeared that most of the translocated abalone moved out of the site within six months of release. Anecdotal sightings of large tagged abalone off the south-east corner (to windward) of the core study area occurred through the study period. The area where tagged abalone were sighted outside the site, was slightly more complex, although with more bull kelp (*Durvillea potatoratum*). Interestingly translocated abalone were rare within the bull kelp beds at all other sites. These observations suggest site choice will be a strong driver of the success of any translocation effort.

Several other site factors may also have contributed to the patterns observed. The Gardens Translocation site was very complex with large boulders and a broad range of cryptic habitats available to all size classes of abalone. This site was also largely separated from neighbouring reef patches by large expanses of sand (> 50m). These two factors, habitat complexity and isolation, most likely contributed to the greater persistence within the core study area of the translocated abalone over the 27 month study period, notwithstanding the effects of the June 2007 storm. Conversely, the Pebbly Beach translocation site was not bordered by sand, and while there was some degree of complexity through the presence of 1.5m rock ribbing traversing north-south through the study area, and some block structure, there was cryptic habitat only for juveniles up to approximately 60mm. Shelter for larger juveniles and sub-adults was largely in the form of corners and ledges on the rock rib and block structures. Nevertheless, the translocated abalone that stayed within the site remained attached to the rock rib for the first 12 months, until the June 2007 storm, and a smaller group persisted there through to the end of the study period at 27 months.

4.4.2 *Can translocated abalone impact positively on the local population*

One of the primary objectives of this study was to test the efficacy of translocation of mature abalone for the purpose of stock rebuilding. The connectivity study using population genetic techniques (Chapter 7) creates a strong argument that the scale of benefit of any translocation of *Haliotis rubra* will be almost entirely local, even if the translocation activity is highly successful. The remaining objectives relate to understanding the resilience of populations to fishing and/or other perturbations, and factors or circumstances that might contribute to, or prevent population recovery. Several patterns evident in the abalone density surveys and population size structure data hint at additional density related threshold effects that may affect resilience or ability of populations to recover from depletion episodes.

At four of the six sites (Gardens Translocation, Fancy Reef Control, Fancy Reef Translocation, Pebbly Beach Translocation), juvenile & sub-adult (< 120mm SL) abundance within the core study areas increased over the 27 month study period following the Translocation. The majority of the size classes where there was an increase over the study period would have settled and recruited to the site prior to the translocation event. This leads to two possible explanations. One possibility is that there were one or more recruitment events at these four sites, but not the remaining two

sites. The alternate and more likely possibility is that the presence of the larger adults, absent prior to the translocation, contributed to the change in one of several ways. The positive effect of the larger adults might have been to increase survival by providing some shelter from predators. Or, the presence of the larger adults may have contributed to a clustering of the juvenile classes in the crevices behind the adults, or near to the adults such that they were simply more visible to the observers. Resolution of these alternate positive processes will be evident in the longer term, when the juveniles reach a size where they emerge from the cryptic habitat and inhabit open reef surfaces.

The Fancy Reef Control site is an anomaly in the patterns described above, in that it did not receive an addition of several thousand abalone, yet it is clear that this site was influenced by a local recruitment event. However, of the Fancy Reefs and Pebbly Beach sites, the Fancy Reefs Control site had a large population of legal sized abalone prior to the translocation (Figure 13), and thus may have had sufficient adult biomass to have some positive effect. Weather conditions at the time of the survey of individual sites may also have played a role in the patterns observed, as only rarely were the surveys conducted under good conditions.

At the two remaining sites, juvenile and sub-adult abundance either decreased (Gardens Control) or remained the same (Pebble Beach Control). At the former, mortality associated with the June 2007 storms is most likely responsible for the decline. At the latter, the absence of a viable reproductive population clearly continues to limit recovery, and the contrast in change of the population size structure between the Pebble Beach Control and Pebble Beach Translocation sites is an important observation. Final judgement of the efficacy of translocation to rebuild depleted populations however, cannot be determined for several more years, after which the translocated abalone have had several opportunities to reproduce, and for the subsequent recruits to be visible to observers.

4.4.3 The impact of translocation on population density and size structure in the context of the magnitude of natural events

The addition of 2200 abalone at each of the Treatment sites was clearly a significant event at these sites within the period of study. The addition of the abalone at the Translocation significantly altered the short term density of abalone, and resulted in a change in the population size structure evident for at least two years. However, within

this two year period, two natural events appeared to have an effect of a similar magnitude at several sites - a positive effect from a natural recruitment or immigration event, and a negative event in the form of a severe storm. A cohort appeared at the Fancy Reef Control site from a recruitment event prior to the period of this study, and led to sub-adult size classes dominating this site at the end of the study period in Aug 2008. This cohort may have originated within the core study area, or migrated into the study area (see Chapter 5, section 5.3.1.2) from the reef area adjacent to the site. By May 2008, abalone abundance in the core study area at the Fancy Reef Control site was greater than at all other sites, including the three Translocation sites (Figure 10).

The June 2007 storm had a marked effect on the population size structure at the Gardens Control and Gardens Translocation sites. The modal size class of 130mm to 140mm at the Gardens Translocation site (Figure 14) declined from more than 300 individuals in early June 2007 to less than 150 individuals six months later in January 2008. A similar magnitude decline was observed at the Gardens control site between the October 2006 survey and the July 2007 survey (Figure 14). The field team visited the sites four days after the peak of the storm for another component of this project (larval collector study). The effects of the storm were primarily sand scour and shifting of significant quantities of sand, and loss of canopy forming kelps. The effect was much more apparent at the protected Gardens Reef sites, although damage was surprisingly mild at the two more exposed locations of Fancy Reef and Pebbly Beach. The effect of the storms on the abalone populations were difficult to quantify at the Gardens due to the complex habitat structure at these reefs, but appeared to be largely confined to the larger emergent size classes (Figure 14). At three of the four quadrants at the Gardens Translocation site, the full extent of the storm effects on the population structure were not observed until January 2008 due to timing of the surveys (Figure 14).

5. AGGREGATION DYNAMICS AND MOVEMENT POST-TRANSLOCATION

5.1 Introduction

Abalone are known to live in aggregations, and the concept of re-aggregation of abalone after fishing is well entrenched in the literature and general abalone folklore. A recent study on short term abalone movement at Magistrates Point, Maria Island Marine Reserve, found average daily movement rates of less than 30cm/day, and more than 50% of the 200 animals followed over two months remained within 1m of their initial position (Landsdell 2006). The study did not determine if there was movement away and back to home sites at night, though still provides insight into the sedentary nature of abalone. If this is true generally for blacklip abalone, the formation of new aggregations by remnant emergent abalone following fishing is unlikely, especially in depleted abalone populations. Given that exploited haliotid stocks worldwide are considered to be at significant risk of Allee effects due to their aggregative behaviour (Hobday & Tegner 2001, Gascoigne & Lipcius 2004), and the focus of fishers on these aggregations, exploited haliotid species are often considered at high risk of population collapse as a consequence of Allee effects.

Despite aggregations being an important biological/ecological component of population dynamics, and the target of fishers, relatively little is known about the dynamics of abalone aggregations. For example, is the membership of aggregations stable? are the location of aggregations static? do aggregations always form in the same area? Is high density a precursor to the formation of an aggregation.

Previous studies of abalone spatial distribution have largely used Nearest Neighbour methods to determine if abalone distribution is random, uniform, or clumped. However, these methods, cannot reveal the dynamics of individual aggregations. In this study, we were primarily interested in the number and size of aggregations, and the primary objectives were a) to determine whether the aggregation structure at a site changed in response to the addition of a large number of adults associated with the translocation, and b) whether the pattern of aggregation (size, number, location) changed through time.

5.2 Methods

5.2.1 Aggregation surveys

To monitor the change in aggregation structure of translocation and control populations, surveys were scheduled prior to the translocation event, and at six month intervals for the duration of the study. Weather conditions frequently prevented surveys from being done on the scheduled time period, and the final survey schedule reflects the window of opportunities presented. For the 12 month post-translocation survey scheduled for May 2007, the survey was completed in three separate trips, conducted over a period of 6 weeks, due to frequent storms and few fair weather opportunities. The Pebbly Beach sites (Translocation and Control) were surveyed in the last week of May 2007. The two most protected reefs, Fancy Reef Control and Gardens Translocation sites, were partially completed (75%) in the first week of June 2007 before the survey was abandoned due to unsafe diving conditions. These sites were completed in the first week of July, along with the Gardens Control and Fancy Reef Translocation sites. Between the surveys in June and July 2007, a severe Easterly storm pushed swells in excess of 4m directly onshore at all study sites. Substantial effects were observed at both the Gardens sites, apparent through loss of kelp canopy and shifting of sand, and surprisingly minor effects observed at the two more exposed locations of Fancy Reefs and Pebbly Beach. While the pattern of site completion for this survey was not ideal, safe diving conditions dictated which sites could be surveyed on any given trip.

At each site, the centre baseline was re-established, and left in place until the aggregation survey was complete (several days). To ensure a complete census was achieved, a systematic search regime was applied within each of the four quadrants (NE, NW, SE, and SW). Each quadrant was divided into six 5m x 15m segments. The centre baseline was marked at 2.5m intervals, enabling divers to determine their position along the baseline. A 15m lead-rope transect line was clipped to the centre baseline at the start of each quadrant, and swum out, perpendicular to the baseline using a compass bearing. The end of the 15m weighted transect line was clipped to the stipe of *Phyllospora* or *Ecklonia* to hold it in place temporarily. A second transect line was then clipped 2.5m along the baseline, following the same procedure, creating a defined lane to search within. The 15m transect lines were colour coded in 5m blocks, so that the position of an abalone or aggregation could be easily identified as within the inner,

middle, or outer 5m block. When a lane was completed the first weighted line was moved along the baseline to create the next searching lane, and the process repeated until the quadrant was complete or the diver reached the planned dive time.

Within each search lane, divers systematically searched the bottom, recording the estimated size of each abalone encountered, the habitat position (open rock, boulder junction, vertical or horizontal crack, vertical or horizontal ledge, vertical wall), the abalone tag number (where present) and if it was isolated or part of a patch (aggregation). The segment and the block (inner, middle, outer) was also recorded. The decision rule as to whether an abalone was part of a patch was based on the methods of Andrew et al (2000), where neighbouring abalone were considered to be part of the same patch if the distance between them was less than two average shell lengths. In this study, the average shell length was set at 150mm.

As a primary objective of this study was to follow trends in the movement and aggregative behaviour of abalone within each study site, abalone were not removed from the substrate for measurement in order to minimise behavioural changes or movement of individuals associated with handling. For this reason, abalone size was estimated to the nearest 5mm with the aid of a stainless steel ruler (200mm) attached to the back of a small writing slate. Length increments (10mm) were also etched on abalone bars to aid size estimation. While sacrificing resolution on abalone size, this procedure allayed concerns that chipping and handling abalone could modify abalone behaviour, and or result in lethal or sub-lethal injury, and subsequent loss of individuals.

5.2.2 Temporal patterns in the patch size-frequency distribution of abalone within the core study areas

Frequency distributions of aggregation size were calculated to determine if the aggregation structure of populations changed in response to the translocation event, or changed over time. The addition of large numbers of adult abalone was expected to alter the frequency distribution of patch-size classes of abalone, specifically an increase in the number of large patches or aggregations of abalone (> 10 abalone) was expected. To test for differences in the distribution of abalone patch-sizes, a sequence of Anderson-Darling k -sample tests were conducted using the R package “*adk*” (Scholz 2008) to determine if the distributions observed at each survey period came from a

common continuous distribution. The test strategy employed, used an initial omnibus k -sample test across all sampling periods. Where a significant result was found, two additional 2-sample tests were conducted to compare specific hypotheses; a) the observed distributions before (T1) and 6 months after (T2) the translocation came from a common underlying distribution, and, b) the observed distributions 6 months (T2) and 27 months (T5) after the translocation come from a common underlying distribution.

For presentation purposes, Class Sizes used for all frequency distributions graphs were patch sizes of 1 to 10 in single steps, with all patches containing greater than 10 abalone grouped into a single class of >10 abalone. i.e this provided a distribution that presented the frequency of solitary abalone, the number of patches containing two abalone, three abalone etc.

In addition, frequency distributions were calculated that identified the proportion of the total population that was observed in a patch (class) size of 1, 2, 3 etc. For example if there were 400 abalone at a site, with 100 abalone observed as solitary individuals, and 20 observations of patches containing only two abalone, these two classes represent 25% and 5% of the total population respectively. The proportion of the total population in each patch size class provides an indication of the relative importance of different patch size classes to the overall population. i.e. are aggregations greater than 10 rare, and are they a negligible/important component of the total population. The rarity of large aggregations is an indicator of the importance of aggregations to overall population dynamics.

5.2.3 Patch size and spatial distribution of abalone aggregations

As the aggregation surveys recorded the grid cell reference of every patch of abalone, it is possible to describe the spatial distribution of abalone within each core study area, and the spatial distribution of aggregations. Using the grid cell reference data, any changes in the distribution of abalone within the site, and whether the location of aggregations is static or dynamic. For simplicity, the spatial pattern of aggregations is grouped into two classes a) small aggregations (patches containing between 6 and 10 abalone), and b) large aggregations (patches of more than 10 abalone). The assessment of spatial distribution of aggregations was restricted to the Gardens Control, Gardens Translocation, Fancy Reef Control and Pebbly Beach Translocation sites. The Fancy

Reef Translocation and Pebbly Beach Control sites had insufficient aggregations to make an assessment. To simplify description of patterns, the following notation will be used – NE quadrant, cell I will be referred to as NE-I.

5.2.4 Patterns of individual movement in relation to patch membership

A key question concerning the formation and maintenance of aggregations is whether patch “membership” changes, and whether aggregations or patches are formed by the addition of solitary individuals to existing groups. Using the small amount of mark-recapture data of tagged individuals, any change in the size of the patch any individual was a member could be monitored over time i.e. did a solitary individual join a larger patch? or did an individual in a large patch become solitary?

The extent of within-site movement of tagged abalone was examined to determine the distances moved over successive time periods. This was achieved by creating a spatial grid (6 cols, 12 rows, 5m cell size), and using the centroid of each cell as the spatial location of an individual. While this is a relatively coarse estimate of position, it provides an indication of the larger movements between successive surveys.

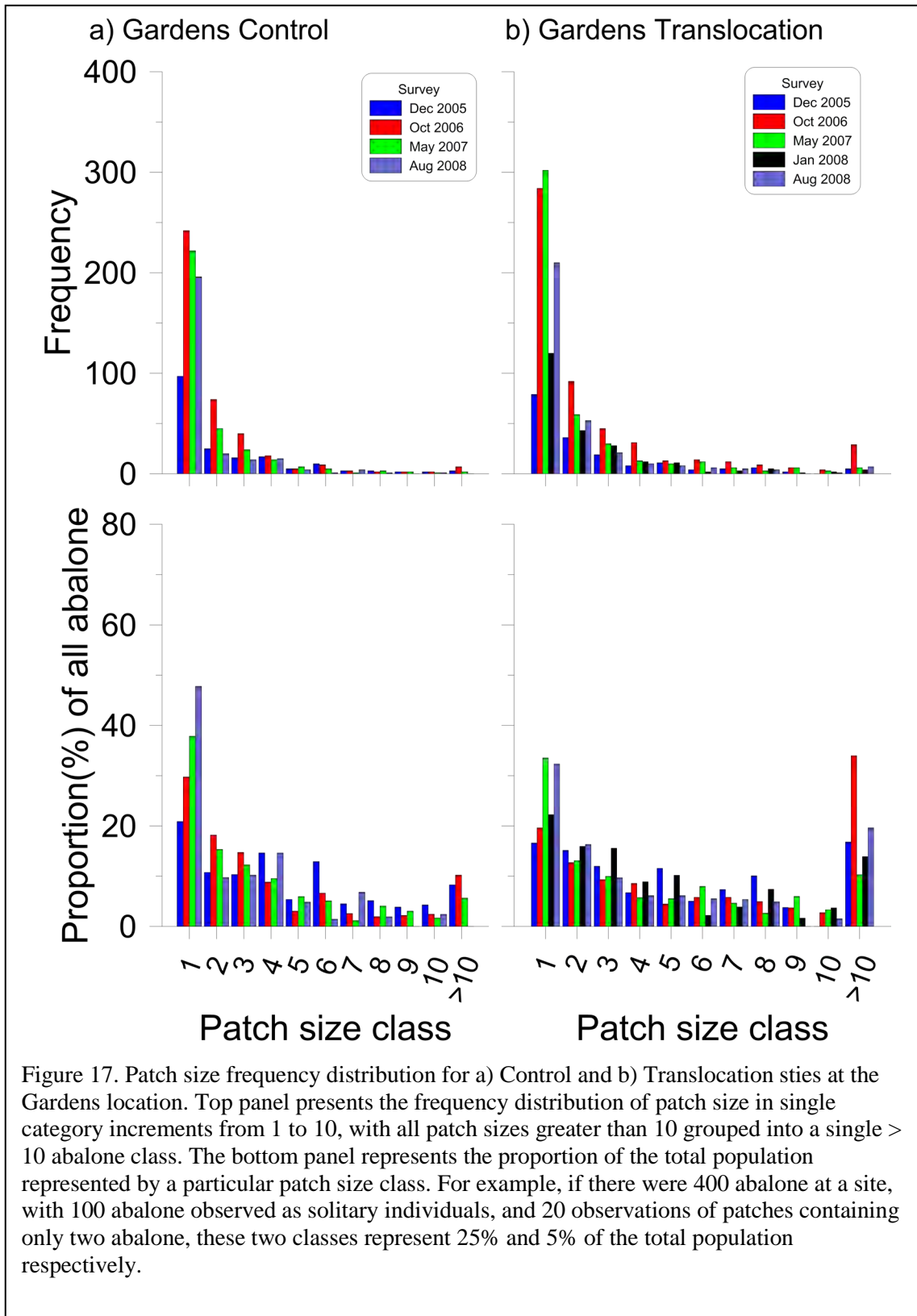
5.3 Results

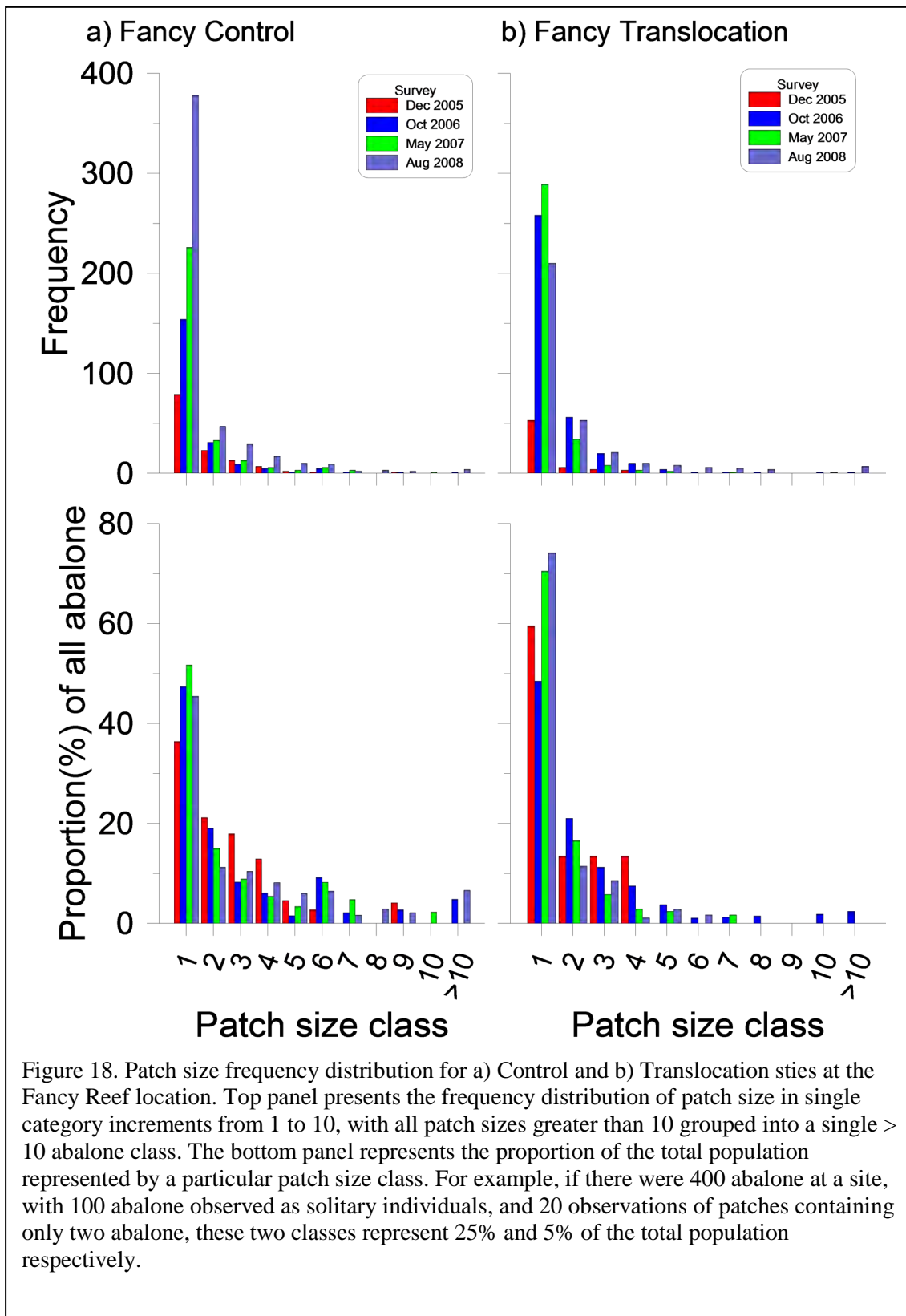
5.3.1 Temporal patterns in the patch size frequency distribution of abalone

Anderson-Darling k sample analyses revealed that significant differences in patch size frequency distribution among time periods were present at five of the six sites, with the exception being the Pebbly Beach Translocation site (Table 6, Figure 17, Figure 18, Figure 19). At the remaining two Translocation sites, further pair-wise testing of the patch-size frequency distribution between T1 (before translocation) and T2 (after translocation) surveys, revealed no-significant effect of the addition of 2200 adult abalone on the patch-size frequency distribution at the Gardens and Fancy Reef Translocation sites. In contrast, at the neighbouring Control sites for these two locations there were significant differences in the patch size frequency distribution between time periods T1 and T2 (Table 6, Figure 17, Figure 18). There was a highly significant change in the underlying patch size frequency distribution between time period T2 (after translocation), and T5 (final aggregation survey) at four of the six sites.

