2-2016

Bioeconomic evaluation of commercial-scale stock enhancement in abalone

Anthony M. Hart

Lachlan W.S. Strain

Recommended Citation

Sandoval-Castillo, J., Robinson, N., Strain, L.W.S., Hart, A.M., Beheregaray, L.B. 2016. Stock enhancement in Greenlip Abalone :(5) Population Genomics. In: Bioeconomic evaluation of commercial-scale stock enhancement in abalone, eds. A.M. Hart and L.W.S. Strain, Fisheries Research Report 269, Department of Fisheries, Western Australia, 148 pp.

Fisheries Research Report No. 269, 2016

Bioeconomic evaluation of commercial-scale stock enhancement in abalone

Anthony M. Hart and Lachlan W. S. Strain (eds.)

Government of **Western Australia**
Department of **Fisheries**

An Australian Government Initiative

CRC AUSTRALIA

Australian Government
Fisheries Research and Development Corporation

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

Fisheries Research Division
Western Australian Fisheries and Marine Research Laboratories
PO Box 20 NORTH BEACH, Western Australia 6920

8602/15

Fish for the future

Suggested citation format

Entire report:

Hart, A.M. and Strain, L.W.S. (eds). 2016. Bioeconomic evaluation of commercial-scale stock enhancement in abalone. Australian Seafood CRC Report No. 2009/710. Fisheries Research Report No. 269. Department of Fisheries, Western Australia.

Individual chapter reports:

Sandoval-Castillo, J., Robinson, N., Strain, L.W.S., Hart, A.M., Beheregaray, L.B. 2016. Stock enhancement in Greenlip Abalone :(5) Population Genomics. *In:* Bioeconomic evaluation of commercial-scale stock enhancement in abalone, eds. A.M. Hart and L.W.S. Strain, *Fisheries Research Report* 269, Department of Fisheries, Western Australia, 148 pp.

The Australian Seafood CRC is established and supported under the Australian Government's Cooperative Research Centres Program. Other investors in the CRC are the Fisheries Research and Development Corporation, Seafood CRC company members, and supporting participants.

Office: Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042

Mail: Postal Box 26, Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042

Phone: Tollfree 1300 732 213 Phone 08 8201 7650 Facsimile 08 8201 7659

Website: www.seafoodcrc.com

ISSN: 1035-4549 (Print) ISBN: 978-1-877098-12-3 (Print)

ISSN: 2202-5758 (Online) ISBN: 978-1-877098-13-0 (Online)

Table of Contents

1 Non-Technical Summary

2009/710 Bioeconomic evaluation of commercial-scale stock enhancement in abalone

Principal Investigator: Dr Anthony Hart

Address: Department of Fisheries, 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) 92030199

Project Objectives

1. To estimate long-term growth and survival of enhanced Greenlip Abalone.
2. Undertake a bioeconomic analysis of large-scale stock enhancement in Greenlip Abalone.
3. To evaluate appropriate wild-stock management protocols that facilitate stock enhancement.
4. Develop bio-security protocols for stock enhancement.
5. To research and develop a commercial-scale stock enhancement manual.

Outcomes Achieved

The evaluation of commercial-scale stock enhancement was undertaken to determine its viability in the Western Australian Greenlip Abalone fishery. The key outcome of the project is that commercial-scale abalone stock enhancement is environmentally and economically achievable. The bioeconomic analysis revealed significant economic potential of a stock enhancement program for Australian *Haliotis laevigata* fisheries.

This analysis was possible through large-scale Greenlip Abalone releases into natural habitats within a commercial fishery. These releases of hatchery-reared abalone were able to significantly increase the biomass (density) of abalone and successfully produce individuals that entered the commercial fishery by reaching legal minimum length. This provided a greater understanding of the carrying capacity of abalone habitat and the ecological processes that influence wild abalone fisheries and stock enhancement.

The project also successfully developed a technical manual that standardises the transport and release protocols for abalone stock enhancement. The manual allows training of personnel so that commercial-scale stock enhancement can be logistically possible in Australian abalone fisheries.

The comprehensive genomic analysis of population genetic diversity and population connectivity of Greenlip Abalone stocks in Western Australia indicated levels of genome-wide diversity were similar in all populations. When the fraction of the genomic dataset not under natural selection was considered, the analyses indicated high population connectivity, with average differences in genetic diversity between sites of about 1% (Fst = 0.01). Analysis of the section of the genome under natural selection however, defined five genetically distinct groups of populations for Greenlip abalone. Geographic distance was not the most important explanation of these adaptive groups. Instead, the influence of different selective environments in shaping the connectivity, settlement and local persistence of abalone was stronger. Significant associations between the distribution of these adaptive groups and the spatial variation of key environmental parameters, including differences in temperature and oceanographic variables were found.

Commercial fishers will benefit from this research through a better understanding of stock enhancement's fundamental principles as a fisheries management strategy. The project outcome to implement commercial-scale stock enhancement to increase the value and profitability of the Western Australian Greenlip Abalone fishery was unsuccessful. This was due to industry concerns over biosecurity and a conservative approach to the outputs of this project. However, a sea-ranching commercialisation model was developed with a commercial aquaculture partner and commercial-scale sea-ranching has been implemented under an aquaculture license. One of the main hurdles to achieving commercial fishery enhancement in Australia was a disease issue through the presence of highly virulent herpes-like-virus (Abalone Viral

Ganglioneuritis – AbHV-1, AVG) in wild stocks in Victoria and Tasmania. If the commercial wild abalone industry changes their risk profile to this threat, all processes will be in place to facilitate commercial-scale stock enhancement in the Western Australian Greenlip Abalone fishery.

List of Outputs Produced

1. Formal assessment of the effect of stock enhancement on existing Western Australian abalone populations and their environment.
2. Rigorous scientific information on the natural ecology and ecological processes involved in stock enhancement of Greenlip Abalone, including long-term growth and survival estimates, habitat limitation and estimates of carrying capacity.
3. Bioeconomic model constructed evaluating the economic viability of abalone stock enhancement within Australia.
4. Spatial and temporal enhancement targets established for abalone stock enhancement in Western Australia.
5. Detailed stock enhancement manual produced, standardising the procedures for all aspects of commercial abalone enhancement programs.
6. Comprehensive genomic analysis of genetic diversity and connectivity in Greenlip Abalone stocks in Western Australia. Recommendations for capturing genetic diversity and adapted genotypes for increasing the chances of Greenlip Abalone stock enhancement success.
7. Risk assessments into the bio-security protocols of stock enhancement in Western Australia published, detailing procedures to minimise risk to the environment and existing stocks (Jones and Fletcher, 2012).
8. Department of Fisheries Western Australia policy for stock enhancement as a management strategy developed and published (Policy on restocking and stock enhancement in Western Australia FMR No. 261 and Abalone Aquaculture Policy 2013).

Summary

Seeding of hatchery-produced marine animals into productive fisheries, known as stock enhancement, is becoming a sought-after fisheries management strategy around the world. Stock enhancement is based on the principle that fish stocks are generally recruitment limited and that the carrying capacity of the ecosystem is rarely reached. This indicates that the system can accommodate a greater number of fish compared to what is naturally produced (recruited) from the breeding stock. Therefore, hatchery-produced animals can be released into wild populations to “fill the space” between the natural recruitment and the carrying capacity of the system, thereby increasing the overall biomass and subsequently the catch and profitability of fisheries.

However, stock enhancement programs worldwide have had limited success, which has led to a cautious approach and the scientifically rigorous protocol called the “Responsible

Approach" to stock enhancement. This approach details fundamental principles of stock enhancement, including natural ecological processes, economic performance and development of governance, as in the past a lack of understanding of these principles has hindered the practice of stock enhancement.

Abalone enhancement programs are no exception to this, with many studies around the world struggling to have commercial success. Given abalone fisheries are high value, low volume and in Australia have been sustainably managed over a long period of time, Australian abalone fisheries are well placed to incorporate stock enhancement as a fisheries management strategy. Therefore, the focus of this project was to evaluate the potential of commercial-scale stock enhancement of Greenlip Abalone (*Haliotis laevigata*) in Western Australia. In doing this, the project aimed to address some of the current knowledge gaps in abalone stock enhancement within Australia by conducting a series of large-scale juvenile Greenlip Abalone releases into natural habitats within a commercial fishery.

These stock enhancement experiments allowed investigation into biological data such as long-term growth and survival estimates, which indicated that released juvenile abalone (Age 1.5 and ~ 30 mm) reach legal minimum length (140 mm) at approximately 5 years of age and there is clear evidence of these abalone being commercially fished. Release mortality is considered critical as initial (6 month) survival differs significantly among sites but not beyond this time period. Given the effect of habitat variation on abalone survival, the development of a population survey technique that measured density, as a function of available habitat was important to assess population and ecological responses of enhancement.

In examining the carrying capacity of the ecosystem, releases of juvenile abalone were able to initially increase densities significantly (up to 800%), however after 2.5 years they stabilised at 8 per m^2, which was still an increase of 400% above baseline densities. This was the predicted carrying capacity with the enhanced abalone cohort represented 50% of the population and demonstrates that the system is recruitment limited and can accommodate greater abalone biomass. Given no environmental effects from enhancement were detected other than the increase in abalone density, it suggests that as long as release densities are controlled within natural limits, successful stock enhancement can be attained for this species with minimal ecological impacts.

This greater understanding of natural population processes and new quantitative approaches to determining viable habitat and associated release densities, allowed a bioeconomic evaluation of commercial-scale stock enhancement to be conducted. This bioeconomic analysis also took into account current fishery assessment information and economic data collected from the commercial industry. The analysis was initially conducted on a Western Australian fishery with enhancement targets defined as a function of natural recruitment and compared to current harvest strategies. It was then applied to the Australian Greenlip Abalone fishery as a whole and revealed significant economic potential for stock enhancement programs. To achieve this potential an integration of enhancement inputs and harvest strategy outputs is essential, where they are considered part of the same fisheries management system.

Given the bioeconomic evaluation indicated that abalone stock enhancement within Australia is potentially viable; a methodology for the commercial enhancement of juvenile Greenlip Abalone into the areas of the Western Australian abalone fishery was investigated. This methodology standardised the logistics, schedule and techniques of enhancement into a manual for the training and education of organisations and personnel throughout Australia, that plan on utilising stock enhancement as an abalone fishery management strategy.

New diagnostic genomic tools were developed to study natural population genetic structure and monitor the success of stock enhancement in a commercial Greenlip abalone fishery within Western Australia. Samples from 372 Greenlip abalone collected from 13 locations from across the WA fishery were analysed using the new tools, and produced 69,720 high quality genomic markers in the form of SNP's (single nucleotide polymorphisms). The screening of genome-wide variation in samples collected from the wild shows 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, five genetically distinct groups of populations for Greenlip abalone were identified. Significant associations between the distribution of these adaptive groups and the spatial variation of key environmental parameters, including differences in temperature and oceanographic variables were found. This finding will help managers select which abalone populations are likely to perform best in specific environments (i.e. likely fitness), consequently improving the chances of successful stock enhancement programs.

Overall these results suggest that if the general principles of abalone stock enhancement, including ecological processes and the carrying capacity of the system, genetic, economic parameters, governance (policy) and bio-security are understood and brought together, then commercial-scale enhancement of Greenlip Abalone is feasible in Australian abalone fisheries.

Keywords: Greenlip Abalone, *Haliotis laevigata*, stock enhancement, abalone seeding, demographic parameters, BACI experiment, carrying capacity, bioeconomic model, procedural manual, genomics, population genetic structure.

2 Acknowledgements

Firstly the authors would like to acknowledge the financial contribution the Australian Seafood Cooperative Research Centre (CRC) made towards funding this research through the project 2009/710 “Bioeconomic evaluation of commercial-scale stock enhancement in abalone”. Also to the Department of Fisheries Western Australia for supporting the research throughout its entirety, from its infancy back in 2004 to the completion of this report.

To all the Department of Fisheries WA staff that contributed towards this research, from the fieldwork (diving) component of the preliminary enhancement trials through to the data analysis and bioeconomic modelling, it was greatly appreciated. This includes but is not limited to Frank Fabris, Mark Davidson, Jamin Brown, David Murphy, Stephen Leporati, Fiona Parker, Sam Hair, Sabine Daume, Alex Hesp and Nick Caputi.

The population genomic study was a collaboration between Department of Fisheries WA, Flinders University and Nofima through the Seafood CRC projects 2009/710 “Bioeconomic evaluation of commercial scale stock enhancement in abalone” and 2012/714 “PDRS: Use of next generation DNA technologies for revealing the genetic impact of fisheries restocking and ranching”. The investigators of this project sincerely thank Jonathan Sandoval-Castillo and Luciano Beheregaray from Flinders University and Nick Robinson from Nofima for conducting the excellent genomic population analysis of Greenlip 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 Seafood CRC Final Report.

The authors’ thanks go to the Western Australian Abalone Industry Association in particular Ian Taylor and Brad Adams for facilitating this innovative work. As well as all the industry divers who participated in the site selection, juvenile release experiments and provided the economic data for fishing costs, namely Darren and Nathan Adams, Jay Clauson, John Lashmar, Michael and Peter Rickerby, and Steve and George Beres.

Appreciation goes to the two commercial abalone farms in Western Australia, for the hatchery culture of *Haliotis laevigata* required for the enhancement experiments conducted throughout this research. Firstly, to the former Great Southern Marine Hatchery in Albany and its managers Steve Parsons and Rick Lambert with assistance from Sabine Daume, Fiona Graham and Mark Davidson; secondly, 888 Abalone in Bremer Bay and its directors Peter and Craig Kestel, as well as its farm manager Shane Smith.

Lastly, thanks also to Professor Kai Lorenzen (University of Florida) for detailed discussion, evaluations and useful tips on the “EnhanceFish” software and bioeconomic modelling, as well as guidance on stock enhancement policy development.

3 General Introduction

3.1 Background

Stock enhancement, and in particular enhancement of high value sedentary shellfish is being increasingly recognised as a novel management strategy that could increase economic yield of a fishery (Caddy and Defeo, 2003). This particularly applies to abalone fisheries with most abalone-producing countries with access to aquaculture techniques, experimentally trialling stock enhancement and assessing it's viability for use as a fishery management strategy. Stock enhancement of abalone actually originated from research into restoring collapsed fishing stocks (Hammasaki and Kitada, 2008) and is principally an assisted recruitment program for natural abalone populations.

Overall, the success of stock enhancement as a fishery management strategy across varying fisheries around the world has been limited (Molony et al., 2003). In the past this has generally been the case for abalone fisheries, even with a number of countries examining different approaches and scales of stock enhancement programs. For example, Japan has had a long running, large-scale abalone enhancement program (approx. 40 years), which has produced very little effect on overall fisheries production (Hammasaki and Kitada, 2008). However, success has occurred within isolated locations enhanced by this program (Kojima, 1995, Masuda and Tsukamoto, 1998), indicating localised small-scale enhancement can be possible. Other countries including South Africa, New Zealand and Australia have conducted small-scale novel approaches to stock enhancement by looking at, enhancing abalone beyond their current natural distribution (Sweijd et al., 1998), preliminary identification of suitable release habitat (Schiel, 1993), seeding using abalone larvae (Shepherd et al., 2001) and utilising experimental, artificially constructed habitats (Dixon et al., 2006). Even though these and many other abalone stock enhancement studies have had varying degrees of success, the important outcome has been the range of natural population factors identified that require significant assessment before abalone stock enhancement can be possible on a commercial-scale.

Given this general lack of success in many marine stock enhancement programs worldwide, it has prompted a cautious approach to future development (Kitada and Kishino, 2006) and the promotion of a scientifically rigorous protocol called the "Responsible Approach" to stock enhancement (Blankenship and Leber, 1995; Lorenzen et al., 2010). This approach details fundamental stock enhancement principles including evaluation of release densities, examination of ecological processes, assessment of economic performance and development of governance, as well as identifying inherent threats to the system. Poor knowledge of these principles and the processes of natural population regulation and fisheries management will ultimately hinder the practice of stock enhancement. Therefore, in any commercial-scale application, it is essential that variability in parameters such as growth, mortality and recruitment of the target populations be taken into account when evaluating stock enhancement programs.

The carrying capacity of an aquatic system encompasses these ecological processes and can be seen as the theoretical basis for successful stock enhancement. Generally the carrying

capacity of fish stocks are rarely reached except in exceptional years and therefore additional recruits can be accommodated, up to the carrying capacity limits of the system (Hart et al., 2007). It is this difference between the current recruitment limited abalone populations and the potential carrying capacity of the system that stock enhancement targets. However, for stock enhancement to be successful a strong understanding of species ecology and the carrying capacity of the ecosystem is necessary so that ecological processes such as density-dependence can effectively regulate enhancement parameters including size at release (Hillborn, 1998; Bell et al., 2005). In fact, a review by Hammasaki and Kitada (2008) indicated understanding local carrying capacity and the natural ecology of the species was the key to improving the large-scale Japanese enhancement program.

Recent studies on abalone stock enhancement have continued to investigate the important ecological processes affecting the successful implementation of enhancement programs. For example, in New Zealand Roberts et al. (2007) identified the critical habitats for release, and now stock enhancement of juvenile *Haliotis iris* (»12 mm) is considered feasible if the sites and habitat are carefully selected. A preliminary assessment of stock enhancement in Western Australia also identified habitat as having a significant effect on the survival of juvenile abalone released and with more robust estimates of ecological parameters, commercial-scale stock enhancement could be possible (Hart et al., 2007).

As the fundamentals of the "Responsible Approach" are applied to new abalone stock enhancement programs there becomes greater understanding of the ecological process, specifically the carrying capacity of the system. However, there is also a need for robust evaluation of the bioeconomics of stock enhancement in abalone with long-term growth and survival, accurate economic data and a proper assessment of the ecological impacts of such an activity. If this evaluation indicates stock enhancement is economically viable, an enhancement program would provide the fishery with a biomass level that may only ever be achieved in an exceptional year of natural recruitment, and in the longer term, it will rebuild stock numbers towards virgin levels, thus increasing catch rates and ultimately economic efficiency and profitability of the abalone fishery.

3.2 Need

Abalone fisheries currently contribute 15% ($200 million) of the total annual GVP of Australian fisheries (Mayfield et al., 2012). In Western Australia the Greenlip Abalone (*Haliotis laevigata*) is the most valuable abalone species of the three commercially exploited, with the limited entry fishery first established in 1970 and 166 tonnes (whole weight) of Greenlip Abalone landed in 2013 (Hart et al., 2014). During these 42 years the catch has fluctuated slightly but with the high market value of abalone and a greater importance placed on research, the fishery has seen the implementation and improvement of various harvest strategy output controls, such as Total Allowable Commercial Catches (TACCs), size limits, management areas and licence holder allocations, to more accurately and sustainably manage the fishery.

A recent review of the Greenlip Abalone fishery management produced an annualised standardised catch per unit effort (SCPUE) index (Hart et al., 2009). This SCPUE is now the primary indicator for the fishery and forms the basis of the decision rule framework for

annual quota setting. The standardisation of the CPUE takes into account factors such as the abalone diver, sub-area, year and month of fishing as well as technological improvements such as GPS and internet weather prediction services. Given the need to continually improve the way fisheries are managed to provide a sustainable marine resource into the future, the Department of Fisheries Western Australian along with the Western Australian Abalone Industry Association (WAAIA) are constantly investigating and researching new abalone fisheries management strategies.

Stock enhancement as a management strategy remains one of the few viable alternatives for increasing the biomass and profitability of an abalone fishery without compromising the current fishery in terms of access or allowable catches (Hart et al., 2007). The attributes that make Australian abalone fisheries ideally suited for stock enhancement are that they have been subject to long-term sustainable management (Mayfield et al., 2012) and that they are low volume, high value sedentary invertebrate fisheries (Bell et al., 2005; Caddy and Defeo, 2003). Given the benefits of stock enhancement numerous experimental studies have been undertaken in Australia on the two major commercial species, Blacklip (*Haliotis rubra*) and Greenlip Abalone (Shepherd et al., 2001; Dixon et al., 2006; Goodsell et al., 2006; Heasman, 2006; Hart et al., 2007; Chick, 2010).

In 2006, 2008 and 2011 the Department of Fisheries Research Division in collaboration with the WAAIA commenced multiple large-scale field study trials to assess the potential of stock enhancement as a management strategy. This project aims to complete these existing large-scale, long-term field studies, while producing robust estimates of growth and survival to harvestable sizes, estimation of ecological effects, compilation of accurate economic data and undertake a bioeconomic analysis of enhancement, therefore enabling a comprehensive commercial-scale evaluation to be completed. If the trials and evaluation are successful in demonstrating the bioeconomic viability of enhancement, the WAAIA can commercialise these results and establish a stock enhancement program in the Western Australian Greenlip Abalone fishery.

3.3 Objectives

1. To estimate long-term growth and survival of enhanced Greenlip Abalone.
2. Undertake a bioeconomic analysis of large-scale stock enhancement in Greenlip Abalone.
3. To evaluate appropriate wild-stock management protocols that facilitate stock enhancement.
4. Develop bio-security protocols for stock enhancement.
5. To research and develop a commercial-scale stock enhancement manual.

3.4 References

Bell, J. D., P. C. Rothlisberg, J. L. Munro, N. R. Loneragan, W. J. Nash, R. D. Ward and N. L. Andrew. Restocking and stock enhancement of marine invertebrate fisheries. Adv. Mar. Biol., 49: 1-370 (2005).

Blankenship, H. L. and K. M. Leber. A responsible approach to marine stock enhancement. Am. Fish. Soc. Symp., 15: 167–175 (1995).

Caddy J. F. and O. Defeo Enhancing or restoring the productivity of natural populations of shellfish and other marine invertebrate resources. FAO Fisheries Technical Paper No. 448, FAO, Rome, Italy, 159 pp (2003).

Chick, R. C. Stock enhancement of local populations of blacklip abalone (*Haliotis rubra*, Leach) in New South Wales, Australia. PhD Thesis, James Cook University of North Queensland, Australia. 233 pp (2010).

Dixon, C. J., R. W. Day, S. M. H. Huchette and S. A. Shepherd. Successful seeding of hatchery-produced juvenile greenlip abalone to restore wild stocks. Fish. Res., 78: 179-185 (2006).

Goodsell, P. J., A. J. Underwood, M. G. Chapman and M. P. Heasman. Seeding small numbers of cultured black-lip abalone (Haliotis rubra Leach) to match natural densities of wild populations. Mar. Freshw. Res., 57: 747-756 (2006).

Hammasaki, K. and S. Kitada. The enhancement of abalone stocks: lessons from Japanese case studies. Fish and Fisheries, 9: 1-18 (2008).

Hart, A. M., F. P. Fabris and S. Daume. Stock enhancement of *Haliotis laevigata* in Western Australia – a preliminary assessment. Fisheries Research Report No. 166, Department of Fisheries, Western Australia (2007).

Hart, A., F. Fabris and N. Caputi. Performance indicators, biological reference points and decision rules for Western Australian abalone fisheries (*Haliotis sp*.): (1) standardised catch per unit effort. Fisheries Research Report No. 185, Department of Fisheries, Western Australia (2009).

Hart, A., F. Fabris and J. O'Malley. Greenlip/Brownlip Abalone Fishery Status Report. In: Status Reports of the Fisheries and Aquatic Resources of Western Australia 2013/14: The State of the Fisheries eds Fletcher, W.J., and K. Santoro, Department of Fisheries, Western Australia, pp 242-247 (2014).

Heasman, M. P. In pursuit of cost-effective fisheries enhancement of the New South Wales blacklip abalone, Haliotis rubra (Leach) fishery. J. Shellfish. Res., 25: 211-224 (2006).

Hillborn, R. The economic performance of marine stock enhancement projects. Bull. Mar. Sci., 62: 661-674 (1998).

Kitada S and H. Kishino. Lessons learnt from Japanese marine finfish stock enhancement programs. Fish. Res., 80: 101-112 (2006).

Kojima, H. Evaluation of abalone stock enhancement through the release of hatchery-reared seeds. Mar. Freshwater Res., 46: 689-695 (1995).

Lorenzen, K., K. M. Leber and H. L. Blankenship. Responsible approach to marine stock enhancement: An update. Rev. Fish. Sci., 18(2):189–210 (2010).

Masuda, R. and K. Tsukamoto. Stock enhancement in Japan: review and perspective. Bull. Mar. Sci., 62:337-358 (1998).

Mayfield, S., C. Mundy, H. Gorfine, A. M. Hart and D. Worthington. Fifty years of sustained production from the Australian abalone fisheries. Rev. Fish. Sci., 20(4): 220-250 (2012)

Molony, B. W., R. Lenanton, G. Jackson and J. Norris. Stock enhancement as a fisheries management tool. Rev. Fish Bio. Fish., 13:409-432 (2003).

Roberts, R. D., E. F. Keys, G. Prendeville and C. A. Pilditch. Viability of abalone (*Haliotis iris*) stock enhancement by release of hatchery-reared seed in Marlborough, New Zealand. J. Shell. Res., 26(3): 697-703 (2007).

Schiel, D. R. Experimental evaluation of commercial-scale enhancement of abalone Haliotis iris populations in New Zealand. Mar. Ecol. Prog. Ser., 97: 167-181 (1993).

Shepherd, S. A., P. A. Preece and R. G. W. White. Tired nature's sweet restorer – ecology of abalone stock enhancement in Australia. In: Workshop on rebuilding abalone stocks British Columbia (A. Campbell, Ed.). Can. Spec. Publ. Fish. Aquat. Sci., 130: 84-98 (2001).

Sweijd, N., Q. Snethlage, D. Harvey and P. Cook. Experimental abalone (*Haliotis midae*) seeding in South Africa. J. Shell. Res., 17(3): 89-904 (1998).

4 Stock Enhancement in Greenlip Abalone: (1) Long-Term Growth and Survival

Anthony M. Hart[1], Lachlan W. S. Strain[1], Frank Fabris[1], Jamin Brown[1] and Mark Davidson[1]

[1]Western Australian Fisheries and Marine Research Laboratories, PO Box 20, North Beach, Western Australia, 6920, Australia.

Please note that this chapter has been published as the following manuscript:

Hart AM, Strain L, Fabris F, Brown J, Davidson M (2013). Stock enhancement of greenlip abalone: (1): Long-term growth and mortality. *Rev. Fish. Sci*. 21: 299-309

4.1 Abstract

A cohort of *Haliotis laevigata*, spawned from wild broodstock, was monitored from settlement at a hatchery until recruitment into the fishery (Age 5+). Animals were released into the wild at 31 mm (± 4 SD), targeting an enhancement size-class of 135 - 145 mm shell length. Release densities were tailored to match wild-stock densities using a size-dependent mortality model. A total of 7500 animals were released into 24 sites, and each site was precisely mapped to control release densities. Environmental and husbandry factors were also quantified. Initial survival rates (6 months post release) differed significantly among sites (range: 11 – 67%), but not beyond this time period. Legal minimum length (140 mm) was achieved, on average, at 5 years of age or 3.5 years post release and there was clear evidence of fishing mortality on the seeded cohort by Age 6+. Cumulative survival at Age 5 varied between 20% at the better sites, and 6% at the worst sites, with an average of 13%. Water depth was significantly positively correlated with growth (r = 0.47; $p < 0.05$), but no other ecological variables influenced growth or survival. Husbandry factors were implicated in sites with poor survival, but this was not confirmed statistically. The cohort successfully entered the fishery and was harvested at a fishing mortality (F) of 0.36, which was similar to the fishing mortality estimates for the entire fishery.

Keywords: abalone seeding, fisheries enhancement, demographic parameters.

4.2 Introduction

Stock enhancement has been a sought after strategy in abalone fisheries management for a number of decades. Initially researched as a measure for restoring collapsed abalone stocks (Hammasaki and Kitada, 2008), it has been experimentally trialled in almost all abalone-producing nations of the world with numerous conferences and varying degrees of progress (e.g. Campbell, 2001), but no outstanding examples of success. In Japan between 20 and 30 million hatchery-bred juveniles were released annually between the mid-1960s and 2005 (Hammasaki and Kitada, 2008). These provided negligible overall benefit for fishery production, however individual successes were achieved. Recapture rates from individual cohorts exceeded 50% in some localised cases (Kojima, 1995) and a review of the Japanese enhancement program concluded that the future lay in understanding local carrying capacity and the natural ecology of the species (Hammasaki and Kitada, 2008).

Elsewhere attempts have been of an experimental nature and focused at a 'proof of concept' scale rather than concerted efforts to increase fishery production. South African attempts with *Haliotis midae* have involved a novel introduction of juveniles beyond their current distribution, within areas containing fossil evidence of prior habitation (Sweijd et al., 1998). Positive results were obtained, but subject to key ecological constraints such as presence of suitable habitat (De Waal and Cook, 2001). Shepherd et al. (2001) considered larval releases in *Haliotis laevigata* and *H. rubra*, but noted habitat limitation in the crustose coralline algal (CCA) community. Larvae preferentially settle in this limited habitat, utilise it for up to 3 months, and survival is strongly density-dependent.

Unless animals are released above the size at which density-dependence process are influential, enhancements are likely to be unsuccessful, so a strong understanding of species ecology and carrying capacity of the ecosystem is necessary (Hillborn, 1998; Bell et al., 2005). Recent experiments on New Zealand abalone (*Haliotis iris*) have identified the critical habitats for release (Roberts et al., 2007), a factor not established with certainty in earlier studies (Schiel, 1993). Stock enhancement in *H. iris* using juvenile animals (»12 mm) is now considered feasible if the sites and habitat are carefully selected (Roberts et al., 2007), and release densities are designed to match natural densities (Goodsell et al., 2006). This also assumes detailed knowledge of the natural mortality schedules of juveniles and adults.

Regarding *Haliotis laevigata* (Greenlip Abalone), an important commercial species in Australia, enhancement experiments have focused on larval release (Shepherd et al., 2001), and juvenile releases in experimentally constructed (Dixon et al., 2006) and natural habitats (Hart et al., 2007). Larval seeding was not recommended because of CCA habitat limitation, but promising results with juveniles (20 - 35 mm) were found with releases onto experimental boulder habitats (Dixon et al., 2006) and natural habitats (Hart et al., 2007). In this study we seek to extend these pilot studies by evaluating long-term growth and survival of a cohort of *H. laevigata* released into the wild at age 18 months (»30 mm shell length) and monitored until Age 6. The method used was multiple mark recapture (MRR), and release densities were determined with a size-dependent mortality model that incorporated differential mortality between life history stages (Lorenzen, 2006). The long-term MRR study was accompanied by a population and ecological study designed under BACI (Before-After-Control-Impact) principles (Hart et al., 2013). Together these two studies represent a significant advancement in our knowledge of stock enhancement in abalone and provide some novel quantitative approaches to determining viable habitat and associated release densities.

4.3 Materials and Methods

4.3.1 Study site, spawning and culture methods

Enhancement experiments were carried out on Haliotis laevigata stocks in the Augusta region of Western Australia (Figure 1.1). This region comprises an important part of the entire Western Australian Greenlip Abalone fishery, producing approximately 30% of the total catch. Hatchery-reared juveniles from wild broodstock were used in the grow-out experiment. Broodstock were spawned on the 4th October 2004 at Great Southern Marine Hatcheries (GSMH) in Albany, Western Australia and cultured according to standardised abalone aquaculture protocols for this species (Daume and Ryan, 2004; Daume et al., 2004; Strain et

al., 2006). Disease-free certification of abalone to be released was achieved from the Department of Fisheries Fish Health Unit prior to enhancement experiments.

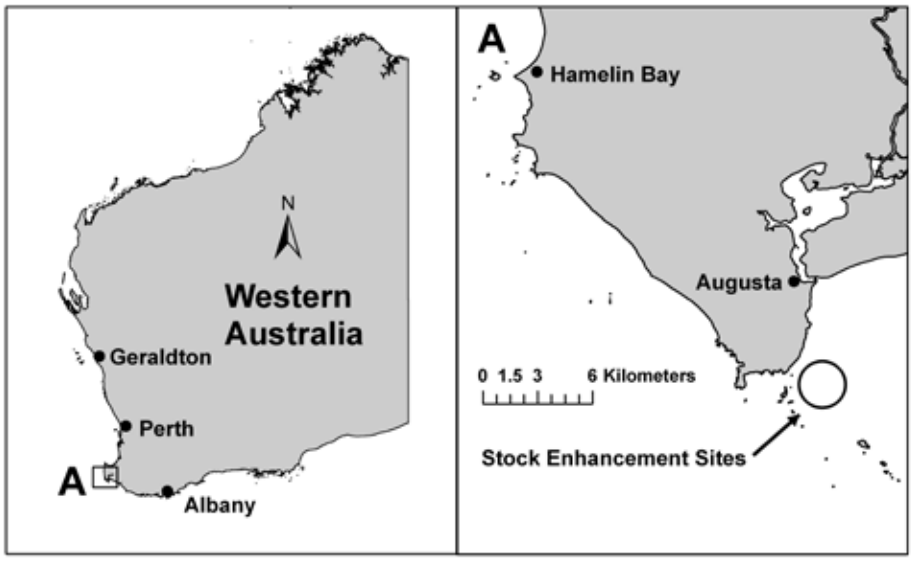

Figure 1.1. Location of study sites for stock enhancement experiments on Greenlip Abalone (*Haliotis laevigata*), near Augusta, Western Australia.

4.3.2 Optimising release densities

Estimation of release densities was undertaken with a modelling approach. The approach utilised knowledge of three key ecological parameters; (1) size-dependent mortality, (2) size-dependent growth, and (3) target enhancement density at *n* years post-release, where *n* was 4 years is this study. The objective was to match natural densities so as to minimize the risk of density-dependent effects influencing survival.

Size-dependent estimates of growth in *Haliotis laevigata* were obtained from published studies (Shepherd and Hearn, 1983; Shepherd, 1988; Shepherd et al., 1992; Wells and Mulvay, 1995; Officer, 1999). Growth is highly variable for this species, but for the purposes of developing the release density model, assumed mean annual growth increments for animals released at 31 mm (Age 1.5) were 39, 35, 20, and 15 mm respectively, arriving at the target enhancement size of 140 mm at 4 years post-release.

A single-parameter size-dependent mortality model (Lorenzen, 2006) was used as it required only one input parameter (M1 – natural mortality at unit length [1 cm]) and could be easily manipulated to represent both cumulative and point estimates of mortality (i.e. M for a certain size) that were equivalent to published estimates of M in this species. The mortality model was $M(L_{cm}) = M1/L_{cm}$ where M1 is the natural mortality at unit length (1 cm), and $M(L_{cm})$ is the natural mortality at length L (Lorenzen, 2006). Values of M1 between 2 and 5 were investigated (Figure 1.2A) as these corresponded with published estimates of adult Greenlip mortalities of between 0.13 and 0.4 (Shepherd and Baker, 1998). Predicted cumulative survival at four years post-release (Figure 1.2A) varied between 36% (M1 = 2) and 5% (M1 = 5). The survival predicted by an M1 of between 3 & 4, corresponding to cumulative survival between 8% and 15% was considered the most likely scenario. The release density model for *Haliotis laevigata* was as follows:

$$D_{r,x} = \frac{D_{t,y}}{C_{s,y}}$$ **Equation 1.1**

where $D_{r,x}$ is the density of release (# per m^2) for size class x (»31 mm shell length), $D_{t,y}$ is the target density (# per m^2) for size class y (140 mm shell length), and $C_{s,y}$ is the cumulative survival at y years post-release, assumed to be the time taken for release size *x* to grow to the target size *y*. Densities of abalone (³ 140 mm) prior to enhancement were estimated at 1.3 per m^2 (± 0.3) using a new habitat survey method (Hart et, al., 2013). A figure of approximately twice this density (2.5 per m^2) was chosen as $D_{t,y}$.

Translating the model into release densities, a release of 35 per m^2 would result in a $D_{t,y}$ of 2.5 per m^2 at 4 years post-release, if the survival trajectory is based on M1 = 4 (Figure 1.2B). It was assumed that the mean area of habitat targeted by each release was 4 m^2; this resulted in a release of 140 animals (35 x 4) as a maximum density release. The minimum density release was 70 animals (18 per m^2).

4.3.3 Site selection and experimental design

Sites were selected with the assistance of commercial abalone divers to ensure that appropriate habitat was targeted. A total of 24 sites were selected, 12 for the maximum density release (35 per m^2) and 12 for the minimum density (18 per m^2). Three experimental release points were positioned within these sites; one at the permanent mooring (0°, 0m), and the other two at known locations along two fixed transects of 30 x 1m quadrates radiating from the permanent mooring (Figure 1.3). All sites were surveyed three times (6, 12 and 18 months post-release). Following this, 4 representative sites were selected and monitored for a total of 4.5 years, between the initial survey in April / May 2006, and December 2010. Preliminary analyses detected no difference in densities post-seeding and all seeded sites were pooled for analysis purposes.

4.3.4 Tagging, transport, release, and recapture methods

Seventy percent of animals released were tagged for mark-recapture analysis, with two tagging methods employed. Initially juvenile abalone were tagged using a numbered tag attached to a steel spring (Figure 1.4A). The spring was slipped over the growing edge of the shell, and incorporated into the shell matrix by the animals' shell deposition process. Tags were applied 8 weeks prior to release to ensure they were embedded within the shell. At 24 to 48 hours prior to release, abalone were loaded into seeding devices constructed from PVC (dimensions 300 x 100 x 50 mm; Figure 1.4B), and transported from the hatchery to the boat ramp using large seawater tubs (400 L), aerated with O_2. Tubs were loaded up onto one research and six commercial abalone dive vessels, each of which had a pre-defined release schedule to achieve (»3 to sites per vessel). Release sites were randomly allocated amongst personnel to enable a comparative analysis of survival. The release procedure involved swimming to the chosen release point and placing the release devices into suitable habitat (Figure 1.4B).

Recapture surveys for the juvenile tagged animals involved locating all three-release positions at each site (see Figure 1.3), and conducting a thorough search up to an approximate radius of 10m from the release position. Average search time was 41 minutes (± 16 SD) and

depended largely on the complexity of the habitat. Tag loss and mortalities were also quantified into 3 categories (see data analysis section).

After 18 months survival rates had stabilised, and a subset of four representative sites were selected for longer term monitoring at 12-month intervals. At these sites animals (n = 120) were re-tagged *in-situ* with a marine epoxy resin tag (Figure 1.4C) to minimise further tag loss, as the initial spring tags were beginning to be unreadable. The brand of epoxy resin used was Emerkit 2 part epoxy putty.

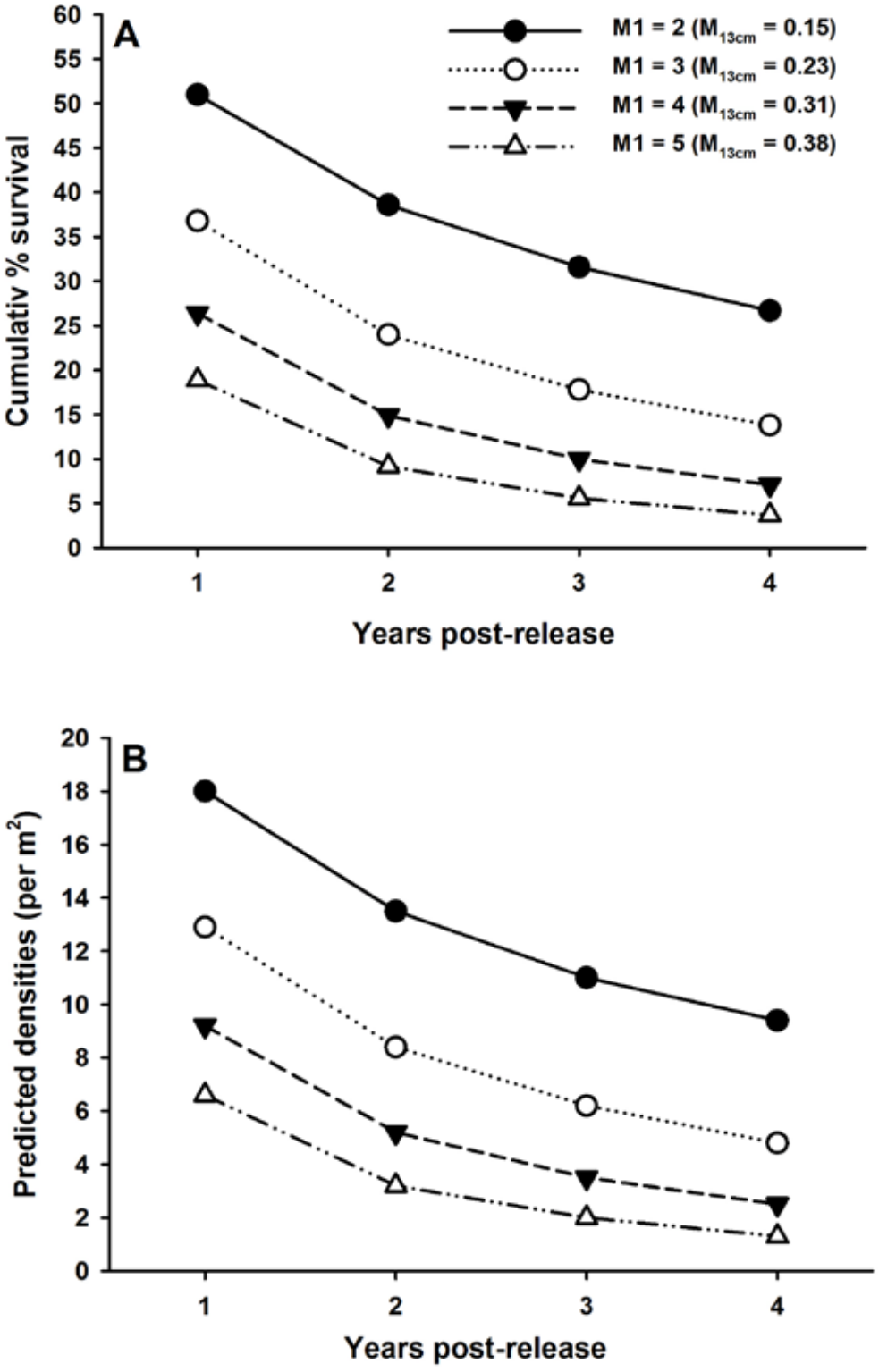

Figure 1.2. (A) Predicted cumulative survival (for 30 mm release size of *Haliotis laevigata*) under the natural mortality model $M(L_{cm}) = M1/L_{cm}$, and assumed growth parameters; (B) Predicted post-release densities of *Haliotis laevigata* for a release density of 35 m^{-2} (140 animals into 4 m^2 of habitat), under varying M1.

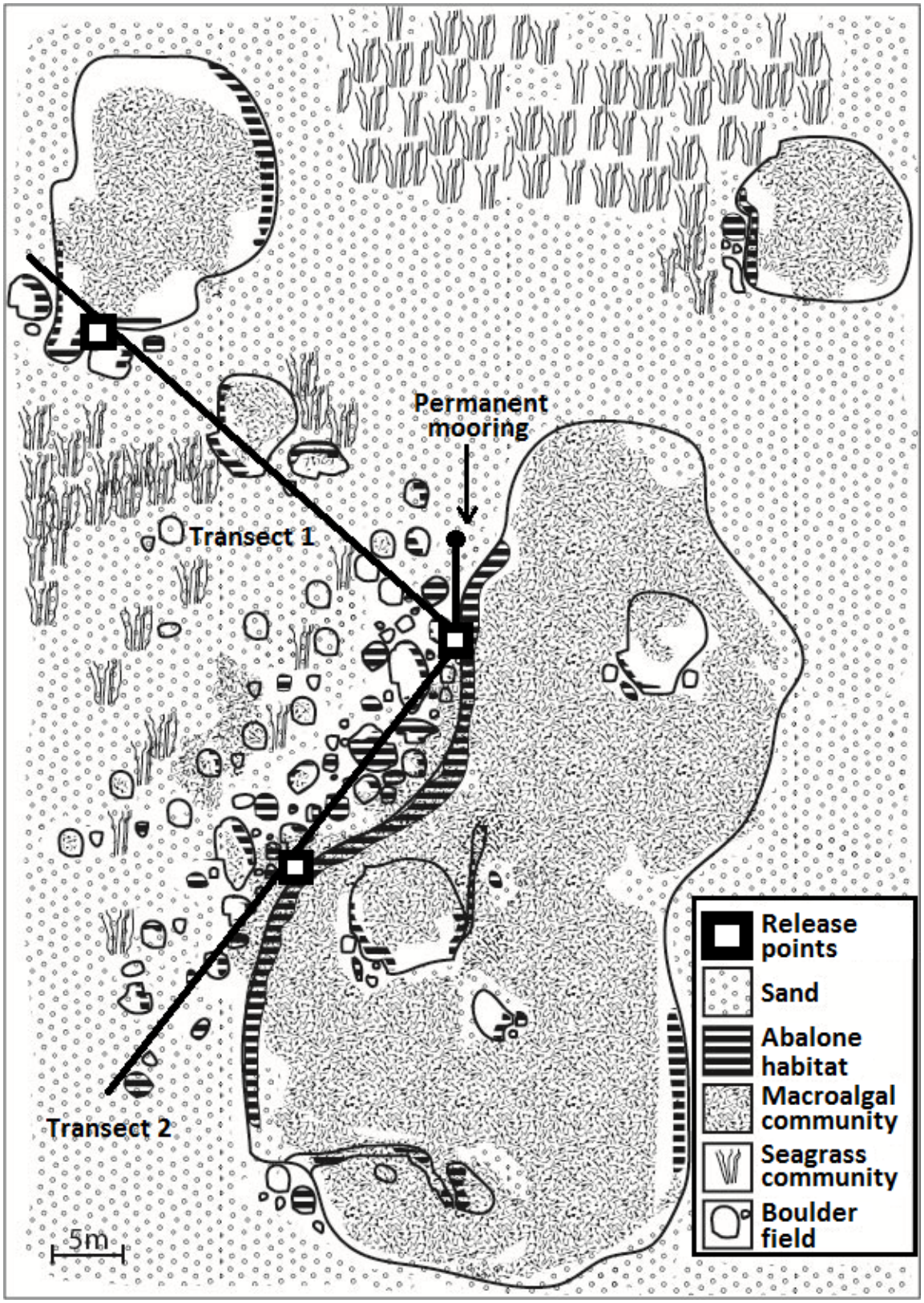

Figure 1.3. Site schematic of experimental reef with a typical distribution of abalone habitat, and location of the three release positions and the two fixed survey transects.

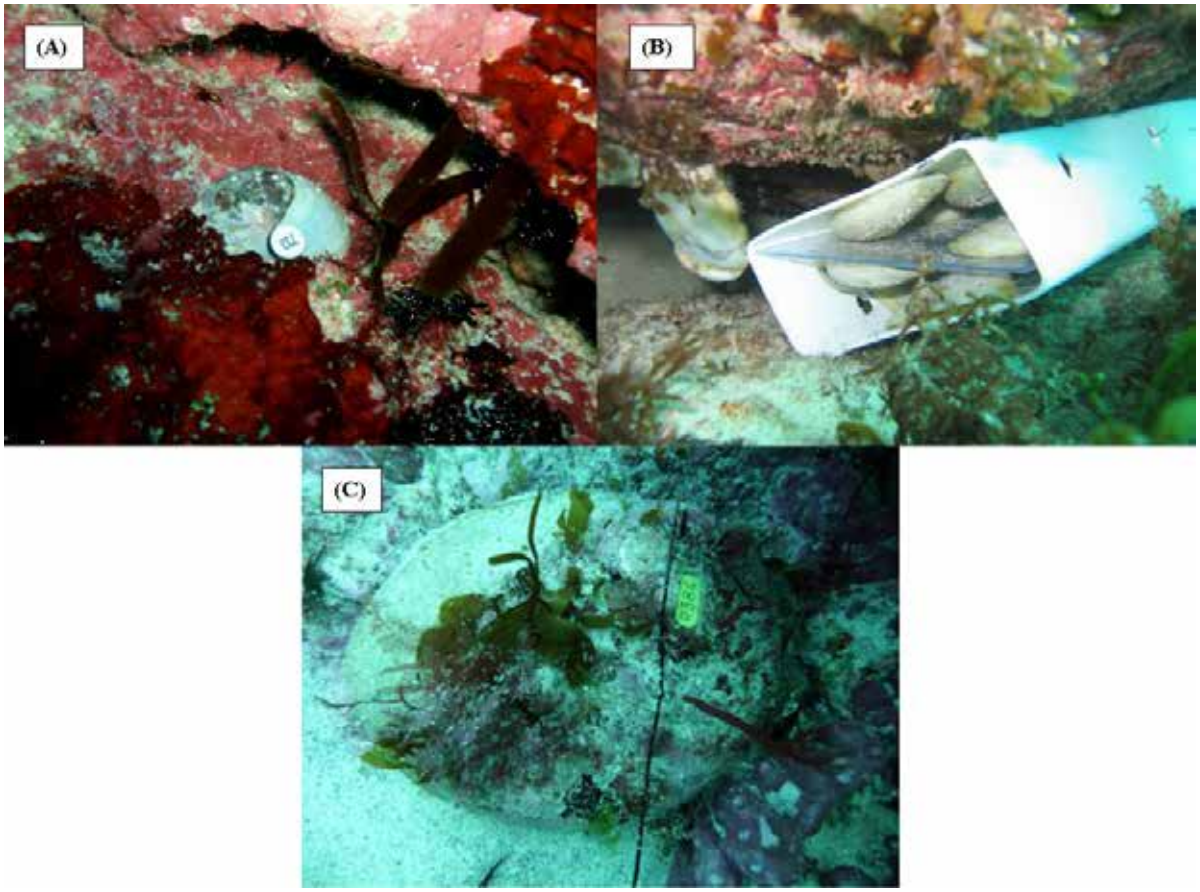

Figure 1.4. Newly released *Haliotis laevigata* with a spring tag (A); newly released *H. laevigata* exiting exit device and entering the natural habitat (B); seeded abalone at 3.5 years post–release with an epoxy tag (embedded in resin) (C).

