TOWARDS UNDERSTANDING GREENLIP ABALONE POPULATION STRUCTURE

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2010/013 Towards understanding greenlip abalone population structure

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OBJECTIVES:

1. Quantify greenlip abalone population genetic structure within key fishing areas; and
2. Assess genetic connectivity within and among greenlip abalone populations in key fishing areas.

NON-TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

There are four primary outcomes from this project. First, stakeholders – fishery managers, commercial and recreational fishers and researchers – have been provided with detailed information on greenlip abalone (*Haliotis laevigata*; hereafter termed greenlip) genetic diversity, genetic structure and connectivity within and among populations. These findings should be incorporated into future management arrangements for these fisheries. Second, key differences in population structure and connectivity between greenlip and blacklip abalone (*Haliotis rubra*; hereafter termed blacklip) have been identified. This outcome is important because it demonstrates that species-specific management arrangements are likely to be required to account for differential metapopulation size and structure of these two species. Notably, these differences between the sympatric and con-generic blacklip and greenlip highlight that, even for species that are typically characterised by spatial structure at small scales, it is difficult to generalise about ecological processes and the potential consequences of similar life history characteristics. Third, the fine-scale resolution of greenlip connectivity across the most productive reef for this species in Australia – Tiparra Reef – yielded evidence to support the long-held hypothesis that the smaller greenlip in the southern areas of this reef contribute substantial larval numbers to the heavily-fished parts of the reef towards the north and west. However, the southern areas were not the only source of recruits to Tiparra Reef. Finally, a comprehensive set of validated microsatellite loci are available for genetic analyses on greenlip. These complement a similar set for blacklip.

Sustainable harvests, the ultimate goals of fishers and fishery managers throughout the world, require substantial understanding of key biological information (e.g. growth rate, reproduction, longevity) and population dynamics (recruitment, population growth, migration, mortality) of the target species. Increasingly, however, the number of stocks exploited by a fishery and the connectivity between fished stocks is becoming recognised as an integral component of modern fisheries science and management. This is because such stock identification enables management to occur at spatial scales that more appropriately reflect the actual population structure of the species, thereby facilitating more effective fisheries management.

Fine-scale population structure is common in many inshore marine species, particularly sedentary invertebrates with limited larval dispersal. Populations (or stocks) of such species tend to be characterised by a complex spatial structure evident at fine spatial scales, whereby local populations are effectively isolated. The assessment and management of these spatially complex stocks is challenging, and as a result, assessment and management processes often occur at spatial scales considerably greater than the spatial complexity of the stocks. This disparity has been blamed for the failure to maintain sustainable resources in numerous sedentary invertebrate fisheries.

Abalone are a typical example of a benthic invertebrate species with spatially-structured stocks. Australian abalone fisheries currently provide about 50% of the global wild-harvest production and show little evidence of the well documented declines in abalone production observed elsewhere. Despite having biological and ecological features strongly indicating the need for small-scale spatial management, the management of abalone fisheries in Australia generally occurs over relatively large spatial scales (from 100 – 1000 km). Perseverance with this approach may ultimately compromise the sustainability of the Australian abalone resources, highlighting the need for consideration and development of more biologically-relevant management units underpinned by stock identification. Stock identification provides information that enables management to occur at spatial scales that more appropriately reflect the actual population structure of the species. While several approaches to stock identification have been used, genetic methods remain among the most effective and appropriate tools to use.

This project used a genetic approach based on microsatellite DNA analysis to (1) quantify greenlip population structure within the principal fishing grounds across south-eastern (SE) Australia, and (2) assess genetic connectivity among these populations. Adult greenlip were collected from South Australia, Victoria and

Tasmania, thus spanning four large biogeographical regions (Great Australian Bight, Spencer Gulf, Bonnie Upwelling and North West (NW) Tasmania). A hierarchical sampling design was used. The three levels were regions, locations within regions, and replicate sites within locations. At Tiparra Reef (Spencer Gulf biogeographic region), the most productive fishing ground for greenlip in Australia, sampling was more intensive. This was to test the commercial fishers' hypothesis that the seldom harvested shorter, domed greenlip on the southern parts of the reef were the prime larval source supporting the intensive commercial harvest of the large, flat greenlip in the northern and western areas of the reef. DNA was extracted from all samples and analysed using 15 microsatellite loci that had suitable levels of polymorphism for a population genetic study. Results were interpreted using a range of statistical methods available in Genepop 4.2, Microchecker, LOSITAN, HeirFstat, GeneClass2 and GenalEx.

Greenlip sampled were genetically diverse with no evidence of reduced genetic diversity or bottleneck effects expected in an exploited species, suggesting that harvests and management practices have effectively mitigated loss of genetic diversity. Genetic diversity also decreased with increasing latitude and longitude, consistent with expected reduced diversity in populations at the southern end of their distributional range. The lack of significant genetic differences among the samples across Tiparra Reef suggest this important fishing ground represents a single, panmictic population, rather than multiple, spatially-structured metapopulations. Thus, most adults sampled across Tiparra Reef were assigned to the location where they were collected and assignment tests on the 45 juveniles sampled showed that >70% likely originated from Tiparra Reef, again indicating strong self-recruitment. There was evidence indicating that the southern parts of the reef are a source of recruits to the commercially-fished northern and western areas, however they are not the only source of recruits, as 67% of juveniles were assigned to sites other than those on the south of Tiparra Reef. Overall, greenlip on Tiparra Reef appear to comprise a single population with strong connections to those greenlip at Cape Elizabeth, thereby conforming to a larval-pool structure.

Genetic subdivision across SE Australia indicated that greenlip do not comprise a single, large, panmictic population. Differentiation was most evident at the two largest scales: among biogeographic regions (i.e. hundreds of kilometres) and among locations within regions (i.e. tens of kilometres). These yielded a strong pattern of isolation by distance. However, different processes were likely to be occurring within each region and scales of connectivity among greenlip populations are unlikely to be

easily identified. Nevertheless, assignment tests again confirmed that larval dispersal only occurs relatively rarely, even among locations within regions, as >90% of adults sampled were assigned to the site where they were collected.

Overall, we estimate that populations generally encompass reefal areas of around 30 km^2, are largely maintained through self-recruitment, and that distances of up to 130 km are effective barriers to larval dispersal. These findings differ substantially from those obtained previously for blacklip, with the spatial scale of greenlip stock structure and connectivity being two orders of magnitude larger than that for blacklip. There are two plausible explanations for the different patterns of connectivity between greenlip and blacklip: the contrasting scale of connectivity may be related to structural differences (e.g. algal density) on the reef habitats supporting these populations, or the patterns found may highlight species level differences in spawning and larval ecology, leading to different scales of larval dispersal. The differences identify the need for species-specific management approaches even among closely related, spatially-structured stocks.