Table 6. Anderson-Darling k sample analyses to test whether patch size-frequency distributions from different surveys have a common underlying distribution. The test strategy involves an initial omnibus k -sample test across all sampling periods. Where a significant result was found, two additional 2-sample tests were conducted to compare a) the observed distributions before (T1) and 6 months after (T2) the translocation, and b) the observed distributions 6 months after (T2) and 27 months (T5) after the translocation.

Site		T	p	
Gardens Control	k -samples	19.09	0.0001	***
	$T1$ vs $T2$	5.44	0.003	***
	$T2$ vs $T5$	16.9	0.0001	***
Gardens Translocation	k -samples	19.8	0.0001	***
	$T1$ vs $T2$	1.22	0.1	<i>ns</i>
	$T2$ vs $T5$	12.9	0.0001	***
Fancy Reef Control	k -samples	4.78	0.002	***
	$T1$ vs $T2$	4.05	0.008	***
	$T2$ vs $T5$	-0.72	0.54	<i>ns</i>
Fancy Reef Translocation	k -samples	18.33	0.0001	***
	$T1$ vs $T2$	0.26	0.26	<i>ns</i>
	$T2$ vs $T5$	25.06	0.0001	***
Pebbly Beach Control	k -samples	2.06	0.04	*
	$T1$ vs $T2$	1.21	0.1	<i>ns</i>
	$T2$ vs $T4$	3.69	0.01	**
Pebbly Beach Translocation	k -samples	0.98	0.15	<i>ns</i>
	$T1$ vs $T2$			
	$T2$ vs $T5$			





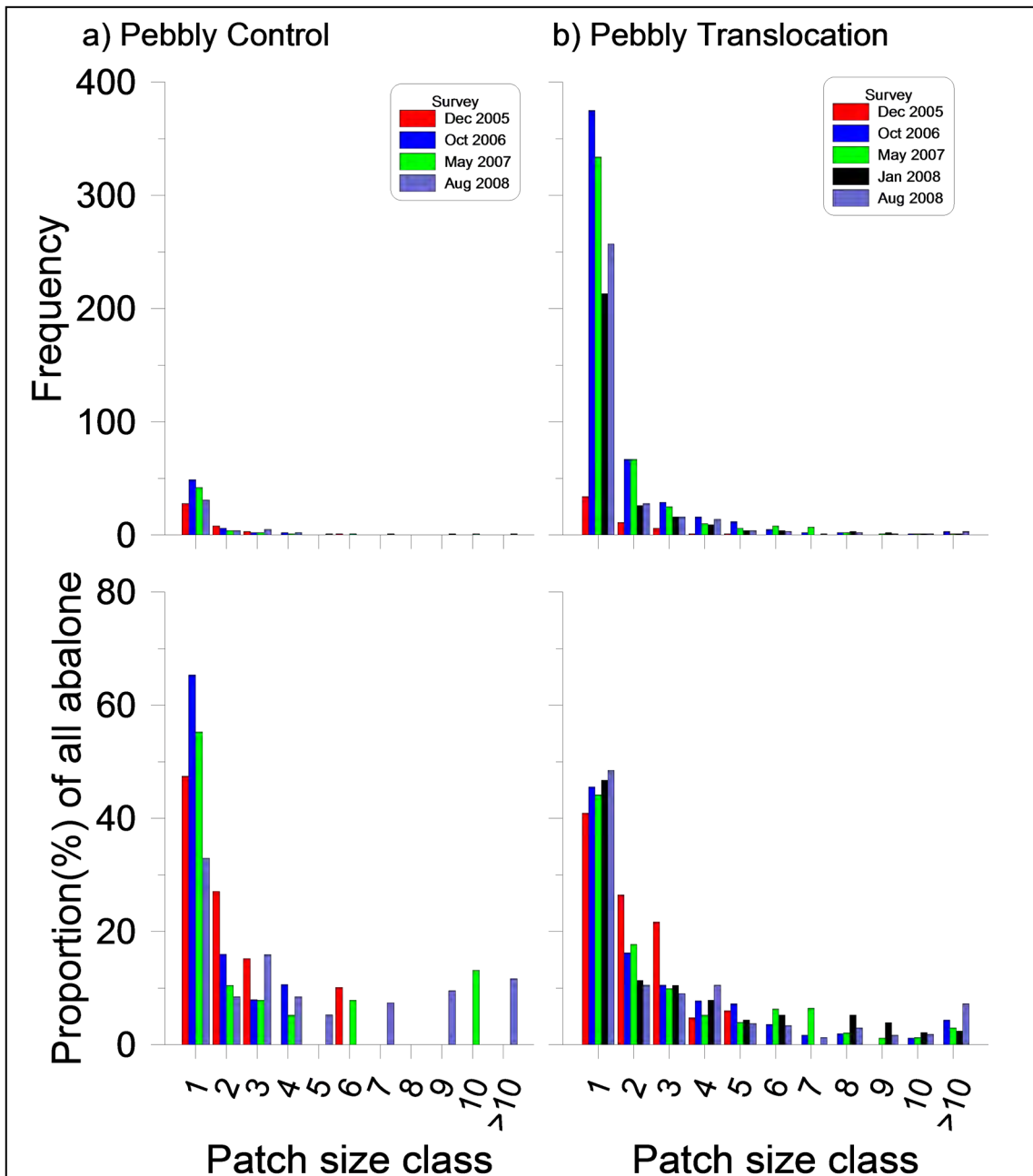


Figure 19. Patch size frequency distribution for a) Control and b) Translocation sites at the Pebbly Beach location. Top panel presents the frequency distribution of patch size in single category increments from 1 to 10, with all patch sizes greater than 10 grouped into a single > 10 abalone class. The bottom panel represents the proportion of the total population represented by a particular patch size class. For example, if there were 400 abalone at a site, with 100 abalone observed as solitary individuals, and 20 observations of patches containing only two abalone, these two classes represent 25% and 5% of the total population respectively.

5.3.1 Patch size and spatial distribution of abalone aggregations

The pattern of temporal change in the number and location of small (6 to 10 abalone) and large (> 10 abalone) aggregations appears to be site specific (Figure 20, Figure 21, Figure 22, Figure 23). There does however appear to be a positive relationship between the local abundance (in this case abundance within a quadrant of the core 60m x 30m study area) and the number of small and large aggregations.

5.3.1.1 Gardens Sites

At the Gardens Control site, few abalone were observed in the South-east quadrant throughout the study period (Panel a, Figure 20). Most small and large aggregations were found in the northern half of this site (Panel b, Figure 20). Several smaller transient aggregations appeared in the SW quadrant at the initial survey (blue dots) six months prior to the translocation and the third survey (green dots), 12 months after the translocation, but these had largely disappeared by the final survey (purple dots) (Figure 20). During October 2006, seven large aggregations (> 10 abalone) were observed at this site, whereas during the final survey in August 2008, no aggregations of > 10 abalone were observed (Figure 20).

The location of abalone aggregations and the number of small and large aggregations at the Gardens Translocation site also changed markedly over the 32 month study period. There was also a substantial shift in the pattern of abundance of abalone among quadrants associated with the translocation event at this site. Prior to the translocation, there were more abalone in the SW quadrant, whereas six months after the translocation the majority of abalone were found in the SE quadrant (Panel a, Figure 21). At the end of the study (27 months after the translocation), the pattern of abundance across the quadrants had returned to that observed prior to the translocation. The location of small and large aggregations largely followed the pattern of abundance across the quadrants through time, although spatial patterns in the locations of aggregations did not appear to be stable. Small aggregations of abalone were consistently observed at several locations during most surveys of the Gardens Translocation site; broadly these were associated approximately with a) NW-H/I, b) SW-G/H/J/K, and c) SE D/E/G/H (panel b., Figure 21). Several areas within the Gardens Translocation site appeared to harbour large aggregations (greater than 10 abalone) consistently throughout the study in three locations. These were at locations a) NW-L, b) NW-N, c) SW-B/C, and d) SW-N (panel c, Figure 21).

5.3.1.2 Fancy Reef sites

The abundance of abalone at the Fancy Reefs Control site increased throughout the study (see Chapter 4 Figure 10, Figure 15), however while there was a broad increase in abundance across the site, the increase primarily occurred in the south-east quadrant (panel *a*, Figure 22). Small aggregations were observed at all survey periods in NE-O and SE-D, and in the area of NW-F. At the final survey, five small aggregations were observed in cells SE-E and SE-F. Prior to this final survey, no aggregations of six or more abalone were observed in these two cells. In the final survey a large aggregation of > 10 abalone had developed in the area of cell NW-F, and cell NE-O, neither of which had previously been observed (panel *c*, Figure 22).

5.3.1.3 Pebbly Beach sites

The abundance of abalone at the Pebbly Beach Translocation site (panel *a*, Figure 23) was extremely low at the commencement of the study (see chapter 4, Figure 10, Figure 16). Only two aggregations of between 5 and 10 abalone were recorded prior to the translocation (panel *b*, Figure 23), located in the lower south-west corner. However, aggregations were not seen here at any further stage in the study, although numerous aggregations formed elsewhere at the site. After the translocation, aggregations formed predominantly in the central area of the eastern side of the site. The spatial pattern in location of aggregations observed six months after the translocation did not however, persist through to the final survey (panel *b*, Figure 23). Small aggregations were consistently observed in three areas, 1) NE-Q, 2) SW-F and 3) SE-L). The number of large aggregations was low (panel *c*, Figure 23), although these were mostly located in areas where there were also small aggregations present, or small aggregations were present at previous surveys (Figure 23).

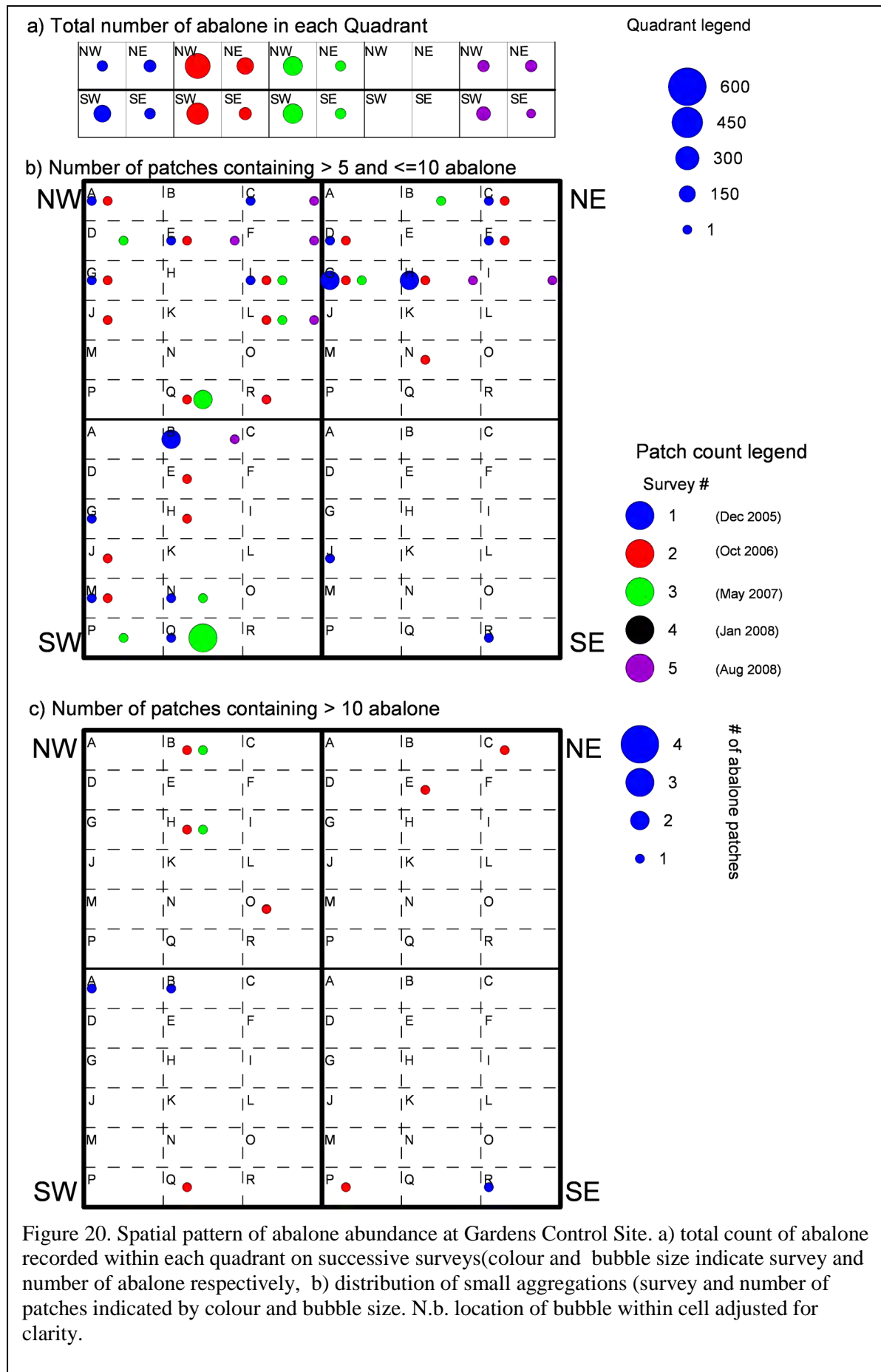


Figure 20. Spatial pattern of abalone abundance at Gardens Control Site. a) total count of abalone recorded within each quadrant on successive surveys (colour and bubble size indicate survey and number of abalone respectively, b) distribution of small aggregations (survey and number of patches indicated by colour and bubble size. N.b. location of bubble within cell adjusted for clarity.