4.3.5 Environmental and husbandry factors

Factors that influence growth and survival of seeded abalone include environmental variables such as water depth, availability of food and habitat, ecological variables (presence of conspecifics and density of competitors and predators), and the competency / experience of the person undertaking the enhancement. A total of ten environmental and ecological variables were quantified at each release site (Table 1.1) and correlated with growth and survival. See Hart et al. (2011b) for more details about these factors.

4.3.6 Data analysis - Growth

Variation in mean growth (mm month^{-1}) and survival (proportion) after 18 months was analysed using a 1-way ANOVA. A growth model was fitted to the known age (abalone spawned on 5th October 2004) and length using maximum-likelihood estimation techniques. The model was a Gompertz growth equation (Equation 1.2) with parameters: L_t – length (mm) at age t; $L_¥$ – maximum theoretical length; K – growth coefficient; t_0 – theoretical length (mm) at Age 0.

$$L_t = L_¥ e^{[-e(-K(t-t_0))]} \qquad \textbf{Equation 1.2}$$

4.3.7 Data analysis - Survival

Survival rates for each site were obtained from the multiple-recapture history of each tagged individual. The software employed to estimate survival was the "MARK" software (White and Burnham, 1999) obtained from http://www.phidot.org/software/mark. MARK estimates probabilities of survival and recapture using maximum likelihood techniques. Re-encounters can be from dead recoveries (e.g. the animal is harvested), live recaptures (e.g. the animal is re-trapped or re-sighted), or from some combination of these sources. Data from the three release points per site were pooled to increase sample size and precision in estimates of growth, survival and recapture probability. The estimated 6-month survival at each site was then *post-hoc* corrected for tag loss. Tags found were either “readable” tags (R), “unreadable” (U), or “not present” (NP), meaning that, although found, had not been recorded as part of the original release. “Unreadable tags” (U) were those tags still attached but with illegible numbers. The tag loss correction factor = 1 + [(U+NP)/(R+U+NP)]. Mean value of the correction factor was 1.11 (± 0.1), however as a precautionary measure, the more conservative correction factor of 1.05 was used. Survival estimates from individual sites were *post-hoc* combined into 3 groups (High, Medium, and Low), based on 6-month survival results, and subjected to a 2-factor ANOVA, with Site and Time as the factors. Fishing mortality was estimated between the Age 5 and Age 6 time periods using tag-recapture data, and the average natural mortality rate (M = 0.2).

4.3.8 Data analysis – Environmental and husbandry influences on survival

The relevance of environmental and husbandry variables in explaining growth and survival were analysed using correlation and multiple regression. The dependent variables chosen were growth and survival at 6 months for each site, and the explanatory variables investigated included depth, area of abalone habitat, existing abalone population densities, density of predators and competitors and % cover of red (Rhodophyta) and brown (Phaephophyta) algae (Table 1.1). See Hart et al., (2013) for a complete description of these methodological variables. A 1-way ANOVA was used to compare mean survival between different personnel undertaking the enhancement, after exploratory multiple regression analysis indicated this was potentially the only significant factor affecting survival.

Table 1.1. Environmental and ecological variables used to examine variability in growth and survival of Greenlip Abalone Haliotis laevigata.

Variable	Ecological Category	Description	Notes
AD	Conspecific	Existing abalone density (per m^2 of habitat)	Total density and density of breeding adults and juveniles
HT	Habitat	Area of abalone habitat (m^2)	Expressed as m^2 of habitat per $30m^2$ of transect; averaged from 2 surveys (May and November 2006)
Depth	Habitat	Depth of release site (m)	Release sites varied from 6 to 18 m depth
SD	Competitor	Density index of staircase abalone (*Haliotis scalaris*)	Presence /absence recorded for each $1m^2$ quadrat per $30m^2$ transect, resulting in a maximum index of 30; averaged per site
PD	Competitor	Density index of purple sea urchin (*Heliocidaris erythrogramma*)	Presence /absence recorded for each $1m^2$ quadrat per $30m^2$ transect, resulting in a maximum index of 30; averaged per site
LD	Competitor	Density index of the keyhole limpet (Fissurellidae) *Scutus antipodes*	Presence /absence recorded for each $1m^2$ quadrat per $30m^2$ transect, resulting in a maximum index of 30; averaged per site
WD	Predator	Density of wrasse sp. (Labridae).	Density (per m^2) of 7 wrasse species, primarily *Psudolabris parilus* and *Opthalmolepsis lineolata*
RD	Predator	Density index of eagle ray (*Myliobatis australis*)	Number sighted per transect / site
RC	Food	Rhodophyta (red algae) percent cover	% cover estimated for each $1m^2$ quadrat per $30m^2$ transect
PC	Food	Phaeophyta (brown algae) percent cover	% cover estimated for each $1m^2$ quadrat per $30m^2$ transect
P	N/a	Person undertaking the seeding	Each person randomly allocated 3 sites (9 release points). Comparisons undertaken between research and industry personnel

4.4 Results

4.4.1 Growth

Growth rate of *Haliotis laevigata* released at 31 mm (mean length) differed significantly among sites (df = 24, 656; F = 5.9 P < 0.001). Mean growth rate at 18 months post release was 36 (± 3 SD) mm year^{-1}, however the range at individual sites varied between 30 and 40 mm year^{-1}. The long-term growth curve was described well by a Gompertz growth function, but there was considerable variation around the mean growth and large variability in L¥ (Figure 1.5A). Truncation of larger sizes above 150 mm (»6 years of age) was a result of fishing mortality, estimated at 30% (F = 0.36) for the released cohort (Figure 1.5A). Average age at legal minimum length (140 mm) was 5 years, or 6 years to current commercially harvested size (155 mm; Figure 1.5A). Despite being of the same cohort, the variable between-site growth meant that age at which animals achieved the minimum legal size of 140 mm at "fast" growth sites was 1 year earlier than those released onto a slower growing site (Figure 1.5B). The lag was nearer two years for the fishery minimum size of 155 mm (Figure 1.5B).

4.4.2 Survival

Survival rate of *Haliotis laevigata* released at 31 mm differed significantly among the main effects of sites and time; however the key result was a Site × Time interaction (Table 1.2). Between 0 and 6 months post-release, there was a significant difference in survival between sites, with a mean proportional survival of 0.38 ± 0.04 (Figure 1.6A). At the highest 8 sites, survival was 0.58 ± 0.04, however at the lowest 8 sites, survival at 6 months was 0.19 ± 0.04 (Figure 1.6A). Survival at all sites was significantly greater between 6 and 12 months, compared to 0 to 6 months, however the increase was smaller for the high survival sites (0.58 ® 0.76) compared to the low survival sites (0.19 ® 0.80; Figure 1.6A). Overall, there was no significant difference in site survival between 6 and 18 months.

Cumulative survival at 3.5 years post-release varied between 0.20 at the highest sites, and 0.06 at the lowest sites, with an average of 0.13 (Figure 1.6B). At 4.5 years post release, or 6 years of age (»150 mm), average cumulative survival was 0.1 or 10%. This equates to an M1 of between 3 and 4, for the size-dependent mortality model (Figure 1.2A), and is within the range of expected natural mortality in wild stocks.

4.4.3 Environmental and husbandry factors influencing growth and survival

A significant positive correlation was found between growth (mm month^{-1}) and water depth, but no other variables were found to influence growth or survival of *Haliotis laevigata* (Table 1.3). Habitat area and depth were the most influential ecological variables overall. Habitat area was significantly positively correlated with initial total density of abalone, density of the elephant snail (*Scutes antipodes*) and % cover of Phaeophytes, but negatively correlated with depth (Table 1.3). Depth was significantly negatively correlated with density of *H. laevigata*, and density of the elephant limpet (*Scutes antipodes*), and % cover of Phaeophytes, but positively correlated with growth of *H. laevigata*. Urchin density and abalone densities were positively correlated. Finally, although not statistically significant ($F_{(df\ 6,17)}$ = 2.3; p = 0.09), a

higher mean survival of 0.51 was achieved by R (research personnel), I1 (industry diver 1), and I3, compared to 0.17 for I4 (Figure 1.7).

4.4.4 Total mortality (Z), fishing mortality (F), and correlation with growth

Z and F on the enhanced cohort between age 5 and age 8 were 0.42 and 0.27 respectively (Figure 1.8). However there was a significant variation between sites, with F particularly varying between 0.06 at Site 28 and 0.52 at Site 1 (Figure 1.8). Fishing mortality was highly correlated with site-specific growth (r = 0.89).

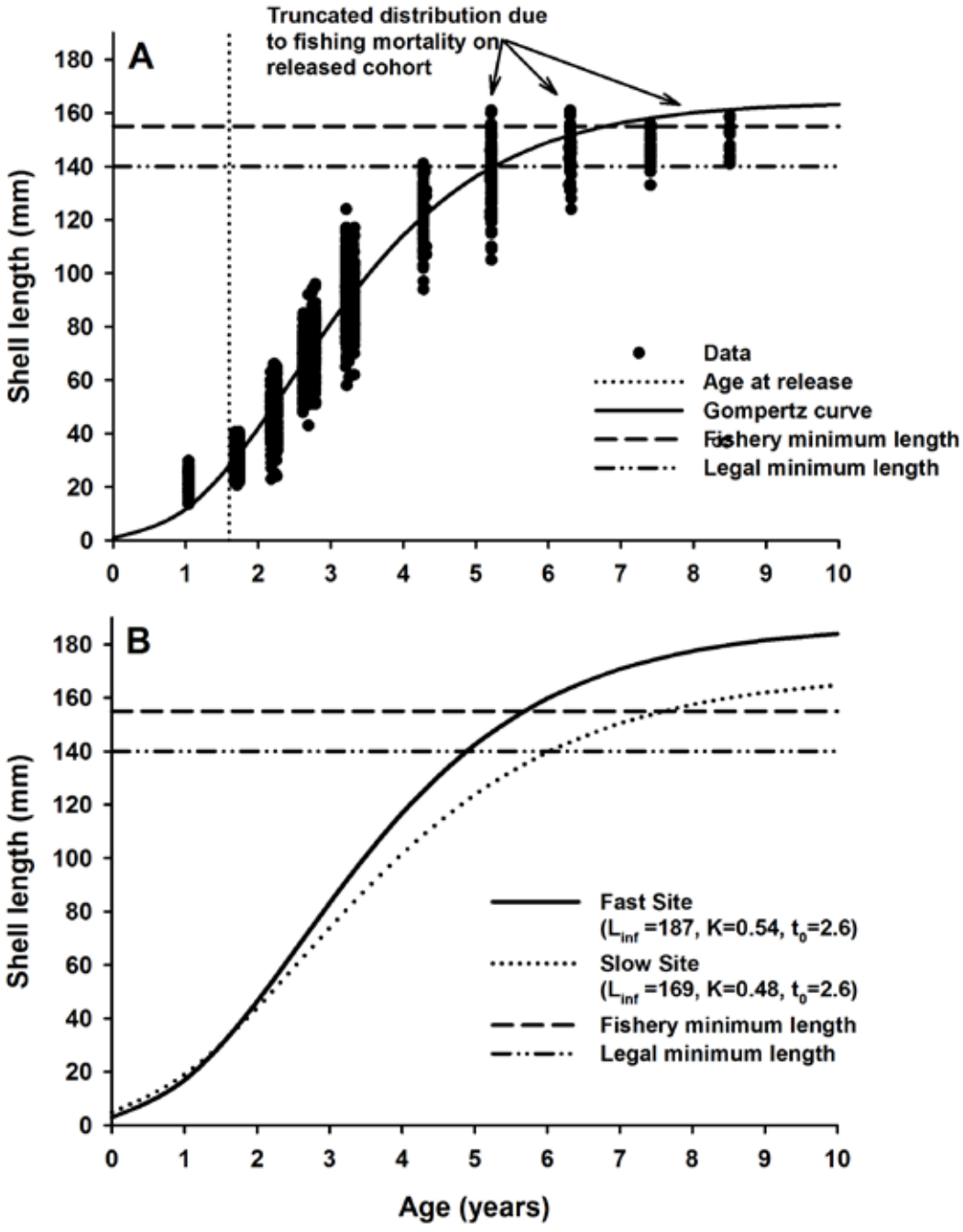

Figure 1.5. Age and length data and Gompertz growth curve for all sites (A); Comparison of growth curve at a fast and slow growing site (B).

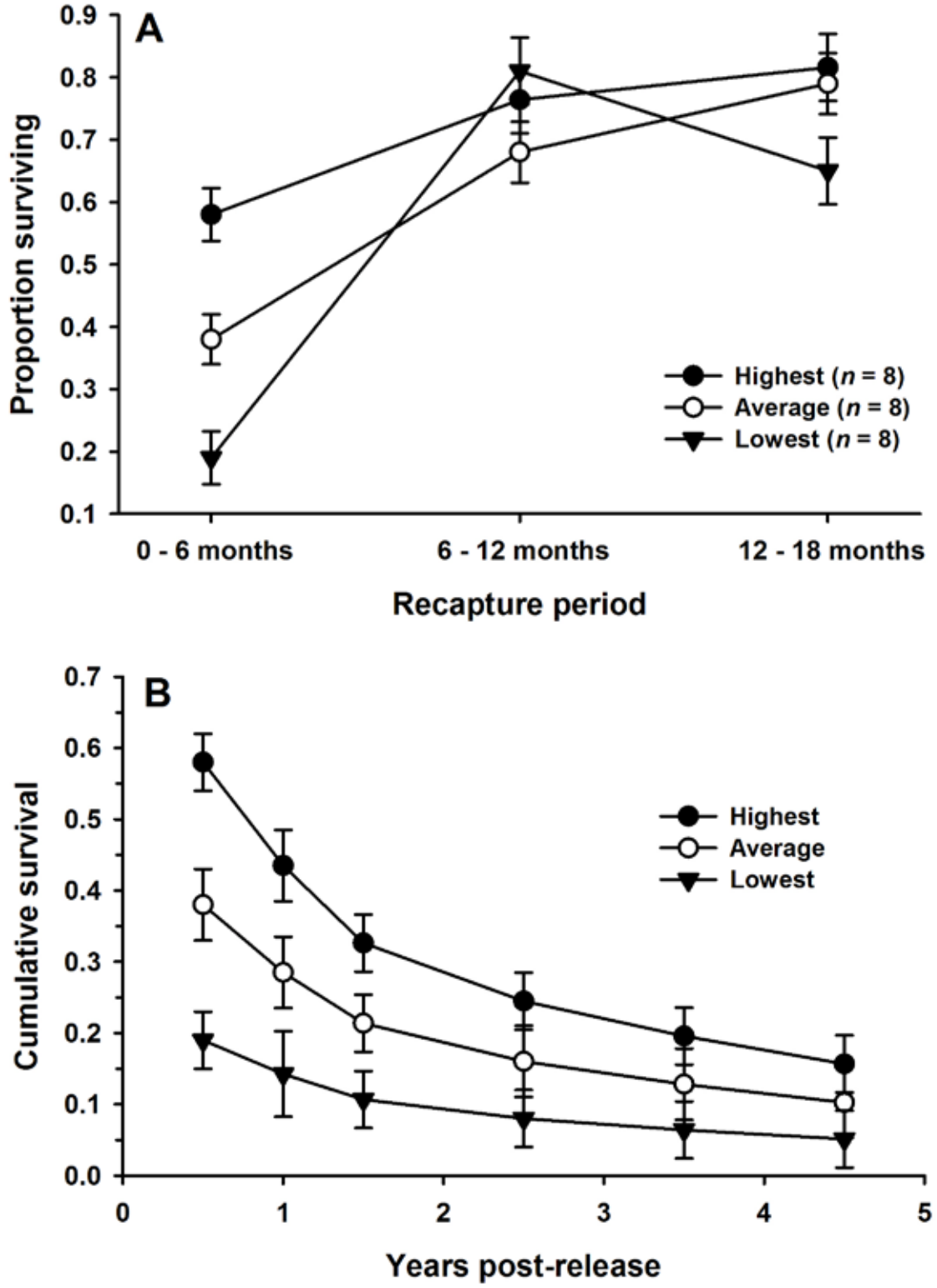

Figure 1.6. Survival curves for *Haliotis laevigata* released at 31 ± 4 mm (A). Survival (± SE) by recapture period between highest, average and lowest sites; Long-term cumulative survival at the highest, average, and lowest sites (B) (n = 8).

Table 1.2. ANOVA results for the effect of recapture period and Site on proportion surviving of *Haliotis laevigata*.

Source of variability	df	MS	F	P
		Proportion surviving		
Recapture period	2	0.93	9.29	<0.001
Site	2	0.13	68.1	<0.001
Recapture period ´ Site	4	0.09	6.32	<0.001
Residual	47	0.01		

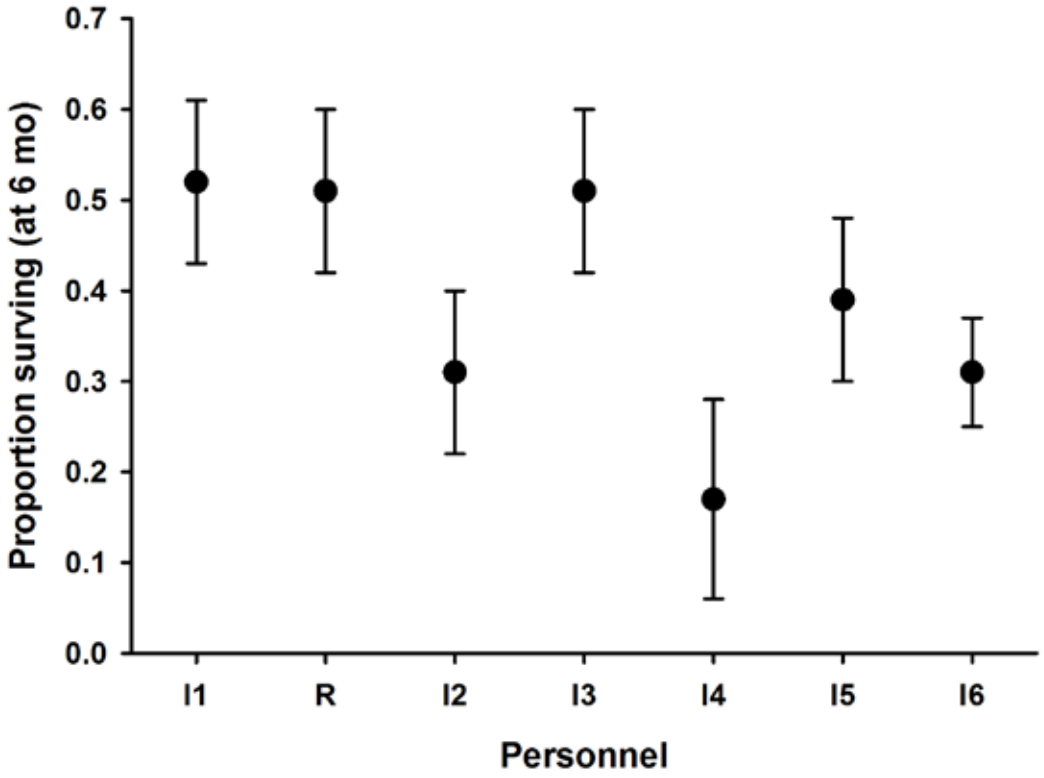

Figure 1.7. Proportion of *Haliotis laevigata* surviving after 6 months according to personnel undertaking the enhancement (± SE). I1 - I6 – Industry divers 1 to 6; R = Research Staff; Number of sites (*n*) = 3 for all except I6, where *n* = 7

Table 1.3. Correlation matrix of ecological variables with growth (mm month^{-1} at 18 months post-release) and survival (proportion surviving at 6 months post-release) of seeded *Haliotis laevigata*. See Table 1.1 for description of variables.

	Survival	Growth	AD	HT	Depth	SD	PD	LD	WD	RD	RC
Growth	0.28										
AD	-0.11	-0.37									
HT	-0.21	-0.19	0.40*								
Depth	0.15	0.47*	-0.54*	-0.54*							
SD	0.15	0.11	-0.01	0.16	0.03						
PD	0.03	-0.24	0.45*	0.32	-0.39	0.70*					
LD	-0.27	-0.07	0.19	0.66*	-0.42*	0.33	0.37				
WD	0.06	0.37	-0.22	-0.09	0.46*	0.46*	0.23	-0.14			
RD	0.39	-0.10	0.03	0.21	-0.32	0.25	0.33	0.15	-0.21		
RC	-0.06	0.10	-0.41*	0.05	0.13	0.06	-0.01	0.30	0.20	0.00	
PC	-0.07	-0.26	0.26	0.54*	-0.55*	0.20	0.31	0.34	-0.21	0.10	-0.02

* significant at 0.05

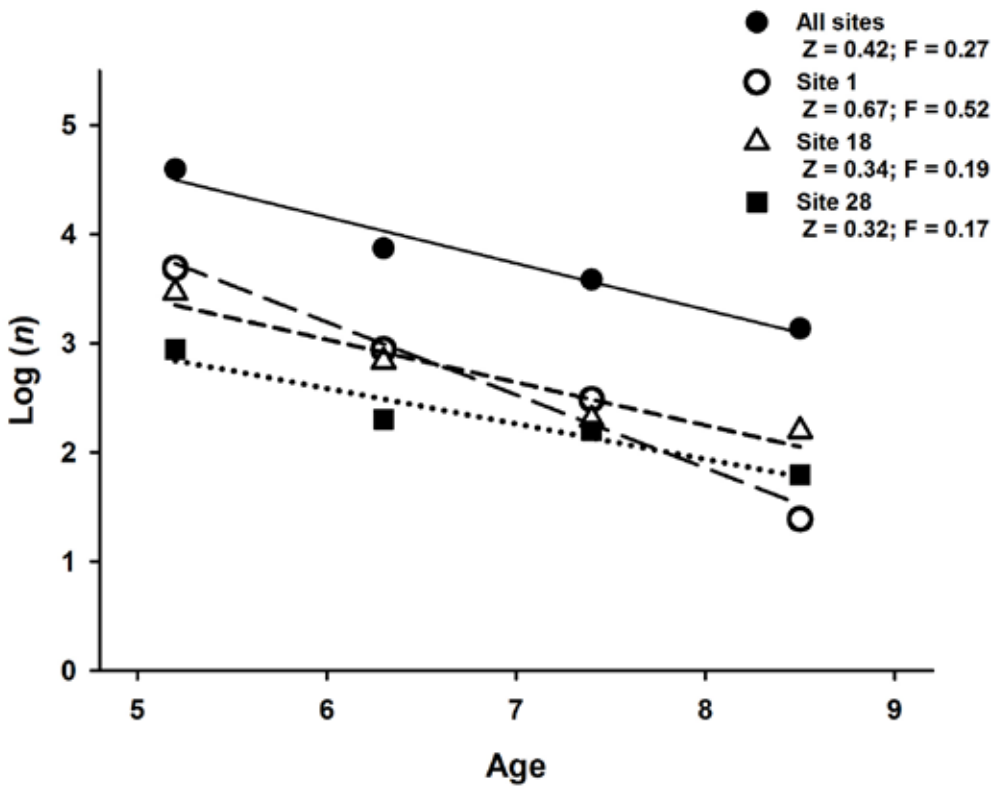

Figure 1.8. Total mortality (Z) and fishing mortality (F) estimates for the enhanced cohort of *Haliotis laevigata* between age 5 and age 8. Log (*n*) is the natural log-transformed estimate of the number of tagged animals (*n*) remaining in the population. Data is from *in-situ* field surveys at 4 sites. M assumed at 0.15.

4.5 Discussion

A single age cohort of *Haliotis laevigata*, spawned in October 2004, and seeded onto natural wild stock habitats, was successfully tracked for 6 years until it entered the commercial fishery. During this time, a rigorous assessment of growth and survival at juvenile and adult stages demonstrated that stock enhancement in this species is viable and can significantly increase stock densities (see Hart et al., 2013). Comparative studies have monitored released cohorts for up to two years (Roberts et al., 2007), with 12-18 months being a common time period (Sweijd et al., 1998; Dixon et al., 2006). The main limitation to the shorter time period is that the cryptic nature of juvenile abalone creates a bias in short term estimates of survival. This study demonstrated that the probability of recapture decreases rapidly below 70 mm size, which is consistent with earlier findings (Shepherd, 1990). Because individually tagged animals were tracked sequentially for a number of years as they matured from their cryptic to their exposed phase, it was possible to calibrate for this behavioural effect and improve the survival estimate (Strain, unpublished data).

Growth of *Haliotis laevigata* was variable, but consistent with published estimates. Wells and Mulvay (1995) estimated legal size (140 mm) to be attained at 5 to 6 years, which was confirmed by this study (Figure 1.5B). The advantage in this study was that age was known, compared to the tag-increment analysis of Wells and Mulvay (1995) and all other studies. Officer (1999) also found close agreement between tag-increment analysis and age-length analysis for *H. laevigata* in Tasmania. In comparison with other *H. laevigata* populations, the mean 36 mm year^{-1} growth rate for 30 to 100 mm animals was higher than average. Shepherd et al. (1992) show most populations to exhibit 20 – 30 mm year^{-1} growth for this size class. The juvenile and adult growth pattern was best described by a Gompertz growth model, as has been found for other Australian abalone species (Bardos, 2005; Hancock, 2004; Troynikov et al., 1998). Despite this, the most common growth model used in abalone is still the von Bertalanffy growth curve, although an inverse-logistic growth model has recently been advocated as a better fit of the large variability in abalone growth (Haddon et al., 2007).

From a stock enhancement perspective, the most important aspect of growth is duration to harvest. In this study, seeded Greenlip Abalone first entered the commercial fishery (³ 155 mm) at Age 5 or 3.5 years post-release, as confirmed by tag-recapture. However, fishing mortality on this cohort was site-specific and highly correlated with growth. Overall, 72% of the cohort had recruited to the fishery by age 8 (Z = 0.42), but at the fast growing site 87% had recruited (Z = 0.67), compared to only 47% at the slow growing sites (Z = 0.21). This would affect the economics of enhancement, although in a situation where natural levels of recruitment were being enhanced, the use of size-limits to maximise the biomass yield only, rather than also maintaining egg production, becomes an important option. Preliminary bioeconomic analysis (Hart, unpublished data), suggests that reducing size-limits in this fishery can result in significant economic gains, but at the expense of egg conservation.

Survival rates of *Haliotis laevigata* achieved were similar to other recent studies of abalone enhancement. Dixon et al. (2006) who also released *H. laevigata* around 30 mm, reported large variability, with a range (after 9 months) from 23 to 57%. Of the 24 sites in our study, the overall mean survival after 6 months was 38%, with survival approaching 60% at the top

1/3 (n = 8) of sites, and 20% in the bottom 1/3 of sites. The two studies are not directly comparable as Dixon et al. (2006) constructed artificial habitats, whereas this work is based on natural habitats, but it is encouraging that similar results were obtained. Sweidj et al. (1998) working on the *H. midae*, reported survival estimates of 30% after 6 months, however recognised that they were underestimates. Based on our quantification of recapture rates of juvenile abalone in the cryptic phase, we postulate that Sweidj et al. (1998) survival results could have been higher if they had been able to monitor the cohort through tagging of individuals, and had a longer post-release survey period. This concern has been echoed in many earlier studies (Rogers-Bennet and Pearce, 1998; Tegner and Butler, 1985).

Shepherd (1998) carried out a long-term (14 years) study of juvenile mortality in wild stocks of *Haliotis laevigata*, which provides a benchmark for our results. M (year^{-1}) in wild stocks of 10 – 50 mm juveniles was found to be variable, with a mean of 0.98 (SE 0.09), and a range of 0.2 – 3.2 (Shepherd, 1998). The comparative value in our study is mean cumulative survival at 12 months post release, which was 0.30 (± 0.06 SD). This translates to an M of 1.2 (year^{-1}), which was higher than the mean M for natural stocks but within the range of natural variability. For this age group the top 8 sites had an M of 1.1, which was similar to M in natural stocks, while the worst 8 sites had an M of 2.2, which was higher than generally experienced in wild stocks.

Roberts et al. (2007) also found encouraging results with *Haliotis iris* in New Zealand. Survival after 20 months varied widely, but averaged around 14%. Estimated survival to harvest varied between 2 and 19%, but was similar to the range found in this study (6 – 20%). An important point is that most of this variability results from mortality during the first few months of release, and focus must be on optimising survival at the point of release. The key finding of Roberts et al. (2007) was that economic viability was likely as long as release sites and habitat are chosen carefully; in the case of *H. iris* it was the 'under-boulder' crevasse habitat that was important. This was also the finding summarised by Hamasaki and Kitada (2008) in their major review of the Japanese abalone enhancement program. In particular, they identified issues such as local carrying capacity and optimising density of releases. These are examined for *Haliotis laevigata* in further detail in a companion paper (Hart et al., 2013).

There are three key reasons why survival estimates on parity with natural survival rates were obtained in this enhancement study. The first is a use of a size-dependent mortality model (Lorenzen, 2006) to ensure that release densities were tailored to match natural densities, as recommended by Goodsell et al. (2006). Secondly, long-term multiple recapture monitoring of tagged individuals enabled robust survival estimates that included estimates of the probability of recapture at each survey period and size-class. This feature of our study will be examined in more detail by modelling the probability of survival as a function of size at release (L. Strain, pers. comm.). The third reason is the critical attention paid to quantifying the habitat and ensuring that releases were made using protective devices (McCormick et al., 1994) into cryptic locations within areas known to have viable natural populations. These factors had the cumulative effect of ensuring strong experimental control, which is often difficult to achieve logistically in stock enhancement in the marine environment. Both

Roberts et al. (2007) and Dixon et al. (2006) who utilised artificial habitats to estimate survival, emphasised the importance of selecting the correct habitat. A clear example of this is in New Zealand *H. iris* populations. The release habitat was identified to be 'under boulder' habitat in the intertidal area, estimated to be, on average, only 2 m wide. Therefore, commercial-scale stock enhancement would require large stretches of coastline to be accessed.

In this study, environmental and ecological variables were generally not significant in influencing growth or survival. This is in contrast to many other studies, including an earlier pilot study, where habitat area was found to significantly influence survival (Hart et al., 2007). Similarly, DeWall and Cook (2001) found a positive correlation between survival and size of boulders. However we did find a positive correlation between habitat area and existing wild stock densities, as well as correlation between different environmental variables (Table 1.3), indicating that environmental factors are important in structuring populations. The relative unimportance of ecological factors on growth and survival may have been because release densities in this study were below the carrying capacity, and animals were at a size in which density dependence effects on survival are negligent (Shepherd, 1998). In a companion paper (Hart et al., 2013) we examine carrying capacity to see what densities can be theoretically achieved in *Haliotis laevigata*. Predation is often identified as a major limiting factor in abalone stock enhancement (Tegner and Butler, 1985), however that study provided no evidence that their release site contained a functioning adult population, i.e. there were no emergent animals. Densities of predator wrasses and eagle rays in this study were not significantly correlated with survival. Urchin density was positively correlated with abalone densities, as was found with South African abalone (*Haliotis midae*; Tarr et al., 1996), but contrasts with the negative correlation between urchins and abalone in NSW, Australia (Andrew and Underwood, 1992) and California (Karpov et al., 1998). This fact is examined in more detail in a companion paper (Hart et al., 2013).

Often it is the longer-term effects of environmental variables that matter, but are more difficult to detect. This was highlighted by a 30-year study on the interaction of hatchery-bred and wild pink salmon stock in Prince William Sound, Alaska (Wertheimer et al., 2004). At a time when environmental conditions appeared to be negatively impacting on the abundance of wild stocks, hatchery releases for stock enhancement more than compensated, doubling the maximum historical catch and contributing more than 70% of the harvest (Wertheimer et al., 2004). Although the implications of this result are controversial and disputed (see Hillborn and Eggers, 2000), it does highlight the potential benefits of stock enhancement in times of changing environmental conditions.

The only factor that possibly influenced survival was a husbandry factor, which can be translated into the competency of the person undertaking the release. Our release methodology involved the use of trained research personnel who had mapped each of the release points and had a very good understanding of the habitat, and six other commercial abalone industry divers with varying experience and competency. These individuals were given GPS points, maps, and instructed to swim to sites and release their devices. The data suggest that three of the personnel had consistent and better results. Although this was not

statistically significant ($p = 0.09$), anecdotally there was recognition of the importance of training required to ensure correct identification of habitat and placement of release devices.

In summary we conclude that with careful consideration of local habitat capacity and controlled release densities, hatchery-bred juveniles of *Haliotis laevigata* stocked into the wild at an appropriate length will attain equivalent survival and growth to natural populations. If these experimental findings can be translated into commercial-scale enhancement programs, significant effects on fishery harvests are expected. The three main areas of further research are bioeconomics of enhancement, optimal size and release densities, and commercial-scale development of release methodologies. This must include large-scale quantification of available habitat, and adequate training of personnel involved in the release of juveniles. Currently however, the main stumbling block to further development of abalone stock enhancement in Australia is a disease issue. The presence of highly virulent herpes-like-virus (Abalone Viral Ganglioneuritis – AbHV-1) in wild stocks in Victoria and Tasmania is causing significant concern to the industry and community in all abalone-producing areas (Hooper et al., 2007; Corbeil et al., 2010; Savin et al., 2010). A comprehensive risk assessment of the threat of AVG, and appropriate mitigation strategies, is needed before commercial-scale enhancement initiatives are considered.

4.6 Acknowledgements

Thanks to Sabine Daume and Fiona Graham who assisted with the hatchery culture and release of the animals; Great Southern Marine Hatchery, the Western Australian Abalone Industry Association (WAAIA) in particular Ian Taylor and Brad Adams for facilitating this exciting work, and to all the industry divers who participated in the site selection and juvenile release, namely Brad Adams, Darren, and Nathan Adams, Jay Clauson, John Lashmar, Michael and Peter Rickerby, and Steve and George Beres. This study was funded by the Department of Fisheries, Western Australia and the Australian Seafood CRC (Cooperative Research Centre).

4.7 References

Andrew, N. L. and A. J. Underwood. Associations and abundance of sea urchins and abalone on shallow subtidal reefs in southern New South Wales. Aust. J. Mar. Freshw. Res., 43: 1547–1559 (1992).

Bardos, D.C. Probabilistic Gompertz model of irreversible growth. Bull. Math. Biol., 67: 529–545 (2005).

Bell, J. D., P. C. Rothlisberg, J. L. Munro, N. R. Loneragan, W. J. Nash, R. D. Ward, and N. L. Andrew. Restocking and stock enhancement of marine invertebrate fisheries. Adv. Mar. Biol., 49: 1–370 (2005).

Campbell, A. (Ed). Workshop on Rebuilding Abalone Stocks in British Columbia. Can. Spec. Publ. Fish. Aquat. Sci., 130 (2001).

Corbeil, S., A. Colling, L. M. Williams, F. Y. Wong, K. Savin, S. Warner, B. Murdoch, N. O. Cogan, T. I. Sawbridge, M. Fegan, I. Mohammad, A. Sunarto, J. Handlinger, S. Pyecroft, M. Douglas, P. H. Changs, M. S. Crane. Development and validation of a TaqMan PCR assay for the Australian abalone herpes-like virus. Dis. Aquat. Org., 92: 1-10 (2010).

Daume, S., S. Huchette, S. Ryan and R. W. Day. Nursery culture of Haliotis rubra: The effect of cultured algae and larval density on settlement and juvenile production. Aquaculture, 236: 221–239 (2004).

Daume, S. and S. Ryan. Nursery culture of the abalone Haliotis laevigata: Larval settlement and juvenile production using cultured algae or formulated feed. J. Shellfish Res., 23:1019-1026 (2004).

De Waal, S. and P. Cook. Quantifying the physical and biological attributes of successful ocean seeding sites for farm-reared juvenile abalone (Haliotis midae). J. Shell. Res., 20(2): 857-861 (2001).

Dixon, C. J., R. W. Day, S. M. H. Huchette and S. A. Shepherd. Successful seeding of hatchery-produced juvenile greenlip abalone to restore wild stocks. Fish. Res., 78: 179-185. (2006).

Goodsell, P. J., A. J. Underwood, M. G. Chapman, and M. P. Heasman. Seeding small numbers of cultured black-lip abalone (Haliotis rubra Leach) to match natural densities of wild populations. Mar. Freshw. Res., 57: 747-756 (2006).

Haddon, M., C. Mundy and D. Tarbath. Using an inverse-logistic model to describe growth increments of blacklip abalone (Haliotis rubra) in Tasmania. Fish. Bull., 106: 58-71 (2007).

Hammasaki, K. and S. Kitada. The enhancement of abalone stocks: lessons from Japanese case studies. Fish and Fisheries, 9: 1-18 (2008).

Hancock, B. The biology and fishery of Roe's abalone Haliotis roei Gray in south-western Australia, with emphasis on the Perth fishery. PhD thesis, University of Western Australia. 184 p (2004).

Hart, A. M, F. P. Fabris, and S. Daume. Stock enhancement of Haliotis laevigata in Western Australia – a preliminary assessment. Fisheries Research Report No. 166, Department of Fisheries, Western Australia (2007).

Hart, A.M, F. Fabris, L.W.S. Strain, M. Davidson, and J. Brown. Stock enhancement in greenlip abalone part II: population and ecological effects. Rev. Fish. Sci., 21(3-4): 310-320 (2013).

Hillborn, R. The economic performance of marine stock enhancement projects. Bull. Mar. Sci., 62: 661-674 (1998).

Hillborn, R. and D. Eggers. A review of the hatchery programs for pink salmon in Prince William Sound and Kodiak Island, Alaska: Trans. Am. Fish. Soc., 129: 333-350 (2000).

Hooper, C., P. Hardy-Smith, J. Handlinger. Ganglioneuritis causing high mortalities in farmed Australian abalone (Haliotis laevigata and Haliotis rubra). Aust. Vet., J. 85: 188-193. (2007).

Karpov, K. A., M. J. Tegner, L. Rogers-Bennett, P. E. Kalvass, and I. K. Taniguchi. Interactions among red abalones and sea urchins in fished and reserve sites of northern California: Implications of competition to management. J. Shell. Res., 20(2): 743-754 (2001).

Kojima, H. Evaluation of abalone stock enhancement through the release of hatchery-reared seeds. Mar. Freshwater Res., 46: 689-695 (1995).

Lorenzen, K. Population management in fisheries enhancement: gaining key information from release experiments through use of a size-dependent mortality model. Fish. Res., 80: 19–27 (2006).

McCormick, T. B., K. Herbinson, T. S. Mill, and J. Altick. A review of abalone seeding, possible significance and a new seeding device. Bull. Mar. Sci., 55: 680-693 (1994).

Officer, R.A. Size limits for greenlip abalone in Tasmania. TAFI Technical Report Series No. 5, 48 p. Tasmanian Aquaculture and Fisheries Institute, Hobart (1999).

Roberts, R. D., E. F. Keys, G. Prendeville, and C. A. Pilditch. Viability of abalone (Haliotis iris) stock enhancement by release of hatchery-reared seed in Marlborough, New Zealand. J. Shell. Res., 26(3): 697-703 (2007).

Rogers-Bennet, L., and J. S. Pearce. Experimental seeding of hatchery reared juvenile red abalone in northern California. J. Shell. Res,. 17(3): 877-880 (1998).

Savin, K. W., B. G. Cocks, F. Wong, T. Sawbridge, N. Cogan, D. Savage, and S. Warner. A neurotropic herpesvirus infecting the gastropod, abalone, shares ancestry with oyster herpesvirus and a herpesvirus associated with the amphioxus genome. Vir. J., 7: 308 (2010).

Schiel, D. R. Experimental evaluation of commercial-scale enhancement of abalone Haliotis iris populations in New Zealand. Mar. Ecol. Prog. Ser., 97: 167-181 (1993).

Shepherd, S. A., and W. S. Hearn. Studies on Southern Australian Abalone (Genus Haliotis). IV: Growth of H. laevigata and H. rubra. Aust. J. Mar. Freshw. Res., 34: 461-475 (1983).

Shepherd. S. A. Studies on Southern Australian Abalone (Genus Haliotis). VIII: Growth of juvenile H. laevigata. Aust. J. Mar. Freshw. Res., 39(2): 461-47 (1988).

Shepherd. S. A. Studies on Southern Australian Abalone (Genus Haliotis). XII: Long-term recruitment and mortality dynamics in an unfished population. Aust. J. Mar. Freshw. Res., 41: 475-492 (1990).

Shepherd, S. A., S. M. Clarke, and A. Dalgety. Studies on Southern Australian Abalone (Genus Haliotis). XIV: Growth of H. laevigata on Eyre Peninsula. J. Malac. Soc. Aust., 13: 99-113 (1992).

Shepherd. S. A. Studies on Southern Australian Abalone (Genus Haliotis). XIX: Long-term juvenile mortality dynamics. J. Shell. Res., 17(3): 813-825 (1998).

Shepherd, S. A., P. A. Preece, R. G. W. White. Tired nature's sweet restorer – ecology of abalone stock enhancement in Australia. In: Workshop on rebuilding abalone stocks British Columbia (A. Campbell, Ed.). Can. Spec. Publ. Fish. Aquat. Sci., 130: 84-98 (2001).

Strain, L., M. Borowitzka and S. Daume. Growth and survival of juvenile greenlip abalone (Haliotis laevigata) feeding on germlings of the macroalga Ulva sp. J. Shell. Res. 25: 239–247 (2006).

Sweijd, N., Q. Snethlage, D. Harvey, P. Cook. Experimental abalone (Haliotis midae) seeding in South Africa. J. Shell. Res. 17(3): 89-904 (1998).

Tarr, R. J. Q., P. V. G. Williams, and A. J. Mackenzie. Abalone, sea urchins, and rock lobster: a possible ecological shift may effect traditional fisheries. S. Afr. J. Mar. Sci. 17: 319-323 (1996).

Tegner, M. J., and R. A. Butler. The survival and mortality of seeded and native red abalone, H. rufescens on the Palos Verdes Peninsula. Calif. Fish Game 71: 150-163 (1985).

Troynikov, V. S., R. W. Day, and A. M. Leorke. Estimation of seasonal growth parameters using a stochastic Gompertz model for tagging data. J. Shell. Res. 17(3): 833-838 (1998).

Wells, F. E., and P. Mulvay Good and bad fishing areas for Haliotis laevigata: A comparison of population parameters. Mar. Freshw. Res. 46: 591-598 (1995).

Wertheimer, A. C., W. R. Heard, and W. M. Smoker. Effects of hatchery releases and environmental variation on wild-stock productivity: consequences for sea-ranching of pink salmon in Prince William Sound, Alaska. In: Stock enhancement and sea ranching: Developments, pitfalls, and opportunities, pp. 307-326 (K. M. Leber, S. Kitada, H. L. Blankenship, and T. Svasand, Eds.). Blackwell Publishing (2004).

White, G. C., and K. P Burnham. Program MARK: survival estimation from populations of marked animals. Bird Study 46: 120–139 (1999).

5 Stock Enhancement in Greenlip Abalone: (2) Population and Ecological Effects

Anthony M. Hart[1], Frank Fabris[1], Lachlan W. S. Strain[1], Mark Davidson[1] and Jamin Brown[1]

[1]Western Australian Fisheries and Marine Research Laboratories, PO Box 20, North Beach, Western Australia, 6920, Australia.

Please note that this chapter has been published as the following manuscript:

Hart AM, Fabris F, Murphy D, Brown J, Strain M, Davidson M, (2013). Stock enhancement of greenlip abalone: (2): Population and ecological effects. *Rev. Fish. Sci*. 21: 310-320.

5.1 Abstract

A series of stock enhancement experiments were carried out on *Haliotis laevigata* populations. Methodologies included a large-scale Before-After-Control-Impact (BACI) experiment (42 sites), a carrying capacity experiment, which involved a high-density release at 2 sites, and a detailed survey of abalone populations and ecological parameters. Increased densities were detected for most age classes, although fishing mortality began obscuring the effect by Age 5+. Age 4+ animals showed the clearest result, with no density differences between enhanced and control sites at 6, 12, and 18 months post-release, and then a 300% increase at enhanced sites at 30 months post-release. Overall, a single release of Age 1+ animals in May 2006 had doubled the total density by November 2008. In the carrying capacity experiment, densities initially increased rapidly (by up to 800%), however had stabilised at a 400% increase after 2.5 years, at around 8 per m^2. This was the predicted carrying capacity, with the enhanced cohort representing 50% of the population. A PERMANOVA analysis of ecological similarity detected no effect of enhancement on the environment, although changes in algal % cover were detected at both control and enhanced sites. Overall our study suggests that, as long as release densities are controlled within natural limits, successful stock enhancement can be attained for this species, with minimal ecological impacts.

Keywords: BACI experiment, PERMANOVA, carrying capacity

5.2 Introduction

Stock enhancement in fish populations is an assisted recruitment program, and poor knowledge of the processes of natural population regulation will hinder the practice. Competitive interactions between wild and cultured fish, for example, for the available mysid food source for Japanese flounder (Yamashita and Kurita, 2007), or temporally variable habitat suitability for juvenile sea cucumbers (Purcell and Simutoga, 2008), or unpredictable variability in natural recruitment are all components of fish population systems that need understanding. The capacity for a target population to respond to stock enhancement in these systems relies on knowledge of the natural responses and the extent to which these can be controlled by either accurate observation or experimental manipulation. Moreover, often the most fundamental parameters, e.g. density, are not easily understood due to methodological

constraints. Haliotids are a case in point with a review in the 1990s concluding that few assessments had been successful due to an inability to properly measure the spatially complex metapopulations (McShane, 1998). Abundance indices like catch per unit effort (CPUE) in commercial fisheries are often considered unreliable (McShane, 1998; Prince and Hillborn, 1998; Dowling et al., 2004).

The species of interest in this study (Greenlip Abalone – *Haliotis laevigata*) is no exception to the general case. Numerous indices of density have been developed (reviewed by McGarvey, 2006), however none of these were found to be particularly suitable to detecting a response to the experimental manipulation of density at the fine spatial scale required for stock enhancement in this species. Pilot study results (Hart et al., 2007) indicated that accurate coverage of positions of release by the stock survey method was required in order to detect the effect, as movement was small and habitat disaggregated. Random placement of surveys therefore had a very high probability of not encountering the habitat that had been targeted, thus lowering the ability to detect a stock response. This methodological limitation was overcome by developing a population survey technique that measured density as a function of available habitat, rather than total area surveyed, and combining it with a formal mark-release-recapture (MRR) experiment in which individually tagged animals were monitored for 6+ years (Hart et al., 2013). Using this methodology within an environmental impact assessment framework, population and ecological responses to stock enhancement were investigated for *Haliotis laevigata*. The objectives were to establish the magnitude of the population response, investigate carrying capacity of the habitat, and assess if there had been an ecological response to enhancement. Coupled with the formal MRR study of Hart et al. (2013), these two studies provide new quantitative approaches to determining viable habitat and associated release densities in Haliotids.

5.3 Material and Methods

5.3.1 Site selection and release methodology

Enhancement experiments were carried out on *Haliotis laevigata* stocks in the Augusta region of Western Australia (Figure 2.1). This region comprises an important part of the Western Australian Greenlip Abalone fishery, producing approximately 30% of the total catch. To ensure that appropriate habitat was targeted, study sites were chosen with the assistance of commercial abalone divers. We utilised hatchery-bred F1 juveniles reared from wild-caught adults. These were tagged and released into the experimental sites at a mean length of 31 mm, about age 18 months and subject to a detailed mark-release-recapture study up to 4 years post-release. See Chapter 1 (Hart et al., 2013) for more details of culture and enhancement methodology and MRR results.

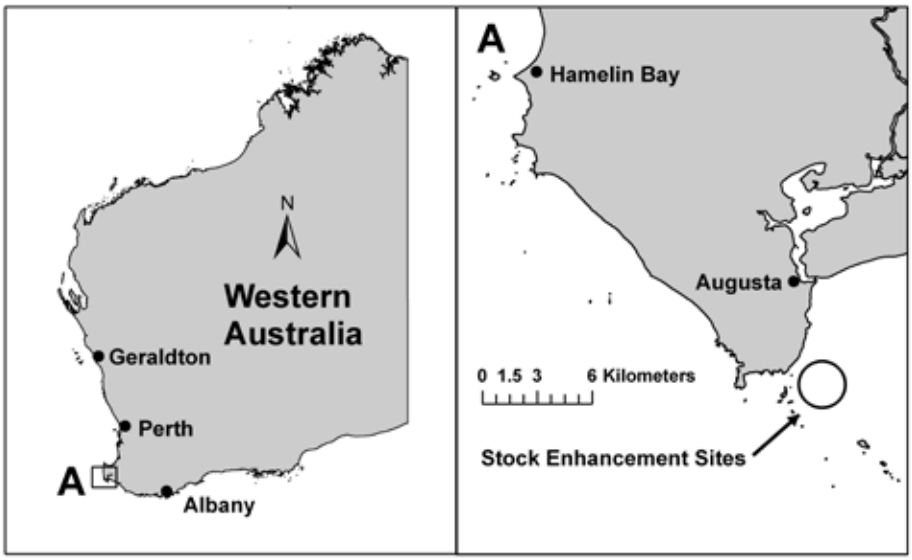

Figure 2.1: Location of study sites for stock enhancement experiments on Greenlip Abalone (*Haliotis laevigata*), near Augusta, Western Australia.