The number and size of stocks exploited by a fishery and connectivity between spatially structured stocks is recognised as an integral component of modern fisheries science. In this study, we identified a spatial structure of stocks (metapopulations) that is not reflected in the historic management arrangements (i.e. broad-scale catch controls). Given the large-scale reductions in global abalone production since the 1980s, the more recent decreases in the total Australian catch and difficulty in sustainably harvesting similar species with a high degree of stock structure, the mismatch between metapopulation and existing fishery management boundaries suggests the current management of this fishery warrants review.

KEYWORDS: greenlip abalone; microsatellite; genetic diversity; stock structure; spatial management.

2 ACKNOWLEDGEMENTS

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3 BACKGROUND

Fine-scale population structure is common in many inshore marine species (Swearer *et al.* 2002; Orensanz *et al.* 2005), particularly sedentary invertebrates with limited larval dispersal whose populations (or stocks) tend to be characterised by a complex spatial structure evident at fine spatial scales (Strathmann *et al.* 2002; Orensanz *et al.* 2005). Aggregations of these species form discrete populations that are effectively isolated from conspecifics by reproduction and migration (Berryman 2002; Morgan and Shepherd 2006). They also often have variable life history parameters (McShane *et al.* 1988; Orensanz and Jamieson 1995; Withler *et al.* 2003; Orensanz *et al.* 2005). Assessment and management of spatially complex stocks is challenging, which has resulted in assessment and management processes occurring at spatial scales considerably greater than those suggested by the spatial complexity of the stocks (Prince 2005). The mismatch between the scale of the component stock units and the scale of assessment and management has been termed the 'tragedy of scale' (Prince and Hilborn 2003). This disparity has been blamed for the failure to maintain sustainable resources in numerous sedentary invertebrate fisheries (Perry *et al.* 2002; Orensanz *et al.* 2005).

Abalone (Family Haliotidae, Genus *Haliotis*) are gastropod molluscs that support valuable fisheries in many parts of the world (Hamasaki and Kitada 2008). Abalone fisheries in Australia are the source of around 50% of the global harvest of wild abalone (Gordon and Cook 2004), and to date there is little evidence of declines in Australian abalone catches, unlike those documented elsewhere (Prince 2004). One of the key features of abalone biology that is important for effective fishery management is their fine-scale, complex population structure and limited larval dispersal (Prince 2005; Morgan and Shepherd 2006; Saunders *et al.* 2008, 2009; Prince *et al.* 2008; Saunders and Mayfield 2008; Miller *et al.* 2009). However management of abalone fisheries in Australia generally occurs over large spatial scales (from 100 – 1000 km of coastline) on a State-by-State basis through the use of minimum harvest lengths, total allowable catches and individual quotas (Prince and Shepherd 1992). These regional-scale management approaches do not consider the spatial complexity of abalone stocks, or the spatial variability in life-history parameters among populations (Prince 2005). Perseverance with this approach may ultimately compromise the sustainability of Australia's abalone resources, and suggests the need for consideration and development of more biologically-relevant management units (MU; Taylor and Dizon 1999; Palsbøll *et al.* 2006). Unfortunately, even though there is growing evidence of the need for fine-scale assessment and

management, strategies and programs to move beyond relatively broad-scale management are rare.

One of the earliest examples of a reduction in the spatial scale of abalone fishery management was the introduction of separately-managed, 'fish-down' areas (FDAs) in the South Australian Southern Zone in 1994. The changes in the spatial management of these populations recognised the biological and morphological variability among blacklip abalone (*Haliotis rubra*; hereafter termed blacklip) populations in this zone. FDAs were designed to encompass components of the fishery within which the blacklip populations were considered to be 'stunted' (Tyrer 1995; Mayfield *et al.* 2009). This was followed by a FRDC-funded project (2004/019 *Towards optimising the spatial scale of abalone fishery management*; Mayfield and Saunders 2008), from which a principal outcome was the identification of a 'morphometric marker', based on the ratio between shell length and shell height, for discriminating among blacklip stocks and predicting their biological characteristics. At about the same time, the Western Abalone Divers Association Inc. (WADA), the industry association representing divers and license holders in the Western Zone of the Victorian Abalone Fishery, began assessing and managing their blacklip fishery at a reef-code scale. To achieve this, they use a harvest policy framework underpinned by a 'rapid assessment' of abalone population 'health', visually determined from the shape and appearance of blacklip shells from the commercial catch (Prince *et al.* 2008). Extension and development of this process has led to increasingly complex, spatial management of the resource, including reef-specific total catch limits, daily catch limits and minimum legal sizes (MLS). This WADA initiative, undertaken primarily through a series of workshops, was augmented and extended into other jurisdictions through a concurrent FRDC project (FRDC 2005/024 *Abalone industry development: local assessment and management by industry*; Day et al. 2010).

Stock identification is an integral component of modern fisheries science and management (Begg and Waldman 1999; Palsbøll *et al.* 2006). This is because stock identification facilitates effective fisheries management by providing information that enables management to occur at spatial scales that more appropriately reflect the actual population structure of the species. Considerable effort has been expended over the past decade in understanding the stock structure and dynamics of blacklip. In contrast, the stock structure of greenlip abalone (*Haliotis laevigata*; hereafter termed greenlip) has received little attention. This is despite current commercial catches of greenlip exceeding 700 t/yr across southern Australia (Mayfield et al.

2012). For a range of reasons, including environmental differences between the reefs typically occupied by blacklip (shallow water and complex, heterogenous reef) and greenlip (deeper water and flat, uniform reef), connectivity among greenlip populations is expected to differ substantially to that for blacklip. While this suggests that it is inappropriate to apply the stock structure model developed for blacklip to greenlip, there are few data to support this hypothesis.

Several approaches to stock identification have been undertaken. While these include morphology (Cadrin 2000; Mayfield and Saunders 2008), parasites (Zischke *et al.* 2009) and otolith biochemistry (Fowler *et al.* 2004), genetic methods remain among the most effective and appropriate tools to use (Ward and Elliott 2001; Palsbøll *et al.* 2006; Temby *et al.* 2007; Miller *et al.* 2009). Indeed, Palsbøll *et al.* (2006) suggest an approach for identifying MU on the basis of the observed estimates of genetic divergence.

This project uses a genetic approach – microsatellite analyses – to (1) quantify greenlip population structure within the principal fishing grounds, and (2) assess genetic connectivity among these populations. Microsatellites are short sections of repeated DNA sequence (e.g. CACACACA) that tend to occur in the non-coding regions of DNA. The repeat sequence is typically between 5 and 40 repeats in length (referred to as a microsatellite locus) and are commonly found in the nuclear genome of most taxa (Selkoe and Toonen 2006). Microsatellite regions mutate frequently during DNA replication either losing or gaining a repeat sequence (e.g. CA) resulting in a change in the number of repeats and thus the length of the repeat string (alleles), which is then passed on to offspring. As individuals within a population will recombine their microsatellites during sexual reproduction, this maintains a set of microsatellite alleles that are characteristic for that population. That set of microsatellite alleles will be distinct from other populations which are not connected by either larval dispersal or adult migration. Because microsatellite alleles have relatively high mutation rates, and the mutations are normally not-lethal, they provide the necessary allelic diversity to examine genetic processes acting on ecological time scales (Selkoe and Toonen 2006).

