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Recovering a collapsed abalone stock through translocation. Seafood CRC Project No. 2011/762

Lachlan W.S. Strain

Jamin M. Brown

Anthony M. Hart

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GOVERNMENT OF
WESTERN AUSTRALIA

Fisheries Research Report No. 292

Recovering a collapsed abalone stock through translocation

Seafood CRC Project No. 2011/762

Lachlan W. S. Strain, Jamin M. Brown and Anthony M. Hart

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This project was conducted by the Department of Primary Industries and Regional Development, Western Australia
WA Fisheries and Marine Research Laboratories
39 Northside Drive, Hillarys WA, 6025
PO Box 20, North Beach WA, 6920
Tel: +61 8 9203 0111
Email: library@fish.wa.gov.au
Website: www.fish.wa.gov.au

In collaboration with several organisations:
Flinders University, Sturt Road, Bedford Park, SA 5042, Australia.
Nofima, PO Box 5010, 1432 AS, Norway.
888 Abalone Pty Ltd, White Trail Road, Bremer Bay, WA 6338, Australia.

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1 Non-Technical Summary

2011/762 Recovering a collapsed abalone stock through translocation

Principal Investigator: Dr Lachlan Strain

Address: Department of Primary Industries and Regional Development, Western Australia

Western Australia Fisheries and Marine Research Laboratories

39 Northside Drive, Hillarys WA, 6025

PO Box 20, North Beach WA, 6920

Ph: (08) 9203 0111

Fax: (08) 9203 0199

Project Objectives

1. To establish founder populations of Roe's Abalone in areas of mass mortality.
2. To evaluate the genetic structure of existing and founder populations.
3. To compare natural and assisted recovery rates of Roe's Abalone populations.
4. To evaluate the genetic contribution of existing and founder populations to stock recovery.
5. Develop spawning protocols for Roe's Abalone and conduct a pilot juvenile stock enhancement release (this objective was incorporated during the latter stages of the project).

Outcomes Achieved

Key outcomes:

- Assisted recovery programs are a viable fisheries management tool in depleted abalone populations (scale and timeframe dependent).
- Establishment of multiple founder populations of effective breeding size created through translocation of mature wild Roe's Abalone to assist the recovery of the Area 8 fishery in Western Australia.
- Juvenile Roe's Abalone present at a founder population, indicating that the number and density of translocated abalone at a release site were sufficient for recruitment to occur.
- No natural recovery observed within the depleted Roe's Abalone stock.

- Restocking using hatchery-reared juvenile Roe's Abalone possible at remote founder populations.
- Genomic techniques successful at identifying locally adapted genotypes that would increase the success rate of translocation and restocking of Roe's Abalone.

These key outcomes provide the foundation for developing a commercial-scale assisted recovery program, and meet the long-term need of industry and managers in Western Australia, and other abalone producing states contemplating recovery programs.

Additional outcomes:

- Established long-term monitoring sites for this assisted recovery program and any future large-scale release programs, plus the ongoing stock assessment of the Area 8 Roe's Abalone fishery.
- Developed capacity in the abalone aquaculture industry for commercial-scale production of Roe's Abalone for either restocking and stock enhancement programs or aquaculture (export) markets.
- Improved and refined live transport methods for abalone of all sizes, particularly to remote and inhospitable areas within Australia.

Outcomes still under development:

- The genetic contribution of remanent and founder populations to stock recovery has not been evaluated due to the limited number of recruits found. However, the genomic protocols have been developed to complete this aspect.
- Commercial-scale assisted recovery program required to recover the Area 8 Roe's Abalone fishery.

List of Outputs Produced

1. Formal comparison of natural and assisted recovery strategies for an abalone fishery depleted through an extreme environmental event.
2. Detailed translocation protocols developed for the transport and release of both juvenile and adult Roe's Abalone to remote areas of the species distribution.
3. Scientific information on the natural ecology and ecological processes involved in the translocation/restocking of Roe's Abalone, including estimates of translocation survival and natural mortality, as well as important behavioural characteristics and their effect on recovery success.
4. Comprehensive Roe's Abalone aquaculture spawning and rearing protocols.
5. Comprehensive genomic analysis of genetic diversity and connectivity in Roe's Abalone populations within Western Australia. Recommendations for capturing genetic diversity and adapted genotypes for increasing the success of stock recovery initiatives.

Summary

A Roe's Abalone (*Haliotis roei*) fishery in Western Australia (Area 8) suffered catastrophic mortality (99.9%) due to an anomalous environmental event in the summer of 2011. During this extreme marine heatwave there was a sustained period of elevated sea surface temperatures that rose to lethal levels for this species and effectively wiped out an entire stock at its northern distribution. Natural recovery within the foreseeable future was considered unlikely, thus providing a unique opportunity to test fishery restoration strategies for abalone. Over the course of this assisted recovery program (5.5 years) no natural recovery was observed in the region most affected by the mortality event.

The objective of this study was to investigate the viability of recovering a collapsed Roe's Abalone population through the creation of founder populations. Given the extent of the mortality event north of the Murchison River, near Kalbarri, Western Australia, reef platforms devoid of abalone were identified as release sites to create founder populations. For these founder populations to be viable breeding populations, the aim was to achieve at least 500 adult Roe's Abalone at densities greater than 3 per m^2 on the reef platforms.

Nearly 9,000 adult Roe's Abalone were translocated over 7 events spanning a 2-year period. The animals were harvested from 2 source locations (Lucky Bay and the Perth Metropolitan Fishery) and released at 5 sites in the Kalbarri Cliffs study zone. To facilitate this process research was undertaken on translocation methodology to harvest, transport and release large numbers of mature animals into the remote and inhospitable region. This included evaluating different transport methods, such as comparing "hand release" versus mechanical release. Several release modules were tested to improve the survival of released animals while also increasing the number of Roe's Abalone that could be released during a translocation event. The adult Roe's Abalone had a translocation survival that ranged from 0.24% to 35%, depending on the transport or release method used. The transport method "full water with aeration" resulted in the highest translocation survival (35%). The prevailing weather (swell, tide and wind) conditions at the time of release were considered important for not only translocation survival but also personnel safety during release and survey accuracy, as the reef platforms are extremely exposed and potentially dangerous.

Information on the ecology of Roe's Abalone, such as natural mortality and behavioural characteristics were obtained through the translocations, including how these processes affected the assisted recovery program. The natural mortality rate (M) of translocated Roe's Abalone at 3-year post-release was estimated to be 0.31 $year^{-1}$ (0.03 SE). A distinct characteristic of the translocated abalone was the migrating and clustering behaviour, with cluster densities averaging 103.9 ± 15.9 abalone.m^{-2} and the clusters accounting for nearly 50% of the surviving abalone at the release sites.

Importantly, founder populations of effective breeding size were able to be created through translocation of adult Roe's Abalone, with some sites surveyed having 800 abalone at 2.88 abalone.m^{-2} on the reef platform. At one founder population recruitment was recorded. Even though it was only a few recruits, this result is a significant step forward for the assisted recovery program as it proves recruitment is possible at founder populations created with

translocated adult Roe's Abalone. Both founder populations and areas that were not restocked are being monitored annually to determine long-term recovery rates.

Restocking using hatchery-reared juvenile Roe's Abalone was also examined as a method to aid the recovery of decimated Roe's Abalone populations. To achieve this, detailed spawning protocols were developed to produce the required juveniles, as Roe's Abalone had not been spawned on this scale in aquaculture before. Roe's Abalone were able to be produced on a commercial scale and 77,364 hatchery-reared juveniles were released during 5 restocking events at the Kalbarri Cliffs study zone over a 3-year period. The translocation survival of the juvenile Roe's Abalone 1-year post-release was less than 1% for each of the 5 restocking events. The recapture rate would have also been low due to the small size of the juveniles and the variability in prevailing weather conditions at time of survey. The low survival was hypothesised to two main factors: (a) the high wave energy and temperature environment the juveniles were released into, and (b) the difference between the genetic adaptive population clusters of the source and sink populations. All juveniles were progeny of broodstock from the southern adaptive cluster and were released into the northern adaptive cluster (Kalbarri Cliffs study zone), with significant genetic differentiation between the two localities (see below).

New diagnostic genomic tools to study natural Roe's Abalone population genetic structure and monitor the success of the assisted recovery program at the Kalbarri Cliffs study zone were developed with the collaborative Seafood CRC project 2012/714. Samples from 428 adult Roe's Abalone collected from ten locations covering a large range of the species distribution (Kalbarri Cliffs in WA to Spencer Gulf in SA) were analysed using the new tools, and produced a total of 31,008 high quality genomic markers in the form of SNPs (single nucleotide polymorphisms). The screening of genome-wide variation in samples collected from the wild showed that 'neutral' SNPs (i.e. DNA markers that are not under the influence of natural selection) support the existence of one single abalone population with high connectivity across the geographic range sampled. However, when the SNP markers under natural selection were examined, three genetically distinct groups of populations for Roe's Abalone were identified (north [Kalbarri Cliff to Lucky Bay], southwest [Greenough to Augusta] and south [Albany to Spencer Gulf]). Significant associations between the distribution of these adaptive groups and the spatial variation of key environmental parameters, including differences in annual maximum temperature were found. These results are critically important for the restocking initiative of Roe's Abalone at the Kalbarri Cliffs given it occurred at the species northernmost distribution.

This genomic analysis has provided an outstanding resource and detailed knowledge base that will assist the management of abalone fisheries, restocking initiatives and aquaculture in WA. Firstly, thousands of DNA markers were identified and characterised; these markers will be useful for monitoring the genetic health of Roe's Abalone stocks. Secondly, high genetic connectivity was detected across the sampling area but with more than one adaptive group detected. This finding will aid managers in specifying which abalone will improve the chances of success of specific stock recovery programs.

Given the current course of climate change and the prediction that extreme environmental events are likely to become more regular and intense, it's imperative that stock recovery

initiatives like this are able to assist in the restoration of not only commercial fisheries but populations of at-risk marine species. Overall this research showed that translocation of Roe's Abalone can create founder populations in a remote area, and while recruitment was low at these founder populations, no natural recovery has been observed at the Kalbarri Cliffs study zone. Therefore, assisted recovery (specifically translocation of mature adults) appears to be most viable method of recovering the Roe's Abalone populations moving forward, and this project provides the foundations for a long-term, large-scale assisted recovery strategy for the Area 8 Roe's Abalone fishery.

Keywords: Stock Recovery, Translocation, Restocking, Survival, Aquaculture, Marine Heatwave, Roe's Abalone, *Haliotis roei*.

2 Acknowledgements

The authors would like to acknowledge the financial contribution the Australian Seafood Cooperative Research Centre (CRC) made towards funding this research through the project 2011/762 “Recovering a collapsed abalone stock through translocation”. Also to the Department of Primary Industries and Regional Development, Western Australia, for supporting the research following the closure of the Area 8 Roe’s Abalone fishery due to the mortality event cause by the marine heatwave in 2011. To all the Department staff that contributed towards this research, including Frank Fabris, David Murphy, Stephen Leporati, Naomi Greenham, Jonathan Baulch and Nick Caputi, it was greatly appreciated.

The authors’ thanks go to the West Coast Abalone Divers Association (WCADA) for providing considerable in-kind contributions through its members, in terms of commercial abalone fishing expertise to help establish the founder populations. Particularly thanks to John Craike for his invaluable assistance navigating in the remote locations, site selection, harvesting, transport and release of Roe’s Abalone. Thanks also to a number of “Kalbarri locals” that helped with the translocation events over the years. This research would not have been possible without the support of Calum and Belinda Carruth at Murchison House Station for allowing us access through the station to reach the remote reef platforms of the Kalbarri Cliffs study zone.

Appreciation goes to the commercial abalone farm 888 Abalone Pty Ltd, for the collaborative work on hatchery production and culture of Roe’s Abalone. Without this collaboration, the research on developing spawning protocols for Roe’s Abalone and the production of hatchery-reared juvenile abalone stock would not have been possible. So to 888 Abalone and its directors Peter and Craig Kestel, and the managers at the time of this project, Shane Smith and Vincent Encena, thank you. The sections in this report on Roe’s Abalone hatchery and nursery production were based on trials conducted at both 888 Abalone in Bremer Bay and the Western Australian Fisheries and Marine Research Laboratories in Perth.

The population genomic study was a collaboration between Department of Primary Industries and Regional Development WA, Flinders University and Nofima through the Seafood CRC projects 2011/762 “Recovering a collapsed abalone stock through translocation” and 2012/714 “PDRS: Use of next generation DNA technologies for revealing the genetic impact of fisheries restocking and ranching”. The authors of this report sincerely thank Jonathan Sandoval-Castillo and Luciano Beheregaray from Flinders University and Nick Robinson from Nofima for conducting the excellent genomic population analysis of Roe’s Abalone in Western Australia. The sections on genomic analysis within this report have been extracted from the Seafood CRC Final Report on the project 2012/714 and adapted to suit this final report. The citation for the Seafood CRC Final Report 2012/714 is below.

Sandoval-Castillo J., Robinson N., Strain L., Hart A. and Beheregaray L.B. (2015) Use of next generation DNA technologies for revealing the genetic impact of fisheries restocking and ranching. Australian Seafood CRC Report No. 2012/714. Flinders University, Adelaide, 47pp.

3 General Introduction

3.1 Background

In Western Australia (WA) the abalone resource is separated into the Roe's Abalone (*Haliotis roei*) fishery and the Greenlip/Brownlip Abalone (*H. laevigata* and *H. conicopora*) fishery. The scale of the WA Roe's Abalone fishery is unique within Australia given the only other commercial fishery for Roe's Abalone is a small-scale experimental/exemption fishery in South Australia (Western Zone Fishery with 11 t annual catch limit (Strain and Heldt 2019)). Roe's Abalone are distributed from Shark Bay in WA south around to western Victoria (Geiger and Owen 2012), although they are not uniformly distributed throughout this range and are most abundant on the south-west coast around Perth and Cape Naturaliste (Figure 3.1). They tend to inhabit the shallow coastal waters on intertidal reef platforms and shallow adjoining subtidal reef to about 10 m depth. In WA, Roe's Abalone have a maximum size of 89 mm shell length and reach size at maturity around 40 mm, but growth in Roe's Abalone varies significantly between populations (Hart et al. 2013).

The Roe's Abalone fishery is part of both the Abalone Managed Fishery (AMF, commercial) and the Western Australian Recreational Abalone Fishery, with the current management of these in accordance with the Abalone Resource of Western Australia Harvest Strategy 2016–21 (DoF 2017a). The AMF is divided into 8 spatial management areas with Roe's Abalone quota allocated in 6 of these areas (Figure 3.2). Commercial catches are managed primarily through Total Allowable Commercial Catches (TACCs) set annually for each management area. This is achieved through the harvest control rule, which uses the key performance indicator of a 3-year moving average of standardised catch per unit effort (SCPUE) against specified limit, threshold and target reference levels, to ensure sustainable take in the fishery (DoF 2017a).

The Roe's Abalone commercial fishery began first in WA around the mid 1960's but was part-time and focused on the Perth Metropolitan Fishery. After 1969 there was rapid expansion in both the Roe's and Greenlip/Brownlip Abalone fishery's with Roe's Abalone catch peaking at 170 t in 1971, after which it fluctuated before settling around 90 to 120 t between 1979 and 2010 (Figure 3.3). Since then the commercial catch has declined to 49 t in 2016 due to a combination of environmental (marine heatwave), economic (cost of accessing and prevailing weather conditions in regional areas) and market (competition against aquaculture product) conditions (Strain et al. 2018).

The recreational fishery is managed in three zones, Northern, Western and Southern (Figure 3.4), with a mix of input and output controls, including an abalone recreational fishing licence, size limits, daily bag and possession limits, and temporal and spatial closures. The focus of the recreational fishery is in the Western Zone which together with Area 7 of the AMF forms the Perth Metropolitan Roe's Abalone fishery. The Perth Metropolitan Fishery is the only abalone fishery in Australia managed through a stock prediction model, which uses the predicted recruitment (Age 1+) and a temperature factor to set the Total Allowable Catch (TAC) (Hart et al. 2018). The TAC is then separated into TACC and Total Allowable Recreational Catch

(TARC) using the available biomass in each habitat and each sector's patterns of usage (DoF 2017a).

Recreational catch in the Roe's Abalone fishery is significant with 46% of the 2016 season total catch (91 t) taken by recreational fishers (Strain et al. 2018). The recreational catch has also reduced on average since 2010, as catches generally ranged between 40 and 60 t from 1992 when estimates first became available (Figure 3.3). As the main component of the recreational fishery is located adjacent to the Perth metropolitan area there is high fishing pressure, subsequently strict management arrangements are enforced (described above). This highly restrictive management was further tightened as a result of stock sustainability issues attributed to adverse environmental conditions post 2010, principally with the TARC being set at 20 ± 2 t in the Western Zone.

The adverse environmental conditions that affected the Roe's Abalone populations post 2010 came in the form of an extreme (category 4) marine heatwave (Hobday et al. 2018) followed by a sustained period (several years) of elevated sea water temperature off the WA coast. Marine heatwaves are extreme warming events that have the potential to devastate marine ecosystems. The frequency of these heatwaves appears to be increasing in coastal waters (Lima and Wethey 2012) and given the ecological impacts associated (e.g. Caputi et al. 2016; Wernberg et al. 2016; Lenanton et al. 2017), these heatwaves are of significant concern for marine fisheries. In February-March of 2011 the sea surface temperatures (SST) increased to record levels off the west and south-west coast of WA. During this event the SST rose to more than 3^{o}C above long-term monthly averages (Figure 3.5) and for a 2-week period it peaked at 5^{o}C above normal (Pearce et al. 2011; Feng et al. 2013).

The 2011 marine heatwave affected numerous WA fish stocks other than Roe's Abalone. Some stocks experienced negative impacts such as declines in abundance of Ballot's Saucer Scallop and Blue Swimmer Crab in Shark Bay, Ballot's Saucer Scallop near the Abrolhos Is., Western Rock Lobster around Kalbarri and the recruitment of Yellow-eye Mullet on the west and south coast (Caputi et al. 2014). Other stocks experienced positive impacts, for example, increases in recruitment of silver bream on the west coast and abundances of tropical finfish species in the southern extent of their ranges (Caputi et al. 2014). Since this marine heatwave considerable work has been completed to identify the effects and potential risks of climate change on WA fish stocks and the implications for management (e.g. Caputi et al. 2015a & b).

The abalone stocks in the Kalbarri region (northern extent of the species distribution) suffered a devastating mortality event as a result of the marine heatwave, given this region was exposed to the peak effect of the heatwave (Figure 3.5). While the devastating mortality event occurred throughout the Area 8 fishery (Figure 3.2), it primarily affected a 40-mile stretch of coast north of the Murchison River. Surveys of Roe's Abalone populations immediately after the marine heatwave estimated survival rates to be 1 in 10,000 (0.01%) or less north of the Murchison River (Bald Face), while survival varied in regions south of the Murchison River like Lucky Bay (80-90%) and Port Gregory (5-10%) (Figure 3.6). Subsequently, Area 8 of the Roe's Abalone fishery was identified as one of the two most urgent management priorities for WA authorities stemming from the marine heatwaves impact (Pearce et al. 2011).

The variation in mortality rates of Roe's Abalone populations down the west coast of WA indicated spatial closures of affected areas should be considered to manage the fishery (Figure 3.6). The region north of the Murchison River historically produced 90% of the commercial catch in Area 8 prior to the mortality event. (Table 3.1). Given the Roe's Abalone population distribution in this part of WA as identified by the historical commercial catch and the extent of the mortality event, it meant there was limited stock biomass available to fishers. Consequently, both the commercial and recreational fisheries were closed immediately for the entire region. This closure took place from Moore River, north to the WA/Northern Territory border and is still in place.

The effect of the marine heatwave was not constrained to the mortality events in the northern distribution of Roe's Abalone in WA but was also felt in the Perth Metropolitan Fishery and regions further south. In the Perth Metropolitan Fishery there was a decline in large animals and recruitment immediately following the marine heatwave, with a decline in spawning biomass occurring in later years (Hart et al. 2018). In the Capes region (Area 6) anecdotal reports from commercial fishers indicated some small-scale localised areas of mortality following the marine heatwave (pers. comm. WCADA). Given the exposed nature of Roe's Abalone habitat to the elements and their sedentary nature, Roe's Abalone are unable to avoid unfavourable environmental conditions. Therefore, this species can be considered a key indicator of climate change, particularly regarding increasing ocean temperatures.

Before the marine heatwave in 2011 there were no known major environmentally limiting factors impacting on the Roe's Abalone population. The fishery was sustainably managed and even with fluctuations from year to year was expected to continue at pre 2010 harvest levels. The current stock status of Area 8 is deemed "inadequate" due to environmental conditions and as such the commercial and recreational fisheries are closed indefinitely. Given there are no Roe's Abalone populations left in the region north of the Murchison River, other management options were assessed to aid the recovery of the stocks. Restocking of Roe's Abalone was seen as having no negative impacts on the current fisheries performance and management arrangements. The only impact restocking could have on the wild population would be to establish founder populations that may aid in the populations recovery over time.

3.2 Need

This restocking initiative was in response to a catastrophic mortality event of a Roe's Abalone fishery in WA, due to an anomalous environmental event in the summer of 2010/11 (Pearce et al. 2011). During this event a sustained period of elevated SST rose to lethal levels for Roe's Abalone and effectively wiped out an entire stock in the Kalbarri region (Figure 3.6). The population (stock) has subsequently been closed to fishing to protect any remaining animals and promote natural recovery. Unfortunately, the severe extent of the mortality (>99.9%) means that natural recovery was seen as unlikely within the foreseeable future.

The need for this initiative was to examine whether recovery of this fishery can be assisted using the latest knowledge in translocation, restocking and enhancement methodologies. This incident provided an opportunity to test an important management strategy, namely will the establishment of founder populations be a viable tool for fishery restoration, particularly in

stocks like abalone that have localised recruitment? This can be determined by comparing natural and assisted recovery rates and evaluating the genetic contribution of existing and founder populations. Such a study is relevant to all Australian abalone fisheries and an integral part of understanding how fisheries populations can be sustained in a changing environment. Particularly, given one of the key predictions is increased environmental variability and average SST, with the lower west coast of WA classified as one of the hotspots of SST increases (Hobday and Pecl 2014), therefore having consequential effects of range contractions or extension of species at the edges of their natural range. This particular case represented a perfect example of this effect, as this abalone fishery (Area 8) was located at the northern end of the species range, and therefore vulnerable to this extreme environmental event. However, the rare occurrence of such an extreme event means that population recovery could potentially be possible prior to the next event.

Figure 3.1: Distribution of Roe's Abalone (*Haliotis roei*) around Australia (Shark Bay, WA to western Victoria).

Figure 3.2: Map showing the boundaries of the management areas in the commercial Abalone Managed Fishery in Western Australia. The Roe's Abalone fishery operates in Areas 1, 2, 5, 6, 7 and 8, other areas are associated with the Greenlip/Brownlip Abalone fishery.

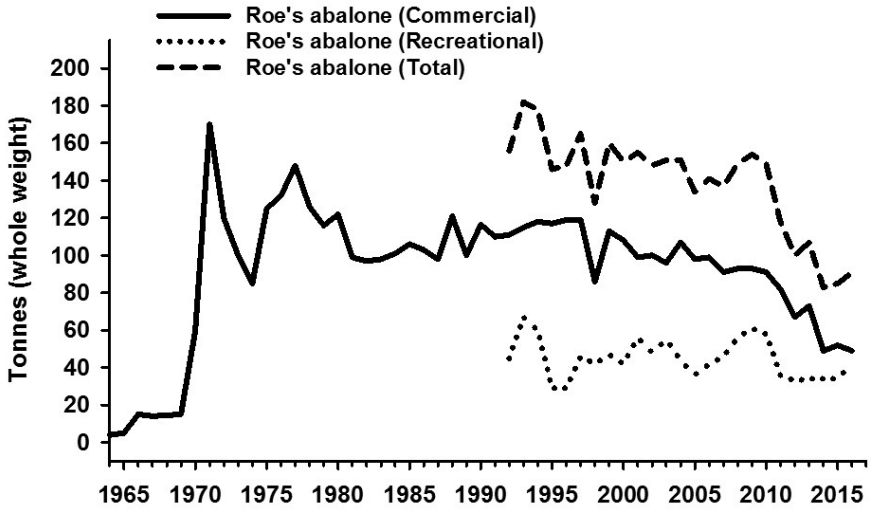

Figure 3.3: Historical commercial catch estimates (tonnes whole weight) from the Roe's Abalone fishery in Western Australia. Historical commercial catches (1969 to 1985) sourced from Prince and Shepherd (1992) and figure updated from Hart et al. (2013).

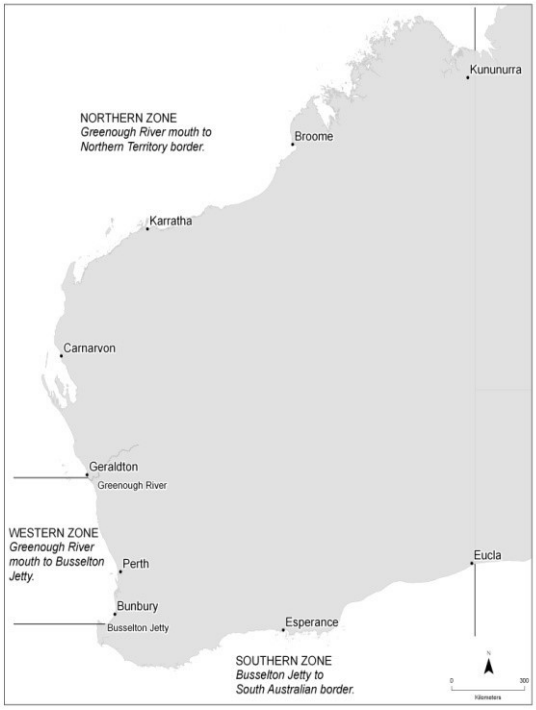

Figure 3.4: Map showing the boundaries of the three zones within the Western Australian Recreational Abalone Fishery; the Western Zone, the Northern Zone and the Southern Zone.