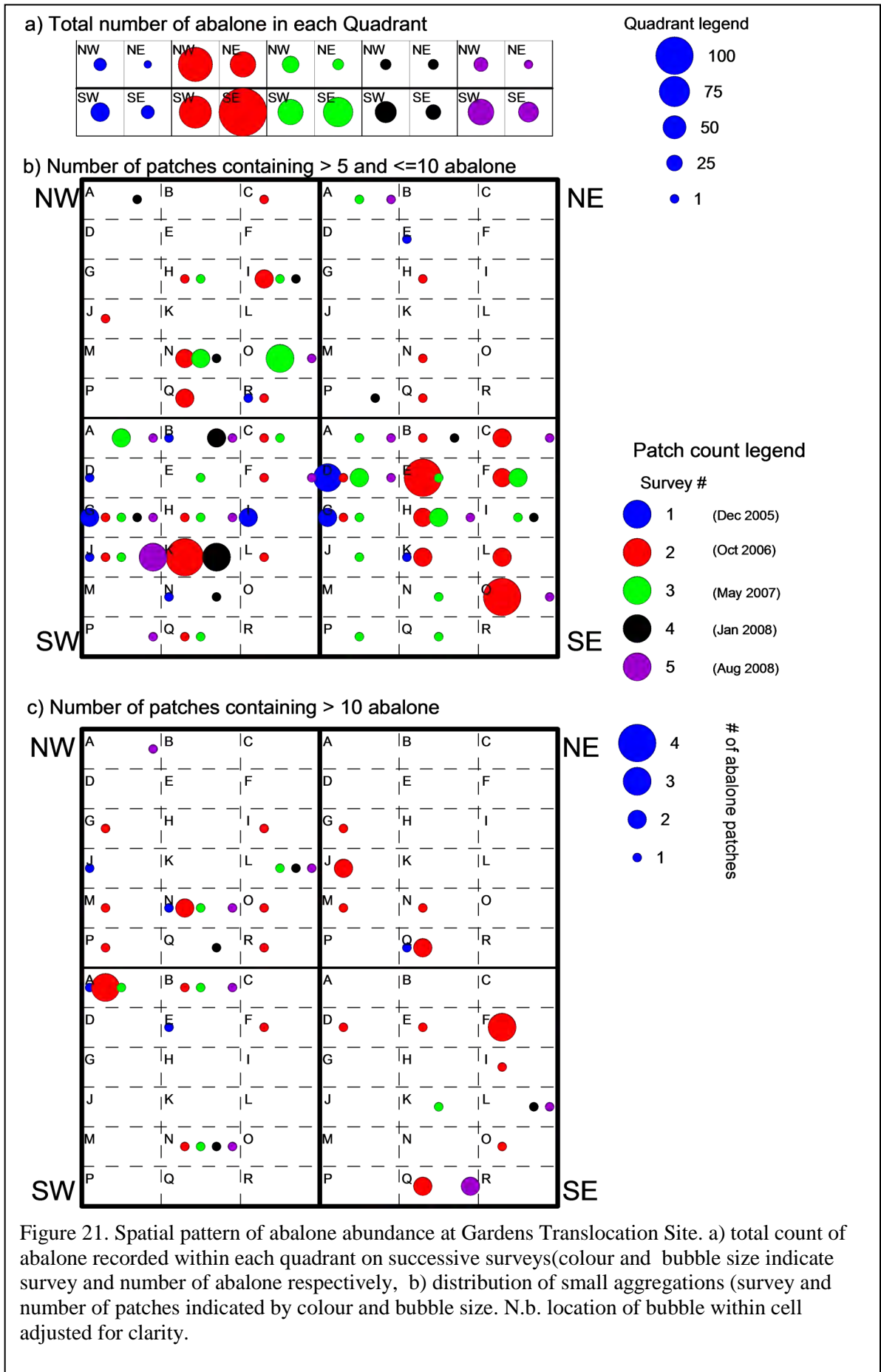
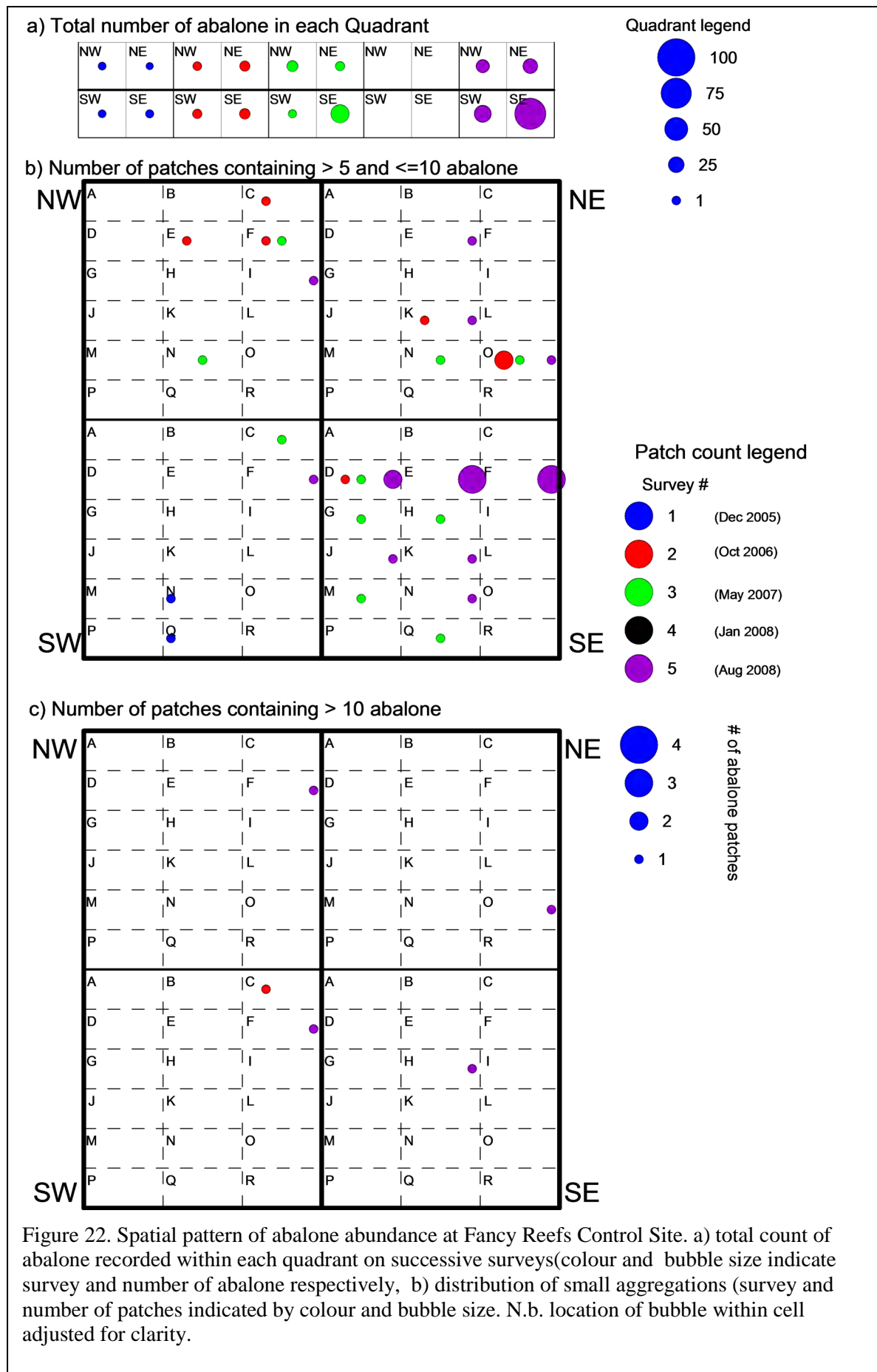
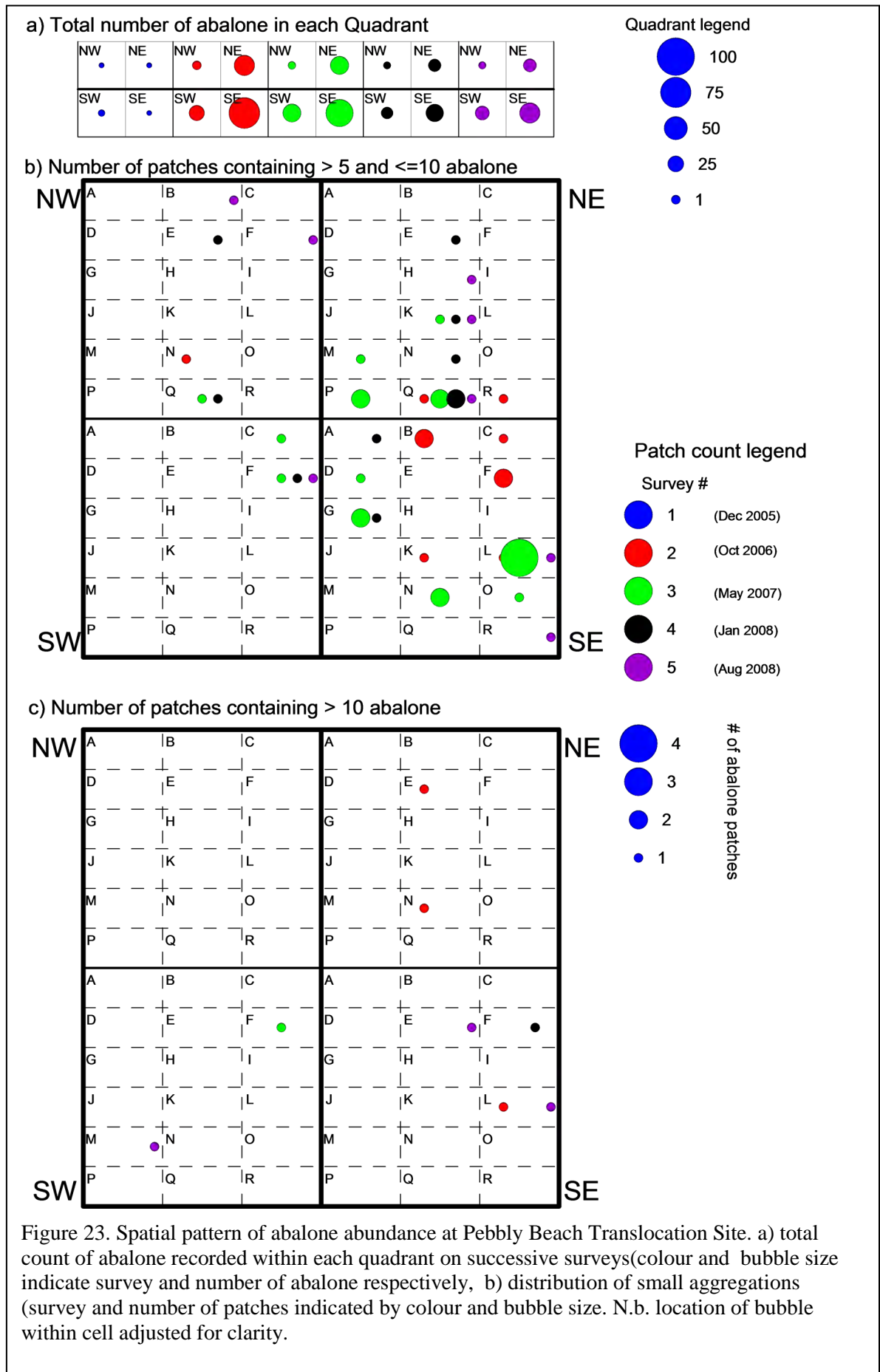


Figure 21. Spatial pattern of abalone abundance at Gardens Translocation Site. a) total count of abalone recorded within each quadrant on successive surveys (colour and bubble size indicate survey and number of abalone respectively, b) distribution of small aggregations (survey and number of patches indicated by colour and bubble size. N.b. location of bubble within cell adjusted for clarity.





5.4 Discussion

5.4.1 *Aggregation structure is independent of density*

The pattern of aggregation of abalone in the context of what proportion of abalone occur as solitary individuals, or in larger clusters or aggregations appeared to be site specific, and largely independent of density. For example despite major increases in the density of abalone associated with the translocation event, the underlying distribution of patch-size frequency did not change (Table 6).

There did appear to be a relationship between the proportion of abalone occurring as solitary individuals and habitat complexity, suggesting habitat is a more important driver of aggregation structure than density alone. The habitat complexity at each of the three locations varied from a highly complex, large boulder and crevice habitat at the Gardens location, small boulder habitat, and few crevices at the Fancy Reef location, to a ridge and gully substrate with very little cryptic habitat at the Pebbly Beach location. The paired Control and treatment sites at each location were chosen such that the habitat was comparable within locations. The Pebbly Beach sites had the lowest habitat complexity, and the highest proportion of abalone occurring as solitary individuals (> 60%), whereas the Gardens sites (highest substrate complexity) had the lowest proportion of abalone occurring as solitary individuals (~ 40%) and a greater number of abalone occurring within large aggregations.

5.4.2 *Spatial pattern in aggregation location*

The observation of persistent aggregations in some areas of those sites where aggregations were common, supports the above conclusion that habitat structure is an important driver of the location and number of aggregations in sub tidal reef systems. This may have been a large part of the rapid emigration of translocated abalone from the Fancy Translocation site within 6 months of release, to areas adjacent to the core study area. The habitat at the Pebbly Beach Translocation site where aggregations persisted throughout the study was typically elevated rock structures with frequent short angular ledges providing smooth horizontal and vertical faces with some protection. The highly localised increase in abundance in the south-east quadrant at the Fancy Reef Control site over the duration of the study is also likely to be driven by habitat complexity, as the western side of this site was dominated by shallow bull-kelp swept platforms, or small boulder areas, whereas the eastern side had more large boulders without bull-kelp.

6. THE RELATIONSHIP BETWEEN ADULT DENSITY, LARVAL SETTLEMENT AND JUVENILE ABUNDANCE IN THE ABALONE *HALIOTIS RUBRA*

6.1 Introduction

As over fishing continues to increase globally, one of the primary challenges facing scientists and resource managers is how to facilitate the recovery of collapsed populations. While the closure of a fishery is perhaps the simplest option, it is clear that the ability of many fisheries to rebound even in the absence of continued fishing is low, and likely to take considerable time and thus the need for stock enhancement measures is increasing with the growing decline in fisheries resources, and the science behind rebuilding populations remains an important area of ecological research. .

Re-building collapsed populations through the translocation of reproductively mature adults, has been explored in a variety of ecosystems and fisheries (eg abalone, scallops, trochus, southern rock lobster), although we are still a long way from such approaches becoming mainstream. The key ecological principle that underpins adult translocation as a re-building strategy is the Allee effect (Allee 1931) which predicts that population fitness will decline with population size. Indeed for marine invertebrates, there is mounting evidence that factors including fertilisation success, larval settlement and juvenile recruitment are negatively correlated with the size and density of spawning populations (Levitan and Sewell 1998, Lundquist and Botsford 2004, Bell et al 2008). Clearly if the number of adults in a population can be increased through the translocation of reproductively mature individuals, then factors such as reproduction and recruitment that will be critical for population recovery can be enhanced. However, although translocations of marine invertebrates have been trialled in a few locations (e.g. abalone: Hamasaki & Kitada 2008, trochus: Purcell & Cheng 2010), most studies have focused on survival of translocated individuals, and hence the effectiveness of translocation for the longer term objective of enhancing natural larval production and subsequent settlement remains poorly understood.

Poor recovery of abalone fisheries in NE Tasmania has been linked to Allee effects including recruitment failure associated with declining fertilization success (Keesing & Babcock, 1997), the fact that abalone populations appear to be largely self-recruiting

(Temby et al 2007; Miller et al 2009) and that larval dispersal is likely to be limited (McShane et al 1988, Prince et al 1988, Miller et al 2009). Thus as part of the stock enhancement trial in North Eastern Tasmania to assess whether translocation of reproductively mature abalone would facilitate population recovery we wanted to determine if increasing the abundance or density of adults would result in a concurrent increase in larval settlement and recruitment, and hence facilitate recovery in over-fished populations.

6.2 Methods:

6.2.1 Deployment of larval collectors

Artificial settlement substrata were used to assess the numbers of larvae settling into six sites in NE Tasmania; three treatment sites where ~2000 adult abalone were translocated to increase adult density and three control sites where adult abalone densities were left unchanged. The larval collectors were modified from Rodda et al (1997). Each collector measured 30x30cm and comprised 7 sheets of Geosheet® cusped High Density Polyethylene (HDPE) drainage sheet (Geofabrics Australasia Pty Ltd) threaded onto a stainless-steel rod and held together by two 6mm polyethylene sheets (Figure 24). The three-dimensional surface of the plastic sheets provided a range of surface orientation and light regimes to optimise larval settlement. The total area available for settlement on each of the collectors was estimated to be 1.48m².

Collectors deployed in the first three sampling periods were attached to a heavy (40kg) stainless steel metal plate and placed directly on the substrate below the kelp canopy (Figure 24). Due to corrosion between different metal types, a new system was established for the final two deployment periods whereby a small hole was drilled into the dolerite rock substrate using a pneumatic hammer drill. A stainless steel expansion socket with a female thread was fitted to the hole, and fitted with a 150mm long stainless steel threaded rod. A hole in the middle of the collector enabled the collector to be placed over the rod and firmly secured to substrate using a locking nut. This system also allowed easy retrieval and replacement of collectors. In both designs, the collectors could easily be detached and reattached from the anchoring system by a simple pin or bolt. For both attachment methods, the collector sat above the substrate but remained within the open understorey space (see Figure 24).

Collectors were deployed on five occasions; once prior to the translocation (Oct 05-Jan 06; 100 days) and four times following the translocation (Jul 06-Oct 06, 83 days; Oct 06-Mar 07, 156 days; and Mar 07-Jul 07, 120 days, Jul 07 to Oct 07, 90 days). Ten replicate collectors were deployed at all sites at each deployment period (Table 7) with the exception of the pre-translocation period when collectors were only deployed at the three experimental sites and not at the control sites. Some collectors were lost during storm events, and this reflected in fewer replicates being retrieved and processed in most sampling periods.

Table 7. Number of Collectors retrieved during each sampling period. Number in brackets indicates the number of collectors where zero abalone was found. N.b. only the eight collectors retrieved from the Gardens Translocation Site in the last deployment were processed due to time constraints. (*ns* indicates site not sampled; *np* indicates samples not processed).

Site	Oct 05 - Jan 06	Jul 06 - Oct 06	Oct 06 - Mar 07	Mar 07 - Jul 07	Jul 07 - Oct 07
Gardens Control	<i>ns</i>	6 (6)	7 (5)	5 (1)	<i>np</i>
Gardens Trans	10 (9)	6 (1)	8 (4)	9 (0)	8 (1)
Fancy Control	<i>ns</i>	9 (6)	9 (4)	2 (1)	<i>np</i>
Fancy Trans	<i>10 (10)</i>	8 (2)	7 (6)	<i>1 (0)</i>	<i>np</i>
Pebbly Control	<i>ns</i>	4 (4)	3 (3)	5 (4)	<i>np</i>
Pebbly Trans	10 (8)	8 (6)	10 (9)	4 (3)	<i>np</i>
Total (retrieved)	30	41	45	26	57

6.2.2 Processing of larval collectors

On removal from the anchoring system, collectors were placed immediately into purpose-designed plankton mesh bags (110 microns) and returned to the surface. Collectors were then either frozen intact and transported back to the laboratory for later processing, or processed on arrival back onshore by soaking for at least 10 minutes in 5% ethanol (to act as an anaesthetic) then rinsing with sea water over a 150 µm sieve.

All sediment and biological material rinsed from each larval collector was split into three fractions (200-250µm, 250-500µm, >500) and preserved in 95% ethanol. Each fraction was then sorted under a dissecting microscope and all abalone counted and measured (shell length). Due to the different length of time that collectors were deployed for during each period and the variable retrieval success, recruitment was standardised as the number of larvae/m²/day across the study period for comparison of settlement among sites.

To determine if there was a relationship between recruitment and adult density across our sites, we compared recruitment with adult density at each site



Figure 24. Image of larval collector (Type I) in situ at Pebbly Beach Translocation site. Note the short stipe length of *Phyllospora comosa* at this site. The head of the collector was designed to sit below the kelp canopy at all sites, to maximise the possibility of intercepting larvae retained below the canopy.

6.3 Results

6.3.1 Patterns of recruitment across translocation treatments

Retrieval rate of collectors was moderate. Of the 210 deployments across the study period (Table 7), 199 collectors were retrieved and 149 were processed. From these, we recorded a total of 1218 abalone post-larvae, although the number from each time/site/collector was highly variable (Table 8) with ~65% of all collectors having no abalone larvae (Table 7), 15% containing a single larva and only 3 collector deployments having >100 larvae. Very few post-larvae were found on collectors deployed prior to the translocation (~0.001 larvae/m²/day at Gardens and Pebbly Beach). Abalone post-larvae were generally recorded at all sites post-translocation (ranging from 0.001-0.23 larvae/m²/day), although the majority of larvae (94%) were

recorded from the Gardens Translocation site (Table 8). Recruitment also appeared to be patchy in time, with 89% of all post-larvae recorded during the March 2007 to July 2007 sampling period (Table 8).

The pulse of post-larvae arriving at the Gardens Translocation site suggests a single successful recruitment event at this site (Table 8, Table 9). The larval collectors from this sample period were retrieved approximately 4 days after the major storm event on the 28th of June 2007. The majority of these post-larvae were greater than 300 microns and therefore highly unlikely to have been associated with post-storm spawning as has been observed in Japan (Sasaki and Shepherd, 1995, Onitsuka et al, 2010). The presence of post-larvae at most sites in most sampling periods suggests there is year round low level successful recruitment which may supplement single, large recruitment events.

Table 8. Number of post-larvae recorded at each site, for each sampling period (pooled across collectors). *ns* = not sampled; *np* = not processed.

Site	Oct 05 - Jan 06	Jul 06 - Oct 06	Oct 06 - Mar 07	Mar 07 - Jul 07	Jul 07 - Oct 07	Total (Periods pooled)
Gardens Control	<i>ns</i>	0	2	30	<i>np</i>	32
Gardens Trans	2	30	13	1056	47	1148
Fancy Control	<i>ns</i>	3	10	2	<i>np</i>	15
Fancy Trans	0	12	1	1	<i>np</i>	14
Pebbly Control	<i>ns</i>	0	0	2	<i>np</i>	2
Pebbly Trans	2	3	1	1	<i>np</i>	7
Total (Sites Pooled)	4	48	27	1092	47	1218

Table 9. Mean number of post-larvae/collector at each site and time period. (standard errors inside parentheses). *ns* = not sampled; *np* = not processed.

	Oct 05 - Jan 06	Jul 06 - Oct 06	Oct 06 - Mar 07	Mar 07 - Jul 07	Jul 07 - Oct 07
Gardens Control	<i>ns</i>	0.0 (0.0)	0.3 (0.2)	6.0 (2.4)	<i>np</i>
Gardens Trans	0.2 (0.2)	5.0 (2.8)	1.6 (0.7)	117.3 (48.5)	5.9 (1.4)
Fancy Control	<i>ns</i>	0.3 (0.2)	1.1 (0.4)	1.0 (1.0)	<i>np</i>
Fancy Trans	0.0 (0.0)	1.5 (0.5)	0.1 (0.1)	1.0 (0.00)	<i>np</i>
Pebbly Control	<i>ns</i>	0.0 (0.0)	0.0 (0.0)	0.4 (0.4)	<i>np</i>
Pebbly Trans	0.2 (0.1)	0.4 (0.3)	0.1 (0.1)	0.3 (0.3)	<i>np</i>

There was no apparent relationship between abalone larval recruitment and factors including the deployment period (pre- vs post-translocation) or treatment (Translocation vs. Control), although due to the low numbers of recruits to most collectors we have little power to detect trends (Table 9). Recruitment was also highly patchy, even between adjacent collectors in the same site, suggesting small-scale processes greatly influence abalone recruitment (Table 9).