5.3.2 Habitat and density estimates

To account for the fine-scale patchy distribution of *H. laevigata*, and facilitate accurate estimates of habitat to control release densities, a new survey method was developed. The method was adapted from a technique developed for *Haliotis rubra* in Victoria (Gorfine et al., 1998; Hart et al., 1997) and involved quantifying habitat area and numbers of *H. laevigata*. The sample unit was a 30 m^2 transect divided into 1 m^2 quadrats. This length was appropriate to our fixed release sites, however a randomised sample survey method would likely utilise longer transects lengths of 100 m (McGarvey, 2006).

Observers swam out a rope marked at each 1 m interval and quantified abalone abundance, size and habitat area within each quadrat. Tagged animals encountered in survey transects were recorded to enable a differentiation between wild and seeded animals. The area of suitable habitat in each 1 m^2 quadrat was quantified according to criteria developed specifically for this species (Table 2.1). Suitable abalone habitat was defined as habitable surfaces (generally granite or limestone) of sufficient quality and area to allow effective attachment for at least 1 abalone of 40 mm shell length or above. Smaller juveniles are usually cryptic, while the larvae settle preferentially on non-geniculate coralline algae, and require different habitat and sampling requirements (McShane, 1995; Daume et al., 1999). Comparisons were made between observers to help standardise the search criteria and new observers were trained in the habitat survey criteria prior to sampling. The relationship between the two density measures (per habitat m^2; per total m^2) was examined using precision (SE / $\bar{x}$) and logistic regression analysis. Confidence limits for parameters of the logistic expression were obtained by bootstrap analysis (n = 10,000).

Table 2.1. Habitat survey criteria for *Haliotis laevigata*. Codes are applied to each 1 m^2 quadrat within the larger sample unit (a 30 m^2 transect is used here). The midpoint is the value used to estimate habitat area (m^2) per transect.

Habitat Code	Area (m^2)	% of quadrat	Midpoint (m^2)
0	0	0	0
1	0 – 0.1	1-10%	0.05
2	0.1 – 0.2	11-20%	0.15
3	0.2 – 0.3	21-30%	0.25
4	0.3 – 0.5	31-50%	0.4
5	0.5 – 1.0	51-100%	0.75
6	>1.0		1.1

5.3.3 Experimental design – large scale BACI experiment

A large-scale BACI (Before-After- Control- Impact) sampling design was used to test for the effect of enhancement on densities of *Haliotis laevigata*. A total of 42 sites (21 control and 21 "impact"/ seeded) sites were randomly selected prior to enhancement, with care taken to ensure a sufficient range of habitats were present to represent the population, for example, depth of both seeded and control sites varied between 6 and 18 m. Twenty-nine sites (8 control, 21 seeded) were randomly dispersed amongst each other with a minimum distance of 200 m between sites, whilst the remaining 13 control sites were situated between 1 and 4 km away. It was assumed, for analysis purposes, that this minor spatial separation did not contravene the assumption of random mixing of experimental units. The baseline surveys also supplied information on habitat characteristics and natural densities that was used to estimate the two release densities of 18 and 35 animals per m^2 (Hart et al., 2013). Specific parameters obtained from the baseline surveys were (a) mean habitat area targeted by each release device (»4 m^2), and (b) target densities of *Haliotis laevigata* of shell length ³ 140 mm (1.3 per m^2). Mean habitat area targeted by each release device was derived from the median of the frequency distribution of the number of 1 m^2 quadrats with contiguous habitat. See Hart et al. (2013) for details of release methodology.

Two fixed transects radiating from a permanent mooring were established at all control and seeded sites (Figure 2.2). An essential criterion at seeded sites was that the release habitat must be encountered (Figure 2.2). All control and seeded sites were surveyed four times; before (1 to 6 months prior to enhancement) and 6, 30, and 54 months (4.5 years) post-enhancement. Seeded sites were surveyed more often (also at 12, 18, and 24 months post-enhancement), to monitor the enhanced cohort. Both abalone abundance and ecological parameters were measured (Table 2.2).

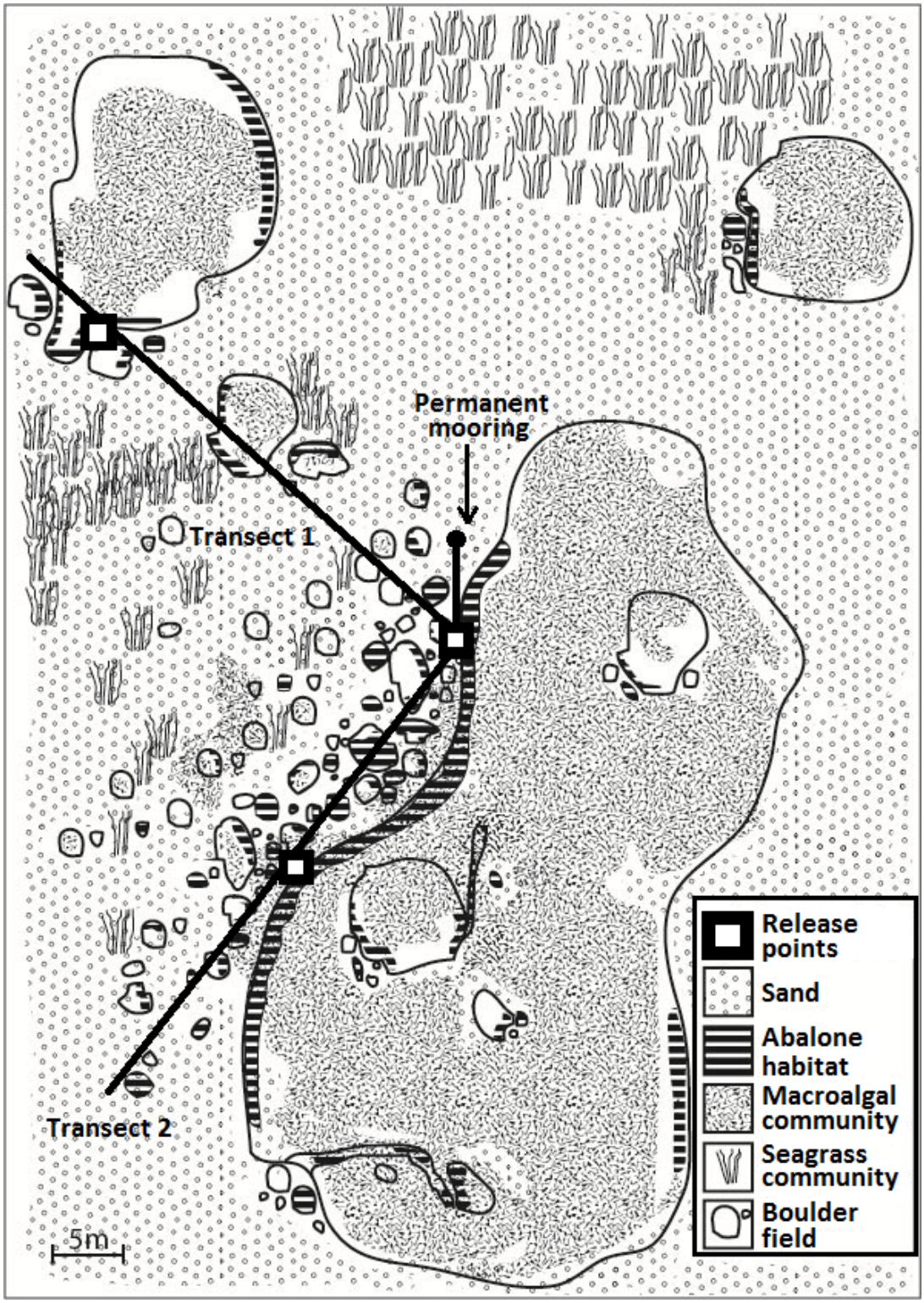

Figure 2.2. Site schematic of experimental reef with a typical distribution of abalone habitat, and location of the three release positions and the two fixed survey transects.

5.3.4 Experimental design – carrying capacity experiment

To evaluate when density dependence is likely to negatively impact stock survival, a 'carrying capacity' study was undertaken. Defined here as "maximum observed density per m^2", the carrying capacity of *Haliotis laevigata* habitat was evaluated using the field survey data prior to enhancement (Figure 2.3), followed by a high-density experimental release at 2 sites. The objective of the high-density release was to increase stock densities to the maximum theoretical density levels, as predicted by the logistic regression analysis as (»8 per m^2; see Results). If this was achieved, it was deemed to represent a reasonable estimate of the "average" carrying capacity of Greenlip Abalone habitat.

Two sites were mapped prior to release and habitat area estimated using the habitat survey method described above. Target densities (7 – 9 per m^2; see Figure 2.3) and release densities (5,900 animals of 30 mm mean shell length into 45-50 m^2 of habitat; »120 m^{-2}) were estimated using the methodology of Hart et al. (2013). As with the main BACI experiment, fixed transects (n = 4 or 5) were established at each site and surveyed four times; before (1 month prior to enhancement), and 6, 18, and 30 months post-enhancement.

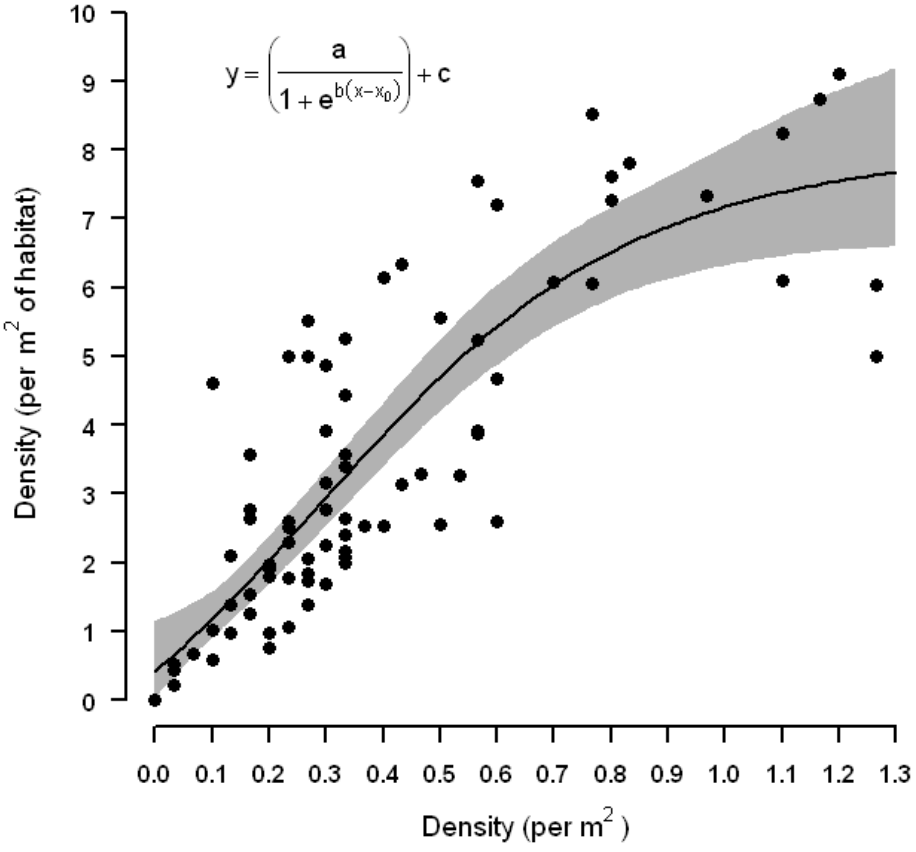

Figure 2.3. Comparison between density of *Haliotis laevigata* per m^2 of available habitat (vertical axis), and density per total m^2 surveyed, using a logistic regression. Parameter estimates are a = 12.9, b = -3.73, c = -4.75, and x_0 = 0.20. The maximum theoretical density is 8.2 per m^2 of habitat (6.7 – 11.3 CL). Data from n = 42 sites (84 x 30 m^2 transects). Grey shading represents 95% CL, obtained from bootstrapping (n = 10,000).

5.3.5 Experimental design - ecological effects of enhancement

The BACI design used to test for ecological effects was similar to that used to test for density effects, except that fewer control sites and times were measured due to logistical limitations. A total of 36 sites (27 seeded and 9 control) were surveyed prior to (May 2006), and at 18 months post-enhancement (November 2007). After quantifying abalone abundance to estimate density effects, observers' measured ecological parameters within each 1 m^2 quadrat on the 30 m^2 transects. Relevant variables included density of conspecifics, competitors and predators, food and habitat availability (Table 2.2). Densities of roving vertebrate predators (wrasses and stingrays) were estimated after completing the benthic surveys. Observers swam back along the 30m^2 transect and estimated density of 7 wrasse species within a 4 m width, and recorded any sightings of eagle rays within visible distance (Table 2.2).

5.3.6 Data analysis

For the main BACI experiment on density effects, abundance and habitat data from the 2 x 30 m^2 transects per site were combined, with each site representing an independent replicate within a treatment. The data was analysed with a 2-Factor ANOVA (Time x Treatment), with the raw data transformed to log (x +1). *Post-hoc* analyses were carried out with the Tukey's HSD test (Day and Quinn, 1989). Data from the carrying capacity experiment were also analysed with a 2-Factor ANOVA (Time x Site).

Ecological effects of abalone enhancement were investigated by multivariate techniques following the approach of Clarke and Warwick (2001). The software utilised were the PRIMER v6 (Clarke and Gorley, 2006) and PERMANOVA+ (Anderson et al., 2008) packages. Specifically, ecological similarity between control and seeded sites, one month prior to, and 18 months after experimental releases, were examined with non-metric multidimensional scaling (MDS) and permutational MANOVA (PERMANOVA) using a two-way crossed design (Time x Treatment). To normalise distributions prior to analysis, selective data transformations were undertaken, dependent on the underlying raw data distribution. Of the ecological variables listed in Table 2.2, density indices were either Öx or log (x+1) transformed, while the habitat and brown algal (Phaeophyta) abundance variables did not require transformation. Where significant effects were obtained, the multivariate tests were followed by ANOVA tests of individual ecological variables to identify which were important.

Table 2.2. Variables used to examine ecological effects of stock enhancement of Greenlip Abalone *Haliotis laevigata*. Variables were quantified for each 1m^2 quadrat within the 30m^2 sample transect.

Variable	Ecological Category	Description	Methodology Notes
AD	Conspecific	Existing abalone density (per m^2 of habitat)	Total density and density of breeding adults and juveniles
HT	Habitat	Area of abalone habitat (m^2)	Expressed as m^2 of habitat per 30m^2 of transect;
Depth	Habitat	Depth of release site (m)	Release sites varied from 6 to 18 m depth
SD	Competitor	Density index of staircase abalone (*Haliotis scalaris*)	Presence /absence recorded for each 1m^2 quadrat per 30m^2 transect, resulting in a maximum index of 30; averaged per site
PD	Competitor	Density index of purple sea urchin (*Heliocidaris erythrogramma*)	Presence /absence recorded for each 1m^2 quadrat per 30m^2 transect, resulting in a maximum index of 30; averaged per site
LD	Competitor	Density index of the keyhole limpet (Fissurellidae) *Scutus antipodes*	Presence /absence recorded for each 1m^2 quadrat per 30m^2 transect, resulting in a maximum index of 30; averaged per site
WD	Predator	Density of wrasse sp. (Labridae).	Density (per m^2) of 7 wrasse species, primarily *Psudolabris parilus* and *Opthalmolepsis lineolata*. Area surveyed: 4 ´ 30 m = 120 m^2
RD	Predator	Density index of eagle ray (*Myliobatis australis*)	Number sighted per transect / site
RC	Food	Rhodophyta (red algae) percent cover	% cover estimated for each 1m^2 quadrat per 30m^2 transect; averaged per site
PC	Food	Phaeophyta (brown algae) percent cover	% cover estimated for each 1m^2 quadrat per 30m^2 transect; averaged per site

5.4 Results

5.4.1 Habitat and density surveys

There was no significant difference in precision between the two survey methods (t = -1.1; p > 0.05). Mean precision (from n = 2 transects per site) of the habitat area method was 0.35 (± 0.05 SE) compared to 0.29 (± 0.04 SE) for the total area method. There was a significant positive correlation between the two estimates of abalone density (Spearman rank r = 0.75; P < 0.01), however the relationship between the two was not linear over the entire density range, and was described adequately with a logistic equation (Figure 2.3). Maximum theoretical density was 8.2 per m^2, with a 95% confidence range of 6.7 to 11.3 per m^2 (Figure 2.3). The mean area of abalone habitat (per 30 m^2 transect) was 3.3 m^2 (± 0.26 SE) or 11% of area surveyed at each fixed site. Maximum density per m^2 of habitat (7 - 9) was achieved at a range of total area densities between 0.6 – 1.3 per m^2 (Figure 2.3).

5.4.2 Large-scale BACI experiment

Densities were significantly higher on seeded compared to control sites for total densities and all age-classes, except for 6+ age class animals (Table 2.3). However the principal result was a significant interaction between time and treatment for most age-classes (Table 2.3). *Post-*

hoc tests revealed that the clearest indication of a stock enhancement effect was evident in the Age 2+, 3+ and 4+ size classes, as well as total density (Table 2.4). Densities of Age 5+ animals did significantly increase post-enhancement (Table 2.4), but there was no overall differences between seeded and control sites.

The progression of the seeded cohort through the population is evident from viewing densities of individual size/age classes (Figure 2.4) and population size-frequency (Figure 2.5). Age 2+ densities were the first to respond (Figure 2.4a); initially no differences were detected, however at 6 months post release (Nov 2006), Age 2+ densities had doubled. At 30 months post release (November 2008), Age 2+ densities at seeded sites had also increased, however by November 2010 Age 2+ densities had declined to pre-enhancement levels (Figure 2.4a). For Age 3+ densities, there was no difference between enhanced and control sites for the first 2 time periods, however at 18 months post-release (Nov 2007), densities increased significantly on enhanced sites and remained high for at least a year (Figure 2.4b). Age 4+ animals showed the clearest effect of enhancement, with three time periods of no difference between enhanced and control sites, followed by a three-fold increase at enhanced sites at 2.5 years post-release (Nov 2007; Figure 2.4c). The pattern is less clear Age 5+ animals, although a significant increase was detected at seeded sites (Figure 2.4d; Table 2.4). There was no evidence of enhancement in Age 6+ animals (Figure 2.4g). Overall, this single cohort release of Age 1+ animals in 2006 was sufficient enough to double to total density by November 2008 (2.5 years post-release; Figure 2.4f). The overall increase in density appears to have been around 3 abalone per m^2, although this declined over the final 2 years of the study (Figure 2.4f).

Table 2.3. ANOVA results for the effect of Time, Treatment (Seeded, Control), and Site on density (per m2) of Haliotis laevigata. Data has been log (x+1) transformed.

Source of variability	df	MS	F	P	MS	F	P	MS	F	P
		Total density			Age 2+ (41 - 80 mm)			Age 3+ (81-110 mm)		
Time	3	2.30	5.13	<0.001	0.68	5.52	0.001	2.66	14.7	<0.001
Treatment	1	3.62	8.06	0.005	1.26	10.23	0.002	2.04	11.2	0.001
Time ´ Treatment	3	1.50	3.34	0.019	0.52	4.18	0.006	0.53	2.9	0.03
Residual	335	0.45			0.12			0.18		
		Age 4+ (111 - 135 mm)			Age 5+ (136 - 149 mm)			Age 6+ (150+ mm)		
Time	2	2.45	9.60	<0.001	0.74	3.44	0.02	0.09	0.45	ns
Treatment	1	1.21	4.74	0.03	0.41	1.90	ns	0.68	3.25	0.07
Time ´ Treatment	2	1.10	4.30	0.005	0.62	2.88	0.04	0.50	2.38	0.07
Residual	126	0.26			0.22			0.21		

Table 2.4. Summary of Tukey's HSD *post-hoc* test results (p-values) for the effect of stock enhancement on Greenlip Abalone density. Test A = Significant density increase (p < 0.05) at seeded sites over time, Test B = Significant density difference between seeded and control sites, post-enhancement. ns = not significant (p > 0.05).

Age / Size-class	Test A	Test B
2+ (41 - 80 mm)	<0.001	0.001
3+ (81 – 110 mm)	<0.001	0.001
4+ (111 – 135 mm)	<0.001	0.002
5+ (136 – 149 mm)	0.004	ns
6+ (³ 150 mm)	ns	ns
Total density	<0.001	0.003

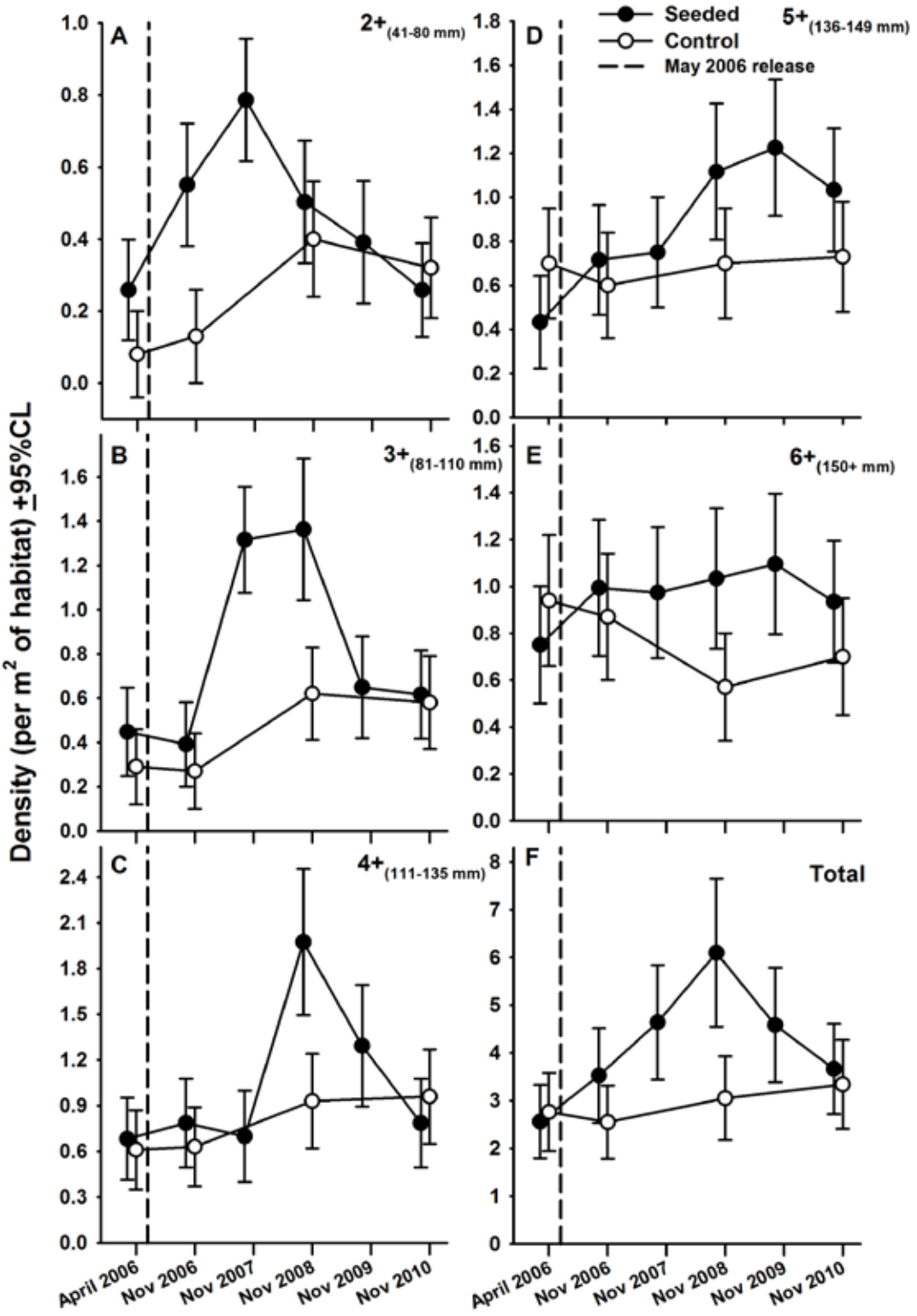

Figure 2.4. Densities (per m^2 of habitat) of *Haliotis laevigata* over time at seeded and control sites. Individual graphs correspond to Age classes from 2+ to 6+ for the seeded animals, and the equivalent sized-animals in the wild stocks, whose ages are unknown.

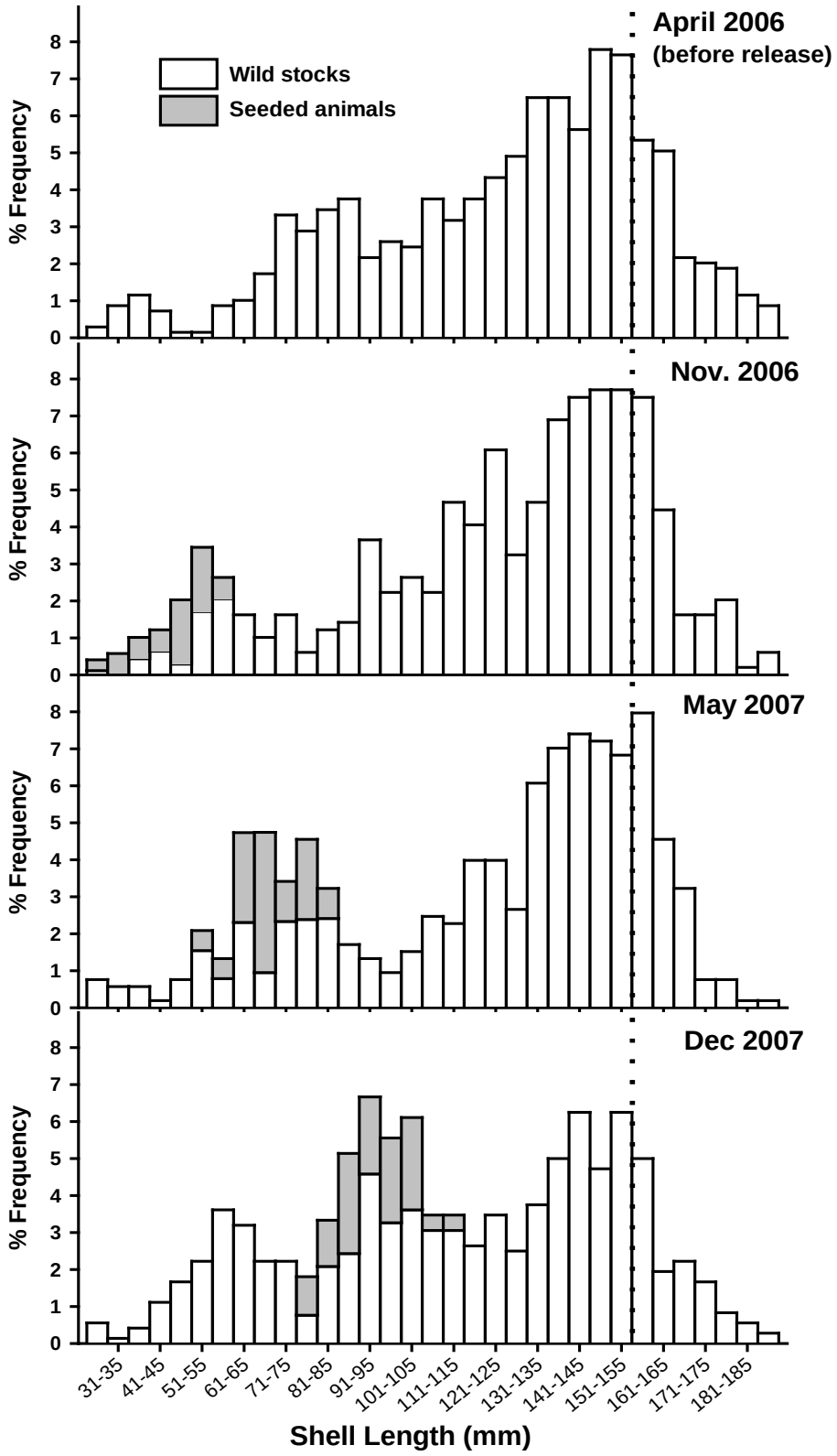

Figure 2.5. Percent frequency of wild stock and seeded *Haliotis laevigata* at BACI experimental sites between April 2006 and December 2007. Dashed line indicates minimum size at commercial harvest.

5.4.3 Carrying capacity experiment

In the high-density release experiment (> 100 m^{-2}), there was a significant effect of site and time on total densities, however the main result was a site by time interaction ($p < 0.05$). At Site A, total density increased to 18 m^{-2} at 6 months post-release, and then declined to 8 m-2 by 30 months post release (Figure 2.6). A different pattern was seen at Site B, with densities reaching 9.5 m-2 at 6 months post-release, and slowly reducing to around 8 m^{-2} by 30 months post-release (Nov 2009; Figure 2.6). This result confirmed the theoretical predictions from the initial surveys (Figure 2.3), and suggests an average carrying capacity of around 8 per m^2. Overall the seeded cohort comprised approximately 60% of the total population at both 6 months (November 2008), and 18 months (November 2009) post-release, and this had declined to 50% of the total population by November 2010 (Figure 2.7).

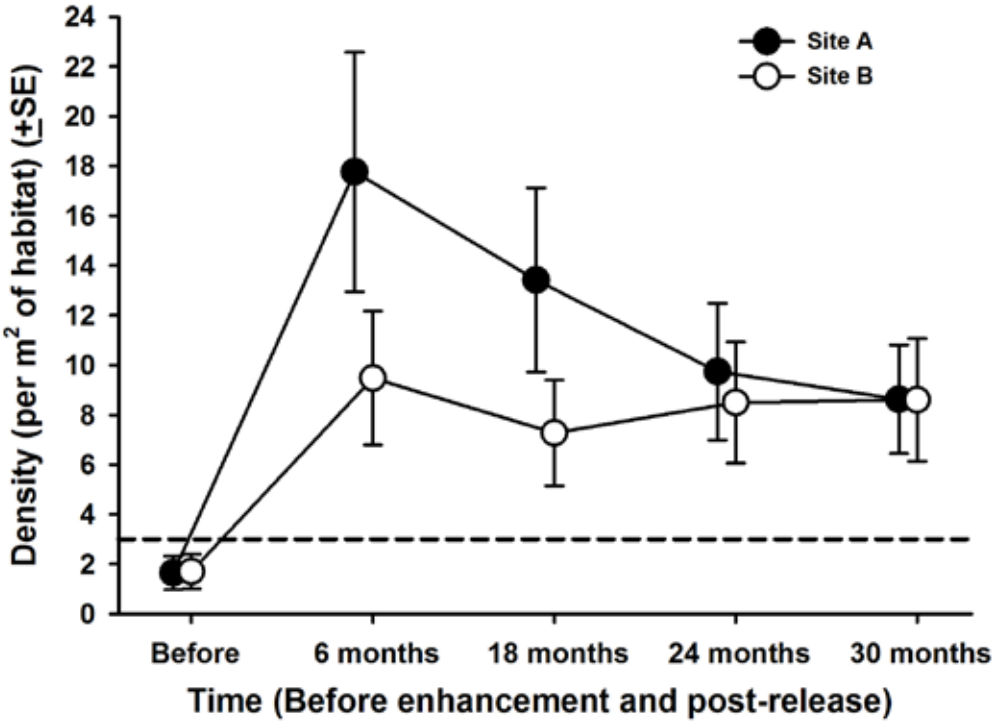

Figure 2.6. Total densities before and after enhancement in the carrying capacity experiment. Dashed line indicates average total density on commercially fished reefs.

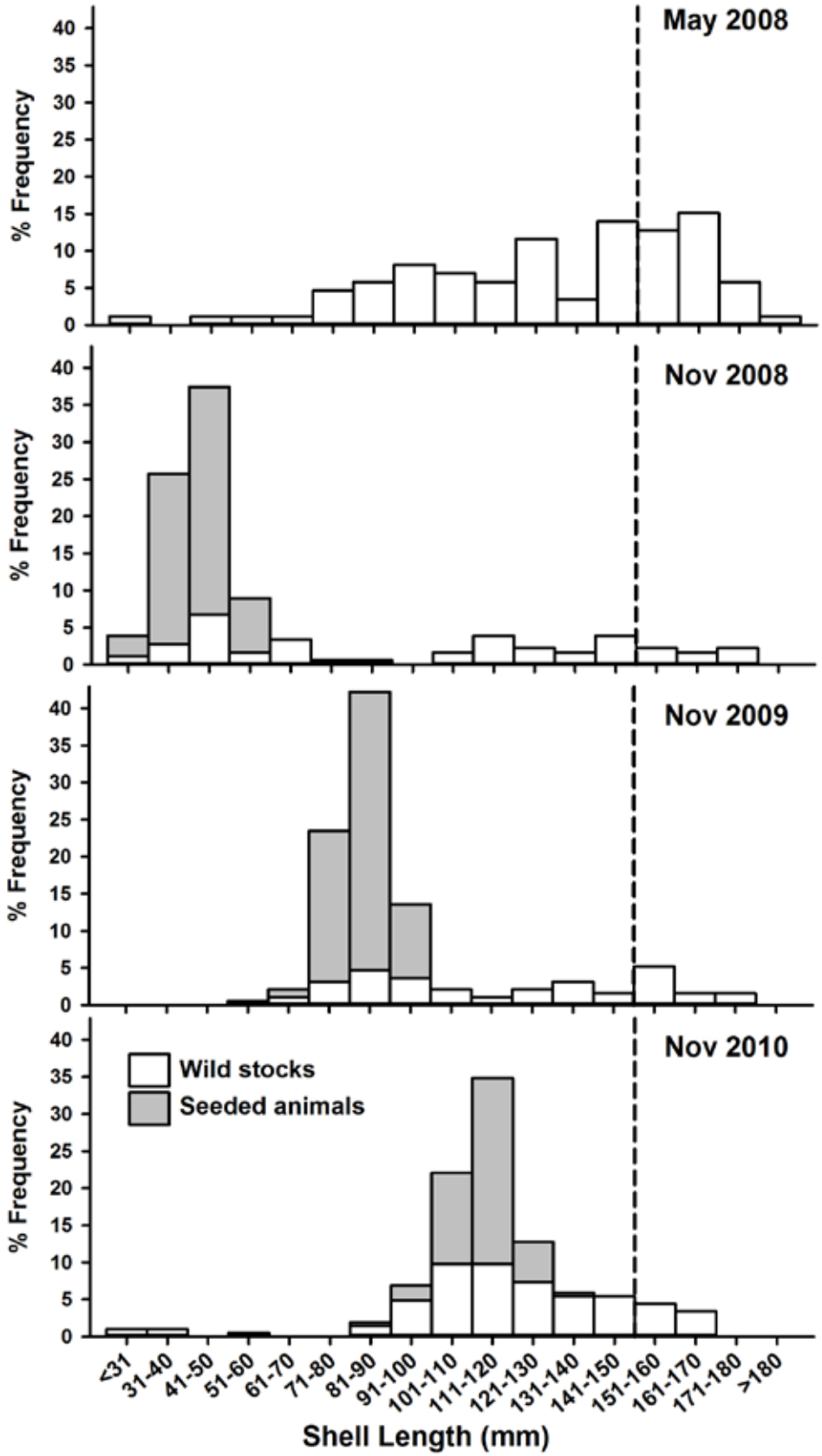

Figure 2.7. Shell-length distribution of wild stock and seeded *Haliotis laevigata* at sites subject to the carrying capacity experiment, between May 2008 and December 2010. Dashed line indicates minimum size at commercial harvest.

5.4.4 Ecological effects of enhancement

A PERMANOVA analysis of ecological similarity detected no significant difference between control and enhanced sites, and no site × time interaction (Table 2.5). However there was a significant effect of time (Table 2.5). MDS plots of ecological similarity show that enhanced and control sites are similar to each other before (Figure 2.8a) and after (Figure 2.8b) seeding. However, there is dissimilarity due to time (Figure 2.8c). Analysis of individual ecological variables revealed no statistical differences in time or treatment, with the exception of % cover of Phaeophyta (Table 2.6). Brown algal percent cover increased at both seeded and control sites after seeding (Figure 2.9a). A similar pattern was seen for Rhodophyta % cover (Figure 2.9b), although the result was not statistically conclusive ($p = 0.09$; Table 2.6). While not statistically significant ($p = 0.06$), mean % cover of Rhodophyta was higher on control sites, particularly at 18 months post-enhancement (Figure 2.9b).

Table 2.5. PERMANOVA results for the effect of Time (Before, After – 18 months post enhancement) and Treatment (Seeded, Control), on ecological similarity (Bray-Curtis coefficient) of benthic communities. Data for ecological variables (see Table 2.2) comprising the resemblance matrix have been transformed where necessary (see methods for details). Type III sums of squares has been computed.

Source of variability	df	SS	MS	Psuedo-F	P
			Bray-Curtis coefficient		
Time	1	1374	1374	4.35	0.007
Treatment	1	448	448	1.42	0.232
Time ´ Treatment	1	11.2	11.2	0.04	0.965
Residual	68	21485	316		
Total	71	23566			

Table 2.6. ANOVA results for the effect of Time (Before, After) and Treatment (Seeded, Control), on ecological variables. Data transformed where necessary (see methods).

Source of variability	df	MS	F	P	MS	F	P	MS	F	P
		Habitat area			*Haliotis scalaris*			*Heliocidaris erythrogramma*		
Time	1	0.43	0.03	ns	0.27	0.14	ns	0.18	0.94	ns
Treatment	1	36.6	2.77	ns	1.79	0.94	ns	0.11	0.55	ns
Time´Treatment	1	2.80	0.21	ns	0.03	0.01	ns	0.01	0.02	ns
Residual	68	13.20			1.92			0.20		
		Keyhole limpet *Scutus antipodes*			Red algal % cover			Brown algal % cover		
Time	1	0.62	0.23	ns	1.22	2.88	0.09	852	7.21	0.009
Treatment	1	0.02	0.01	ns	1.49	3.53	0.06	111	0.94	ns
Time´Treatment	1	0.07	0.03	ns	0.25	0.59	ns	0.91	0.01	ns
Residual	68	2.66			0.42			118		

ns = $p > 0.05$

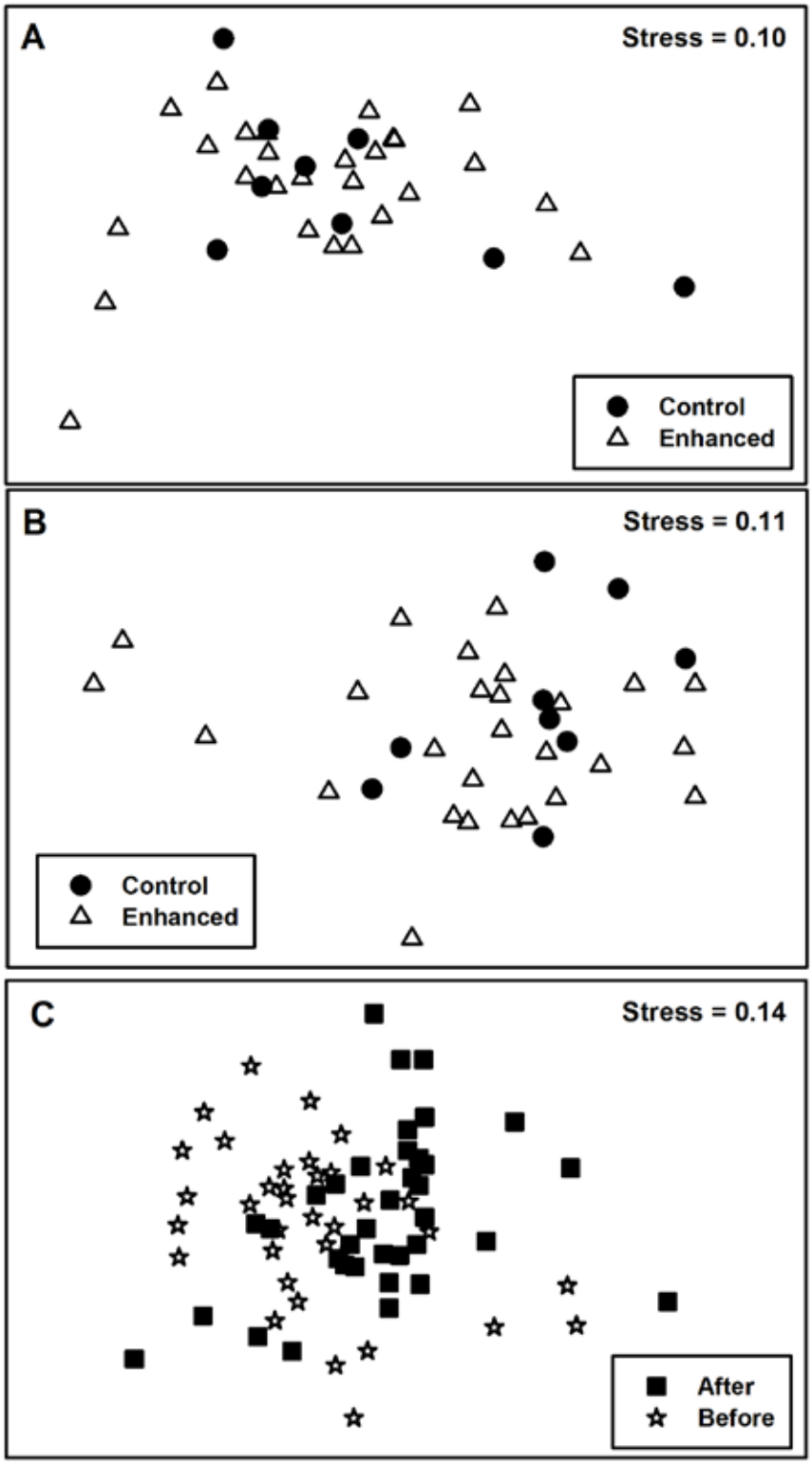

Figure 2.8. Non metric MDS plots of ecological similarity at survey sites on Greenlip Abalone habitat. (A) Before enhancement (enhanced vs control sites); (B) 18 months post enhancement (C) All sites, before vs after.

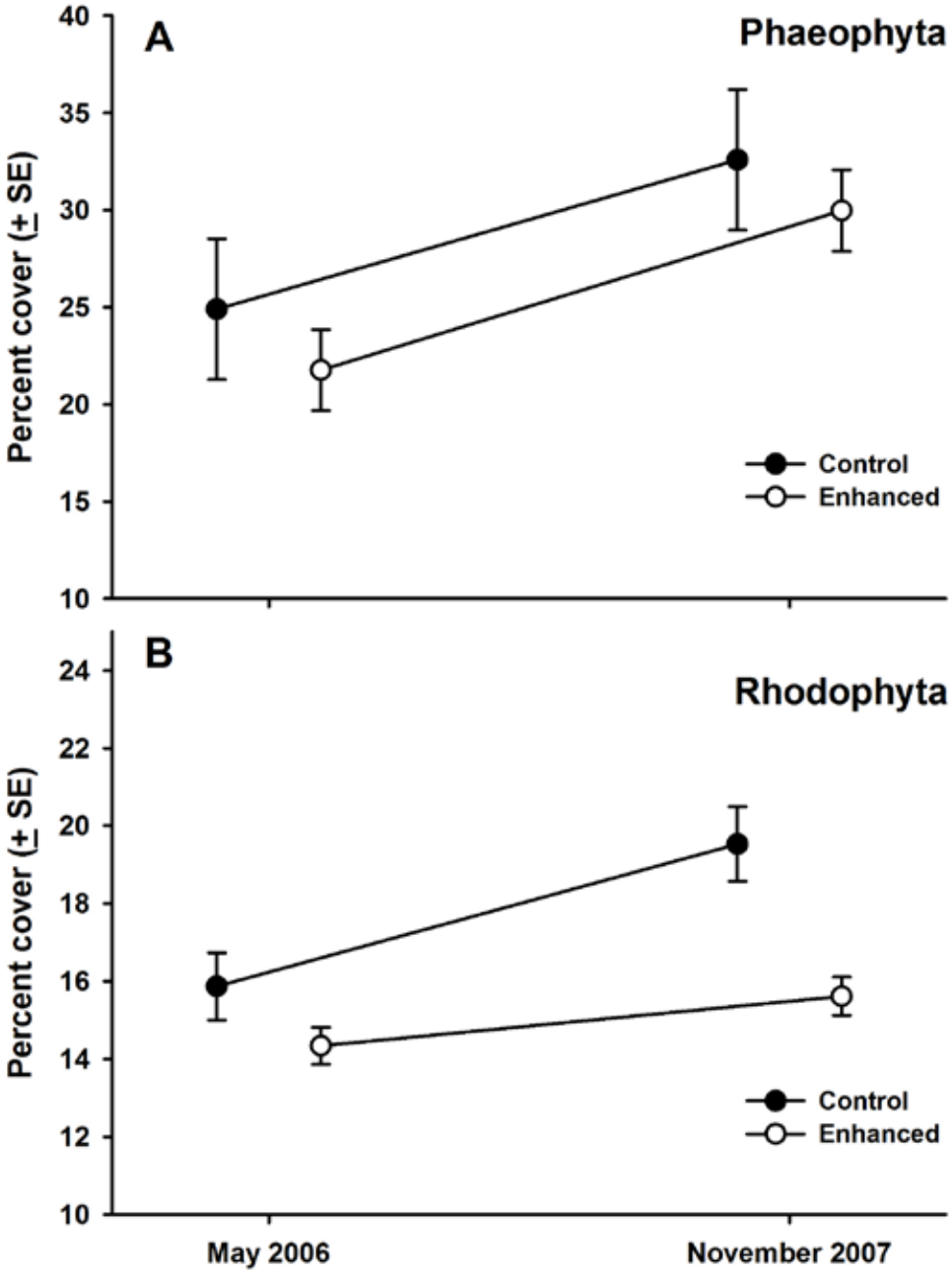

Figure 2.9. Percent cover of major algal groups in May 2006 (before enhancement) and November 2007 (18 month posts-enhancement). Phaeophyta (a); Rhodophyta (B).

5.5 Discussion

This study has revealed three findings that contribute substantially to development of the science of stock enhancement in *Haliotis laevigata*. Firstly, a significant increase in abalone density occurred that was clearly attributed to enhancement. More importantly, the overall magnitude of the density increase in the main BACI experiment peaked at approximately 2.5 to 3 m^{-2}, similar to the target density of 2.5 m^{-2}, as estimated by the release model (Hart et al., 2013). Secondly, a theoretical carrying capacity of 8.2 m^{-2} (range: 6.3 – 11.2 m^{-2}) was estimated and confirmed by experimentation. Whilst the results are preliminary, they propose a clear upper limit on release densities that can be tested by further experimentation and provide guidance for future releases. For example, a commercial release proposing to triple the densities of Age 5+ from 0.8 to 2.4 m^{-2}, would set a target density of 1.6 m^{-2}. Assuming a mean survival of 13% at 3.5 years post-release (Hart et al., 2013) this target requires a release density of 12.3 m^{-2}. Thirdly, no immediate ecological effects of enhancement were detected, despite seasonal changes in community composition being detectable at both control and experimental sites. This can be largely attributed to target densities being within natural capacity limits of the habitat. Overall these results provide the foundations for a set of quantitative principles that can be used to facilitate the integration of stock enhancement into wild fishery management. For example, the next logical step is to translate catches and quota (measured in tonnes) to density estimates using estimates of fishing mortality and size-structure information of wild stocks. Once this is achieved with statistical robustness, a new harvest quota model can be developed that links density of the catch with density of recruitment, predicted from contributions of stock enhancement, and natural recruitment, noting that natural recruitment in this species exhibits log-normal variability (Shepherd, 1990). In this respect, the results of this work fit into the paradigm proposed by Miller and Walters (2004), namely that stock enhancement experiments should be used to advance ecological understanding (predictive capacity) and to guide stocking and management practice.

Fine scale habitat availability was clearly demonstrated to influence density of *H. laevigata*, with implications for an understanding of the effects of experimental manipulation of population densities. Previously established threshold levels for population density do exist for this species. Shepherd and Partington (1995) posit a figure of 0.2 – 0.3 m^{-2} for adult densities, based on Ricker-style stock recruitment curve, below which increased vulnerability to recruitment failure is likely. This figure was supported by experimental analysis of fertilization success as a function of distance between conspecifics (Babcock and Keesing, 1999). However, our analysis showed that the 0.3 m^{-2} threshold density, when adjusted for available habitat, encompassed a density range of 1 – 5 m^{-2} (Figure 2.3) indicating that a re-assessment of the 0.3 threshold figure is warranted, as it is primarily a function of the method used to generate it. From a stock enhancement perspective, the maximum possible density is the critical limiting factor and a theoretical maximum of 8 m^{-2} was predicted by the habitat density analysis (Figure 2.3), and confirmed by experimental evaluation (Figure 2.6). These results suggest that examining abalone densities as a function of the available habitat is a more accurate way to assess the effects of enhancement and that there are explicit limits in which enhancement can be effective.