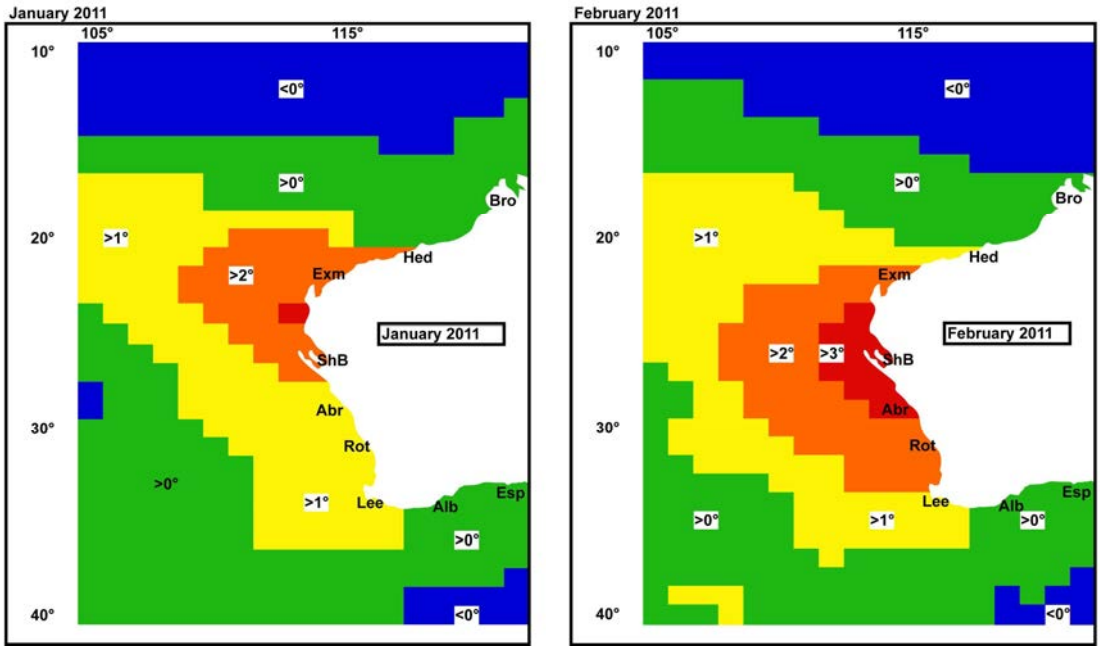

Figure 3.5: Monthly SST anomalies in the south-eastern Indian Ocean in January and February 2011 (at the peak of the heat wave) derived from the Reynolds SST dataset. Each coloured block, which is nominally 100 km * 100 km square, represents the difference between the monthly SST for that block and the long-term average for the month; the colour codes are: red >3°C anomaly; orange 2-3°C; yellow 1-2°C; green 0-1°C; blue <0°C (Sourced from Pearce et al. 2011).

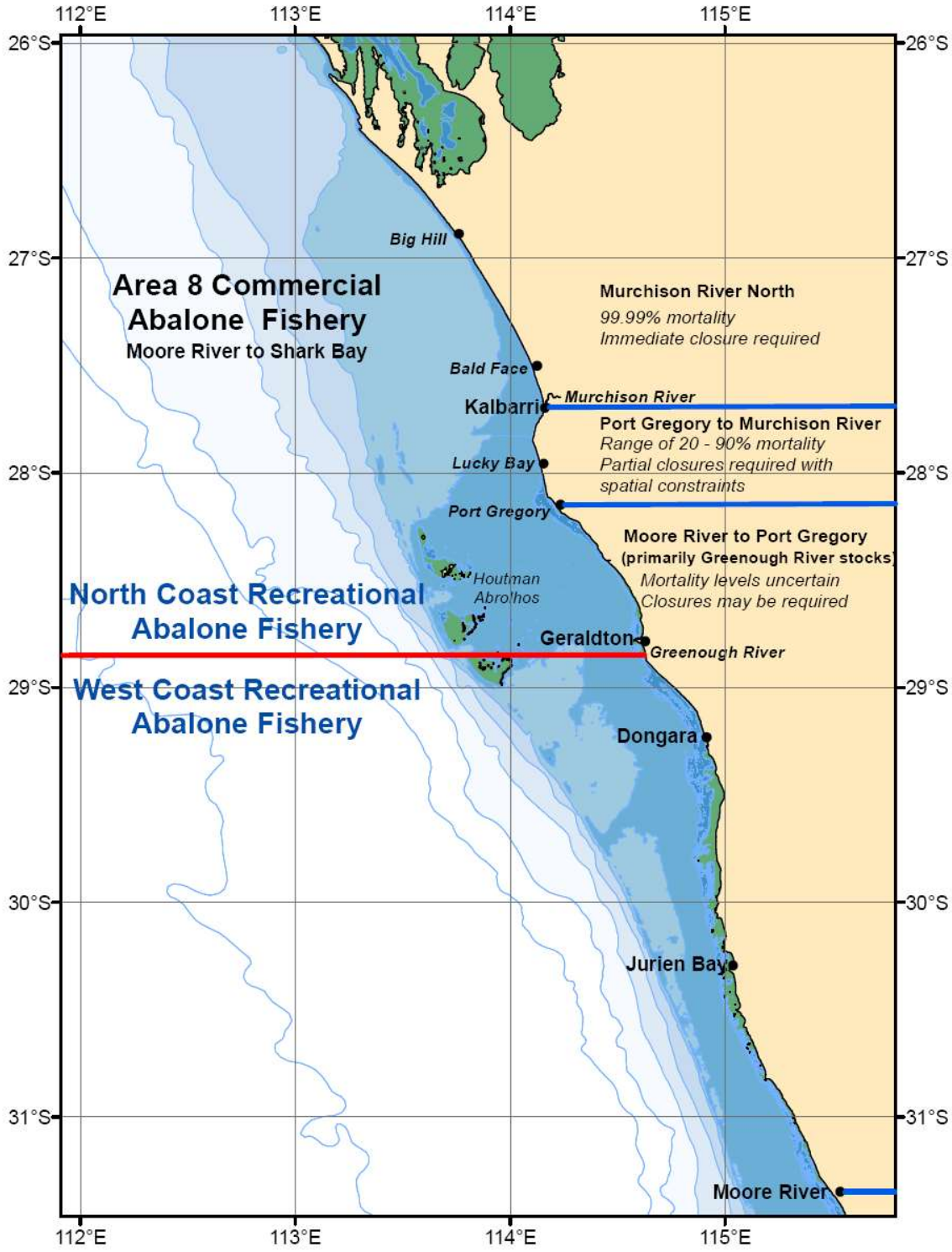

Figure 3.6: Map showing the mortality levels in various regions and the potential management options considered for the affected area within the Abalone Managed Fishery (Area 8) and the Western Australian Recreational Abalone Fishery (North and West Zones).

Table 3.1: Historical Roe's Abalone commercial catch (t) by region from north to south in Area 8 of the Abalone Management Fishery over the 20 years prior to the 2011 marine heatwave (1991-2010).

Area 8 Regions	Catch (t)	Percentage (%)
Big Hill to WA/NT Border	6	2
Murchison River to Big Hill	329	88
Port Gregory to Murchison River	9	2
Moore River to Port Gregory	29	8*
Total	373	100

* 5% of this region comes from the Greenough River stocks

3.3 Objectives

The primary objective of the project was to investigate the viability of recovering a collapsed Roe's Abalone population through the translocation of mature wild abalone together with restocking hatchery-reared juvenile abalone.

1. To establish founder populations of Roe's Abalone in areas of mass mortality.
2. To evaluate the genetic structure of existing and founder populations.
3. To compare natural and assisted recovery rates of Roe's Abalone populations.
4. To evaluate the genetic contribution of existing and founder populations to stock recovery.
5. Develop spawning protocols for Roe's Abalone and conduct a pilot juvenile stock enhancement release.

Note that the fifth objective was incorporated during the latter stages of the project.

Report Structure

To help address the objectives of the study, the report is structured according to a chapter format and is partitioned into the following distinct components (headings) for ease of navigation:

- **Stocking Founder Populations**: a restocking initiative to establish founder populations by translocating adult Roe's Abalone compared with natural population recovery. This section addresses objective 1 and 3 above.
- **Aquaculture Spawning and Restocking**: experimentation on aquaculture spawning and rearing protocols, and the release of the juvenile hatchery-reared Roe's Abalone at the founder populations. This section addresses objective 5 above.
- **Genomic Analysis**: a genomic study of Roe's Abalone to evaluate the natural population genetic structure and monitor the success of the restocking initiative. This section addresses objectives 2 and 4 above.

4 Stocking Founder Populations

This section addresses objectives 1 and 3, which are to establish founder populations of Roe's Abalone in areas of mass mortality and compare natural and assisted recovery rates of Roe's Abalone populations.

4.1 Material and Methods

4.1.1 Site Selection

To establish founder populations, reef platforms with suitable abalone habitat were identified from within a 30 km stretch of coastline, north of Kalbarri in WA. The reef platforms selected for sites were done so in order to accommodate an average of approximately 1-1.5 km between the founder populations. The rationale for this spacing was to maximise the probability of offspring encountering both adult abalone and suitable habitat for settlement after the short larval period (7-10 d), so as to provide connectivity between founder populations.

Control sites were established and surveyed to monitor the natural recovery of the abalone population. The control sites were located at the north and south end of the translocation sites to examine the direction of larval drift and whether it was capable of connecting populations greater than 1 km apart. Therefore, the locations of control sites were dictated by an equal allocation north and south of the translocation sites and greater than 3 km separation from both the nearest founder population and the other control site.

Translocation and control sites were identified through the assistance of experienced commercial fishers, with all sites having been commercial fished prior to the mortality event. The sites were selected according to 5 criteria;

1. Productivity – previous catch
2. Ease of access – by land and sea
3. Total abalone habitat area
4. Workability of the platform for releases and surveys
5. Distance to next founder population

Reef platforms that met the above criteria were surveyed, with particular attention paid to the platform size so that the abalone habitat was close to or less than 300 m^2. This area was used as a maximum to create founder populations of more than 1000 mature abalone at a density of greater than 3 abalone.m^{-2}. Given that there is a >95% decrease in fertilisation rates as abalone densities drop from 2 abalone.m^{-2} to 0.2 abalone.m^{-2} (Babcock and Keesing 1999), and viable breeding populations of greater than 500 animals are required for effective reproduction (Ryman and Laikre 1991; Tringali and Bert 1998), reef platforms capable of supporting but confining these parameters were utilised.

The final translocation and control sites selected within the study zone, which stretched along nearly 28 km of coastline located approximately 40 km north of Kalbarri, are illustrated in Table 4.1 and Figure 4.1. The 14 individual reef platforms (sites) with a combined habitat area

of 3,350 m^2 ranged in spatial separation within the study zone from 136 m to 12.2 km, with large sections of coastline either inaccessible or containing non-conducive habitat for abalone (Table 4.1). The sites were spread as evenly as possible along the study zone to encompass the main commercial fishing grounds (landing locations and access points). The four sites at either end of the 28 km Kalbarri Cliffs study zone were selected as controls. The two control sites (KTC-01 and KTC-02) established at the southern end of the study zone were the only reef platforms within considerable distance that had suitable abalone habitat and accessibility (Table 4.1). This was evident by the variation in distances between sites, particularly the 12.2 km between KTC-01 and KTC-02 given the 9.5 km stretch of coastline with no suitable abalone habitat. Even though the control sites were designed with a spatial separation of approximately 3 km, this was not always possible given the regions difficult terrain and remoteness (at least 1 km separation was maintained).

Table 4.1: The translocation (KT) and control sites (KTC; in bold) identified within the Kalbarri Cliffs study zone showing the fish ground, distance between sites and site area.

Site Code	Site Name	Fish Ground	Distance (m)	Site Area (m^2)
KTC-01	Bald Face (Sth)	Bald Face	-	100
	9.5 km of non-abalone habitat			
KTC-02	One Rock	4th Fence	12,200	300
KT-01	Ry's Stick		1,300	280
KT-02	Pilchard Patch		1,400	200
KT-03	Moon Craters		136	200
KT-04	Sth Old Shack	Old Shack	521	300
KT-05	Nth Old Shack		446	120
	3 km gap (inaccessible)			
KT-06	Pilchard Patch Sth	Paper Track	3,200	250
KT-07	Car Park		778	160
KT-08	Dune on Beach		345	240
KT-09	Pilchard Patch Nth		949	250
KT-10	5th Fence	5th Fence	738	400
KTC-03	Nth 5th Fence		3,000	250
KTC-04	Sth Hajduk	Hajduk	2,700	300
Total			27,713	3,350

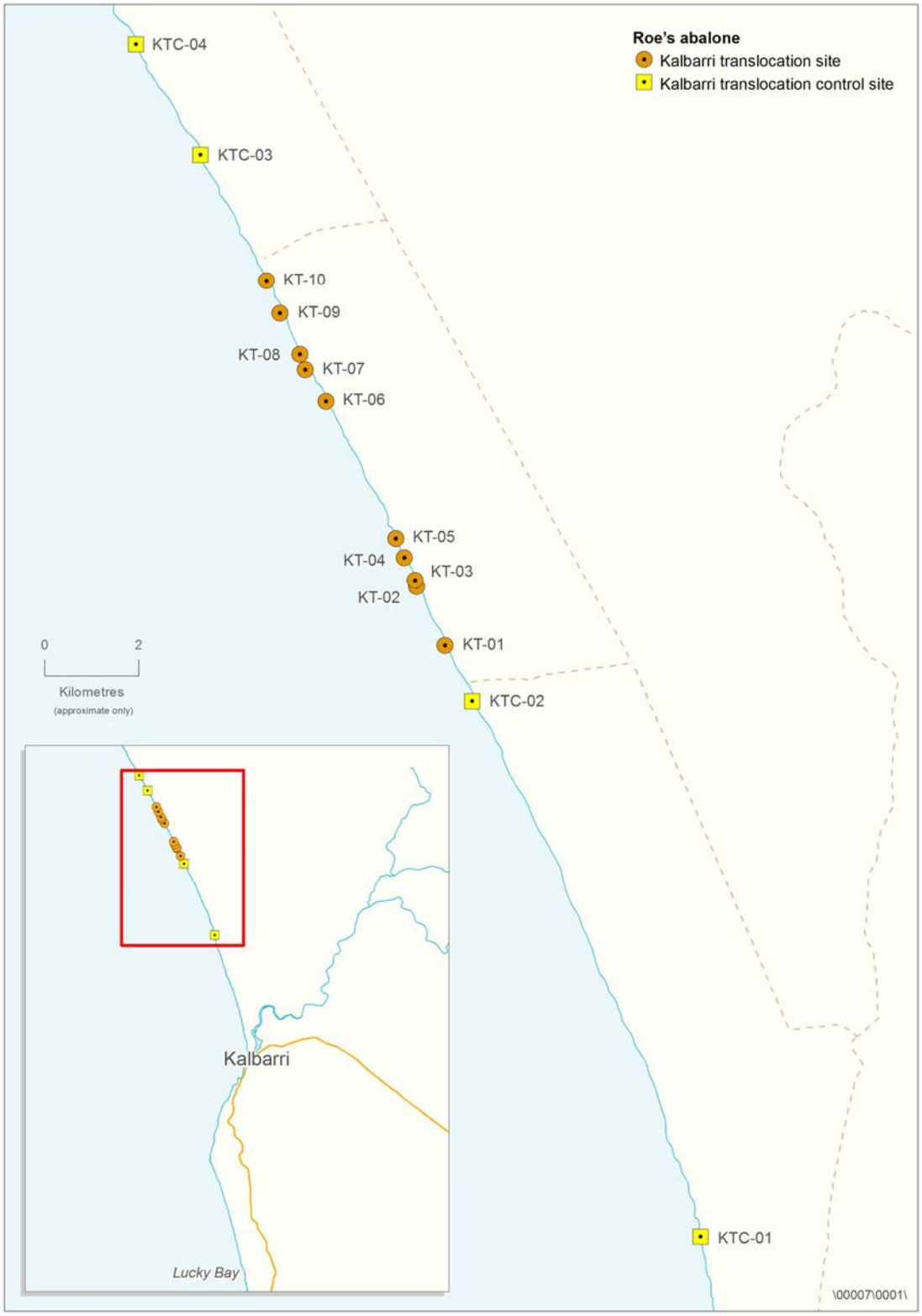

Figure 4.1: A map of the study zone north of Kalbarri with the 10 translocation (KT) and 4 control (KTC) sites identified, as well as one of the source population locations at Lucky Bay.

4.1.2 Survey Techniques

All translocation and control sites had baseline surveys conducted (October 2011 to March 2012) to establish the severity of the mortality event and determine the number and size of remnant Roe's Abalone populations. Monitoring of all sites occurred at least once a year, with the translocation sites re-surveyed immediately prior to any Roe's Abalone being released onto the reef platforms. All surveys were carried out at two periods of the year, February to March and October to December. The environmental conditions; tide, swell, wind and temperature were recorded for the time at which each release of Roe's Abalone and site surveys were conducted.

Two survey methods were utilised to assess translocation success and stock recovery. A transect survey was performed at both control and translocation sites, whilst a count survey was used at the translocation sites post release of abalone. The two survey methods were utilised given the variability in site/platform size, number and size of abalone released/present and the area the survey method sampled. Density of abalone was calculated from the number of abalone present on the platform within a given area (m^2) as defined by the survey method.

Transect Survey Method

After the site area had been defined, the midpoint of the platform on the landward side was marked (GPS position) and indicated the start of the master transect (Figure 4.2). A 10 m transect rope was run perpendicular to the ocean from the midpoint to the outer edge of the platform (ocean side). This section of the platform was surveyed given the settlement zone for this species is highly restricted to a thin band of habitat (5-10 m) on the outer edge of limestone platforms (Keesing et al. 1995). The transect consisted of four, 0.25 m^2 quadrats positioned south and west of regular increment marks on the rope and weighted down by three, 9 lb dive weights (either end and one in the middle). All abalone within each quadrat were recorded and measured using a Roe's Abalone gauge. Depending on the depth of the water on the platform a scope was generally required to count the abalone, as shown in Figure 4.3.

At the completion of the master transect the increment marks on the transect rope were used to measure the determined distance to the start of next transect and then between subsequent transects. Transects had even spacing between them that was dictated by the size of the platform, with 2 transects north and 2 transects south of the master transect. Several of the translocation sites had a reduced number of abalone released and as such the area of platform seeded was reduced to achieve the same stocking density across all translocation sites. At these sites two additional transects were incorporated (one north and one south) equal distances between the master and the next closest transect (Figure 4.2).

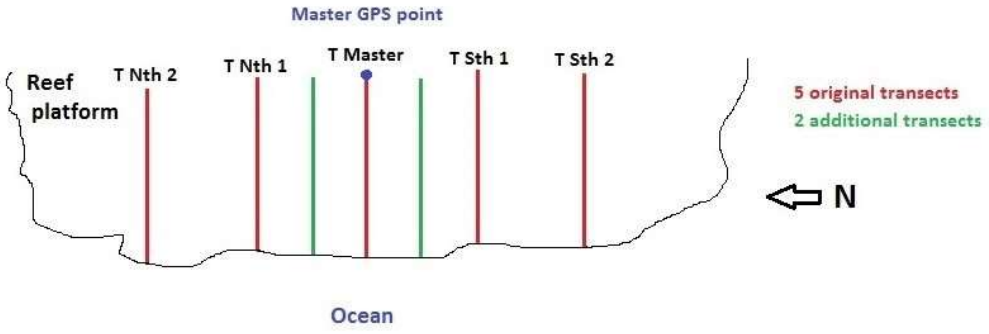

Figure 4.2: A diagram representing the layout of the transect survey method performed at all sites within the Kalbarri Cliffs study zone to record the number of Roe's Abalone present on the reef platforms.

Figure 4.3: A photo illustrating the transect survey method including the scope and metal 0.25 m^2 quadrat used to survey Roe's Abalone on the reef platforms at all sites within the Kalbarri Cliffs study zone.

Count Survey Method

At the translocation sites a total count of all abalone present on the reef platform was conducted. The site was divided into 3 sections from east to west across the platform (Figure 4.4). These sections were then traversed and any Roe's Abalone present, counted and the total number of abalone recorded. During this survey, clusters of Roe's Abalone were identified. Clusters were defined as having at least 5 abalone congregated together in close proximity (i.e. within 10 cm of each other). Each cluster meeting this criterion then had the number of abalone present and the size (dimensions) of the cluster recorded.

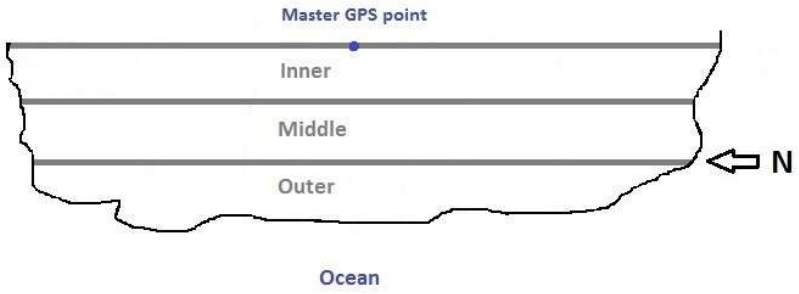

Figure 4.4: A diagram representing the layout of the count survey method performed at translocation sites post-release of Roe's Abalone.

4.1.3 Environmental Conditions

To quantify the prevailing weather conditions at the translocation sites within the Kalbarri Cliffs study zone when either translocations or site surveys were conducted, an index was developed based on the index used to estimate the catch in the Perth Metropolitan Recreational Abalone Season (Hancock and Caputi 2006). Given the translocation sites consist of reef platforms on an exposed coastline, wave and tide height as well as wind were considered the driving environmental conditions. Wave heights (both wind-driven and swell height) and tide levels (actual, predicted and residual) for the Geraldton recording station were obtained from the Geraldton Port Authority. Wave heights for Cape Naturaliste and Jurien were acquired from the Department of Transport, while wind direction and strength as well as ambient air temperature were recorded from the Kalbarri weather station, Bureau of Meteorology.

4.1.4 Roe's Abalone Translocation

The founder populations created at the translocation sites were comprised of Roe's Abalone (>50 mm shell length) sourced from the nearest viable populations. The source location was dependent on the extent of the marine heatwaves effect on populations south of the study zone. The final source locations were chosen so as to not place greater pressure on already affected Roe's Abalone populations. The translocated animals were harvested from the source locations by current commercial practices, then transported to the translocation sites within the study zone and released during the best possible weather conditions. Two source locations were utilised and all details of the translocation methodologies are summarised in Table 4.2.

Pilot Transport Experiment (Pilot 1)

Adult Roe's Abalone were collected from a commercially productive reef within the Perth Metropolitan Fishery (Area 7). Divers on Surface Supply Breathing Apparatus (SSBA) using a modified abalone iron, "chipped" (prised) the abalone from the substrate of both the subtidal and platform sections of the reef (depth 0-3 m). The modified abalone iron utilised was a flathead screwdriver that had the head flattened further to create a splayed end. The abalone were then checked for condition, stacked into shallow plastic trays lined with hessian that had been thoroughly washed and soaked in seawater (Table 4.2). The abalone filled trays were then stacked into a large cooler box and periodically (45 min) doused with seawater (10 L). Ice bricks were placed on the bottom of the cooler box to lower the ambient temperature within, to help reduce the stress the abalone experienced during transportation. The abalone were then

removed from the trays and stocked into two, 400 L coffin tanks at the Western Australian Fisheries and Marine Research Laboratories (WAFMRL) aquaculture facility to monitor the abalone condition post simulated transportation. The tanks were supplied flow through, filtered (25 µm) seawater at 5 $L.min^{-1}$ and the water aerated constantly from three airlines on the bottom of the tank. Maintenance of the tanks occurred twice a week, which involved draining the seawater, removing any dead animals if required, cleaning the tanks and refilling. Abalone were fed directly after tank maintenance, a mixture of green and red macroalgal species (*Ulva*, *Gracilaria*, *Laurencia*, *Plocamium*, etc.) collected weekly from nearby reef platforms.

Source Location for Founder Populations – Lucky Bay

The source location at Lucky Bay is situated approximately 70 km south of the Kalbarri Cliffs study zone (Figure 4.1). The methods for translocating Roe's Abalone from Lucky Bay to the Kalbarri Cliffs (Table 4.2) can be separated into 3 sections; harvest, transport and release.

Harvest at Lucky Bay

To harvest the adult Roe's Abalone from the reef platforms at Lucky Bay, commercial fishers waded onto the platform and using the modified abalone iron chipped the abalone from the platform substrate. The fishers took great care in handling the abalone (not cutting the muscular foot, etc.) to limit the stress the abalone suffered during harvest. The small number of damaged Roe's Abalone were not used for translocation.

Transport Method

Once harvested from the reef platform and checked for condition, the adult Roe's Abalone were stacked into shallow plastic trays (approximately 225 abalone per tray). The transport method followed that of the Pilot Transport Experiment (above) with a few minor alterations. Containers of seawater (20 L) for dousing during transport were filled at Lucky Bay, while the cooler box was drained of seawater before every dousing period so that no abalone were left sitting in static seawater (reduced oxygen concentration) at the bottom of the cooler box.

Release Method

Once the adult Roe's Abalone had been transported to the translocation site within the Kalbarri Cliffs study zone, the trays with stacked abalone were transferred from the cooler box into a hessian bag that had been soaked in seawater. The abalone were transferred to the translocation site via quad bike and placed into a cool rock pool to acclimatise to the seawater temperature. Surveys of the translocation site were conducted while the abalone were acclimatising and once completed, the abalone were removed from the plastic trays within the hessian bag and placed on the reef platform. Placement of abalone onto the platform was carefully done by hand, into appropriate topography such as smooth cups, moon holes, shallow runs and depressions within the site area.

Pilot Module Release Experiment (Pilot 2)

A release module was tested that consisted of modifying a commercial Octopus trigger trap, which has 3 self-closing pots attached to a metal cradle (Hart et al. 2016). The modification included attaching a ramp to the trap opening to allow the abalone a surface to move down onto the substrate, holes in the trap to allow greater water movement through the trap and the removal of the trap closing mechanism. Adult Roe's Abalone collected from within the Perth

Metropolitan Fishery were placed in the release module, with 60 abalone in one pot and 120 in another pot. The release module was placed on the reef platform where abalone collection had occurred and after 1 h the trap doors opened, then monitored over a 24 h period (Table 4.2).

Source Location for Founder Populations – Perth Metropolitan Fishery

The source location within the Perth Metropolitan Fishery is situated about 750 km south of the Kalbarri Cliffs study zone. The methods for translocating Roe's Abalone from the Perth Metropolitan Fishery to the Kalbarri Cliffs study zone (Table 4.2) can be separated into 4 sections; harvest, holding in aquaculture, transport and release.

Harvest at Burns Beach

Adult Roe's Abalone were harvested from Burns Beach, a productive reef platform within the Perth Metropolitan Fishery. The abalone were chipped by divers on SSBA (as in the Pilot Transport Experiment), checked for condition (cuts etc.) and size, then placed in onion bags (60 abalone per bag) and transported under seawater soaked hessian by boat to the WAFMRL.

Holding in Aquaculture

The harvested adult Roe's Abalone were removed from the onion bags and stocked into 400 L coffin tanks at the WAFMRL. Husbandry followed the protocols described in the Pilot Transport Experiment.