6.3.2 Relationship between mature abalone density and post-larval recruitment

An exponential relationship was evident between adult density at a site and larval recruitment/m²/day (Figure 26). However, while a significant proportion of the variance in recruitment was explained by adult abalone density ($R^2 = 0.69$), this should be considered with great caution due to an absence of data in the intermediate abalone density range and the single data point with high recruits associated with high adult densities.

6.3.3 Size-structure of abalone post-larvae at the Gardens Translocation site

The Gardens Translocation site was the only site where reasonable levels of recruitment were observed. For this site, we compared the size-frequency of post-larvae across the deployment periods. In three of the four post-translocation deployment periods, most post-larvae were from a single size class (Figure 26) consistent with a single pulse of recruitment onto the collectors. From July-Oct 2007 there was a much greater range in the size of post-larvae with similar numbers of individuals observed in size classes ranging from 400-1400 microns.

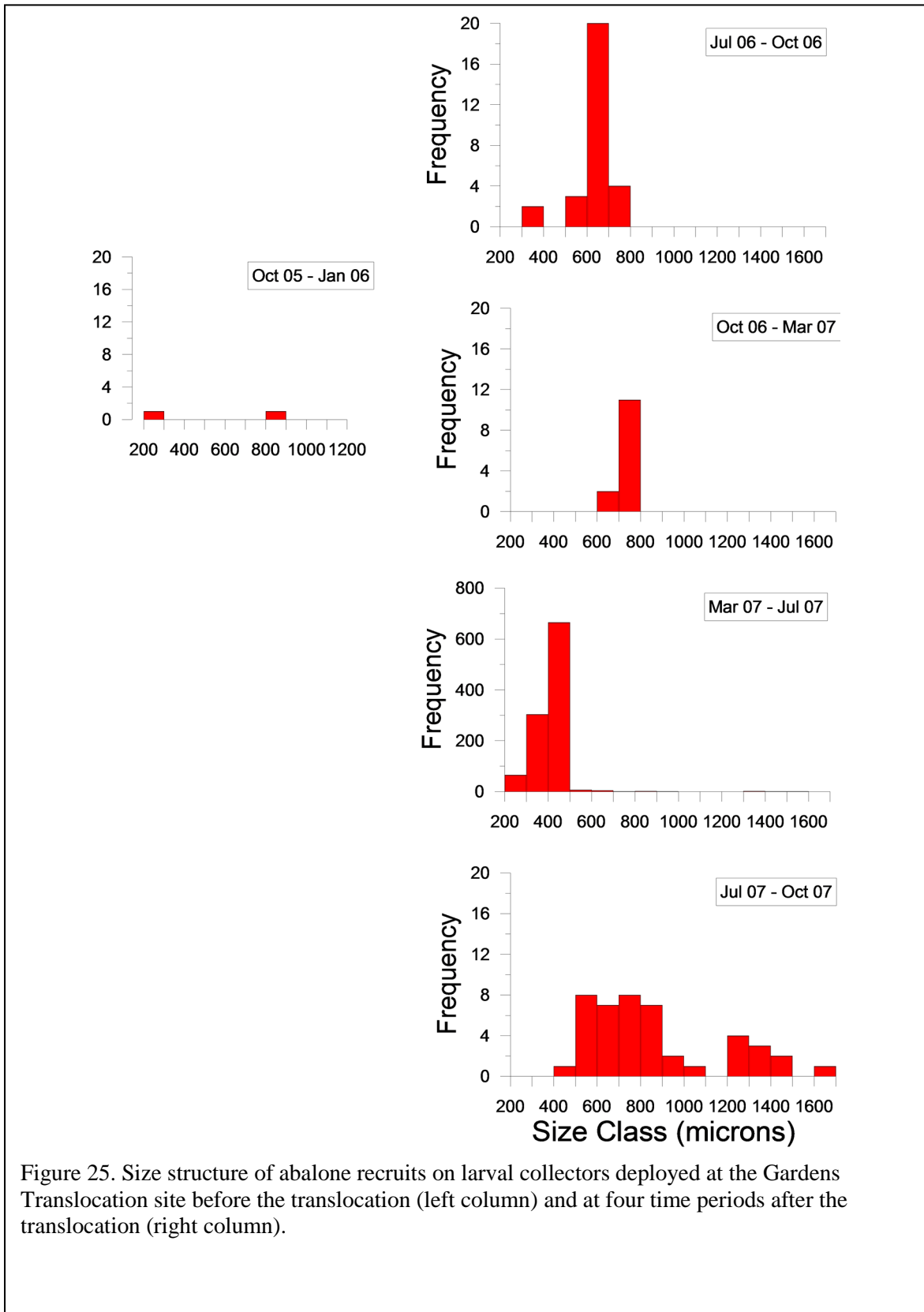
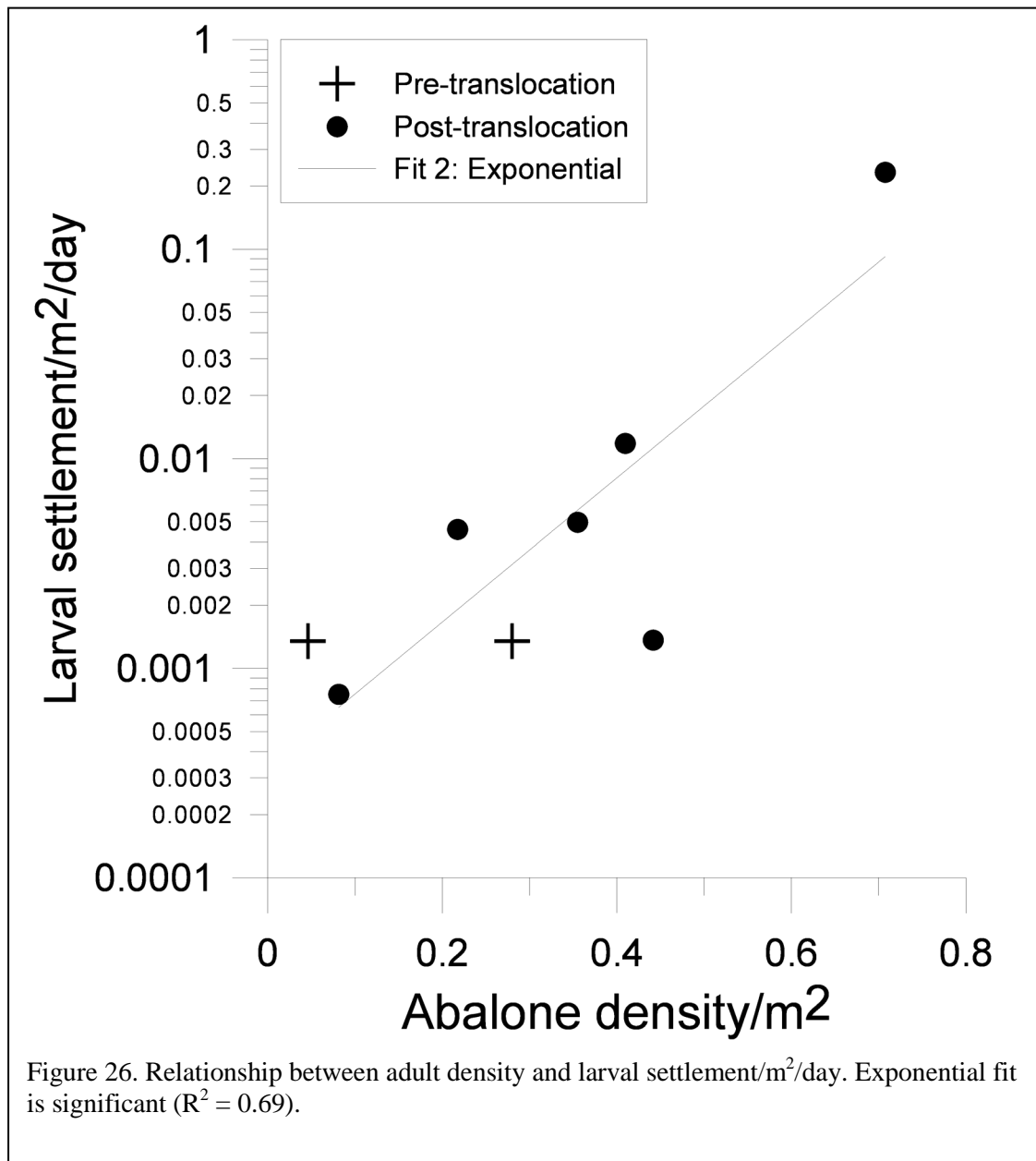


Figure 25. Size structure of abalone recruits on larval collectors deployed at the Gardens Translocation site before the translocation (left column) and at four time periods after the translocation (right column).



6.4 Discussion

Levels of recruitment to larval collectors in this study were highly variable both among sites, and within sites. There was no clear relationship between density of mature adult abalone and larval recruitment, although the highest level of recruitment was observed at the site with the highest adult density. It may be that a threshold density of reproductive abalone exists beyond which adult density is sufficient to negate Allee effects and settlement is high. Unfortunately we do not have sufficient data to properly test this hypothesis as high recruitment was only observed at a single location, and at a

single sampling period. Given that dispersal of abalone larvae is highly localised (Chapter 7) and that high, but consistent variation among replicate collectors within sites is likely, experiments to describe the relationship between abalone population density and larval recruitment will require detailed consideration of both the above factors. The existence or not of a stock recruitment relationship for abalone has been the focus of many previous studies (Prince et al, 1987; Mcshane et al, 1988, Sheppard & Partington 1995) although has not yet been confirmed or described. Our results support the notion that there may well be a stock-recruitment relationship in abalone, and that there may exist a density threshold below which recruitment failure occurs. We caution however, that our results are suggestive rather than conclusive and encourage further research in this field.

Patterns of recruitment across collectors were highly variable, although patterns within sites were not always consistent across sampling periods. Additionally, as post-larval density was very low in some sampling periods even at the Gardens Translocation site, we were unable to determine whether the patchy nature of recruitment at the small scale (i.e. metres among collectors within sites) is a function of local adult density or local topography, and either of these influences could be a key driver of observed variation in recruitment among replicate collectors within sites. Further investigation of adult density/recruitment relationships should explicitly consider the local topography issue in the experimental design.

Of note is the fact that the study of larval recruitment to experimental collectors was the most resource intensive of all components of this translocation project. Field work was intensive and frequently disrupted by weather, although new methods for secure and rapid deployment/retrieval of larval collectors on rocky reefs were developed during the study. Processing of sample material obtained from the collectors was very time consuming, with material from a single collector sample typically requiring between 16 and 24 hours to process. Further research on techniques to reduce the time commitment to sorting collector samples is essential prior to any further experiments using this approach to measuring recruitment success. A very large number of zero data points also occurred, which raises some questions about the approach typically taken in terms of the larval collector design. Research in the area of collector design and size is also essential prior to further field-based research on abalone recruitment.

7. GENETIC DIVERSITY AND GENE FLOW IN COLLAPSED AND HEALTHY ABALONE FISHERIES

Note: This chapter has been published in the scientific literature. The complete reference is: Miller, K., Maynard, B. and C. Mundy (2009). Genetic diversity and gene flow in collapsed and healthy abalone populations. *Molecular Ecology* 18: 200-211.

7.1 Introduction:

The sustainability of exploited populations is a primary goal in the management of natural resources. In the marine realm, this can be especially challenging as most extractive industries operate remotely (i.e. trawling, trapping) and due to the nature of the marine environment, exact estimates of population size, the geographic extent of populations, and other important demographic parameters are almost impossible to obtain. While management of some fisheries address biological sustainability requirements through quota systems, or designation of no-take areas to protect brood stock, many marine fisheries around the world continue to decline or have collapsed through over-fishing (Hilborn *et al.* 2003). Indeed current estimates indicate 29% of the world's fisheries have collapsed and are no longer economically viable, and these levels are predicted to rise (Worm *et al.* 2006).

One of the major issues associated with fisheries management is optimising yield without compromising ecosystem or population processes (Fogarty *et al.* 1991; Caddy & Seijo 2005; Worm *et al.* 2006). Population decline beyond a critical point may be detrimental for at least two reasons. Firstly, Allee effects, whereby fitness declines with population size, may result in reproductive and recruitment failure and severely constrain recovery potential of a population (e.g. Levitan & Sewell 1998). Secondly, over-fishing may lead to reduced genetic diversity in a population (Hauser *et al.* 2002) and small populations will also be susceptible to further loss of genetic diversity through drift (e.g. Allendorf & Luikart 2007).

In the absence of active intervention or enhancement, the recovery of collapsed fisheries will rely entirely on new recruitment. Understanding of the likely source and abundance of new recruits has become, therefore, a critical goal of fisheries science and management. For some fisheries, such as large fin-fish, direct-tagging of individuals to understand emigration or immigration is possible (e.g. Davis & Stanley 2001; Block *et*

al. 2005). However for commercially important benthic invertebrate species the primary dispersal phase is usually a microscopic, pelagic larva which is difficult to tag, and close to impossible to track. For such species, the application of genetic techniques has proven critical for understanding connectivity among populations (Hellberg *et al.* 2002).

Marine molluscs of the family Haliotidae, collectively referred to as abalone, represent one such group. Adult abalone are benthic, with small home ranges (Prince 1989) and the planktonic larvae are the primary dispersal phase (McShane, 1992). Abalone are economically important, with commercial fisheries existing in seven countries, and form an important global industry worth around \$500 million. However, several major abalone fisheries have collapsed in recent decades with no, or only marginal, recovery. The most notable collapse was the Californian commercial abalone fishery which once produced >2000 t/yr but which was closed in 1997, and has still not recovered (Karpov *et al.* 2000). In fact four of the five target species within that fishery are either listed as endangered or considered “species of concern” (Micheli *et al.* 2008). Genetic and ecological studies have shown that, for many abalone species, larval dispersal is likely to be limited, and this has been cited as the major reason for slow recovery following over-fishing (e.g. Prince *et al.* 1987; Prince *et al.* 1988; McShane *et al.* 1988; Miner *et al.* 2006; Temby *et al.* 2007; Gruenthal & Burton 2008).

In Tasmania, Australia, black-lip abalone (*Haliotis rubra*) represents the largest wild abalone fishery in the world, supplying more than 25% of the global catch. Historically, the Tasmanian fishery production has remained relatively constant. However, despite conservative management, several depletion episodes over the past three decades have resulted in contractions of the fishery, with some reef systems failing to recover to prior productivity levels (Tarbath *et al.* 2007). What remains a mystery in Tasmania, is why some areas recover while others remain depleted. Do the different Tasmanian populations of *H. rubra* experience different dispersal or recruitment regimes? Or is the collapse of some regions linked to other biological or physical phenomena such as environmental change or loss of genetic variation?

Limited dispersal of *H. rubra* larvae is well accepted, although there is likely to be at least some occasional long-distance dispersal that has effectively maintained genetic homogeneity around Tasmania (Elliott *et al.* 2002; Temby *et al.* 2007). However, the

frequency, directionality and distance of those dispersal events remain unknown. In this study, we compare genetic variation in two key areas of the Tasmanian abalone fishery - one that continues to be highly productive, and another that has failed to recover from a depletion episode in the mid 1980s - to determine if there is any evidence of a link between larval dispersal processes and recovery in the two regions. We also assess gene flow among abalone populations in the two regions to determine the level and directionality of connections among abalone populations and hence the role of dispersal in the future recovery of collapsed fisheries.

7.2 Materials and Methods:

7.2.1 Study sites and fishing background:

Two regions were targeted for this study. The Actaeons region in South East (SE) Tasmania (Fishing Blocks 13 and 14, Figure 27), represents a resilient, highly productive part of the abalone fishery. Annual catches from this region have been relatively consistent since 1975, averaging $289.9 \pm 16.6(\text{SE})$ and $177.4 \pm 12.5(\text{SE})$ tonnes for Blocks 13 and 14 respectively (Figure 27). An abalone fishery reserve at George III Rock has existed in this region since 1985.

In contrast, parts of the North East (NE) region of Tasmania (Fishing Block 30, Figure 27) have exhibited a classic boom and bust pattern, with annual catches peaking in the mid 1980s (298 tonnes) and subsequently declining to <10% of the long-term average (Figure 27). Block 30 was closed to commercial fishing in 2006 to facilitate recovery. Interestingly, this depleted area is adjacent to one of the most productive fishing grounds in the NE region (Fishing Block 31, Figure 27).

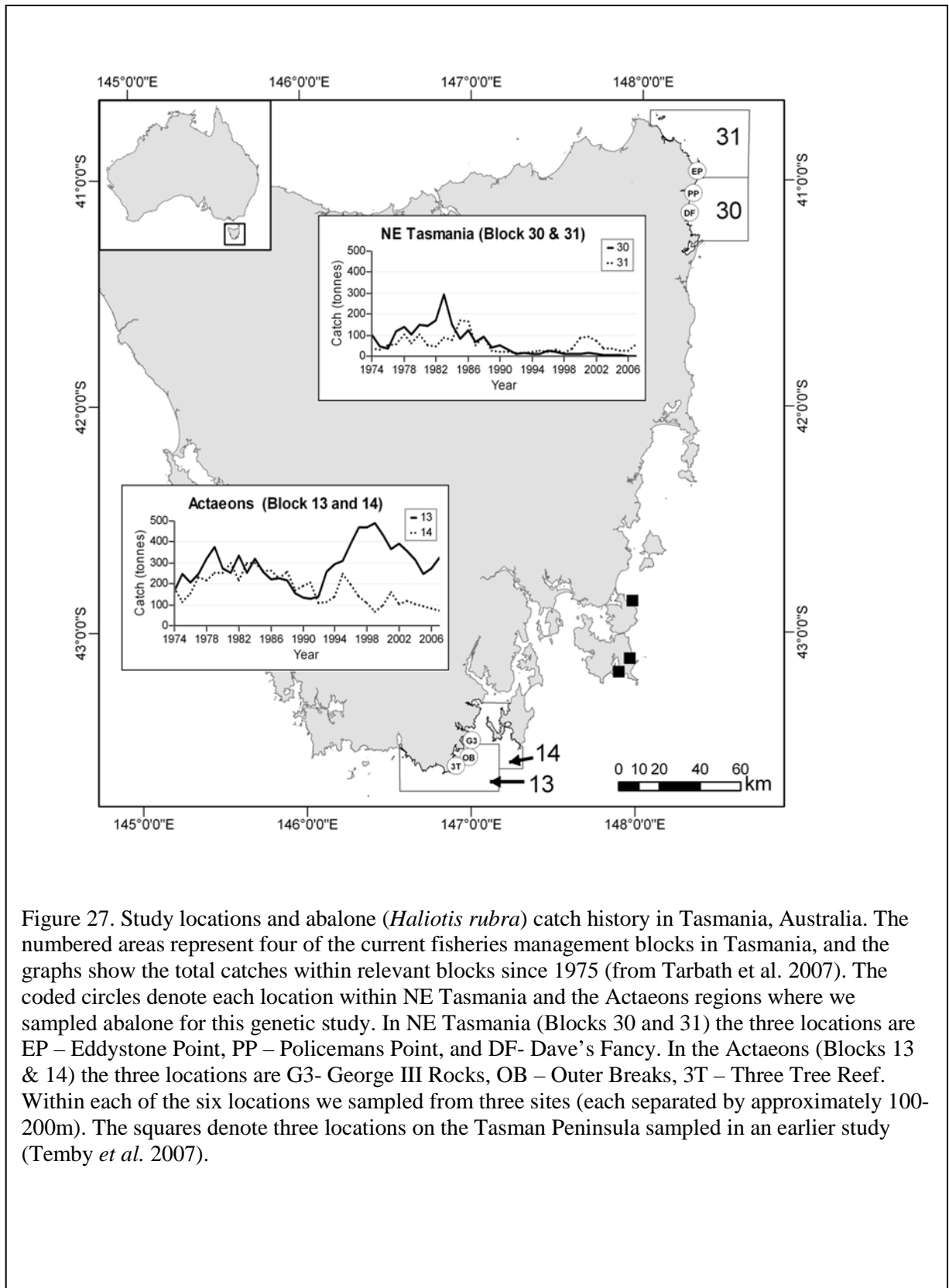


Figure 27. Study locations and abalone (*Haliotis rubra*) catch history in Tasmania, Australia. The numbered areas represent four of the current fisheries management blocks in Tasmania, and the graphs show the total catches within relevant blocks since 1975 (from Tarbath et al. 2007). The coded circles denote each location within NE Tasmania and the Actaeons regions where we sampled abalone for this genetic study. In NE Tasmania (Blocks 30 and 31) the three locations are EP – Eddystone Point, PP – Policemans Point, and DF- Dave’s Fancy. In the Actaeons (Blocks 13 & 14) the three locations are G3- George III Rocks, OB – Outer Breaks, 3T – Three Tree Reef. Within each of the six locations we sampled from three sites (each separated by approximately 100-200m). The squares denote three locations on the Tasman Peninsula sampled in an earlier study (Temby et al. 2007).