The significant effect of stock enhancement corroborates the results of the mark-recapture experiments (Hart et al., 2013), and provides an unambiguous example of successful enhancement in abalone at an experimental scale. A major strength of this study was the longer term monitoring of one cohort from birth until maturity and recruitment into the fishery. A number of recent small-scale experimental studies on *Haliotis iris* (Roberts et al., 2007), *Haliotis laevigata* (Dixon et al., 2006), and *H. midae* (Sweijd et al., 1998) have shown promising results, compared to some earlier work (Rogers-Bennet and Pearse, 1998; Tegner and Butler, 1985) but lacked the spatial and temporal scale to be conclusive. In this study, and its companion paper (Hart et al., 2013), the combination of large scale BACI design (42 sites), tagging to enable a distinction between wild and seeded stocks, estimates of survival probability, and a test of carrying capacity all provided multiple strands of evidence. Overall however, it is our view that careful in-water experimental control, significant use of GPS and detailed habitat maps, and permanent site monitoring were all essential ingredients.

The question of ecological effects has not previously been considered in detail, as most studies are short-term in nature. Dixon et al., (2006) recorded 100% abalone mortality at two sites, and attributed this to starfish predation. Shepherd (1998), from a 14 year study, concluded that crabs and wrasses were the major predators of juveniles. No correlation was found between wrasse density and survival in this study. Observations of mortality due to octopus predation were noted at one site, however the difficulty of quantifying octopus abundance precluded an in-depth analysis. Overall our analysis of ecological similarity detected no clear difference between control and seeded sites; however there was slight the suggestion differences in red algae abundance, post release (Figure 2.9b). Given that the preferred diet of *H. laevigata* is red algae (Shepherd and Steinbeck, 1992), this result may bear further investigation. However a comprehensive analysis of the ecological role of *Haliotis rubra* on reef ecosystems in Victoria found limited evidence of any impact of fishing, indicating that ecological effects of abundance changes are likely to be highly localised and undetectable at a population scale (Hamer et al., 2010). Being opportunistic feeders that rely on drift algae as a primary food source, abalone are not generally considered to play a major role in the structuring or performance of their ecological community.

Further work is needed in the area of release densities that optimise survival. For example, Hart et al. (2013) detected a large range in survival between sites that was not adequately explained by the two release densities or any ecological parameters. Husbandry factors were implicated, but initial work suggests that size at release is an important factor (Strain, unpublished data). While survival was not explicitly estimated in the carrying capacity experiment, the final release density at the completion of the experiment (»8 per m^2) suggests a survival at 30 months of 6 – 8%. This is lower than predicted from the release density model (see Hart et al., 2013) and may represent a density-dependent response, as discovered by Dixon et al. (2006) on experimentally constructed reefs.

5.6 Acknowledgements

Thanks to Sabine Daume and Fiona Graham who assisted with the hatchery culture and release of the animals; Great Southern Marine Hatchery, the Western Australian Abalone Industry Association (WAAIA) in particular Ian Taylor and Brad Adams for facilitating this

exciting work, and to all the industry divers who participated in the site selection and juvenile release, namely Brad Adams, Darren, and Nathan Adams, Jay Clauson, John Lashmar, Michael and Peter Rickerby, and Steve and George Beres. This study was funded by the Department of Fisheries, Western Australia and the Australian Seafood CRC (Cooperative Research Centre).

5.7 References

Anderson, M. J., R. N. Gorley, and K. R. Clarke. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E: Plymouth, UK. 214 p. (2008).

Babcock, R. and J. K. Keesing. Fertilisation Biology of the Abalone Haliotis laevigata: Laboratory and Field Studies. Canadian Journal of Fisheries and Aquatic Science, 56: 1668-16781999.

Clarke, K. R., and R. M. Warwick. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E; Plymouth, UK. (2001).

Clarke, K. R., and R. N. Gorley. PRIMER v6: User Manual/Tutorial. PRIMER-E; Plymouth, UK. 190 p. (2006).

Daume S., S. Brand-Gardner, and W. J. Woelkerling. Preferential settlement of abalone larvae: diatom films vs. non-geniculate coralline red algae. Aquacult., 174: 243-254 (1999).

Day, R. W. and G. P. Quinn. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr., 59(4): 433 – 463 (1989).

Dixon, C. J., R. W. Day, S. M. H. Huchette, and S. A. Shepherd. Successful seeding of hatchery-produced juvenile greenlip abalone to restore wild stocks. Fish. Res. 78: 179-185. (2006).

Dowling, N. A., S. J., Hall and R. McGarvey. Assessing population sustainability and response to fishing in terms of aggregation structure for greenlip abalone (Haliotis laevigata) fishery management. Can. J Fish. Aquat. Sci., 61: 247-259 (2004).

Gorfine, H., D. A. Forbes, and A. S Gason. A comparison of two underwater census methods for estimating the abundance of the commercially important blacklip abalone, Haliotis rubra. Fish. Bull., 96: 438-450. (1998).

Hamer, P. A., G. P. Jenkins, B. A. Wormersly, and K. A. Mills. Understanding the ecological role of abalone in the reef ecosystem of Victoria. Final report to the Fisheries Research and Development Corporation. Project No. 2006/040. Department of Primary Industries, Queenscliff. 132 p. (2010).

Hart, A. M., H. K. Gorfine, and M. P. Callan. Abundance estimation of blacklip abalone (Haliotis rubra) I. An analysis of diver-survey methods used for large-scale monitoring. Fish. Res., 29: 159-169 (1997).

Hart, A. M, F. P. Fabris, and S. Daume. Stock enhancement of Haliotis laevigata in Western Australia – a preliminary assessment. Fisheries Research Report No. 166, Department of Fisheries, Western Australia (2007).

Hart, A. M., L. W. S. Strain, F. P. Fabris, J. Brown, and M. Davidson. Stock enhancement in greenlip abalone part I: long-term growth and survival. Rev. Fish. Sci. 21(3-4): 299-309 (2013).

McGarvey, R. Assessing survey methods for greenlip abalone in South Australia. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Research Report Series No. 184. 195 pp. (2006).

McShane, P. E. Recruitment variation in abalone: Its importance to fisheries management. Mar. Freshw. Res., 46: 555-530 (1995).

McShane, P. E. Assessing stocks of abalone (Haliotis spp.): methods and constraints. Can. Spec. Pub. Fish. Aquat. Sci., 125: 41 –48 (1998).

Miller, J. M., and C. J. Walters. Experimental ecological tests with stocked marine fish. In: Stock enhancement and sea ranching: Developments, pitfalls, and opportunities, pp. 142 – 152 (K. M. Leber, S. Kitada, H. L. Blankenship, and T. Svasand, Eds.). Blackwell Publishing (2004).

Prince, J., and R. Hilborn. Concentration profiles and invertebrate fisheries management. Can. Spec. Pub. Fish. Aquat. Sci., 125: 187-196 (1998).

Purcell, S. W. and M. Simutoga. Spatio-temporal and size-dependent variation in the success of releasing cultured sea cucumbers in the wild. Rev. Fish. Sci., 16(1-3): 204 – 214 (2008).

Roberts, R. D., E. F. Keys, G. Prendeville, and C. A. Pilditch. Viability of abalone (Haliotis iris) stock enhancement by release of hatchery-reared seed in Marlborough, New Zealand. J. Shell. Res. 26(3): 697-703 (2007).

Rogers-Bennet, L., and J. S. Pearce. Experimental seeding of hatchery reared juvenile red abalone in northern California. J. Shell. Res. 17(3): 877-880 (1998).

Shepherd. S. A. Studies on Southern Australian Abalone (Genus Haliotis). XII: Long-term recruitment and mortality dynamics in an unfished population. Aust. J. Mar. Freshw. Res. 41: 475-492 (1990).

Shepherd. S. A. Studies on Southern Australian Abalone (Genus Haliotis). XIX: Long-term juvenile mortality dynamics. J. Shell. Res. 17(3): 813-825 (1998).

Shepherd S.J., Steinberg, P.D. Food preferences of three Australian abalone species with a review of the algal food of abalone. In: Abalone of the World, biology, fisheries, and culture (S. A. Shepherd, M, J. Tegner, S. A. Guzman del Proo, Eds).pp. 169 –181. Fishing News Books, Blackwell Scientific (1992).

Shepherd, S. A., P. A. Preece, R. G. W. White. Tired nature's sweet restorer – ecology of abalone stock enhancement in Australia. In: Workshop on rebuilding abalone stocks British Columbia (A. Campbell, Ed.). Can. Spec. Publ. Fish. Aquat. Sci., 130: 84-98 (2001).

Shepherd, S. A., and D. Partington. Studies on Southern Australian Abalone (Genus Haliotis). XVI. Recruitment, Habitat and Stock Relations. Mar. Freshw. Res., 46: 669 – 680 (1995).

Sweijd, N., Q. Snethlage, D. Harvey, P. Cook. Experimental abalone (Haliotis midae) seeding in South Africa. J. Shell. Res. 17(3): 89-904 (1998).

Tegner, M. J., and R. A. Butler. The survival and mortality of seeded and native red abalone, H. rufescens on the Palos Verdes Peninsula. Calif. Fish Game 71: 150-163 (1985).

Yamashita, Y., and Y. Kurita. An appropriate stocking size of juvenile Japanese flounder, Paralichthys olivaceus, in consideration of carrying capacity. Bull. Fish. Res. Agen., 19:33 (2007).

6 Stock Enhancement in Greenlip Abalone: (3) Bioeconomic Evaluation

Anthony M. Hart[1], Lachlan W. S. Strain[1], Alex S. Hesp[1]

[1]Western Australian Fisheries and Marine Research Laboratories, PO Box 20, North Beach, Western Australia, 6920, Australia.

Please note that this chapter has been published as the following manuscript:

Hart AM, Strain LWS, Hesp A (2013). Stock enhancement of greenlip abalone: (3): Bioeconomic evaluation. *Rev. Fish. Sci*. 21: 354-374.

6.1 Abstract

This study presents a bioeconomic evaluation of the effect of stock enhancement on biomass, net present value (NPV), profitability, and gross value of product (GVP) of the Australian Greenlip Abalone (*Haliotis laevigata*) fishery. Enhancement targets were defined as a function of natural recruitment (*Nr*), and compared with current harvest strategies. The model (*EnhanceFish* program) was conditioned on a Western Australian fishery, and then applied to Greenlip Abalone stocks throughout Australia. Two levels of releases (50% *Nr* and 100% *Nr*) at varying fishing mortality (*F*), size-at-harvest, and size-at-release, were evaluated in detail. Model validation was also undertaken by comparing the model-derived spawning biomass (*SSb*) with an alternative estimate (SSb_f) obtained using in-water surveys and a different growth model. Economic profitability and increased spawning biomass were achieved for most stock enhancement scenarios and optimal profitability occurred with a 10 – 20% decrease in *F* from current levels, a 10% decrease in minimum legal length, and an annual enhancement of *Nr* juveniles to match natural recruitment. More radical scenarios such as an annual release of 150% *Nr* combined with a 30% decrease in size-at-harvest resulted in greater profitability (+175%), but presented a higher risk of wild stocks being replaced with hatchery genotypes. Sensitivity analysis revealed that mortality, size at release, and harvest price were the critical parameters, whilst costs of production and fishing were less important. At the national scale, an enhancement scenario involving an annual release of 6.1 million, 4 cm juveniles (»Age 2) resulted in a 60% increase in GVP ($25 to $40 million), a 120% increase in profitability ($12 to $26 million), and NPV ($190 to $420 million; 6% discount), and a 25% increase in *SSb*.

Keywords: Net Present Value (NPV), population model, recruitment, Gross Value Product (GVP)

6.2 Introduction

Lack of success with many marine stock enhancement programs worldwide has prompted a cautious approach to future development (Kitada and Kishino, 2006) and the promotion of a scientifically rigorous protocol called the “Responsible Approach” to stock enhancement (Blankenship and Leber, 1995; Lorenzen et al., 2010). Key ingredients of this approach include: (1) experimental evaluations of impacts of different release densities; (2) examinations of effects of fundamental ecological processes such as density-dependence, habitat limitation and environmental effects on recruitment; (3) assessments of the economic

performance of restocking, and development of sound governance and polices to deal with resource allocation issues and (4) inherent threats, such as disease or pest species introductions. Often, however, the high variability of natural recruitment and the intrinsic stochastic nature of wild fish populations can render experimental evaluations of the efficacy of stock enhancement uninformative (Kitada and Kishino, 2006). In any commercial-scale application, it is therefore essential that variability in growth, mortality, and recruitment of the target populations be taken into account when evaluating stock enhancement programs.

Abalone fisheries currently contribute 15% ($200 million) of the total annual GVP of Australian fisheries (Mayfield et al., 2012) and numerous experimental stock enhancement studies have been undertaken on the two major commercial species, blacklip (*Haliotis rubra*) and Greenlip (*H. laevigata*) abalone (Shepherd et al., 2001; Dixon et al., 2006; Goodsell et al., 2006; Heasman, 2006; Hart et al., 2007; Chick, 2010; Hart et al., 2013a,b). Attributes that make these fisheries ideally suited for stock enhancement are that they have been subject to long-term sustainable management (Mayfield et al., 2012) and that they are low volume, high value sedentary invertebrate fisheries (Bell et al., 2005; Caddy and Defeo, 2003).

This study presents a bioeconomic evaluation of commercial-scale stock enhancement in Greenlip Abalone fisheries in Australia based on data from long-term field experiments (Hart et al., 2013a,b). We initially applied the bioeconomic model *EnhanceFish* (Lorenzen and Medley, 2006) to a well-known "test" fishery, validated the model by comparing spawning biomass estimates against an alternative technique that used field surveys and mark-release-recapture data, undertook sensitivity analyses to assess the effects of variation in mortality, size at release, cost of fishing, cost of enhancement, and value of harvest on profitability, and then applied the model to all Australian *Haliotis laevigata* fisheries. The objective was to establish if stock enhancement was likely to be commercially viable for this species and to identify the key features and limiting factors of successful commercial-scale enhancement.

6.3 Material and Methods

6.3.1 Biomass Units

In this paper, unless otherwise stated, biomass is expressed in whole weight (kg or tonnes) to help facilitate easier interpretation of results by readers not familiar with Greenlip fisheries in Western Australia and South Australia, for which quotas (Total Allowable Catches), length-weight relationships, and economic data are typically expressed as a function of meat weight, which is the weight of the foot muscle once it has been removed from the abalone shell. Consequently, many of the analyses employed data based on meat weight, and results were converted to whole weight using a conversion rate of whole weight (kg) = 2.667 x meat weight (kg), as currently employed by the Department of Fisheries, Western Australia (Department of Fisheries, 2011).

6.3.2 Bioeconomic model

Bioeconomic evaluations were undertaken using the *EnhanceFish* modelling program developed by Lorenzen (2005) and Lorenzen and Medley (2006), with software and manuals sourced from www.aquaticresources.org/enhancefish.html. *EnhanceFish* uses a dynamic pool fisheries model to quantitatively assess impacts of stock enhancement on biological and

economic parameters (Lorenzen and Medley, 2006). Outcomes can be evaluated against a range of fisheries management options, such as varying levels of fishing effort and size-at-harvest. The model extends traditional dynamic pool models to accommodate stock enhancement by incorporating numerous parameters including; density dependent effects in the pre-recruit stage, population regulation in the recruited phase via size-dependent mortality and density-dependent growth, and biological differences and interactions between hatchery and wild fish (Lorenzen, 2005). Full mathematical details on the model can be sourced from Lorenzen (2005).

6.3.3 Model exploration (Test fishery)

The *EnhanceFish* model was used to explore the consequences of stock enhancement for a Greenlip Abalone fishery in south western Australia (near Augusta, 34° 22' S, 115° 10' E), for which the biological attributes of the targeted stocks and economics of that fishery are well understood (Table 3.1). For estimating Net Present Value (NPV), a discount rate of 6% was applied (Table 3.1). Prior to undertaking economic analysis, estimates were made using "base-case" values for biological parameters such as virgin spawning biomass (*Vb*), current spawning biomass (*SSb*), and natural recruitment (*Nr*) of animals at *SSb*. Key assumptions common to all analyses for the south-western Australian fishery included a recruitment steepness parameter value of 4 (Myers, 1999), a current average annual catch of 18 t meat weight (48 t whole weight), an instantaneous rate of fishing mortality (F) of 0.69 year^{-1}, a minimum legal shell length (MLL) for retention of 15.4 cm, and level of natural mortality at 1 cm (M_1) of 2.7 year^{-1} (Table 3.1).

The natural mortality parameter (M_1), which scales natural mortality (M) by size (Lorenzen, 2000), was estimated by fitting, in Excel using least squares regression, the model $M_L = M_1 / L_{cm}$, where M_L is the natural mortality at length L_{cm} and M_1 is the natural mortality at 1 cm, to size-dependent estimates of M sourced from mark-recapture studies in the literature (Shepherd, 1998; Dixon et al., 2006; Hart et al., 2013a).

6.3.4 Model validation

Three principal assumptions that control the estimates of spawning biomass (*SSb*) from the *EnhanceFish* model are the 2-parameter (k, L_∞) von Bertalanffy growth function, the 1-parameter (M_1) size-dependent mortality function, and the estimate of fishing mortality (F). In reality, however, abalone growth often does not strictly follow a von Bertalanffy growth pattern and, depending on the species, use of alternative growth models such as the Gompertz (Troynikov et al., 1998; Bardos, 2005), Gaussian (Rogers-Bennett, 2007) or inverse logistic model (Haddon et al., 2008, Helionidotis et al., 2011) have been recommended. In this study on Greenlip stocks from south-western Australia, a Gaussian growth model was fitted to tag recapture data and compared with the von Bertalanffy and Gompertz growth functions (see later).

To assess the validity of the estimate of *SSb* obtained from *EnhanceFish*, an alternative estimate of spawning stock biomass (hereafter SSb_f) was derived. The method utilised commercial catch data (total numbers and associated length compositions for three fishing seasons; Hart, unpublished data), field survey density data (Hart et al., 2013b), growth parameter values from the Gaussian model, an estimate of natural mortality (average of

values reported in the literature for Greenlip Abalone for animals > 11 cm, see Figure 3.2a) and estimates of total mortality derived using length-converted catch curves (Pauly, 1986).

6.3.5 Model validation: growth

The most appropriate growth model for Greenlip Abalone of the following three candidate models was selected: the von Bertalanffy, Gompertz and Gaussian growth models. Fitting the von Bertalanffy growth model to tagging data, $\Delta\hat{L}$, the expected change in length of an individual between initial capture and recapture was estimated as

$$\Delta\hat{L} = (L_\infty - L_t)(1 - e^{-k\Delta t}) \qquad \textbf{Equation 3.1}$$

where L_∞ is the average maximum length of individuals in the population, k is the growth coefficient, L_t is the length of the individual at the time of initial capture and Δt is the period of time between capture and recapture (Haddon, 2001; Helidoniotis et al., 2011). Fitting the Gompertz growth model, $\Delta\hat{L}$ was estimated as

$$\Delta\hat{L} = L_\infty . \left(\frac{L_t}{L_\infty} \right)^{e(-g.\Delta t)} - L_t \qquad \textbf{Equation 3.2}$$

where g is a growth constant (Helidoniotis et al., 2011). Fitting the Gaussian model, $\Delta\hat{L}$ was estimated as

$$\Delta\hat{L} = Ae^{-(L_t - u)^2 / 2\sigma^2} \qquad \textbf{Equation 3.3}$$

where A is the maximum growth (mm, year^{-1}), u is the size at maximum growth (mm) and σ is the standard deviation of the distribution of maximum growth *vs* size (Rogers-Bennett et al., 2007). All models were fitted by maximising the value of the log-likelihood function, expressed as

$$LL = \sum_{i=1}^{n} \log_e \left\{ \frac{1}{\sigma\sqrt{2\pi}} e \left[\frac{\left(\Delta L_i - \Delta\hat{L}_i \right)^2}{2\sigma^2} \right] \right\} \qquad \textbf{Equation 3.4}$$

where ΔL_i and $\Delta\hat{L}_i$ are the observed and predicted growth increments, respectively, for individual i, and σ is the standard deviation of the normal random errors for L_i (Helidoniotis et al., 2011).

A comparison of the fit of each growth model to the data is provided in Figure 3.1. For further analyses, the Gaussian growth model was selected as it provided the best statistical fit to the data (Figure 3.1), and had lower AIC_{min} (Akaike's Information Criterion) value, defined as $AIC_{min} = 2LL+2K$, where K is the total number of growth parameters in the respective growth model (including the variance) and $2LL$ is twice the log-likelihood at its optimum (Burnham and Anderson, 2002). AIC for each curve was as follows: von Bertalanffy (9387), Gompertz (9061), Gaussian (7756). To account for uncertainty when using the Gaussian growth curve, resampling, with replacement was used to produce 5000 data sets from the original tagging data to which the Gaussian growth curve was fitted. The

point estimate and upper and lower 95% confidence limits for each growth parameter was taken as the median, 2.5 and 97.5 percentile values, respectively, for the 5000 growth parameters resulting from the resampling analysis.

A growth curve describing the relationship between length *vs* age of abalone (for use in catch curve analysis, see below) was constructed by initially specifying a value of zero length at age zero and then using the Gaussian growth model fitted to the tag increment data to estimate the mean length of individuals at age one and then, in a recursive manner, also at ages 2, 3…. *n* (Rogers-Bennet et al., 2007). Note that this method provides estimates of length at each integer age. An estimate of length at any decimal age, or age at any specified length (as required for catch curve analysis) can, however be determined by fitting a spline curve to the constructed growth curve and using interpolation methods to estimate the value from that fitted spline curve.

6.3.6 Model validation: mortality

For catch curve analysis, the frequencies of abalone in successive 2 mm length classes in catches taken by commercial fishers were calculated. The catch curve equation was of the form

$$Ln\left[N_i\left(\frac{dl_i}{dt} \right) \right] = -Zt + b \qquad \textbf{Equation 3.5}$$

where Z is total mortality, N_i is the number of abalone in length class i, dl_i / dt is the growth rate (cm year^{-1}) of length class i at age t, estimated from the Gaussian growth model. For each length class, the value of t corresponding to its mid-point was estimated using the cubic spline curve and interpolation methods. Uncertainty in Z was accounted for by resampling, with replacement, the lengths of abalone in commercial catch samples, fitted to the 5000 growth parameters previously derived, to produce 5000 estimates for Z. This analysis was applied for three years (*i.e.*, 2011, 2010 and 2009) in which length-frequency data were available from commercial catch samples.

6.3.7 Model validation: field surveys of spawning biomass

A post-fishing field survey of spawning biomass per unit habitat area (*SSb* m^{-2}) was undertaken in each year between 2004 and 2010 at some time during the months of November and December, when approximately 70-90% of the annual commercial harvest had been taken (see Hart et al. (2013b) for full details of survey methods). Estimates of *SSb* m^{-2} were initially calculated separately for the different years of sampling (n = 20 – 30 survey sites per year) but, as these did not differ significantly (df = 3,164; F = 0.45, p = 0.72), the data for all years were pooled to produce an overall estimate for the full study period. The average *SSb* m^{-2} was 0.98 kg m^{-2}.

The alternative estimate of total spawning stock biomass (SSb_f), in kg, was

$$SSb_f = \left(SSb\, m^{-2} \right) H_f \qquad \textbf{Equation 3.6}$$

where H_f is the habitat area (m^2) fished, calculated as

$$H_f = N_a / D_a \qquad \textbf{Equation 3.7}$$

In the above equation, N_a is the numbers of fully recruited harvest-sized abalone (≥ 160 mm shell length) in the population at the time of the survey (post-fishing) and D_a is an estimate for the mean density of harvest-sized abalone at the time of the survey. D_a was calculated as the back-transformed average of $\log_e(x+1)$ transformed values for abalone densities at each survey site. To account for uncertainty, 5000 values of D_a were calculated by randomly resampling, with replacement, the density values recorded at each site. The value of N_a was determined as

$$N_a = N_b.e^{-Z} \qquad \textbf{Equation 3.8}$$

where N_b is the number of animals in the population before fishing, and Z is the instantaneous rate of total mortality (year^{-1}), as estimated from the length-converted catch curve analysis (Equation 3.5).

N_b is an unknown quantity but can be solved numerically by minimising the sum of squared deviations between the numbers of animals in the observed catch (C_k) *vs* expected catch (C_e). The former quantity included catches taken by commercial fishers, as calculated from compulsory daily records, and by recreational fishers, *i.e.* ~4% of the commercial catch, as estimated from phone-diary surveys (see Hart et al., 2009). Using the Baranov catch equation, C_e is estimated as

$$C_e = \frac{F}{Z}\left[1 - e^{-Zt}\right] N_b \qquad \textbf{Equation 3.9}$$

where F is the instantaneous fishing mortality rate (year^{-1}), which is derived by subtracting an estimate for instantaneous rate of natural mortality (M, year^{-1}) from Z, and t is the period (year^{-1}) from the start of the commercial fishing season to the time of the survey. Uncertainty in M was considered by generating 5000 estimates for this parameter from an assumed normal distribution, with an estimated mean of 0.15 and a standard deviation of 0.04, as determined for animals > 11 cm (Figure 3.2a).

For each of the three recent years in which catch length-frequency data was available (2011, 2010, 2009), the analysis for estimating SSb_f was repeated 5000 times, in each case using a different estimate for D_a , SSb m^{-2}, Z and F, as determined from previous analyses. For each year, the point estimate and lower and upper 95% confidence limits for SSb_f were taken as the median value and 2.5 and 97.5 percentiles, respectively, of the 5000 estimates resulting from the analysis.

6.3.8 Enhancement scenarios

The likely outcomes of different enhancement scenarios were evaluated by defining enhancement targets (*i.e.* density at release) as a function of natural recruitment (Nr) at the current level of spawning biomass, SSb, where Nr is a size-dependent parameter. This allowed size-dependent mortality and growth patterns to be accounted for, and in the case of smaller lengths at stocking (L_S < 3 cm; Table 3.1), density-dependent effects were also modelled. For example, when comparing a release density of Nr, which is the natural recruitment at SSb, across two L_S (2 cm, 4 cm; see Figure 3.7b of results), it meant comparing

outcomes from a release of 1.4 million x 2 cm animals with those from 0.48 million x 4 cm animals (Figure 3.2b).

Nr was estimated within *EnhanceFish* by specifying the size-at-recruitment and iteratively testing alternative values of the steepness parameter (r_m; Table 3.1) and current average catch (C_c; Table 3.1) to see their effect on average fishery yield at the current value of *F* and length-based gear selectivity parameters (L_c; Table 3.1). This generates a stock-recruitment curve (Figure 3.2b), and an estimate of stock biomass *vs F* curve (Figure 3.5a), from which *Nr* can be estimated.

EnhanceFish was next used to assess the effect of enhancement on *SSb*, fishery yield, profitability (Resource Rent), Gross Value of Product (GVP), and Net Present Value (NPV). The two enhancement scenarios chosen for in-depth analysis were release densities of 50% *Nr* and 100% *Nr*, minimum size-at-harvest being 15 and 14 cm, respectively, and a size-at-release of 4 cm (Table 3.1). These were compared with *SSb*, fishery yield, profitability, GVP, and NPV from the no enhancement scenario.

Sensitivity analyses exploring the effect of varying mortality, size-at-release, costs of production and enhancement, value of harvest, and cost-of-fishing on the profitability of enhancement were investigated (see Table 3.1 for values tested for each variable).

6.3.9 Effect of enhancement on wild stock spawning biomass

The key paradigm in sustainable fisheries is maintenance of wild stock spawning biomass above a threshold level. Theoretical studies suggest that minimum levels of 35 – 40% of virgin biomass will be sufficient protection of the breeding stock for most fisheries (Zhoue et al., 2012; Clark 2002). The main challenge in a successful enhancement fishery is not only maintaining total spawning biomass, but minimising the replacement of the wild genotype with the hatchery genotype.

The *EnhanceFish* model estimates *SSb* in an enhanced fishery as a combination of naturally recruited biomass and the biomass derived from stocked animals. Naturally recruited biomass is further partitioned into two components, the wild genotype and the hatchery genotype, which are linked through a heritability parameter (h^2; Table 3.1), describing the rate at which the hatchery genotype evolves into the wild genotype (Lorenzen & Medley, 2006). An h^2 of 0.2 was assumed for this analysis (Table 3.1, Table 3.2) from selective breeding experiments (e.g. Kube et al., 2007, Robinson et al., 2012).

We analysed the extent of wild genotype replacement under our chosen enhancement scenarios (50% *Nr*, 100% *Nr*) for our test fishery. The spawning biomass for the minimum replacement trajectory was estimated by combining the two components of naturally recruited biomass (wild and hatchery). Conversely, the spawning biomass for the maximum replacement trajectory was estimated by considering the wild component only. The replacement scenarios for the two release densities were compared with a biological reference point (Zhou et al., 2012) of 40% virgin *SSb* (*VSSb*).

6.3.10 Model exploration (Australian fishery)

EnhanceFish was next used to investigate the biological and economic impacts of an Australia-wide stock enhancement program for *Haliotis laevigata* (Table 3.2). The main

differences from the Augusta test fishery were the values of the growth, maturity, economic, and yield parameters. The growth parameters (k, L_{∞}) were averaged from published data for all populations where L_{∞} was greater than 14.5 cm as this is the mean minimum harvest size across all fisheries. The model was conditioned to an average Australia-wide harvest of 700 t whole weight (260 t meat weight; Mayfield et al., 2012), and assumed a control cost ($ per kg) of administering the fishery as 7% of GVP. The true cost varies between states. Economic data was available for the Western Australian fishery (this manuscript), and the South Australian fishery (Econsearch, 2011). Estimates of F for Australian fisheries were sourced from the literature (Chick et al., 2009; Hart et al., 2013a; Mayfield et al., 2008 and references therein). Average F was 0.41 ± 0.11 SD (n = 29).

Table 3.1. *EnhanceFish* bioeconomic model parameters, baseline values, and ranges used in sensitivity analysis for the Augusta *Haliotis laevigata* fishery.

Parameter	Baseline value	Range	Description
Growth, morphometry, and reproduction			
L_{∞}	18.5 cm		Asymptotic length at biomass ® 0
k	0.28		von Bertalanffy growth rate
g	4.0 ´ 10^{-6} cm kg^{-1}		Density dependent growth / competition coefficient
a	2.0 ´ 10^{-5}		Length-meat weight coefficient
b	3.363		Length-meat weight exponent
L_m	9.7		Length at 50% maturity (cm)
p	-4		Steepness of maturity function
rp	1		Relative reproductive performance of stocked fish
Life History and evolution parameters			
L_0	0.1		Length at settlement (cm)
A_0	0.05		Age at settlement (years)
L_r	4	2 – 5.5	Length at recruitment (cm)
A_r	2	1 - 2.5	Age at recruitment (years)
h^2	0.2		heritability of life-history traits
Natural mortality and Stock Recruitment (Beverton & Holt)			
M_{1w}	2.66	2.0 – 3.0	Mortality of wild phenotype at L = 1 cm
M_{1s}	3	2.3 – 4.0	Mortality of stocked phenotype at L = 1 cm
a	29.13	various[$]	Maximum recruits per unit *SSb* (@ L_r = 4 cm)
b	512,000	various[$]	Maximum average recruitment (@ L_r = 4 cm)
r_m	4		steepness parameter (maximum annual reproductive rate) - Myers (1999)
Fishing parameters and economics			
C_c	18,000		Current average catch (kg meat weight)
F	0.69	0 – 1.6	Fishing mortality
L_c	15.4	14 – 16	Gear selection length (minimum harvest size; cm)
q	-4		Steepness of gear selectivity curve

g_1	40	30 - 50	Cost of fishing ($ kg^{-1}; meat weight); equivalent to $11 - $19 kg^{-1} whole weight
p	110	70 – 130	Ex-vessel price of fish ($ kg^{-1}; meat weight)
j	5.5		Management cost ($ kg^{-1}; meat weight)
d	6%	3 - 10	Discount rate. For NPV analysis
Stock enhancement parameters and costs			
D_S	0.24; 0.48	0.2 – 1.0	Density of stocking (millions) under the two enhancement scenarios (*50% Nr*; *100% Nr*). *Nr* is natural recruitment at current spawning biomass
L_S	4	2 – 5.5[$$]	Length-at-stocking (cm; shell length)
g_2	0.33	0.27 – 0.47 [$$$]	Hatchery production + enhancement costs ($ cm^{-1}) Enhancement costs are $0.07 cm^{-1}

[$] *EnhanceFish* uses the input values of C_c and r_m to estimate *a* and *b*, and estimates vary with the size-at-recruitment (L_r).

[$$] Density dependent effects of stocking were modelled for L_S between 2 and 3 cm, but not for lengths ³ 3cm. See Lorenzen (2005) for mathematical details.

[$$$] Enhancement costs include cost of release devices, packing, transport, and deployment (Strain, unpublished data)

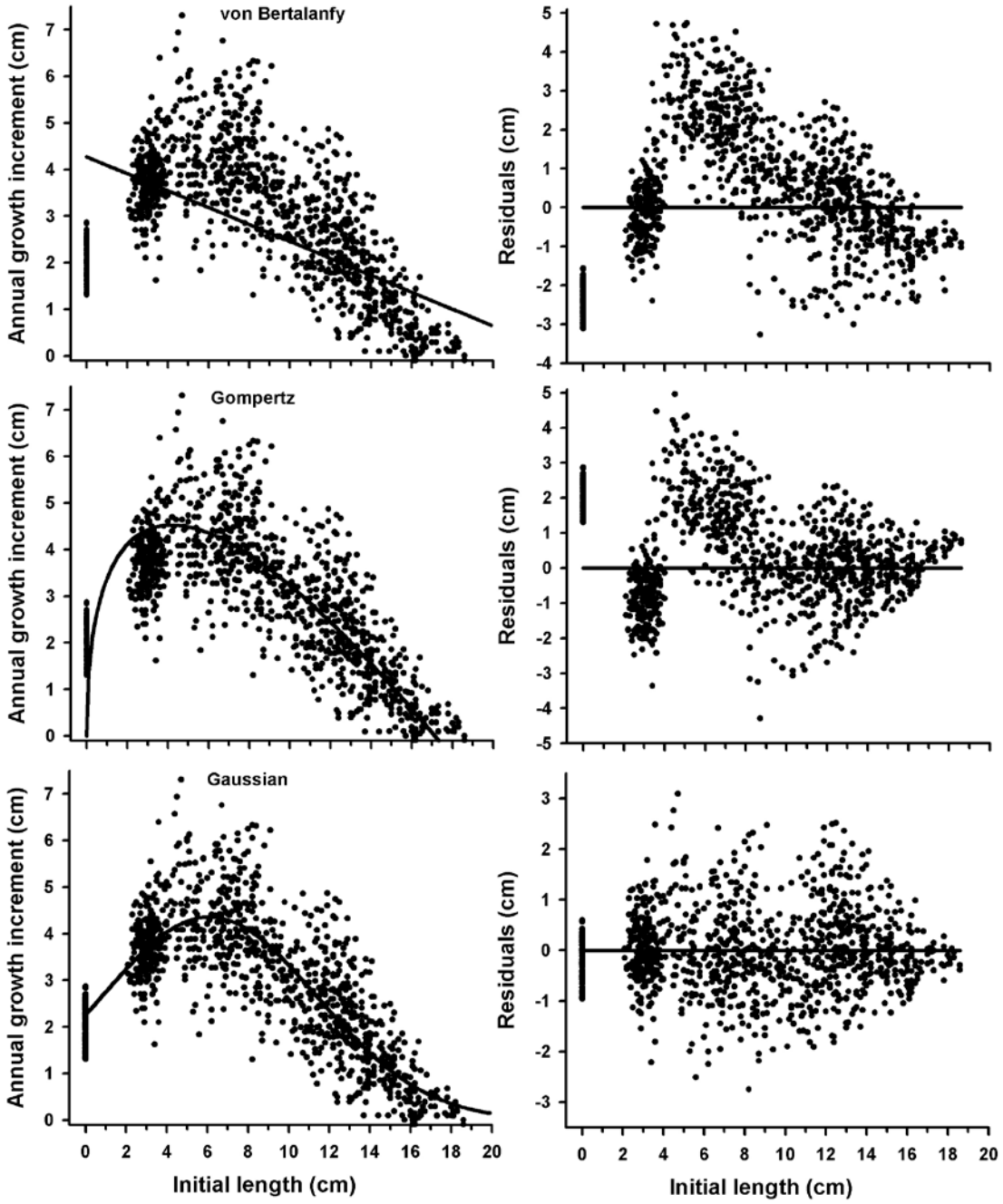

Figure 3.1. Growth model fits to annual tag-increment data, and residual analysis for three growth models (von Bertalanffy, Gompertz, Gaussian). Data are from *Haliotis laevigata* populations in Augusta, Western Australia. Growth increments for initial length of 0 cm (*n* = 105) obtained from hatchery-bred animals prior to their release into the wild at Age 1; all other increments (*n* = 998) from *in-situ* growth in the wild.

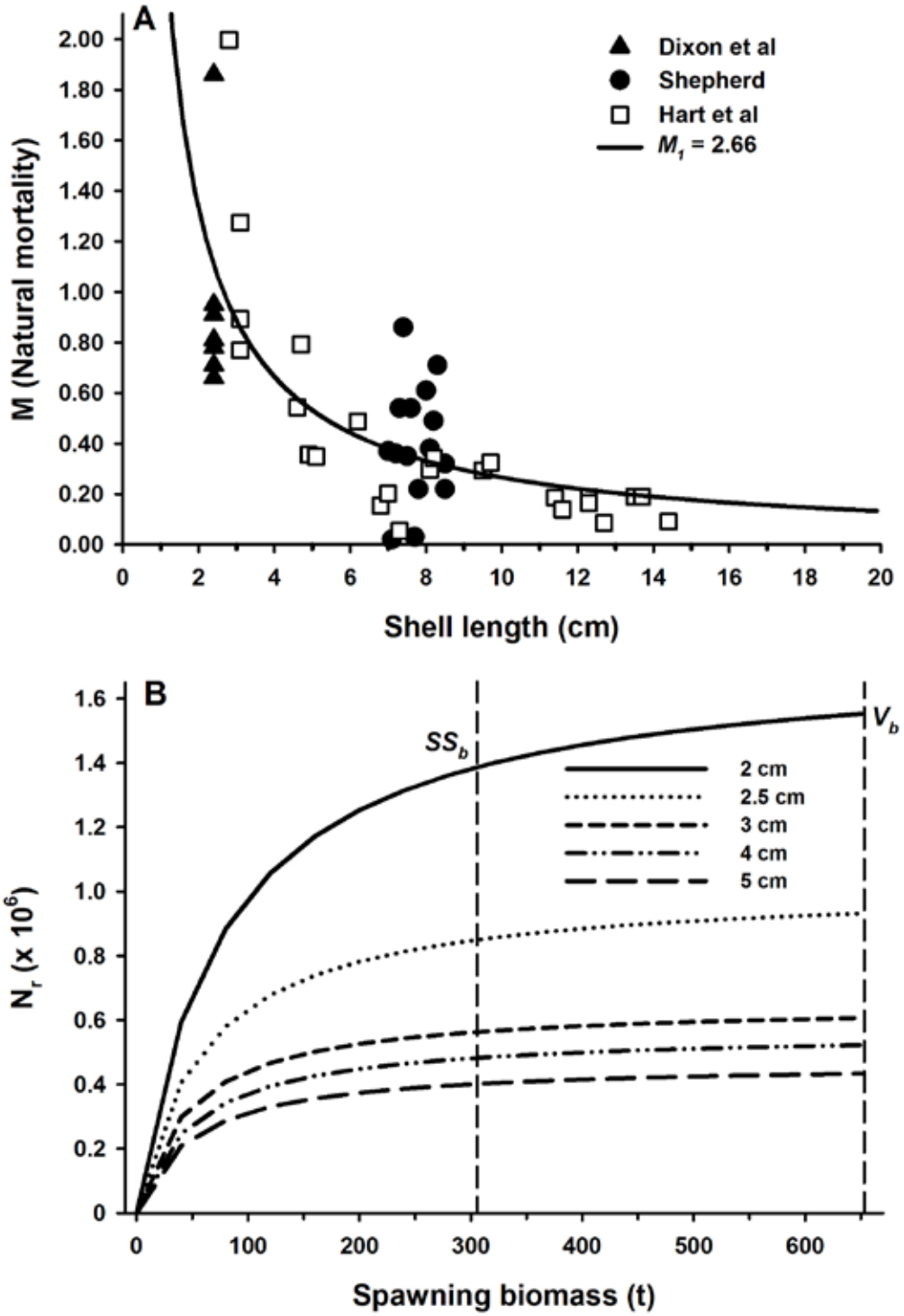

Figure 3.2. Population model development for the Augusta *Haliotis laevigata* fishery. (a) Natural mortality (M) as a function of shell length, with the least-squares solution for the mortality model (M_1 = 2.66). Mortality data derived the literature (Dixon et al., 2006; Hart et al., 2013a, Shepherd, 1990). (b) Recruitment (*Nr*) as a function of spawning stock biomass (*SSb*; t whole weight) for varying length-at-recruitment (cm). Curve is of the form *Nr* = aSSb* / 1+ b**SSb*, with estimates of a* and b* obtained in *EnhanceFish* (see Table 3.1 for details). *Vb* is virgin biomass, and density-dependent effects on recruitment were incorporated for lengths < 3 cm.**

Table 3.2. *EnhanceFish* model parameters and values used in bioeconomic analysis of stock enhancement for the Australian *Haliotis laevigata* fishery.

Parameter	Value	Description
Growth, morphometry, and reproduction		
$L\infty$	17.5 cm	Asymptotic length at B ® 0
k	0.35	von Bertalanffy growth rate
g	3.0×10^{-7} cm kg^{-1}	Density dependent growth / competition coefficient
a	2.0×10^{-5}	Length-meat weight coefficient
b	3.363	Length-meat weight exponent
L_m	8	Length at 50% maturity (cm)
p	-4	Steepness of maturity function
r	1	Relative reproductive performance of stocked fish
Life History and evolution parameters		
L_0	0.1	Length at settlement (cm)
A_0	0.05	Age at settlement (years)
L_r	4	Length at recruitment (cm)
A_r	2	Age at recruitment (years)
h^2	0.2	heritability of life-history traits
Natural mortality and Stock Recruitment (Beverton & Holt)		
M_{1w}	2.7	Mortality of wild phenotype at length = 1 cm
M_{1s}	3	Mortality of stocked phenotype at length = 1 cm
a^*	24.691	Maximum recruits per unit *SSb* (@ L_r = 4 cm)
b^*	7,478,000	Maximum average recruitment (@ L_r = 4 cm)
r_m	4	Myers (1999) steepness parameter (maximum annual reproductive rate)
Fishing parameters and economics		
C_c	260,000	Current average catch (kg meat weight; 700 t whole)
F	0.41	Fishing mortality (effort) under assumed growth curve parameters
L_c	14.5	Gear selection length (minimum harvest size; cm)
q	-4	Steepness of gear selectivity curve
g_1	40	Fishing costs (\$ kg^{-1}; meat weight); equivalent to \$15 kg^{-1} whole weight
p	110	Ex-vessel price of fish (\$ kg^{-1}; meat weight)
j	7	Administrative cost (\$ kg^{-1}; meat weight)
d	6%	Discount rate. For NPV analysis
Stock enhancement parameters and costs		
D_S	3.06; 6.12	Density of stocking (millions) under the two enhancement scenarios (*50% Nr*; *100% Nr*)
L_S	4	Length-at-stocking (cm; shell length)
g_2	0.33	Hatchery production + enhancement costs (\$ cm^{-1}) Enhancement costs are \$0.07 cm^{-1}

6.4 Results

6.4.1 Preliminary explorations using the EnhanceFish model

The *EnhanceFish* model estimated virgin spawning biomass (*Vb*), current spawning biomass (*SSb*), and natural recruitment (*Nr*) at *SSb* for the Augusta fishery as 653 tonnes, 300 tonnes, and 0.48 million recruits (at 4 cm), respectively (Figure 3.2b). Estimates of *Nr* were produced for several sizes at recruitment. For example, an *Nr* of 1.4 million x 2 cm animals was equivalent to an *Nr* of 0.48 million x 4 cm for the estimated *SSb* of 300 tonnes (Figure 3.2b). The estimate for the natural mortality parameter M_1 was 2.66 year^{-1} (Figure 3.2a) and this value was used for the wild-stock component in the model (Table 3.1).

6.4.2 Estimates of SSb, using EnhanceFish and an alternative approach

The four main outputs of the alternative approach included the number of harvest-size animals prior to the commencement of the fishing season (N_b; Figure 3.3a), fishing mortality (F; Figure 3.3b), habitat area fished (H_f; Figure 3.3c) and SSb_f (Figure 3.3d). Median estimates for N_b were similar between 2010 and 2011 (220 - 230,000), but about 25% lower (170,000) in 2009 (Figure 3.3a). Estimates for F for the three fishing years, derived using length composition data from commercial catches varied between 0.64 and 0.67 year^{-1} (Figure 3.3b). These were very similar to the estimate of 0.69 year^{-1} used in the *EnhanceFish* model (Table 3.1). Median estimates of H_f ranged between 0.33 and 0.45 km^2, with a smaller area fished in 2009 (Figure 3.3c). This resulted in a lower spawning biomass estimate for that year, compared to 2010 and 2011 (Figure 3.3d).

The median value of the estimates of SSb_f for 2011 (410 tonnes, 95% confidence limits = 304-560 t) was slightly less than for 2010 (454 tonnes, 95% confidence limits = 363-593 t) but substantially greater than for 2009 (316 tonnes, 95% confidence limits = 217 - 453 t). The median value of SSb_f for all years, combined, was 394 t, which was 30% greater than the *EnhanceFish* estimate of 300 t (Figure 3.3d).

6.4.3 Bioeconomic reference points – Hypothetical abalone fishery

To illustrate key points of the bioeconomic evaluation, yield, revenue, and cost curves for a hypothetical *Haliotis laevigata* fishery are shown in Figure 3.4. If this hypothetical stock was harvested at a minimum size of 15.4 cm and F was 0.69 year^{-1}, this would result in an average fishery yield of 47 tonnes (Figure 3.4a). For the same level of fishing effort, an enhancement program that involves the release of 0.4 million x 4 cm animals could yield 72 tonnes (66% increase) (Figure 3.4a).

Analysis of revenue and cost curves enables a more robust analysis (Figure 3.4b). The line on Figure 3.4b marked AB highlights the level of F corresponding to optimal profitability for the fishery, i.e. the maximum economic yield (MEY). The level of F corresponding to optimal profitability for a non-enhanced fishery (0.33 year^{-1}) is 48% of the current estimate for the Augusta fishery of 0.69 year^{-1}. This equates to around a $200K loss in profitability per year. If the fishery were enhanced, however, optimal profitability, highlighted by the line marked CD (Figure 3.4b), would occur when F was reduced by only 20% (to 0.55 year^{-1}).

More broadly, however, the analyses indicate that, at the current sizes over which this species is fished, the two types of fishery (non-enhanced and enhanced) are profitable over a wide range of values for *F* (Figure 3.4b). For the enhanced fishery, the values of *F* over which the fishery is predicted to be profitable were 0.2 to 2.6 year^{-1}. The open access equilibrium points (where cost = revenue) were reached at an F of 2.0 for the base case fishery and 2.7 for the enhanced fishery, which are considerably greater than current F (Figure 3.4b).

6.4.4 Augusta fishery – Base case evaluation

Assuming an average minimum size of 15.4 cm for which Greenlip is fished and a value for *F* of 0.69 year^{-1}, *EnhanceFish* estimated that the current spawning biomass of the Augusta stock is 300 t (Figure 3.5a), which equates to 46% of the estimated virgin biomass (653 t). Estimated profitability for this scenario was $1.1 million (Figure 3.5b). Estimated GVP and NPV were $1.9 million (Figure 3.5c) and $17 million (Figure 3.5d), respectively.

The optimum economic scenario was a 40% reduction in *F* from 0.69 to 0.40 year^{-1}, combined with a reduction in minimum size fished from 15.4 to 14 cm. This resulted in a 15% reduction in spawning biomass (300 to 260 t; Figure 3.5a), a 30% increase in profitability from $1.15 to $1.5 million (Figure 3.5b), a 5% increase in GVP from $1.9 to $2.0 million, and a 20% increase in NPV of total profit over the long term from $17 to $21 million (Figure 3.5d). Based on the currently assumed stock-recruitment relationship for the Augusta *Haliotis laevigata* fishery, the 10% reduction in spawning biomass to 260 t would have negligible effect on average recruitment (Figure 3.2a).