Transport Method

Two transport systems were used to translocate the adult Roe's Abalone from the Perth Metropolitan Fishery to the Kalbarri Cliffs study zone;

1) Trays with Hessian. Once the abalone had been harvested from the coffin tanks and checked for condition, they were then stacked in shallow plastic trays, the trays lined with hessian soaked with seawater and placed in the cooler box (Figure 4.5a). This transport method followed the procedure described in the Pilot Transport Experiment. However, there were some minor alterations with the transport occurring at night rather than during the day, and the dousing with seawater occurred every 90 min for the first 5 h of transport, then hourly for the next two hours and every 45 min between Kalbarri and the Kalbarri Cliffs study zone.

2) Full Seawater with Aeration. The adult Roe's Abalone were harvested from the coffin tanks, checked for condition and then placed in onion bags (60 abalone per bag). The abalone filled onion bags were distributed into 4 crates, which were stacked in the cooler box filled with filtered seawater (25 µm). Constant aeration was provided to the seawater by a 12-volt air pump via two airlines on the bottom of the cooler box (Figure 4.5b). Transport occurred at night with the pumps function checked regularly and after 4 h of transport, 50% of the seawater within the cooler box was exchanged with fresh seawater from containers that had been filled at the WAFMRL.

Release Method

The release method differed depending on which of the 2 transport methods had been used (Table 4.2). The release of abalone transported via the shallow plastic trays lined with hessian method followed the same procedure as the Roe's Abalone translocated from Lucky Bay to the Kalbarri Cliffs study zone.

a) b)

Figure 4.5: The two transport systems used to translocate Roe's Abalone from the Perth Metropolitan Fishery to the Kalbarri Cliffs; a) trays with hessian and b) full seawater with aeration.

A different approach was used for the release of abalone transported via the full seawater with aeration method. Upon arrival at the fishing grounds within the Kalbarri Cliffs study zone, all abalone were transferred in their onion bags from the cooler box into seawater soaked hessian sacks and placed in a crate. The crates were then transported via quad bike to the translocation site and placed into cool rock pools. Site surveys were conducted as the abalone acclimatised, upon completion the abalone were removed from the crates, hessian sack and onion bags and released by hand into appropriate topography within the translocation sites defined boundaries. The modified Octopus trigger trap release module was trialled with one third of the Roe's Abalone loaded into 2 release modules (Figure 4.6a) and then these modules placed on the reef platform in positions that allowed the abalone to migrate out onto the substrate by themselves (Figure 4.6b). The day after release the modules were checked to see if the Roe's Abalone had managed to move out of the module, if any abalone had not left the module these were removed and placed on the reef platform by hand.

a) b)

Figure 4.6: The modified Octopus trigger trap release module trialled to release adult Roe's Abalone at the translocation sites in the Kalbarri Cliffs study zone; a) loading the abalone into the module, b) module placed in position on the reef platform.

Table 4.2: Summary of all translocations of mature wild Roe's Abalone completed to the Kalbarri Cliffs study zone and the pilot experiments into methodology development, shown in a chronological order.

Translocation	Site	Release Date	Source Location	Harvest Days Prior	Transport Day	Transport Start Time	Transport End Time	Release Completed	Time Out Of Water
Pilot 1	-	-	Perth Metro	0		1000	1500		5:00
1	KT-10	27/10/2011	Lucky Bay	0	Same Day	0545	1240	1315	6:55
		28/10/2011	Lucky Bay	0	Same Day	0540	1115	1215	5:35
2	KT-01	25/03/2012	Lucky Bay	0	Same Day	0640	1400	1515	7:20
3	KT-07	2/11/2012	Lucky Bay	0	Same Day	0550	1130	1240	5:40
4	KT-09	16/11/2012	Lucky Bay	0	Same Day	0600	1130	1330	5:30
5	KT-05	5/12/2012	Lucky Bay	0	Same Day	0520	1130	1300	6:10
6	KT-01	13/02/2013	Perth Metro	3	Day Prior	1900	0715	1000	12:15
Pilot 2	-	9/09/2013	Perth Metro	0	-	-	-	-	-
7	KT-01	21/11/2013	Perth Metro	73	Day Prior	2000	0720	0910	0:20

Table 4.2 continued: Summary of all translocations of mature wild Roe's Abalone completed to the Kalbarri Cliffs study zone and the pilot experiments into methodology development, shown in a chronological order.

Translocation	Release Method	Transport Device	Transport Method	Abalone Size	Abalone Number	Method Ref (page)
Pilot 1	-	250L cooler / 4 trays	Hessian	-	192	20
1	Hand	250L cooler / 4 trays	Hessian	Adult	902 901	21
2	Hand	400L cooler / 7 trays	Hessian	Adult	1463	21
3	Hand	400L cooler / 4 trays	Hessian	Adult	836	21
4	Hand	400L cooler / 4 trays	Hessian	Adult	852	21
5	Hand	400L cooler / 4 trays	Hessian	Adult	807	21
6	Hand	400L cooler / 7 trays	Hessian	Adult	1481	21
Pilot 2	Octopus Trap	-	-	Adult	180	21
7	Hand / Octopus Trap[#]	400L cooler / 4 crates	Full Water	Adult	1700	22

[#]Animals released by hand the following day.

4.2 Results and Discussion

4.2.1 Baseline and Control Site Surveys

During site selection, baseline surveys were conducted at 11 of the 14 sites with only 1 adult Roe's Abalone found at KT-02. The 4 control sites were re-surveyed every 6 months for 4 years then annually for 2 years, with only 3 adult Roe's Abalone found at 2 of the control sites over the 5.5 year period (Table 4.3). Given the 4 isolated Roe's Abalone, found over multiple sites and a 5.5-year sampling period, were all adults (>50 mm shell length) and therefore remnant animals, this indicated that no natural recovery of abalone populations within the Kalbarri Cliffs study zone has occurred. Note, three of the 14 sites identified were not surveyed at all (KT-04, KT-06 and KT-08), while 2 sites were not utilised post baseline surveys (KT-02 and KT-03). This was due to accessibility issues and concerns over personnel safety on the reef platform in this harsh and remote location.

Table 4.3: Number of adult Roe's Abalone (*Haliotis roei*) present (density of abalone on platform (m^2)) on 10 surveys completed at the control sites within the Kalbarri Cliffs study zone.

Survey	Date	KTC-01	KTC-02	KTC-03	KTC-04
Baseline	Mar 2012	0	0	0	0
1st	Nov 2012	0	0	0	0
2nd	Mar 2013	0	1 (0.003)	0	0
3rd	Nov 2013	0	0	0	0
4th	Mar 2014	0	0	0	0
5th	Nov 2014	0	0	0	1 (0.003)
6th	Feb 2015	0	0	0	0
7th	Nov 2015	0	0	0	0
8th	Dec 2016	0	0	0	0
9th	Dec 2017	0	1 (0.003)	-	-
Total		0	2	0	1

4.2.2 Roe's Abalone Translocation

Translocation Survival

Overall 5 founder populations were established with nearly 9,000 adult Roe's Abalone translocated from two source locations, Lucky Bay (5,761 abalone) and the Perth Metropolitan Fishery (3,181 abalone). The translocation survival of the released abalone for each translocation event varied greatly from 0.24 to 35.36 % (Table 4.4). Given none of these 5 translocation sites had Roe's Abalone present at the baseline survey and only 4 remnant abalone were found during the 5.5 years' worth of control surveys, all abalone surveyed on the translocation site platforms were considered translocated abalone. To estimate the translocation survival, the total count results were standardised to a 1-year time at liberty. The survival was

standardised to deal with the variation in time at liberty between release and the first recapture for different translocations, as well as the variation in weather conditions between the 2 sampling periods each year. The first recapture was not always used for the translocation survival estimate and two assumptions were made when selecting which total count to analyse. The first assumption was to utilise the recapture closest to 1-year time at liberty for all translocations, so as to reduce the variation in the time at liberty when comparing translocation events. The second assumption was to utilise the recapture surveys that occurred in the October to December sampling period. This was done because the October to December sampling period provided significantly better weather conditions in which to conduct the surveys. The average tide experienced when either translocating or surveying at the sites within the Kalbarri Cliffs study zone during the October to December sampling period was significantly lower than that during the February to March sampling period, while the average swell was not significantly different between the two sampling periods (Table 4.5). This was supported by 80-90% of the commercial fishing days recorded occurring in October to December when the Area 8 fishery, which includes the Kalbarri Cliffs study zone, was open to fishing pre-mortality event (Strain, unpublished data). Actual survival could be slightly higher as probability of recapture was never 100%, given the possibility of abalone migration off the front of the platform to a limited amount of habitat that was inaccessible to survey.

The variation in translocation survival was not site dependent with three translocations performed at KT-01 producing survival rates of 0.24, 8.58 and 35.36%, using both transport methods and abalone from both source locations (Table 4.4). The translocations using adult Roe's Abalone sourced from Lucky Bay ranged in survival from 4.37 to 28.51% and may have been affected by weather conditions during harvest and release. The Roe's Abalone translocated from the Perth Metropolitan Fishery to KT-01 resulted in the lowest (0.24%) and highest (35.36%) translocation survival, indicating an effect from the different transportation methods (Table 4.4).

Table 4.4: Survival (%) of adult Roe's Abalone (*Haliotis roei*) 1 year post-release, compared with the number of abalone translocated from the two source locations to the sites within the Kalbarri Cliffs study zone. See methods section (Table 4.2) for full description of transport methods.

Translocation	Source Location	Transport Method	Release Site	Abalone Released	Survival (%)
1	Lucky Bay	Hessian	KT-10	1803	9.52
2	Lucky Bay	Hessian	KT-01	1463	8.58
3	Lucky Bay	Hessian	KT-07	836	28.51
4	Lucky Bay	Hessian	KT-09	852	4.37
5	Lucky Bay	Hessian	KT-05	807	12.84
6	Perth Metro	Hessian	KT-01	1481	0.24
7	Perth Metro	Full Water	KT-01	1700	35.36

Table 4.5: The average tide (m) and swell (m) experienced during surveys of the sites within the Kalbarri Cliffs study zone for the two sampling periods. Data are actual tide and swell recorded by the Geraldton Port Authority at the time of surveys, averaged if multiple surveys occurred on the same day. One-way ANOVA indicated significant differences (p<0.05) between sampling periods. Mean ± std. error (n=14).

Sampling Period	Tide (m)	Swell (m)
February – March	0.86 ± 0.06	0.84 ± 0.04
October – December	0.54 ± 0.04	0.80 ± 0.08
df	1, 12	1, 12
F	20.961	0.235
p value	<0.05	0.632

Weather Influence on Translocation Survival

The prevailing weather conditions influence during the release of adult Roe's Abalone onto the reef platforms was examined due to the range in survival recorded 1-year post-release. The index used to define the weather conditions was a scaled, ranking system and assessed the severity of the actual tide, swell and wind during the time of release at the translocation sites within the Kalbarri Cliffs study zone (Hancock and Caputi, 2006). This weather index was compared to the translocation survival for all releases (Figure 4.7a) and produced a negative linear relationship that was not significant (F=2.063, p=0.210). Given the 7 translocations included abalone from 2 different source locations using different translocation methodologies, the weather index was also compared for the 5 translocations of abalone sourced from Lucky Bay and transported using the same methodology (Hessian) (Figure 4.7b). This produced a stronger negative relationship (exponential) than for all translocations but was still not significant (F=2.053, p=0.247). This index has been used to examine the influence weather has on the recreational Roe's Abalone catch in the Perth Metropolitan Fishery, producing a significant relationship where the catch decreases linearly with an increase in the weather index (Hart et al. 2013). This indicated that the conditions people experience when fishing for Roe's Abalone directly affect their catch rate on the reef platforms.

Even though the comparison between weather index and translocation survival was not significant due to the small sample size, the influence of the weather on the success of the translocations was still considered an important factor by the personnel conducting the releases. The reef platforms where the releases occurred are situated in an extremely harsh environment and exposed to severe weather conditions (swell, etc.), so personnel's safety in this remote area was of paramount concern. Therefore, the weather conditions experienced at the Kalbarri Cliffs study zone still played an important role in when translocations could occur, and measures were taken to conduct translocations in the best possible weather conditions to not only ensure personnel safety but potentially improve the survival of released abalone in the future.

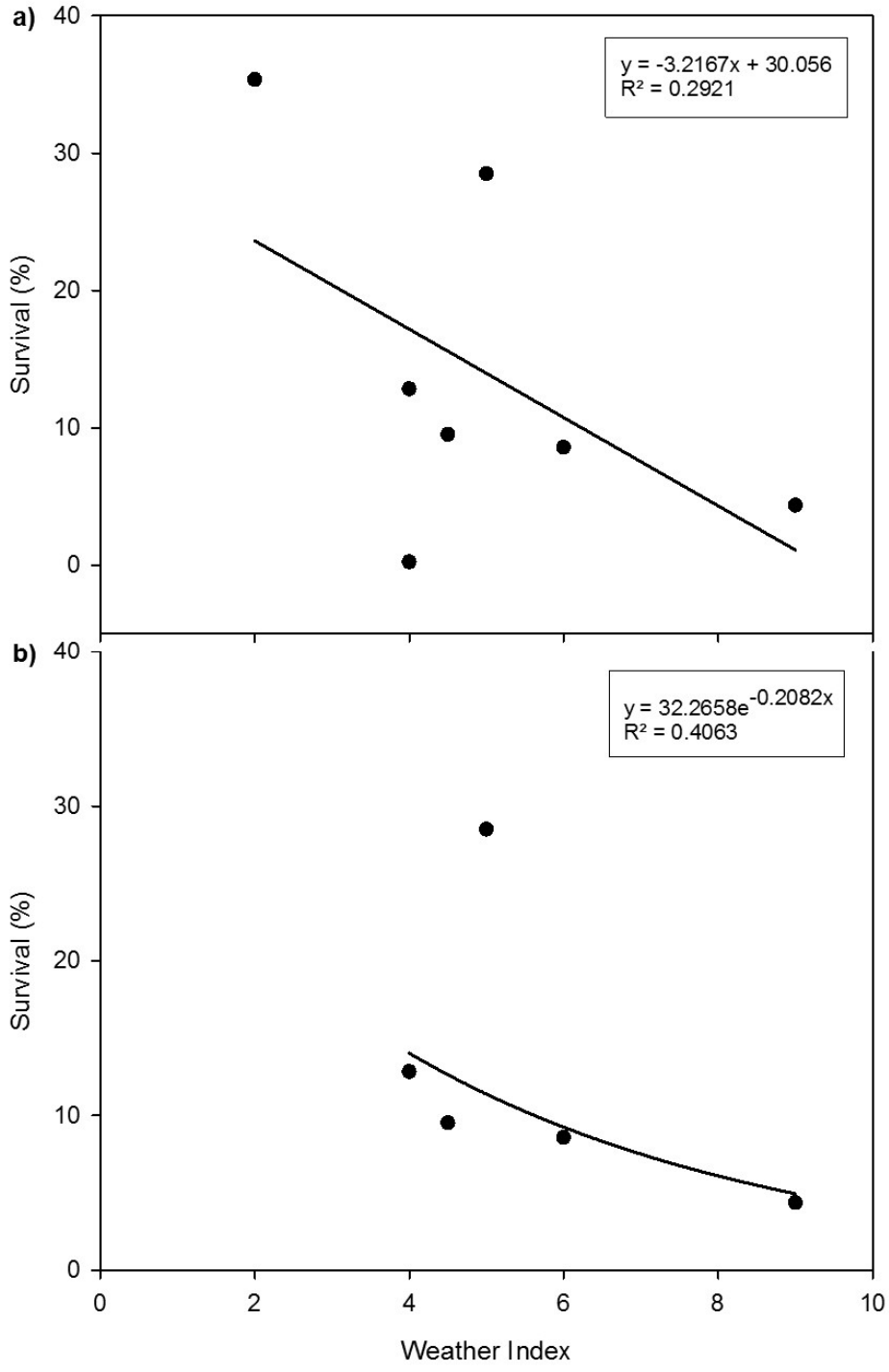

Figure 4.7: Survival (%) of adult Roe's Abalone (*Haliotis roei*) 1 year post-release as a function of the Weather Index (low index=good conditions) observed during release at the sites within the Kalbarri Cliffs study zone. a) Translocations using both transport methods, trays with hessian and full seawater with aeration, b) Only translocations using abalone sourced from Lucky Bay using the trays with hessian transport method.

Comparison of Translocation Methods

To develop appropriate translocation methodologies, pilot experiments were conducted to examine aspects of both the transport and release methods (Table 4.2). In the Pilot Transport Experiment, the trays with hessian transport method was tested prior to the translocation events, with all adult Roe's Abalone used in the replicated transport conditions surviving and none showed adverse effects for 7 d in culture. The pilot Module Release Experiment examined the use of a module to improve the efficiency of adult Roe's Abalone release. This was based on experience from the first 6 releases, in that the hand release method relies on a very limited number of days a year with suitable weather conditions to release Roe's Abalone at the sites within the Kalbarri Cliffs study zone. This release module was designed to withstand less favourable weather conditions and therefore, allow abalone to be released at the translocation sites on a greater number of days per year. However, the modified Octopus trigger trap module was only able to be stocked with 100 abalone in each pot, as the door could not be opened when the pots were stocked with 120 abalone. The module remained in position on the Perth Metropolitan Fishery reef platform and only 18 abalone were still inside or on the module 24 h after release. The outcomes of these pilot experiments influenced the transport and release methodology for the translocation events depending on the source population locations and logistical requirements.

The translocation methodology (Table 4.2, KT-10) of trays with hessian was able to translocate adult Roe's Abalone to the harsh and remote conditions at the Kalbarri Cliffs study zone. Prior to the translocation there were no Roe's Abalone present at KT-10 but 1 year post-release they were present on the reef platform (Table 4.4). However, as each translocation was performed and new information gathered the methodologies for harvest, transport and release evolved over time. The evolution of these methods can be seen in the method summary table (Table 4.2), and was particularly evident with the Perth Metropolitan Fishery source location, full seawater with aeration translocation to site KT-01 and the resultant increase in translocation survival (Table 4.4).

The trays with hessian transport method was considered suitable for the translocations from Lucky Bay. This was because there was nowhere to adequately fill a large cooler box (400 L) with clean filtered seawater, the weight would have been an issue on the sandy beach and the distance to the translocation sites was not far given it was the closest population to the Kalbarri Cliffs study zone. However, when the same methodology was used to transport adult Roe's Abalone from the Perth Metropolitan Fishery to the Kalbarri Cliffs study zone, only 0.24% of the abalone survived to 1 year post-release (Table 4.4). In fact, when the abalone were taken out of the cooler box for release some had already decomposed, while a large portion had a ridged muscular foot and were in poor condition. The full seawater with aeration transport method was developed to overcome this issue and proved to be successful with a 35.36% survival of adult Roe's Abalone after 1 year (Table 4.4). The abalone appeared to be healthy and active when they arrived at the translocation site and the water turbidity inside the cooler box less than the previous translocations. Given the abalone transported from the Perth Metropolitan Fishery were required to be collected at least a day prior to transport, the amount of time the abalone were out of their natural environment (held in aquaculture facilities) could adversely affect their survival (Table 4.2). However, this didn't appear to be the case as the

translocation with animals out of their natural environment for the longest time period resulted in the highest survival (35.36%), while releases completed on the same day as harvest produced a range in survival from 4.37 to 28.51% (Table 4.2 and Table 4.4).

Effect of Emersion Time on Survival

The key difference between the two transport methods was the amount of time the abalone spent out of water dependent on the source population locations. During the translocations from Lucky Bay to the Kalbarri Cliffs study zone the abalone spent on average 6:50 h out of water (only being doused periodically). Between the WAFMRL (source location Perth Metropolitan Fishery) and the Kalbarri Cliffs study zone the abalone spent 12:15 h out of water when transported with the trays and hessian method, and 0:20 h in the full seawater with aeration method (Table 4.2). The time spent out of water was compared with the instantaneous mortality based on the translocation survival and produced a significant relationship (exponential) with an R^2 of 0.87 (Figure 4.8, F=13.55, p<0.05). This indicated that when the transport method of full seawater with aeration can be utilised (dependent on source population location and logistics) it was considered the most suitable transport method for translocation.

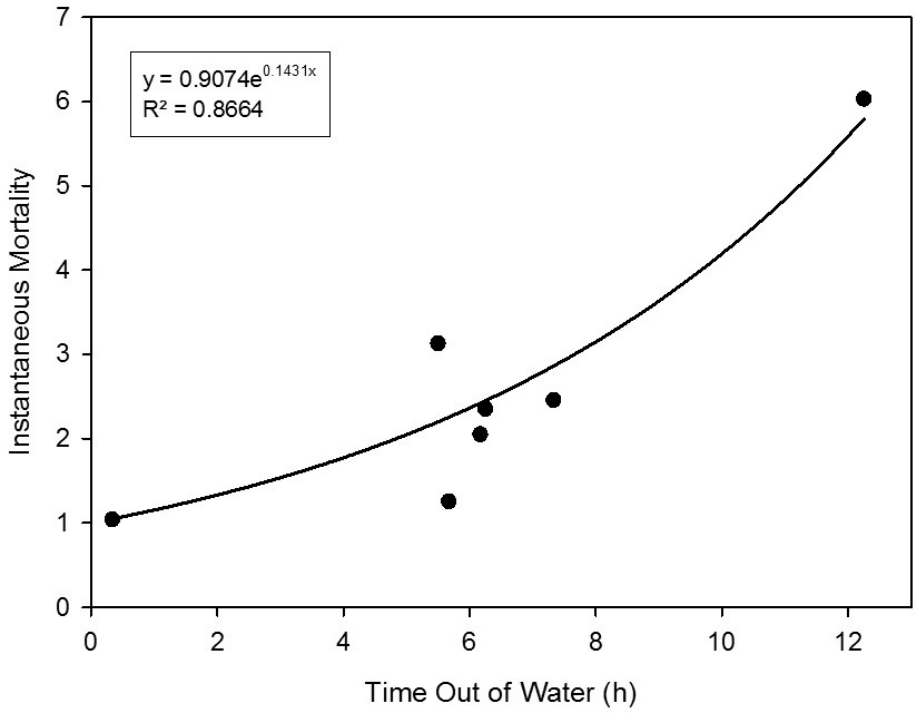

Figure 4.8: Instantaneous mortality based on the translocation survival (1 y post release) as a function of the time the adult Roe's Abalone (*Haliotis roei*) spent out of water during transport from the source locations to the release sites within the Kalbarri Cliffs study zone.

Effect of Release Method on Survival (hand versus module)

The use of a release module to improve the translocation survival and allow a greater number of days to be accessible for release at the Kalbarri Cliffs study zone did not result in the intended improvements in methodology (Table 4.2, translocation 7). Of the 560 adult Roe's Abalone that were loaded into the 2 Octopus trigger trap modules used at KT-01, 70% were still residing in the modules 24 h later. To compound this issue both modules had been washed 20 m up the platform and were sitting upside down in only 100 mm of seawater. The animals remaining in the models appeared healthy and were released by hand onto the reef platform. Therefore, hand release remains the most appropriate method of releasing adult Roe's Abalone onto the reef platforms and more experimentation into release modules needs to be considered.

Natural Mortality

Natural mortality of the adult Roe's Abalone translocated to the Kalbarri Cliffs study zone was estimated over 1 to 2 years following the translocation survival period. Post translocation the effect on adult Roe's Abalone of the harvest, transport and release methods were demonstrated through the translocation survival estimate. As this survival estimate was derived from surveys standardised to 1-year post-release, it was considered long enough duration to encompass any lag in the effects the abalone experienced from translocation.

Decline in founder populations' numbers after 1 year post-release was considered natural mortality (or emigration) and can be seen in Figure 4.9 for all translocation sites where adult abalone were released. All sites exhibited a varying degree of decline in total count numbers over time. Even though the numbers of adult Roe's Abalone translocated to KT-09 declined on average over time, there were increases in the total count between survey periods (Figure 4.9). Given no Roe's Abalone were present during the baseline surveys and only one translocation was performed at this site, the variation in abalone numbers could be attributed to sampling error and/or emigration/immigration. This may have occurred because of the platforms greater exposure to the prevailing weather conditions than other sites, as well as the variation in these conditions between the two survey periods each year. This fluctuation makes it difficult to accurately estimate natural mortality at that site, which was illustrated in the low R^2 of the linear regression (Table 4.6).

The linear regression analysis performed on the decline in abalone numbers over time was only significant for the translocation site KT-07 (Table 4.6). However, the R^2 of the linear regression for the translocation sites KT-01 (pre and post Perth Metro), KT-05 and KT-10 were all greater than 0.61. The slope (coefficient a) of the linear regression produced an estimate of natural mortality for all the translocation sites and ranged from 0.24 to 0.4 y^{-1} (Table 4.6). The average natural mortality estimated for Roe's abalone at the translocation sites was 0.31 y^{-1} (except KT-09). There is only limited information on natural mortality for Roe's Abalone populations and an estimate for the Perth Metropolitan Fishery of 0.43 y^{-1} (unpublished data) was near the upper end of the range estimated for the translocation sites at the Kalbarri Cliffs study zone.