7.2.2 *Sample collection and population data:*

A total of 602 abalone was collected for microsatellite analysis. Sampling was according to a spatially replicated, hierarchical design so as to elucidate the spatial scale of population structure; 30-35 abalone were collected from each of three sites, within each of three locations across two geographic regions in Tasmania (18 sites in total; Figure 27). Sites were areas typically 20x20m in size, and replicate sites within each location were 100-200m apart. The locations within each region were separated by 7-10km (Figure 27). Collections were made in June 2006 (NE Region) and January 2007 (Actaeons, SE Region). Two of the locations in the North East Region (Dave's Fancy Reef and Policemans Point) were classed as "collapsed" (no longer capable of supporting a commercial fishery), whereas the remaining four locations (Eddystone Point in the NE, and George III Rock, Outer Breaks and Three Tree Reef in the Actaeons) were classed as "healthy" populations (i.e. continue to support a commercial fishery or in a no-take fishery exclusion area).

Within each site, abalone were collected from as small an area as possible, typically 20m x 20m, and in the collapsed populations our collections included all emergent individuals within the area. A small sliver of foot muscle was removed from each abalone and preserved immediately in ethanol for genetic analysis. Abalone were then returned to the sampling site. Foot muscle tissue samples were stored at -20°C prior to DNA extraction.

The length of each abalone was measured to the nearest mm using a SciElex electronic measuring board (www.scielex.com.au). Abalone size data were used to classify the reproductive status of individuals whereby all animals ≤ 80 mm length were considered immature (Tarbath *et al.* 2001). Immature animals in the NE Region were classed in one of four cohorts based on estimated annual growth rates of 20mm (Haddon *et al.* 2008).

7.2.3 *Genotyping*

Genomic DNA was extracted from each abalone sample using Qiagen DNEasy kits, and according to the manufacturer's protocol. For each abalone we amplified seven microsatellite loci representing a mix of di- (*cmrHr1.14* and *cmrHr1.24*, (Evans *et al.* 2000)), tri- (*Hrub2.B01*, (Baranski *et al.* 2006b)), and tetra- (*cmrHr2.14* (Evans *et al.* 2000), *Hrub6.C04*, *Hrub7.B11*, *Hrub9.H11* (Baranski *et al.* 2006a)) nucleotide repeats.

The seven loci are distributed among \geq six different putative chromosomes mapped by Baranski *et al.* (2006a), and are therefore unlikely to be linked.

PCR amplifications for all seven loci were in a final volume of 25 μ L and contained 2.5 mM MgCl₂ (Promega), 5 pmol (locus *cmrHr1.24*) or 10 pmol (all other loci) of each primer (Sigma Genosys), 0.2 mM of each dNTP (Promega), PCR buffer (10 mM Tris-HCl, 50 mM KCL, 0.1% Triton X-100), 2 units TAQ polymerase (Promega) and ~50-100 ng of genomic DNA template. All forward primers were 5' end-labelled with WellRED dyes (D2, D3 or D4). PCR products were sized by comparison to an internal size standard labelled with D1 on a Beckman Coulter CEQ8000XL automated sequencer. Loci with different labels and/or non-overlapping size ranges were co-complexed on the sequencer in the following combinations; a) *cmrHr1.24* / *Hrub7.B11* / *Hrub6.C04* , b) *Hrub2.B01* / *Hrub9.H11* , c) *cmrHr2.14* / *cmrHr1.14* . Forty-eight representative samples, in two different loading ratios, for each co-plex combination, were initially analysed in order to spectrally calibrate sequencing software and assess genotyping error rates. Scoring discrepancies were only detected at locus *7.B11*, data from which was not analysed further.

Alleles were scored according to the PCR fragment size. For all loci, the size of fragments and inferred number of repeats was compared against GenBank sequence and allele sizes recorded in other published studies that have utilised these loci (Evans *et al.* 2000; Elliott *et al.* 2002; Baranski *et al.* 2006a; Temby *et al.* 2007). In some instances, allele sizes recorded in our genotyping varied by 1 or 2 bases to that expected based on sequence data and/or the sizes recorded in other studies. This seems to be a common occurrence across microsatellite studies performed in different laboratories, at different times, labelled with different dyes, and analysed on different systems with varying gel or capillary temperatures and migration algorithms (e.g. Presson *et al.* 2006; Pasqualotto *et al.* 2007). To enable a direct comparison specifically between the data generated here and that of Temby *et al.* (2007) from the Tasman Peninsula, alleles size adjustments were made at loci *cmrHr1.14* (+1base), *cmrHr1.24* and *cmrHr2.14* (+2bases).

7.2.4 Comparison of genetic diversity between collapsed and healthy populations

Where populations have declined due to fishing pressure, we might expect to see reduced levels of genetic variation. We tested the hypothesis that genetic diversity is

lower in collapsed vs. healthy populations by ANOVA, and based on allelic diversity (the total number of alleles across loci (tN_a) and the average number of alleles/locus (mN_a)), the number of rare alleles (where frequency ≤ 0.05) and the expected heterozygosity (H_E).

We then assessed the genetic structure within all *H. rubra* populations in two ways. Firstly we determined if the frequency of genotypes at each locus matched expectations of Hardy-Weinberg equilibrium for randomly mating populations with χ^2 tests using the software Genepop v3.4 (Raymond & Rousset 1995), and following Bonferroni correction of significance levels to allow for multiple tests. We subsequently calculated values of Wright's fixation index (f) to determine if departures from equilibrium represented heterozygote deficiencies (i.e. $f > 0$) or excesses (i.e. $f < 0$). Where heterozygote deficiencies were present, we tested for the presence of null alleles using Micro-checker (Van Oosterhout *et al.* 2004). Where there was evidence of null alleles, we adjusted allele frequencies (based on the Oosterhout correction algorithm) labelling the null allele size as the largest allele + 1 repeat. The adjusted data set was then used for the remaining data analyses.

7.2.5 Connectivity and the scale of population subdivision

We used F -statistics, calculated as Weir and Cockerham's θ , to examine levels of genetic differentiation among all sites using FStat. Mean F_{ST} was calculated by jack-knifing over loci. Departures from panmixis among sites was tested using 95% CI calculated by bootstrapping over loci. Because microsatellites invariably lead to low F_{ST} estimates due to high amounts of within-population genetic variation, we also calculated a standardised measure of F_{ST} (F'_{ST}) according to the methods described by Hedrick (2005) and Meirmans (2006). Additionally we used hierarchical F -statistic analyses to partition genetic variance; among sites within location (F_{SL}), among locations within regions (F_{LR}), and among locations (F_{LT}). We also analysed our data in combination with the data of Temby *et al.* (2007) for three loci *cmrHr1.14*, *cmrHr 1.24* and *cmrHr 2.14* to extend the geographic spread of our results (to include the Tasman Peninsula, Figure 27) and to include an additional Tasmanian region where abalone populations are fished sustainably.

7.2.6 Migration and directionality of gene flow

We used assignment tests to determine the proportion of individuals self-recruiting to a population as well as to identify which individuals were first-generation migrants into each population (here we consider migration to have occurred through larval dispersal and hence migrants to be individuals that have recruited into a population following the dispersal of a larva from a different population). Assignment tests were done in the software package GeneClass2 (Piry *et al.* 2004) using the resampling algorithm of Paetkau *et al.* 2004 (with exclusion probability of 0.01) and were based on the “L_home” likelihood estimation (to account for the fact that not all possible source populations were sampled), and with the probability of assignment based on a threshold of $p < 0.05$ and on 10,000 simulated individuals. Data from the two regions (NE Tasmania and Actaeons) were analysed separately as we considered it unlikely that there would be direct larval dispersal between the two regions based on the results of F_{ST} analyses.

Individuals identified as first generation migrants to each site were removed from the data set and then reassigned back to the remaining sample to determine their most likely population of origin. Where the re-assignment indicated an abalone may have originated from more than 1 population, we considered the source population to be that with the highest probability of assignment. This data was then used to determine frequency and directionality of larval dispersal among sites. Using shell size as a proxy for age, we then determined the proportions of each generation (that would have recruited in the previous 3-6 years) that originated from self-recruitment or migration within the NE region. As most individuals sampled in the Actaeons were mature and could not be reliably aged based on shell size it was not possible to do this assessment in the SE region.

For the assignment tests we excluded Policemans Point Site 2 in the NE region as we found no reproductive adults within this site (all animals seen and collected were $< 80\text{mm}$ length (Figure 28) and we therefore assumed that all individuals were highly likely to be migrants. Instead, for Policemans Point Site 2 we used assignment tests to determine the likely source population for those individuals that were $< 40\text{mm}$ diameter, and that were likely to have recruited to the site 12-24 months prior to our collections, and during the period in which the area was closed to fishing. For these abalone we considered it highly unlikely that the parents would have been removed from the site by

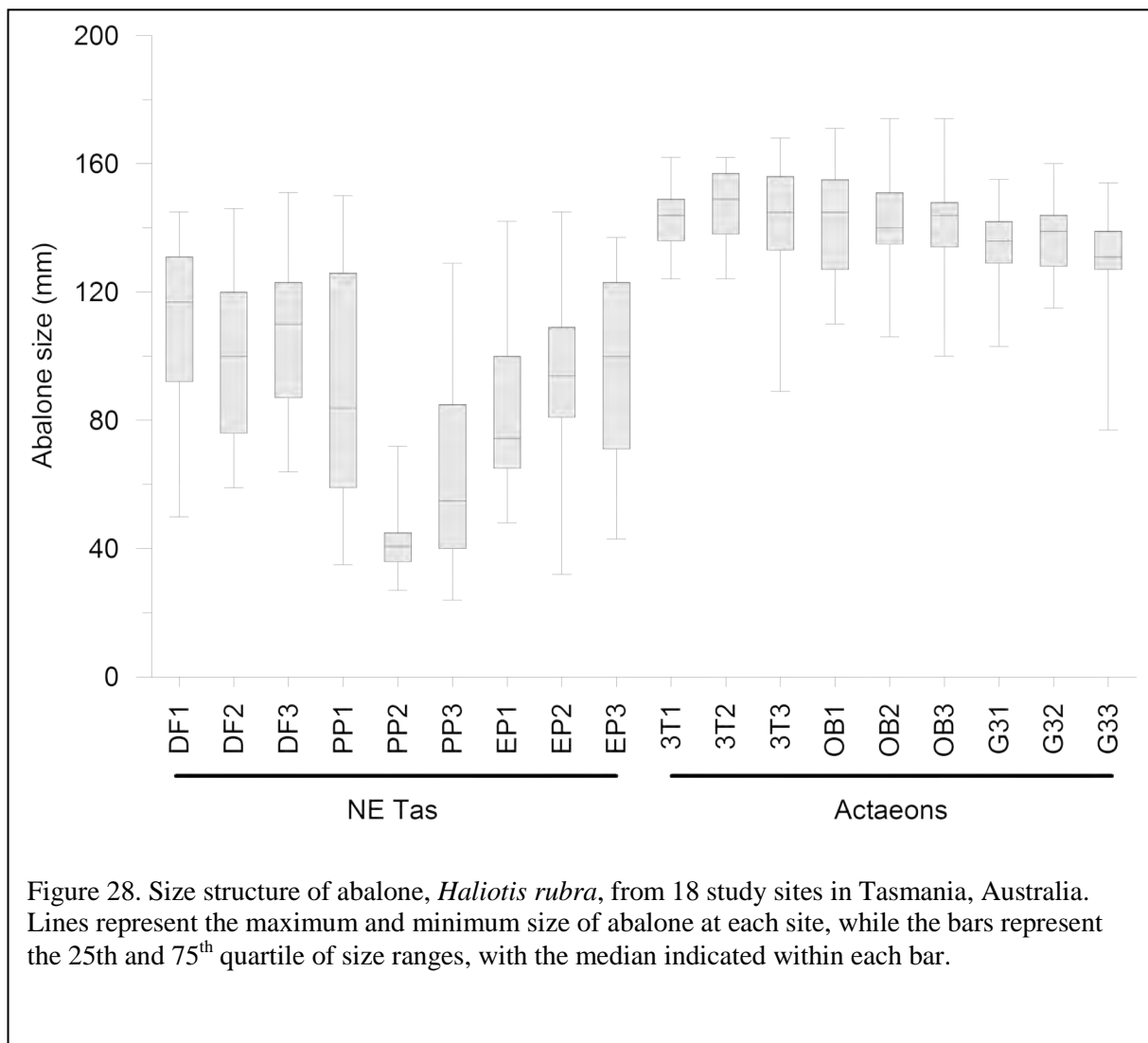
fishers, and excluding natural mortality, the parents of these individuals were therefore likely to be from a different site. We used the results from this assignment test to determine the source and directionality of larval dispersal to Policemans Point Site 2.

7.3 Results

7.3.1 Abalone size and age

The average size of abalone was smaller in the NE than in the SE populations (Figure 28), and this is in part due to a higher proportion of smaller animals in the sample associated with less cryptic reef types. Growth rates and size at maturity are also slightly lower in the warmer waters of northern Tasmania (Tarbath *et al.* 2001) in comparison with the cooler southern areas. With the exception of Policemans Point Site 2, the maximum size of animals was similar across all sites, although few small animals were collected in the Actaeons region (Figure 28) primarily due to the abundance of emergent adults and low probability of encountering cryptic juveniles during random collections in healthy populations.

At Policemans Point Site 2, all individuals were <80mm in length, and therefore classified immature. Almost half (46%) of these were from a cohort likely to have recruited between 1 and 2 years prior to collection (2004/5), and another 43% from the previous year's cohort. It appears that recruitment prior to 2003 at this site was limited, given that our collections included all emergent animals (and most cryptic individuals) within the area.



7.3.2 Genetic diversity in collapsed vs. healthy populations

Although we expected that genetic diversity would be lower in areas where abalone had been heavily fished, in fact we found significantly higher levels of genetic variability in the collapsed populations compared with the healthy populations (Table 7). Both the total number of alleles (tN_a) and average alleles/locus (mN_a) were significantly higher in collapsed than in healthy populations ($tN_a - F_{1,4} = 8.4$, $MS_{Residual} = 13.05$, $P = 0.04$; $mN_a - F_{1,4} = 8.4$, $MS_{Residual} = 0.363$, $P = 0.04$) although there was no significant difference in the levels of expected heterozygosity between collapsed and healthy populations ($H_e - F_{1,4} = 0.03$, $MS_{Residual} = 0.001$, $P = 0.87$). Surprisingly, more than half (57%) of all private alleles were found in populations considered to be collapsed, even though they represented only 35% of the total sample. A similar pattern was evident when we considered rare alleles, whereby collapsed populations had

significantly more rare alleles (mean=3.72±0.4SE) than healthy populations (mean=2.99±0.2SE) ($F_{1,4} = 8.3$, $MS_{\text{Residual}} = 0.69$, $P=0.045$).

Values of Wrights fixation index (f) were low across most abalone populations. Heterozygote excess was apparent in only 24 of 108 single-locus x population tests, and heterozygote deficits in 82 cases. However, in only 21 of these (all heterozygote deficits) were significant departures from Hardy-Weinberg equilibrium detected ($p<0.05$) and only three of these remained significant after Bonferroni correction of significance levels. All three of these were heterozygote deficits at locus *Hrub6.CO4* (Table 8). Null alleles were predicted to exist at three loci; *Hrub6.CO4*, *CmrHr2.14* and *CmrHr1.14*.

7.3.3 Connectivity among abalone populations

We found significant genetic subdivision among all abalone populations sampled, consistent with limited gene flow among sites ($F_{ST}=0.026$, $p<0.01$). When F_{ST} values were standardised to account for high within-population variation, levels of subdivision were moderate ($F'_{ST}=0.065$, Table 3). Notably, however, most of this subdivision is being driven by a single locus – *CmrHr1.24* (Table 3) – suggesting this locus may well be under selection (e.g. Slatkin 1995). When we recalculated F -statistics based only on five loci (i.e. excluding *CmrHr1.24*) we still found low but significant subdivision among all sites ($F_{ST}=0.009$, $p<0.01$, $F'_{ST}=0.027$, Table 3).

Hierarchical F -statistic analysis indicates that most of the subdivision occurs at our smallest sampling scale i.e. among sites within locations ($F_{SL} = 0.026$, $p<0.01$), and this is the case whether locus *CmrHr1.24* is included or excluded from the analysis (Table 9). Interestingly, although there is no significant subdivision between the two regions based on the six-locus analysis ($F_{RT} = 0.0003$), when we removed locus *CmrHr1.24* from the analysis we did detect small but significant subdivision between NE Tasmania and the Actaeons ($F_{RT} = 0.002$, $p<0.05$) (Table 9).

Table 10. Genetic diversity measures for abalone (*Haliotis rubra*) populations sampled in two regions of Tasmania, Australia. N=number of individuals genotyped, tN_a =the total number of alleles across loci, mN_a = the average number of alleles/locus, H_E =expected heterozygosity.

	<u>NE Tasmania</u>									<u>Actaeons</u>								
	Dave's Fancy			Policemans Point			Eddystone Point			Three Tree Reef			Outer Breaks			Geroge III Rock		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
N	35	34	34	35	34	35	30	35	35	32	31	32	32	35	33	31	31	28
tN_a	52	49	40	44	44	47	40	42	44	41	44	45	39	45	48	39	44	40
mN_a	8.7	8.2	6.7	7.3	7.3	7.8	6.7	7.0	7.3	6.8	7.3	7.5	6.5	7.5	8.0	6.5	7.3	6.7
private alleles	3	2	1	3	0	3	0	1	2	2	2	1	0	1	0	0	0	0
rare alleles/locus	5.0	4.7	2.3	3.2	3.5	3.7	2.2	3.7	3.0	2.3	2.8	3.7	3.2	3.0	3.8	2.0	3.7	2.5
H_E	0.57	0.62	0.53	0.57	0.57	0.58	0.57	0.49	0.59	0.59	0.63	0.58	0.59	0.57	0.58	0.60	0.53	0.58

Table 11. Values of Wright's fixation index (f) for *Halitotis rubra* at 18 sites in Tasmania, Australia. Positive values represent heterozygote deficits and negative values represent heterozygote excess. Significant departures from Hardy-Weinberg equilibrium are denoted as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Figures in bold remain significant after Bonferroni correction of significance levels.