6.4.5 Augusta fishery – Enhancement scenarios

For the two enhancement scenarios investigated, i.e. 50% *Nr* and 100% *Nr* (Figure 3.6), the optimal *F* from an economic perspective of 0.55 year^{-1} is 20% less than the current estimated value for this parameter. Fishing at a minimum harvest size of 14 cm produced a higher biological and economic yield (Figure 3.6d, f, h) compared to a 15 cm minimum harvest size (Figure 3.6c, e, g). The optimum economic scenario was an annual release of 100% *Nr* (0.48 million x 4 cm animals) combined with a decrease in *F* from 0.69 to 0.55 year^{-1} and a decrease in minimum size fished to 14 cm (from 15.4 cm). This resulted in a 40% increase in spawning biomass (300 to 430 t; Figure 3.6b), an 85% increase in profitability from $1.15 to $2.1 million (Figure 3.6d), a 75% increase in GVP from $2.0 to $3.5 million (Figure 3.6f), and a 94% increase in NPV from $17 to $32 million (Figure 3.6h).

6.4.6 Augusta fishery – Sensitivity analysis

Mortality had the largest net effect on profitability (Figure 3.7a). For animals released at 4 cm, the break-even survival was around 8%, which corresponded to an M_1 value of 4.0 year^{-1} (Figure 3.7a). Although greater profitability was achieved with twice the number of releases (100% *Nr vs* 50% *Nr*), such doubling of releases did not achieve a doubling of profits (Figure 3.7a).

With respect to size-at-release, the two enhancement scenarios (100% *Nr vs* 50% *Nr*) were profitable for sizes at release between 2.5 and 5.5 cm (Figure 3.7b). Profit increased with size between 2 and 3 cm, and was constant between 3 and 4 cm (Figure 3.7b). It was $1.8 million (~60% above base case scenario) for smaller release density (50% *Nr*), and $2.2 million for

the larger release density (Figure 3.7b). For size-at-release between 4 and 5.5 cm, profitability declined with increasing size, but at a faster rate for the larger density at release.

Both the costs of enhancement (Figure 3.7c) and of fishing (Figure 3.7e) had a minimal effect on profitability, for the range of costs currently experienced in Australian hatcheries ($0.27 – $0.47 cm^{-1}) and in the harvesting industry ($11 - $19 kg^{-1} whole weight). For example, in the case of a release density of 50% *Nr*, profitability of an enhancement program for abalone costing $0.25 cm^{-1} was only 11% higher ($1.92 million compared to $1.73 million) than animals costing $0.45 cm^{-1} (Figure 3.7c). Similarly, for the higher release density (100% *Nr*), profitability on a cost of $0.25 cm^{-1} was $2.5 million (16% higher) compared to $2.15 million for a cost of $0.45 cm^{-1} (Figure 3.7c).

Value of harvest had a significant effect on profitability of stock enhancement (Figure 3.7d). For the range of harvest values experienced in the Australian Greenlip Abalone fisheries over the last decade ($26 - $48 kg^{-1}), there was 3-fold effect on profitability of both the current fishery ($0.5 to $1.5 million) and an enhancement fishery (Figure 3.7b). Both the current fishery and the enhancement fishery were profitable over the range of likely harvest values, with enhancement adding significantly to profitability in each scenario.

The extreme range of economic costs was also briefly investigated. Under the lowest harvest price ($26 kg^{-1}), highest fishing cost ($19 kg^{-1}), and average enhancement costs ($0.33 cm^{-1}), a scenario arose where the Augusta fishery without enhancement could not achieve a break-even point at a low *F* (0.3 $year^{-1}$), but became slightly profitable with a stock enhancement program of either 50% *Nr* or 100% *Nr*.

6.4.7 Augusta fishery – Effect of enhancement on wild stock spawning biomass

For all four scenarios (with different values for *Nr* and harvest size) at the optimal economic *F* for enhancement (0.55 $year^{-1}$), the maximum replacement trajectory resulted in wild stock biomass (*SSb*) falling below the biological reference point (40% *VSSb*) (Figure 3.8). Under the minimum replacement trajectory *SSb* was maintained above 40% virgin for both 50% *Nr* and 100% *Nr* if harvesting was carried out at a larger minimum size of 15 cm (Figure 3.8a, c). When the minimum size of harvest was 14 cm, *SSb* fell slightly below the reference point for both release densities (Figure 3.8b, d).

6.4.8 Australian Greenlip Abalone Fishery – Base case evaluation

Virgin spawning biomass was estimated at 9300 t (Figure 3.9a). Spawning biomass throughout Australia, assuming $F = 0.41$ $year^{-1}$ and an average minimum size fished of 14.5 cm, was 4200 t (Figure 3.9a), around 45% of the estimated virgin biomass. Estimated profitability for this scenario was $16 million (Figure 3.9b). Estimated annual GVP and NPV were $28 million (Figure 3.9c) and $250 million (Figure 3.9d), respectively.

The MEY scenario for the Australian *H. laevigata* fishery, namely that which achieved the highest profitability, required a reduction in minimum size fished from 14.5 to 13 cm, and a 20% reduction in *F* from 0.41 to 0.33 $year^{-1}$ (Figure 3.9b). This resulted in a 10% reduction in spawning biomass (4200 to 3800 t; Figure 3.9a), a 25% increase in profitability from $16 to

$20 million (Figure 3.9b), no change in GVP from $28 million, and a 15% increase in NPV from $250 to $290 million (Figure 3.9d).

6.4.9 Australian Greenlip Abalone Fishery – Enhancement scenarios

Optimal *F* from an economic perspective was 0.45 year^{-1}, a 10% increase over current *F* (Figure 3.10d). However the optimum was a shallow peak so a wide range in *F* resulted in near optimum profitability (e.g. 0.3 to 0.6 in Figure 3.10d). Fishing at a minimum size of 13 cm (Figure 3.10b, d, f, h) produced a higher biological and economic yield compared to a minimum harvest size of 14 cm (Figure 3.10a, c, e, g). The optimum economic scenario was an annual release of 100% *Nr* (6.5 million x 4 cm) combined with an increase in *F* from 0.41 to 0.45 year^{-1} and a minimum size fished of 13 cm. This resulted in a 40% increase in spawning biomass (4200 to 5900 t; Figure 3.10b), a 100% increase in profitability from $16 to $33 million (Figure 3.10d), a 95% increase in GVP from $28 to $55 million (Figure 3.10f), and a 110% increase in NPV from $250 to $530 million (Figure 3.10h).

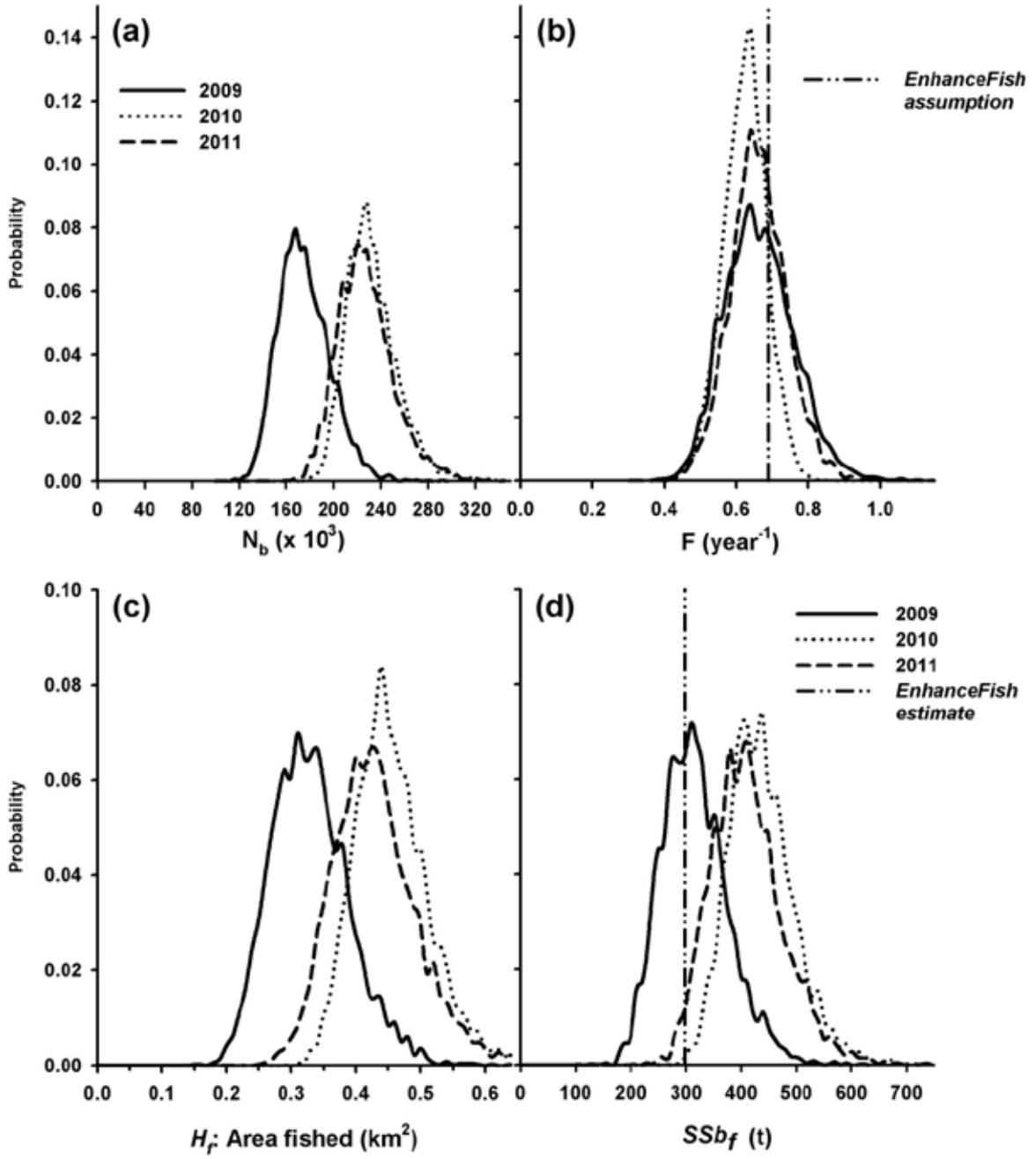

Figure 3.3. Probability distribution of estimates of (a) number of harvest-size animals pre-fishing (N_b); (b) fishing mortality (F); (c) area fished; and (d) spawning biomass (SSb_f) in the Augusta *Haliotis laevigata* fishery from our alternative model, compared with *EnhanceFish* inputs and estimates.

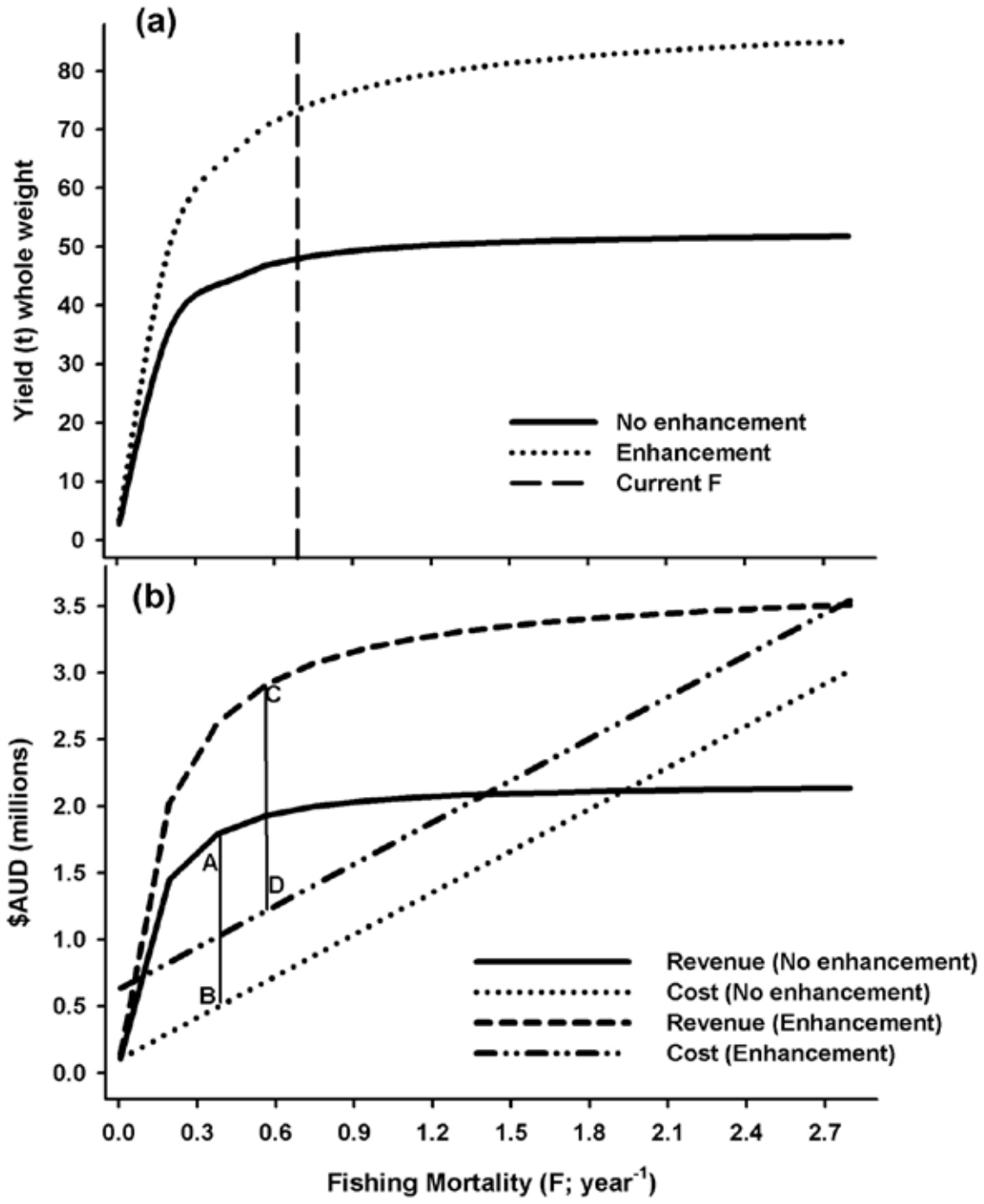

Figure 3.4. (a) Yield and (b) bioeconomic reference points for a hypothetical *Haliotis laevigata* fishery before and after stock enhancement. In (a) the fishery is fished at a minimum harvest length of 15.4 cm and an F of 0.69. In (b), AB represents the optimum resource rent (profit) for no enhancement, and CD is the optimum resource rent for enhancement (of 400,000 x 4 cm juveniles).

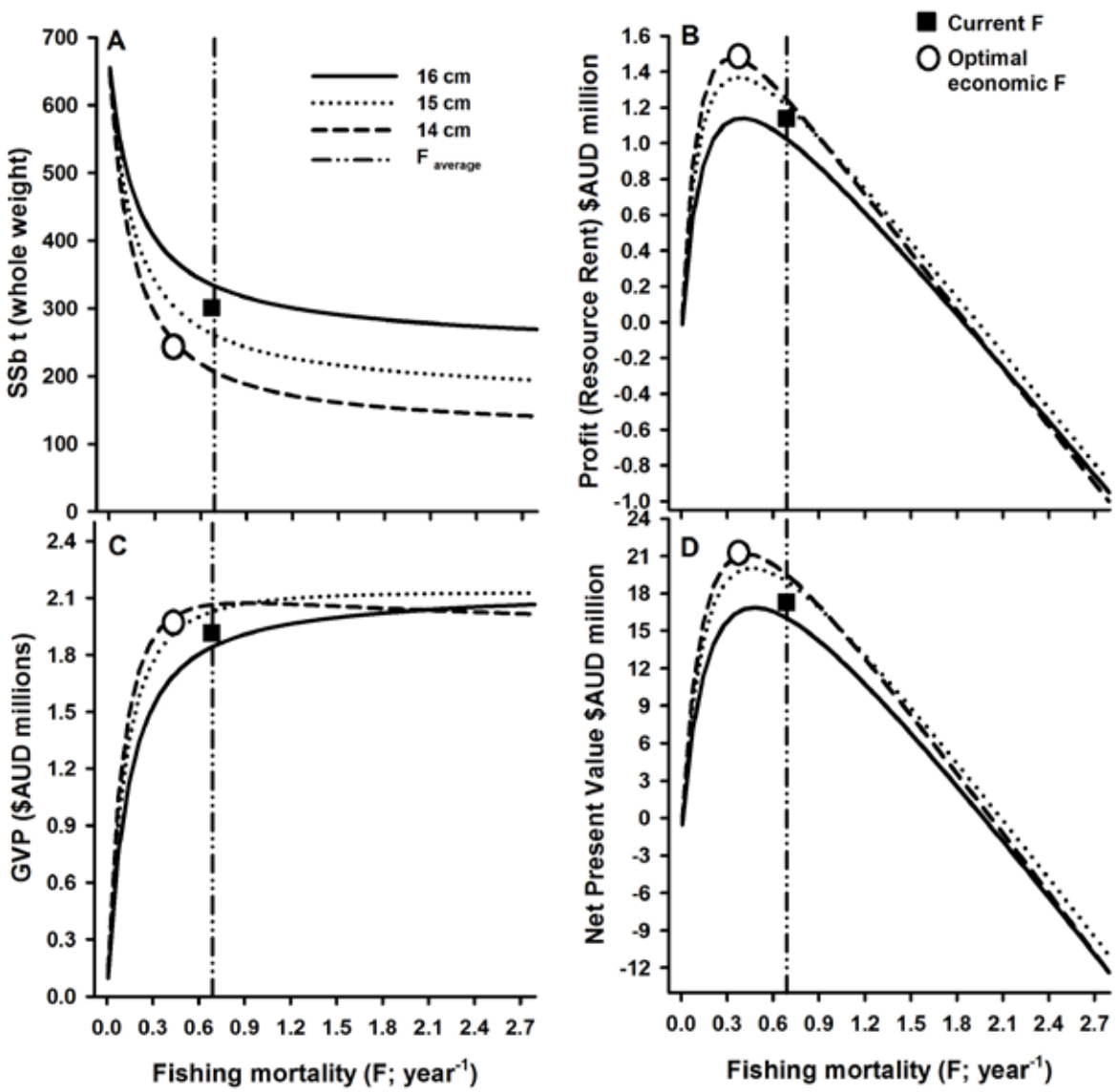

Figure 3.5. Augusta *Haliotis laevigata* fishery (base case scenario). Effects of F and minimum size at harvest (16, 15, 14 cm) on (a) spawning biomass (t; whole weight), (b) profit, (c) GVP, and (d) NPV.

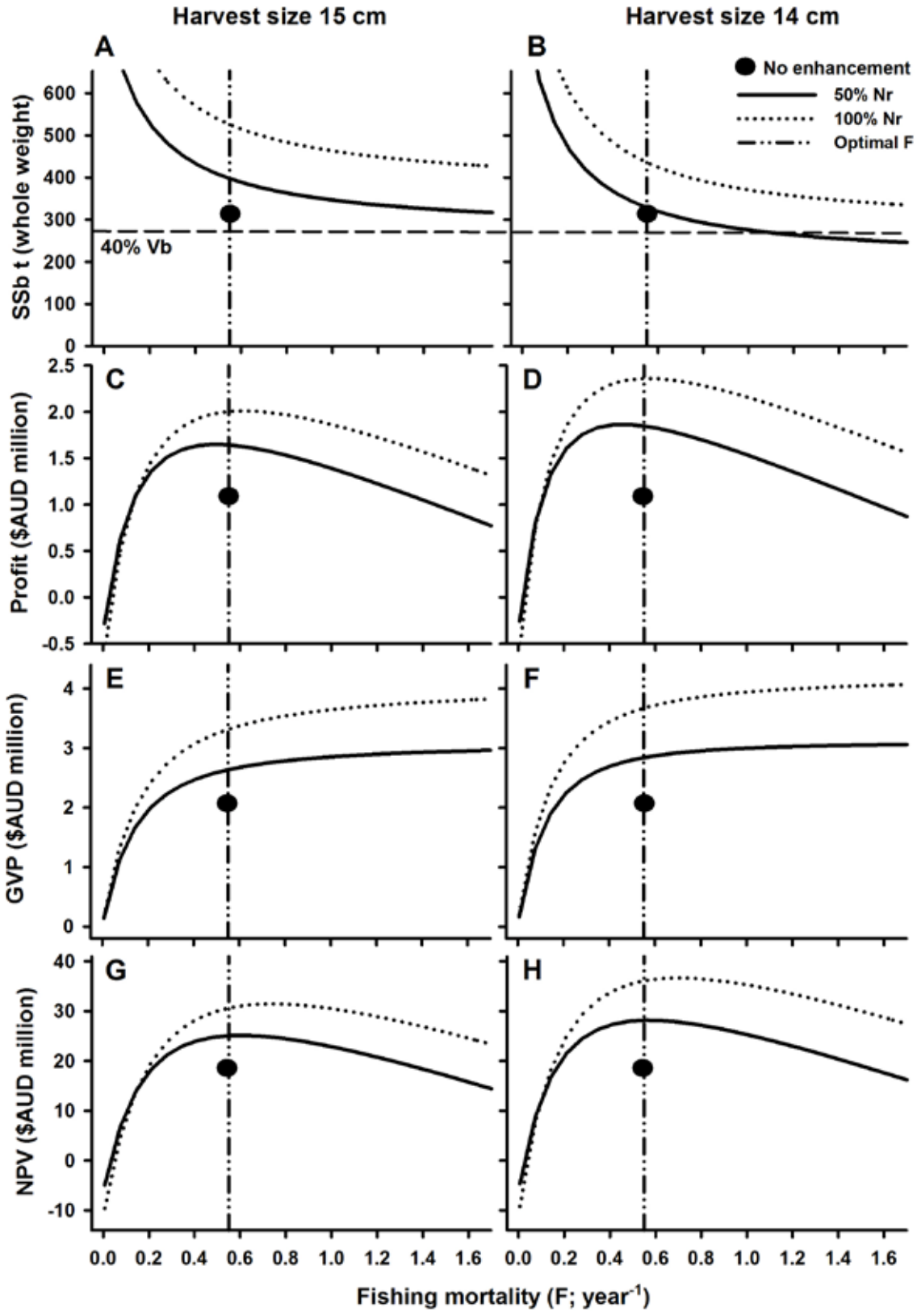

Figure 3.6. Augusta *Haliotis laevigata* fishery (enhancement scenario). Effects of F and density of release (50% *Nr*, 100% *Nr*) on total spawning biomass (a, b), profit (c, d), GVP (e, f) and NPV (g, h). Optimal F is 0.55. 40% of Virgin spawning stock biomass (40% *VSSb*) is shown in a, b. Outputs are for two minimum harvest lengths [15 cm (a, c, e, g), and 14 cm (b, d, f, h)]. Animals released at 4 cm length.

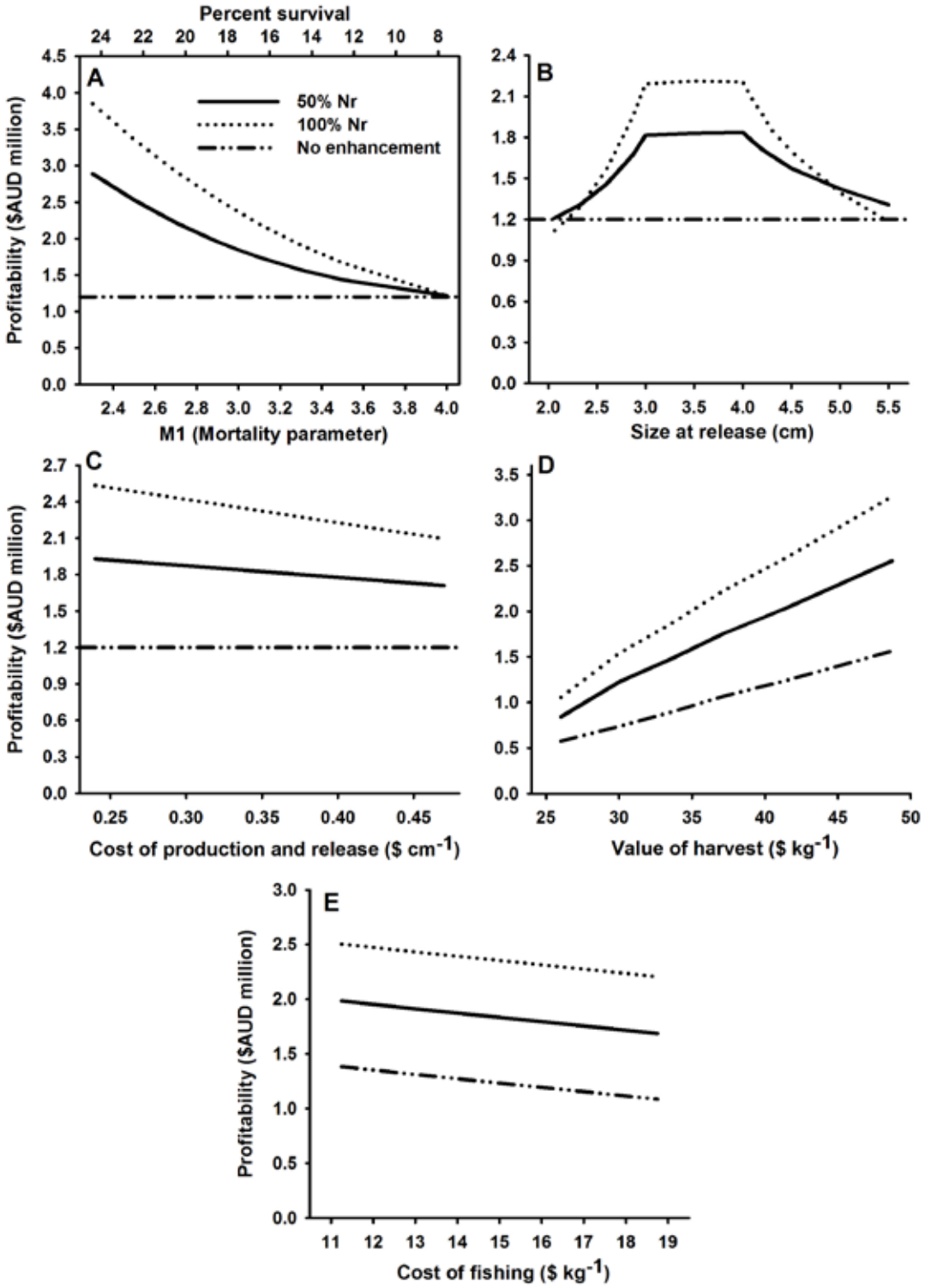

Figure 3.7. Sensitivity analysis of the effect of changes in (a) M1 (mortality parameter), (b) size-at-release, (c) cost of enhancement ($ cm^{-1}), (d) value of harvest ($ kg^{-1}), and (e) cost of fishing ($ kg^{-1}), on the profitability of enhancement in the Augusta *Haliotis laevigata* fishery. F was set at 0.55 for a 14 cm harvest size. Size-at-release is assumed to be 4 cm, except for (b) where the enhancement targets of 50% *Nr* and 100% *Nr* are different numbers for different size-at-release (see Figure 3.1).

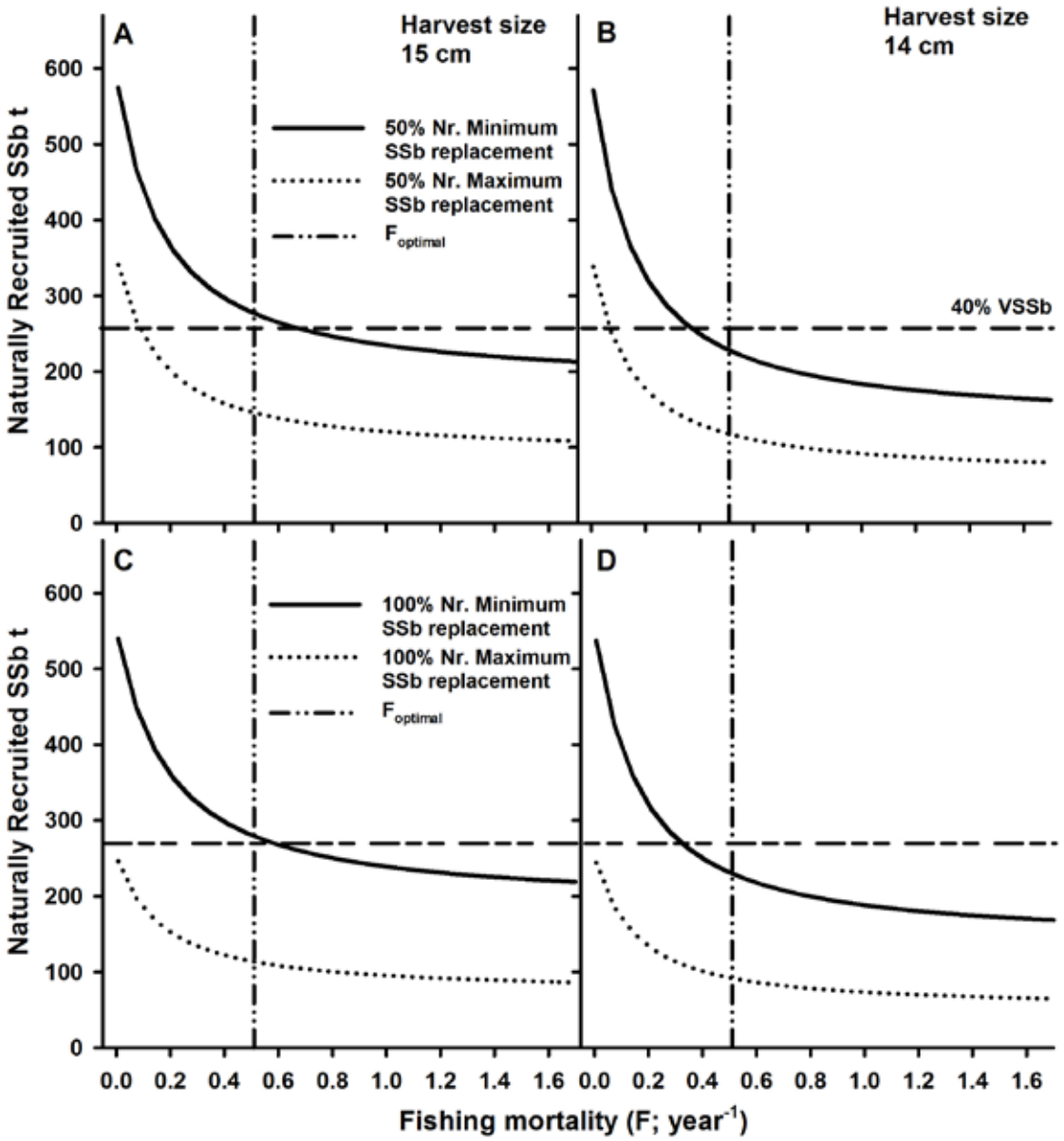

Figure 3.8. Effects of F, density of release, and minimum size of harvest (a,c = 15 cm; b,d = 14 cm) on naturally recruited spawning biomass in the Augusta *Haliotis laevigata* fishery. Minimum equates to the minimum replacement of wild stock with hatchery genotypes and is the best-case scenario. Maximum equates to the maximum replacement of wild stock with hatchery genotypes and is the worst-case scenario. 40% *VSSb* is the biological reference point of 40% virgin biomass. F optimal (0.51) is from an economic perspective.

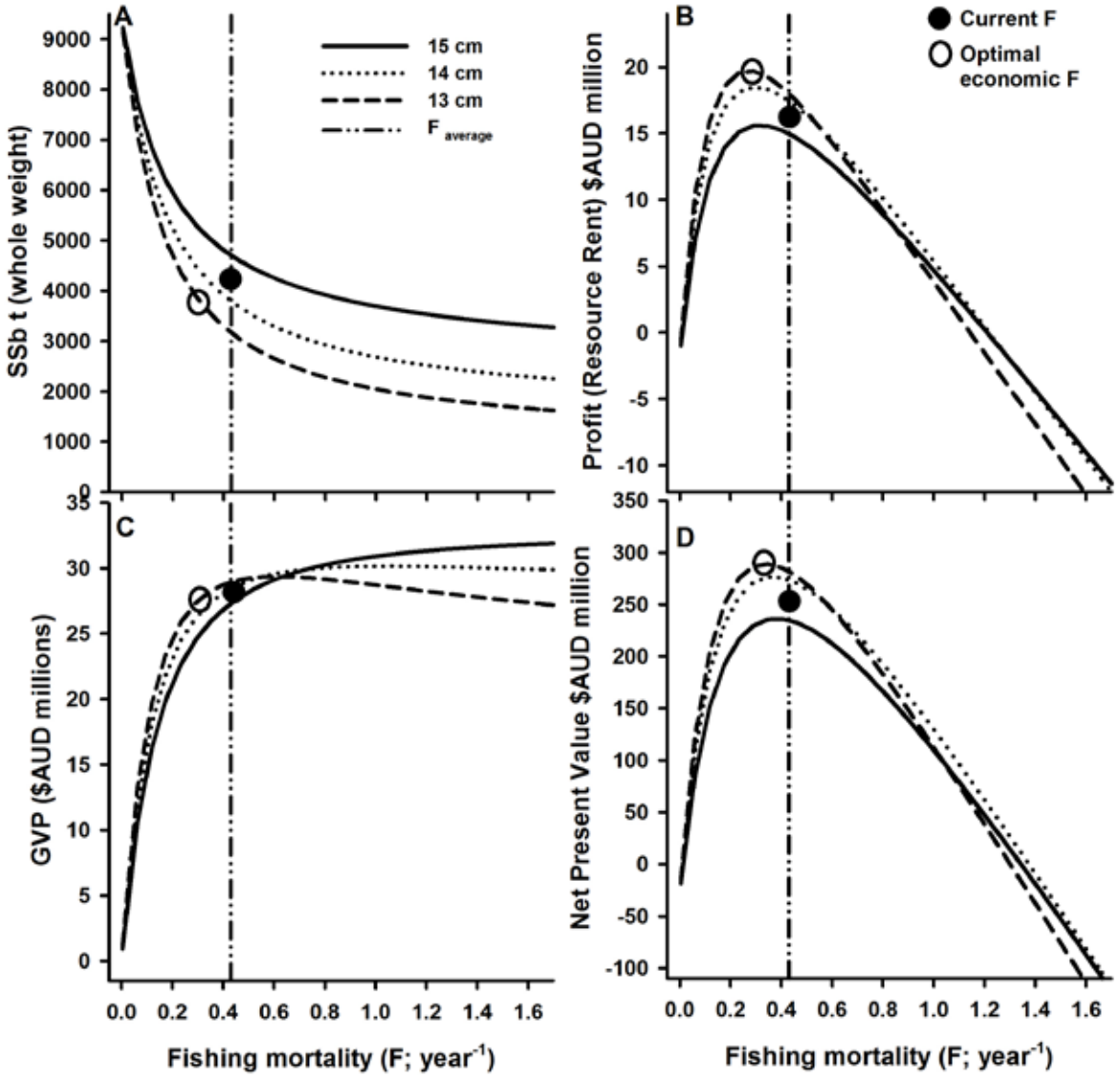

Figure 3.9. Australian *Haliotis laevigata* fishery (base case scenario). Effects of F and minimum size at harvest (15, 14, 13 cm) on (a) spawning biomass (t; whole weight), (b) profit, (c) GVP, and (d) NPV.

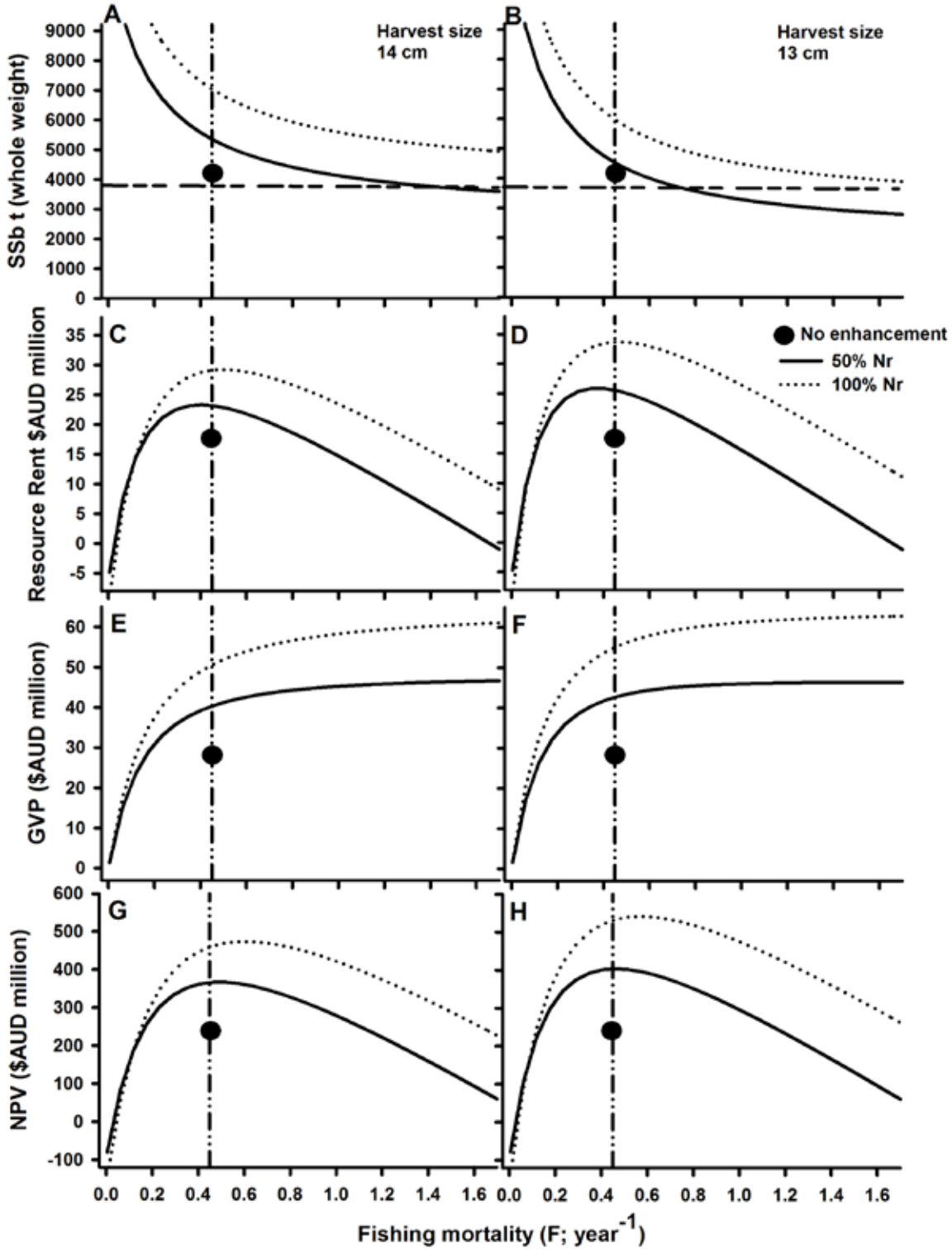

Figure 3.10. Australian *Haliotis laevigata* fishery (enhancement scenario). Effects of F and density of release (50% *Nr*, 100% *Nr*) on total spawning biomass (a, b), profitability (c, d), GVP (e, f) and NPV (g, h). Optimal F is 0.45. 40% of Virgin spawning stock biomass (40% *VSSb*) is shown in a, b. Outputs are for two minimum harvest lengths [14 cm (a, c, e, g), and 13 cm (b, d, f, h)]. Animals released at 4 cm length.

6.5 Discussion

Stock enhancement in *Haliotis laevigata* fisheries was found to be economically viable under a wide range of scenarios of release numbers and sizes at release. Even at the less profitable end of the range in economic parameters (high fishing costs, low harvest value), stock enhancement in a fishery operating at a loss was predicted to move the business over the break-even point, resulting in a small profit. Also, scenarios are likely to exist that would be more economically optimal than either of those examined in this study, although some of these may be less biologically plausible that the ones considered. For example, a theoretical annual release of 150% *Nr* combined with a minimum size fished of 11 cm is predicted to result in a 175% increase in profitability, but such a result is not biologically plausible due to the large replacement of natural spawning biomass that occurred. Use of more conservative scenarios in terms of release size were thus considered to allow realistic explorations of the potential for enhancement, noting that radical scenarios may be considered when functioning enhancement programs have been developed, according to the responsible approach (Blankenship and Leber, 1995; Lorenzen et al., 2010).

Although this study provides the first positive assessment for Australian abalone fisheries, Roberts et al. (2007) reached a similar conclusion of economic viability for the New Zealand abalone *Haliotis iris*, after about 20 years of research. Earlier assessments on Australian species concluded there was limited potential, citing carrying capacity issues, slow growth rates and high juvenile mortality as major limiting factors (Shepherd et al, 2000; Prince, 2004). An assessment of the economics of enhancement for *Haliotis rubra* (Chick, 2010) concluded that survival had to be greater than 4%, and harvest price greater than \$34 kg^{-1} to achieve positive financial return under the most realistic scenarios, however that assessment considered only small sizes of release (0.7 cm, 1.5 cm) and a discount rate of 10%, compared to 6% in this study. The choice of discount rate in NPV analysis can have a big impact on the outcome of economic assessment, however when discounting flows of benefits from natural or environmental resources, a recent perspective is that smaller discount rates will more accurately reflect the needs of both current and future generations (Sumaila and Walters, 2005). In a review of abalone stock enhancement in the Japan, Hamasaki and Kitada (2008) found the programs were profitable in terms of recovering the cost-of-release; their analysis was only based on the ratio of the value of landings (from released abalone) to the release cost and thus did not include fishing or management costs. Similarly, the economic model used in the *H. iris* study (Roberts et al., 2007) treated stock enhancement as a stand-alone economic entity and did not account for other factors such as the impact of alternative fisheries management policies. Our contention is that stock enhancement must be integrated into the fisheries management strategy if it is to be commercially viable. This is because its economic performance depends directly on management policies such as harvesting rights, quota allocation, minimum-size-limits and fishing effort or mortality indicators. There are also compelling bio-security and operational reasons for this integration as will be discussed further below.

This study has made a number of significant contributions to literature on abalone stock enhancement. Firstly, the estimation of densities of release were based on the natural

recruitment rate (*Nr*), which was ascertained by first developing a population dynamics model for the fishery of interest. Although *Nr* will vary from year to year, the estimate of spawning biomass produced by the *EnhanceFish* model (*SSb*), which is dependent on the estimate of 'average' *Nr*, was in general agreement with spawning biomass obtained from field surveys (SSb_f: Figure 3.3d). Thus, the use of *Nr* to guide release strategies for Greenlip Abalone stock enhancement programs appears to be a viable approach. The advantage of this strategy is that it acknowledges the natural limits to recruitment of abalone, and places stock enhancement immediately within a fisheries management context where future harvest options are broadened significantly. For example, the need for very stringent size-limits to protecting the breeding capacity of abalone stocks in Australia could be lessened; allowing improvements in yields, or size-dependent harvest quotas could be adjusted in response to market demands. At a practical level, the estimation of annual enhancement targets (e.g. 6.1 million for an Australia-wide program releasing 4 cm juveniles) puts an immediate cap on the scale of hatchery production and operational logistics of deployment, which provides more certainty for commercial development. The estimated release densities for *H. laevigata* could be supplied by a small number of purpose-built hatcheries in each of the Australian producing states.

The dynamic interplay between density of release, minimum harvest size, and fishing mortality, and their combined effect on spawning biomass and economic indicators (Figure 3.6) highlight the need for an integrated enhancement and harvest strategy. For example, substantially improved profitability can be gained simply by fishing abalone at smaller sizes (Figure 3.6d). This occurs because a greater proportion of the biomass created by the enhanced cohorts is translated into fishing yield as opposed to spawning biomass (Figure 3.6a *vs* b). However, fishing at a lower size is likely to result in greater replacement of wild spawning biomass with hatchery genotypes (Figure 3.8a *vs* b) thereby increasing the risk associated of negative effects on fitness of wild populations (Araki and Schmidt, 2010). In a review of 21 studies that assessed effects of rearing on the fitness of hatchery fish (Araki and Schmidt, 2010), 12 studies found a negative effect on fitness, six studies found no effect, and 1 found a positive effect. Although biased towards the salmonid/trout family, the conclusion from that review was that hatchery-reared animals are likely to be less reproductively successful than wild counterparts. Thus, the potential economic profitability of an enhancement fishery represents a trade-off between the risk of wild stock replacement, as well as density of release, size-at-harvest, and harvest strategy (high *F vs* low *F*). In this regard, the issues involved in managing interactions between wild and cultured fish is an expanding area of concern (Lorenzen et al., in press).

Sensitivity analysis revealed that for *H. laevigata*, cost of fishing and cost of enhancement had only minor effects on profitability (Figure 3.7c and e) relative to mortality, size-at-release, and value of harvest. For a 4 cm animal, the economic break-even point for survival was 8% (Figure 3.7a), suggesting that R&D effort would be best focused on reducing mortality of released stock and effects of size-at-release. The results relating to size-at-release, in particular (Figure 3.8b), pose a more complex problem than previously identified for abalone fisheries. Density-dependent processes were modelled to act on the smaller release sizes (< 3 cm) and reduced relative survival, i.e. three times the number of 2 cm

animals compared to 4 cm animals have to be released to achieve the same biomass target (Figure 3.2b). Above a release size of 4 cm, costs of production outstrip increases in survival and profitability declines, confirming initial speculations of Hart et al. (2007), which contrast conclusions made for *H. iris* (Roberts et al., 2007) that 1 to 1.5 cm seed is the most cost-effective size-at-release.

One of the most important innovations that could arise from an integrated enhancement fishery will be spatially explicit harvest strategies conditioned on density, rather than a size limit and/or catch quota, which are sub-optimal due to variable growth and mortality. Although compensatory density-dependence processes exert fundamental underlying controls on fish populations (Rose et al., 2001), they are poorly understood in abalone fisheries (Bardos et al. 2006) and in need of high-quality experimental investigations at a scale relevant to fisheries management. For example, dependence on limited habitat at the juvenile stage has been cited as the main bottleneck to successful abalone enhancement (Shepherd et al., 2001). However, experimental investigations (Shepherd, 1998; Roberts et al., 2007; Dixon, 2011) have not confirmed this unequivocally and we are currently exploring the hypothesis that adult habitat is the limiting factor in *H. laevigata* populations (Strain, unpublished data). Technological developments such as reef scale assessment (Prince et al., 2008), and accurate GPS mapping of fishing effort, reef habitat, and size-structure of the catch (Mundy, 2012) can provide accurate records that enable a density-dependent approach to harvesting, and consequent elucidation of underlying mechanisms of population control. This approach will require greater cooperation at all levels of the fishing industry and between the industry, management, and research sectors than currently exists, and will require significant cultural change. However the principle of co-management is a key element of the management approach in all Australian abalone fisheries (Mayfield et al., 2012), and with strong leadership and sufficient incentive (Gutierrez et al., 2011), an integrated enhancement program could be part of a proactive management approach in Australian abalone fisheries.

This study has combined evaluation modelling with robust experimental data to predict significant economic outcomes for a stock enhancement program focused on *H. laevigata*. The predictions rely on a number of assumptions including, for example, the use of a deterministic, equilibrium model, which assumed constant growth and recruitment. In the defence of the *EnhanceFish* model, our validation model, which incorporated uncertainty, alternative growth, recruitment and mortality functions, and an independent estimate of spawning biomass based on field surveys, provided similar estimates of spawning biomass (Figure 3.3). Furthermore, the long-term field experiments described in Hart et al. (2013a,b) showed abundance increases in areas subject to experimental stock enhancement and recruitment of released juveniles into the fishery, beginning at 3.5 years post–release (age 5). There are, however, other significant issues and risks that need to be addressed to facilitate a viable large-scale enhancement strategy including:

1. *H. laevigata* fisheries are comprised of spatially disaggregated stocks, each subject to potentially different management and legislative frameworks. Enhancement strategies would need to focus at the appropriate level in each jurisdiction.

2. An integrated release and harvest strategy would require more defined and coordinated levels of exploitation than currently exists in Australian abalone fisheries. Reef-scale assessment and accurate GPS mapping of fishing effort and reef habitat can facilitate this, however, such a significant development in management strategy requires policy initiatives that promote inclusive and collaborative harvesting and involvement of wild industry divers in the enhancement process.
3. The possibility of substantial economic gain must facilitate development of rigorous breeding protocols in facilities dedicated to stock enhancement, to ensure conservation of existing wild stock diversity and best practice disease management. This would likely result in a greater cost of enhancement (e.g. \$0.45 cm^{-1} *vs* \$0.3 cm^{-1}), but in an integrated enhancement program, increased enhancement costs were predicted to have only a minor effect on overall profitability (Figure 3.7c).
4. Stock enhancement represents a merging of two currently separate industries in Australia, aquaculture and wild fisheries.

However the recent spectre of disease in wild stocks in Victoria and Tasmania (Corbeill et al., 2010; Savin et al., 2010; Hooper et al., 2007), and the implication of inadequate aquaculture bio-security practices in its transmission, has resulted in a prevailing scepticism that the disease risk can be controlled. In response to this, two recent risk assessments concluded that bio-security control measures can facilitate the stocking of hatchery-reared animals of equal or higher health status to that of aquatic animals already living in the considered "open systems" (Jones and Fletcher, 2012; Stevens, 2012). Acceptance of this finding by wild fishery stakeholders will be necessary if stock enhancement is to be pursued as a viable management option.