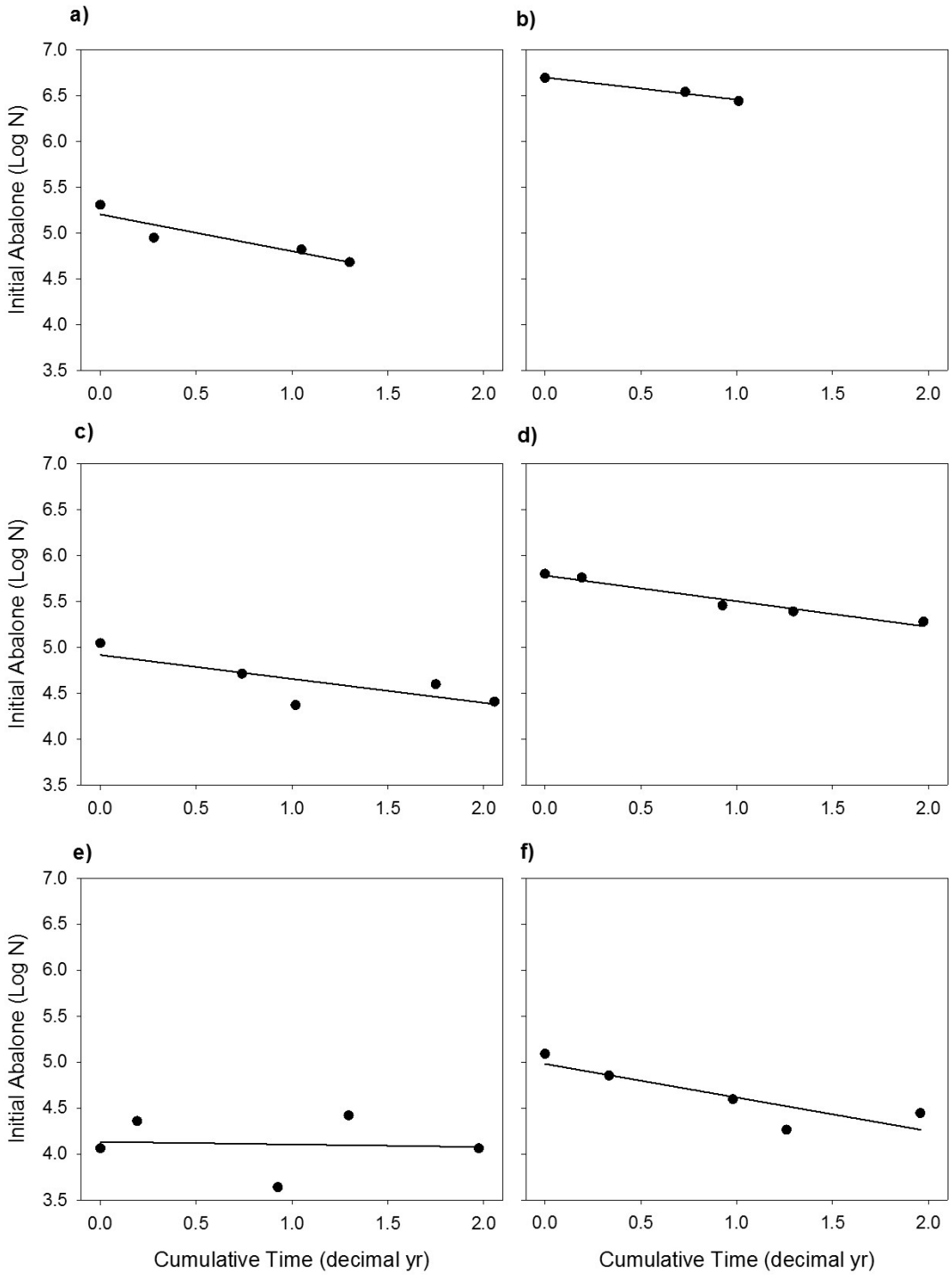

Figure 4.9: Number of abalone (Log N) surveyed at each site after the translocation of adult Roe's Abalone (*Haliotis roei*) to the Kalbarri Cliffs study zone (year=0 represents first re-survey approximately 1 year post-release). The slope (coefficient $\boldsymbol{a}$ in $\boldsymbol{y = ax + b}$, Table 4.6) of the regression line indicated the natural mortality at the translocation sites, a) KT-01 before Perth Metro release, b) KT-01 post Perth Metro release, c) KT-05, d) KT-07, e) KT-09 and f) KT-10.

The decline in adult Roe's Abalone numbers over time has serious implications for establishing founder populations, particularly if they are not yet at an effective breeding population size (Ryman and Laikre 1991; Tringali and Bert 1998). Given recruitment is unlikely to occur at founder populations below this level, the only way to maintain an effective breeding population is to perform multiple translocations of adult Roe's Abalone to a site. Otherwise through a combination of the mortality suffered due to translocation, and natural mortality of the surviving animals, the numbers of abalone will continue to decline until the founder populations are extinct.

Table 4.6: Natural mortality ($\boldsymbol{M}$, y^{-1}) of adult Roe's Abalone (*Haliotis roei*) at the translocation sites within the Kalbarri Cliffs study area, monitored over the 2nd to 4th years post translocation. Linear equation, R^2 and *p* value calculated from a regression analysis of Figure 4.9 data, while coefficient $\boldsymbol{a}$ in the linear equation was used as the $\boldsymbol{M}$ estimate.

Site	Linear Equation	R^2	*p* value	M (y^{-1})
KT-01 (pre Perth Metro)	y= -0.4018x + 5.2036	0.8492	0.0785	0.40
KT-01 (post Perth Metro)	y= -0.2418x + 6.6983	0.9839	0.0810	0.24
KT-05	y= -0.2603x + 4.9144	0.6136	0.1171	0.26
KT-07	y= -0.2794x + 5.7812	0.9574	<0.05	0.28
KT-09	y= -0.0282x + 4.1315	0.0054	0.9065	0.03
KT-10	y= --0.3653x + 4.9791	0.7429	0.0603	0.37
Average				0.26 ± 0.05
Average (less KT-09)				0.31 ± 0.03

Abalone Densities, Clustering Behaviour and Recruitment

After translocation the densities of adult Roe's Abalone on the reef platforms were assessed by three metrics; transect, total count and cluster count. The density of abalone recorded across all translocation sites varied for each of these metrics with the transect metric averaging 1.32 abalone.m^{-2}, the total count averaging 1.40 abalone.m^{-2} and the cluster count averaging 103.9 abalone.m^{-2} (Table 4.7). A statistical comparison between the results of the transect and total count survey method was made using a "paired *t*-test" for each translocation site and the method average, with only a significant difference (p<0.05) shown at one translocation site (KT-07). Given the density of abalone sampled through the transect and total count survey methods were not significantly different for 4 out of the 5 sites and the method average, they represent a similar measure of abalone density no matter how many abalone have been released and whether that was on one or multiple occasions. Therefore, the numbers and subsequently density of adult Roe's Abalone at the translocation sites can be determined by either method. The cluster count metric was not compared with the transect and total count metric as it only samples high abundance areas and not the entire reef platform at the translocation sites.

The variation in abalone densities between translocation sites (e.g. KT-01 to KT-10, Table 4.7) can be attributed to the number of adult Roe's Abalone released at each site and the translocation survival (Table 4.4). Therefore, the density of abalone at each site are not directly

comparable. As a comparison, the Perth Metropolitan Fishery has a much greater range in densities for adult Roe's Abalone of a similar size to those translocated to the Kalbarri Cliffs study zone. The yearly densities recorded from 1997 to 2012 by fisheries-independent sampling using a transect method (translocation transect survey method was based on this method), ranged from 4 to 8 abalone.m^{-2} for Age 3+ (51-60 mm), 5 to 12 abalone.m^{-2} for Age 4+ (61-70 mm) and 1 to 5 abalone.m^{-2} for Age 5+ (71+ mm) (Hart et al. 2013).

The proportion of abalone recorded on the platforms by the three metrics gave an indication as to how the number of abalone related to the density on the platform. If the total count was assumed to sample 100% of abalone at the translocation site, then the transect survey sampled 4.07% and the cluster count 48.41%. Obviously this was not entirely the case given survey conditions and observer ability could affect number of abalone re-captured. However, the important points are that the transect survey resulted in the same abalone density as the total count while sampling less than 5% of the population, and the cluster count metric included nearly 50% of abalone at the translocation sites.

The cluster count metric was utilised after the first release when abalone exhibited the behavioural characteristic of migrating together on the reef platforms (Shepherd 1986), as shown by the abalone clustered together in the centre of Figure 4.10. This clustering behaviour produced multiple clusters at all translocation sites, with the number of clusters ranging within the 5 founder populations. The Roe's Abalone densities presented by the cluster count metric were considerably higher than those by the transect and total count metrics (Table 4.7).

The number of abalone within the clusters and the size of the clusters varied greatly across the reef platforms and produced abalone densities ranging from 7.5 to 400 abalone.m^{-2} (Figure 4.11a). A few of these clusters had densities exceeding 250 abalone.m^{-2}, while other clusters had up to 450 animals present, and some clusters covered a substrate area of up to 34 m^2. However, the majority of clusters were over a small substrate area (<1 m^2) and had abalone densities ranging from 10 to 245 abalone.m^{-2} (Figure 4.11b). These clusters often had lower numbers of abalone present but some did have up to 66 abalone present. As more adult Roe's Abalone are released onto these translocation sites the number and size of the clusters will increase and begin to merge, eventually producing founder populations.

This behaviour showed that nearly 50% of the abalone released congregated together in clusters and these were found at densities averaging 103.90 ± 15.95 abalone.m^{-2}. Therefore, the clustering behaviour is considered extremely important when it comes to effective breeding population densities. Given founder populations require breeding adult densities of greater than 3 per m^2, all of the translocation sites have adult Roe's Abalone clustered in densities that are substantially greater than the required breeding density.

The translocation site KT-01 has been able to reach an effective breeding population. It had over 800 mature Roe's Abalone surveyed on the platform (2.88 abalone.m^{-2}, total count), with 700 of these in various clusters and one of the clusters having up to 450 animals over a 34 m^2 area at a density of 13.2 abalone.m^{-2}. This founder population should be at a stage where recruitment could be possible and juvenile Roe's Abalone may be present in the future. In fact, recruitment has occurred at one of the founder populations (KT-10) with juvenile Roe's Abalone surveyed (30 mm shell length) 4 years after translocation event 1 (Table 4.2). These

recruits were found before any hatchery-reared juvenile Roe's Abalone were restocked at this site (Table 5.1). This demonstrates that translocated adult Roe's Abalone can create viable founder population at the Kalbarri Cliffs study zone with the capacity to produce recruitment.

Table 4.7: The mean Roe's Abalone densities (abalone.m^{-2}) as determined by three metrics; transect, total count and cluster count, for all five translocation sites individually and as a metric average. Means and mean ± std. error (*n*=3 to 6).

Metric (m^2)	KT-01	KT-05	KT-07	KT-09	KT-10	Average
Transect	3.47	1.34	1.11	0.48	0.20	1.32 ± 0.58
Total Count	1.54	1.75	2.97	0.49	0.24	1.40 ± 0.49
Cluster Count	94.72	83.98	79.14	166.51	95.14	103.90 ± 15.95

Figure 4.10: An example of translocated adult Roe's Abalone (*Haliotis roei*) exhibiting clustering behaviour on the reef platform at a site within the Kalbarri Cliffs study area.

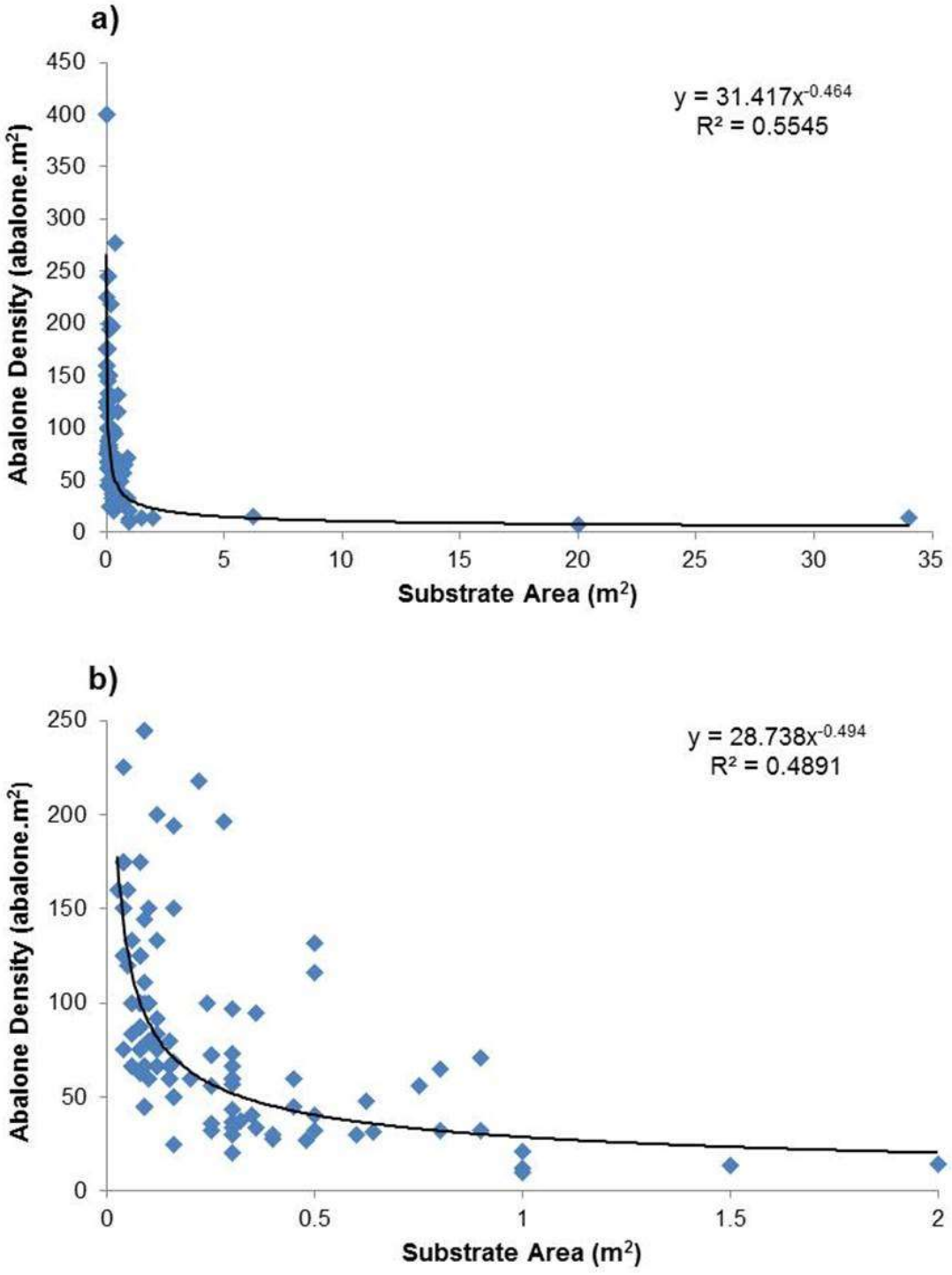

Figure 4.11: Density of adult Roe's Abalone (*Haliotis roei*) present in individual clusters from all translocation sites within the Kalbarri Cliffs study zone. a) All clusters recorded, b) Data refined to illustrate smaller clusters (<250 abalone.m^{-2} and <2 m^2 substrate area). Regression line indicates relationship between abalone density and substrate area (formula and R^2 shown).

5 Aquaculture Spawning and Restocking

This section addresses objective 5, which is to develop spawning protocols for Roe's Abalone and conduct a pilot juvenile stock enhancement release.

5.1 Material and Methods

5.1.1 Broodstock Collection

Two types of wild Roe's Abalone broodstock were used for the initial spawning experiments, recently collected (RC) broodstock and long-termed conditioned (LTC) broodstock. The RC broodstock were harvested immediately prior to spawning induction, whereas LTC broodstock had previously been collected, residing a minimum of one year within the commercial hatchery. The RC broodstock were collected from the intertidal reef in Short Beach, Bremer Bay, Western Australia, during August, as this was when spawning of Roe's Abalone has been reported to be at its peak (Wells and Keesing 1986; Wells and Bryce 1987). The same methods as those utilised for harvesting adult Roe's Abalone from the Perth Metropolitan Fishery source location were used for broodstock collection. Of the RC broodstock, 54 were selected for the spawning trials consisting of 36 females and 18 males, while 72 LTC broodstock consisting of 48 females and 24 males were utilised. All male and female Roe's Abalone broodstock (>40 mm shell length) used for induction of spawning were selected using ocular inspection of the gonad condition. It was noted that most of the RC broodstock, especially the females had low condition while the LTC broodstock were in good condition.

5.1.2 Spawning and Larval Rearing

Induction of Spawning

The RC and LTC Roe's Abalone broodstock were stocked into twelve, 10 L plastic tubs at a density of 12 females and 8 males (separated) per tub. These animals were induced to spawn using a combination of two desiccation times (30 and 60 min) followed by a 1 h thermal shock (3^{o}C above ambient seawater temperature) and UV irradiated seawater. After this induction period the seawater heater was turned off to allow the water temperature to return to ambient and the UV lamps kept on overnight, till the trial was terminated after cessation of spawning.

Fertilisation

Once the male Roe's Abalone started spawning a sample of fresh sperm was taken and placed on a haemocytometer for observations of its motility and the sperm density calculated. The eggs released by female Roe's Abalone were siphoned through a 350 µm mesh into a 20 L bucket (filling it to 10 L) and agitated to evenly distribute. A 1 ml subsample was taken using a pipette, placed into a Sedgewick Rafter cell and counted. The eggs were left for 10 min to settle out and then the bucket drained to leave at least 2 L of seawater, concentrating the eggs for fertilisation.

The female eggs were fertilised with fresh sperm at a ratio of 10 sperm.egg^{-1}, but this was altered depending on the sperm motility. The appropriate amount of fresh sperm was added to the eggs and mixed gently. The eggs were exposed to the sperm for 30 seconds and then 1 µm filtered, UV sterilised seawater added to fill the bucket to 20 L. This should effectively stop

fertilisation and in some cases prevent polyspermy. The egg/sperm mixture was left for 20 min to allow the fertilised eggs to settle out and then the bucket drained of 12 L to eliminate excess sperm. The female eggs were poured gently into a 75 µm mesh and placed inside a shallow bucket for washing. The eggs were washed five times using 1 µm filtered, UV sterilised seawater. A 1 ml subsample was then taken and the fertilisation rate assessed by counting the number of fertilised (formation of first polar bod or occurrence of first cell division) versus unfertilised eggs in the sample. The fertilised eggs were poured into a larval rearing tank filled to 1500 L with 1 µm filtered, UV sterilised seawater. The seawater was supplied at a rate of 3 to 5 $L.min^{-1}$ at ambient water temperature (18°C) and gently aerated. The eggs were then left undisturbed in the larval rearing tank until hatch out.

Larval Rearing and Settlement

The embryonic and larval developmental stages were documented using a stereomicroscope and digital timer. A micrometre eyepiece was used to take measurements of egg diameter and larval shell length and width. A 1 L subsample of fertilised Roe's Abalone eggs maintained at 18°C provided ready access to egg samples for observations on embryonic development.

Newly hatched abalone larvae (trochopores) swam up to the water surface and formed spirals. The trochopores were then swum across to a clean, larval rearing tank filled to 1500 L with 1 µm filtered, UV sterilised seawater. This was done for a minimum of 2 h to ensure at least 80 to 90% of the trochopores were transferred to the new larval rearing tank. One micron filtered, UV sterilised seawater was then supplied at a rate of 1 to 3 $L.min^{-1}$ along with gentle aeration, while a 75 µm banjo sieve fitted to the drain in the middle of tank allowed the seawater to pass through but retain the trochopores in the tank.

At 43 h post fertilisation the Roe's Abalone trochopores were slowly drained into a 75 µm mesh and gently rinsed at least five times with 1 µm filtered, UV sterilised seawater and then poured into a 20 L bucket filled to 10 L. The trochopores were evenly homogenized in the bucket using water flow. A 1 ml subsample was placed into a Sedgewick Rafter cell and the trochopores counted. After the count was complete the trochopores were then placed into a clean larval rearing tank with the seawater maintained at 18°C. This process was completed for 4 days or to a total of 145 h post fertilisation, upon which time the trochopore developed the third or fourth cephalic tentacle tubules signifying that it was ready for settlement or metamorphosis.

Nursery tank settlement plates were prepared for the larvae by inoculating with *Ulvella lens* spores a week prior to induction of Roe's Abalone spawning. In addition, benthic diatoms were encouraged to grow on the plates by adding Abasol (Manutec, SA), a water-soluble aquaculture fertiliser at 0.06 $g.L^{-1}$ to the nursery tank. A day before the abalone were expected to exhibit pre-settlement behaviour and development of the cephalic tentacle tubules, an empty, clean nursery tank was filled with 5 µm filtered seawater. Chlorine (sodium hypochlorite, 12.5%) was then added at a rate 0.08 mL per litre of seawater to sterilise the tank and seawater. The next day, 2 M sodium thiosulfate was added to neutralise the chlorine at 0.025 $mL.L^{-1}$ with strong aeration for several hours. Chlorine strips were then used to confirm that the chlorine had been neutralised. The settlement plates seeded with *U. lens* were then transferred into the sterilised nursery tank and minimum aeration provided.

Competent Roe's Abalone larvae were collected into a 75 µm sieve and rinsed once with filtered, UV sterilised seawater. The abalone larvae were evenly distributed into the nursery tanks with static seawater (no flow through) and limited aeration. The nursery tank was covered with shade cloth and left undisturbed for three to five days. Once it was observed that there were no free swimming abalone larvae in the water column, flow through 5 µm filtered seawater was provided at 0.5 $L.min^{-1}$ with moderate aeration.

5.1.3 Nursery and Grow-out Culture

Nursery Culture

The nursery system consisted of coffin tanks containing metal baskets of 20 vertically arranged PVC settlement plates. The nursery tank was kept shaded for at least 30 d and Abasol added after 14 d post settlement with full aeration provided from this point onwards. An estimate of the number of abalone settled on the plates was completed at 30 d post settlement by counting the abalone post larvae on both sides of ten randomly selected settlement plates. The average number of abalone per settlement plate was calculated and then extrapolated to determine the number of abalone per nursery tank. Pyrethrum was used to eliminate copepods every two weeks from as early as 14 d post settlement. Abasol was continuously used at least three times a week to improve diatom and *U. lens* growth until 90 d post settlement. The Abasol was then replaced by Micro Algae Fertiliser (MAF, Manutec SA) and added at 0.06 $g.L^{-1}$ to the nursery tanks three times a week during the later stages of nursery rearing. The settlement plates were rotated (flipped) every two weeks from 30 d post settlement, which alternated the exposure of the plate ends to sunlight and promoted even growth of diatoms and *U. lens*. Random samples of Roe's Abalone juveniles (n=50) were collected and measured using a micrometre eyepiece.

Transportation of Abalone

The juvenile Roe's Abalone reared in nursery culture at the commercial abalone farm (888 Abalone Pty Ltd) were collected and transported to the WAFMRL facility. They were harvested from the nursery system by dissolving benzocaine in the seawater (0.5 $mL.L^{-1}$ of 10% in ethanol solution), then graded, weighed and loaded into shade cloth bags before being placed in coffin tanks with high water flow and heavy aeration overnight, to flush the benzocaine from their system. The following day the shade cloth bags filled with abalone were loaded into crates and placed into the full seawater with aeration transportation system (see Transport Method in Source Location for Founder Populations – Perth Metropolitan Fishery from Section 4.1.4).

Grow-out Culture

The juvenile Roe's Abalone were grown out for 5 months in weaner tanks at the WAFMRL facility. The weaner tanks were a shallow, round tank with a stepped bottom and supplied with filtered (25 µm) seawater at 10 $L.min^{-1}$ (Daume et al. 2007; Strain 2012). The juvenile abalone had been graded into 2 size classes at the commercial abalone farm and 2,750 abalone of the 17 mm shell length size class were stocked into each weaner tank (5 tanks), while 3,000 abalone of the 13 mm shell length size class were stocked into 1 weaner tank. At the beginning of the grow-out culture period the spray bars in the weaner tanks were set up for circular flow (whirlpool) as the tanks were designed, but this was altered after two weeks to spray bars with

holes on both sides to provide a more even water flow and reduce the clustering behaviour of the abalone in the shallow areas of the tank with the greatest water movement. For the first 2.5 months the juvenile Roe's Abalone were fed an artificial diet (course crumb, Adam and Amos, Mt. Barker, South Australia) by hand, every second day at a rate of 2% body weight per day (dry food, live abalone), with the tanks cleaned the following day.

Due to the clustering behaviour exhibited by the juvenile Roe's Abalone, structures were incorporated to provide habitat in 4 of the tanks stocked with the larger size class animals. At this stage the abalone in these 4 tanks were also transitioned from the artificial diet to a natural diet of green and red macroalgal species (*Ulva*, *Gracilaria*, *Laurencia*, *Plocamium*, etc.). These abalone were fed fresh macroalgae weekly at 10% body weight per day (blotted wet weight macroalgae, live abalone) while the tanks were cleaned twice a week. The remaining tank of the larger size class and the tank of the smaller size class juvenile Roe's Abalone were maintained on the artificial diet and cleaning regime throughout the grow-out culture period. The tank design and diet changes were continued for the remaining 2.5 months the juvenile Roe's Abalone were in grow-out culture at the WAFMRL facility.

5.1.4 Restocking Hatchery-Reared Juvenile Roe's Abalone

To conduct releases of juvenile hatchery-reared Roe's Abalone into the Kalbarri Cliffs study zone, modifications to the Roe's Abalone Translocation (Section 4.1.4) methodologies were performed and can be separated into the 3 sections; harvest, transport and release. All hatchery-reared abalone were disease tested prior to release by a suite of histological and pathological analyses at the DPIRD Fish Health Unit. The survey methods used at the translocation and control sites (see 4.1.2) were continued to monitor the hatchery-reared abalone post release.

Harvest at the WAFMRL Facility

In preparation for the juvenile hatchery-reared Roe's Abalone to be harvested, feeding was stopped 2 d prior and the weaner tanks thoroughly cleaned the day prior. For harvest, Epsom salts were dissolved in the tanks creating a super saline solution to agitate the abalone and cause them to detach from the tank substrate. The abalone were then collected, weighed and loaded into onion bags (200 abalone per bag) before being placed into coffin tanks with high water flow and heavy aeration to flush the Epsom salt from their system.

Transport Method

At least 12 h post-harvest and the day prior to release, the juvenile abalone within the onion bags were loaded into crates and placed into the full seawater with aeration transportation system (see Transport Method in Source Location for Founder Populations – Perth Metropolitan Fishery from Section 4.1.4). The only minor alteration to the transportation method was that the seawater was not exchanged at any point during transport.

Pilot Juvenile Abalone Module Release Experiment (Pilot 3)

Three different release modules were tested given, hand release's reliance on optimal weather conditions, issues with the original Octopus trigger trap release module for release of adult Roe's Abalone, and to deal with the greater number and smaller size (shell length) of the juvenile Roe's Abalone. All modules used square PVC pipe to hold the juvenile abalone, with

the first module utilising a Lintel base (Figure 5.1a), the second incorporating a tyre (Figure 5.1b) and the third an Octopus trigger trap metal base (Figure 5.1c). All three devices weighed approximately 50kg and were left overnight with no abalone inside, on the reef platform at the translocation site KT-10 (Figure 5.1d) within the Kalbarri Cliffs study zone (Table 5.1).