Locus	<u>NE Tasmania</u>								
	Dave's Fancy			Policemans Point			Eddystone Point		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
6.CO4	0.44 ***	0.39 *	0.18 *	0.5 ***	0.43 ***	0.33 **	0.26 *	0.2 *	0.25 *
CmrHr2.14	0.12	0.12	0.33	-0.09	-0.02	0.18 *	0.06	0.26	0.25 *
9.H11	0	-0.06	-0.07	0.02	-0.05	-0.05	-0.01	0.06	0.01
CmrHr1.24	-0.07	0.08	0.12	0.13	0.03	-0.11	0	0.12	-0.06
CmrHr1.14	-0.08	0.16	0.28	0.34 *	0.04	0.13	0.01	-0.04	0.12
2.B01	0.06	-0.1	0.1 *	0.04	0.04	0.01	0.02	-0.03	0.03
Locus	<u>Actaeons</u>								
	Three Tree Reef			Outer Breaks			Geroge III Rock		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
6.CO4	0.25	0.37 ***	0.02	0.26	0.22	0.46 ***	0.19 *	0.22	0.72 ***
CmrHr2.14	0.06	0.24 *	0.07	0.12	0.13	0.14	-0.06	0.14	0.09
9.H11	-0.06	0.03	0.16	0.03	0.05	0.04	0.07	0.08	0.13
CmrHr1.24	0.25	-0.12	-0.03	0.06	0.09	-0.08	0.03	0.07	0.07
CmrHr1.14	-0.12	0.29 ***	0.09	0.04	0.05	0.15	-0.16	0.17	0.32
2.B01	-0.01	-0.04	0.11	0.14 ***	0.24	0.12	0.23	0.05 *	-0.01

Table 12. . Results from F -statistic analyses of 18 *Haliotis rubra* populations from two regions in Tasmania - NE Tasmania and Actaeons. Overall¹ - F_{ST} over all loci calculated based on six loci. Overall² - F_{ST} over all loci calculated excluding locus *CmrHr1.24*. Significant genetic subdivision is denoted as * $p < 0.05$, ** $p < 0.01$.

	<i>6.CO4</i>	<i>CmrHr2.14</i>	<i>9.H11</i>	<i>CmrHr1.24</i>	<i>CmrHr1.14</i>	<i>2.B01</i>	Overall ¹	Overall ²		
F_{ST}	0.023	0.005	0.001	0.2	0.012	0.001	0.026	**	0.009	**
F'_{ST}	0.056	0.017	0.004	0.28	0.019	0.009	0.065		0.027	
<i>Hierarchical analysis</i>										
F_{SL}	0.023	0.006	0.0004	0.165	0.011	0.005	0.026	**	0.008	**
F_{LR}	0	0.0005	0.0001	0.001	0	0.005	0.0002	ns	0.000	ns
F_{RT}	0.0005	0.0009	0.0017	0	0	0.004	0.0003	ns	0.002	*

An additional F -statistic analysis including abalone populations from the Tasman Peninsula extended the generality of these findings, although it was based on only three loci. There was significant subdivision among sites ($F_{ST}=0.048$, $p<0.01$), and hierarchical analysis again indicated that most of the subdivision was at the smallest scale (among sites within locations $F_{SL} = 0.047$, $p<0.01$) with little variation either among locations or between regions (Table 10). Again much of the subdivision was driven by locus *CmrHr1.24* although removal of this locus did not alter the conclusions or level of significance overall ($F_{ST}=0.022$, $p<0.01$), and as seen in the two-region analysis, did result in significant subdivision at the regional scale ($F_{RT} = 0.003$, $p<0.01$). As this final comparison is based only on two loci, results need to be treated with caution, however it is pertinent that the same conclusions can be drawn from the two-locus data set from 3 regions as the five-locus data set from two regions.

Table 13. Results from F -statistic analysis among 27 *Haliotis rubra* populations from three regions in Tasmania - NE Tasmania, Actaeons and Tasman Peninsula. The analysis incorporates data on Tasman Peninsula abalone from Temby *et al.* (2007). Significant genetic subdivision is denoted as ** $p<0.01$.

	<i>CmrHr2.14</i>	<i>CmrHr1.24</i>	<i>CmrHr1.14</i>	overall	
F_{ST}	0.009	0.131	0.036	0.048	**
F'_{ST}	0.03	0.197	0.062	0.093	
<i>Hierarchical analysis</i>					
F_{SL}	0.009	0.117	0.038	0.047	**
F_{LR}	0	0.002	0.019	0.005	ns
F_{RT}	0.001	0	0.005	0	ns

7.3.4 Migration

Assignment tests indicated that abalone populations were largely self-seeding. 90-100% of individuals in the Actaeons region and NE Tasmania region (excluding Policemans Point Site 2) were assigned to their parent population (Table 11, Table 12).

Interestingly, of the 12 individuals identified as first generation migrants in the Actaeons region, 7 of those were assigned to sites at George III Rock, suggesting the abalone no-take reserve is an important source of larval recruits (Table 12). Two individuals migrated from Three Tree Reef to George III Rock while two other individuals could not be reliably assigned to any of the sampling sites, and may have originated from an unsampled population. (Table 12).

In the NE region, 11 individuals were identified as first generation migrants. Four of these were assigned to Policemans Point Site 3, and three others to Dave's Fancy Site 1 and Eddystone Point Sites 1 and 3 respectively. Four individuals could not be assigned to any of the sampled populations. Of the 16 abalone that had recently recruited to Policemans Point Site 2 (i.e. those ≤ 40 mm), most of these appear to have originated from the north (69%), with 11 assigned to sites at Eddystone Point. The remaining five abalone were all assigned to Dave's Fancy Site 2 (Table 11).

Table 14. Assignment of abalone, *Haliotis rubra*, to site of origin. Results show the proportion of individuals collected from each site that was assigned to each potential source population for the NE region of Tasmania. Italicised values represent the proportion of individuals sampled at each site that originated from the site (i.e. self-recruitment). Note the results for Policemans Point Site 2 are from a separate assignment test that assumed all abalone at the site were migrants due to the absence of reproductive adults at this site.

Sample population	<u>Source population</u>								
	DF1	DF2	DF3	PP1	PP3	EP1	EP2	EP3	unassigned
Dave's Fancy 1	<i>97.1</i>							2.9	
Dave's Fancy 2		<i>97.1</i>			2.9				
Dave's Fancy 3			<i>94.1</i>		2.9				2.9
Policemans Point 1				<i>91.4</i>		2.9			5.7
Policemans Point 2		31.3				50.0		18.8	
Policemans Point 3					<i>100.0</i>				
Eddystone Point 1						<i>100.0</i>			
Eddystone Point 2		2.9			5.7		<i>91.4</i>		
Eddystone Point 3								<i>97.1</i>	2.9

Table 15. Assignment of abalone, *Haliotis rubra*, to site of origin. Results show the proportion of all individuals from each site that was assigned to each potential source population for the Actaeons region in SE Tasmania. Italicised values represent the proportion of individuals sampled at each site that originated from the site (i.e. self-recruitment).

Sample population	<u>Source population</u>									
	3T1	3T2	3T3	OB1	OB2	OB3	GIII1	GIII2	GIII3	unassigned
Three Tree Reef 1	<i>96.9</i>									3.1
Three Tree Reef 2		<i>93.5</i>					3.2			3.2
Three Tree Reef 3			<i>90.6</i>				6.3			3.1
Outer Breaks 1				<i>96.9</i>			3.1			
Outer Breaks 2					<i>97.1</i>		2.9			
Outer Breaks 3						<i>93.9</i>	3.0		3.0	
George III Rock 1							<i>100.0</i>			
George III Rock 2			3.2						<i>96.8</i>	

Interestingly, for the first-generation migration events identified through the assignment tests, there were no instances where larvae have dispersed successfully between sites within each of the locations. All migration events recorded were between sites in different locations i.e. across 7-20km (Figure 29). In addition, for each of the two regions studied, the proportion of larvae that disperse rather than self-recruit is relatively consistent among years. In the NE region, nine of the first-generation migrants could be confidently aged based on size. Of these, 1, 2, 4 and 2 individuals would have recruited over the preceding 3, 4, 5 and 6 years respectively. This represents 2, 3, 8 and 5% of each year-class across our entire sample (an average of 4.5% migration in each year).

7.4 Discussion

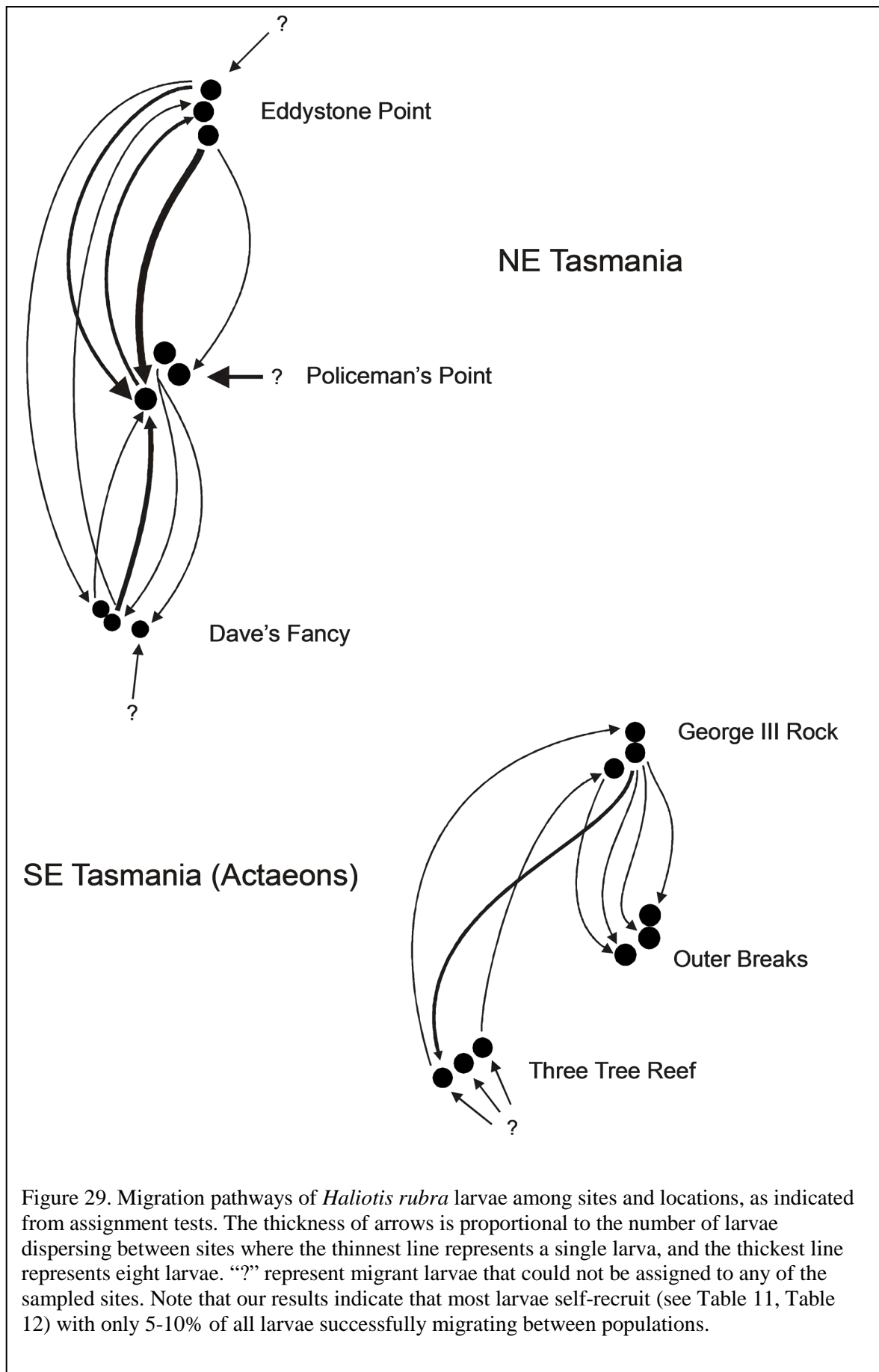
The failure of abalone populations in NE Tasmania to recover from depletion is unlikely to be linked to differing patterns of gene flow or larval dispersal within different regions. We found similar scales of population subdivision, based on microsatellite DNA data, both within the healthy fishery in the Actaeons region as well as the depleted NE region. These results are also consistent with our earlier findings from a third region, the Tasman Peninsula (Temby *et al.* 2007), indicating that small-scale population subdivision (i.e. across hundreds of metres) is most likely widespread in *H. rubra*. Why some regions are apparently more resilient to fishing pressure than others remains unclear as all three regions in Tasmania have also been subject to identical management regimes since the inception of the fishery (early 1960s). Clearly resilience is linked to factors other than differential larval dispersal or fishery management.

7.4.1 Scale of larval dispersal in *Haliotis rubra*

Our results indicate that *H. rubra* populations are largely self-seeding. Certainly self-recruitment is considered common in the marine environment and has been recorded across a diverse range of taxa (Swearer *et al.* 2002). Local recruitment in *H. rubra* as inferred from genetic data is consistent both with our knowledge of its larval biology (McShane 1992) and empirical field studies of dispersal (Prince *et al.* 1987; McShane *et al.* 1988). What is most surprising, however, is that while most larvae appear to recruit to the parent population, there appears to be no short-distance larval dispersal

(100s of m between adjacent sites) but at least low levels of larval exchange at the meso-scale (across 7-20kms among locations).

Modelling studies by McShane *et al.* (1988) suggest that once abalone larvae exit the kelp canopy into the water column, they are likely to be advected 10s of km away from the parent reef. This phenomenon may well explain why we find little genetic evidence that larvae disperse to adjacent sites, but appear to travel occasionally between adjacent locations. Additionally, models of dispersal distance in the New Zealand con-gener *Haliotis iris*, also indicate abalone larvae will disperse over tens of km (Stephens *et al.* 2006). Critically, however, our genetic data indicate that successful meso-scale dispersal events are rare. Back-calculation of the time since dispersal for each of our first-generation migrants based on the relationship between age and size suggest that, although low levels of migration occur within regions every year, successful dispersal may only occur once every few years between any two sites.



McShane *et al.* (1988) hypothesised that the likelihood of an abalone larva that is advected from the natal reef encountering and re-entering any reef system will be low, and this is certainly supported by our results. In fact more larvae appear to successfully disperse between adjacent locations (i.e. 7-10km) than between distant locations (15-20km) (Figure 29) emphasising the declining probability of successful recruitment with increased dispersal distance. Indeed our overall findings are consistent with the concept of bimodality of dispersal in marine larvae (e.g. Raimondi and Keough 1990; Krug 2001) whereby most larvae settle rapidly at competency and within the natal habitat, while others disperse but with associated risks including higher mortality and ultimately low successful long-distance dispersal rates.

Directionality of dispersal among locations shows no obvious trends except in the Actaeons where most migrants originate from George III Rocks. We currently have no knowledge of the small-scale hydrodynamics in the Actaeons region, so cannot determine if this is related to local current patterns. However, the relatively large apparent export of larvae from George III Rocks may be related to the fact that this is a fishery no-take area that contains higher densities of mature abalone than any other site in the region (Mundy *et al.* 2006) and hence may contribute proportionally more individuals to the larval pool. Thus marine protected areas may be important at least for local replenishment of abalone stocks and to contribute to the productivity of a fishery.

7.4.2 *Does overfishing lead to increased genetic diversity?*

Despite our prediction that collapsed populations will suffer from loss of genetic variation, our results show that the depleted populations in NE Tasmania actually have higher numbers of private alleles, rare alleles and total alleles than the healthy populations in either the Actaeons or NE Tasmania. We propose two reasons for this unexpected result. Firstly, as our data show, *H. rubra* populations are largely self-seeding. Thus we would expect healthy abalone populations, like many marine invertebrates (Knowlton & Jackson 1993) to be subject to some level of inbreeding and have naturally reduced levels of genetic variation; indeed heterozygote deficits were observed in some of our populations. The small amount of migration that we propose occurs among locations may, however, be sufficient to reduce the effects of inbreeding depression and partially allay the effects of drift.

Secondly, where populations have been depleted and the number of adults diminished, local reproductive output will be low (Allee effect) and concurrently, local recruitment will also be reduced. Under these circumstances, we suggest that migrant larvae will represent a higher proportion of total larval recruitment. Given that the source of these migrants is likely to be varied both in space and through time (Figure 29), the end result may be a local population with a more diverse genetic composition than those populations that are largely self-recruiting. Thus although over-fishing in itself will not result in increased genetic diversity, the changed ratio of migrant to local larvae may well result in an apparently higher genetic diversity in recovering populations, at least in the short term. Obviously it will be interesting to test if, once these depleted populations have rebuilt to normal adult densities, whether a subsequent reduction of genetic diversity occurs following multiple generations of local recruitment and inbreeding.

7.4.3 Implications for management of abalone fisheries

Our study raises two issues that are central to the sustainable management of abalone fisheries, as well as having implications for many other benthic invertebrate fisheries worldwide. Firstly, that populations are largely self-seeding suggests the decline of local populations of *H. rubra* will be linked to Allee effects, and especially reproductive and recruitment failure associated with a decline in adult abundance (e.g. Levitan & Sewell 1998; Babcock & Keesing 1999). Quite possibly, the collapse of abalone fisheries globally e.g. Canada, California and Japan (Bell *et al.*, 2005) may also be linked to similar Allee effects suggesting management must address the prevention of Allee effects, rather than continue with the current focus on determination of a total allowable catch (TAC) independent from the ecological dynamics of the fishery. Even a conservative TAC is unlikely to prevent Allee effects because economic drivers and the tragedy of the commons encourage fishers to fish an area intensively, rather than to leave some animals behind (Prince 2003).

Secondly, successful dispersal of abalone larvae occurs only rarely (on average <5% of a cohort will be migrants); and typically migrant larvae will disperse in the order of ten kilometres. Provided healthy abalone populations exist within a 10-20km radius of a collapsed population, it is probably safe to assume that gradual recovery through the recruitment of migrant larvae will occur. This is evident in NE Tasmania where, because only a relatively small area of the coast suffered severe population decline,

there appears to be sufficient healthy populations nearby that are providing a source of larvae (e.g. Eddystone Point to the north) - but notably this is an effect we are only now beginning to see after more than 20 years. Thus for the abalone fishery in NE Tasmania, like other fisheries worldwide (Hutchings 2000), recovery without intervention will be possible, but may be on a timescale unacceptable to fishery managers and fishers.

For fisheries that have been less “lucky”, and where whole regions have collapsed, the absence of a larval source at the meso-scale may well limit population recovery. For example in Canada, widespread depletion of the Pinto abalone *Haliotis kamtschatkana* has occurred and populations continued to decline 7 years after the fishery was closed in 1990 (Jamieson 1999). Thus, in addition to management strategies that prevent Allee effects, fisheries will also need to be managed at the meso-scale to avoid regional depletion. In Tasmania, the abalone fishery is currently managed in geographic zones (i.e. East, West and North Tasmania), and reported at smaller management units (Blocks – see Figure 27) that each cover more than 10km of coastline. If over-fishing occurs at the scale of each of these blocks, then there is probably little chance of swift recovery. Resilience of abalone fisheries clearly lays both at the local scale, where most recruits originate, but also at the meso-scale (10s of km) where more distant populations provide sporadic recruits. The relatively high number of successful migrants from the fisheries no-take area at George III Rock to other SE locations illustrates the importance of healthy local populations in combination with marine protected areas for maintaining regional fisheries (Le Quesne *et al.* 2007).

Despite the important role that localised recruitment and sporadic larval dispersal will play both in the decline and recovery of Tasmania abalone populations, our results still fail to explain the differential recovery of a few locations in the NE region relative to other parts of the Tasmanian fishery. Habitat complexity may have an antagonistic effect on the dynamics of depletion in abalone populations. For example the presence of cryptic habitat for juvenile stages is positively correlated with juvenile recruitment (Shepherd and Partington 1995). The collapsed NE reefs comprise a mix of low complexity mudstone (Policemans Point) and more complex granite boulders (Fancy Reefs) suggesting no obvious relationship between habitat complexity and resilience in this area. Furthermore, while changes in subtidal assemblages have been seen following

the collapse of abalone populations in California (Miner *et al.* 2006), broad-scale surveys in Tasmania have found only a very weak correlation between community composition and abalone abundance ($r^2=0.02 - 0.3$; J. Valentine unpubl. data). Differential fishing pressure may also play a role, especially in areas around the Fancy Reefs which are easily accessible and adjacent to a major tourist destination, and so potentially subject to higher levels of recreational fishing pressure than many other reefs.