Previous abalone genetics studies have focused primarily on blacklip (Brown 1991; Huang *et al.* 2000; Conod *et al.* 2002; Elliott *et al.* 2002; Baranski *et al.* 2006a,b; Temby *et al.* 2007; Mayfield and Saunders 2008; Miller *et al.* 2009; Appleyard et al. 2009) and have confirmed the fine-scale population genetic structure for blacklip, and evidence of limited gene flow among even adjacent populations (Temby *et al.* 2007;

Miller *et al.* 2009). In contrast, greenlip have rarely been considered (Brown and Murray 1992; Maynard *et al.* 2004). The more recent of these studies explored the population structure of greenlip around Port Philip Bay using microsatellites and found some evidence of genetic differentiation, but no link between genetic and geographic distances expected under a stepping-stone model of larval dispersal. Brown and Murray (1992) showed significant population subdivision among South Australian greenlip populations, as well as between Tasmania and South Australia, including evidence of isolation-by-distance. They suggest that greenlip neighbourhood size may be small, but highlighted the need for further sampling. Although these studies have shown some evidence of genetic separation for greenlip populations, neither sampled at the spatial scale required to address the question of genetic connectivity among adjacent, putative greenlip populations within and among key fishing areas. The sampling design used here is therefore appropriate to the spatial scale identified in the questions posed by abalone fishers in South Australia and Tasmania.

4 NEED

The principal need is to enhance understanding of greenlip population genetic structure, and the degree to which nearby populations are connected, in order to optimally manage exploitation of greenlip. Greenlip support valuable fisheries across southern Australia. Total catch is >700 t with a landed value of ~$27M with most of the catch harvested in South Australia (Mayfield et al. 2012).

The majority of abalone-related funding has addressed research needs for blacklip. This research has focussed on stock structure and dynamics, developing assessment and management approaches to overcome spatial complexity, and stock rebuilding strategies. Recent projects (FRDC 2004/019, 2005/024, 2005/029), have clearly demonstrated that (1) blacklip populations are effectively isolated from conspecifics at fine spatial scales (Miller *et al.* 2009), and (2) each has typically variable life-history parameters (e.g. growth rates) that influence productivity and response to fishing.

Historically, little effort has been directed towards understanding variation or inter-dependence among greenlip populations. Connectivity among greenlip populations is expected to be substantially different to that observed for blacklip, due, in part, to environmental differences (e.g. current, swell, kelp) in reef systems they inhabit. However, there are few data to support this assertion. If, as expected, patterns of connectivity among greenlip populations differ from blacklip, this will require a different approach and different scales of fishery management and assessment.

Understanding greenlip population structure is a high priority in South Australia, Tasmania and Western Australia. Development of improved techniques for assessment, definition of metapopulation boundaries and reducing the spatial scale of fishery management are high research priorities of the South Australian Abalone Fishery Management Plan. Similarly, developing harvest models that incorporate fine-scale fishery management to guide harvest practices and optimise yield is a research priority in Investment Platform 3 in the Abalone Council Australia Strategic Plan.

5 OBJECTIVES

The overarching goal of this project was to address knowledge gaps relating to greenlip population structure, genetic diversity and connectivity across key fishing areas using microsatellite analysis.

There were two objectives:

1. Assess genetic connectivity within and among greenlip abalone populations in key fishing areas; and
2. Quantify greenlip abalone population genetic structure within key fishing areas.

6 METHODS

6.1 Sample collection

Adult greenlip (>100 mm shell length; n = 2,368) were collected across SE Australia from South Australia, Victoria and Tasmania (Figure 6-1). We sampled up to ten locations within each biogeographical region (Great Australian Bight, Spencer Gulf, Bonnie Upwelling and North West (NW) Tasmania), and within locations we sampled 30-40 individuals from each of three sites. This yielded a three-level, hierarchical experimental design comprising regions, locations within regions and replicate sites within locations. A small tissue sample comprising a sliver of mantle (approximately 5 mm by 2 mm) was removed from each greenlip using a scalpel and was preserved immediately in >90% molecular-grade ethanol for subsequent genetic analysis. Additional (fixed tissue) samples were opportunistically provided by colleagues. These were from Baudin Rocks (one site, n=30) and Port Phillip Bay (three sites, n = 30/site) (Figure 6-1), and were used wherever possible to supplement the data set.

As Tiparra Reef (Spencer Gulf biogeographical region; Figure 6-1) has been the most productive fishing ground for greenlip in Australia, this area was sampled more intensively to (1) better understand connectivity and gene flow across this important fishing ground; and (2) test the hypothesis that the net movement of larvae was from the south to the north and west. The latter enabled testing of the commercial fishers' hypothesis that the seldom harvested shorter, domed greenlip on the southern parts of the reef were the prime larval source supporting the intensive commercial harvest of the large, flat greenlip in the northern and western areas of the reef. Thus, adult greenlip were collected from five locations (Figure 6-1a) across the reef, each separated by approximately 2-4 km and within each of these locations we collected 30-35 abalone from each of three replicate sites approximately 150-300 m apart. We also sampled 45 juveniles (43 – 90 mm shell length) from three sites (West Bottom; Coalground; Lighthouse), all located in the northern and western fished areas.