6.6 Conclusions

In conclusion, this bioeconomic analysis has revealed significant economic potential of a stock enhancement program for Australian *H. laevigata* fisheries, with the possibility of 100% increases in MEY and NPV. A necessary step to achieving this potential is an integrated enhancement and harvesting strategy where inputs (juveniles released) and outputs (catch quotas, size limits etc.) are considered as part of the same fishery management system. Another key ingredient is dedicated aquaculture facilities for stock enhancement. These facilities would operate on annual production and release quotas based on methodologies proposed in this study and have the economic advantage of more certainty, e.g. annual production targets calculated from natural recruitment rates rather than market demands, and faster grow-out schedules (1 – 2 years) compared to current abalone aquaculture in Australia for the food export market (3 – 4 years). They would also provide biological certainty of the best quality offspring from notoriously variable growth and survival rates currently experienced in abalone aquaculture (Daume and Ryan, 2004; Kawamura et al., 1998). When combined with best practice genetic and disease risk management, an integrated enhancement and harvesting program will be well positioned to address the significant future challenges for Australian abalone fisheries, which include declining catch and profitability, range contractions driven by ocean warming, and increasing population and access rights issues (Mayfield et al., 2012).

6.7 Acknowledgements

This work was funded by the Australian Seafood Cooperative Research Centre (CRC) under the project 2009/710 “Bioeconomic evaluation of commercial-scale stock enhancement in abalone”, and the Department of Fisheries Western Australia. Our thanks go to the Western Australian Abalone Industry Association (WAAIA) in particular Ian Taylor and Brad Adams, and to all the industry divers who participated in the site selection and juvenile release experiments, as well as providing the economic data of fishing costs. Thanks also to Kai Lorenzen for detailed discussion and useful tips on the *EnhanceFish* software and Rowan Chick, Simon de Lestang, Steve Mayfield, Brett Molony and two anonymous reviewers for their comments.

6.8 References

Araki, H., C. Schmid. Is hatchery stocking a help or a harm? Evidence, limitations and future directions in ecological and genetic surveys. Aquacult., 308: (Suppl. 1), S1 – S11 (2010).

Bardos, D.C. Probabilistic gompertz model of irreversible growth. Bull. Math. Biol., 67: 529-545 (2005).

Bardos D.C., R.W. Day, N.T. Lawson, and N.A Linacre. Dynamical response to fishing varies with compensatory mechanism: An abalone population model. Ecol. Model., 192: 523–542 (2006).

Bell, J. D., P. C. Rothlisberg, J. L. Munro, N. R. Loneragan, W. J. Nash, R. D. Ward, and N. L. Andrew. Restocking and stock enhancement of marine invertebrate fisheries. Adv. Mar. Biol., 49: 1–370 (2005).

Blankenship, H. L., and K. M. Leber. A responsible approach to marine stock enhancement. Am. Fish. Soc. Symp., 15: 167–175 (1995).

Burnham, K.P., and D.R. Anderson. Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York (2002).

Caddy, J.F., and O. Defeo. Enhancing or restoring the productivity of natural populations of shellfish and other marine invertebrate resources. FAO Fisheries Technical Paper, No. 448. FAO, Rome, Italy (2003).

Chick, R.C. Stock enhancement of local populations of blacklip abalone (*Haliotis rubra*, Leach) in New South Wales, Australia. PhD Thesis, James Cook University of North Queensland, Australia. 233 p.

Chick, R.C., N. Turich, S. Mayfield, and J.J. Dent. Western zone abalone (*Haliotis laevigata* & *H. rubra*) Fishery (Region A). Fishery assessment report for PIRSA. SARDI aquatic Sciences Publication No. F2007/000561-3. SARDI Research Report Series No. 376. 99p (2009).

Corbeil, S., A. Colling, L. M. Williams, F. Y. Wong, K. Savin, S. Warner, B. Murdoch, N. O. Cogan, T. I. Sawbridge, M. Fegan, I. Mohammad, A. Sunarto, J. Handlinger, S. Pyecroft, M. Douglas, P. H. Changs, M. S. Crane. Development and validation of a TaqMan PCR assay for the Australian abalone herpes-like virus. Dis. Aquat. Org. 92: 1-10 (2010).

Daume, S. and S. Ryan. Nursery culture of the abalone *Haliotis laevigata*: Larval settlement and juvenile production using algae or formulated feed. J. Shellfish Res., 23: 1019-1026 (2004).

Department of Fisheries, 2011. State of the Fisheries and Aquatic Resources Report 2010/11. (Fletcher, W.J., and K. Santoro, Eds.) Department of Fisheries, Western Australia 359p.

Dixon, C. J. Experimental approaches for understanding density dependence of greenlip abalone (*Haliotis laevigata*) populations. PhD Thesis, Department of Zoology, University of Melbourne, Australia. 138 p (2011).

Dixon, C. J., R. W. Day, S. M. H. Huchette, and S. A. Shepherd. Successful seeding of hatchery-produced juvenile greenlip abalone to restore wild stocks. Fish. Res. 78: 179-185. (2006).

Econsearch. Economic indicators of the South Australian Abalone Fishery 2009/10. A report prepared for Primary Industry and Resources South Australia. Available from www.econsearch.com.au/pages/completed-projects/fishing-aquaculture/ fish10.php (2011).

Goodsell, P. J., A. J. Underwood, M. G. Chapman, and M. P. Heasman. Seeding small numbers of cultured black-lip abalone (*Haliotis rubra* Leach) to match natural densities of wild populations. Mar. Freshw. Res. 57: 747-756 (2006).

Gutierrez N.L., Hilborn, R., Defeo O. Leadership, social capital, and incentives promote successful fisheries. Nature. 470: 386-389 (2011).

Haddon, M., C. Mundy, D. Tarbath. Using an inverse-logistic model to describe growth increments of blacklip abalone (*Haliotis rubra*) in Tasmania. Fish. Bull. 106: 58 – 71 (2007).

Hammasaki, K., and S. Kitada. The enhancement of abalone stocks: lessons from Japanese case studies. Fish and Fisheries, 9: 1-18 (2008).

Hart, A. M, F. P. Fabris, and S. Daume. Stock enhancement of *Haliotis laevigata* in Western Australia – a preliminary assessment. Fish. Res. Rep. No. 166, Department of Fisheries, Western Australia (2007).

Hart, A., F. Fabris and N. Caputi. Performance indicators, biological reference points and decision rules for Western Australian abalone fisheries (*Haliotis* sp.): (1) standardised catch per unit effort. Fisheries Research Report No. 185, Department of Fisheries, Western Australia (2009).

Hart, A. M., L. W. S. Strain, F. P. Fabris, J. Brown, and M. Davidson. Stock enhancement in greenlip abalone part I: long-term growth and survival. Rev. Fish. Sci. 21(3-4): 299-309 (2013a).

Hart, A. M, F. P. Fabris, L. W. S. Strain, M. Davidson, and J. Brown. Stock enhancement in greenlip abalone part II: population and ecological effects. Rev. Fish. Sci. 21(3-4): 310-320 (2013b).

Heasman, M.P. In pursuit of cost-effective fisheries enhancement of the New South Wales blacklip abalone, *Haliotis rubra* (Leach) fishery. J. Shellfish. Res. 25: 211-224.

Helidoniotis, F., Haddon, M., Tuck, G., Tarbath, D. The relative suitability of the von Bertalanffy, Gompertz and inverse logistic models for describing growth in blacklip abalone populations (*Haliotis rubra*) in Tasmania, Australia. Fish. Res. 112: 13-21. (2011).

Hooper, C., P. Hardy-Smith, J. Handlinger. Ganglioneuritis causing high mortalities in farmed Australian abalone *(Haliotis laevigata* and *Haliotis rubra*). Aust. Vet J. 85: 188-193. (2007).

Jones, B, R., Fletcher. Assessment of the risks associated with the release of abalone sourced from Abalone Hatcheries for enhancement or marine grow-out in the open ocean areas of WA. Fisheries Research Report 227. Department of Fisheries, Government of Western Australia (2012).

Kawamura, T., Roberts, R.D. and Takami, H. A review of the feeding and growth of postlarval abalone. J. Shellfish Res., 17: 615-625 (1998).

Kitada, S, Kishino, H. Lessons learnt from Japanese marine finfish stock enhancement programs. Fish. Res. 80: 101-112.

Kube, P.D., S.A. Appleyard and N.G. Elliott. Selective breeding greenlip abalone (*Haliotis laevigata*): preliminary results and issues. J. Shellfish Res. 26: 821-824 (2007).

Mayfield, S., I.J. Carlson, and R.C. Chick. Central zone abalone (Haliotis laevigata & H. rubra) Fishery (Region A). Fishery assessment report for PIRSA. SARDI aquatic Sciences Publication No. F2007/000611-2. SARDI Research Report Series No. 306. 65p (2008).

Lorenzen, K. Allometry of natural mortality as a basis for assessing optimal release size in fish stocking programs. Can. J. Fish. Aquat. Sci., 57: 2374–2381 (2000).

Lorenzen, K., Leber, K.M., Blankenship, H. L. Responsible approach to marine stock enhancement: An update. Rev. Fish. Sci. 18(2):189–210 (2010).

Lorenzen, K. Population dynamics and potential of fisheries stock enhancement: Practical theory for assessment and policy analysis. Phil. Trans. Royal Soc. B, 360: 171–189 (2005).

Lorenzen, K. & Medley, P.A.H. *EnhanceFish* Manual (Beta release). London: Imperial College, (2006)

Lorenzen, K., M. C. M. Beveridge, M. Mangel. Cultured fish: integrative biology and management of domestication and interactions with wild fish. Biol. Rev. (in press).

Mayfield, S., Mundy, C., Gorfine, H., Hart, A.M., Worthington, D. Fifty years of sustained production from the Australian abalone fisheries. Rev. Fish. Sci. 20(4): 220-250 (2012).

Mundy, C. Using GPS technology to improve fishery dependent data collection in abalone fisheries. Final Report to the Fisheries Research and Development Corporation, Project Number 2006/029. 124 pp. Hobart, Tasmania: Institute for Marine and Antarctic Studies, University of Tasmania (2012).

Myers, R.A., K.G. Bowen, and N.J. Barrowman. Maximum reproductive rate at low population sizes. Can J. Fish. Aquat. Sci. 56: 2404-2419 (1999).

Officer, R.A. Size limits for Greenlip Abalone in Tasmania. Tasmanian Aquaculture and Fisheries Institute, Technical Report Series No. 5. 48 p (1999).

Prince, J.D. The decline of global abalone (Genus *Haliotis*) production in the late twentieth century. Is there a future? In: Stock enhancement and sea ranching: Developments, Pitfalls, and Opportunities (K.M. Leber, S. Kitada, H.L. Blakenship, T. Svasand, Eds). Blackwell Science Ltd, Oxford, UK, p 427 –443 (2004).

Prince J.D, H. Peters, H. Gorfine, and R.W. Day. The novel use of harvest policies and rapid visual assessment to manage spatially complex abalone resources (Genus *Haliotis*). Fish. Res. 94: 330–338 (2008).

Roberts, R.D., E.F. Keys, G. Prendeville, and C.A. Pilditch. Viability of abalone (*Haliotis iris*) stock enhancement by release of hatchery-reared seed in Marlborough, New Zealand. J. Shell. Res. 26(3): 697-703 (2007).

Robinson, N., B. Smith, I. Cooke and J. Strugnell. A snail's pace: a preliminary analysis of the effects of stress and genetics on movement of *Haliotis*. Aquaculture 376-379: 25-35 (2013).

Rogers-Bennett, L., D. Rogers, and S.A. Schultz. Modelling growth and mortality of red abalone (*Haliotis rufescens*) in northern California. J. Shell. Res. 26: 719–727 (2007).

Rose, K.A., J.H. Cowan Jr., K.O. Winemiller, R.A. Myers, and R. Hilborn. Compensatory density dependence in fish populations: importance, controversy, understanding and prognosis. Fish and Fisheries. 2: 293–327 (2001).

Savin, K. W., B. G. Cocks, F. Wong, T. Sawbridge, N. Cogan, D. Savage, and S. Warner. A neurotropic herpesvirus infecting the gastropod, abalone, shares ancestry with oyster herpesvirus and a herpesvirus associated with the amphioxus genome. Vir. J. 7: 308 (2010).

Shepherd. S. A. Studies on Southern Australian Abalone (Genus *Haliotis*). XII: Long-term recruitment and mortality dynamics in an unfished population. Aust. J. Mar. Freshw. Res. 41: 475-492 (1990).

Shepherd. S. A. Studies on Southern Australian Abalone (Genus *Haliotis*). XIX: Long-term juvenile mortality dynamics. J. Shell. Res. 17(3): 813-825 (1998).

Shepherd, S. A. and J. L. Baker. Biological reference points in an abalone (*Haliotis laevigata*) fishery. In: Proceedings of the North Pacific Symposium on Invertebrate Stock assessment and Management (G.S. Jamieson and A. Campbell, Eds.) Can. Spec. Publ. Fish. Aquat. Sci., 125: 235-245 (1998).

Shepherd, S. A. and L. D. Brown. What is an abalone stock: implications for the role of refugia in conservation. Can. J. Fish. Aquatic. Sci. 50: 2001-2009.

Shepherd, S. A., P. A. Preece, R. G. W. White. Tired nature's sweet restorer – ecology of abalone stock enhancement in Australia. In: Workshop on rebuilding abalone stocks British Columbia (A. Campbell, Ed.). Can. Spec. Publ. Fish. Aquat. Sci., 130: 84-98 (2001).

Stevens, R. D. Disease risk assessment for abalone stock enhancement. FRDC project 2011/46. Fisheries Research and Development Corporation, Australia.

Sumaila, U. R., and C. Walters. Intergenerational discounting: a new intuitive approach. Ecol. Econ. 52: 135-142 (2005).

Troynikov, V.S., R.W. Day, and A. Leorke. Estimation of seasonal growth parameters using a stochastic Gompertz model for tagging data. J. Shell. Res. 17: 833–838 (1998).

Zhou, S., Y. Shaowu, J. T. Thorson, A.D.M. Smith, M. Fuller. Linking fishing mortality reference points to life history traits: an empirical study. Can. J. Fish. Aquat. Sci. 69: 1292-1301 (2012).

7 Stock Enhancement in Greenlip Abalone: (4) Commercial-Scale Stock Enhancement Manual

Lachlan W. S. Strain[1], Anthony M. Hart[1], Frank Fabris[1], Mark Davidson[1]

[1]Western Australian Fisheries and Marine Research Laboratories, PO Box 20, North Beach, Western Australia, 6920, Australia.

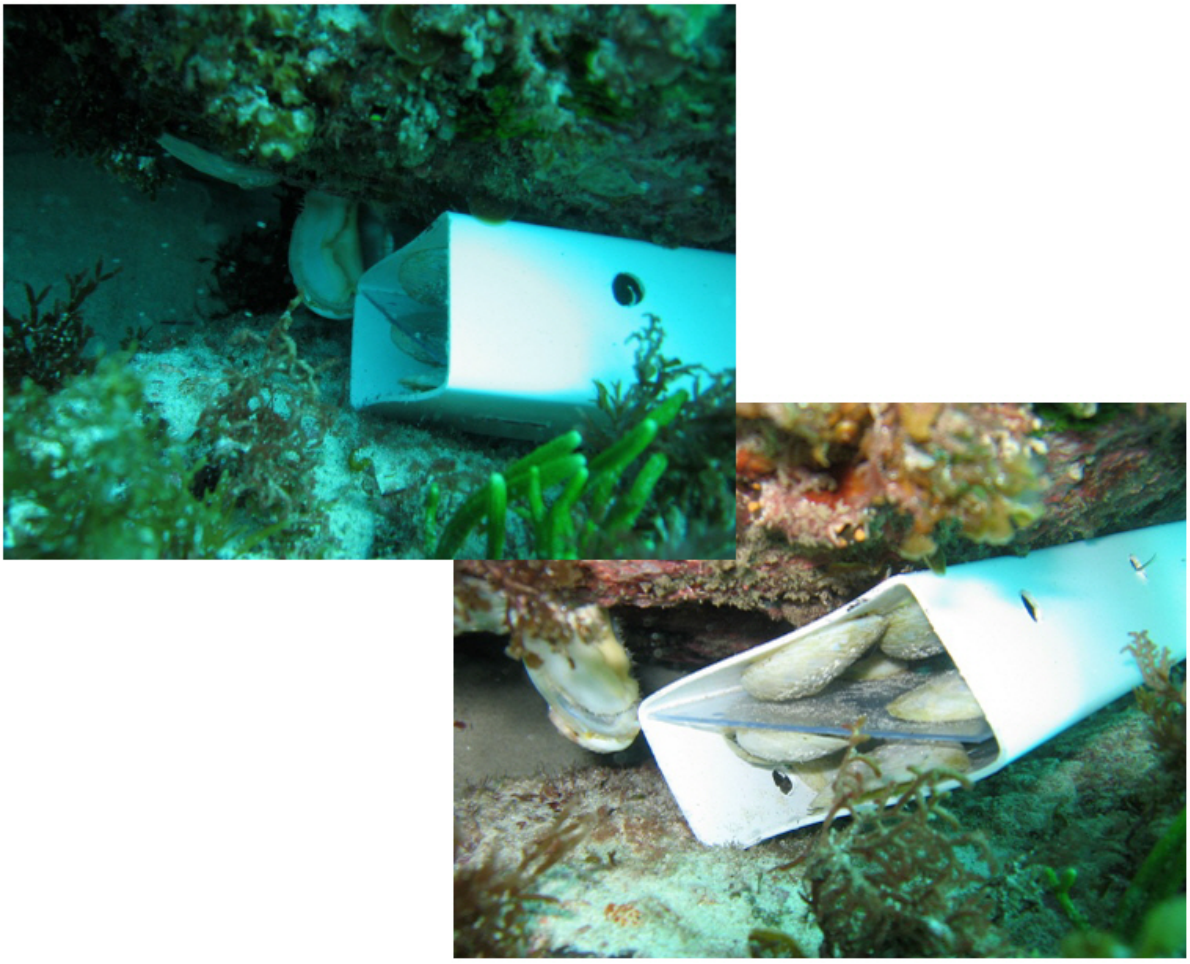

Figure 4.1. Greenlip Abalone entering the natural habitat from a deployed release device.

7.1 Scope

This manual aims to develop a standardised methodology for commercial-scale stock enhancement of Greenlip Abalone into areas of the Western Australian abalone fishery. It does this by examining all aspects of the enhancement procedure from spawning and nursery protocols in the hatchery, to the packaging and transport of abalone and finally the deployment of abalone by divers into natural habitat.

Experimental trials of abalone enhancement have clearly shown that cumulative survival at harvest (Age 5) is highly variable, with ranges between 6 and 20% (Hart et al., 2013a). Moreover, this survival has been shown to be statistically dependent on habitat and husbandry techniques, such as placement of abalone within the habitat mosaic and protection from predators. At a commercial-scale such variation has significant economic impacts, so to achieve commercial-scale survival rates similar to those observed at the experimental scale it requires industry training/extension and a detailed set of protocols to ensure quality control. To ameliorate the significant economic impacts in scaling up from experimental to commercial-scale enhancement, while maximising survival and economic viability, there is a need to standardise a set of transport and release protocols, including the design of abalone release devices and the vessel containers, into a training manual for personnel involved in stock enhancement.

This document therefore, outlines the operational requirements to ensure that organisations have an understanding of the procedures involved in stock enhancement and the potential hazards. Organisations may utilise this document as an educational tool to train staff undertaking any aspect of abalone stock enhancement and employ best practices. It is expected however, that the guiding principles will be adapted to situations specific to different species and locations and operations.

7.2 Guiding Principles

The guiding principles in this manual are:

1. Incorporate stock enhancement into Western Australian abalone fisheries.
2. Utilise current technology in abalone hatcheries.
3. Involve commercial fishers and practices in the release abalone into the natural habitat.
4. Develop a protocol that can be managed on an experimental-scale and be increased effectively to a commercial-scale.
5. Each section can be utilised independently of the manual as a whole.

7.3 Hatchery Requirements

Responsible hatchery protocols with strict bio-security measures are required to produce abalone for stock enhancement programs. These protocols are an overview of how hatcheries should operate to provide best practice in abalone stock enhancement. Even though hatcheries can produce large numbers of abalone these protocols are not set for any specific

number of abalone. Therefore, the protocols can be scaled depending on the size of stock enhancement program undertaken.

The aim of the hatchery stage is to produce Greenlip Abalone to the desired size at release. The hatchery requirements assume:

1. An abalone size at release of 30 – 40 mm shell length (Hart et al., 2013c).
2. Abalone will be released at »18 months of age.
3. Hatchery spawning will take place at the same time as natural populations spawn each year (Oct-Nov).
4. Abalone are to be harvested from the nursery system at 10 - 15 mm, graded and placed in the grow-out system.
5. A further grading will occur by the time the animals are 18 months.
6. Removal of poor quality individuals will occur during grading to produce a smaller variance in abalone size and therefore release the best quality abalone (fastest growers).
7. Disease testing of the abalone is to take place 2 weeks prior to any release.
8. Hatchery schedule shall optimise genetic variation of each cohort to be released by maintaining a well-mixed population of juveniles.

7.3.1 Bio-security Protocols

Hatcheries are to follow strict bio-security protocols at all times. Each hatchery will have their own current bio-security protocols but these must be amended to incorporate stock enhancement principles. The bio-security at hatcheries will be directed by the relative management organisation of aquaculture and stock enhancement for that jurisdiction. Bio-security protocols in Western Australia need to be approved by the Department of Fisheries Western Australia and adhere to the Policy on Restocking and Stock Enhancement in Western Australia (FMP No. 261) and Abalone Aquaculture Policy. All personnel are required to read and familiarise themselves with the bio-security protocols before commencing any section of the stock enhancement manual.

7.3.2 Broodstock Collection

1. Broodstock to be collected under an appropriate breeding and genetic management schedule based on the outcomes of population genetics studies (Chapter 5).
2. Collection of broodstock to occur prior to natural population spawning (August – November) and with sufficient time to allow conditioning in the hatchery.
3. Divers are to chip healthy, “fast growing” adult abalone (no damage) and check sex and gonad development.
4. Abalone that are selected are to be held in live tanks while at sea.
5. Broodstock transported from collection location to abalone hatchery.

a. Transported in a large cooler box full of seawater under heavy aeration.

6. Abalone tagged as per local authority regulations.
7. Broodstock placed in isolation area of hatchery for bio-security protocols.

a. Tanks receive flow through seawater (10 $L.min^{-1}$ at ambient temperature) with aeration.

8. Abalone held until required for spawning under an appropriate conditioning regime.

7.3.3 Spawning Protocols

These protocols are an overview of the spawning process and every hatchery should utilise their specific Standard Operating Procedures. Hatcheries will have different systems and procedures so these protocols are designed to cover the principles of spawning and not the specifics of each task (e.g. Daume, 2007).

Sterilisation

Before spawning

1. Turn seawater off at mains and empty all lines and filters to spawning room.
2. Clean lines using a commercial cleaner (Hypo-chloride, Vortex, etc.).
3. Flush lines with fresh water, open lines and air-dry.
4. Clean all spawning equipment and tanks with commercial cleaner (Hypo-chloride, Cip-safe, Decon 90, etc.).
5. Rinse with fresh water and air dry.

Spawning Day

1. Turn seawater on and rinse all lines, equipment and tanks thoroughly before use.
2. Run spawning system before placing abalone into the tubs.
3. Fill the UV unit then turn on.
4. Set heater to »3^{o}C above ambient seawater temperature.

Spawning

1. Select broodstock from isolation tanks and clean before use.
2. Sex and check gonad development of all broodstock and place in spawning tubs.
3. Utilise largest possible number (> 50 animals) and sex ratio of broodstock to maintain the genetic variability within and between populations (Chapter 5).
4. Desiccate abalone for about 30 to 45 min.
5. After desiccation, fill tubs with heated, UV seawater at a flow rate of »0.5 $L.min^{-1}$ and adjust aeration.
6. Slowly reduce temperature on heater back to ambient.

Sperm Collection

1. Males will generally spawn first.

2. Siphon sperm from the top of the spawning tub into a jug.
3. Check motility of sperm under a microscope.
4. Count sperm on a Haemocytometer and determine the number of sperm per mL.

Egg Collection

1. Siphon eggs from the bottom of the spawning tub into a bucket through a 300µm screen to remove faeces.
2. Suspend the eggs in the water column and collect a sample.
3. Count the eggs on a Sedgewick Rafter slide and determine the number of eggs per mL.

Fertilisation

1. After counting eggs, determine the amount of sperm to be added.

a. Assume a ratio of 10 - 15 sperm.egg^{-1}.

2. Add fresh sperm to the bucket containing eggs and agitate, fertilise for about 15 min.
3. Rinse the eggs free of excess sperm by pouring over a screen (75µm) into a bucket, changing the water for 15 min.
4. Suspend the fertilised eggs in the water column and sample to determine the fertilisation rate.

Fertilisation Rate

1. After 2 h count the number of dividing Zygotes under a microscope and determine the fertilisation rate as % of the number of eggs per mL^{-1}.

Hatching

1. Place the fertilised eggs gently into the hatch tub (number depends on size of tub).
2. Eggs should settle and form an evenly distributed monolayer on the tub bottom.
3. Add very gentle aeration just below surface of the water.
4. Attach a banjo (filter) securely to the outlet.
5. Turn water on at a slow flow rate (»5 water exchanges per 24 hr) and allow overflowing from near surface.
6. Hatch out will occur around 20 hr after fertilisation.
7. Turn water and aeration off just before this and then leave for 1 h after hatch out begins.

Larval Rearing

1. Siphon trochophores (free swimming larvae) from near the surface of the hatch tub into a bucket.
2. Attach a banjo to the larval rearing tank and half fill the tank.

3. Gently add the larvae to the larval rearing tank at density of »20 larvae.ml^{-1}.
4. Suspend the remaining water in the hatch tub, take a sample and drain the water as it contains egg casings, unhatched eggs and mainly slow larvae.
5. Calculate the Hatch Rate
6. For the first 24 - 30 h of larval rearing use static water (no flow) in the larval rearing tank with very low aeration.
7. After the larvae have developed an operculum (24 - 30 h), gentle flow through seawater can be used.
8. Water change then occurs every second day.
9. Remove the banjo from the outflow and swim the larvae (healthy larvae raft at the top) through the overflow pipe onto a screen submerged in a tub.
10. Wash the larvae thoroughly on the screen and then carefully wash into a bucket.
11. Collect a sample and record the water volume in the bucket.
12. Estimate Larvae Density
13. Gently add the larvae into a clean larval rearing tank (setup as before) with slow flow through seawater and aeration.
14. Drain the remaining water from the larval rearing tank.
15. Repeat water changes until larvae are ready for settlement.
16. Determine Larval Density at each water change.

Hatch Rate

1. Count the number of unhatched eggs.
2. Calculate the number of unhatched eggs as a % of the total number of eggs in the hatch tub.
3. Subtract this from the fertilisation rate giving the percentage hatch rate.

Larval Density

1. Count the number of larvae.
2. Determine the number of larvae per mL to get a total larval count.

Settlement

1. On last day of larval rearing (»5 - 6 d after hatching at 17°C) check under the microscope if third tubule on their cephalic tentacle is developed and if they are displaying settlement behaviour (spending time on their foot crawling and crawling).
2. Collect larvae as per water exchange and estimate Larval Density.
3. Calculate volume required to seed each nursery tank with larvae (»0.2 larvae.mL^{-1}).
4. Attach a banjo sieve on the nursery tank outlet.

5. Prior to settlement all nursery tanks are conditioned with algae ready for abalone settlement.
6. Gentle add the larvae to the surface of the water in the nursery tank.
7. Introduce very low flow water and aeration.

7.3.4 Nursery Protocols

These protocols are an overview of the nursery rearing process and every hatchery should utilise their specific Standard Operating Procedure. Hatcheries will have different nursery systems and procedures so these protocols are designed to cover the principles of nursery rearing and not the specifics of each task (e.g. Daume and Ryan, 2004; Strain et al., 2006; Daume et al., 2007; Strain, 2012).

System Design

1. Rectangular, nursery tanks have seawater inflow (»10 $L.min^{-1}$) at one end through a filter and an outflow at the other end.
2. Each tank contains metal baskets of 20 vertically arranged PVC plates (»60x30 cm).
3. The seawater is aerated by weighted airlines (seepage hose) spaced evenly along the bottom.
4. All tanks are shaded with shade cloth, depending on the time of year, algae diet and presence of abalone.

Sterilisation

1. Nursery tanks and all contents are scrubbed clean prior to use.
2. Tanks are filled with filtered seawater and sterilised using a commercial cleaning agent (Hypo-chloride).

System Maintenance

1. While in operation, nursery tanks are flushed on a regular basis to remove any dead abalone, abalone faeces and detritus.
2. The baskets with PVC plates inside are rotated 180^{o} about the horizontal at regular intervals to maintain even algae coverage.
3. Nutrients are added to the tanks as required using commercial fertiliser (Abasol, MAF, etc.)
4. Algae species are inoculated in the tanks prior to spawning and then as required.
5. Species used depends on specific hatchery protocols with common species utilised in Australian abalone hatcheries:

a. *Ulvella lens* and diatoms (Naturally occurring and/or *Navicula jeffreyi*).

6. Abalone numbers, shell length (mm) and weight (g) are measured periodically throughout the nursery phase.

7. Based on these measurements grading is carried out if required to maintain an adequate stocking density (e.g. 40, 5 mm abalone per plate).

Algae Diets

Ulvella lens – Using Seed Plates

1. Settlement plates with a dense, even cover of mature *U. lens* (dark green) are selected as seed plates from the nursery system.
2. Seed plates are wiped clean and rinsed with freshwater.
3. The plates are then placed in a nursery tank with filtered flow through seawater (1µm) in complete darkness, until required (at least 1 week).
4. When required the seed plates are interspersed between clean settlement plates in the nursery tanks.
5. Tanks are filled with filtered seawater, receive no water flow with very light aeration and are uncovered.
6. Nutrients (Abasol) are added to the tanks.
7. Once sporulation has occurred the *U. lens* seed plates are removed (»5 - 7 d) and the filtered seawater is turned on.
8. On a regular basis, rotate the plates and add nutrients (Abasol at 0.06 $g.L^{-1}$).

Ulvella lens – Using in Nursery System

1. Nursery system is run as per usual until sporulation is required
2. Tanks with settlement plates covered in *U. lens* are then shaded.
3. After at least a week, the tanks are uncovered, seawater flow is turned off, aeration is lowered and nutrients (Abasol) are added.
4. Once sporulation has occurred (»5 - 7 d) the filtered seawater is turn on and aeration increased.
5. On a regular basis, rotate the plates and add nutrients (Abasol at 0.06 $g.L^{-1}$).

Diatoms – Naturally Occurring

1. Many diatom species occur naturally in the nursery tanks.
2. To increase the density when needed, keep the tank uncovered, turn off the water for 24 hr and add nutrients (MAF at 0.06 $g.L^{-1}$).

When specific diatoms species are required for the nursery tanks by inoculation they are scaled up according to a diatom inoculation protocol (e.g. Strain, 2012)

7.3.5 Grow-out Protocols

These protocols are an overview of the grow-out system and every hatchery should utilise their specific Standard Operating Procedures. Hatcheries will have different grow-out systems and procedures, so these protocols are designed to cover the principles of grow-out and not the specifics of each task.

System Design

1. Slab tanks/raceways are long, shallow concrete tanks with seawater inflow at one end and an outflow at the other end.
2. Each tank contains only enough water to cover the abalone.
3. All tanks are shaded to be in complete darkness.

System Maintenance

1. Abalone are fed a commercially produced artificial diet, specifically designed for the size of abalone.
2. The feed is delivered manually at feed rates specified by the hatchery.

a. Approximately 2 % body weight per day (dry feed, live abalone).

3. The grow-out tanks are flushed depending on feeding schedule to remove abalone faeces and uneaten artificial food.
4. Flushing occurs by a tip tray being filled and then dumped creating a wave effect down the tank.
5. Abalone numbers, shell length (mm) and weight (g) are measured periodically throughout the grow-out phase.
6. Based on these measurements grading is carried out if required to maintain an adequate stocking density.

7.4 Packing and Transportation Requirements

These protocols are an overview of how to pack and transport abalone for commercial-scale stock enhancement programs. The specifics of these protocols will vary depending on the total number of abalone to be released and can be scaled accordingly.

The aim of the transportation stage is to move abalone from the hatchery to the enhancement sites with as little mortality or stress to the abalone as possible. The packing and transportation requirements assumes:

1. A single commercial abalone vessel (7 – 9 m length) is used to deploy 10,000 Greenlip Abalone.
2. Deployment of 10,000 abalone occurs in 1 day.
3. Abalone have reached the desired size for release (40 mm, Hart et al., 2013c).
4. The field requirements for establishing the enhancement sites have been completed.
5. The abalone are to be placed into the release devices the day before being seeded into the fishery.
6. Release devices to be stocked with 60 abalone.

7.4.1 Release Devices

Release devices are the containers the abalone are transported in from the hatchery to the enhancement sites and allow deployment of abalone into the natural habitat. These devices need to be water resistant for at least 48 h and must withstand the pressure of depths <30m. Given the nature of the abalone habitat the devices are being placed in, they also need to be structurally sound to provide protection for the released abalone as they move out into the habitat.

Numerous types of release devices were examined but PVC pipe was considered most suited to achieving all of these requirements, while also being simple to source and safe to work with. PVC pipe also comes in a variety of sizes, thickness and shapes, which make it ideal to be tailored to the number of abalone released, the abalone size at release and the habitat the abalone are being released into. Price does vary slightly between the different sizes and shapes of PVC pipe but given cost of production was not considered critical in the bioeconomic analysis (Hart et al., 2013c) and enhancement costs are \$0.07 cm^{-1} (Table 3.1), this slight variation in price does not translate into a defining factor.

Two different shaped PVC pipe release devices, one rectangular and one cylindrical were experimentally tested during a large-scale Greenlip Abalone release (10,000 abalone) into the Augusta fishery in Western Australia. Release mortality has been shown to be critical for long-term survival as initial release survival (6 month post-release) differs significantly between sites (Hart et al., 2013b). The change in density of abalone at the enhanced sites during this initial release period (6 month post-release) was not significantly different between the sites seeded using the two different shaped PVC pipe release devices ($F_{(df\ 1,8)}$=0.028, p=0.872). Therefore either a rectangular or cylindrical shaped PVC pipe release device can be utilised in stock enhancement programs. However, on a qualitative basis the rectangular PVC pipe release device was considered easier to pack into the transport containers and also place in the natural habitat, making it the preferred choice for the divers conducting the abalone release.

This manual will assume that the release device used to deploy 40 cm abalone into the natural habitat can be a PVC pipe either rectangular (30 cm x 10 cm x 50 cm) or cylindrical (30 cm x 10 cm diameter) with a length of 30 cm. Both of these devices will utilise flyscreen as end covers to allow high water flow through the device and easy removal once deployed.

7.4.2 Packing Abalone into the Release Devices

At least 2 groups of 3 people (6 people) will be required to pack the abalone into the release devices.

Harvesting (3 people)

1. Harvest the abalone by anaesthetising (Benzocaine or salt) and then removing from the grow-out tanks.
2. Grade the abalone to reduce the size variation in the release cohort, and utilise the larger animals to ensure the best quality abalone are released.
3. Place abalone into small tanks lined with shade cloth, receiving high flow through seawater (reduces probability of mortality due of anaesthetic).

4. Measure shell length and weight of the abalone to get an average size for the release cohort.

Loading Device (3 people)

1. Determine the weight of 60 abalone (60 x weight of 1 abalone). This will be used to measure the abalone into the devices rather than counting the abalone individually.
2. Take handfuls or “clumps” of abalone and weight to the target weight (60 abalone).
3. Load the abalone into the release device and seal the ends.
4. Place several packed devices into a mesh bag and stack into the transport container, which is receiving high water flow and high aeration.
5. It should take approximately 3 h to pack 10,000 abalone into the 167 release devices required.

7.4.3 Loading of Transport Containers

1. Transport containers should be loaded during device packing to avoid double handling.
2. Transport containers must be insulated (cooler box) and would be preferable to have built-in palette base for forklift access (Figure 4.2).
3. Number of transport containers required will depend on their internal volume.
    a. If the container is at least 250 L (100 cm x 50 cm x 50 cm) it can theoretically fit the 167 devices.
    b. However, to provide enough water flow and aeration to the abalone in the devices the containers should not be filled completely with devices.
    c. If this is the case then 2 transport containers should be utilised.
    d. This size transport container is easy to work with and allows for placement on vehicles and vessels.
4. Each transport container requires aeration from two air stones produced by a portable aerator.
    a. Use 12-volt aerators powered from the vehicle’s battery.
5. Transport containers are to be drained and then loaded onto a vehicle.
    a. The vehicle will be dependent on the number of abalone to be released
    b. For 10, 000 abalone a utility or a trailer would be sufficient.
6. The transport containers on the vehicle are then filled with filtered seawater and the aerators turned on to supply heavy aeration.

7.4.4 Transportation of Abalone

1. The vehicle should depart the hatchery and travel during the night to maintain the ambient water temperature and ensure delivery to vessel in the early morning.
    a. This will remove the need to regulate the temperature by incorporating ice blocks into the transport containers if it is hot.

2. Departure time will depend on the distance to be travelled from the hatchery to the fishing grounds being enhanced.
3. Two people would be required for transportation.
4. Stops need to occur on longer journeys (every 2 - 3 h) to check transport containers, aerators and water temperature.
5. Vehicle is to arrive at the vessel launch site at dawn to maximise the available daylight for abalone deployment.

Figure 4.2. An example of a system used to transport abalone in their release devices from a hatchery to the enhancement site, utilising 2 transportation containers.

7.4.5 Loading of Abalone onto Vessels for Release

1. Commercial abalone fishing vessels are trailer-able and should be at the launch site before the vehicle transporting the abalone arrives.
2. Release devices with abalone inside are loaded onto the vessel depending on their holding capabilities.
3. The vessel then launches and travels to the enhancement (release) site.

Vessel Holding Capabilities

1. If the vessel has room the transport container can be loaded directly onto the deck.
2. If the vessel has a live tank, the mesh bags containing the release devices can be removed from the transport container and placed into the tank.
3. Once on the water the seawater system on the vessel is to be turned on to provide the transport container or live tank with high flow through seawater.
4. When neither of these options is available then the mesh bags containing the release devices can be placed on the vessels deck and covered with wet Hessian.
    a. If this is the case an in-water storage system is required once at the enhancement site.

In-water storage system

1. This system requires the release devices to be placed in several large mesh bags and suspended in the water column while deployment is occurring (Figure 4.3).
2. A large weight/anchor is dropped from the vessel with a surface line attached.
3. The large mesh bags with the release devices in are then suspended from the surface line by buoys.
4. When required the vessel pulls alongside the surface line, hooks one of the buoys and pulls the large mesh bags aboard.
5. The release devices can then be removed from large mesh bag and are ready for deployment.
6. Meanwhile the remaining release devices are still within the large mesh bags attached to the surface line and suspended in the water column.

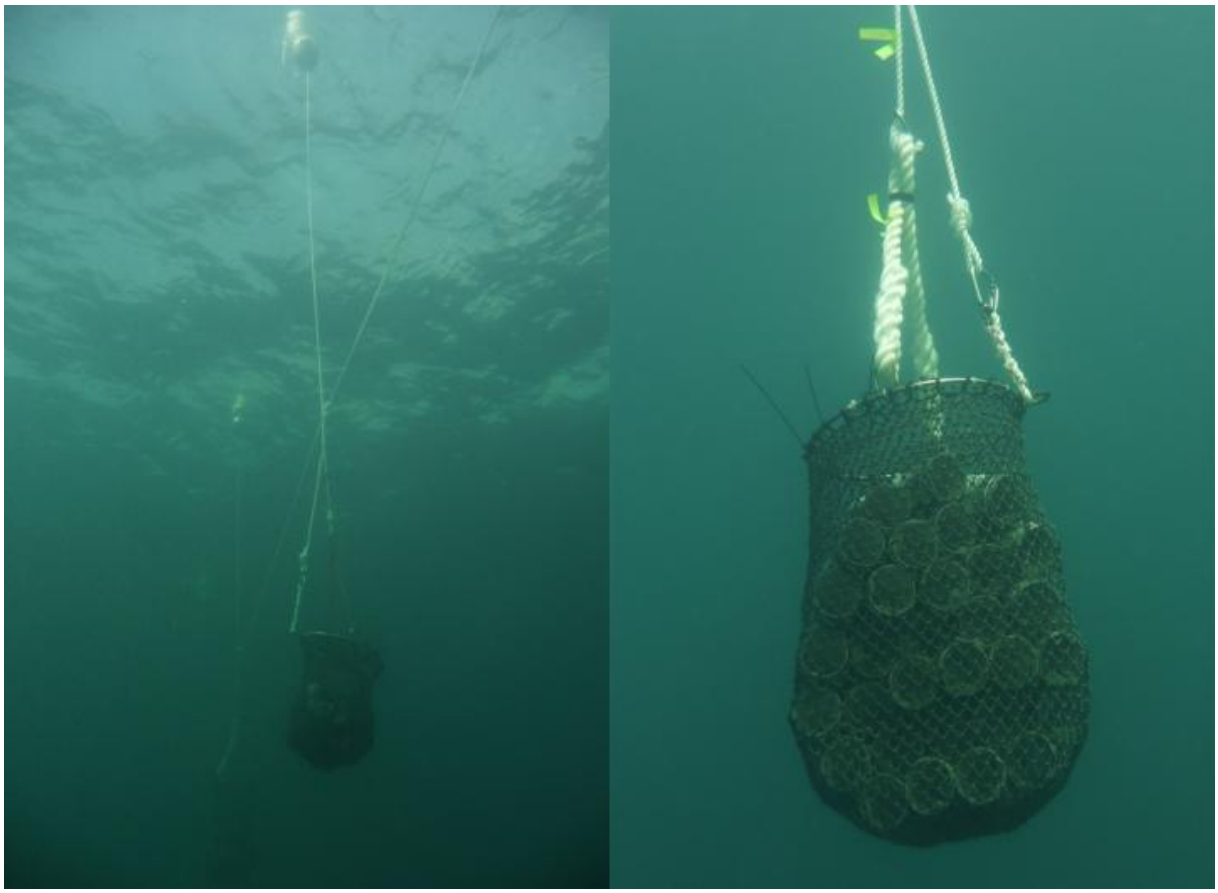

Figure 4.3. Underwater photos of the In-water storage system holding the release devices packed with abalone ready for deployment into natural habitat.

7.5 Field Requirements

These protocols are an overview of the field requirements in conducting an abalone stock enhancement program. These requirements will vary depending on the fishery to be

enhanced, the scale of enhancement and the reason for enhancement. Given the variation in enhancement programs possible these protocols can be scaled accordingly.

The aim of the infield stage is to develop a standardised procedure for the successful deployment of abalone into the natural habitat at appropriate limits. The field requirements assume:

1. Appropriate biological, fisheries and economic data have been collated to inform the field requirements.
2. All areas enhanced are subject to similar ecological responses.
3. A single commercial abalone vessel (7 – 9 m) is used to deploy 10,000 Greenlip Abalone in 1 day.
4. Hatchery and transportation requirements have been completed.

7.5.1 Formulation of Spatial Areas

The boundaries of fishing grounds must be identified prior to enhancement taking place. Firstly, areas with high catches can be identified from annual commercial catches. Then the industry (fishers) must either:

1. Provide the stock enhancement program with GPS marks.
2. Use GPS trackers to enable the enhancement programme to identify spatial boundaries of the area being enhanced.

Option 1 is preferable as all marks can be overlayed and fishing grounds accurately identified and delineated. Option 2 can be more problematic given issues with GPS trackers and the time required in the collection and analysis of the data. Data is to only be handled by the appropriate organisation administering the stock enhancement program, for confidentiality reasons.

The fishing grounds can then be ‘boxed-up’ once they have been identified and the area of each calculated. This area can then be either sampled by a random or even sampling design. An example of a stratified sampling design in a boxed area of a fishing ground is presented in Figure 4.4. Transects are then to be conducted within the boxed area to determine abalone density and the available abalone habitat. These estimates will then inform the enhancement strategies, which can be specifically developed for the targeted areas.

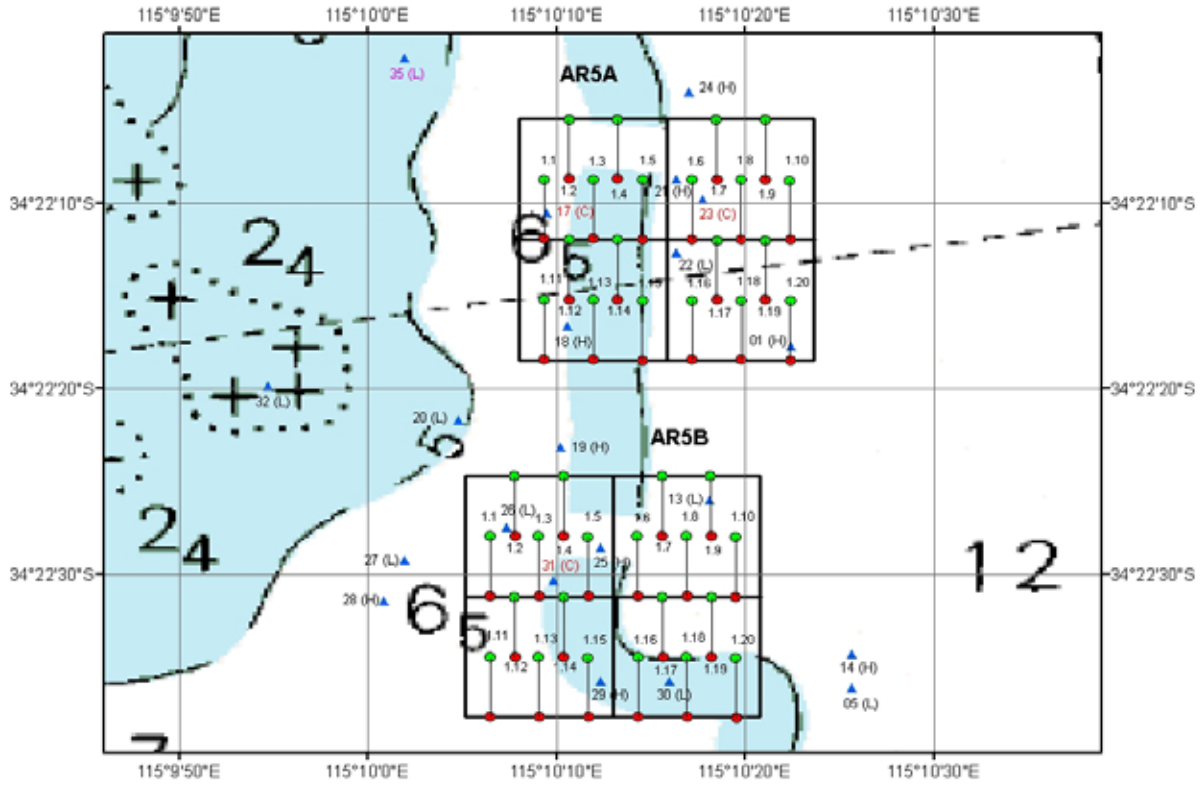

Figure 4.4. Boxed areas of a commercial fishing ground with even spaced transects to determine the abalone density and available abalone habitat.

7.5.2 Release Density and Enhancement Density

To set the release and enhancement densities, knowledge of wild-stock density survival from size at release to minimum fished size, and the available abalone habitat, is required. This information will depend on the length at which the abalone are being fished, as well as their growth and survival. Some common definitions are as follows:

1. Release Density: the number of abalone released per m^2 of available abalone habitat.
2. Enhancement Density: the future target density of legal-size abalone being sought after a certain post-release period (e.g. 3 to 5 years post-release).
3. Wild stock density: the current wild stock density of abalone in the fishery determined from fishery-independent surveys (FIS).
4. Survival: Average cumulative survival of reseeded animals at Age 5 was 13% with a range of 6-20% (Hart et al., 2013a).

A simplified example of determining release and enhancement densities:

1. FIS data indicates an existing wild stock density of 1.5 per m^2 (legal sized abalone), which are assumed to be stable.
2. The enhancement density being sought is 3 per m^2 (an increase of 1.5 per m^2)

3. Using a cumulative predicted survival of 13% over 4 years, a release density of 14 per m^2 will achieve a survival of 2.1 per m^2. When added to the existing stock of 1.5 per m^2, an enhancement density of 3.1 per m^2 shall be achieved.

These basic estimations can be utilised but a much more detailed interpretation of release densities and enhancement targets is presented in a bioeconomic analysis in Hart et al. (2013c) (Chapter 3).

7.5.3 Target Enhancement Level (TEL)

The target enhancement level is the total number of abalone to be released. It depends on the total area of abalone habitat being targeted and how many year-classes are being replaced. It must be explicitly defined for each enhancement event, as it determines the release density.