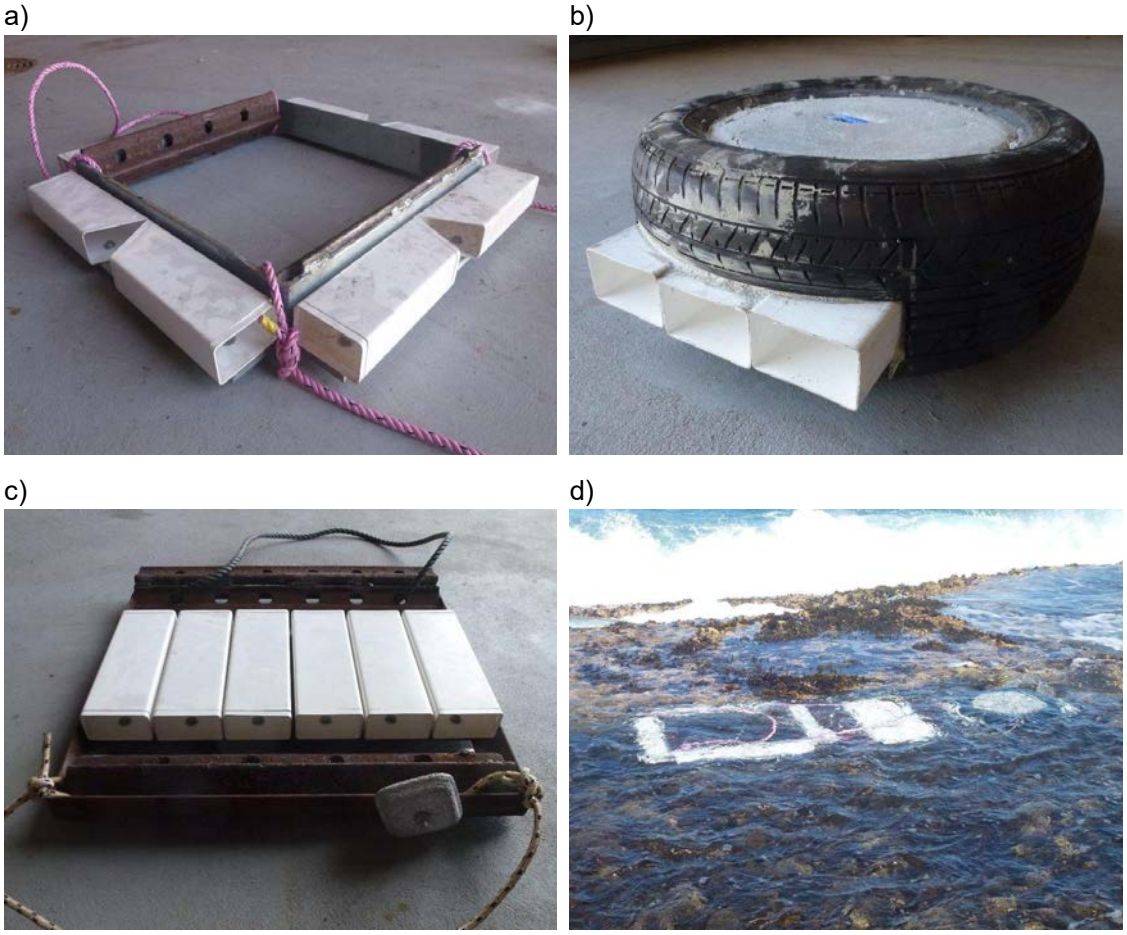

Figure 5.1: Three different modules tested to release juvenile hatchery-reared Roe's Abalone onto the reef platforms at the translocations sites within the Kalbarri Cliffs study area; a) release module with Lintel base, b) release module incorporating a tyre, c) release module with Octopus trigger trap metal base and d) all three release modules in water at KT-10.

Release Method

The release module deemed most appropriate from the pilot experiment was compared to hand placement of juvenile Roe's Abalone (Translocation 8 and 9 in Table 5.1). Juvenile Roe's Abalone were loaded into all 6 of the PVC release pipes on the Octopus trigger trap metal base module and the openings covered with shade cloth to stop the abalone escaping but allowing water flow through the PVC pipes. The modules were placed in position on the reef platform once the site surveys had been completed, then the shade cloth removed to allow the juvenile Roe's Abalone to migrate out of the release modules and onto the substrate (Figure 5.2a and b). Using the release modules allowed greater numbers of juveniles to be released and hence Roe's Abalone were released at 2 different translocation sites on the same day (Table 5.1). This was achieved due to the close proximity of the two sites, by transporting one crate with half of

the abalone via quad bike to each site. The day after release, the modules were checked to see if the juvenile Roe's Abalone had managed to migrate out of the module and attach to the reef platform. If any juvenile abalone remained in the module, they were removed and placed on the reef platform by hand.

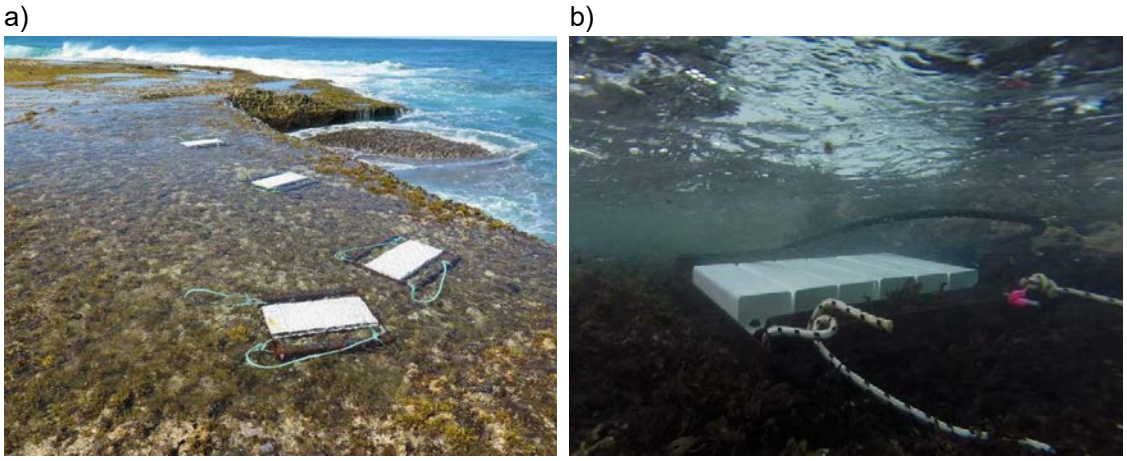

Figure 5.2: The Octopus trigger trap metal base release module with juvenile hatchery-reared Roe's Abalone inside deployed on a translocation site at the Kalbarri Cliffs study area; a) 4 modules on the reef platform, b) underwater view of module.

When using the full seawater with aeration transportation system, the juvenile Roe's Abalone were handled in the same manner as the adult Roe's Abalone until the physical release onto the platform (Section 4.1.4). Hand release of juveniles involved placing clusters as opposed to individuals into appropriate topography such as small holes, depressions, cuts and around translocated adult Roe's Abalone. For Translocation 10, 11 and 12 (Table 5.1) the juvenile Roe's Abalone were all released by hand, while for Translocation 10 and 11 the animals were released at 1 site per day and for Translocation 12 it was 2 sites per day.

Table 5.1: Summary of all restocking of hatchery-reared juvenile Roe's Abalone to the Kalbarri Cliffs study zone and the pilot experiment into methodology development, shown in a chronological order following on from the translocations of mature wild Roe's Abalone.

Translocation	Site	Date	Release Method	Abalone No.
Pilot 3	KT-10	-	Release Module	-
8	KT-01	15/11/2014	Release Module[#]	4123
	KT-05		Release Module[#] / Hand	4123
9	KT-07	27/11/2014	Hand	3600
	KT-09			3600
10	KT-01	15/11/2015	Hand	17143
11	KT-10	17/11/2015	Hand	15645
12	KT-01	4/12/2016	Hand	14565
	KT-10			14565

[#]Animals released by hand the following day.

5.2 Results and Discussion

5.2.1 Spawning Experiment Results

Induction of Spawning

Long term conditioned (LTC) male and female Roe's Abalone spawned readily when induced using desiccation, thermal shock and UV irradiated seawater. However, the majority of recently collected (RC) broodstock did not spawn successfully, particularly the females. It was difficult to quantify the spawning rate and fecundity for both LTC and RC wild Roe's Abalone in this trial as the broodstock were not placed in individual containers. Sympathetic spawning would have occurred in some cases and fresh sperm was used as stimuli for females to spawn. At an approximation about 90% male and 40% female LTC Roe's Abalone successfully spawned. RC Roe's Abalone had a much lower success rate, with about 20% male and 5% female broodstock spawned successfully. Generally spawning occurred for both LTC and RC animals at 3 h for males (Figure 5.3a) and 9 h for females (Figure 5.3b) post induction. A total of 5,874,000 eggs were collected with an average egg diameter of 228 µm.

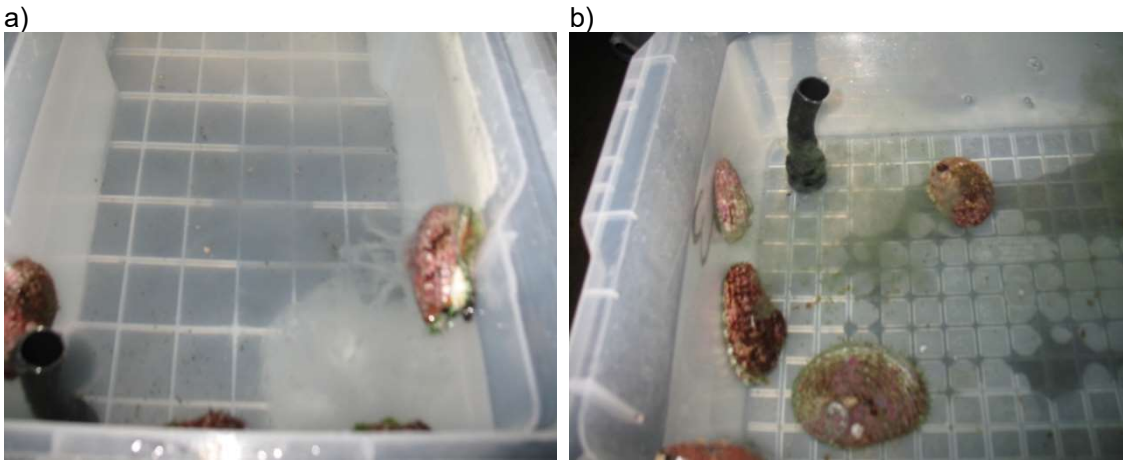

Figure 5.3: The spawning of wild Roe's Abalone (*Haliotis roei*), a) male releasing sperm (white sperm cloud bottom right of photo) and b) females releasing eggs (green egg cloud in centre of photo) (photos supplied by Vincent Encena).

Fertilisation

Roe's Abalone eggs were successfully fertilised using standard artificial fertilisation protocols for Greenlip Abalone (*Haliotis. laevigata*) eggs (Hart and Strain 2016). The volume of fresh sperm suspension and resultant sperm to egg ratio used was optimum (1 egg to 10 sperm) and this resulted in a 92.2 to 98.8% fertilisation rate, with no observations of dissolved egg cases or polyspermy. Formation of the first polar body occurred within 30 min post fertilisation in 18^{o}C seawater.

Larval Rearing and Settlement

Fertilised Roe's Abalone eggs underwent first cell division at 2 h post fertilisation and second to third cell divisions (four to eight cell stage) were observed at 3 h post fertilisation (Figure 5.4a and b). Hatch out occurred at 15 h post fertilisation and Roe's Abalone trochopore measured 228 x 199 µm in size. The complete larval shell and development of the operculum

was attained within 43 h after fertilisation (Figure 5.4c). Hatch out rate was estimated to be 65% and survival rate to trochopore and competence was 24.7 and 14.6%, respectively.

Roe's Abalone veligers reached competence at 145 h post fertilisation in 18ºC seawater. This was characterised by larvae having developed the third or fourth cephalic tentacle tubules and onset of the crawling behaviour (Figure 5.4d). A total of 860,000 veligers (14.6%) were stocked in a nursery tank, with the settlement rate calculated at 1.1% based on the post larvae counts 30 d post settlement This very low settlement rate prompted another spawning run to be conducted in order to produce the required number of juvenile Roe's Abalone for translocation to the Kalbarri Cliffs study zone.

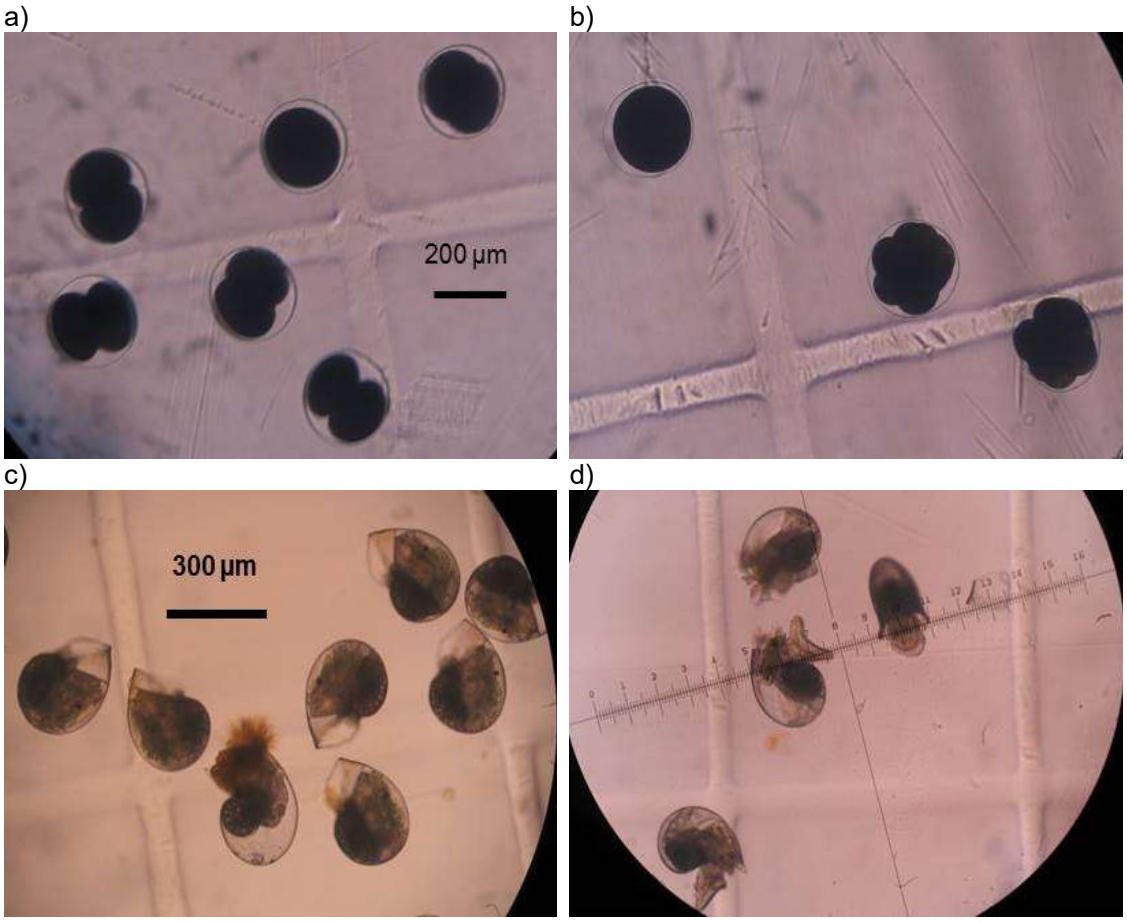

Figure 5.4: Fertilised Roe's Abalone (*Haliotis roei*) eggs undergoing cell division and the development of larvae. a) eggs undergoing first cell division (Scale = 200 µm), b) eggs undergoing second cell division, c) veligers (Scale = 300 µm) and d) veligers with the appearance of the cephalic tentacle tubules exhibiting crawling behaviour (photos supplied by Vincent Encena).

5.2.2 Spawning Protocols

The protocols below have been developed from successful spawning trials of Roe's Abalone at the 888 Abalone hatchery.

Spawning

1. Chlorinate all hatchery equipment prior to use and rinse with fresh water. Directly before using equipment rinse tanks with UV irradiated seawater.
2. Select Roe's Abalone greater than 40 mm in shell length with a ripe gonad from available broodstock. Twice as many females as males will be required.
3. Place male and female broodstock separately in spawning tubs and fill with 1 µm filtered, UV irradiated seawater.
4. When ready to induce spawning remove all seawater from spawning tubs and desiccate the broodstock (exposed to air) for 30 min.
5. After 30 min desiccation, fill the spawning tubs with seawater at 3°C above ambient temperature at a flow rate of 0.3 to 0.5 $L.min^{-1}$ with ozonation for 60 min. Ozone is produced through an ozone producing UVC lamp.
6. After induction the room should be kept in darkness with minimum interruptions.
7. After 60 min of thermal shock, turn the seawater heater off so that the temperature returns slowly to ambient. Maintain a flow rate of between 0.3 and 0.5 L. min^{-1} for the duration of spawning.
8. Fill at least two 1500 L Larval Rearing Tanks (LRT) with 1 µm filtered, UV irradiated seawater ready for eggs as spawning time approaches. Note; this must not be ozonated water for larval rearing.
9. Ripe males may spawn as early as 11 pm on the same day of induction and up to 6 am the next day. Females may start as early as 7 am the following day.
10. Take a sample of the sperm and check motility using a haemocytometer. The more sperm motility the better.
11. **Counting sperm**. Take a 10 ml sample of sperm and mix with a drop of Lugol's iodine solution in a petri dish (don't use to much iodine as it will dilute the sperm). Transfer a few drops of the fixed sample to a haemocytometer and count the sperm (calculate sperm per mL). You want to supply between 10-15 sperm per egg depending on sperm quality.
12. Using a 20 mm clear siphon hose to collect the eggs in a 20 L bucket through a 450 µm screen to catch any faeces. Before siphoning make sure the bucket has had a good rinse with UV irradiated seawater and there are a couple of litres of seawater in the bottom to cushion the eggs.
13. **Counting eggs**. Top up bucket with eggs to 10 L and use a homogenizer to distribute the eggs evenly, then take a 1 ml sample. Place sample on a Sedgewick Rafter slide and count the eggs, then multiply by 10,000 to get the total number of eggs in the bucket.
14. Wait until the eggs settle to the bottom of the bucket (about 10 min) and then gently drain the top 8 L of seawater off.

15. **Add sperm**. Ensure sperm is fresh. Agitate the eggs and pour the sperm in (10-15 per egg) using a little bit of water or an agitator to mix the eggs and sperm thoroughly. Wait for 30 seconds and then quickly fill the bucket up to 20 L. After 15 min assess the eggs to ensure the correct amount of sperm has been added by taking a small sample of eggs and placing them on a slide under the microscope. You should be able to see sperm moving within the egg casing (between 3 and 6 is good). If the egg casings are severely crumpled or dissolved, then you have added too much sperm; conversely if no sperm have penetrated the egg then you haven't added enough sperm or have not left them long enough.

16. **Rinsing eggs**. After eggs are fertilised or after about 15 min, tip the top 7-8 L out of the bucket ensuring no eggs are lost then gently pour remaining eggs over a rinsing screen (75 µm) and into a tub. Rinse thoroughly, completely changing the water at least 5 times. Try to keep the number of eggs in the rinsing screen to less than 2 million at a time otherwise some will be crushed.

17. **Add eggs to LRT**. Make sure to take a sample with a disposable pipette. Ensure that the 75 µm banjo is fitted securely to the LRT. Eggs may be washed directly from the rinsing screen into the LRT. Spread them as evenly as possible around the cone of the tank. Record the number of eggs added to each tank. Put no more than 5 million per LRT (1,500 L tank). Air flow should be fairly low but constant, such that eggs are slowly washed down the cone of the tank before re-entering the water column. The majority of the eggs will be on the cone wall. The water flow should be as light as possible without causing a splash and at a rate of about 6 complete water exchanges in a 24 h period.

18. **Fertilisation Rate**. Quantify fertilisation rate by counting the number of eggs in two or four cell development stages using a Sedgewick Rafter cell. This is easiest about 2 h after fertilisation. Expect around 90% fertilisation rate if the artificial fertilisation protocol has been followed correctly.

Larval Rearing

1. **Hatching**. Hatch-out will occur around 15 h after fertilisation in 18ºC seawater. Be sure to turn the water off just before hatch-out has begun but leave air on but as low as possible. Wait until half of the total eggs in the LRT are hatching (about 20 h) and then shut the air off, except for a tiny bubble every so often. Hatched larvae or trochopores can then be conveniently swum, jugged or siphoned across to a clean LRT. Ensure the water is drawn from slightly below the surface of the hatching tank so that the scum on the surface remains. Only take the best, strongest larvae across to the next tank as any weak larvae will jeopardise the health of other larvae. The best larvae will be located in the top 1/3 of the tank, often seen shoaling and forming whirlpools on the surface.

2. Secure the banjo before allowing the tank to overflow. Only very gentle air flow is required from now on.

3. **First water change**. This needs to be carried out around 30-45 h after hatch out (dependent on water temp) or after the larvae (now called veligers) have developed an operculum. Turn off the air and water, and close the tap on the bottom of the LRT. Healthy larvae will raft to the surface within the first 2 min. After 2 min drop the first 20 L of water down the drain. The larvae in this water are of poor quality and will jeopardise the health of other larvae, there will also be some empty egg casings.

4. Fit the large standpipe to the outlet inside the larvae tank. Commence the drop again this time into a catching screen and container (all of the water should be coming from the top of the LRT). Adjust the flow so that the larvae will collect in a spiral down the pipe, this will make it harder for them to swim away. Before the top of the outlet pipe is reached, turn off the flow and replace with a shorter pipe. Repeat this process until you have used all three stand pipes. The larvae left in the bottom of the larval rearing tank should be discarded. Stand pipes are sized to reach 4/5 up the tank and then 3/5 and 2/5 respectively.

5. Wash the larvae in the catching screen thoroughly by exchanging water 5 times, then transfer to a 20 L bucket for counting (as per the eggs) before transferring to a clean LRT. To count, add 1 drop of Lugol's iodine solution to the slide first before adding the larvae. When counting also asses and record the number of normal larvae and veligers damaged or abnormal. This will give you an idea of how well the water changes are working, as by the 3rd change all larvae should be good (there should be no heads or empty shells left).

6. **2nd water change**. The water needs to be changed every day for now on. The 2nd water change is the same as the 1st water change.

7. **3rd water change**. As per 1st except the outlet stand pipes are not required.

8. **4th water change**. As per 3rd water exchange except by now larvae are crawling and testing the tank surfaces for settlement. Rinse excess larvae from the banjo and cone of the tank during water exchange.

9. **Settling Day**. Assess development of 4th cephalic tubule or the larvae's behaviour (crawling is a sign they are nearing metamorphosis or settlement). Abalone larvae by this time are crawling and very sticky, too sticky to count accurately so work on the previous day's counts.

10. Collect larvae as per water exchange. Determine volume to be apportioned to each settlement tank and divide equally into separate buckets by volume. Expect about 30-40% of eggs spawned to reach settlement. Settlement time will vary depending on seawater temperature but as a rule, it takes 145 h for Roe's Abalone larvae to reach competence in 18^{o}C seawater.

5.2.3 Nursery and Grow-out Culture

Nursery Culture

The juvenile Roe's Abalone were able to grow in nursery culture by consuming the *U. lens* and diatom diet, with examples of various sized abalone displayed in Figure 5.5. The 14 d post larvae had grown to ~350-400 µm shell length (Figure 5.5a) while the 30 d post larvae had a shell length of ~450 µm (Figure 5.5b). After approximately 8 months in nursery culture the juvenile Roe's Abalone had grown to a size of 15.62 ± 0.39 mm shell length and 0.65 ± 0.04 g body weight (Figure 5.5c and Figure 5.6). This resulted in nursery culture growth rates for the juvenile Roe's Abalone of 68.21 µm.day^{-1} in shell length and 2.85 µg.day^{-1} in body weight. These growth rates appear consistent with wild Roe's Abalone growth rates from the Perth Metropolitan Fishery (Hart et al. 2013) and those achieved for Greenlip Abalone grown in the nursery system on the same diet of *U. lens* and diatoms in WA (Daume and Ryan 2004; Daume et al. 2007; Strain et al. 2006).

The whole weight-length relationship of hatchery-reared juvenile Roe's Abalone at 240 d post settlement can be seen in Figure 5.6. The significant regression analysis (F=753.63, p<0.05) produced an a coefficient of 7x10^{-5} and a b coefficient of 3.2886 for the whole weight-length relationship $W = aL^b$. This relationship was not too dissimilar to that for wild Roe's Abalone across their entire size range (0-90 mm shell length) within WA (a = 2-3 x10^{-4} and b = 2.86-3.002, Hart et al. 2013).

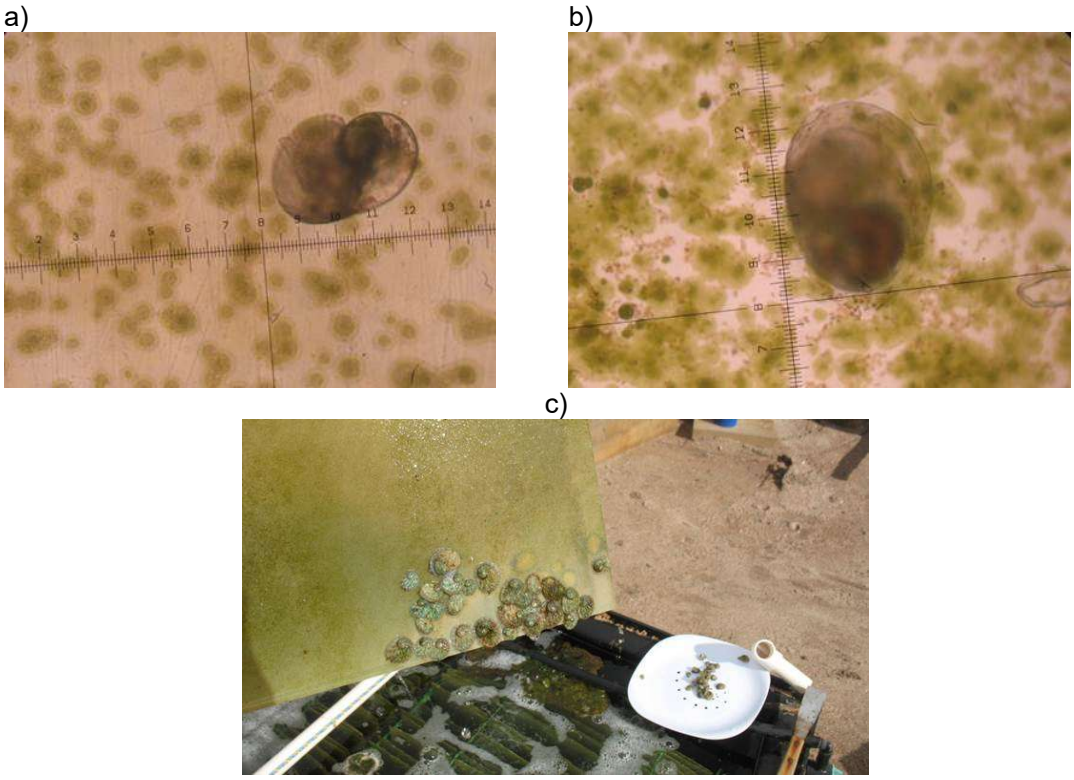

Figure 5.5: Roe's Abalone (*Haliotis roei*) post larvae at 14 d (a) and 30 d (b), as well as juveniles at 8 months (c) post settlement on settlement plates seeded with *Ulvella lens*. Graticule in photo indicates scale (Photos supplied by Vincent Encena).