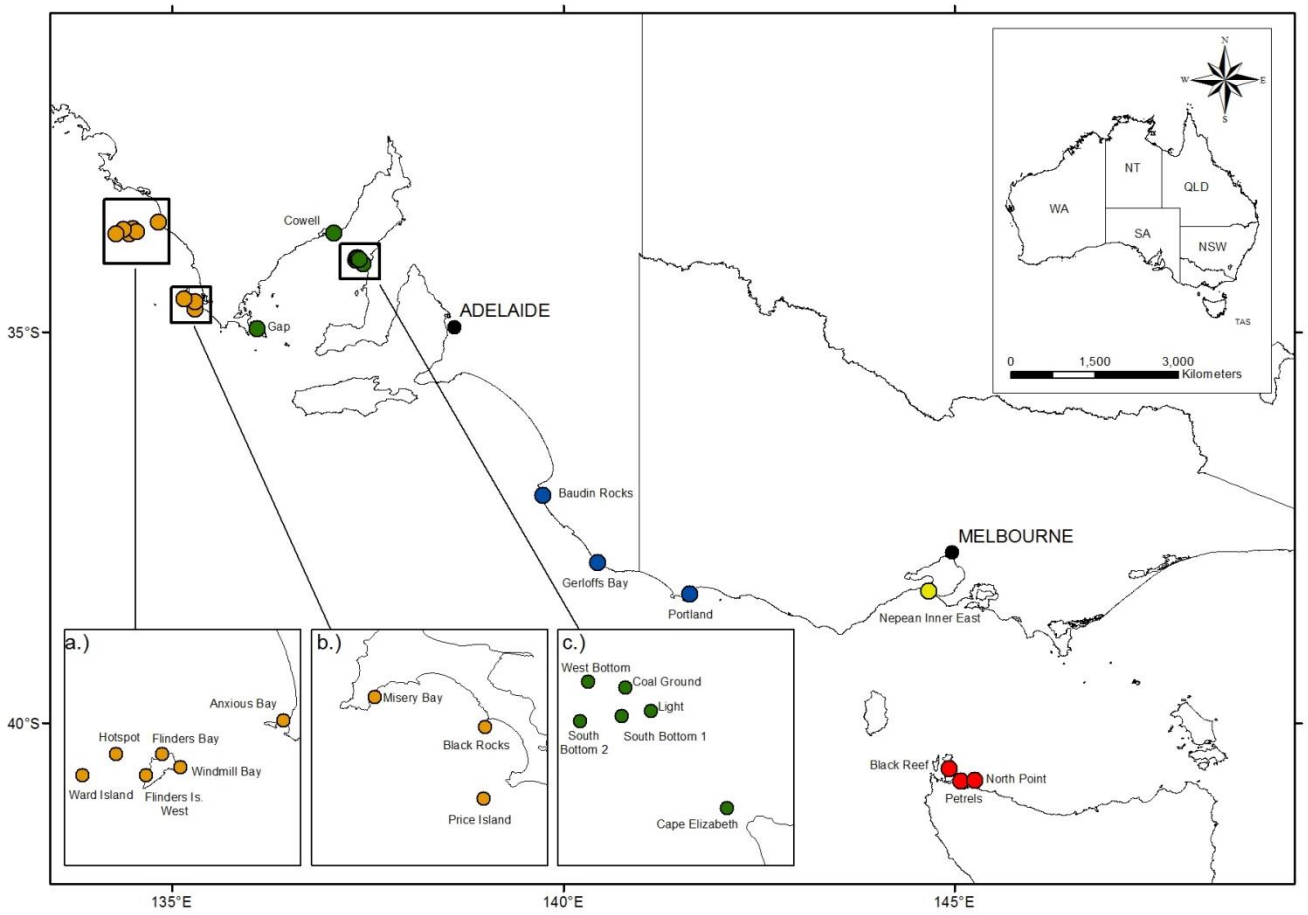

Figure 6-1. Map showing sampling locations for greenlip in SE Australia. Within each location, three replicate sites were sampled with the exception of Baudin Rocks where collections were from only one site. Colours differentiate among biogeographic regions (orange: Great Australian Bight; green: Spencer Gulf; blue: Bonnie Upwelling; red: NW Tasmania and yellow: Port Phillip Bay).

6.2 Sample processing and microsatellite genotyping

DNA was extracted from all samples using the DNeasy extraction kits (Qiagen) according to the manufacturer's instructions and the DNA concentration quantified using a Nanodrop8000 (Thermo Scientific).

Thirty microsatellite loci developed for blacklip (Evans *et al.* 2000; Baranski *et al.* 2006b) were tested on a subset of 25 greenlip to find loci with suitable levels of polymorphism for a population genetic study. Fifteen of these amplified were polymorphic and used for genotyping of all greenlip sampled. Each locus was amplified in separate Polymerase Chain Reactions (PCR) and the alleles analysed using GeneMapper (Applied Biosystems Inc.) and Tandem Ver 1.02 (Matschiner and Salzburger, 2009) software.

6.3 Data analyses

Loci were checked for evidence of linkage disequilibrium in Genepop 4.2 (Raymond and Rousset, 1995), null alleles were tested using Microchecker (Van Oosterhout *et al.* 2004) based on Bonferroni confidence intervals and each locus was assessed for evidence of selection using LOSITAN (Antao *et al.* 2008, Beaumont and Nichols, 1966). As these factors influenced only the magnitude of the statistical significance level, we used the full data set of 15 loci (with four corrected for null alleles) for all subsequent analyses. We summarised genetic diversity in several ways. These were the (1) average number of alleles per locus; (2) allelic richness; (3) observed and expected heterozygosity; and (4) number of private alleles, with each measure being compared among regions using analysis of variance (ANOVA).

A two-level hierarchical F-statistic analysis in HeirFstat (Goudet, 2005) and migrant movements using GeneClass2 were used on samples collected at Tiparra Reef to (1) determine the extent of small-scale population structure; and (2) test the ‘larval-source hypothesis’, respectively. Subsequently, greenlip population structure across SE Australia was also assessed using hierarchical F-statistics calculated in HeirFstat (Goudet 2005): among regions, among locations within regions, and among sites within locations with the replicate sites across Tiparra Reef considered to represent a single location for this analysis. Evidence of isolation by distance was evaluated using a Mantel Test in the ISOLDE program within Genepop. Spatial autocorrelation, performed in GenalEx 6.501 (Peakall and Smouse 2006, 2012) was used to assess the spatial scale of genetic structure across all the sites sampled. Genetic similarity among locations was determined using Principle Co-ordinates Analysis (PCA).

7 RESULTS/DISCUSSION

7.1 Greenlip abalone genetic connectivity and population structure

At all sites, greenlip were genetically diverse. There was an average of 12.9±0.4 alleles per locus observed and the number of alleles at each locus ranged between 8 and 78. Consequently, there was no evidence of reduced genetic diversity or bottleneck effects that might be expected in a population that was heavily exploited (Allendorf and Luikart 2007), despite greenlip having been intensely harvested since the mid 1960s (Mayfield *et al.* 2012). This suggests that harvest amd management practices have effectively mitigated loss of genetic diversity. There were, however, two distinct trends. First, genetic diversity decreased with increasing latitude and longitude (Figure 7-1). Second, there were substantial differences in genetic diversity among biogeographic regions, with significant differences in the total number of alleles and allelic richness observed (Table 7-1). For example, populations in NW Tasmania had significantly lower allelic richness than those from the Great Australian Bight or Spencer Gulf (Table 7-1) and fewer alleles were also found in samples from the Bonnie Upwelling compared with the Great Australian Bight. These patterns most likely reflect reduced diversity in populations at the southern end of their distributional range as observed in other marine invertebrates (Miller and Ayre 2008).