Clearly there is no simple explanation for the delayed recovery of abalone populations at some sites in the NE region of Tasmania, but further studies on the roles of habitat complexity coupled with accessibility may well shed more light on this dilemma. Importantly though, our genetic results indicate that ongoing larval dispersal from nearby healthy reefs should eventually result in the natural recovery of these populations, although it may well take many more decades before abalone densities approach those that existed prior to collapse of the fishery.

8. LABORATORY TRIALS OF TECHNIQUES FOR TAGGING LARVAL ABALONE

8.1 Introduction

In conjunction with assessing the genetic diversity and gene flow of *Haliotis rubra* (blacklip abalone) within the research area, a series of trials were conducted to develop techniques for tagging abalone larvae. The premise was that if connectivity or a lack thereof between adjacent abalone populations could be established through genetic analyses, then a field study using tagged larvae might be able to further quantify the degree or magnitude of this connectivity.

To determine the extent of connectivity between populations of abalone using tagged larvae, the tag or marker applied must meet three essential criteria; 1) The marker must be applied to the larvae as close as possible to the point of fertilisation; 2) It must not have any adverse effect on the development of the larvae; 3) the marker must be retained through metamorphosis and for a sufficient period of time after settlement in order for the tagged post-larva to be identified. If a chemical tag can meet these criteria, then it should be possible to identify tagged larvae released from a known point at a known time, if those larvae disperse and settle in neighbouring reef areas.

Previous success with tagging larvae of the sea urchin *Heliocidaris erythrogramma* with chemical stains provided the framework for the trials with *Haliotis rubra* and *Haliotis laevigata* (see Lyall 2004). The techniques applied to tag larvae of *H. erythrogramma* were thought to be transferrable to larvae of *H. rubra* and *H. laevigata* given the similarities of the mode of larval development of these species. All three species have lecithotrophic (ie. non-feeding) larvae with a short planktonic phase (5-7 days) and do not undergo drastic morphological change during metamorphosis and settlement.

Due to recent bio-security concerns, these experiments were run entirely in hatchery/laboratories, to ensure there was no risk of transfer of pathogens from hatcheries to the wild.

8.2 Methods

8.2.1 *Laboratory trials of Nile Red as a chemical tag for blacklip (*Haliotis rubra*) and greenlip (*Haliotis laevigata*) abalone larvae*

Nile red is a lipid stain that has been used extensively for studying the composition and metabolism of lipids in organisms (Greenspan *et al.* 1985, Castell and Mann 1994, Carman *et al.* 1991, Priscu *et al.* 1990). Nile Red fluoresces within the range of the visible spectrum when irradiated with ultra-violet (uv) and near-uv light. Its fluorescence colour ranges from golden yellow to deep red, depending upon the spectral composition of the source of irradiation and the type of lipid stained (Greenspan *et al.* 1985). Nile Red was chosen because lecithotrophic larvae typically have a high lipid content (Emlet and Hoegh-Guldberg 1997).

In December 2006, two experiments were conducted at the AbTas abalone hatchery, Garden Island, trialling Nile Red for tagging blacklip abalone. Experiment 1 involved four treatments (control, high concentration stain, low concentration stain, and pre-fertilisation stain). The pre-fertilisation treatment was included to determine if exposing unfertilised eggs to the lipid stain affected fertilisation success and subsequent survival. There were 5 x 2L replicate culture jars/treatment, with each jar containing ~2000 larvae (1 larva/mL).

Because of bacterial infections in some of the culture jars in Experiment 1, a second experiment was run using larval rearing chambers supplied by the hatchery. In Experiment 2 there were three treatments (control, high stain concentration, low stain concentration), each with 5 replicates containing 25,000 larvae/replicate (approximately 1.5 larvae/mL).

In both experiments, a random sample of ~50 larvae were taken daily from each replicate culture until metamorphosis. Each larva within the sample was scored for 1. retention of the tag (marked/potentially marked/unmarked), and 2. for the presence of any abnormalities in development (scored as normal/abnormal), by comparing the stage of development of the larva and its morphology with that of the timeline for development and morphology described in Hahn (1989) and Ino (1952). Survivorship for each replicate culture was assessed from day 3 onwards (as it was too ambiguous to

determine prior to this point) and was extrapolated from the number of survivors counted in each daily sample.

In December 2007, previous tests for staining blacklip abalone larvae using Nile Red were re-evaluated for greenlip abalone (Experiment 3), due to an unavailability of blacklip larvae. The trial conducted was identical to that of Experiment 2 for blacklip abalone with the exception of the addition of a fourth treatment (an extra-high stain concentration was added to control, high stain concentration and low stain concentration).

8.2.2 *Laboratory trials of Calcein-AM as a chemical tag for abalone larvae*

Calcein-am (C-AM) is a membrane permeable form of the fluorescent calcium carbonate stain, calcein. Upon being taken up by a cell, C-AM is hydrolysed to the non-membrane permeable form of calcein and becomes intensely fluorescent. C-AM is used extensively as an internal cell tag in biomedical research, and is in the early stages of application to ecological questions, including tagging. In theory, once inside an abalone egg/embryo, the calcein might be bound up into developing calcified structures and/or lipid stores.

A trial to test the suitability of C-AM for tagging blacklip abalone larvae was conducted during December 2007 (Experiment 4). As there is evidence of active calcein expulsion by membrane receptors in sea star embryos (Roepke *et al* 2006), the trial was structured to test the efficacy of staining abalone oocytes pre and post fertilisation. There were five treatments: a control (= no stain) and four staining treatments, two pre-fertilisation and two post-fertilisation. In both pre- and post-fertilisation treatments, the eggs or embryos were stained for 30 or 60 minutes. The working concentration of the stain was the same for all staining treatments. There were two replicate culture vessels per treatment and stocking density was 5000 larvae per vessel (approximately 0.3 larvae/mL).

The trial was run for the first three days of larval development as this was considered adequate time for formation of the abalone larval shell, and to determine if the calcein was bound up within the larval shell. Samples of larvae were taken daily and scored for tag retention and abnormalities in development (refer to 8.2.1), but survivorship was not evaluated.

8.2.3 *Statistical analyses*

Although data were collected for each day of larval development, it was decided *a priori* to test the effects of marking on abnormality and survivorship only on days of specific interest. Days were chosen to coincide with critical stages during larval development, namely the early embryonic stage (day 0), post-larval hatching (day 3) and post-metamorphosis (day 7).

Separate analyses were undertaken for Experiments 2 (Nile Red/Blacklip), 3 (Nile Red/Greenlip) and 4 (Calcein-AM/Blacklip). Abnormality data were not analysed on day 0 for these experiments as replicate samples were not truly independent on this day, having been marked en masse. Abnormality data from Experiment 4 (Calcein-AM/Blacklip) could only be analysed on day 1 of larval development due to insufficient replication as a result of high variability in survivorship of replicate cultures within treatment groups. The proportions of embryos, larvae and juvenile abalone scored as abnormal were compared between treatments for each day using single-factor Model I ANOVA. Similarly, the proportion of the initial stocking density of abalone larvae remaining alive on each day was compared between treatments using single-factor Model I ANOVA.

In all analyses the relationship between group means and standard deviations indicated that no transformations were required to stabilise variances or normalise distributions. Analyses were undertaken using the JMP software package (v. 7.0).

8.3 **Results**

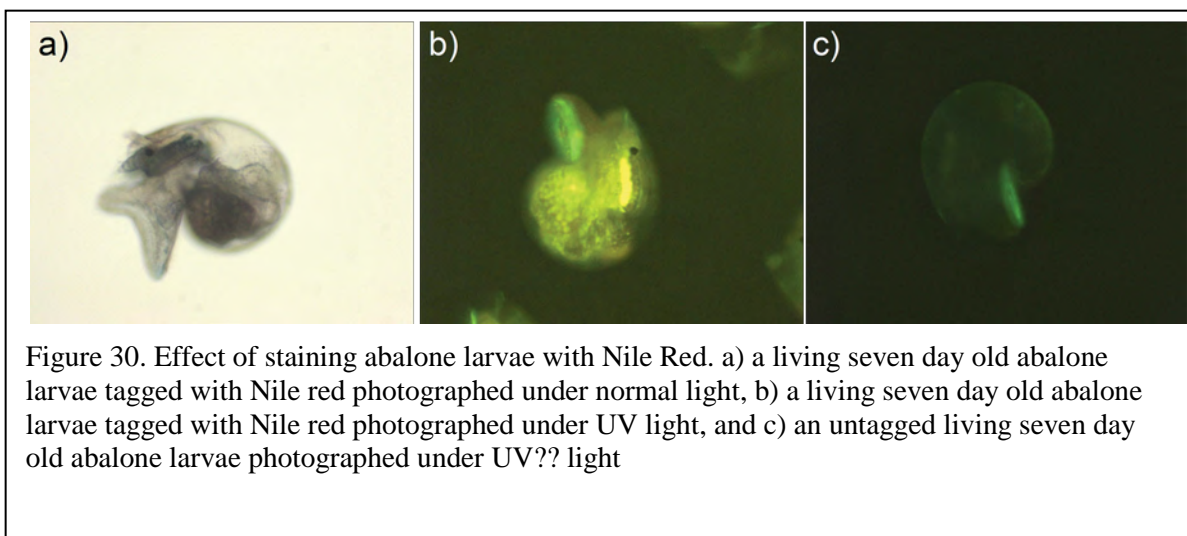
8.3.1 *Laboratory trials of Nile Red as a chemical tag for haliotid larvae*

8.3.1.1 Experiment 1: *Haliotis rubra*

Staining abalone pre-fertilisation was quickly abandoned as it was discovered that marking was less efficient compared to marking post-fertilisation. The remainder of the experiment was discarded due to bacterial infection of the cultures.

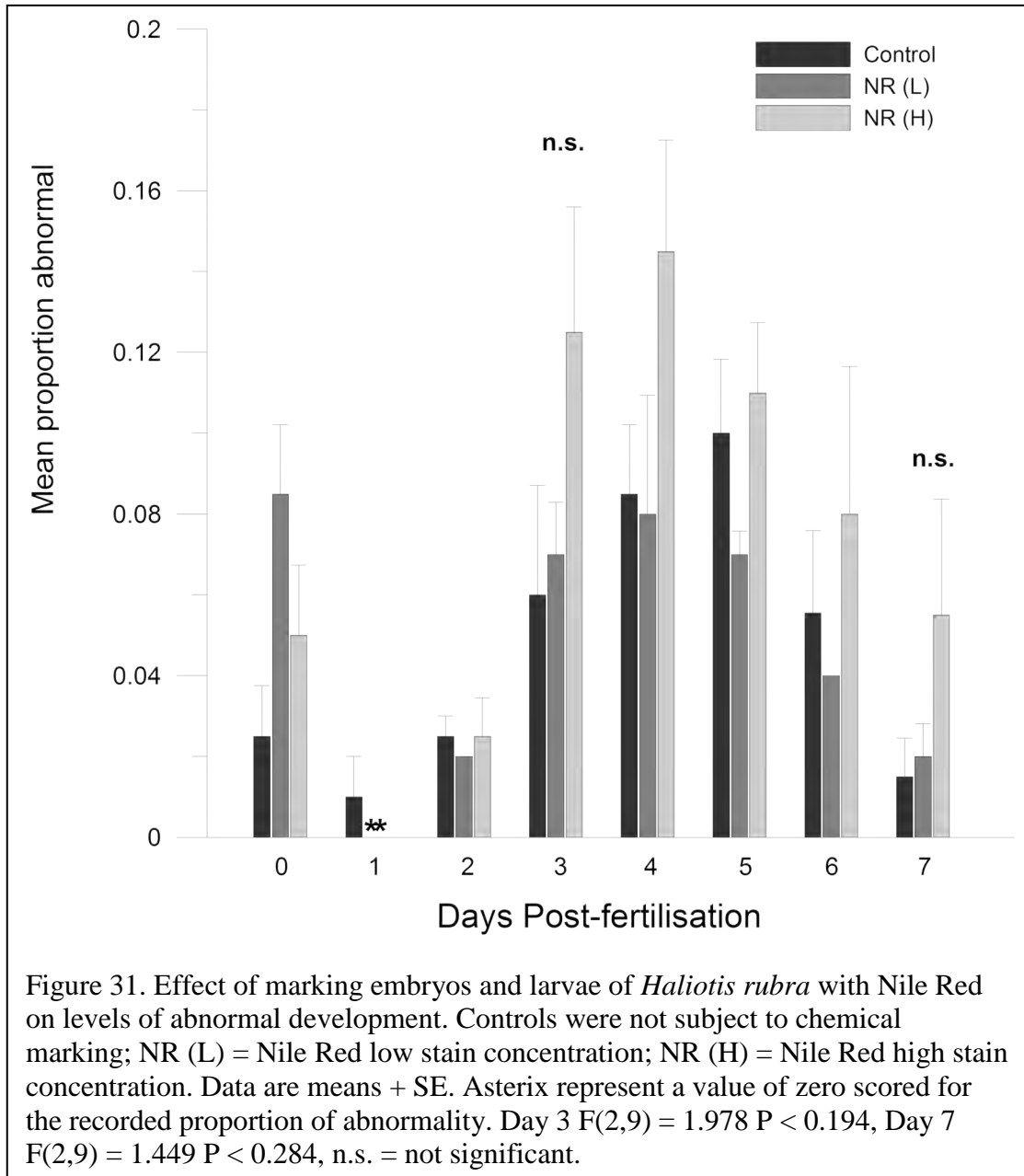
8.3.1.2 Experiment 2: *Haliotis rubra*

Larvae in both low stain concentration NR(L) and high stain concentration NR(H) treatments were unambiguously marked immediately post-fertilisation (day 0). Retention of the tags was variable between the two treatments. All larvae sampled from the NR(H) treatment were scored as marked throughout their development up until settlement (days 0- 7) (Figure 30). All larvae sampled from the NR(L) samples were scored as marked up until days 6 and 7, when they were scored as potentially marked. Tags in larvae from either marking treatment were not clearly evident post-metamorphosis.



Two peaks in the mean proportion of larvae exhibiting abnormal development were observed during the course of larval development in both marked and unmarked (control) treatments in Experiment 2 (Figure 31). These peaks did not entirely coincide with critical stages of *H. rubra* larval development. The level of abnormality was high immediately post-marking, particularly in the marked treatments (NR(L) and NR(H)), however the level of abnormality was low overall (<0.1). There was no significant difference in the level of abnormality observed between control and marked treatments on days 3 and 7 of development (Figure 31).

Mean survivorship decreased with time during the experiment reaching as low as 0.14 (control treatment) by day 7 (Figure 32). This trend was consistent across treatments and while there was no significant difference found between marked and unmarked treatments on day 3, significantly lower mean survival was observed in the control treatment on day 7 ((Figure 32).



8.3.1.3 Experiment 3: *Haliotis laevis*

Larvae sampled from the low stain concentration NR(L), high stain concentration NR(H) and extra-high stain concentration NR(XH) treatments were all unambiguously marked immediately post-fertilisation (day 0). Retention of the tags was variable between the treatments. All larvae sampled from the NR(XH) were scored as marked throughout their development up until settlement (days 0- 7). All larvae sampled from the NR(H) treatment were scored as marked up until days 6 and 7, when they were scored as potentially marked. All larvae sampled from the NR(L) treatment were scored

as marked up until day 4, after which they were scored as potentially marked. No tags from larvae sampled from any of the marked treatments were evident post-metamorphosis.

The mean proportion of larvae exhibiting abnormal development observed during the course of Experiment 3 in both marked and unmarked (control) treatments, was low and consistent over time (Figure 33). There was no significant difference in the level of abnormality observed between control and marked treatments on days 3 and 7 of development (Figure 33).

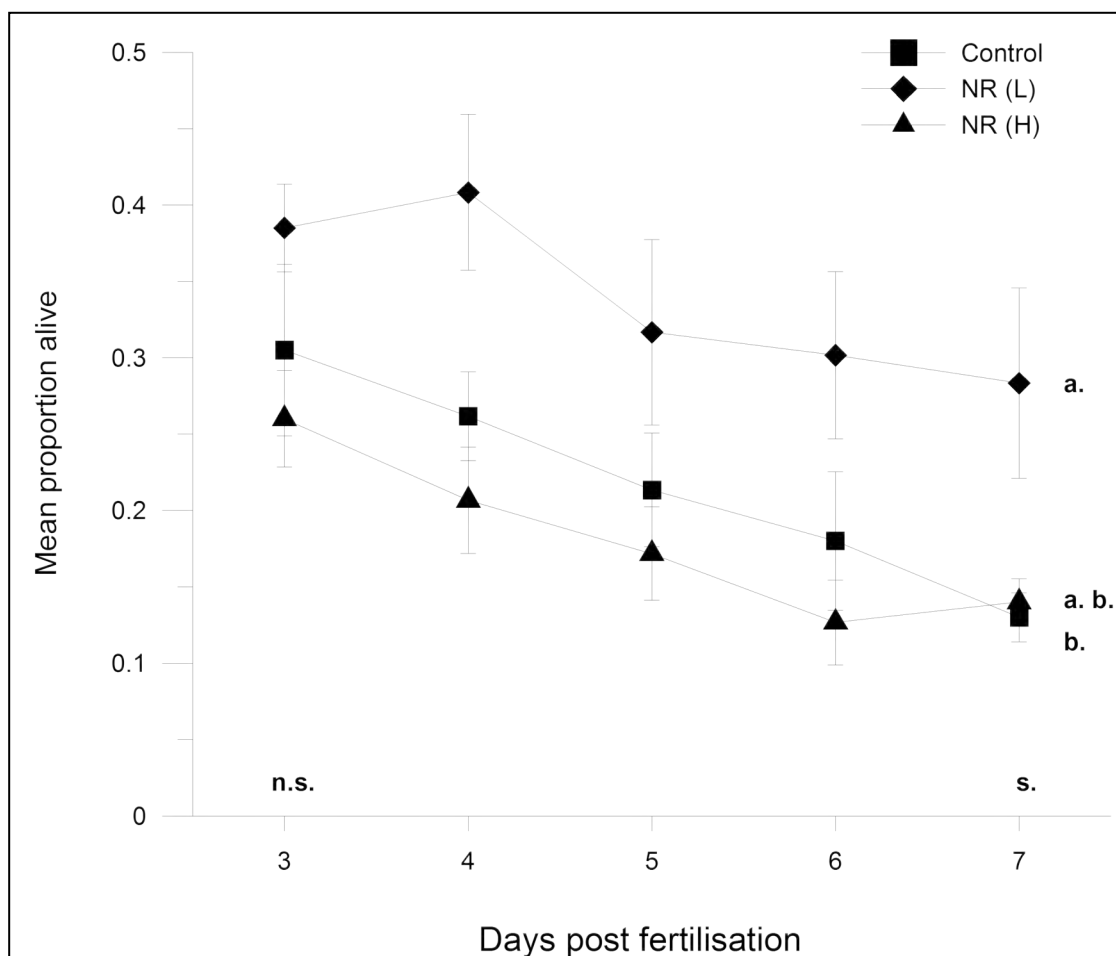
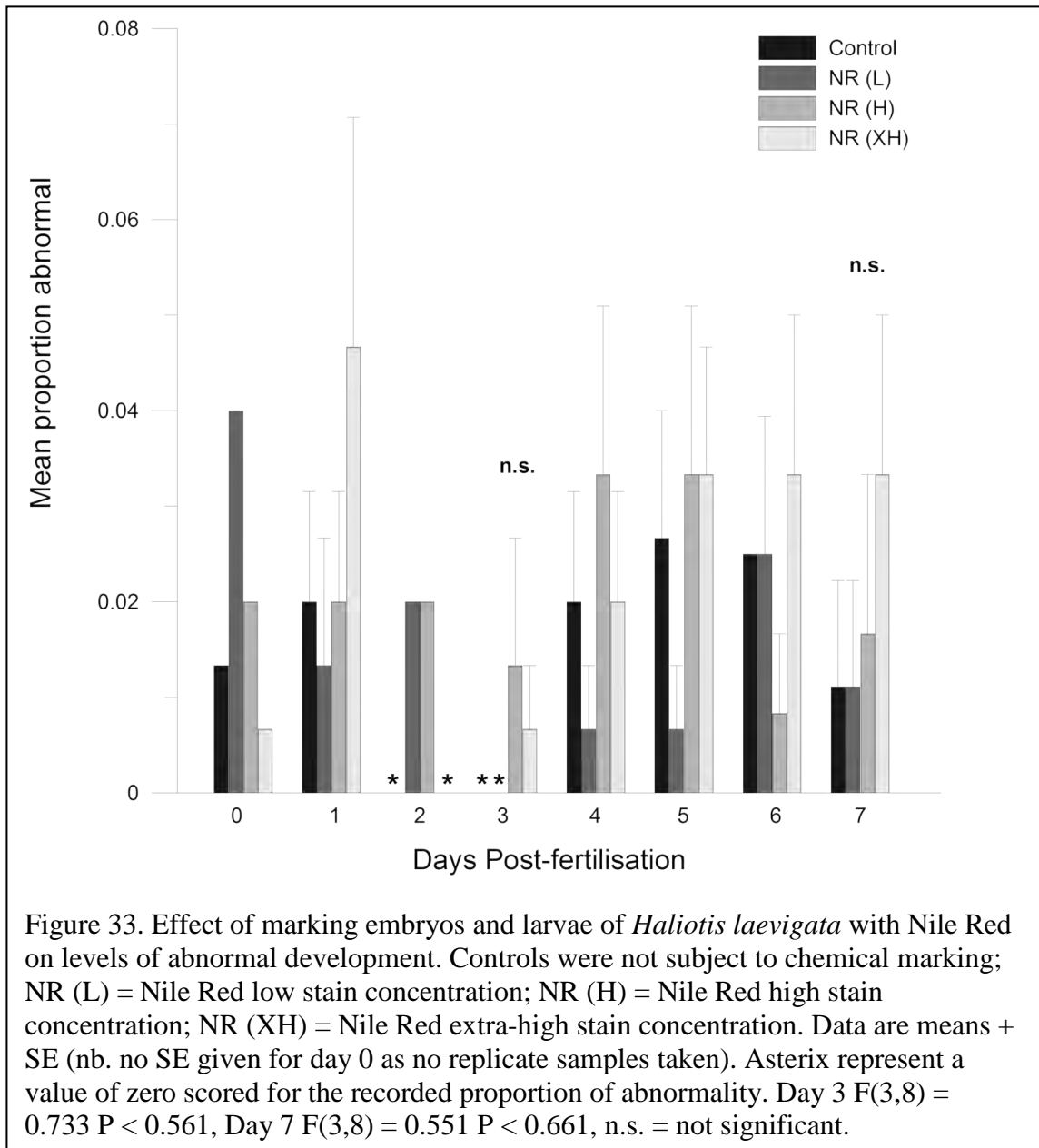