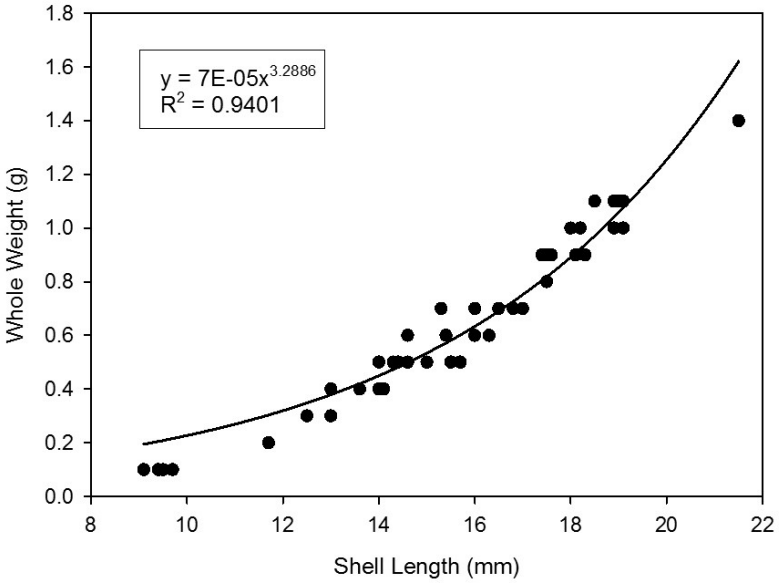

Figure 5.6: Whole weight-length relationship for juvenile Roe's Abalone (*Haliotis roei*) at 229 d post settlement (*n*=50). The equation is $\boldsymbol{W = aL^b}$.

Grow-out Culture

The juvenile Roe's Abalone transferred from the nursery system to the grow-out system exhibited a marked decrease in growth rate and weight gain. When stocked into the grow-out system the abalone growth rates of 14.48 and 24.07 µm.day^{-1} for the 17 and 13 mm size classes respectively, were both well under half what the growth rate had been when in the nursery system (Table 5.2). This could be due to a number of factors, including the harvest and transport of the abalone between systems, the different system designs and the diet being changed from the natural *U. lens* and diatoms diet to an artificial diet. The survival of the juvenile Roe's Abalone was quite high (>90%, Table 5.2), even though weaning between natural and artificial diets for abalone species can result in increases in mortality (Daume et al. 2007; Strain 2012).

Given the slow growth rate and weight gain the juvenile Roe's Abalone achieved in the grow-out system, at the half way point of culture some changes to the system and diet were made. These included transitioning the abalone onto a macroalgal diet and incorporating habitat structures in the tanks to address the clustering behaviour the abalone were exhibiting in the shallow areas with highest water movement. These system and diet changes did not alter the slow growth rates and in fact it slowed them even further, to the point where the growth rate was <1 µm.day^{-1} and the abalone actually lost weight (Table 5.2). These growth rates indicated significant issues with this grow-out system for juvenile Roe's Abalone. Greenlip Abalone in WA of a slightly smaller size (6-9 mm shell length) using exactly the same system design and artificial diet have produced growth rates of up to 80 µm.day^{-1} (Daume et al. 2007; Strain et al. 2007). Further development of the grow-out system design and diet for juvenile Roe's Abalone, to deal with the poor performance under these culture conditions, occurred for the animals associated with translocation events 10, 11 and 12 (Table 5.1), with these experiments to be published following this report.

Table 5.2: The growth rate (μm.day^{-1}), weight gain (μg.day^{-1}) and survival (%) of juvenile Roe's Abalone (*Haliotis roei*) during the 5 month grow-out period. The abalone were stocked at two different size classes (13 and 17 mm shell length) and at the half way point of the grow-out period, the abalone diet was changed from an artificial diet to a whole macroalgae thallus diet in 4 of the tanks stocked with the 17 mm size class.

Diet	Size Class	Growth Rate	Weight Gain	Survival
	mm	μm.day^{-1}	μg.day^{-1}	%
Artificial	17	14.48	1.45	93.05
Artificial	13	24.07	1.91	90.67
Macroalgae	17	0.47	-1.03	90.90
Artificial	17	0	-0.53	96.95
Artificial	13	5.98	1.06	93.53

5.2.4 Restocking Hatchery-Reared Juvenile Roe's Abalone

Even with a successful translocation methodology developed to create founder populations of adult Roe's Abalone within the Kalbarri Cliffs study zone (Section 4.1.4), there were limitations due to the availability of mature Roe's Abalone at source locations in close proximity to the translocation sites. To overcome this issue, the release of hatchery-reared juvenile abalone provided a constant supply of abalone that increased the number of animals released and potentially reduced the pressure on source populations for translocation.

To allow the greater number and smaller size of the juvenile Roe's Abalone to be released efficiently at a translocation site, several release modules were designed and tested (Section 5.1.4). All three modules, Octopus, Lintel and Tyre were placed on the reef platform (KT-10) and after 24 h only the Octopus base module remained in its exact position, while the Lintel base had moved 5 m and the Tyre module disappeared altogether. The Octopus and Lintel base release modules were placed back in position and after another 24 h both remained in position. The Octopus base release module was preferred as it never moved on the reef platform and was more compact and easier to transport to the remote translocation sites.

The juvenile Roe's Abalone harvest and transport methods were similar to the translocation of adult Roe's Abalone from Perth Metropolitan Fishery. During translocation event 8 (Table 5.1) the abalone appeared in good condition when they arrived at the translocation sites, but when loaded into the Octopus base release module PVC pipes they had difficulty adhering due to a clumping behaviour and some abalone were washed away. At the translocation site KT-01 all animals were loaded in the modules, while at KT-05 only half of the abalone were released via the modules due to clumping and the other half released by hand. When the release modules were monitored 24 h later some of juvenile Roe's Abalone had stayed in the modules, with 42% remaining in the modules at KT-01 and 13% at KT-05. The release modules used at KT-05 had moved but were still submerged at low tide. Given a portion of the juveniles remained in the modules and the modules moved on the platform, in translocation event 9 to the sites KT-07 and KT-09 the juvenile abalone were released by hand (Table 5.1). Even though

the juvenile Roe's Abalone are small in size and large numbers are able to be translocated, release by hand in optimum weather conditions was still considered the best release method.

A further 3 translocation events over the next 2 years were conducted to the Kalbarri Cliffs study zone, bringing the total number of juvenile hatchery-reared Roe's Abalone released to 77,364 (Table 5.1). Figure 5.7 shows a cluster of hatchery-reared juveniles (green shell colour) around an adult Roe's Abalone translocated from Lucky Bay. The survival of the juvenile Roe's Abalone 1-year post-release was less than 1% for each of the 5 releases. Given the small size of the juvenile Roe's Abalone and the variability in prevailing weather conditions the recapture rate would also be low when conducting a total count survey.

The low survival could be attributed to the juveniles being cultured on the south coast, then grown-out on the south-west coast before being released at the northern end of the species distribution. Not only would the individuals released be acclimatised to cooler water temperatures but the LTC broodstock used to produce the juveniles were from the adaptive population cluster on the southern coast of Australia (Section 6.2.3). The results of the genomic seascape analysis (Section 6.2.5) indicated a strong influence of SST and that the difference in temperature between locations has promoted adaptive differentiation. So when progeny from the south adaptive cluster were released at the northern extent of the species range (warmest SST) the juveniles struggled to survive given the genetic differentiation between locations.

All juveniles released in the Kalbarri Cliffs study zone were progeny of LTC broodstock from south adaptive cluster. This was due to the spawning success rate between LTC versus RC broodstock (Section 5.2.1) and the urgent need to develop spawning protocols for Roe's Abalone as this species had not been previously cultured at this scale (Section 5.2.2). To alleviate the issues associated with adaptive differentiation between source and release locations, broodstock should be sourced from the Kalbarri Cliffs study zone or at least from the closest possible populations within the north adaptive cluster (for more detail see Section 6.2.6).

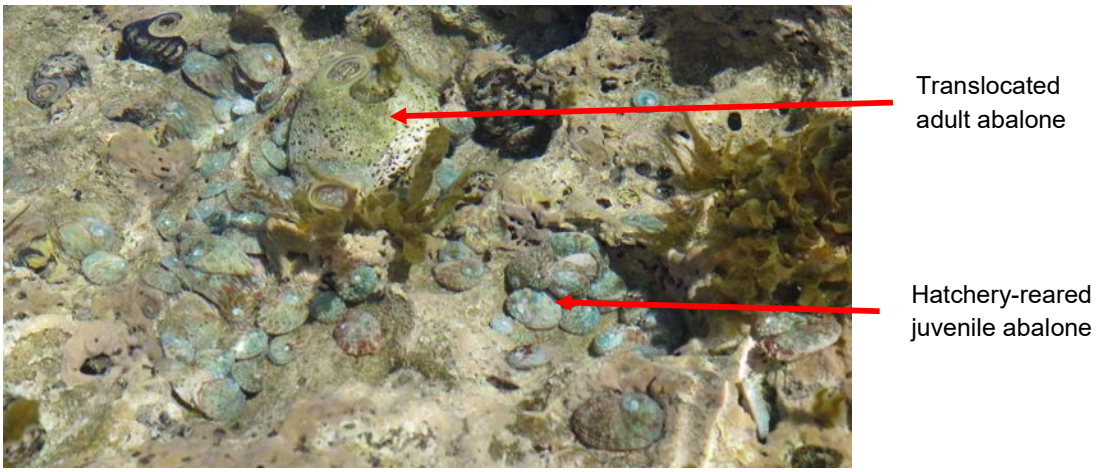

Figure 5.7: Hatchery-reared juvenile Roe's Abalone with the distinctive green shell colour (caused by the artificial diet in the grow-out system) directly after release, surrounding a translocated adult Roe's Abalone.

6 Genomic Analysis

This section addresses objective 2 and 4, which are to evaluate the genetic structure of existing and founder populations and evaluate the genetic contribution of existing and founder populations to stock recovery. Please note that this chapter has been published in greater detail in the following report and that should be used as a citation for this genomic analysis section:

Sandoval-Castillo J., Robinson N., Strain L., Hart A. and Beheregaray L.B. (2015) Use of next generation DNA technologies for revealing the genetic impact of fisheries restocking and ranching. Australian Seafood CRC Report No. 2012/714. Flinders University, Adelaide, 47pp.

6.1 Material and Methods

6.1.1 Sample Collection and Processing

Roe's Abalone were collected from 9 locations around Western Australia and one in South Australia. These locations included a remnant population at the Kalbarri Cliffs, the source population at Lucky Bay, Greenough, three sites in the Perth Metropolitan Fishery (Burns Beach, Waterman's Reserves and Penguin Island), Augusta, Albany, Esperance and Spencer Gulf in SA (Figure 6.1 and Table 6.1). The abalone were collected through commercial fishing practices, processed at the WAFMRL facility and then the extracted samples sent to Flinders University, South Australia for genomic analysis (collaborative project CRC 2012/714).

All locations were sampled between November and January, with 30 to 50 Roe's Abalone of the same size class (mature animals, 50-60 mm shell length) harvested from within 50 m of each other (Table 6.1). The samples were collected whole and placed in a heavy duty plastic bag complete with label (i.e. Location, Date, GPS, Number, etc.), then frozen. Abalone were partially thawed at the time of processing and a small segment of gill tissue (~5 mm^3) was extracted from each animal and placed in a 2 mL labelled vial full of 100% ethanol, then the vials stored in a freezer. For high quality preservation before analysis the ethanol was replaced after several days. The exception to this was the remnant population sampled at the Kalbarri Cliffs study zone were the abalone collected were processed *in-situ* and a sample of the abductor muscle extracted.

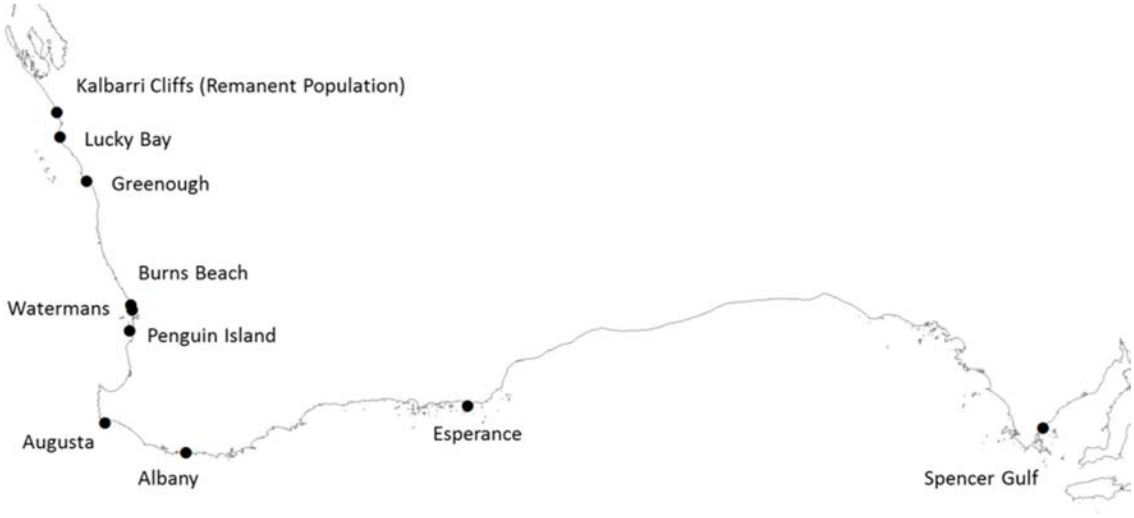

Figure 6.1: Roe's Abalone sampling locations covering most of the species distribution (source CRC 2012/714 Final Report).

Table 6.1: Roe's Abalone sampling locations, location abbreviations and sample size per location (source CRC 2012/714 Final Report).

Location	Abbreviation	Sample (*n*)
Kalbarri Cliff	KC	50
Lucky Bay	LB	50
Greenough	GN	50
Burns Beach	BEA	50
Waterman's	WM	50
Penguin Island	PI	50
Augusta	A	50
Albany	AL	50
Esperance	ES	50
Spencer Gulf	SG	30

6.1.2 Laboratory Protocols

Genomic DNA was extracted from the Roe's Abalone samples using a modified salting out method. These samples were examined using RAD-seq (restriction-site associated DNA sequencing), an approach that simultaneously identifies and types tens of 1000s SNPs (single nucleotide polymorphisms). RAD libraries were prepared where the DNA of each individual was cut using a restriction enzyme (SbfI) producing a set of sticky-end fragments (Figure 6.2A). These fragments were ligated to adapters (P1) that contain a sequence that binds to an Illumina cell, and a short sequence (MID) that will uniquely identify the individual (Figure

6.2B). After tagging the fragments, all the individuals were pooled in the libraries. The libraries were sheared to generate fragments with average length of 500 bases. Sheared fragments were ligated to a second adapter (P2) (Figure 6.2D) and then PCR amplified using P1 and P2 primers (Figure 6.2E). The libraries (420 samples of Roe's Abalone) were sent to the Genome Quebec Innovation Centre (http://gqinnovationcenter.com/index.aspx?l=e) to be sequenced in an Illumina platform. The final large dataset was analysed using the Stacks software pipeline (Catchen et al. 2011) to filter sequences and identify SNPs. Analysis was performed using Flinders University Colossus, a cluster of 1,160 CPU cores and 4.25 TB (4,250 GB) of RAM, as supercomputers are needed to handle the analysis of the large RAD-seq datasets.

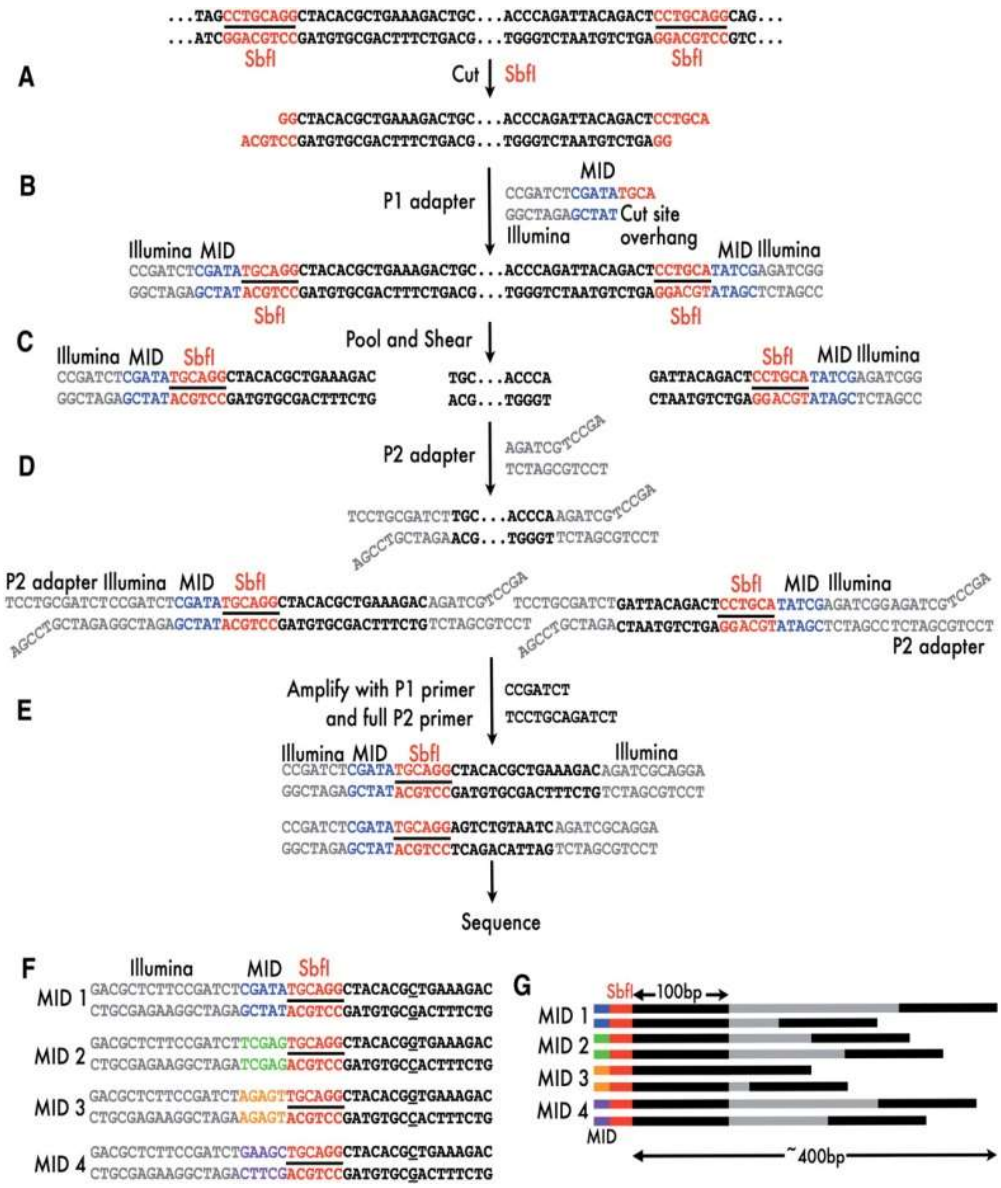

Figure 6.2: A summary of the RAD-seq process (Davey and Blaxter 2010) (source CRC 2012/714 Final Report).

6.1.3 Data Analyses

Categorising loci

There are two main forms of genetic diversity, neutral and adaptive variation. Neutral genetic variation is highly valuable for estimation of demographic parameters, particularly connectivity (i.e. gene flow) and population size. By contrast, adaptive (also known as functional) genetic variation affects the organisms' ability to adapt to new or changing environments. Therefore, in order to extract the maximum information possible from our genomic data, it was important to be able to discriminate between DNA markers (i.e. loci) that are under selection from those that are neutral loci. We assessed the contribution of natural selection to the overall pattern of genetic differentiation between abalone populations using a F_{ST} outlier approach implemented in ARLEQUIN (Excoffier and Lischer 2010). Briefly, this method models the expected distribution of the relationship between F_{ST} (Wright's fixation index) and He (expected heterozygosity) under an island model of migration with neutral markers. The expected distribution was compared to the observed distribution to identify outlier loci that have excessively high F_{ST}. Such outlier loci are considered likely to be subject to the forces of natural selection.

Genomic Analysis

The genetic diversity within localities and the genetic differentiation between localities were estimated using the software ARLEQUIN 3.5 (Excoffier and Lischer 2010). Software STRUCTURE (Pritchard et al. 2000) and ADEGENET (Jombart and Ahmed 2011) were used to determine the optimal number of populations based on our genetic data. STRUCTURE implements a Bayesian clustering algorithm, whereas ADEGENET uses Discriminant Analysis of Principal Components. We tested whether significant genetic differentiation detected between localities could be due to isolation by distance using a Mantel test implemented in GENODIVE 2 (Meirmans and Van Tienderen 2004), which assesses the correlation between a geographic and a genetic distance matrix. To determine the potential influence of hierarchical population structure, we implemented a stratified Mantel test, in which samples were permutated within each of the three clusters detected by STRUCTURE and ADEGENET.

Seascape Analysis

Data for four oceanographic variables (SST, oxygen concentration, pH and nutrient concentration) for 100 years (1914-2014) were obtained from the NOAA World Ocean Data Base Website (http://www.nodc.noaa.gov/OC5/SELECT/dbsearch/dbsearch.html, Table 6.2). For each variable an annual average gridded map at 0.1 degrees' resolution was generated using the DIVA algorithm in ODV 4 (Schlitzer 2010). To explore the effect of extreme temperatures in the genetic structure, we also generated gridded maps for the average of the maximum annual SST (Table 6.2). To illustrate environmental variation between sampling sites we performed a principle component analysis (PCA) with the R package FACTOMINER 1.25 (Lê et al. 2008).

To explore the association between the oceanographic variables and the adaptive genetic differentiation of Roe's Abalone populations ("outliers" data set), we applied two multivariable analytical approaches. First, we used the R software ECODIST 1.2.9 (Goslee and Urban 2007) to perform a Multiple Regression on Distance Matrices (MRDM) analysis. This was an

extension of the partial Mantel test that investigates the relationship between a response distance matrix and any number of explanatory distance matrices. In this case we used the linearized pairwise F_{ST} (F_{ST}/(1-F_{ST})) matrix as the dependent variable and the ecological distance matrices as the independent variables. Second, we used a Canonical Correspondence Analysis (CCA) implemented in the R program VEGAN 2.10 (Dixon 2003). Via constrained ordination diagrams the CCA extracts major synthetic gradients from the response variables in terms of the explanatory variables. In this work locality allele frequencies were used as response variables and the localities specific oceanographic attributes as explanatory variables. Also, a partial CCA was performed using the coordinates.

Table 6.2: Estimated annual average (1914-2014) of five oceanographic variables (pH, Nutrients, Oxygen, SST and Max SST) and the maximum sea surface temperature during the 2010/11 marine heatwave event (SSTHW) for the genetic sample locations (source CRC 2012/714 Final Report).

Location	pH	Nutrients	Oxygen	SST	Max SST	Max SSTHW
Kalbarri Cliff	8.26	2.62	4.28	22.33	24.47	28.44
Lucky Bay	8.26	2.61	4.38	22.25	24.23	28.02
Greenough	8.25	2.31	4.98	20.64	23.48	27.37
Burns Beach	8.20	2.13	5.28	20.60	22.05	25.65
Waterman's	8.20	2.18	5.26	20.59	22.02	25.54
Penguin Island	8.19	2.15	5.20	20.65	22.37	25.20
Augusta	8.25	3.23	5.19	19.89	21.50	23.90
Albany	8.28	2.97	5.14	19.74	20.32	22.20
Esperance	8.32	3.29	5.39	18.68	20.59	20.41
Spencer Gulf	8.15	1.49	5.24	19.67	20.45	18.19

6.2 Results and Discussion

From the nine Illumina lanes ran for the Roe's Abalone samples, ~1.6 billion DNA sequence reads were obtained. Each read was a sequence of approximately 100 base pairs long (i.e. a total of ~160 billion base pairs of DNA data were generated). After filtering the reads, approximately 774 million SbfI RADtags were recognised, of which ~720 thousand were unique sequences. For the Roe's Abalone samples, a total of 31,008 SNP makers were obtained; from which 9,338 SNP were used for further analyses because they were bi-allelic, identified in the existing catalogue, had a coverage depth of over 4 times in at least 80% of the sequenced individuals, and were present in all the sampled localities.

6.2.1 Genetic Diversity

Analysis of the entire SNP data using ARLEQUIN 3.5 (Excoffier and Lischer 2010) showed that levels of genetic diversity were very similar across locations, with slightly higher values near the Perth Metropolitan Fishery (Table 6.3). Despite the catastrophic mortality associated

with the marine heatwave in the northern stock (Area 8), there was no evidence of low genetic diversity or population reduction at any sampled location, including Kalbarri Cliff. Genetic data have low power for detecting a population bottleneck in the first generation after the population reduction. However, due to the very small census population sizes of the remnant population, loss of genetic variation would be detected in the next few generations unless effective restocking programs are implemented.

Table 6.3: Levels of genetic diversity for Roe's Abalone from the ten sampled locations. π=nucleotide diversity, He=expected heterozygosity, PL=percentage of polymorphic loci (source CRC 2012/714 Final Report).

Location		π	He	% PL
Kalbarri Cliff	KC	0.15	0.21	85.53
Lucky Bay	LB	0.14	0.21	84.62
Greenough	GN	0.12	0.22	82.33
Burns Beach	BEA	0.16	0.21	87.95
Waterman's	WM	0.16	0.26	71.40
Penguin Island	PI	0.15	0.21	86.38
Augusta	A	0.14	0.21	84.51
Albany	AL	0.13	0.22	81.05
Esperance	ES	0.12	0.22	81.11
Spencer Gulf	SG	0.15	0.23	82.62

6.2.2 Categorising loci

We detected 553 outlier loci representing ~5.9% of the scanned loci. Subsequent analyses were conducted for the entire dataset (9,338 SNPs), the "outliers" dataset (553 SNPs) and the "neutral" dataset (8,785 SNPs).

6.2.3 Genetic Differentiation

Levels of genetic differentiation for Roe's Abalone were low, but significant (F_{ST}>0.1, p<0.01) between most pairs of locations in all the datasets, with highest values in the "outlier" data set (Table 6.4, Table 6.5, Table 6.6 and Figure 6.3) as estimated using ARLEQUIN. The STRUCTURE and ADEGENET methods both support the existence of one single population for the entire SNP dataset and the "neutral" markers dataset (Figure 6.4A, B and Figure 6.5A, B). On the other hand, the "outliers" dataset suggested the existence of at least three markedly differentiated population clusters (Figure 6.3C, Figure 6.4C, D and Figure 6.5C): 1) the northern part of Roe's Abalone distribution (Kalbarri Cliff and Lucky Bay); 2) the southwest coast of WA (from Greenough to Augusta); and 3) the southern coast of Australia (from Albany to Spencer Gulf) (Figure 6.6 and Figure 6.7).