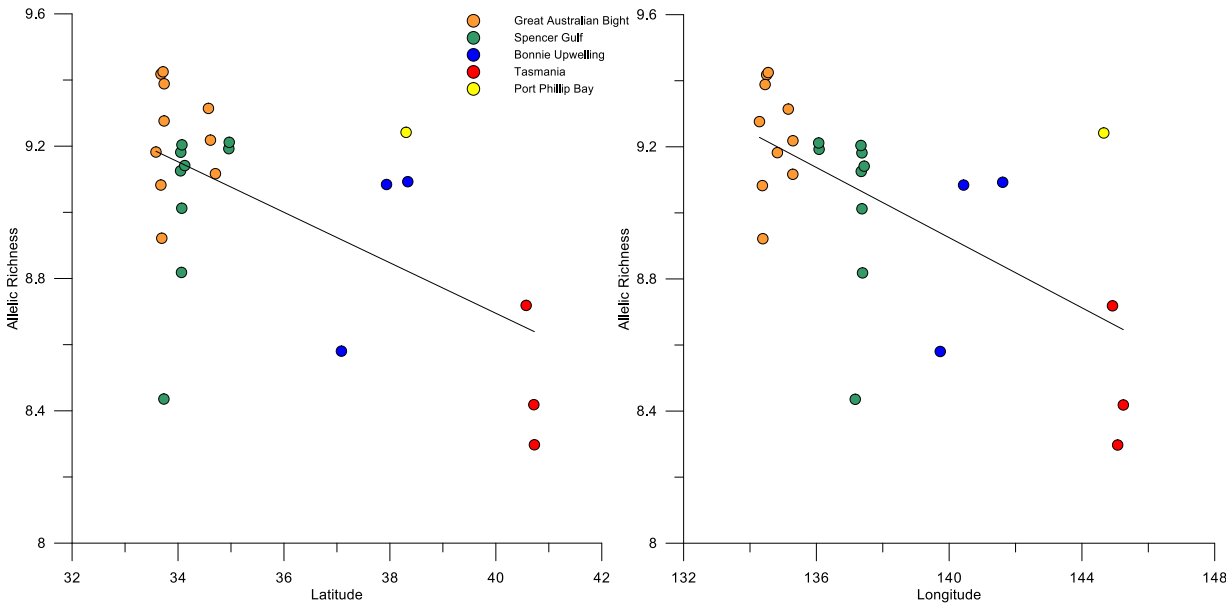

Figure 7-1 Relationship between latitude/longitude and allelic richness in greenlip populations (by location) throughout SE Australia.

Table 7-1 Measures of genetic diversity in greenlip populations averaged across locations (±SE) within each of five biogeographical regions in SE Australia. Only one location was sampled in Port Phillip Bay, hence no means can be calculated and these data were not included in the ANOVA. AR - Allelic richness, HO - Observed heterozygosity, HE - Expected heterozygosity.

Region	N	No. alleles	Private alleles	A_R	H_O	H_E
Bonnie Upwelling	218	173.0 (22.6)	3.0 (0.6)	8.91 (0.17)	0.554 (0.011)	0.605 (0.005)
Great Australian Bight	983	204.6 (2.5)	1.9 (0.4)	9.23 (0.05)	0.558 (0.005)	0.607 (0.004)
Spencer Gulf	839	196.3 (3.7)	1.44 (0.5)	9.04 (0.09)	0.560 (0.004)	0.609 (0.003)
Port Phillip Bay	90	184	0	9.24	0.556	0.610
NW Tasmania	321	180.3 (4.9)	4.0 (3.5)	8.48 (0.12)	0.567 (0.008)	0.606 (0.002)
ANOVA results (df = 3)						
F		4.492	1.145	9.225	0.437	0.117
Prob.		0.014**	0.354	0.000***	0.729	0.949

Our genetic data indicate that Tiparra Reef is a single population. There were no significant genetic differences among the five locations sampled or among sites within locations across Tiparra Reef. However, greenlip on Tiparra Reef were also not genetically different to the nearby Cape Elizabeth, despite the lack of contiguous habitat between them, suggesting greenlip from these areas form a single metapopulation. Whilst there was little power to detect migration events in these data, most adults sampled across Tiparra Reef and Cape Elizabeth were assigned to the location where they were collected (94-98%, Table 7-2). Similarly, assignment of the 45 juveniles sampled from Tiparra Reef to locations throughout the Spencer Gulf showed that 71% (n = 32) and 78% (n = 35) likely originated from Tiparra Reef or from the combined Tiparra/Cape Elizabeth population, respectively (Table 7-3), indicating strong self-recruitment. There was some evidence to support the hypothesis that the southern parts of the reef are an important source of recruits to the commercially-fished northern and western areas. Notably, 15 (33%) of the juveniles sampled from the northern and western sites were assigned to either South Bottom 1 or 2. However, southern Tiparra Reef was not the sole source of recruits to these areas, because a similar proportion of juveniles (35%) were assigned to other areas on Tiparra Reef (42% including Cape Elizabeth) and 22% to other locations within Spencer Gulf (Table 7-3). These data demonstrate that Tiparra Reef does not conform to a source-sink metapopulation model (Shepherd and Brown 1993). Rather, greenlip on Tiparra Reef comprise a single greenlip population with strong connections to those greenlip at Cape Elizabeth, thereby conforming to a larval pool structure. Thus, it is likely that the morphological differences between the northern and southern Tiparra Reef greenlip reflect a phenotypic difference driven by

environmental factors, which is similar to that identified elsewhere for blacklip (Mayfield and Saunders 2008; Saunders *et al.* 2009).

Table 7-2 Assignment of adult greenlip collected from Tiparra Reef and Cape Elizabeth to the most likely source populations within the Spencer Gulf region.

From	To Cape Elizabeth	Coal ground	Light house	South Bottom 1	South Bottom 2	West Bottom	Total Exported
Cape Elizabeth	83	-	1	2	1	3	7
Coalground	2	87	2	-	-	-	4
Lighthouse	1	1	87	-	1	-	3
South Bottom 1	1	-	-	85	1	2	4
South Bottom 2	1	-	2	2	87	1	6
West Bottom	-	1	-	1	-	92	2
Total imported	5	2	4	3	2	3	

Table 7-3 Assignment of juvenile greenlip collected from three sites at Tiparra Reef to the most likely source populations within the Spencer Gulf region.

	Tiparra juveniles			
Assigned to:	Lighthouse n=23	Coalground n=15	West Bottom n=7	Proportion of all juveniles
Tiparra West Bottom	5	0	2	16%
Tiparra South Bottom 1	2	2	0	9%
Tiparra South Bottom 2	4	4	3	24%
Tiparra Lighthouse	3	1	0	9%
Tiparra Coalground	2	4	0	13%
Total from Tiparra	**16**	**11**	**5**	**71%**
The Gap	4	3	2	20%
Cowell	1	0	0	2%
Cape Elizabeth	2	1	0	7%
Total from elsewhere	**7**	**4**	**2**	**29%**

Genetic differences among sites across SE Australia were low, but were significantly different to the values expected in a single, large, panmictic population. Most of the differentiation was evident at the two largest scales: among biogeographic regions (i.e. hundreds of kilometres) and among locations within regions (i.e. tens of kilometres), with sites within locations typically not differentiated from each other. These results were supported by the PCA (73% of the variation in the data set explained) which showed that locations within each biogeographic region generally clustered together (Figure 7-2). There were, however, two notable exceptions – Port Phillip Bay (Bonnie Upwelling) and The Gap (Spencer Gulf) – both of which grouped with the Great Australian Bight locations. Whilst The Gap is adjacent to the Great Australian Bight, the genetic similarity between greenlip from Port Phillip Bay and the Great Australian Bight is more difficult to explain, likely requiring

additional sampling and analysis in future years. Nevertheless, there was a strong pattern of isolation by distance (r^2 = 0.138, P<0.001; Figure 7-3) and significant positive spatial autocorrelation among sites separated by distances up to ~135 km (Figure 7-4).