Figure 32. Effect of marking embryos and larvae of *Haliotis rubra* with Nile Red on survivorship. Controls were not subject to chemical marking; NR (L) = Nile Red low stain concentration; NR (H) = Nile Red high stain concentration. Data are means \pm SE. Day 3 $F(2,9) = 2.413$ $P < 0.1449$, Day 7 $F(2,9) = 5.05$ $P < 0.0338^*$, n.s. = not significant, s. = significant, a. or b. define treatment groups that are not significantly different.

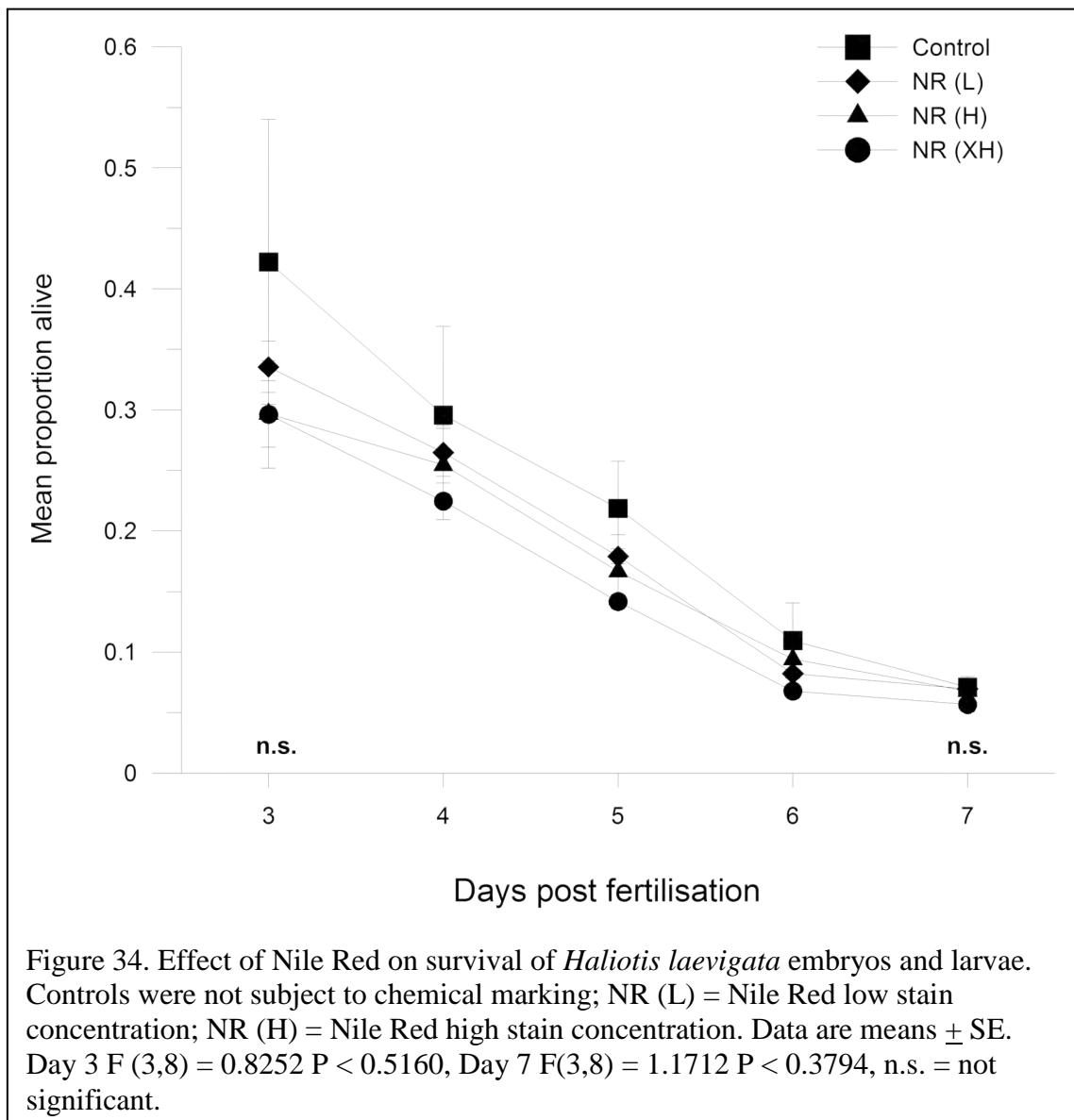


Mean survivorship markedly decreased with time during the experiment reaching as low as 0.06 (NR(XH)) by day 7 (Figure 34). This trend was consistent across treatments and there was no significant difference in mean survivorship between marked and unmarked treatments on day 3 or 7 (Figure 34).

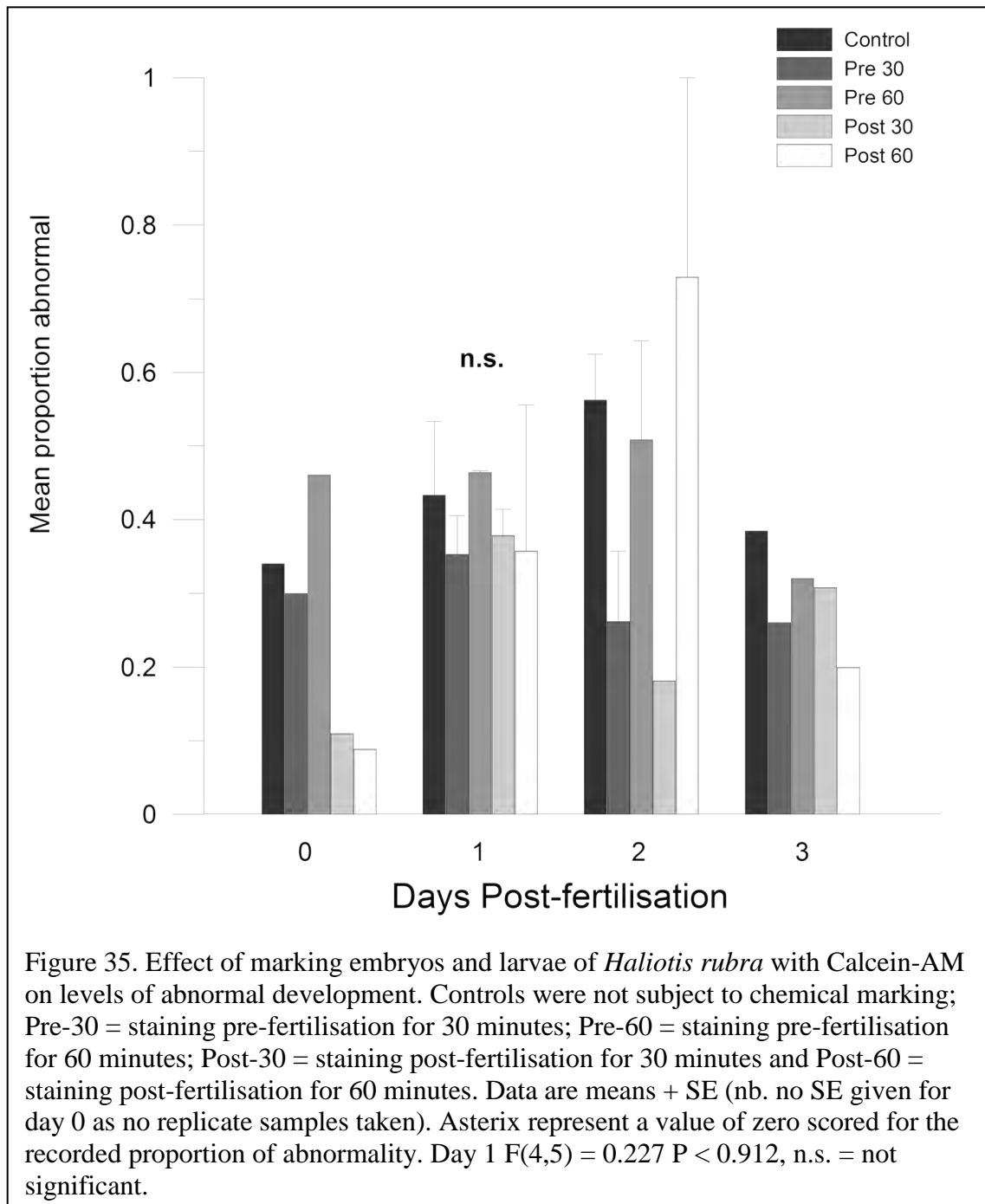
8.3.2 Laboratory trials of Calcein-AM as a chemical tag for blacklip abalone (*Haliotis rubra*) larvae

8.3.2.1 Experiment 4

Low fertilisation rates and highly variable survivorship across replicates and treatments meant there were few larvae to sample from over the three days. Upon examination, embryos appeared to be tagged successfully immediately post marking. The entire embryo was not stained, but rather the tag appeared as patches of faint fluorescent green towards the outer edges of each embryo. There does not appear to be any obvious difference in tag quality/brightness between staining treatments, with the exception of the post-30 specimens that appear to have a slightly fainter tag. Calcein tags were no longer present when day old larvae were examined.



The mean proportion of larvae exhibiting abnormal development observed during the course of Experiment 4 in both marked and unmarked (control) treatments, was consistently high across all treatments and peaked slightly on day 2 (Figure 35). There was no significant difference in the level of abnormality observed between control and marked treatments on day 1 of development (Figure 35).



8.4 Discussion

Nile Red shows some potential as a stain for tagging lecithotrophic haliotid larvae. In experiments with both blacklip and greenlip abalone, larvae were unambiguously stained with no apparent effect on survivorship or development. However, tag retention over the course of larval development was variable and the tag visibility diminished rapidly in larvae of both species post-metamorphosis. Therefore we conclude from this work that Nile Red, whilst an efficient larval stain, is not retained adequately through metamorphosis and the early post-settlement phase post-larvae to be used in larval tagging studies for investigation of larval dispersal and recruitment processes.

The utility of Calcein-AM as an alternate stain for haliotid larvae suggests it is not a viable alternative to Nile Red for tagging larvae. Abalone embryos were not clearly and unambiguously tagged and retention of the tag was poor (< 1 day). It is difficult to draw conclusions of the efficiency of this tag, as a very low working concentration of the stain was used. Staining at higher concentrations may be problematic due to the toxicity of the buffer solution that is used to dissolve the Calcein. In addition, as Calcein-AM is expensive (~\$300 for a few mg of C-AM and buffer) it is unlikely to be a practical method of staining for the ecological-scale experiments that were planned.

Given the unsuitability of both larval tagging methods that were trialled, in combination with ongoing bio-security concerns associated with transfer of biological material from the hatcheries to the wild, it is believed that the experiment involving the release of tagged larvae into the wild should not be pursued at this time.

9. BENEFITS AND ADOPTION

9.1 Cost-benefit analysis – is Translocation worth the effort?

We were successful in translocating >6000 mature abalone to three sites in NE Tasmania, and although there was some mortality, the signatures of these translocations were still apparent as higher abundance of adults in the populations two years later. However we saw little evidence of increased recruitment associated with translocation of adults and the fate of translocated individuals is likely to vary considerably depending on the experimental location. The simplest interpretation of the results thus far, are that although translocation of mature abalone is easily achieved and relatively affordable, there is little evidence yet to support translocation of mature abalone as a remedial management measure for abalone fisheries. The ecological processes involved in stock rebuilding will most likely occur over five to ten year time scales, and thus a detailed cost-benefit analysis is not feasible based on the 3yr timeframe of this project. To achieve a reliable cost-benefit analysis will require surveys of population structure and abalone density at a point at least 6 years following the translocation (i.e. surveys of the Translocation populations relative to the Control populations in 2012) which is when we might be likely to observe any increase in natural recruitment in to the sites. The result of the population genetic component of this study does however have immediate and significant implications for the concept of stock rebuilding in abalone fisheries, whether it is done through translocation of mature adults or reseeded with juvenile abalone.

In addition to the actual operating costs of a translocation event, a detailed cost-benefit analysis requires information from two major areas; costs associated with the source population(s) used for the translocation, and quantitative data on the recovery process. The former addresses the issue of the value of the source animals used in the translocation. The latter is the measurement of a positive effect that is unequivocally aligned with the translocation event. These two issues and that of the spatial scale of benefit are summarised briefly below.

9.1.1 *Value of the source abalone used for translocation*

In this study, there were several local un-fished populations of abalone that could be used to source animals for the purpose of translocation and stock rebuilding. In most

abalone fisheries, there exist populations which grow slower and reach a smaller size than the majority of populations in the fishery. Accessing these populations (as is the case with this study) has little cost to the productivity of the exploited stocks, except those areas adjacent (within a few hundred meters), given larval dispersal is limited (Miller, et al 2009). If however, the source populations are a component of the fishery, then the value of those abalone for an alternate use (rebuilding) must be considered. If the value is taken as equivalent to the beach price at the time of collection, then very clearly, the cost of stock rebuilding will be high (assuming it is successful), and will only be cost-effective if it is amortised over a significant time period (e.g. decades). If the cost is considered as transferred, as these animals will eventually be available to the fishery, then the costs are significantly less. The logic of the decision on how to assign a dollar value to the source animals, and the time-scale over which the costs are amortised are critical steps in any cost-benefit analysis.

9.1.2 Quantitative measures of benefit

The primary measure of success of remedial action associated with stock rebuilding, is an increase in (or presence of) the animals available to the fishery, and that this increase/presence is ongoing. In Australian blacklip abalone fisheries, recruitment to the fishery occurs 7 to 8 years after larval settlement. In Tasmania, blacklip abalone emerge from crypsis approximately at 5 to 7 years of age. This means that a robust and reliable estimate of the success of a translocation (adult or juvenile) can't be achieved until the biological recruits have reached this size.

Estimates of larval supply can confirm that there has been successful spawning and settlement, but an absence of recruits on settlement panels does not infer there has been no spawning or settlement. Additionally, early post-settlement mortality in marine invertebrates is typically high within the first few months, and the first year, and patterns of abundance of later size classes may be distorted by events occurring in this first year. Alternatively, quantitative data on the success of the translocation event can be derived from an estimate of juvenile abundance (2+ or 3+ cohorts). The method most often used or proposed for measuring abundance of juvenile size classes- rolling boulders – is only suitable in habitat where boulders can be moved or collected. In many areas this technique is not possible, either because the boulders are too big, or because the habitat is fractured rock with crevices and grooves, and cannot be manipulated in the same way as small-boulder habitats. Developing a technique to

quantify juvenile abundance in a range of habitat types is critical for early testing of the success of either translocation, or reseeded. A program was included in the original proposal, but was removed in association with the request to decrease the budget.

9.1.3 Scale of benefit

A primary objective of this project was to determine the scale, if any, over which remedial activity such as translocation would occur. The key finding from the population genetic study is that larval dispersal rarely occurs at local scales (i.e. 100's of metres), with occasional dispersal over medium spatial scales (10's of kilometres). Thus larval recruitment from any populations enhanced through translocation of mature abalone will be highly localised, with reefs further than a few hundred meters from the release site unlikely to receive any benefit.

The usefulness of translocation or reseeded as a tool for stock rebuilding must therefore be considered in the context of the size of the area over which remedial action is to be applied. If the area is large, e.g. an entire fishery zone, or scales of 10's of kilometres, it is unlikely that rebuilding through translocation will be cost-effective from a commercial perspective over the entire area. Situations where translocation is likely to be feasible, are where the interest is in enhancing or re-establishing populations of abalone within localised, discrete reef systems.

9.2 Conclusions

This project explored translocation as method for stock recovery. Translocation of mature blacklip abalone can be done efficiently and with minimal transport induced mortality. Despite the ease and success of our translocation exercise, emigration of translocated abalone away from the release site to surrounding available reef habitat is inevitable, and at some sites this may negate the objective of creating an effective spawning population. Blacklip abalone in Tasmania commence transition from a cryptic phase to an emergent phase at the time of reproductive maturity of approximately five years, but are not fully emergent until they reach seven or eight years of age. Thus, expectations of achieving or measuring success must be cognisant of this aspect of abalone biology. The time frame required to determine whether translocation of adult abalone to create an 'instant' spawning population has been effective will be at least seven to ten years. This time-frame is well beyond the typical funding period of research projects.

The clear demonstration of very high levels of self-recruitment by population genetic techniques has significant implications both for blacklip abalone stock rebuilding, and also for management of blacklip abalone populations. In the context of translocation, the spatial scale of benefit of remedial activity will be extremely limited regardless of whether that involves translocation of wild adults, release of juveniles, or release of larvae. We therefore suggest that stock rebuilding using such interventionist methods where the objective is to rebuild depleted stocks over large geographic spatial scales is will be challenging and outside the financial scope of industry.

Translocation of wild abalone can only be used if there is an adequate source of mature abalone that are surplus to the requirements of the fishery. For this reason, it is expected that the circumstances where Translocation of wild abalone for the purposes of stock rebuilding can be undertaken will rarely occur.

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11. APPENDIX 1: INTELLECTUAL PROPERTY

No commercially valuable intellectual property arose from the research. No compelling reason was identified to restrict distribution of results so these have been made publicly available with no protection or confidentiality.

12. APPENDIX 2: STAFF

Project staff were:

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