Table 6.4: Overall genetic diversity: genetic differentiation between samples of Roe's Abalone from ten locations based on 9338 SNPs. F_{ST} values in bold are significant (p<0.001) (source CRC 2012/714 Final Report).

	KC	LB	GN	BEA	WM	PI	A	AL	ES	SG
KC	0.000									
LB	0.001	0.000								
GN	0.002	**0.010**	0.000							
BEA	**0.016**	**0.011**	0.000	0.000						
WM	**0.014**	**0.013**	0.000	0.004	0.000					
PI	**0.014**	**0.012**	0.005	0.004	0.005	0.000				
A	**0.013**	**0.016**	**0.011**	0.004	**0.006**	**0.007**	0.000			
AL	**0.017**	**0.020**	**0.016**	**0.006**	**0.006**	**0.009**	**0.011**	0.000		
ES	**0.009**	**0.016**	**0.015**	0.000	0.000	0.004	**0.009**	0.005	0.000	
SG	**0.011**	**0.013**	**0.007**	**0.008**	**0.007**	**0.008**	**0.007**	0.005	0.000	0.000

Table 6.5: Neutral variation: genetic differentiation between samples of Roe's Abalone from ten locations based on 8785 "neutral" SNPs. F_{ST} values in bold are significant (p<0.001) (source CRC 2012/714 Final Report).

	KC	LB	GN	BEA	WM	PI	A	AL	ES	SG
KC	0.000									
LB	0.000	0.000								
GN	0.000	0.009	0.000							
BEA	**0.012**	**0.007**	0.000	0.000						
WM	**0.011**	**0.010**	0.000	0.003	0.000					
PI	**0.011**	**0.010**	0.004	0.003	0.004	0.000				
A	**0.012**	**0.015**	**0.014**	0.004	**0.005**	**0.007**	0.000			
AL	0.005	**0.012**	**0.013**	0.000	0.000	0.002	0.004	0.000		
ES	**0.010**	**0.013**	**0.009**	0.003	**0.005**	**0.006**	**0.010**	**0.008**	0.000	
SG	**0.007**	**0.009**	0.005	**0.006**	0.005	**0.006**	0.005	0.000	**0.005**	0.000

Table 6.6: Adaptive variation: genetic differentiation between samples of Roe's Abalone from ten locations based on 553 "outlier" SNPs. F_{ST} values in bold are significant (p<0.001) (source CRC 2012/714 Final Report).

	KC	LB	GN	BEA	WM	PI	A	AL	ES	SG
KC	0.000									
LB	**0.018**	0.000								
GN	**0.038**	**0.034**	0.000							
BEA	**0.068**	**0.063**	**0.014**	0.000						
WM	**0.066**	**0.059**	**0.017**	**0.021**	0.000					
PI	**0.058**	**0.050**	**0.018**	**0.021**	**0.013**	0.000				
A	**0.062**	**0.064**	**0.032**	**0.023**	**0.018**	**0.014**	0.000			
AL	**0.084**	**0.078**	**0.045**	**0.037**	**0.022**	**0.027**	**0.027**	0.000		
ES	**0.075**	**0.067**	**0.045**	**0.040**	**0.027**	**0.033**	**0.036**	**0.020**	0.000	
SG	**0.069**	**0.061**	**0.036**	**0.045**	**0.033**	**0.034**	**0.033**	**0.025**	**0.020**	0.000

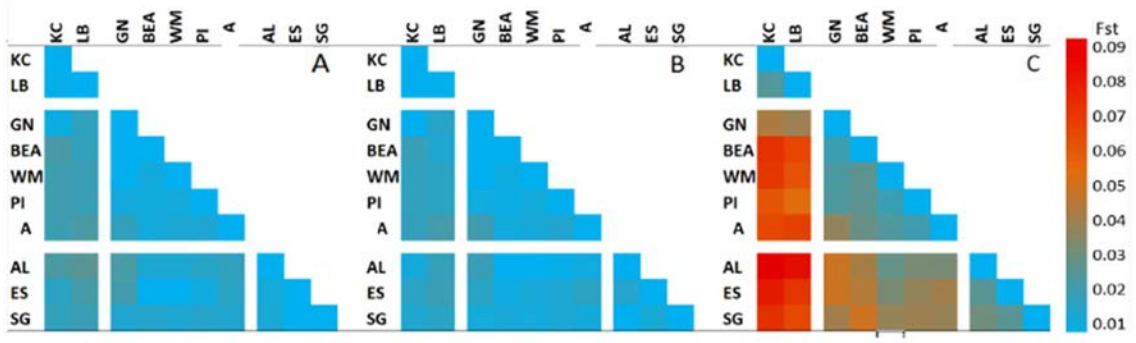

Figure 6.3: Matrix of pairwise genetic differentiation (F_{ST}). (A) Results based on 9338 SNPs (entire dataset), (B) Results based on 8785 "neutral" SNPs, (C) Result based on 553 "outlier" SNPs (source CRC 2012/714 Final Report).

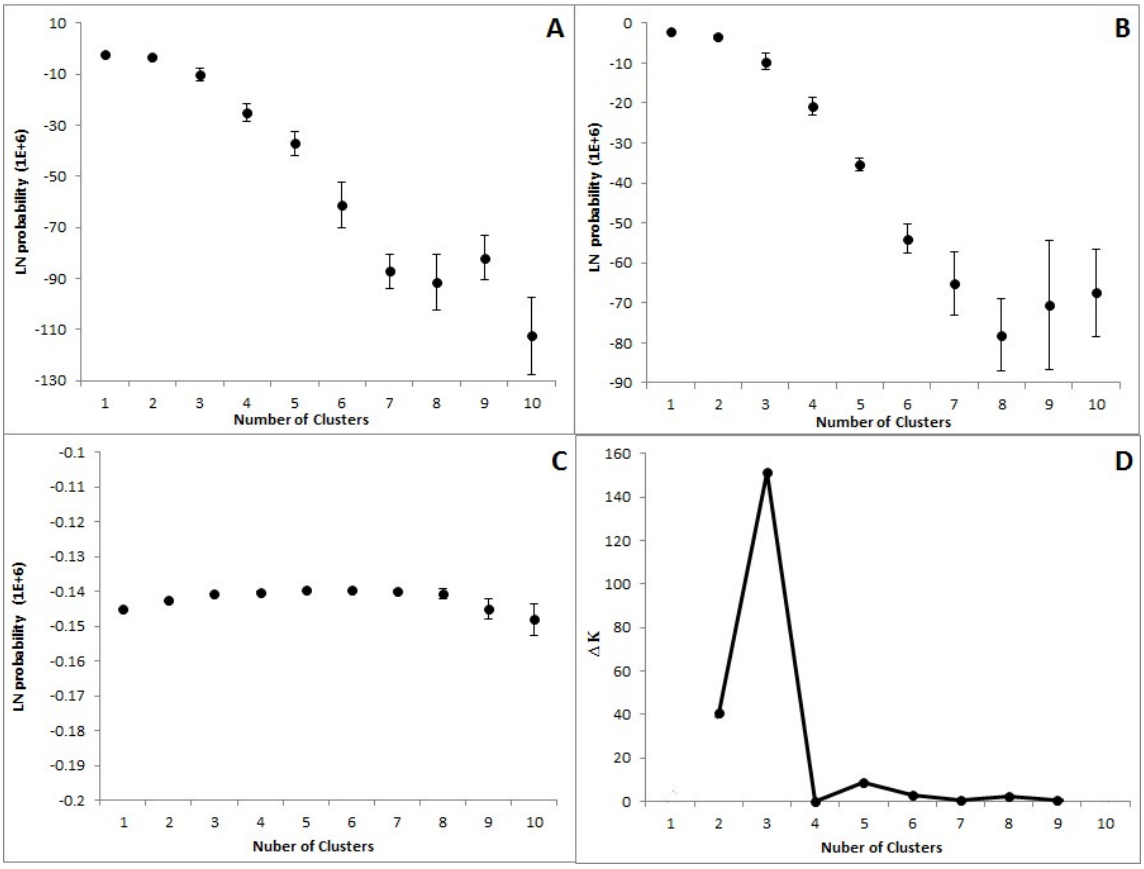

Figure 6.4: STRUCTURE probability of the data as a function of the number of population clusters (A, B, C); and magnitude of ΔK as a function of number of clusters (D). Results are for three data sets: (A) all the 9338 SNPs, (B) 7875 "neutral" SNPs, (C, D) 553 "outliers" SNPs. When the highest probability was difficult to define (as in the outlier data set), the highest ΔK (D) should correspond to the optimal number of clusters (source CRC 2012/714 Final Report).

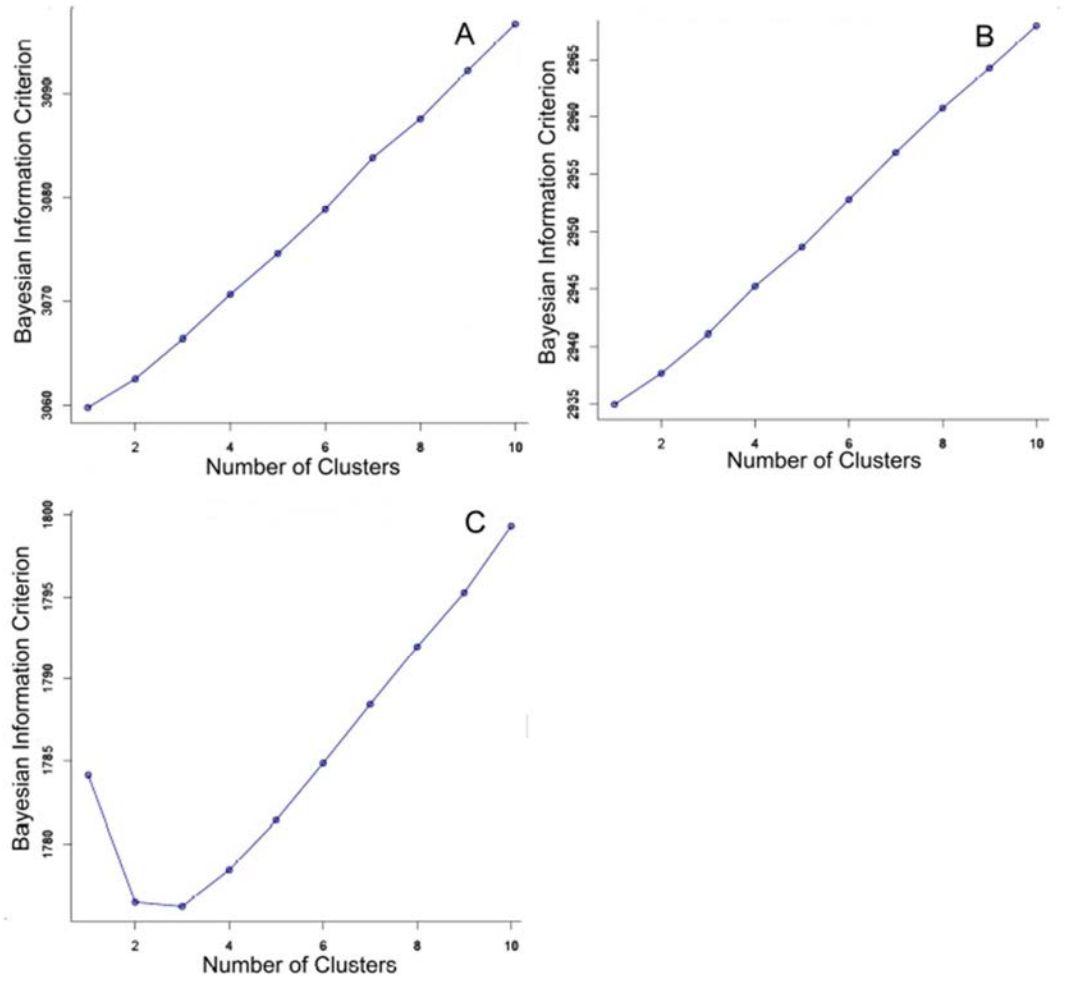

Figure 6.5: ADEGENET Bayesian Information Criterion as a function of number of clusters: (A) using all the 9338 SNPs, (B) using 7875 "neutral" SNPs and (C) using 553 "outliers" SNPs. Ideally, optimal clustering solution should correspond to the lowest Bayesian Information Criterion (source CRC 2012/714 Final Report).

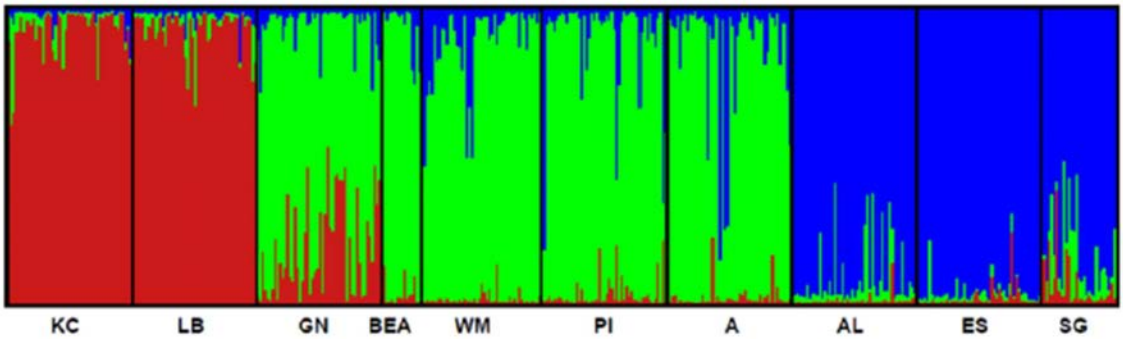

Figure 6.6: STRUCTURE clustering plot for Roe's Abalone based on 553 "outlier" SNPs. K=3 was the optimal number of clusters. The figure was based on colour-coded columns where each line corresponds to an individual and the colours to a specific cluster. Black lines separate each sampling locations (source CRC 2012/714 Final Report).

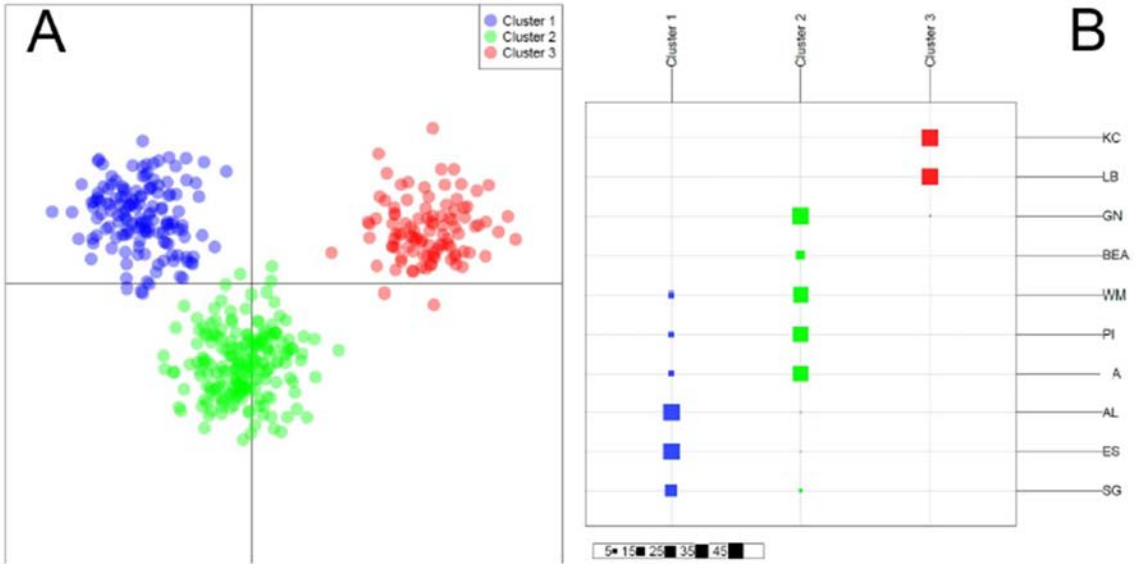

Figure 6.7: (A) ADEGENET discriminant analysis of principal components for 553 "outliers" SNPs of Roe's Abalone. The graphic shows the first two principal components that explain 90% of the genetic variation (PC1=58.8%; PC2=31.2%). (B) Number of samples in each cluster by locality of origin (source CRC 2012/714 Final Report).

6.2.4 Isolation by Distance

Isolation by distance was only found in WA samples in all datasets (Figure 6.8). The stratified Mantel test (in which samples were permuted within each of the three clusters detected by STRUCTURE and ADEGENET) showed that the isolation by distance was only marginally significant for the "neutral" data set (p=0.047) and was not significant for the "outliers" data set (p=0.281).

6.2.5 Seascape Analysis

The PCA of the oceanographic factors revealed three environmentally different regions that are congruent with our three genetic clusters (Figure 6.9). The exception was the Augusta sampling location; while this sampling location was genetically clustered with the south west coast of WA, oceanographically it was clustered with south coast of WA.

Both the MRDM and the CCA revealed a strong influence of SST on the "outlier" genetic pattern (Table 6.7, Table 6.8, Figure 6.10 and Figure 6.11). However, after accounting for the spatial effect, only the MRDM was significant (Table 6.7). The difference between MRDM and CCA results could be due to intrinsic differences between the methods; while MRDM explores the importance of effective separation between the locations, CCA focuses on the relevance of local processes. The results indicated that the individuals are adapted to a temperature range rather than to a specific temperature. Therefore, the difference in temperature between locations has promoted adaptive differentiation and the greater the differences in temperature the greater the genetic differentiation between locations. Additionally, the lack of significance in the partial CCA can be attributed to the dependence of

temperature to latitude rather than to spatial autocorrelation on allele frequency. This hypothesis was supported by the result of the CCA when the latitude and longitude was included as explanatory variables and the temperature as conditional (Table 6.9). If spatial autocorrelation was the main driver of the allele frequency patterns, both latitude and longitude will be significant even after correcting by temperature.

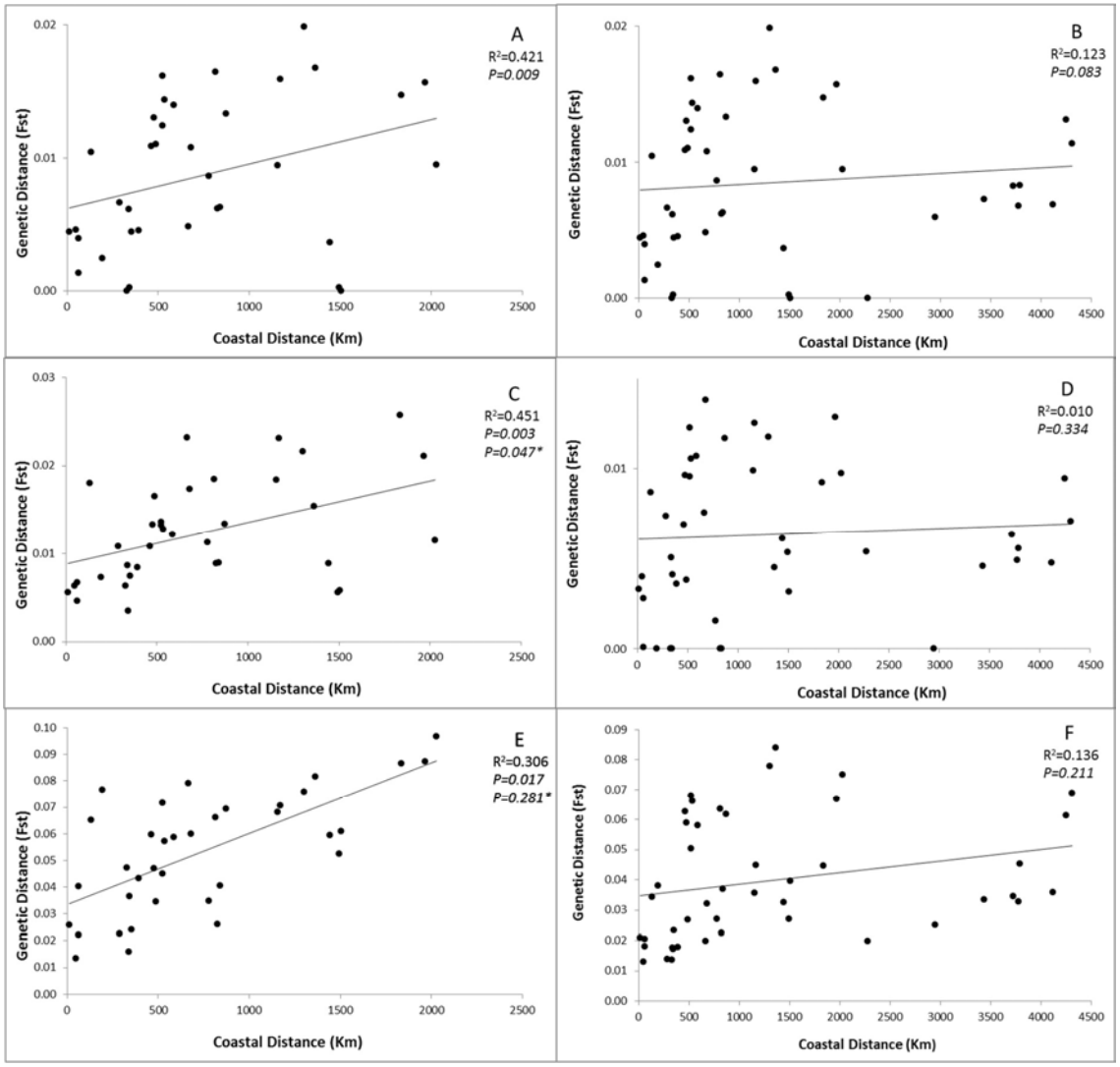

Figure 6.8: Correlation tests between coastal geographical distance and genetic distance F_{ST} (Mantel test) for pairs of Roe's Abalone sampling locations. Western Australia samples (A, C, E); including Spencer Gulf samples (B, D, F). Using three data sets: The whole 9338 SNPs data set (A, B), 8785 "neutral" SNPs (C, D), or 553 "outlier" SNPs (E, F). * *p* values after stratified Mantel test (source CRC 2012/714 Final Report).

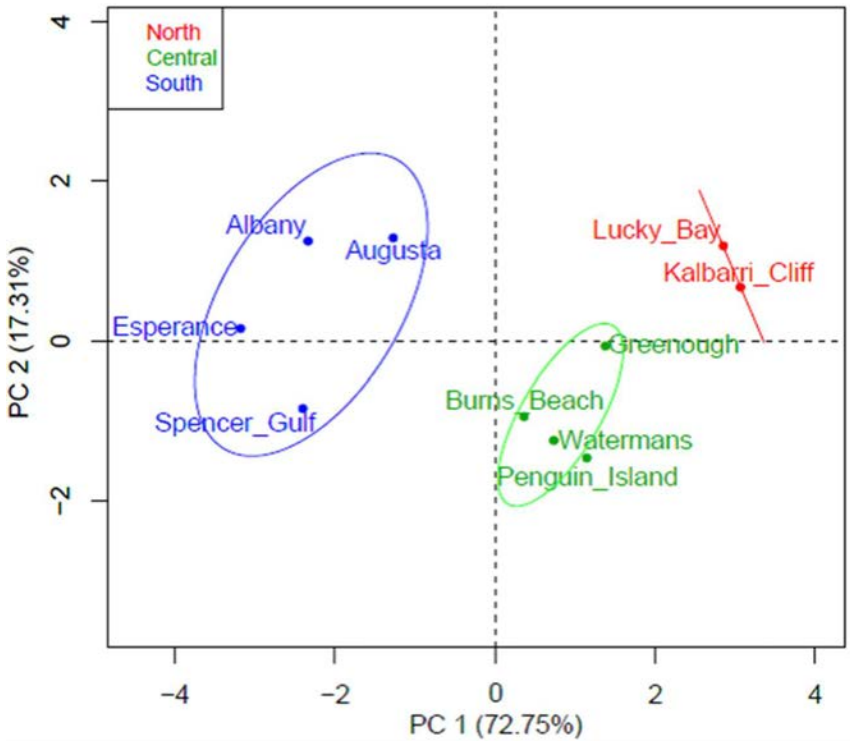

Figure 6.9: Principal Component Analysis based on six oceanographic variables. The scatterplot shows the first two principal components that explain 90.06% of the variation. Dots are coloured according to the most probable environmental groups. Ellipses represent the 95% confidence level of these groups (source CRC 2012/714 Final Report).

Table 6.7: Multiple regressions on distance matrices estimating the correlation of Roe's Abalone genetic distance with oceanographic distances. Included are the full model (all oceanographic variables) and a reduced model (oceanographic variables without collinearity). Significant standardised regression coefficients (***b***) after correction for false discovery rate are in bold (q<0.05) (source CRC 2012/714 Final Report).

Variable	Full Model		Reduced Model	
	b	q	b	q
Temperature	-0.06	0.82		
Maximum Temperature	**0.66**	**0.01**	**0.84**	**0.01**
Marine Heatwave Temperature	0.58	0.12		
Oxygen Concentration	0.33	0.56	0.24	0.18
Nutrients Concentration	-0.28	0.12	-0.22	0.14
pH	0.09	0.56	0.11	0.36
Geographic Distance	-0.50	0.16	-0.10	0.50
Model	**0.84**	**0.00**	**0.82**	**0.00**

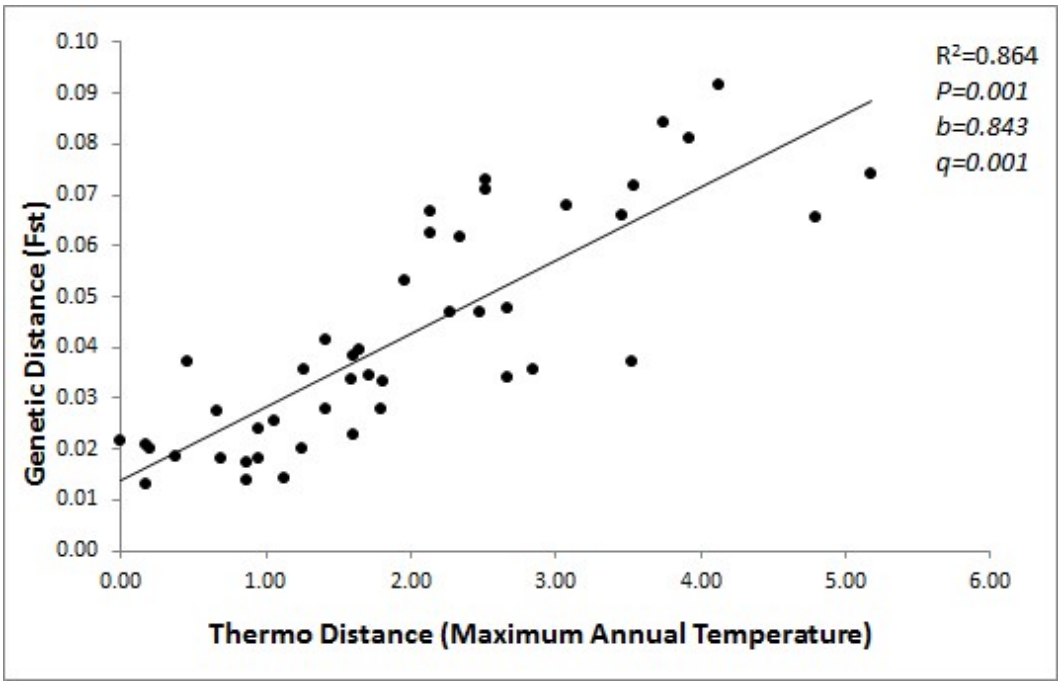

Figure 6.10: Correlation tests between thermal distance and genetic distance for pairs of Roe's Abalone sampling locations. Regression coefficient (R^2) and standardised regression coefficient ($\boldsymbol{b}$) with their associated significance *p* and *q* values (source CRC 2012/714 Final Report).