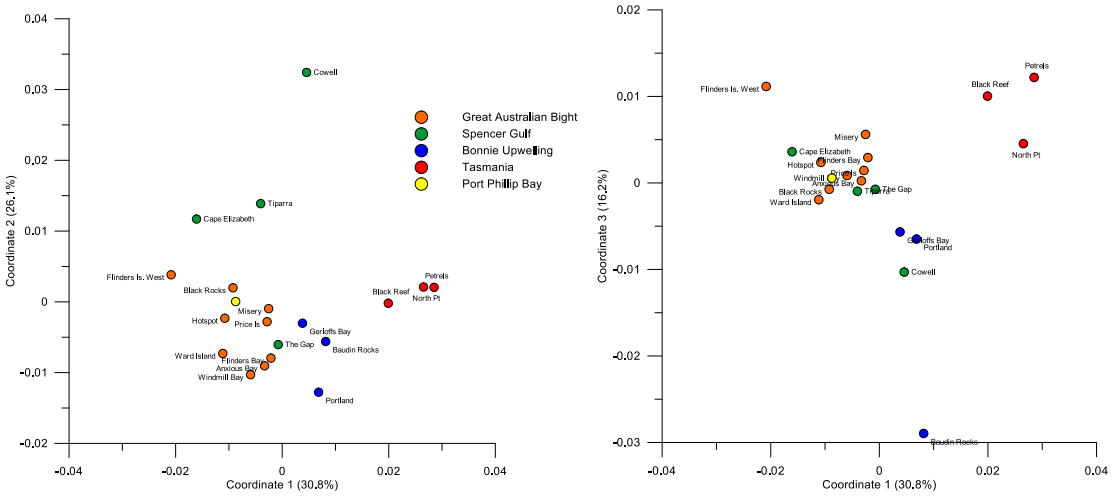

Figure 7-2 Principle Coordinates Analysis by location (replicate sites pooled) based on multi-locus genotypes for greenlip. Sampling locations are colour coded according to biogeographic region.

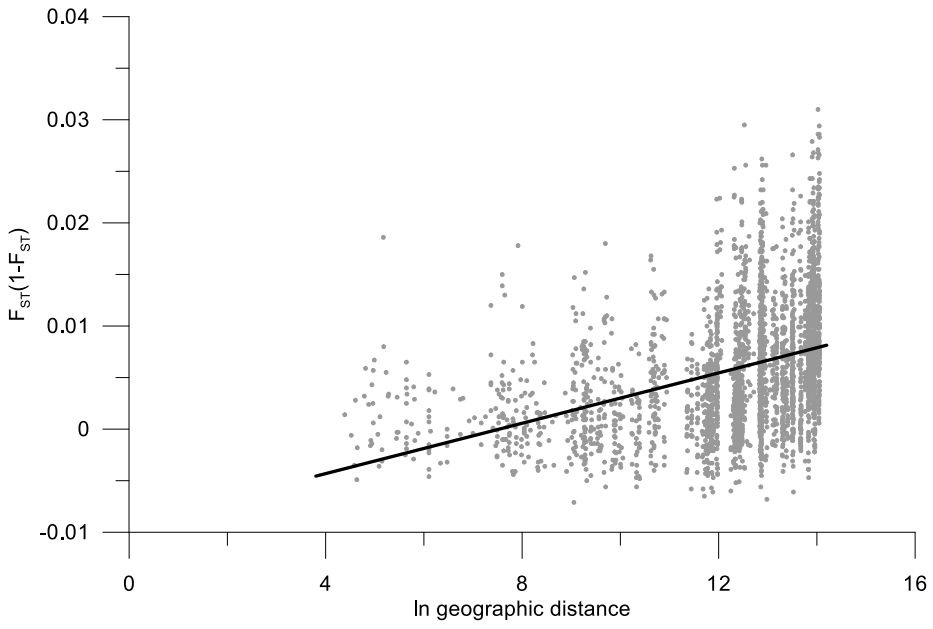

Figure 7-3 Pattern of isolation by distance, based on over-water distances, among greenlip populations sampled from sites across SE Australia.

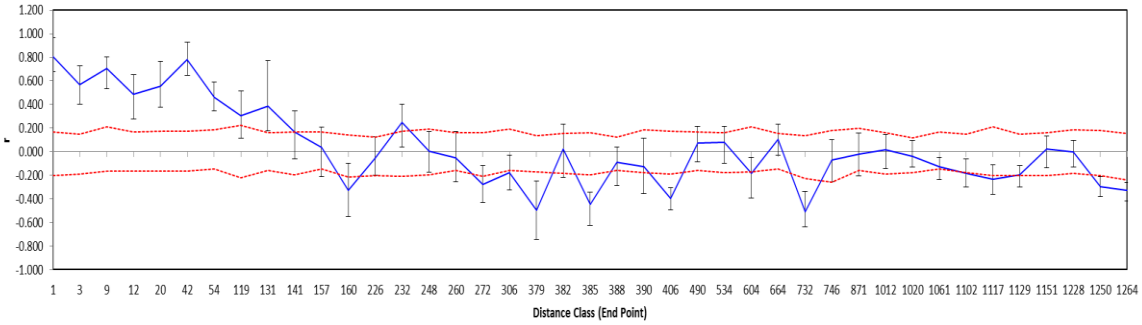

Figure 7-4 Results from spatial autocorrelation analysis, based on over-water distances, across all greenlip sites sampled from across SE Australia. The blue line represents the value of the correlation coefficient *r* at each distance class (±95% CI based on bootstrap resampling) with red lines denoting upper and lower 95% confidence limits around the null hypothesis of no spatial structure.

Pairwise estimates of genetic differentiation, based on pooled data among sites within locations, indicated different processes were likely to be occurring within each region and that scales of connectivity among greenlip populations are not easily predictable. For example, there were no differences among locations in NW Tasmania or the Bonnie Upwelling, whereas within the Spencer Gulf, the abalone at Cowell were differentiated from those at Tiparra Reef and The Gap (Figure 7-5). Relationships among locations in the Great Australian Bight were more variable. Whilst a third (36%) of the pairwise comparisons were significant (Figure 7-5), some locations within clear geographic regions were genetically similar (e.g. the three locations within Avoid Bay and at Flinders Island). Pooling of greenlip across locations within the Great Australian Bight within revised geographical areas yielded three distinct greenlip genetic populations within the Great Australian Bight: (1) Flinders Island area (including Ward Island, Hotspot, Anxious Bay and the three locations around Flinders Island); (2) Avoid Bay (Price Island, Black Rocks and Misery); and (3) The Gap.