An example for calculating the TEL based on replacing the annual harvest from the Augusta Greenlip Abalone fishery in Western Australia with stock enhanced abalone:

1. To replace the current mean annual harvest of Augusta (80,000 animals), a release of between 530,000 (15% survival) and 800,000 (10% survival) is required.
2. Augusta catch is fished from two areas: (1) Outback (80% - 64,000 animals) and (2) Flinders Bay (20% - 16,000 animals).
3. Assuming 500,000 are available for release, hypothetical target enhancement levels are 100,000 (Flinders Bay) and 400,000 (Outback).
4. Estimated habitat area in the Augusta fishery is between 270,000 m^2 (27 hectares) and 400,000 m^2 (40 hectares). This is ascertained by dividing the annual number harvested (80,000) by the fished density (0.2 – 0.3 per m^2).
5. Assume that 4 vessels will be available to release animals at a rate of 10,000 per vessel day (40,000 per day). At this rate, Flinders Bay release will take 2.5 days, and Outback release will take 10 days.

These individual area-release targets will depend on the GPS information obtained from industry divers and the precise abalone habitat and abalone density determined from the pre-enhancement surveys. This would allow the TEL to be based on the actual available abalone habitat, its carrying capacity and spawning biomass, not the annual harvest levels. Therefore, enhancement programs should be developed based on the ecological process occurring in the natural population and not the annual harvest by the commercial fishery.

7.5.4 Abalone Deployment onto Seabed (HDI)

Habitat Deployment Identification (HDI): is the search technique, which divers will be utilising to locate suitable habitat and complete their releases. It is an extremely important component of the enhancement program as habitat and site selection has been shown to significantly affect initial release survival (Hart et al., 2013b). This will in turn affect the number of abalone surviving to a harvestable size. At a commercial-scale such variation in survival has significant economic impacts, therefore site selection and deployment of abalone onto the seabed is critical.

Abalone deployment for all divers will follow a set protocol and should not be deviated from regardless of their experience. If there is an issue during deployment it is best to stop, contact the person in operational control of the field requirements and formulate a solution. Husbandry of the abalone while on the vessel and during deployment is paramount, in order to reduce stress and mortality of the abalone, while also aiding survival once released. Divers must work effectively and efficiently to deploy the release devices in a timely manner while still following the HDI to release the abalone into the best possible habitat.

Deployment Protocol

1. Identify personnel and vessels to undertake enhancement.
2. Ensure that the bio-security policy has been implemented (vessels and dive gear appropriately disinfected prior to use).
3. Ensure that personnel are briefed in their area-specific release targets and release density.
4. Once divers are briefed and release devices are loaded onto the vessel, the vessel proceeds to the enhancement sites.
    a. If an In-water storage system is utilised, it must be deployed as soon as the vessel reaches the enhancement site.
5. Vessel and diver operation during deployment should be similar to the procedures of commercial fishing for abalone.
    a. The skipper of the vessel locates the site by GPS mark.
    b. A diver on Surface Supply Breathing Apparatus (SSBA) descends to the bottom.
    c. Instead of harvesting abalone the diver is enhancing the abalone stocks.
6. Divers should be able to accommodate up to 20 release devices at one time.
7. The diver then begins deploying the devices into the abalone habitat on the seabed following the HDI.
8. Once the diver has deployed their first lot of release devices, they send their bag to the surface and the skipper or deckhand reloads the bag with devices and sends it back down to the diver to continue.
9. This is repeated until all release devices have been deployed.
10. Release devices to be collected at a later date following successful migration of abalone into natural habitats.

All divers must follow dive plans and adhere to dive tables or computers to monitor their diving. Occupational health and safety is of the up most importance and must be adhered to. Depending on the depth of the enhancement sites, two divers may deploy the release devices from a single vessel to enable all 10,000 abalone to be deployed, while the divers stay within their dive plans.

Habitat Deployment Identification (HDI)

This search technique is dependent on the available abalone habitat, number of abalone in the release device and release density utilised.

1. In this deployment each release device contains 60 abalone.
2. Assuming a cumulative mortality of 85 - 90% only 6 - 9 abalone will survive to harvestable size.
3. These 6 - 9 abalone that may reach harvestable size, must be placed over 3 m^2 of abalone habitat to achieve a release density of 2 – 3 abalone per m^2 abalone habitat.
4. Staff must be able to:
    a. Find existing adult abalone stock.
    b. Ask the question: can this piece of habitat support between 6 and 9 adults of legal size? This may involve mentally dividing the habitat into abalone habitat of 3 m^2 areas.
    c. Find a suitable cryptic spot (juvenile habitat = boulders/stones, cracks, etc.) in each 3 m^2 of habitat to place a release device that will allow the juvenile abalone protection to survive.
    d. Ensure release device is protected and anchored down (e.g. place rocks on top if necessary).
    e. Remove the flyscreen mesh end/s to allow the abalone to move out of the release device.
5. Deployment of abalone device into natural habitat is shown in Figure 4.5.

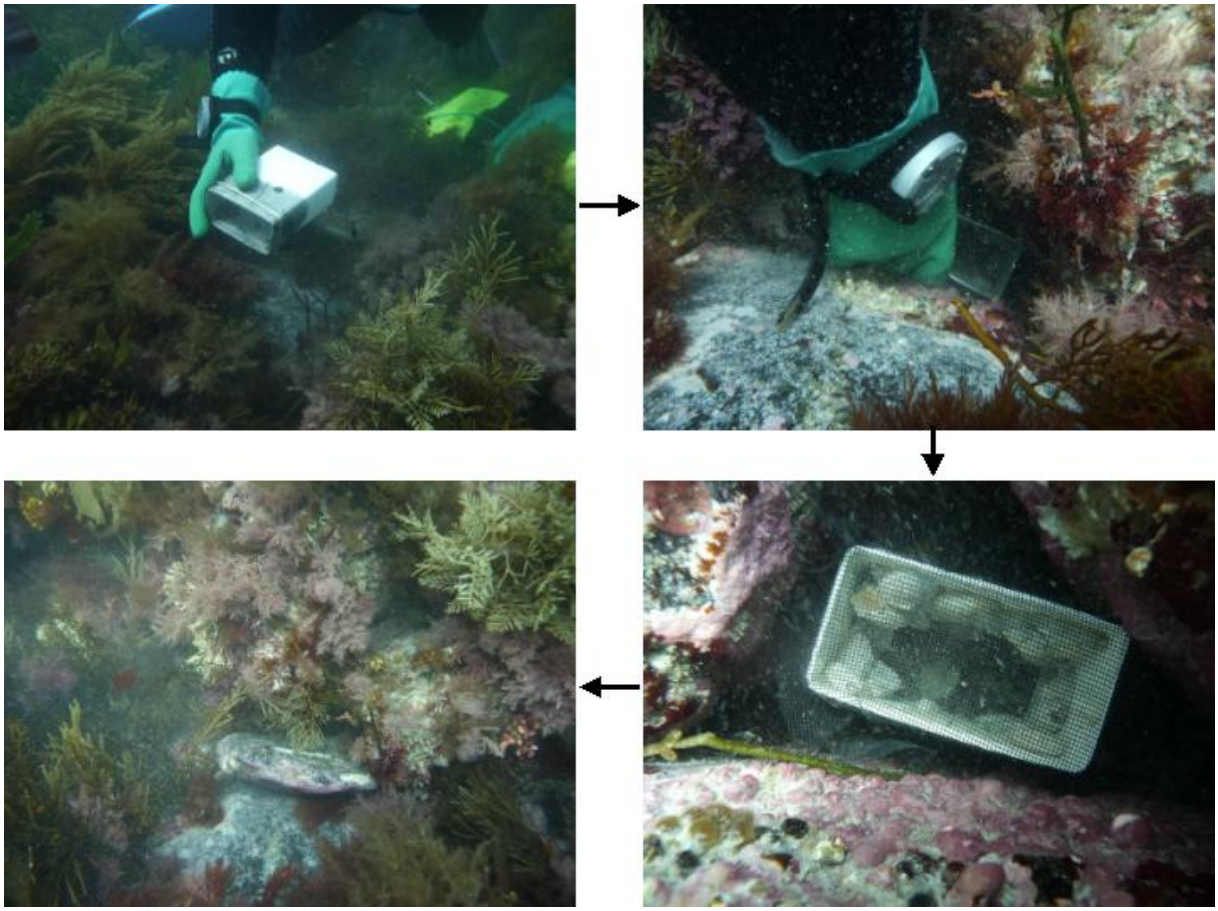

Figure 4.5. Deployment of a release device containing abalone into the natural habitat. It shows the abalone habitat being identified, the release device positioned and then the protection of the release device.

7.5.5 Baseline Fisheries Independent Surveys (FIS)

These surveys are used to provide a base line measurement of the abalone density and size frequency in the natural populations before stock enhancement occurs. They will provide detailed information on the effects of enhancement on the environment over time (Hart et al., 2013b).

1. Complete FIS surveys (prior to placement of release devices) based on a stratified sampling regime (within delineated fish grounds) using random and fixed sites.
2. Post-release FIS surveys (annually) will need to be completed to determine whether a change in densities of abalone can be detected.
3. FIS surveys will be used to predict future harvest-size stock levels, based on abundance of 2, 3, and 4 year olds, so that TACC's can be matched to the enhanced cohorts.

The survey method for the Fishery Independent Surveys will follow standardised protocols appropriate for the species. Protocols for Greenlip Abalone in Western Australia are outlined in Hart et al. (2013b) and include:

1. Each site has two transects performed.

2. Each transect is 30 m long radiating out from a fixed point on a specified bearing.
3. Transects contain 30 x 1 m quadrants.
4. The diver swims the length of the transect and measures all abalone within the quadrants.
5. Divers also recorded a habitat category for each quadrant.
    a. The quantifiable amount of abalone habitat.

7.5.6 Industry Workshops

1. Workshops for the aquaculture industry (hatchery managers and technicians) will cover:
    a. Schedule of stock enhancement.
    b. This training manual and how to use it effectively.
    c. Spawning schedules and required size and number of abalone.
    d. Bio-security protocols and how to adapt the hatcheries protocols to include a stock enhancement program.
    e. Spawning, nursery and grow-out protocols.
    f. Harvesting abalone and packing into the release devices.
    g. Transportation requirements.
2. Workshops for the fishing industry (licensees, divers, quota owners) will cover:
    a. Schedule of stock enhancement.
    b. This training manual and how to use it effectively.
    c. Release area determination processes and release area division.
    d. Release density and enhancement density concepts.
    e. Method for loading vessels.
    f. Husbandry of abalone on board vessels.
    g. HDI (Habitat Deployment Identification) concepts – Discuss and provide video/photos of target habitat (fishing habitat and juvenile habitat) and methods to secure and ‘activate’ release devices.
    h. Explore logistics for integrating commercial fishing with enhancement equipment.
    i. Predator control.

7.6 References

Daume S. and S. Ryan. Nursery culture of the abalone Haliotis laevigata: larval

settlement and juvenile production using cultured algae or formulated feed. Journal of Shellfish Research, 23: 1019-1026. (2004).

Daume S. Improvement and evaluation of greenlip abalone hatchery and nursery production. Final report to Fisheries Research and Development Corporation on Project No. 2003/203. Fisheries Research Contract Report No. 16, Department of Fisheries, Western Australia, 160p. (2007).

Daume S., M. Davidson, S. Ryan and F. Parker. Comparisons of rearing systems based on algae or formulated feed for juvenile greenlip abalone (Haliotis laevigata). Journal of Shellfish Research, 26: 729-735. (2007)

DoF. Policy on restocking and stock enhancement in Western Australia. Fisheries Management Paper [Western Australia] No. 261 2013; 8p. http://www.fish.wa.gov.au/About-Us/Publications/Pages/Fisheries-Management-Papers.aspx

Hart, A. M., L. W. S. Strain, F. P. Fabris, J. Brown, and M. Davidson. Stock enhancement in greenlip abalone part I: long-term growth and survival. Rev. Fish. Sci. 21(3-4): 299-309 (2013a).

Hart, A. M, F. P. Fabris, L. W. S. Strain, M. Davidson, and J. Brown. Stock enhancement in greenlip abalone part II: population and ecological effects. Rev. Fish. Sci. 21(3-4): 310-320 (2013b).

Hart A.M., L.W.S. Strain and A. Hesp. Stock enhancement in greenlip abalone part III: bioeconomic evaluation. Reviews in Fisheries Science, 21(3-4): 354-374 (2013c).

Strain L.W.S., M.A. Borowitzka and S. Daume. Growth and survival of juvenile greenlip abalone (Haliotis laevigata) feeding on germlings of the macroalgae Ulva sp. Journal of Shellfish Research, 25: 239-247. (2006).

Strain L.W.S. Alternative macroalgal diets for juvenile greenlip abalone (Haliotis laevigata) in the later nursery phase. PhD Thesis, Murdoch University, Perth, Western Australia. 206 pp. (2012).

8 Stock Enhancement in Greenlip Abalone: (5) Population Genomics.

Jonathan Sandoval-Castillo[2], Nick Robinson[3], Lachlan W. S. Strain[1], Anthony M. Hart[1], Luciano B. Beheregaray[2].

[1]Western Australian Fisheries and Marine Research Laboratories, PO Box 20, North Beach, Western Australia, 6920, Australia.

[2]Flinders University, Sturt Road, Bedford Park, South Australia, 5042, Australia.

[3]Nofima, PO Box 5010, 1432 AS, Norway.

Please note that this chapter has been published in greater detail in the following report:

Sandoval-Castillo J, Strain L, Hart A, Robinson N, Beheregaray LB (2015) *Use of next generation DNA technologies for revealing the genetic impact of fisheries restocking and ranching*. The Seafood CRC Company Ltd, the Fisheries Research and Development Corporation. Adelaide, Flinders University.

8.1 Abstract

New diagnostic genomic tools were developed to study natural population genetic structure and monitor the success of stock enhancement in a commercial Greenlip abalone fishery within Western Australia. Samples from 372 Greenlip abalone collected from 13 locations from across the WA fishery were analysed using the new tools, and produced 69,720 high quality genomic markers in the form of SNP's (single nucleotide polymorphisms). The screening of genome-wide variation in samples collected from the wild show 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, five genetically distinct groups of populations for Greenlip abalone were identified. These corresponded to geographic regions characterised by differences in oceanography. Significant associations between the distribution of these adaptive groups and the spatial variation of key environmental parameters, including differences in temperature and oceanographic variables were found. The project (CRC 2012/714) provided an outstanding resource and detailed base knowledge that will assist the management of abalone fisheries, stock enhancement and aquaculture in Australia. Firstly thousands of DNA markers were identified and characterised; these markers will be useful for monitoring the genetic health of stocks. Second, high genetic connectivity was detected across the sampling area, but more than one adaptive group were detected with this finding to help managers select which abalone populations are likely to perform best in specific environments (i.e. likely fitness), consequently improving the chances of successful stock enhancement programs.

Keywords: Greenlip Abalone, *Haliotis laevigata*, genomics, population genetic structure, Genotyping-By-Sequencing, double digest RAD-seq, stock enhancement.

8.2 Introduction

Stock enhancement has been examined as a restocking and fisheries management tool in abalone producing countries with varying degrees of success (e.g. Campbell, 2001; Hammasaki and Kitada, 2008). Generally the enhancement program is assessed through some form of tag recapture study that can estimate what proportion the hatchery-reared released animals are of the total population (hatchery and wild). These studies provide information on biological parameters including growth, release mortality, long-term survival, habitat identification, etc. (Roberts et al., 2007; Hart et al., 2013a,b), while an understanding of the carrying capacity and ecological responses to enhancement has been recognised as an important factor for success (Hillborn, 1998; Bell et al., 2005; Hammasaki and Kitada, 2008).

Stock enhancement programs have potential negative genetic effects on population fitness (Waples, 1994), while it is only in the last few decades that attention has been given to these genetic effects and genetics as a tool to monitor the success of enhancement. There is a fundamental need to understand the population genetic dynamics of enhanced populations and the extent of genetic interactions of enhanced and wild animals. Important considerations for the enhanced population are the effects on inbreeding or loss of genetic diversity, recruitment and geographic spread, while the genetic health of individuals used as broodstock and the genetic structuring of source populations play a considerable role in maintaining the genetic fitness of enhanced populations.

Genetic studies are generally lengthy and expensive processes and the cost of genotyping limits the number of loci and animals that can be genotyped for the study, and hence the power of the studies' ability to discriminate effects. However, genomic technologies are rapidly developing and in the last few years a major leap forward in the ability to sequence whole genomes has occurred. Sequencing costs have reduced, accuracy and throughput has improved, and the ability to analyse the terabytes of data produced from a single sequencing run have greatly improved. One powerful technique that has recently emerged has become generally known as Genotyping-By-Sequencing (GBS). GBS uses next generation sequencing where the DNA of individuals is bar-coded such that the SNP (single nucleotide polymorphisms genotypes of these individuals at many different loci can be called direct from the sequence data. Variants of GBS include restriction associated DNA sequencing or RAD-seq (Baird et al., 2008) and double digest RAD-seq (Peterson et al., 2012). Both these techniques involve producing a “reduced representation” DNA library from the genome for genotyping by sequencing purposes (Altshuler et al., 2000). RAD-seq employs restriction enzymes to reduce genome complexity, which greatly simplifies analysis for species with high levels of genetic diversity.

GBS is proving to be quick, extremely specific and highly reproducible, and is finding applications in population genetics, phylogenetics and quantitative genetics (e.g. Cariou et al., 2013; Richards et al., 2013; Xu et al., 2014), including application to commercially important species in the northern Hemisphere such as crops and salmonid fishes (Gagnaire et al., 2013). This study in collaboration with two other Seafood CRC projects (2012/714 and 2011/762) developed GBS (RAD-seq) as a diagnostic tool to study natural population genetic diversity

and structure to assist in examining the potential for a commercial-scale stock enhancement program in the Western Australian Greenlip Abalone fishery.

8.3 Material and Methods

8.3.1 Sample Collection

Greenlip Abalone were collected from 13 locations along the south coast of Western Australia. These locations covered the entire Western Australian Greenlip Abalone fishery and were distributed between all 8 management sub-areas (Figure 5.1 and Table 5.1). The abalone were collected via commercial fishing practices and processed on board a research vessel directly after collection. At all locations, 35 Greenlip Abalone of approximately the same age class (mature animals, 140 – 160 mm shell length) were collected from within 100 m of each other. The genomic samples taken were a small segment of abductor muscle tissue (» 5 mm^3) extracted from each animal and placed in a 2 mL vial full of 100 % ethanol complete with label (i.e. Location, Date, GPS, Number, etc.), then the vials stored in a freezer. For high quality preservation before analysis the ethanol was replaced several times during storage in the freezer. After processing in-situ and storage the extracted samples were sent to Flinders University, South Australia for genomic analysis (collaborative project CRC 2012/714). During genomic analysis not all of the samples per location were utilised and the final number of samples analysed (372 samples total) can be seen in Table 5.1.

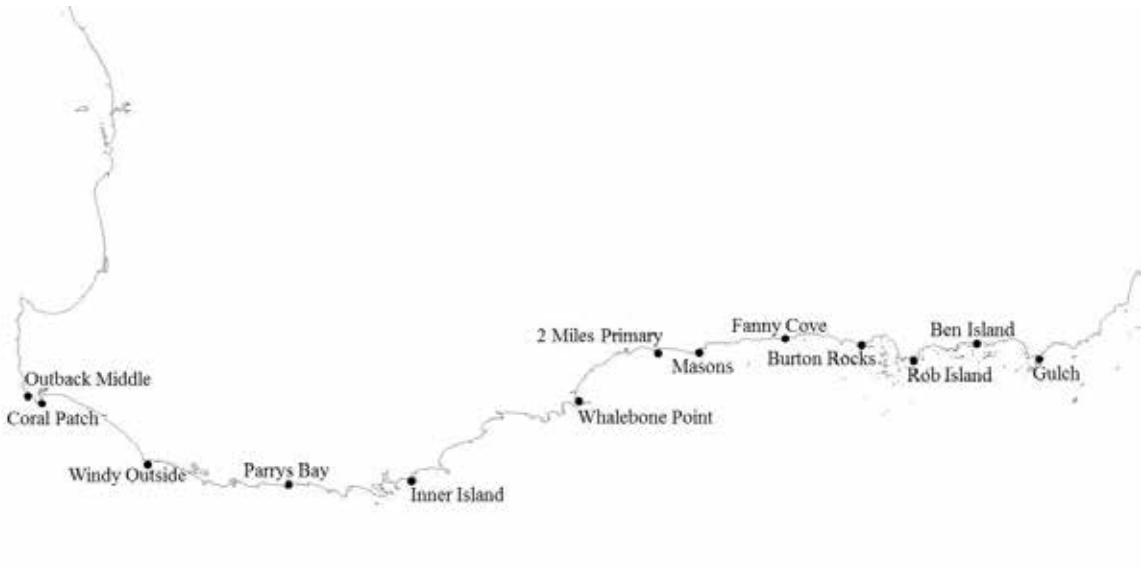

Figure 5.1. Greenlip Abalone sampling locations covering eight sub-areas in the two main management areas of the commercial Greenlip Abalone fishery in Western Australia (source CRC 2012/714 Final Report).

Table 5.1. Greenlip Abalone sampling locations, location abbreviations and genomic sample size per location (source CRC 2012/714 Final Report).

Location	Abbreviation	Sample (n)
Outback Middle	OM	29
Coral Patch	CP	28
Windy Outside	WO	29
Parrys Bay	PB	29
Inner Island	II	29
Whalebone Point	WP	29
2 Mile Primary	MP	28
Masons	MS	29
Fanny Cove	FC	28
Burton Rocks	BR	29
Rob Island	RI	28
Ben Island	BI	29
Gulch	GL	28

8.3.2 Laboratory Protocols

Genomic DNA was extracted from the Greenlip Abalone samples using a modified salting out method. These samples were examined using an improved version of the original RAD protocol (Figure 5.2, described in the collaborative project CRC 2012/714) known as the double digestion RAD-seq (ddRAD-seq) method (Peterson et al., 2012), which includes a restriction digest with two enzymes simultaneously (SbfI and MseI). This protocol eliminates the random shearing and introduces a precise selection of genomic fragments by size. The completed libraries (372 samples of Greenlip Abalone, Table 5.1) 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 4.25TB (4,250 GB) of RAM – supercomputers are needed to handle the analysis of the large ddRAD-seq dataset.

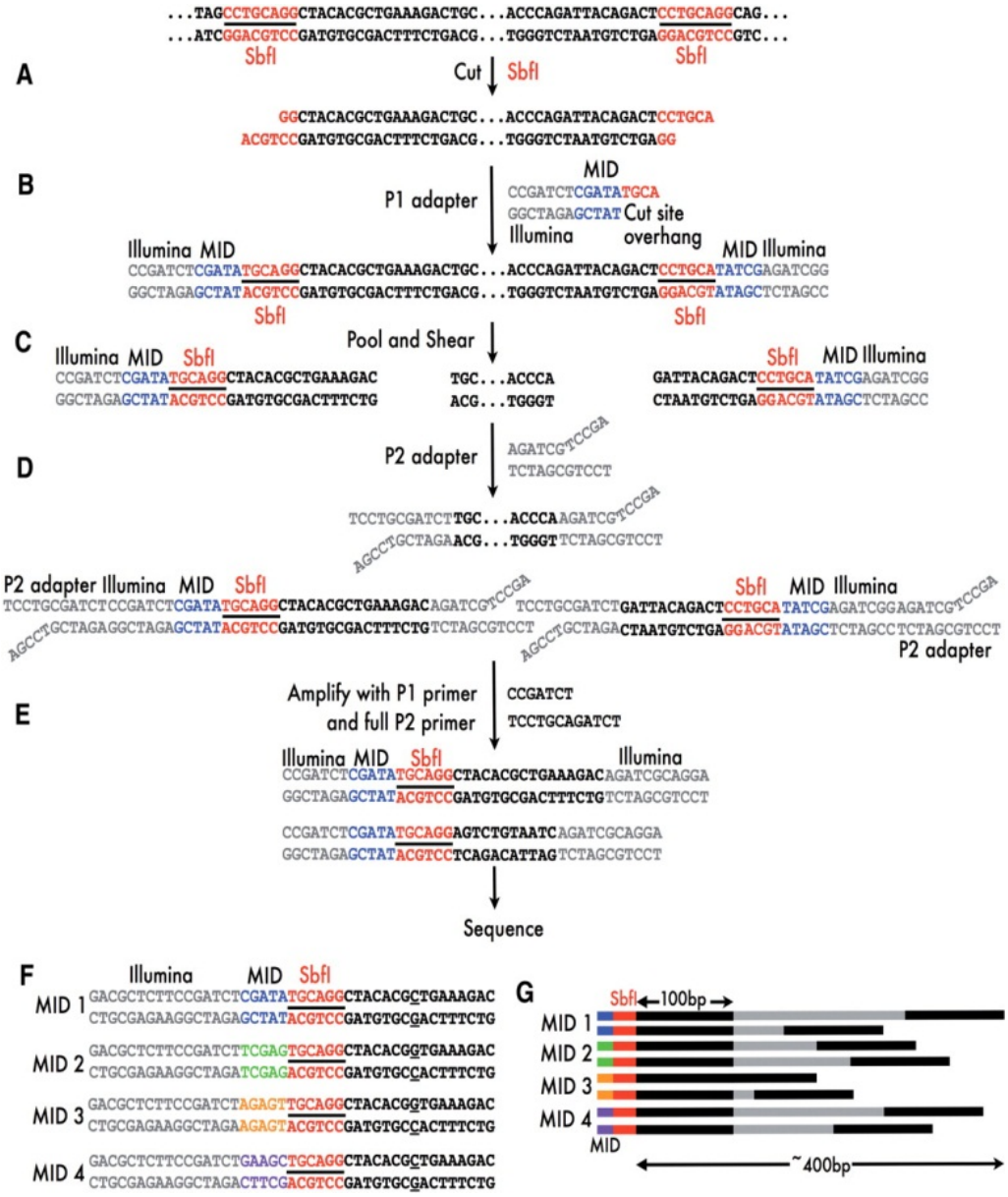

Figure 5.2. A summary of the RAD-seq process (Davey and Blaxter, 2010) (source CRC 2012/714 Final Report).

8.3.3 Data Analysis

8.3.3.1 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 organism's ability to adapt to new or changing environments.

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.

8.3.3.2 Genomic analysis

The genetic diversity within locations and the genetic differentiation between locations 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 locations 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 five groups detected by STRUCTURE and ADEGENET.

8.3.3.3 Seascape Analysis

Data for four oceanographic variables (sea surface temperature, oxygen concentration, pH, and nutrient concentration) for the last 100 years were obtained from the NOAA World Ocean Data Base Website

(http://www.nodc.noaa.gov/OC5/SELECT/dbsearch/dbsearch.html, Table 5.2). 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 sea surface temperature (Table 5.2). To illustrate environmental variation between sampling sites we performed a principal 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 Greenlip 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} ($F_{ST}/1\text{-}F_{ST}$) 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 we used locality allele frequencies as response variables and the locations specific oceanographic attributes as explanatory variables. Also, a partial CCA was performed using the coordinates.

Table 5.2. Estimated annual average of the five oceanographic variables (source CRC 2012/714 Final Report).

Location	pH	Nutrients	Oxygen	SST	Max SST
Outback Middle	8.25	3.17	5.21	20.01	21.55
Coral Patch	8.25	3.37	5.17	19.92	21.49
Windy Outside	8.25	3.27	5.20	19.47	20.66
Parrys Bay	8.24	2.81	5.14	19.76	20.32
Inner Island	8.24	3.06	5.15	19.40	20.32
Whalebone Pt	8.22	2.59	5.12	18.92	21.03
2 Mile Primary	8.26	2.66	5.47	18.79	21.08
Masons	8.28	2.71	5.44	18.53	20.88
Fanny Cove	8.28	2.64	5.38	18.58	20.52
Burton Rocks	8.28	2.77	5.38	18.63	20.38
Rob Island	8.32	3.01	5.37	18.69	20.29
Ben Island	8.31	2.91	5.38	18.79	20.48
Gulch	8.32	3.03	5.39	18.69	20.59

8.4 Results

From the eight paired-end Illumina lanes ran for the Greenlip Abalone samples, »3 billion DNA sequence reads were obtained (i.e. a total of »300 billion base pairs of DNA data were generated). After filtering the reads, over one billion SbfI RADtags were recognised, of which approximately one million were unique sequences. For the Greenlip Abalone samples a total of 69,720 SNPs were obtained, from which 18,803 SNPs were selected. These SNPS were bi-allelic and had a coverage depth of over 4 times in at least 80% of the sequenced individuals.

8.4.1 Overall genetic diversity

The levels of genetic diversity in Greenlip Abalone were very similar across all locations, with marginally higher values for the eastern most locations (Table 5.3). We found no evidence pointing to reductions of genetic diversity, as would be expected if the fishery for this species was overexploited.

8.4.2 Categorising loci – neutral and adaptive variation

We detected 1,026 outlier loci for Greenlip Abalone with a proportion of outliers to the scanned loci of »5.4 %. Subsequent analyses were conducted for the entire dataset (18,803 SNPs), the "outlier" dataset (1026 SNPs_*adaptive variation*) and the "neutral" dataset (17,777 SNPs_*neutral variation*).

Table 5.3. Levels of genetic diversity for Greenlip Abalone from the thirteen sampled locations. π=nucleotide diversity, He = expected heterozygosity, PL = percentage of polymorphic loci (source CRC 2012/714 Final Report).

Location		π	He	% PL
Outback Middle	OM	0.19	0.26	0.7448
Coral Patch	CP	0.19	0.27	0.7505
Windy Outside	WO	0.17	0.28	0.7447
Parrys Bay	PB	0.20	0.27	0.7739
Inner Island	II	0.19	0.27	0.7570
Whalebone Pt	WP	0.20	0.27	0.7677
2 Mile Primary	2MP	0.20	0.27	0.7682
Masons	MS	0.18	0.26	0.7483
Fanny Cove	FC	0.23	0.29	0.7851
Burton Rocks	BR	0.21	0.27	0.8034
Rob Island	RI	0.20	0.26	0.7762
Ben Island	BI	0.19	0.25	0.7777
Gulch	GL	0.17	0.28	0.7402

8.4.3 Genetic Differentiation

Neutral variation (for estimates of population connectivity)

Levels of genetic differentiation for Greenlip Abalone ranged from low to nil between most locations for both the entire and the neutral datasets. The STRUCTURE and ADEGENET methods both supported the existence of a single population based on both the entire SNP and the "neutral" datasets (Figure 5.4A,B and Figure 5.5A,B). Maximum difference in diversity between populations was 2% (FST = 0.02; Fig 5.8a). Average distance was less than 1% (Figure 5.8b).

Adaptive variation (ability to adapt to new environments)

For the "adaptive variation" dataset the levels of differentiation ranged from low to high (Table 5.4, Table 5.5, Table 5.6 and Figure 5.3). This dataset suggested the existence of at least five differentiated adaptive groups (Figure 5.3C, Figure 5.4C,D and Figure 5.5C): 1) the western part of the Greenlip Abalone distribution (from Outback to Windy Outside); 2) the Albany sub-area (Parrys Bay and Whalebone Port); 3) the Hopetoun sub-area (from Inner Island to Mason); 4) the West sub-area (Fanny Cove and Burton Rocks); and 5) the eastern sampling area (from Rob Island to Gulch) (Figure 5.6 and Figure 5.7). Environmental variables, rather than coastal distance are the main reasons for this differentiation (see section 5.4.4).

8.4.4 Isolation by distance

Neutral variation

Isolation by distance was only found in the "neutral" dataset (p=0.01; Figure 5.8b).

Table 5.4. *Overall genetic diversity*: genetic differentiation between samples of Greenlip Abalone from thirteen locations based on 18,803 SNPs. F_{ST} values in bold are significant (p<0.001) (source CRC 2012/714 Final Report).

	OM	CP	WO	PB	WP	II	2MP	MS	FC	BR	RI	BI	GL
OM	0.000												
CP	0.000	0.000											
WO	0.001	0.000	0.000										
PB	0.002	0.001	0.000	0.000									
WP	0.007	0.006	0.006	0.000	0.000								
II	**0.016**	**0.016**	**0.013**	0.004	0.003	0.000							
2MP	**0.015**	**0.015**	0.012	0.002	0.003	0.000	0.000						
MS	**0.012**	**0.013**	**0.016**	0.000	0.001	0.000	0.000	0.000					
FC	**0.014**	0.012	0.000	0.004	0.012	**0.017**	**0.014**	0.012	0.000				
BR	0.007	0.004	0.000	0.000	0.004	**0.014**	0.009	0.007	0.000	0.000			
RI	0.007	0.007	0.004	0.000	0.003	0.010	0.007	0.006	0.001	0.000	0.000		
BI	0.007	0.007	0.009	0.001	0.004	0.011	0.008	0.007	0.004	0.001	0.000	0.000	
GL	0.007	0.005	0.009	0.000	0.001	0.009	0.006	0.010	0.000	0.000	0.000	0.000	0.000

Table 5.5. ***Neutral variation:*** **genetic differentiation between samples of Greenlip Abalone from thirteen location based on 17,777 "neutral" SNPs. F_{ST} values in bold are significant (p<0.001) (source CRC 2012/714 Final Report).**

	OM	CP	WO	PB	WP	II	2MP	MS	FC	BR	RI	BI	GL
OM	0.000												
CP	0.000	0.000											
WO	0.000	0.000	0.000										
PB	0.000	0.000	0.000	0.000									
WP	0.002	0.001	0.000	0.000	0.000								
II	0.003	0.002	0.000	0.000	0.000	0.000							
2MP	0.004	0.003	0.000	0.000	0.000	0.000	0.000						
MS	0.002	0.001	0.004	0.000	0.000	0.000	0.000	0.000					
FC	**0.009**	**0.008**	0.000	0.000	0.004	0.000	0.000	0.000	0.000				
BR	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
RI	0.004	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
BI	0.003	0.003	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
GL	0.002	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 5.6. ***Adaptive variation:*** **of genetic differentiation between samples of Greenlip Abalone from thirteen locations based on 1,026 "outlier" SNPs. F_{ST} values in bold are significant (p<0.001) (source CRC 2012/714 Final Report).**

	OM	CP	WO	PB	WP	II	2MP	MS	FC	BR	RI	BI	GL
OM	0.000												
CP	0.016	0.000											
WO	**0.046**	0.031	0.000										
PB	**0.054**	**0.057**	**0.064**	0.000									
WP	**0.072**	**0.080**	**0.099**	0.011	0.000								
II	**0.185**	**0.203**	**0.233**	**0.117**	**0.082**	0.000							
2MP	**0.157**	**0.169**	**0.189**	**0.082**	**0.060**	0.040	0.000						
MS	**0.149**	**0.165**	**0.186**	**0.074**	**0.054**	0.037	0.012	0.000					
FC	**0.089**	**0.076**	**0.051**	**0.092**	**0.125**	**0.260**	**0.204**	**0.200**	0.000				
BR	**0.068**	**0.053**	0.042	**0.066**	**0.101**	**0.237**	**0.184**	**0.182**	0.012	0.000			
RI	**0.055**	**0.061**	**0.077**	**0.058**	**0.067**	**0.182**	**0.145**	**0.137**	**0.058**	0.045	0.000		
BI	**0.059**	**0.068**	**0.084**	**0.054**	**0.061**	**0.161**	**0.128**	**0.116**	**0.066**	**0.058**	0.021	0.000	
GL	**0.072**	**0.078**	**0.088**	**0.064**	**0.070**	**0.178**	**0.143**	**0.138**	**0.071**	**0.058**	0.029	0.019	0.000

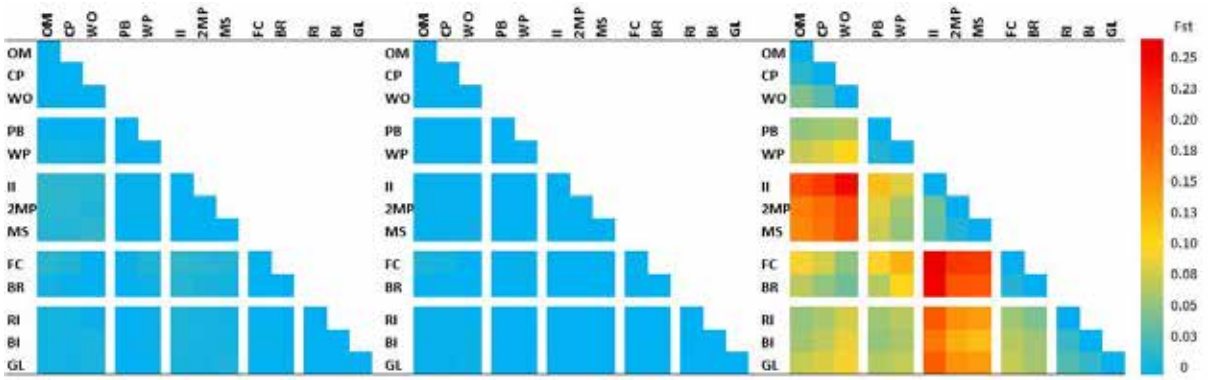

Figure 5.3. Matrix of pairwise genetic differentiation (F_{ST}). Results based on 18,803 SNPs (entire dataset) (A) Results based on 17,777 "neutral" SNPs_*neutral variation* (B); Results based on 1,026 outlier SNPs_*adaptive variation* (C) (source CRC 2012/714 Final Report).

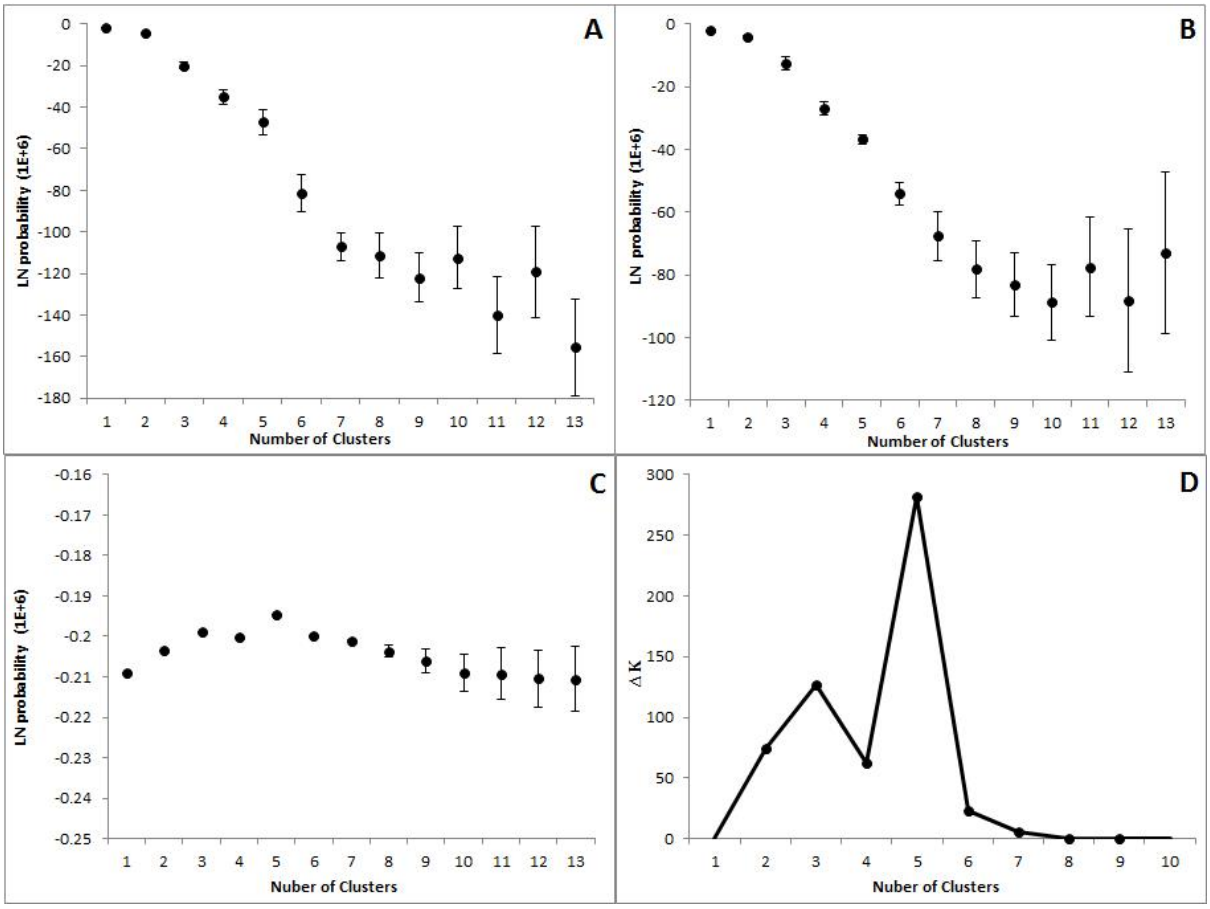

Figure 5.4. STRUCTURE probability of the data as a function of the number of population clusters (A,B,C); and magnitude of ΔK as a function of number of clusters (D). Results shown for the three data sets: all 18,803 SNPs (A); 17,777 "neutral" SNPs (B); 1,026 "outliers" (C,D). When the highest probability 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).

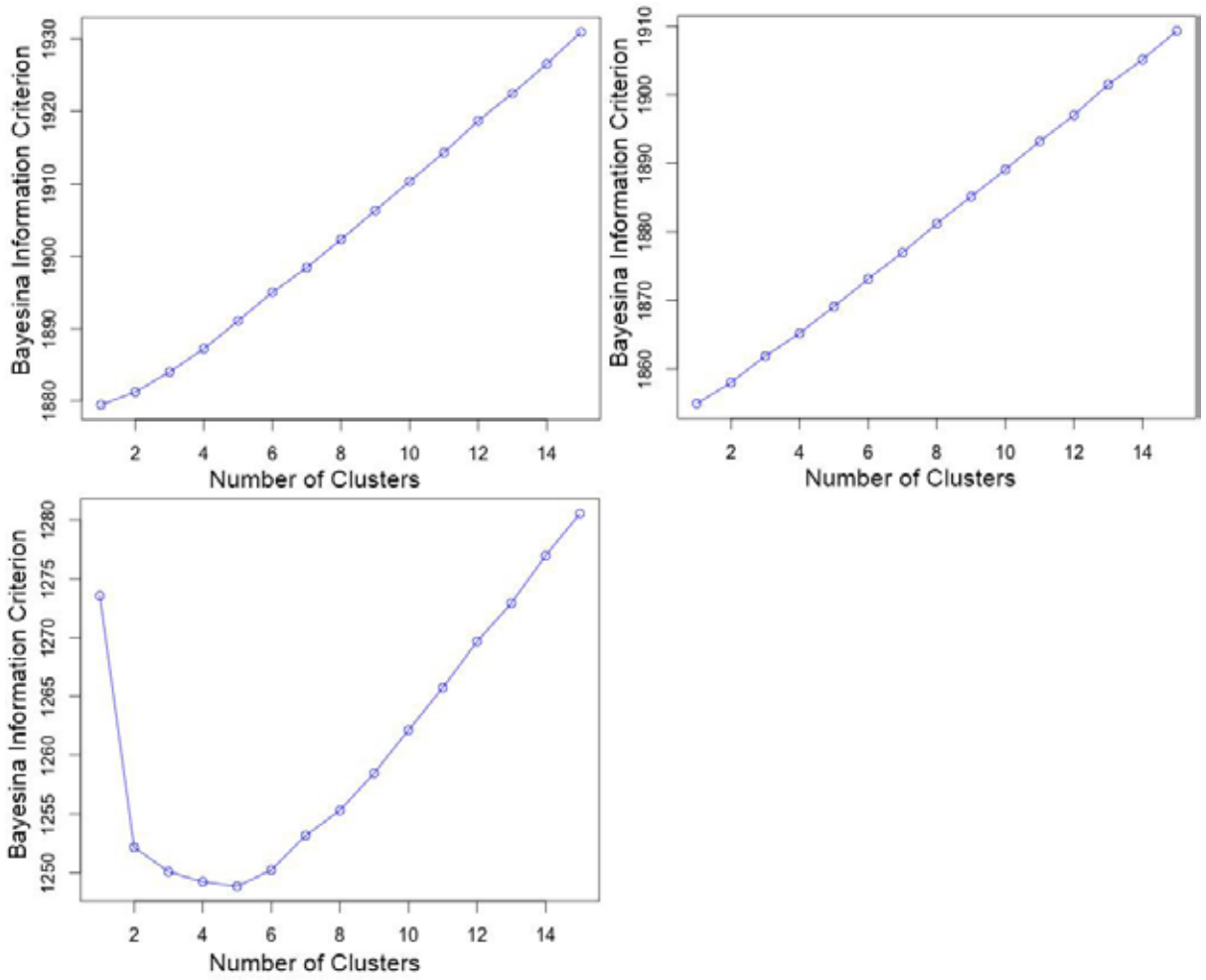

Figure 5.5. ADEGENET Bayesian Information Criterion as a function of number of clusters: using all the 18,803 SNPs (A); using 17,777 "neutral" SNPs- *neutral variation* (B); using 1,026 "outliers" SNPs- *adaptive variation* (C). Ideally, optimal clustering solution should correspond to the lowest Bayesian Information Criterion (source CRC 2012/714 Final Report).

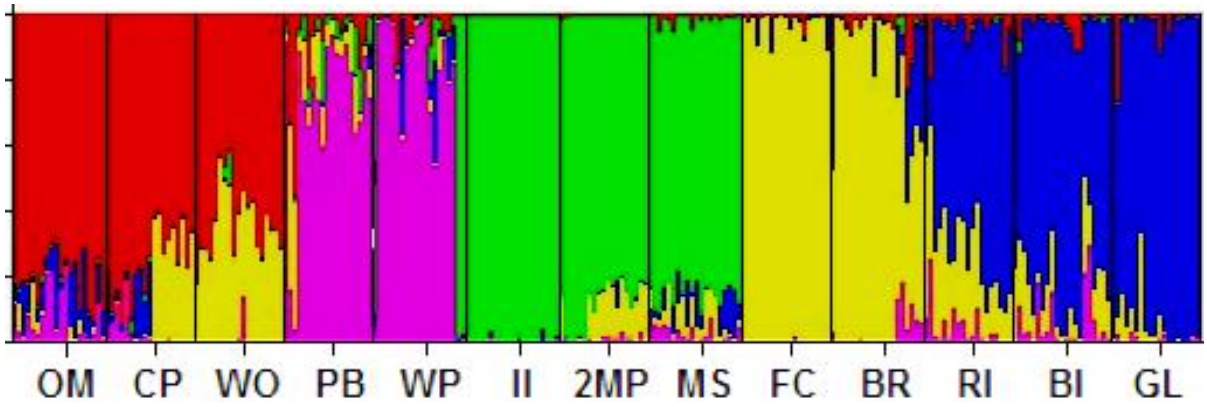

Figure 5.6. *Adaptive variation:* STRUCTURE clustering plot for Greenlip Abalone based on 1,026 "outlier" SNPs. K=5 was the optimal number of clusters. The figure was based on colour-coded columns where each line corresponds to an individual and the colours to a specific cluster. Black lines separate each sampling locations (source CRC 2012/714 Final Report).