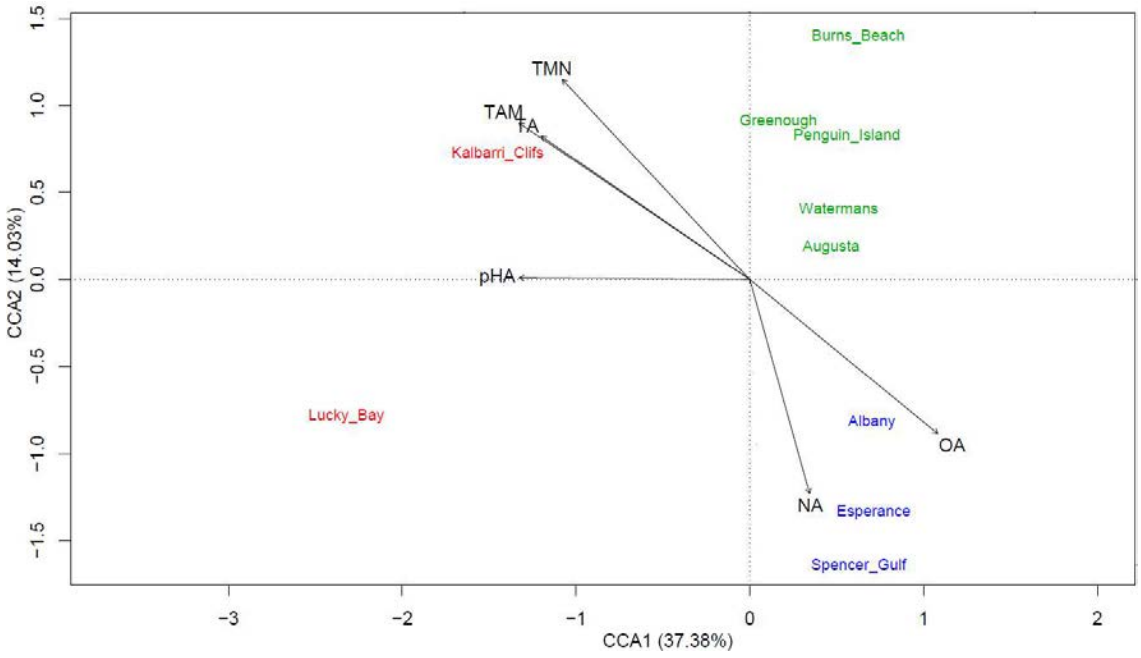

Figure 6.11: Canonical Correspondence Ordination based on six oceanographic variables and 538 "outlier" SNPs. The scatterplot shows sampling sites in relation to the first two canonical components, which explain 51.42% of the variation (source CRC 2012/714 Final Report).

Table 6.8: Canonical Correspondence Analysis (CCA) exploring the relation between Roe's Abalone allele frequencies of 538 "outliers" SNPs and six oceanographic variables. Simple CCA and partial CCA (geographic coordinates as conditionals). Significant canonical coefficients after correcting for false discovery rate are in bold (q<0.05) (source CRC 2012/714 Final Report).

Variables	**CCA**				**Partial CCA**			
	CCA1	CCA2	q	Variation explained (%)	CCA1	CCA2	q	Variation explained (%)
Temperature	**-0.73**	**0.50**	**0.01**		0.07	-0.06	0.46	
Maximum Temperature	-0.81	0.55	0.36		-0.09	0.04	0.45	
Marine Heatwave Max Temperature	-0.66	0.70	0.42		0.02	-0.01	0.54	
Oxygen Concentration	0.66	-0.54	0.28		-0.14	0.00	0.24	
Nutrients Concentration	0.21	-0.75	0.07		-0.41	0.22	0.09	
pH	-0.81	0.01	0.36		-0.62	0.08	0.35	
Geographic Connectivity	0.03	-0.58	0.45		-0.06	0.07	0.71	
Model	**0.37**	**0.14**	**0.02**	86.03	0.32	0.17	0.38	61.56
Conditionals (Latitude, Longitude)								38.44

Table 6.9: Canonical Correspondence Analysis exploring the relation between Roe's Abalone allele frequencies of 538 "outliers" SNPs and geographic space of sampling sites. Simple CCA and partial CCA (temperature as conditional). Significant canonical coefficients after correcting for false discovery rate are in bold (q<0.05) (source CRC 2012/714 Final Report).

Variables	**CCA**				**Partial CCA**			
	CCA1	CCA2	q	Variation explained (%)	CCA1	CCA2	q	Variation explained (%)
Latitude	**-1.00**	**0.01**	**0.00**		-0.16	-0.39	0.23	
Longitude	0.51	-0.86	0.21		-0.60	0.71	0.11	
Model	**0.28**	**0.12**	**0.00**	**40.04**	0.17	0.13	0.10	22.80
Conditional (Temperature)								25.70

6.2.6 Implications for Management and Restocking Initiatives

1. Levels of genome-wide diversity in Roe's Abalone were similar in all populations analysed. The highest diversity was detected in the populations close to Perth.
    - Emphasis should none-the-less be placed on maximising the genetic diversity of restocking efforts by using high founder numbers (e.g. to give genetically effective population sizes, Ne, greater than 500 individuals (Ryman and Laikre 1991; Tringali and Bert 1998).
2. The level of genetic diversity within the remnant Kalbarri Cliff population was comparable to that found within other Roe's Abalone populations along the WA coast (e.g. slightly higher than genetic diversity found within the neighbouring Lucky Bay population).
    - Because the sustained period of elevated water temperatures was a relatively recent event in relation to genomic sampling, there has not been time for loss of genetic diversity to occur from the remnant population. However, due to the low census and small effective population size of the remnant population, it's expected that loss of genetic variation would occur very rapidly in the next generations unless there is significant natural recruitment of abalone from neighbouring populations or extensive restocking programs are implemented to enhance recruitment.
3. The resulting pattern when only the fraction of the genomic dataset that is not under natural selection was considered, shows high connectivity and low population differentiation across the wide region sampled (i.e. from Kalbarri Cliff to Spencer Gulf). This was similar to patterns of connectivity reported for other Australian abalone species based on traditional genetic methods (e.g. Piggott et al. 2008) that are unable to pick up signs of selection.
4. A minimum of three genetically distinct groups can be clearly defined in our dataset when the section of the genome under selection was considered (i.e. functionally important section of the genome). These are: 1. Kalbarri Cliff to Lucky Bay, 2. Greenough to Augusta, and, 3. Albany to Spencer Gulf populations. Natural selection across the range has contributed significantly to the overall pattern of differentiation detected. Levels of genetic differentiation are generally low but highly significant between populations.
5. The seascape analyses support the existence of three environmentally different regions, which are mostly congruent with the three adaptive clusters found in Roe's Abalone. The adaptive differentiation between the three genetic clusters of Roe's Abalone was significantly correlated with a thermal geographic gradient.
    - Management should focus primarily on sourcing animals from close geographical locations (Lucky Bay if possible), as these locations are more likely to share similar environmental conditions to the collapsed population and as such these individuals are more likely to contain useful adaptations

promoting survival in the restocked environment. If Roe's Abalone numbers are low in the nearest geographical locations, and it is therefore not possible/desirable to source animals from these areas, then sourcing animals from other areas further down the coast needs to be considered.

During the restocking initiative all efforts were made to re-establish the Roe's Abalone populations at the Kalbarri Cliffs using individuals sourced from Lucky Bay (Section 4.1.4), given they are from the same adaptive population cluster. However, as the Lucky Bay population also suffered a mortality event (less intense) and the limited biomass present at that location, the Lucky Bay population could not be maintained as a source location. Individuals sourced from Greenough would be the next most appropriate as Greenough shows some genetic admixture with the northern locations and it was part of the most genetically similar cluster. Due to the extent of the marine heatwave, the variable effect on Roe's Abalone populations and the population distribution down the west coast of WA (as identified by the historical commercial catch, Table 3.1), the Perth Metropolitan Fishery should be utilised as the source location for future restocking efforts. This source location is from the next adaptive cluster and is possibly the only Roe's Abalone population with sufficient biomass to sustain the prolonged restocking initiative required to re-establish the Roe's Abalone populations in its northern distribution.

7 Benefits and Adoption

This research was originally designed for the commercial abalone industry in WA but its benefits will flow to the recreational fishing community, aquaculture industry, and fisheries managers and scientists. Not only were the assisted recovery strategies examined as a fisheries management tool to deal with stock issues driven by environmental fluctuations, but given the extent of the Roe's Abalone mortality event in its northern distribution it was important to assess their use as a conservation strategy for abalone species more broadly. The increased frequency of marine heatwaves around the globe and abalone species sessile nature, means range contractions, growth stunting and recruitment failure of abalone populations in the future are of serious concern to authorities around the world.

This project was able to demonstrate proof of concept for assisted recovery through translocation of adult Roe's Abalone and develop the techniques required to implement restocking of hatchery-reared juvenile Roe's Abalone. However, for recovery of the northern stock and reopening of the fishery to occur at some stage in the future, the assisted recovery program would need to be implemented on a commercial scale. The scale required would need to be at a level not yet seen in Australian abalone fisheries.

At present no natural recovery has been evident in the region north of Murchison River, therefore the only option for the commercial and recreational fishing sectors at present would be to continue and significantly increase the assisted recovery program in the Area 8 fishery. Due to the marine heatwaves effect on the Perth Metropolitan Fishery and areas further south, both sectors may see more immediate benefits from this project in the form of restocking initiatives in other regions of WA. The recreational fishing sector strongly supports the notion of restocking and stock enhancement as fisheries management tools, and this research provides the platform to implement Roe's Abalone stocking throughout WA.

In the short-term it is unlikely there will be many social or economic benefits from the assisted recovery program, other than for those community members directly involved in the program. If the program is implemented on a commercial-scale and successful in the long-term there could be substantial social and economic benefits. For example, if the fishery is restored the commercial industry would have economic benefits from an increase in TACCs, while the importance of recreational fishing to the region would have considerable social benefits and the subsequent flow on of associated economic benefits.

The WA abalone aquaculture industry will also significantly benefit from this research given the need for abalone hatcheries to produce juveniles for restocking initiatives. A commercial-scale assisted recovery program for the northern Roe's Abalone stock would create a constant demand for hatchery-reared juvenile abalone. Now that spawning protocols for Roe's Abalone have been developed as part of this project, not only could the aquaculture industry supply restocking or stock enhancement initiatives but there is the potential to produce animals for sale into more conventional markets. While this avenue may require further exploration, at least the option now exists to produce Roe's Abalone in commercial quantities for export markets.

Fisheries managers will find this research important in developing fisheries policy to manage restocking initiatives in WA. This includes the interaction between the abalone fishery and the aquaculture industry, and the issues that interaction poses such as maintaining genetic diversity and disease mitigation. The genomic analysis in this project detailed the genetic differentiation of Roe's Abalone populations throughout its distribution, which was fundamental in informing the genetic management strategy of the Abalone Aquaculture Policy in WA (DoF 2017b).

This research is not restricted to WA and the scientific knowledge obtained on population recovery, genetics, release methodologies and aquaculture production could be transferred to abalone fisheries around Australia and indeed the world. This allows international fisheries scientists and managers to benefit from the results of this project, as restocking initiatives become increasingly recognised as a management tool to deal with fundamental shifts in population dynamics caused by increased environmental variability, habitat loss and fishing pressure.

7.1 Further Development

The establishment of founder populations has been a significant achievement given the logistical challenges the remoteness of the Kalbarri Cliffs study zone poses. Therefore, the outcomes of this project need to be used as the platform for a long-term, large-scale assisted recovery strategy to re-establish the Roe's Abalone population. At present this appears unlikely but significant effort will continue to try and make this a reality. Continuation of monitoring at the release and control sites will be one aspect of the project that will be maintained. This will determine if recruitment continues to occurs at the founder populations and whether there are any signs of natural recovery in the region. This monitoring will form part of the annual stock assessment of the Area 8 fishery conducted by DPIRD scientists to evaluate the stock status and advise fisheries managers of any potential recovery.

In order to transition the techniques developed in this assisted recovery project to the commercial-scale required to fast track the recovery of Roe's Abalone, more research is required into release methodologies. In particular, more experimentation into release modules needs to be considered, given none were deemed an improvement on hand release. As the numbers of animals required to be released increases as part of a commercial-scale recovery strategy, how this occurs in a timely and efficient manner in the remote region would be paramount to the animals being released in the best possible condition to survive.

To facilitate a potential long-term, large-scale assisted recovery strategy a recovery model is currently under development. This model will utilise estimates of translocation survival and natural mortality derived in this project to calculate the number of adult Roe's Abalone required for release to produce and maintain founder populations of effective breeding size (e.g. >500 mature animals at >2 abalone.m^{-2}) at any given time post translocation. If more information on recruitment at the founder populations becomes available this can also be included to model population growth over time. If not, then assumptions based on the recruitment indices from the Perth Metropolitan Fishery could be made, particularly to deal with the discrepancies in scale between the current founder populations and the modelled population's ability to recruit. Also with further work on restocking of juvenile Roe's Abalone

to provide more robust estimates of survival etc., this could also be included in the recovery model as part of the long-term, large-scale assisted recovery strategy.

Before any further restocking of hatchery-reared juvenile Roe's Abalone into the Kalbarri Cliffs study area is conducted, significant research is required into areas such as, conditioning and spawning of north coast broodstock, nursery and grow-out culture, and the methods to release significant numbers of animals. While large numbers of juvenile Roe's Abalone have been produced as part of this project, concerns surrounding the broodstock's source location were identified through the adaptive population differentiation analysis in the genomic study (Section 6.2.3). To deal with this, it was proposed to source broodstock from the northern part of Roe's Abalone distribution, rather than the southwest or south coast of WA. However, there were several issues that meant this was not a possibility; 1) limited numbers of broodstock in the northern adaptive cluster, 2) no suitable aquaculture facilities near the Kalbarri Cliffs study zone, 3) lack of gonad development observed in wild Roe's Abalone in the Perth Metropolitan Fishery which coincided with the years of recruitment failure in this fishery (Hart et al. 2018), 4) spawning protocols for Roe's Abalone were only under development during this project (Section 5.2.2), and 5) the difficulty in getting wild south coast broodstock to spawn (Section 5.2.1). Therefore, significant work into several of these issues needs to be undertaken to continue restocking of hatchery-reared juvenile Roe's Abalone. This is currently occurring through a PhD study looking at the induction cues for spawning wild Roe's Abalone from the Perth Metropolitan Fishery.

Further research also needs to be conducted on the nursery and grow-out systems for Roe's Abalone to deal with the clustering behaviour and diet deficiencies identified in this study. Development of new system designs or diets for juvenile Roe's Abalone in the grow-out phase needs to occur to deal with the poor performance under current culture conditions. This would allow healthy hatchery-reared juvenile Roe's Abalone to be produced and potentially increase their survival and subsequently effectiveness in the restocking initiative.

7.2 Planned Outcomes

The planned outcome for this project was to determine whether assisted recovery programs can be a viable management tool in depleted abalone populations. This was achieved by creating viable breeding populations in the Kalbarri Cliffs study zone, a remote region at the northern distribution of Roe's Abalone that was depleted due to a devastating mortality event caused by anomalous environmental event. This project provides the foundation for developing a commercial-scale assisted recovery program and gives industry and managers in WA, and other abalone producing states contemplating recovery programs, confidence such a program is feasible. Particularly if the assisted recovery program is required in a more convenient location compared with one of the most remote abalone populations in Australia.

The translocation of adult Roe's Abalone established founder populations of effective breeding size, whereby recruitment was possible. While the recruitment was limited, it did demonstrate the assisted recovery program's ability to achieve its most crucial step in facilitating stock recovery. No natural recovery has been observed at the control sites in the Kalbarri Cliffs study zone and even with the limited recruitment present at the founder populations, assisted recovery

appears to be the most likely method to rebuild the population. However, this needs to be followed over time to determine whether natural or assisted recovery will be most appropriate over the long-term.

Through the translocation events, it allowed significant information to be gained into biological characteristics of Roe's Abalone, such as natural mortality and behavioural traits. This information can, not only be used to model a large-scale assisted recovery program, but also improves the limited knowledge of these parameters for Roe's Abalone fishery assessment across WA. The translocation methodologies were created to transport and release large numbers of both adult and juvenile abalone significant distances to isolated and inhospitable terrain. These methodologies are transferable to all abalone species and given their robustness, should be suitable in all conditions.

The development of spawning protocols for Roe's Abalone was an important outcome and an area of research that was added during the later stages of the project. The Roe's Abalone spawning protocols arose from a need to compare translocation of adult with the restocking of hatchery-reared juveniles as a management tool to recover abalone populations. Given the extent of the mortality event there were limitations on the availability of mature Roe's Abalone biomass in close proximity to the affected area, consequently the use of hatchery-reared juvenile abalone was considered an alternative as it provided a constant supply of abalone while also reducing the pressure on source populations. Before this project, Roe's Abalone had been cultured with very limited success and in order to facilitate the release of juvenile Roe's Abalone, significant research had to be carried out to develop reliable spawning protocols. This outcome not only benefited the assisted recovery program but also provided the aquaculture industry with an avenue to explore the potential for Roe's Abalone production.

The comprehensive genomic analysis in this project was a collaboration with the Seafood CRC Project 2012/714. The analysis examined the population genetic diversity and connectivity of Roe's Abalone stocks in WA. When examining the section of the genome under natural selection, three genetically distinct groups were defined. Identifying locally adapted genotypes would increase the success rate of translocating mature wild and restocking hatchery-reared juvenile Roe's Abalone. It also developed the genomic protocols to evaluate the genetic contribution of remanent and founder populations to stock recovery in the future.

The outcomes produced from this experimental recovery project for Roe's Abalone were communicated and disseminated to state and national abalone industry bodies, nation-wide abalone scientists and biologists as well as regional abalone managers. For the WA abalone industry (Abalone Industry Association of Western Australian) this occurred through Annual Management Meetings and Scientific Advisory Group meetings, as well as individual discussions with industry members. Continual communication of the outputs has occurred to fisheries managers at DPIRD to meet the long-term need of developing policies to incorporate assisted recovery programs into WA fisheries. Communication of this projects results and outcomes with the scientific and wider community is detailed below.

7.2.1 List of Publications / Media Produced

Several scientific manuscripts are currently under preparation and will be submitted to international peer-reviewed journals.

The research findings have been presented to the scientific community at an international conference, the 5th International Symposium on Stock Enhancement and Sea Ranching at the University of Technology in Sydney (October 2015).

The media articles that specifically relate to this project produced for websites and radio are listed below:

- ABC radio interview on the Mid West and Wheatbelt plus corresponding website article – "Experimental restocking of abalone off Mid-West coast" – November 2013.
- ScienceNetwork WA website article – "Kalbarri abalone gets helping hand" – January 2015.
- ABC radio interview on the Northern Rural Report and WA Country Hour plus corresponding website article – "High hopes for Kalbarri abalone restocking success, following marine heatwave" – February 2015.
- ScienceNetwork WA website article – "First success for recovering Kalbarri abalone" – April 2016.
- ABC radio interview on the Mid West and Wheatbelt plus corresponding website article – "Restocking program for Roe's Abalone at Kalbarri shows promising results" – April 2016.
- ABC radio interview on the Rural Report and WA Country Hour – 2017
- ABC radio interview on the Rural Report and WA Country Hour – May 2018.
- Media statement and social media video released on the DPIRD's Fisheries website – "Juvenile abalone found on Kalbarri reefs" – May 2018. The web news story is found at http://www.fish.wa.gov.au/About-Us/News/Pages/Juvenile-abalone-found-on-Kalbarri-reefs.aspx, and the YouTube Link is found at https://youtu.be/bnUzQqpE78U.

7.2.2 Public Benefit Outcomes

The recreational fishing sector and wider community have shown significant interest in the recovery effort, even though it was focused on a remote part of the Roe's Abalone fishery. This interest is driven by the recreational fishing sector's strong connection to public fisheries resources and the use of restocking and stock enhancement as fisheries management tools. Importantly, this research provides a positive example of how assisted recovery strategies using translocation and restocking can be implemented in remote fisheries affected by adverse environmental conditions.

During the project there was substantial community interest from the local region to participate in the translocation events. This community involvement not only increased awareness of the assisted recovery program for Roe's Abalone (this project), but also the 2011 marine heatwave and its effects on a range of fisheries in the region and the subsequent management responses.

This community interest is evident in the amount of media attention focused on the Mid-West region of WA (Section 7.2.1). The public support for this research indicates the communities desire to recovery the Roe's Abalone stock in its northern distribution for future generations.

7.2.3 Private Benefit Outcomes

The private benefit outcomes from this project have not come to fruition as the assisted recovery program is yet to demonstrate significant population recovery through large-scale recruitment in the Area 8 fishery. Given the only recruitment and in fact animals within the Kalbarri Cliffs study zone are those at the release sites, recovery is still localised to the founder populations. Therefore, the stock status of Area 8 fishery is still considered inadequate and there is no basis to open the fishery to either sector in the short or long-term. If a commercial-scale assisted recovery program is possible and successful in the long-term, the stock status in region could eventually be deemed adequate through a rigorous stock assessment process. Then the fishery could be opened and managed according to the Recovery Strategy currently under development as part of the Harvest Strategy (DoF 2017a).

7.2.4 Linkages with CRC Milestone Outcomes

This project directly relates to the CRC's Future Harvest Theme Outcomes 1 (Fisheries management delivering maximum benefit from the resource while maintaining stocks above sustainability indicators) and 2 (Novel management strategies in place which increase economic yield from our fisheries). It also targets the CRC's output 1.2 – Enhanced yields from wild-harvest innovations, by achieving the milestones 1.2.1 (Key constrains to increased production characterised and research prioritised in at least one selected fishery) and 1.2.2 (Production interventions implemented in at least one fishery).

7.3 Conclusion

The overall objective of this project was to investigate the viability of recovering a collapsed Roe's Abalone population through the creation of founder populations. This provided a unique opportunity to facilitate research on both translocation and restocking of abalone. The evaluation of these recovery strategies was critical in determining if they are viable fisheries management tools to deal with stock biomass issues driven by environmental fluctuations. This was achieved through the specific objectives, establish founder populations of Roe's Abalone in areas of mass mortality, evaluate the genetic structure of existing and founder populations, compare natural and assisted recovery rates of Roe's Abalone populations, evaluate the genetic contribution of existing and founder populations to stock recovery, and develop spawning protocols for Roe's Abalone and conduct a pilot juvenile stock enhancement release. All but one of these objectives were fully achieved. Given the limited number of recruits found at the founder populations and no natural recovery observed at the control sites, the objective to evaluate the genetic contribution of existing and founder populations to stock recovery was not able to be completed. As monitoring of the founder populations and control sites in the Kalbarri Cliffs study zone continues, the source of new recruits (sufficient numbers required) can be evaluated by the genomic tools developed in this project.

Not only did the translocation of mature wild abalone and restocking of hatchery-reared juvenile abalone in this project facilitate the comparison of natural and assisted recovery

strategies for a depleted abalone population. It also produced a range of important information on assisted recovery programs, aquaculture of Roe's Abalone and Roe's Abalone genetics, including:

- Founder populations of Roe's Abalone can be established in the remote regions of the Area 8 fishery.
- Recruitment is possible at founder populations created through translocation.
- Translocation protocols for the transport and release of both juvenile and adult Roe's Abalone to remote areas of its distribution.
- Estimates of natural mortality and identification of behavioural patterns such as aggregation.
- Detailed spawning protocols but further research required on nursery and grow-out systems.
- Aquaculture production of Roe's Abalone on a commercial scale is viable.
- The genetic diversity and connectivity in Roe's Abalone populations within WA.
- Implications for management regarding restocking initiatives (genetic diversity and adapted genotypes).

The Area 8 Roe's Abalone fishery has shown no signs of natural recovery post the 2011 marine heatwave, particularly in the region north of the Murchison River. In recent years most of the fisheries affected by the marine heatwave have shown some level of natural recovery if not fully recovered. This indicates that the mortality event suffered by the Roe's Abalone populations in its northern distribution was catastrophic and assisted recovery may be the only option for the conservation of these populations. In response to the marine heatwaves effect on fisheries the only other assisted recovery program attempted in WA was on Ballot's saucer Scallops in the Abrolhos Islands. However, this was only a small-scale translocation of broodstock from one part of the Abrolhos to another (Chandrapavan et al. in prep).

The scale of this Roe's Abalone translocation and restocking project in terms of numbers of animals and distance transported is one of the largest for abalone in Australia. Unfortunately, to have any chance of recovering the Area 8 fishery given the extent of the mortality event, the translocation and restocking initiatives would have to be increased dramatically. However, more research is required into the restocking of hatchery-reared juvenile Roe's Abalone in areas such as, conditioning and spawning of north coast broodstock, nursery and grow-out culture, and release of significant numbers of animals. Importantly the monitoring at the Kalbarri Cliffs study zone will continue. This will detect if more recruitment occurs at the founder populations and if any natural recovery is present at the control sites. Thereby evaluating natural versus assisted recovery of the Area 8 fishery over the long-term.

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9 Appendices

Appendix 1. Intellectual property

The results of this project have become public domain and will be published, widely disseminated and promoted with training and extension provided if required. There is no intellectual property associated with this research report and it is not anticipated that any patents will arise from this project.

Appendix 2. List of staff

The following Research Scientists conducted this project.

Dr Lachlan Strain

Dr Anthony Hart

Dr Jonathan Sandoval-Castillo

Dr Nick Robinson

Dr Luciano Beheregaray

Dr Nick Caputi

The following Technical Officers were engaged on this project.

Mr Jamin Brown

Mr Frank Fabris

Mr David Murphy

The following contributed significantly to this project.

Mr John Craike

Mr Shane Smith

Mr Vincent Encena