As with Tiparra Reef, assignment tests suggested larval dispersal only occurs relatively rarely, even among locations within regions. This was because >90% of adults sampled were assigned to the collection location indicating high levels of self-recruitment in greenlip and low levels of dispersal. In addition, patterns of dispersal were generally unpredictable. However, in the Bonnie Upwelling and the Great Australian Bight there was some evidence of westerly movement of larvae. For the latter, some migrants appeared to have originated from either Windmill Bay (13.3%) or Misery Bay (17.8%) with the remainder from locations directly adjacent to these two areas (Flinders Bay (8.9%) and Black Rocks (8.9%)).

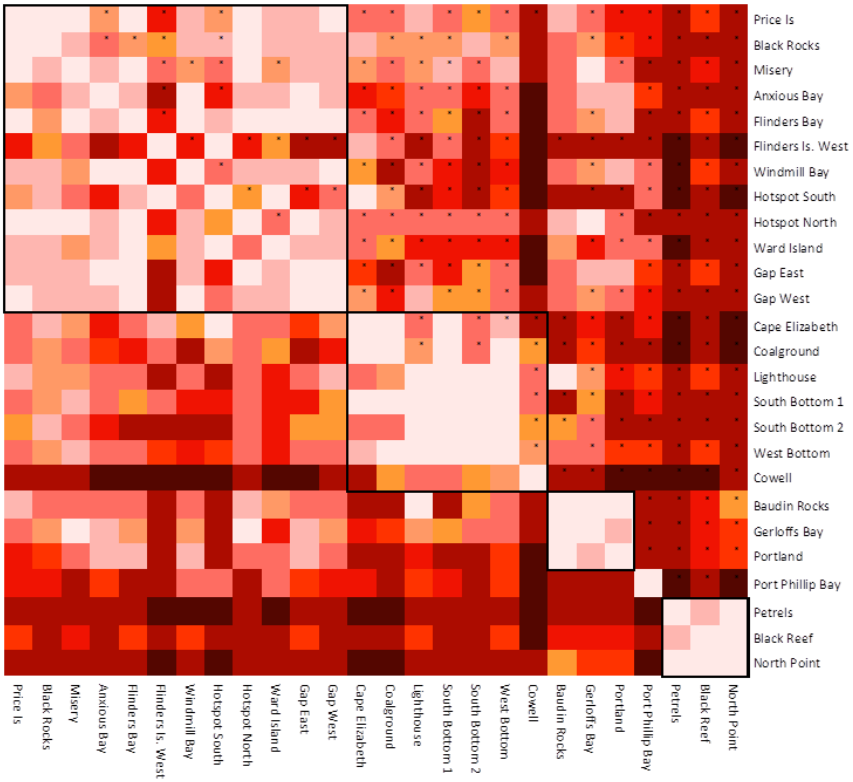

Figure 7-5 Heat map based on pairwise D_{EST} among all greenlip locations sampled across SE Australia. Black dots in the upper half of the matrix denote those pairwise comparisons that showed significant genetic differentiation between locations. Borders encompass pairwise comparisons within biogeographic areas.

7.2 Implications of greenlip abalone stock structure for fisheries management

Sustainable harvests are the ultimate goals of fisheries managers throughout the world and there are increasing requirements to demonstrate sustainable exploitation of resources, either to meet State/National legislation requirements or international accreditation benchmarks (e.g. Marine Stewardship Council). This, in turn, requires greater understanding of the target species' biology, and greater transparency of fishery management processes. Historically, key biological information underpinning sustainable management in fisheries included key life-history characteristics of a species (e.g. growth rate, reproduction, longevity), and population dynamics (recruitment, population growth, migration, mortality). Increasingly, the number of stocks exploited by a fishery and the connectivity between fished stocks is becoming recognised as an integral component of modern fisheries science and management (Begg and Waldman 1999; Palsbøll *et al.* 2006). Such stock identification facilitates more effective fisheries management by providing information that enables management to occur at spatial scales that more appropriately reflect the actual population structure of the species.

Using the data from 15 microsatellite DNA loci, our analyses showed that there was strong evidence of genetic structure, indicative of isolated metapopulations, for greenlip in each of five key biogeographic regions across SE Australia. We estimate that populations encompass reefal areas around 30 km^2, are largely maintained through self-recruitment, and that distances of up to 135 km are effective barriers to larval dispersal. As the metapopulation boundaries do not necessarily relate to the historical/political boundaries that have been the foundation of fisheries management, the current management of this fishery seldom reflects greenlip population genetic structure and associated ecological processes. For example, whilst exploitation in the Tasmanian Greenlip Fishery is managed within four zones each likely to contain a single metapopulation, in South Australia and Victoria the current zonal management arrangements largely do not account for the presence of multiple metapopulations. Our finding that greenlip have a complex metapopulation structure could be used to modify future management strategies thereby enhancing current resource management components. This is important given the large-scale reductions in global production of abalone since the 1980s (Prince 2004), the more recent decreases in the total Australian catch (Mayfield *et al.* 2012) and the difficulty in sustainably harvesting many fisheries species (Perry *et al.* 2002; Orensanz *et al.* 2005). Effort controls such as sub-zonal catch restrictions can be used to ensure effort is managed appropriately across separate stocks within large management zones. While spatial patterns of exploitation by the fishing fleet is often constant, external factors such as fuel price and market preferences can facilitate higher than normal harvests from localised areas, risking longer term reductions in productivity, where harvests deplete metapopulations, regardless of the metapopulation structure.

It is also clear from this study that the spatial scale of greenlip stock structure and connectivity was two orders of magnitude larger than the scale of population subdivision reported for the conspecific blacklip (Miller *et al.* 2009). While blacklip and greenlip occupy a similar geographical range and have similar early life history characteristics, a key difference between the two species is the type of habitat in which they occur. Blacklip live in heterogenous reef environments that are often associated with dense algal communities, where larvae are easily entrained and unlikely to be dispersed. In contrast, greenlip habitat is much more open, which likely facilitates greater dispersal of larvae and hence a larger spatial extent of connected local populations. Consequently, different management approaches for these two species – that dominate the Australian abalone production (Mayfield *et al.* 2012) – are likely to be required.

We have identified that greenlip conform to a stepping-stone or larval pool metapopulation structure and are maintained by local recruitment. Two management options are available to

prevent localised depletion of such species. These are the setting of MLS and spatial management of effort or catch. However, the practical scale of governance in abalone species is much greater than the scale of fishing, the spatial extent of patches, or of local populations (Bedford *et al.* 2013), suggesting spatial management and control of fishing effort has limited capacity to achieve sustainable management objectives for self-recruiting species (but see Prince *et al.* 2008).

Setting an MLS based on reproductive parameters can prevent populations declining below the density threshold levels that are required for fertilisation success (Gascoigne and Lipscius 2004, Bell *et al.*2008). This reduces Allee Effects and likelihood of subsequent population decline. Quantifying threshold levels is problematic (Lundquist and Botsford 2004) and where the biology of exploited species is at risk of Allee Effects, management should be precautionary (i.e. conservative MLS should be set).