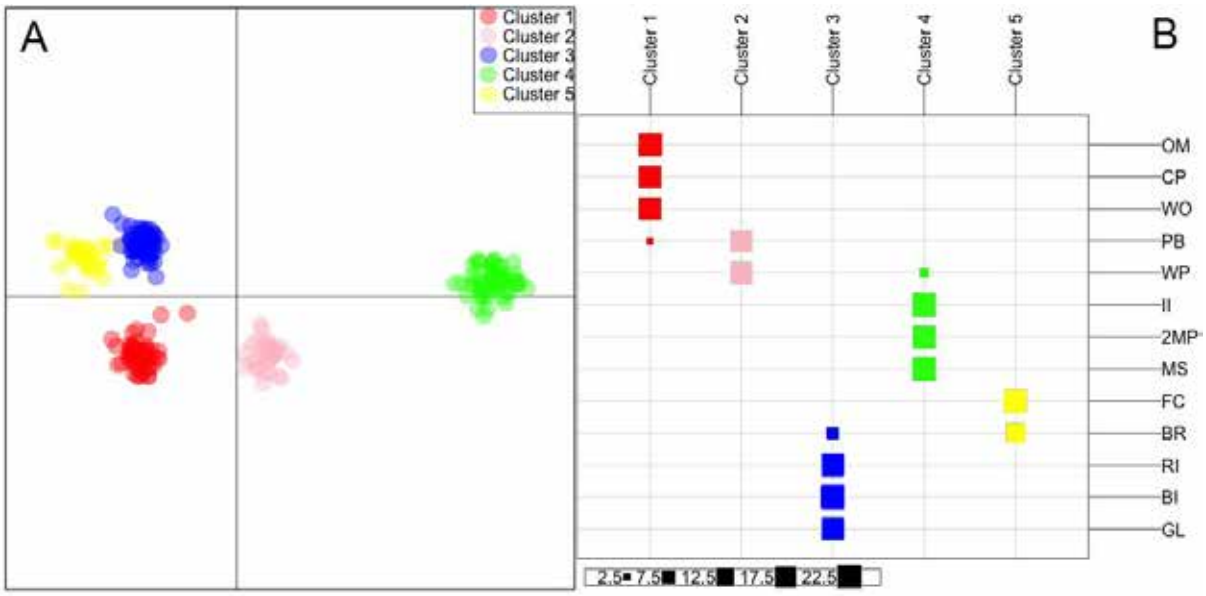

Figure 5.7. ***Adaptive variation:*** **ADEGENET Discriminant analysis of principal components for 1026 "outliers" SNPs of Greenlip Abalone (A). The graphic shows the first two principal components that explain 91.5 % of the genetic variation (PC1=83.0 %; PC2=8.5 %). Number of samples assigned to different clusters by location of origin (B) (source CRC 2012/714 Final Report).**

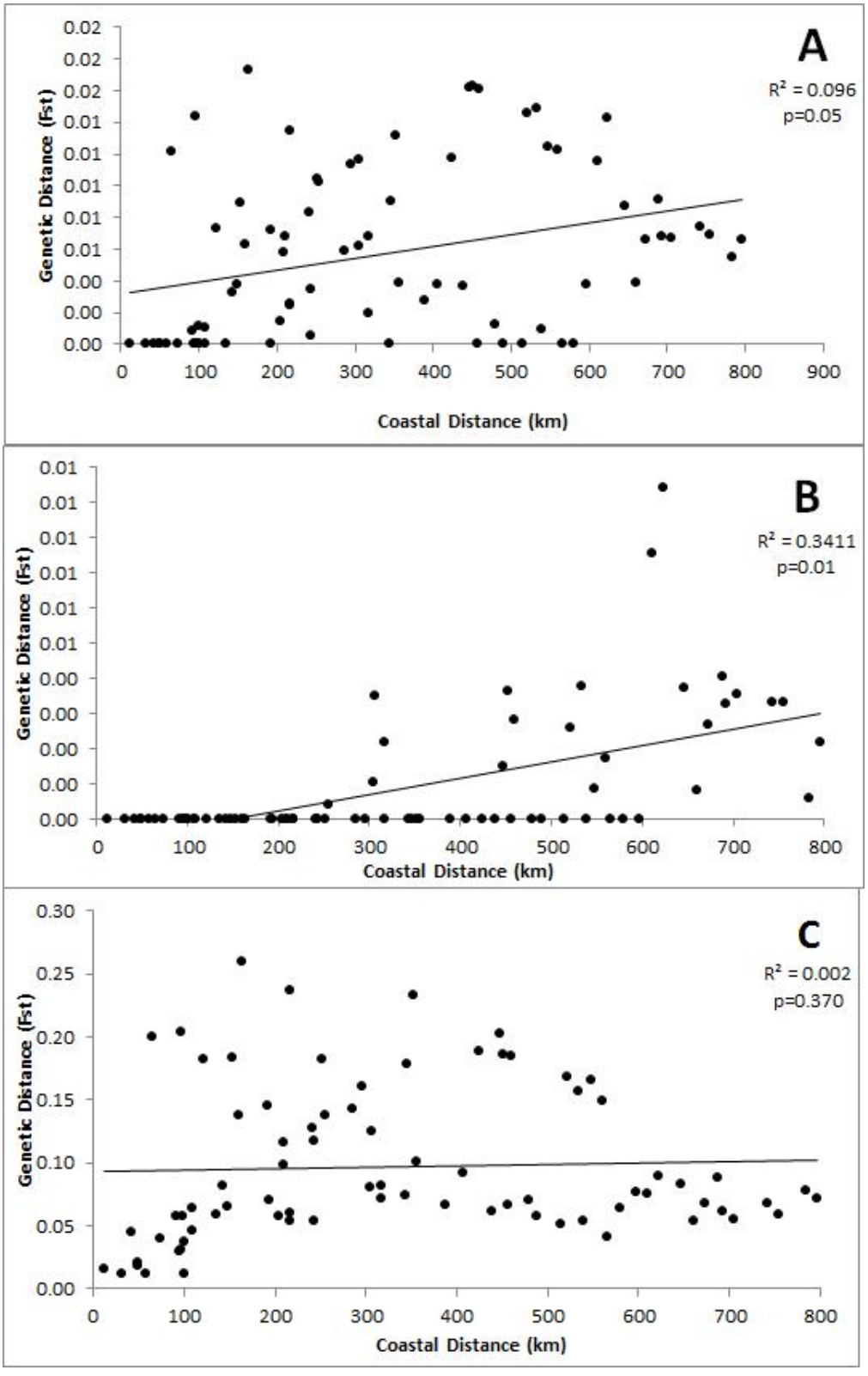

Figure 5.8. Correlation tests between coastal geographical distance and genetic distance F_{ST} (Mantel test) for pairs of Greenlip Abalone sampling locations. The whole 18,803 SNPs data set (A); 17,777 "neutral" SNPs- *neutral variation* (B); 1,026 "outlier" SNPs- *adaptive variation* (C) (source CRC 2012/714 Final Report).

8.4.5 Seascape Analysis – adaptive variations

The PCA of the annual oceanographic data revealed four environmentally different regions that are partially congruent with our five genetic clusters (Figure 5.9). Inner Island samples are very different genetically to those from other locations in the Albany region. However, geographically and oceanographically this location clusters with Parrys Bay and Whalebone Point. In addition, Fanny Cove and Burton Rocks compose a unique cluster but oceanographically they clustered with 2 Mile Primary and Masons. The overall congruence between oceanographic and genetic clusters indicated a strong influence of environmental factors in the genetic structure of the Greenlip Abalone.

The MRDM analysis showed statistically significant correlations of sea surface temperature and oxygen concentration with the "outlier" genetic pattern (Table 5.7). However, when the collinear variables were removed, only oxygen concentration was correlated with levels of genetic differentiation (Table 5.7 and Figure 5.10). These results indicated that differences in oxygen concentration between locations are promoting adaptive differentiation between these groups of populations. The CCA did not show significant correlation with any of the oceanographic variables (Table 5.8), suggesting that the adaptive differentiation observed in Greenlip samples was associated with the difference between locations, with individuals adapted to an oxygen concentration range rather than a specific oxygen concentration.

The weak correlation between adaptive differentiation and oceanographic factors in Greenlip Abalones could be due to the small range of variation in temperature and oxygen concentration observed along the Greenlip Abalone sampling range. The Greenlip Abalone sampling range shows maximum differences of 1.3°C in temperature and 0.4 $mg.L^{-1}$ in oxygen concentration (Table 5.8 and Figure 5.11).

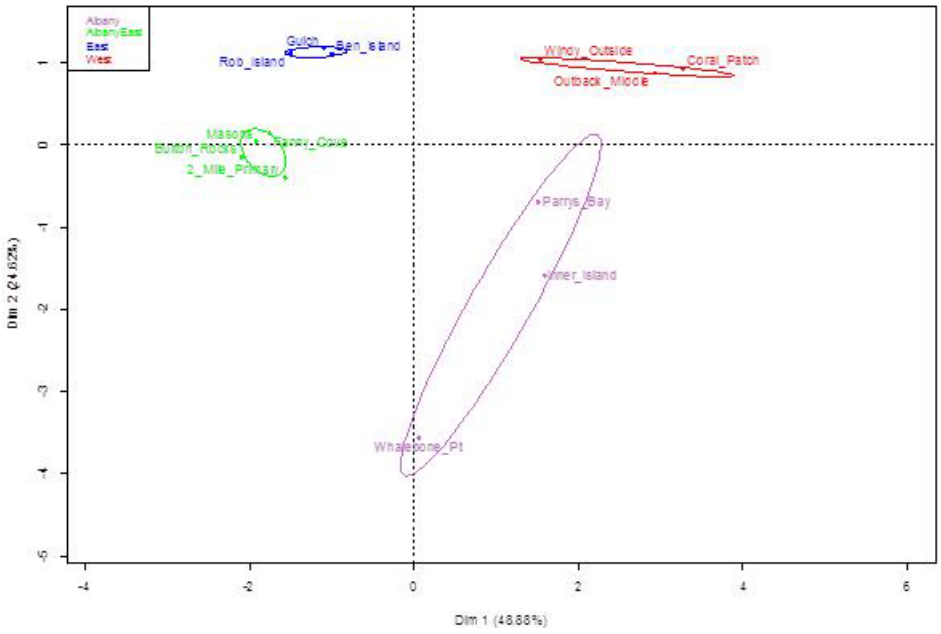

Figure 5.9. Principal Component Analysis based on five oceanographic variables. The scatterplot shows the first two principal components that explain 73.5% of the variation. Dots are coloured according to the most probable environmental groups. Ellipses represent the 95% confidence level of these groups (source CRC 2012/714 Final Report).

Table 5.7. Multiple regression on distance matrices estimating the correlation of Greenlip 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		Reduce Model	
	b	q	b	q
Temperature	**-0.55**	**0.04**	-0.21	0.07
Maximum Temperature	0.11	0.48		
Oxygen Concentration	**0.41**	**0.03**	**0.38**	**0.04**
Nutrients Concentration	0.22	0.18	0.30	0.06
pH	-0.12	0.51	-0.13	0.39
Geographic Distance	0.41	0.22		
Model	**0.23**	**0.04**	**0.22**	**0.03**

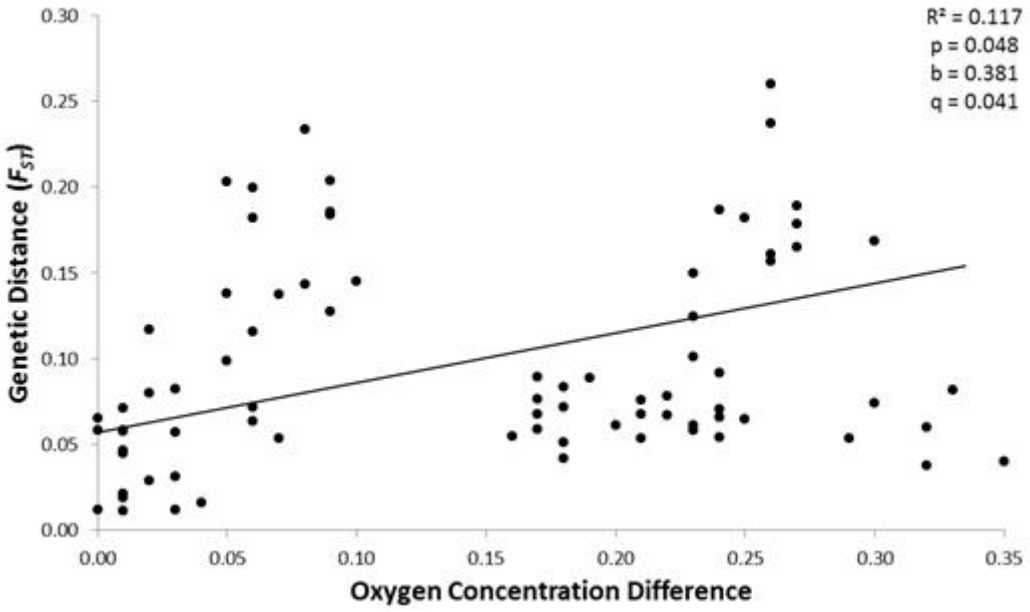

Figure 5.10. Correlation tests between oxygen difference and genetic distance for pairs of Greenlip Abalone sampling locations. Regression coefficient (R^2) and standardised regression coefficient (b) with their associated p and q values (source CRC 2012/714 Final Report).

Table 5.8. Canonical Correspondence Analysis (CCA) exploring the relationship between Greenlip Abalone allele frequencies of 1026 "outlier" SNPs and five oceanographic variables. Shown are results for 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	-0.26	0.87	0.75		-0.19	0.25	0.46	
Maximum Temperature	0.096	0.607	0.25		0.16	0.41	0.15	
Oxygen Concentration	0.023	-0.73	0.37		0.03	-0.21	0.25	
Nutrients Concentration	-0.74	0.527	0.11		-0.61	0.35	0.46	
pH	-0.54	-0.79	0.11		-0.46	0.14	0.14	
Model	0.031	0.014	0.06	53.21	**0.42**	**0.11**	**0.046**	**71.3**
Conditionals (Latitude, Longitude)								17.78

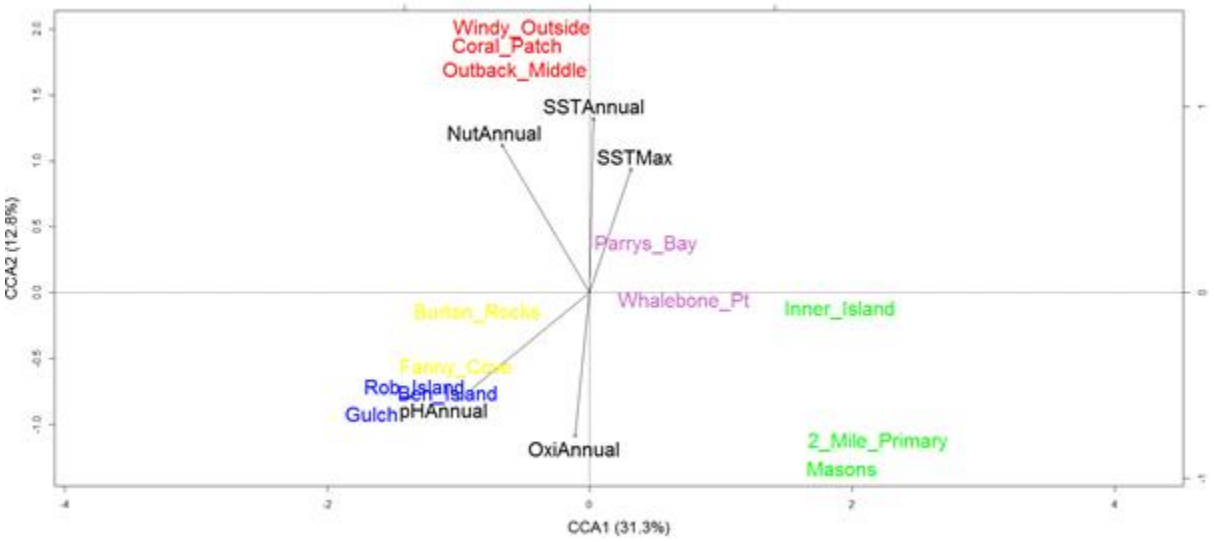

Figure 5.11. Canonical Correspondence Ordination based on five oceanographic variables and 1026 "outlier" SNPs. The scatterplot shows sampling sites in relation to the first two canonical components, which explain 44.1% of the variation (source CRC 2012/714 Final Report).

These results show some concordance with the Western Australian Greenlip/Brownlip Abalone Fisheries Management Subareas (Figure 5.12). Individuals from locations within Augusta and Windy Harbour sub-areas can be used as broodstock for the western populations, while individuals from locations within Town, Duke and Arid sub-areas can be used for stock enhancement in the eastern populations. However, Albany, Hopetoun and West subareas form independent adaptive genetic clusters, therefore broodstock should be taken directly from the populations that are to be enhanced.

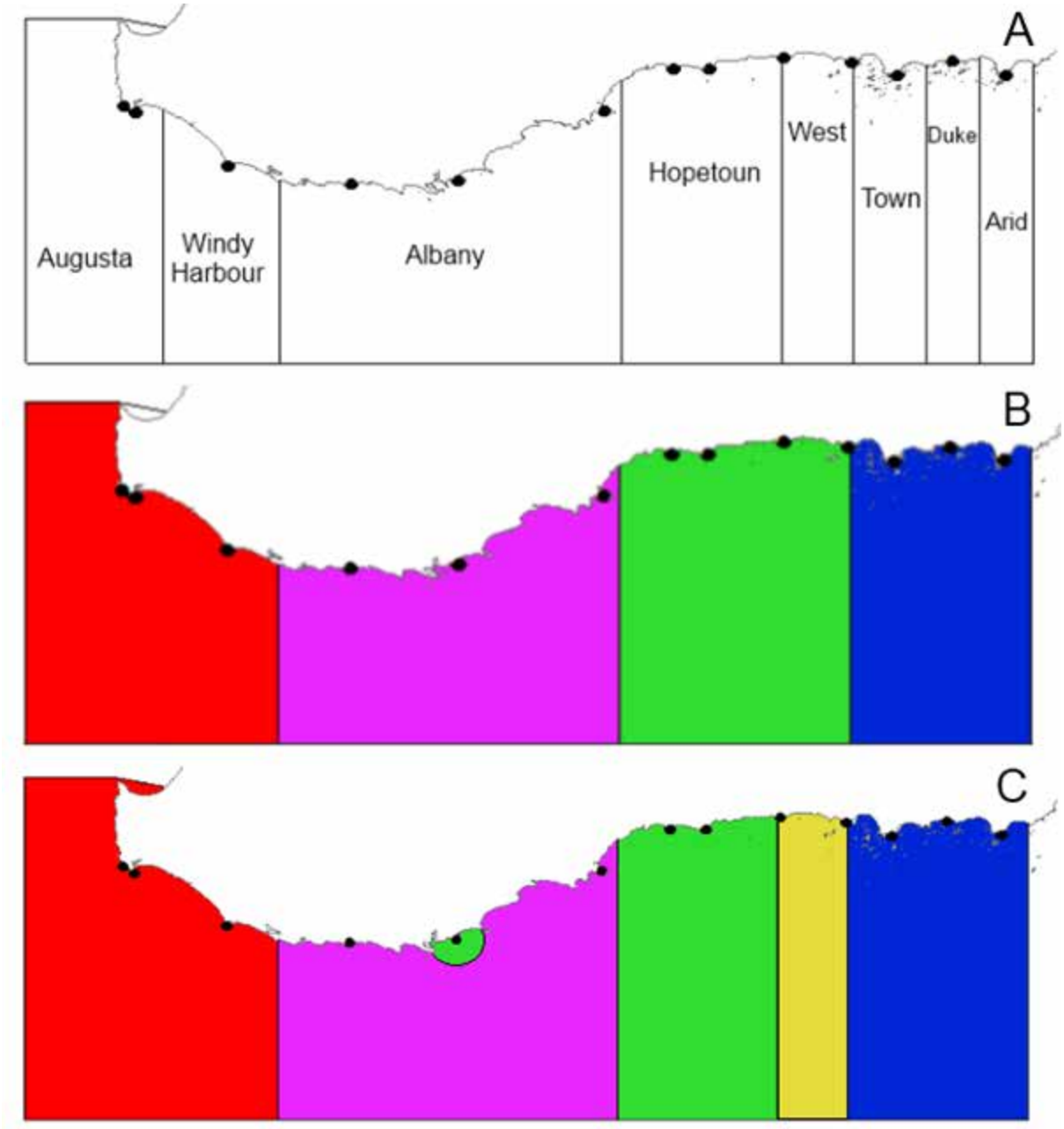

Figure 5.12. **Maps showing A) Management sub-areas for the commercial Western Australian Greenlip/Brownlip Fisheries. B) Oceanographic regions detected by our PCA based on six oceanographic variables. C) Geographic distribution of the Greenlip abalone genetic clusters based on 1,026 "outlier" SNPs (source CRC 2012/714 Final Report).**

8.5 Discussion

The new diagnostic genomic tool developed utilising Genotyping-By-Sequencing (GBS) techniques, was successful at examining the genetic structure of natural Greenlip Abalone populations in Western Australia. The levels of genome-wide diversity in the Greenlip Abalone samples were similar in all populations analysed with the highest diversity detected in the easternmost populations sampled. This genetic diversity was slightly higher than that from Roe's abalone populations in Western Australia examined using the same genomic analysis (Sandoval-Castillo et al., 2015) and similar to the diversity reported for Green abalone from California, USA (Gruenthal et al., 2013). However, the use of large numbers of broodstock is still recommended for the production of hatchery-reared juvenile Greenlip Abalone for stock enhancement, to maintain the genetic variability within and between populations.

The screening of genome-wide variation in Greenlip Abalone samples collected from the wild showed that "neutral" SNPs (i.e. DNA markers that are not under the influence of natural selection) exhibit a pattern of high connectivity, indicating the existence of one single abalone population across the geographic range sampled. This was similar to the pattern reported for Roe's abalone populations from Western Australia examined using the same genomic analysis (Sandoval-Castillo et al., 2015). However, when only the section of genome under selection (outlier SNPs) was considered, five genetically distinct groups can be clearly defined. These are: 1) the western part of the Greenlip Abalone distribution (from Outback to Windy Outside); 2) the Albany sub-area (Parrys Bay and Whalebone Port); 3) the Hopetoun sub-area (from Inner Island to Mason); 4) the West sub-area (Fanny Cove and Burton Rocks); and 5) the eastern sampling area (from Rob Island to Gulch).

These results suggest that Greenlip Abalone show marked genetic structure as a result of local adaptation to environment. The seascape analysis supported the existence of four oceanographically different regions, which are partially congruent with the five adaptive clusters in Greenlip Abalone. The adaptive differentiation between genetic clusters was significantly correlated with geographic variation in oxygen concentration, but not with coastal distance. For example, Figure 5.8c shows that differences between populations separated by 400 km can be less than populations separated by less than 100 km. The correlation between adaptive differentiation and oceanographic factors in Greenlip Abalone was weaker compared to Roe's abalone (Sandoval-Castillo et al., 2015) and could be due to the smaller range of variation in temperature and oxygen concentration observed along the Greenlip Abalone sampling range. The Greenlip Abalone sampling range shows maximum differences of 1.3°C in temperature and 0.4 $mg.L^{-1}$ in oxygen concentration, while the Roe's sampling range includes differences between locations of greater than 3°C in temperature and 1 $mg.L^{-1}$ in oxygen concentration.

The five genetically distinct groups, identified when using only the section of genome under selection, showed some concordance with the Western Australian Greenlip/Brownlip Abalone Fisheries Management Sub-areas. The adaptive genetic groups indicated that the Augusta and Windy sub-areas are one group; the Albany, Hopetoun and West sub-areas are separate groups, while the Town, Duke and Arid sub-areas are all in one adaptive genetic

group. This indicates that the spatial management of the WA commercial Greenlip abalone fishery should be similar to the sub-areas. However, the fishery is currently managed on a larger spatial scale with the Area 3 fishery encompassing the Augusta, Windy and Hopetoun sub-areas, while the Area 2 fishery includes the West, Town, Duke and Arid sub-areas. This difference in population structure and spatial management scales was examined for Greenlip abalone across the south east of Australia (Mayfield et al., 2014). The Greenlip abalone were shown to be genetically diverse with no evidence of reduced genetic diversity due to over exploitation, while the distribution was not a single large panmictic population and differentiation occurred on both a regional (100's km) and a location within region (10's km) scale with a strong pattern of isolation by distance (Mayfield et al., 2014). Therefore, it is important that abalone fisheries management within Australia understand the issues associated with managing Greenlip abalone fisheries on a spatial scale that doesn't align with its natural population scale.

The main implication for stock enhancement in Western Australia arising from these results is that any commercial-scale stock enhancement program should utilise broodstock taken directly from the genetic group to be enhanced. If broodstock cannot be taken from the genetic group to be enhanced, they should be taken from the most genetically similar group available. Since the genetic differentiation detected was probably adaptive, and was therefore expected to result in varying degrees of fitness/performance in the destination environment, the stock enhancement program should be genetically tested to monitor the contribution of different broodstock to the next generation.

The genomic resources that have been produced will help address fundamental questions for the efficiency of stock enhancement of Greenlip Abalone in Western Australia. The catalogue of SNPs for this species can be used as genetic markers for the constant monitoring of the wild populations and are important tools for the fisheries management of the species over its whole distribution. The pattern of adaptive differentiation found in this study should be examined further using transcriptome-outlier annotation. Some of the outlier genes identified could be affecting the fitness of individuals in these different environments and some of the SNPs identified could be causative variants affecting these traits. The identification of genes associated with oxygen through this approach will represent an important step to understanding hypoxia-stress adaptation in abalone.

8.6 Acknowledgements

This population genomic study was funded by the Australian Seafood Cooperative Research Centre (CRC). The study was collaboration between Department of Fisheries WA, Flinders University and Nofima. through the CRC projects 2009/710 "Bioeconomic evaluation of commercial scale stock enhancement in abalone" and 2012/714 "PDRS: Use of next generation DNA technologies for revealing the genetic impact of fisheries restocking and ranching". This chapter has been extracted from the Seafood CRC Final Report on the project 2012/714 and adapted for this Seafood CRC Final Report.

8.7 References

Altshuler D., V. J. Pollara, C. R. Cowles, W. J. Van Etten, J. Baldwin, L. Linton and E. S. Lander. An SNP map of the human genome generated by reduced representation shotgun sequencing. Nature, 407(6803): 513 – 516 (2000).

Baird N.A., P. D. Detter, T. S. Atwood, M. C. Currey and A. L. Shiver. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One, 3: e3376 (2008).

Bell, J. D., P. C. Rothlisberg, J. L. Munro, N. R. Loneragan, W. J. Nash, R. D. Ward and N. L. Andrew. Restocking and stock enhancement of marine invertebrate fisheries. Adv. Mar. Biol., 49: 1-370 (2005).

Campbell, A. (Ed) Workshop on Rebuilding Abalone Stocks in British Columbia. Can. Sp. Pub. of Fish. and Aqu. Sc., 130 (2001).

Cariou M., L. Duret and S. Charlat. Is RAD-seq suitable for phylogenetic inference? An in silico assessment and optimization. Ecology and Evolution, 3(4): 846-852 (2013).

Catchen J.M., A. Amores, P. Hohenlohe, W. Cresko & J. H. Postlethwait. Stacks: building and genotyping loci de novo from short-read sequences. Genes, Genomes, Genetics, 1: 171-187 (2011).

Davey J.L. and M. W. Blaxter. RADSeq: next-generation population genetics. Briefing in Functional Genomics, 9: 416-423 (2010).

Dixon P. VEGAN, a package of R functions for community ecology. J. of Veg. Sc., 14(6): 927-930 (2003).

Excoffier L. and H. E. L. Lischer. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Eco. Res., 10(3): 564-567 (2010).

Gagnaire P.A., S. A. Pavey, E. Normandeau and L. Bernatchez. The genetic architecture of reproductive isolation during speciation-with-gene-flow in lake whitefish species pairs assessed by RAD sequencing. Evolution, 67(9): 2483-2497 (2013).

Goslee S.C. and D. L. Urban. The ecodist package for dissimilarity-based analysis of ecological data. J. of Stat. Soft., 22(7): 1-19 (2007).

Gruenthal K., D. Witting, T. Ford, M. Neuman, J. Williams, D. Pondella II, A. Bird, N. Caruso, J. Hyde, L. Seeb and W. Larson. Development and application of genomic tools to the restoration of green abalone in southern California. Cons. Gen.: DOI 10.1007/s10592-10013-10524-10595 (2013).

Hammasaki, K. and S. Kitada. The enhancement of abalone stocks: lessons from Japanese case studies. Fish and Fisheries, 9: 1-18 (2008).

Hart, A.M., L. W. S. Strain, F. P. Fabris, J. Brown and M. Davidson. Stock enhancement in greenlip abalone part I: long-term growth and survival. Rev. in Fish. Sc., 21(3-4): 299-309 (2013a).

Hart, A.M, F. P. Fabris, L. W. S. Strain, M. Davidson and J. Brown. Stock enhancement in greenlip abalone part II: population and ecological effects. Rev. in Fish. Sc., 21(3-4): 310-320 (2013b).

Hillborn, R. The economic performance of marine stock enhancement projects. Bull. of Mar. Sc., 62: 661-674 (1998).

Jombart T. and I. Ahmed. Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics, 27(21): 3070-3071 (2011).

Lê S, J. Josse and F. Husson. FactoMineR: an R package for multivariate analysis. J. of Stat. Soft., 25(1): 1-18 (2008).

Mayfield, S., K. J. Miller, and C. N. Mundy. Towards understanding greenlip abalone population structure. Final report for the Fisheries Research and Development Corporation. Prepared by

the South Australian Research and Development Institute (Aquatic Sciences), Adelaide. FRDC Project No. 2010/013. 31pp (2014).

Meirmans P.G. and P. H. Van Tienderen. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes, 4(4): 792-794 (2004).

Peterson B.K., J. N. Weber, E. H. Kay, H. S. Fisher and H. E. Hoekstra. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PloS one, 7(5): e37135 (2012).

Pritchard J., M. Stephens and J. Donnelly. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959 (2000).

Richards P.M., M. M. Liu, N. Lowe, J. W. Davey, M. L. Blaxter and A. Davison. RAD-Seq derived markers flank the shell colour and banding loci of the Cepaea nemoralis supergene. Mol. Ecol., 22(11): 3077-3089 (2013).

Roberts, R.D., E. F. Keys, G. Prendeville and C. A. Pilditch. Viability of abalone (Haliotis iris) stock enhancement by release of hatchery-reared seed in Marlborough, New Zealand. J. of Shell. Res., 26(3): 697-703 (2007).

Sandoval-Castillo, J., N. Robinson, L. Strain, A. Hart and L. B. Beheregaray. PDRS: Use of next generation DNA technologies for revealing the genetic impact of fisheries restocking and ranching. Final Report for the Seafood Cooperative Research Centre. Seafood CRC Project No. 2012/714. 47pp. (2015).

Schlitzer R. Ocean Data View (2010).

Waples, R.S. Genetic considerations in recovery efforts for Pacific salmon. Cons. Biol., 8: 884-886 (1994).

Xu P., S. Z. Xu , X. H. Wu, Y. Tao, B. G. Wang, S. Wang, D. H. Qin, Z. F. Lu and G. J. Li. Population genomic analyses from low-coverage RAD-Seq data: a case study on the non-model cucurbit bottle gourd. Plant J., 77(3): 430-442 (2014).

9 Benefits and Adoption

This research benefits a wide range of industry and community sectors but was driven specifically for the Western Australian Abalone Industry Association (WAAIA), which is the commercial abalone fishing industry body in WA. The Western Australian abalone aquaculture industry will also significantly benefit from this research given the need for abalone hatcheries to facilitate stock enhancement. Administrators of fisheries management at the Department of Fisheries Western Australia will find this research critical in developing fisheries management policy to manage the wild abalone fishery and aquaculture industry for the implementation of commercial-scale stock enhancement in WA. This research is not limited to Western Australia and the stock enhancement principles examined here can 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 on abalone stock enhancement. This project also significantly benefits the recreational fishing sector that strongly supports the notion of stock enhancement as a fisheries management tool, but lack successful examples to show guidance in the complexities of implementing an assisted recruitment program.

Abalone stock enhancement is a sought-after fisheries management strategy around the world that can increase catch rates and ultimately the economic efficiency and profitability of an abalone fishery, without compromising the fishery in terms of access or allowable catches. Therefore the Department of Fisheries WA in collaboration with the WAAIA sought to evaluate stock enhancement's viability in the Western Australia Greenlip Abalone fishery. This research has now demonstrated that commercial-scale abalone stock enhancement is economically and environmentally possible in WA. The benefit of this project to commercial fishers is that all principles of an abalone stock enhancement program have been evaluated and a program can be considered ready for implementation. Depending on the level of enhancement undertaken, profitability could increase significantly, with the bioeconomic analysis indicating optimal profitability would be achieved with a range of scenarios. For example, an annual release equal to natural recruitment, in combination with a decrease in fishing mortality of 10 - 20% and a lowering of minimum legal length to ensure optimal yields, would increase annual profitability of a specific Western Australian abalone fishery from $1.15 to $2.1 million and the GVP from $2.0 to $3.5 million.

The Western Australian abalone aquaculture industry also stands to benefit significantly from a stock enhancement program being introduced. The program would create a constant demand for juvenile abalone, which would in turn provide reliable income while also reducing costs by minimising the length of grow-out stage required in production. Even though a commercial stock enhancement program has yet to be undertaken, this project still aids the aquaculture industry through consultation on spawning protocols, minimum breeding numbers and adaptive genetic groups, as well as the development of large-scale, live abalone transport techniques.

This project has also provided rigorous scientific information for fisheries management and contributed heavily to the development of stock enhancement policy. The Department of

Fisheries Western Australia has produced policies on restocking and stock enhancement (FMP No. 261, 2013), as well as abalone aquaculture (2013) during this project. Both of these policies significantly benefited from this research into stock enhancement as a fisheries management strategy.

The genomic resources that have been produced will help address fundamental questions for the efficiency of stock enhancement of Greenlip Abalone in Western Australia. The catalogue of SNPs for this species can be used as genetic markers for the constant monitoring of the wild populations and are important tools for the fisheries management of the species over its whole distribution. Moreover, the distinction of outlier loci would benefit the aquaculture industry, by annotating these loci genes involved in physiological, morphological and behavioural traits can be identified; information that could be used to optimise individual selection and mating programs in order to increase productivity.

Given this project has provided unique insight into the vast array of principles behind stock enhancement, the research will benefit the wider scientific community, particularly fisheries scientists. The information on the ecology of the species and ecological process including, the carrying capacity of the ecosystem will be valuable to other scientists studying abalone stock enhancement or even stock recovery. The positive research findings of this project provide a case study for the successful evaluation of stock enhancement as a fisheries management strategy, which is an important historical breakthrough from the limited success in the past.

9.1 Further Development

There are 2 areas within Objective 3 (To evaluate appropriate wild-stock management protocols that facilitate stock enhancement) of this project where the results of our research necessitate further development.

Firstly, further investigation is required into abalone habitat limitation and effective release density. Even though the carrying capacity of the habitat has been examined by a high release density stock enhancement experiment, which indicated that abalone densities could be increased significantly above what naturally occurs (400% increase after 2.5 years post-release), further research is required into optimal release and enhancement target densities. In the bioeconomic analysis, enhancement releases equating to 50 and 100% of natural recruitment were theoretically tested, with most enhancement scenarios producing an increase in spawning biomass and economic profitability. However, these densities need to be experimentally examined and a large-scale release of juvenile Greenlip Abalone at a range of different densities (5, 15, 25 and 45 per m^2 abalone habitat) is currently being monitored to determine the effect of release densities on wild-stock populations.

Secondly, additional genetic samples should be genotyped across the range of Greenlip Abalone to test for population structure, levels of genetic diversity, selective sweeps and genetic correlations with geospatial data. This information would be useful for making more informed fishery management decisions for the Greenlip populations in WA.

The three scientific manuscripts published in the international journal “Reviews in Fisheries Science” will further disseminate the research produced from this project. Also, once the experimentation and analysis of abalone habitat limitation, stock enhancement release

densities and genetic population structure are completed, they will be published as scientific manuscripts to add value to the three existing studies, and increase the dissemination of this project's findings.

The significant insight of this research into stock enhancement should be commercially applied to improve the knowledge and productivity of abalone fisheries within Australia. Given this project demonstrated that abalone stock enhancement is environmentally, economically and logistically possible, the next logical step is to integrate commercial-scale stock enhancement into the current fishery management practices of a functioning abalone fishery.

9.2 Planned Outcomes

This projects main output was the formal assessment of the effect of stock enhancement on existing Western Australian abalone populations and their environment. Our research indicates that commercial-scale stock enhancement has economic potential and is logistically possible in the Western Australian Greenlip Abalone fishery, without negatively effecting the current wild-stock populations. This output will contribute significantly to the planned outcome of commercially viable stock enhancement. In fact, numerous outputs produced from this project contribute to this planned outcome potentially being achieved. These include an understanding of the natural ecology of the species and the ecological processes involved in stock enhancement, a bioeconomic evaluation of stock enhancement, establishment of spatial and temporal enhancement targets, a stock enhancement manual, risk assessments into bio-security protocols, genomic analysis of Western Australian Greenlip Abalone and consultation on fisheries management policy. All of these outputs cover elements of stock enhancement that need to be understood and combined effectively to implement successful abalone stock enhancement programs. When enhancement is conducted on a commercial-scale these outputs will have contributed to the second planned outcome in this project of increasing the profitability of the Western Australian Greenlip Abalone fishery.

The series of large-scale Greenlip Abalone releases conducted as part of this project provided detailed biological insight into the ecological process in fisheries management and stock enhancement. These insights allowed long-term growth and survival estimates to be produced, examination of habitat limitation and the carrying capacity of the ecosystem. This output of greater understanding of the natural ecology of the species and the ecological processes involved in stock enhancement, directly contributes to the third planned outcome in the project of increased understanding of the carrying capacity of abalone habitat.

From this research another output was the production of a stock enhancement manual to standardise the procedures for commercial abalone enhancement programs. This manual examined all aspects of the enhancement procedure from spawning protocols in the hatchery to the packaging and transport of abalone and the release by divers into natural abalone habitat. This output therefore achieved the three planned outcomes in the project variation by: 1) standardising a set of transport and release protocols, 2) standardising the design of abalone release devices and vessel containers and 3) producing a training manual for personnel involved in stock enhancement.

As part of this project in collaboration with the Seafood CRC Project 2012/714, a comprehensive genomic analysis of population genetic diversity and population connectivity of Greenlip Abalone stocks in Western Australia was completed. This analysis provided recommendations for capturing genetic diversity and locally adapted genotypes for increasing the chances of stock enhancement success. It provides the genomic resources for the monitoring of Greenlip Abalone wild and hatchery stocks and the potential for the improvement of mass selection and mating schemes in aquaculture activities.

All of the outputs produced in this project have been widely communicated and disseminated to the relevant stakeholders. Regular meetings, annual general meetings, milestone reports and updates have been provided to the Western Australian Fishing Industry Council and the Western Australian Abalone Industry Association, which are the peak bodies representing the wild abalone fishing industry in WA, as well as to individual industry members. Continual communication of the outputs has occurred to the managers and policy officers at the Department of Fisheries Western Australia to help inform and develop the fisheries management aspect of incorporating stock enhancement into the WA abalone fishery.

The research findings have also been presented to the scientific community at two international conferences, the 4th international Symposium on Sea Ranching and Stock Enhancement at Shanghai Ocean University in China (October 2010) and the 8th International Abalone Symposium in Hobart, Australia (May 2012). Some of these findings have been published in the scientific peer-reviewed journal "Review in Fisheries Science".

However, the main and fundamental outcome of this project was the implementation of commercial-scale stock enhancement to increase the value and profitability of the Western Australian Greenlip Abalone fishery. Unfortunately this outcome has not been delivered as yet due to the conservative response of the commercial abalone industry to the outputs of this project. One of the main hurdles to achieving this outcome and implementing commercial abalone stock enhancement in Australia has been a disease issue. The presence of highly virulent herpes-like-virus (Abalone Viral Ganglioneuritis – AbHV-1, AVG) in wild stocks in Victoria and Tasmania has caused significant concern to the industry and community in all abalone-producing areas (Corbeill et al., 2010; Hooper et al., 2007; Savin et al., 2010). To address these concerns, comprehensive risk assessments of the threat of AVG and appropriate mitigation strategies have been produced for Western Australia (Jones and Fletcher, 2012; Stevens, 2012).

Until there is a change in risk profile from the commercial abalone industry in Western Australia the main outcome of this project will not be delivered. However, if this change does occur there has already been a commercialised model developed for abalone stock enhancement in Western Australia. This model could affect a "responsible" increase in abalone stocks to be significantly above current levels and in doing so create a new industry based on mass culture of abalone for stock enhancement. It would also establish a new harvest regime based on increased profitability of fishing, and gross value of production from an increased Total Allowable Catch (TAC), with the target being a 100% increase in TAC within 6 – 8 years of full commercialisation. To do this the model would include techniques for economical large-scale deployment of genetically robust juveniles into wild abalone

populations, create and disseminate cost-effective abalone stock enhancement protocols and establish a viable research and management policy to facilitate commercial scale stock enhancement. The implementation of the commercialisation model could potentially result in increased biomass of abalone, gross value of production and profitability for stakeholders in the Abalone industry. This would establish an international precedent for successful marine stock enhancement in abalone and allow acceptance of stock enhancement in the wider community as a useful and viable fisheries management tool.

An unexpected outcome of this project was the development of a Greenlip Abalone sea ranching initiative in the same region as the large-scale stock enhancement releases were conducted. Sea ranching of abalone on artificial habitat structures utilises similar principles to stock enhancement and a large part of this projects outputs are directly transferrable to ranching. In fact results from this project have been used to aid the development of the sea ranching initiative and are now being used to compare the effectiveness of sea ranching and stock enhancement as methods for increasing production of Greenlip Abalone in Western Australia (Melville-Smith, 2013).

9.2.1 List of Publications Produced

Hart AM (submitted). Commercial-scale invertebrate fisheries enhancement in Australia: experiences, challenges and opportunities. Marine Policy.

Hart A .M., L. W. S. Strain, F. Fabris, J. Brown and M. Davidson. Stock enhancement in greenlip abalone part I: long-term growth and survival. Reviews in Fisheries Science, 21(3-4): 299-309 (2013a).

Hart A. M., F. Fabris, L. W. S. Strain, M. Davidson and J. Brown. Stock enhancement in greenlip abalone part II: population and ecological effects. Reviews in Fisheries Science, 21(3-4): 310-320 (2013b).

Hart A. M., L. W. S. Strain and A. Hesp. Stock enhancement in greenlip abalone part III: bioeconomic evaluation. Reviews in Fisheries Science, 21(3-4): 354-374 (2013c).

Strain L. W. S., A. M. Hart, F. Fabris and M. Davidson. Stock enhancement in greenlip abalone part IV: commercial-scale stock enhancement manual. Fisheries Research Report, Department of Fisheries Western Australia. (in prep).

Jones, J. B. and W. J. Fletcher. Assessment of the risks associated with the release of abalone sourced from abalone hatcheries for enhancement or marine grow-out in the open ocean areas of WA. Fisheries Research Report No. 227, Department of Fisheries Western Australia, 24p. (2012).

9.2.2 Public Benefit Outcomes

This research was primarily directed at commercial sectors, specifically the Western Australian wild abalone fishery and aquaculture industry. However, the recreational fishing sector and wider community have shown keen interest in utilising stock enhancement and stock recovery to manage public fisheries resources. This research provides a positive case study for stock enhancement as a management strategy and gives the wider community evidence that stock enhancement can help to improve their fishery resource. Therefore, if the second planned outcome in this project of increasing the profitability of the Western Australian Greenlip Abalone fishery through stock enhancement can be achieved, it will provided benefits to the community and recreational sector by continuing the sustainable management of the fishery in the future.

9.2.3 Private Benefit Outcomes

The private benefit outcomes from this project through implementing commercially viable stock enhancement have not been achieved as yet and the reasons for this have been detailed at the start of the Planned Outcome section above.

9.2.4 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 meets the strategic objectives of Investment Platform 2: Optimising the harvest, of the WAFIC/Seafood CRC strategic plan as well as Objective 2 (Establish management tools and models that enable targeted harvesting of fish to optimise market returns) of the Abalone Council of Australia Investment Platform 3 (Optimising Harvest) strategy. It also targets the CRC's milestones 1.2.2 (Production interventions implemented in at least one fishery) and 1.2.3 (Annual production characterised and interventions optimised in at least one fishery).

9.3 Conclusion

The overall objective of this project was to evaluate the potential of commercial-scale stock enhancement of Greenlip Abalone in the Western Australian fishery. This information is critical in determining if stock enhancement is a viable fisheries management strategy and whether it can be incorporated into current systems. The specific aims of the project were to estimate long-term growth and survival of enhanced abalone, conduct a bioeconomic analysis, evaluate wild-stock management and develop bio-security protocols, while also standardising the methodology of commercial abalone stock enhancement for future use in Australian abalone fisheries. These objectives were all successfully achieved.

By conducting large-scale Greenlip Abalone releases into natural habitats within the commercial fishery we were able to obtain an understanding of the natural ecology of the species and the ecological processes involved. Estimates of growth and survival indicated that habitat critically affected initial (6 month post-release) survival and water depth was positively correlated with growth. The released abalone reached legal minimum length (140 mm shell length) and successfully entered the commercial fishery. The carrying capacity of the ecosystem was also affected, as specific releases of hatchery-reared abalone were able to increase the biomass by 400% (2.5 years post-release) above what's naturally occurring, without any other environmental effects from enhancement being detected. Therefore, the natural system is considered recruitment limited and a greater abalone biomass can be accommodated, indicating stock enhancement of abalone would be a positive addition to the fishery.

From this greater understanding of the carrying capacity and natural population processes, a bioeconomic analysis was conducted to evaluate abalone stock enhancement. This bioeconomic analysis considered the effect of enhancement on biomass, net present value, profitability and gross value of product. Most stock enhancement scenarios modelled for the Western Australian Greenlip Abalone fishery achieved economic profitability and an increase

in spawning biomass, with optimal profitability occurring through a 10 - 20% decrease in fishing mortality, a 10% decrease in minimum legal length and an annual enhancement of juveniles to match natural recruitment. A stock enhancement program for Australian Greenlip Abalone fisheries also showed significant economic potential with the possibility of 100% increases in maximum economic yield and net present value. This signifies that stock enhancement is economically possible in Australian abalone fisheries.

To logistically conduct a commercial-scale stock enhancement program in Western Australia, a training manual was developed to standardise the hatchery, transport and release protocols. This manual also provides a streamlined approach to processes including habitat identification and assessment, release and enhancement density calculation and fisheries independent surveys. In doing so it becomes the basis for the training and education of organisations and personnel involved in stock enhancement.

Maintaining adequate genetic diversity in wild populations when under taking commercial-scale stock enhancement is critically important to its success. The genomic study conducted developed genotyping by sequencing protocols that were very efficient for assessing genome wide diversity in Greenlip Abalone. Levels of genome-wide diversity were moderate to high in all Greenlip Abalone populations analysed. The “neutral” fraction of the genomic dataset shows a pattern of isolation by distance with higher connectivity between proximate locations than between distant locations. Five distinct adaptive groups were clearly defined in the dataset targeted by natural selection of the genome, these are: 1) the western part of the Greenlip Abalone distribution, 2) the Albany sub-area, 3) the Hopetoun sub-area, 4) the West sub-area and 5) the eastern most part of the sampling area.

Even though abalone stock enhancement has been shown to be environmentally, genetically, economically and logistically possible in Western Australia, it will be unable to be utilised as a fisheries management strategy until appropriate management and bio-security measures are in place. This research on stock enhancement has also provided rigorous scientific information to inform fisheries management and aided in the development of fisheries policy and bio-security protocols. Including the Department of Fisheries Western Australia policies on restocking, stock enhancement and abalone aquaculture, this should provide adequate governance for stock enhancement in Western Australia. Substantial risk assessments have been conducted into the bio-security protocols of stock enhancement in order to minimise the risk to the environment and existing stocks (Jones and Fletcher, 2012; Stevens, 2012).

Overall this research showed that if the general principles of abalone stock enhancement, including ecological processes and the carrying capacity of the system, economic parameters, governance (policy) and bio-security are understood and brought together, then commercial-scale enhancement of Greenlip Abalone is possible in Australian abalone fisheries.

9.4 References

Corbeil, S., A. Colling, L. M. Williams, F. Y. Wong, K. Savin, S. Warner, B. Murdoch, N. O. Cogan, T. I. Sawbridge, M. Fegan, I. Mohammad, A. Sunarto, J. Handlinger, S. Pyecroft, M. Douglas, P. H. Changs and M. S. Crane. Development and validation of a TaqMan PCR assay for the Australian abalone herpes-like virus. Dis. Aquat. Org., 92: 1-10 (2010).

Hooper, C., P. Hardy-Smith and J. Handlinger. Ganglioneuritis causing high mortalities in farmed Australian abalone (Haliotis laevigata and Haliotis rubra). Aust. Vet. J., 85: 188-193. (2007).

Jones, J. B. and W. J. Fletcher. Assessment of the risks associated with the release of abalone sourced from abalone hatcheries for enhancement or marine grow-out in the open ocean areas of WA. Fisheries Research Report No. 227, Department of Fisheries Western Australia, 24p. (2012).

Melville Smith R, Adams B, Wilson NJ, Caccetta L (2013). Sea ranching trials for commercial production of greenlip (Haliotis laevigata) abalone in Western Australia: an outline of results from trials conducted by Ocean Grown Abalone Pty Ltd. Final Report for FRDC Project No. 2012/220. Fisheries Research and Development Program and Curtain University.

Savin, K. W., B. G. Cocks, F. Wong, T. Sawbridge, N. Cogan, D. Savage, and S. Warner. A neurotropic herpesvirus infecting the gastropod, abalone, shares ancestry with oyster herpesvirus and a herpesvirus associated with the amphioxus genome. Vir. J., 7: 308 (2010).

Stevens, R. N. Disease risk assessment for abalone stock enhancement. Final report to Fisheries Research and Development Corporation on Project no. 2011/046. Western Australian Fishing Industry Council. 32p. (2012).

10 Appendices

Appendix 1. Intellectual property

The results of this project have become public domain and have been widely published, disseminated and promoted. 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 Anthony Hart

Dr Lachlan Strain

Dr Alex Hesp

Dr Sabine Daume

Dr Jonathan Sandoval-Castillo

Dr Nick Robinson

Dr Luciano Beheregaray

Dr Nick Caputi

The following Technical Officers were engaged on this project.

Mr Frank Fabris

Mr Mark Davidson

Mr Jamin Brown

Mr David Murphy

Ms Fiona Parker

Mr Sam Hair

The following contributed significantly to this project.

Mr Ian Taylor

Mr Brad Adams

Professor Kai Lorenzen