However, in our study, patterns of connectivity among local populations within each of the greenlip metapopulations varied and, thus, identification of a general rule to underpin consistent management across all metapopulations was problematic. Consequently, the differences in the dynamics and scale of population processes between blacklip and greenlip and among greenlip metapopulations in SE Australia highlight the difficulty in generalising about ecological processes and the potential consequences of similar life history characteristics. Thus, knowledge of stock structure and connectivity should be used collectively with data on life history and population dynamics to determine the relative importance of input (e.g. number of fishers, fishing effort) and output (e.g. MLS, TACC) controls thus forming an integral part of the management of fisheries.

8 BENEFITS AND ADOPTION

This project has enhanced our understanding of greenlip population genetic structure, genetic diversity and connectivity among populations. These results will provide stakeholders (industry, management, research) in the Australian greenlip fisheries (principally, South Australia and Tasmania) with critical information to review management of their greenlip fisheries in the context of within-zone spatial management. Revision of management arrangements for the fishery will provide the opportunity for better, more efficient resource use. In turn, this should provide flow-on economic benefits through sustained harvests and licence values. Ideally the approaches developed here will be extended to the greenlip fisheries in Victoria and Western Australia.

9 FURTHER DEVELOPMENT

There are three activities that should be undertaken to strengthen our understanding of greenlip and blacklip genetic and population structure across southern Australia.

1) The genetic tools developed in this study need to be extended to greenlip in Western Australia and eastern Bass Strait using a similar hierarchical method to that employed here. This would enable a greater understanding of greenlip genetic structure and diversity across the second-largest fishery (Western Australia) for this species in Australia.

2) The status of the populations within the Bonnie Upwelling is unclear. Based on the conflicting results from the F_{ST} and D_{EST} comparisons, more intensive sampling across this region is required to resolve the metapopulation status of greenlip at Baudin Rocks, Gerloffs Bay and Portland, and to resolve the apparent anomaly of Port Phillip Bay.

3) Differences between blacklip and greenlip population structure could represent differences between species or habitats. As the blacklip and greenlip genetic studies completed to date have no spatial overlap, expansion of the blacklip program across the areas currently assessed for greenlip population genetic structure is required to resolve the different patterns of connectivity identified for these sympatric species. This will be aided by amalgamating the genetic information on greenlip and blacklip from throughout Australia and will permit identification of (1) differences and similarities between species; and (2) the need to consider species-specific management.

10 PLANNED OUTCOMES

There were two principal outcomes from this project. These were: (1) knowledge of greenlip abalone population genetic diversity and structure across key fishing areas; and (2) confirmation of different levels of connectivity among greenlip populations to blacklip. These outcomes will improve future harvest strategies and management arrangements for the fishery. The principal beneficiaries will be the stakeholders (fishers, managers and researchers) in the South Australian and Tasmanian abalone fisheries. These stakeholders will gain an enhanced understanding of greenlip population dynamics. This will provide environmental benefits (i.e. ability to more closely match spatial scales of stocks with spatial scales of assessment and management). In turn, this will improve management advice for abalone fisheries (maximising the yield and value without compromising the sustainability). This will provide economic benefits (i.e. sustained harvest and licence values). Approaches

developed here will be extended to abalone in Victoria and Western Australia. The potential impacts of the research are (1) improved management practices, and (2) enhanced resource sustainability. The information and knowledge developed through this project has been widely distributed among researchers, industry and managers in South Australia and Tasmania. This was achieved by providing regular updates to these stakeholders during the project, including formal presentation of the findings in Adelaide (4 July 2013) and Port Lincoln (5 July 2013) by Dr Craig Mundy. In addition, Dr Karen Miller presented these findings at the Australian Marine Sciences Association conference, also in July 2013. The final report has also been widely distributed, ensuring that the information will reach as wide an audience as possible (see Appendix 3).

11 CONCLUSION

The two objectives of this study were to (1) assess genetic connectivity within and among greenlip abalone populations in key fishing areas; and (2) quantify greenlip abalone population genetic structure within key fishing areas. Both of these objectives were achieved.

We sampled greenlip hierarchically and then used microsatellite DNA loci to determine genetic diversity and genetic structure across four key biogeographic regions in SE Australia. Data from 15 microsatellite DNA loci were used in our analyses. At all sites, greenlip were genetically diverse with no evidence of reduced genetic diversity or bottleneck effects that might be expected in a heavily-exploited population. Whilst genetic diversity decreased with increasing latitude and longitude, and there were substantial differences in genetic diversity among biogeographic regions, these findings were consistent with reduced diversity at the southern end of their distributional range. Overall, genetic subdivision across SE Australia was low, but significantly different to the values expected in a single, large, panmicitc population. Most of the differentiation was evident at the two largest scales: among biogeographic regions (i.e. hundreds of kilometres) and among locations within regions (i.e. tens of kilometres), with sites within locations typically not differentiated from each other.

The genetic evidence demonstrates that Tiparra Reef, a productive fishing ground for the South Australian Greenlip Fishery, is a single, panmictic population, that includes those greenlip from nearby Cape Elizabeth. This confirms high levels of self-recruitment in greenlip and low levels of dispersal. While the patterns of dispersal were generally unpredictable, there was some evidence of westerly movement of larvae in the Bonnie Upwelling and the Great Australian Bight.

Our analyses showed that there was strong evidence of genetic structure, indicative of isolated metapopulations, for greenlip in each of four key biogeographic regions across SE Australia. We estimate that populations encompass reefal areas around 30 km^2, are largely maintained through self-recruitment, and that distances of up to 135 km are effective barriers to larval dispersal. Importantly, these findings differ substantially from those obtained previously for blacklip because the spatial scale of greenlip stock structure and connectivity was two orders of magnitude larger than that for blacklip (Miller *et al.* 2009). This difference may be related to the nature of the habitat in which these species occur (blacklip: in heterogenous reef environments with dense algal communities that would entrain larvae; greenlip: open habitat, lower algal densities and larger currents that would disperse larvae).

As effective fisheries management requires identification and maintenance of stocks, the apparent mismatch between metapopulation and fishery management (political) boundaries suggests the current management of this important fishery requires revision to ensure management scales reflects greenlip population genetic structure and associated ecological processes.

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13 APPENDIX 1: INTELLECTUAL PROPERTY

No Intellectual Property identified. This report and resulting manuscripts are intended for wide dissemination and promotion.

14 APPENDIX 2: STAFF

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15 APPENDIX 3: REPORT DISTRIBUTION LIST

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Dr Anthony Hart	WA Fisheries
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Dr Rick McGarvey	SARDI Aquatic Sciences
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Mr Geoff Ellis	Executive Officer: EZAIA
Mr Bob Pennington	President: SA CZ abalone association
Mr Jonas Woolford	President: AIASA
Mr Michael Coates	Executive Officer: AIASA
Dr Norman Ragg	Cawthron Institute
Dr Jim Sinner	Cawthron Institute
Mr Ian Cartwright	Thalassa Consulting
Mr Dean Lisson	Chair: Abalone Council Australia
Mr Joey McKibben	Tasmanian Abalone Council
Mr Greg Woodham	Tasmanian Abalone Council