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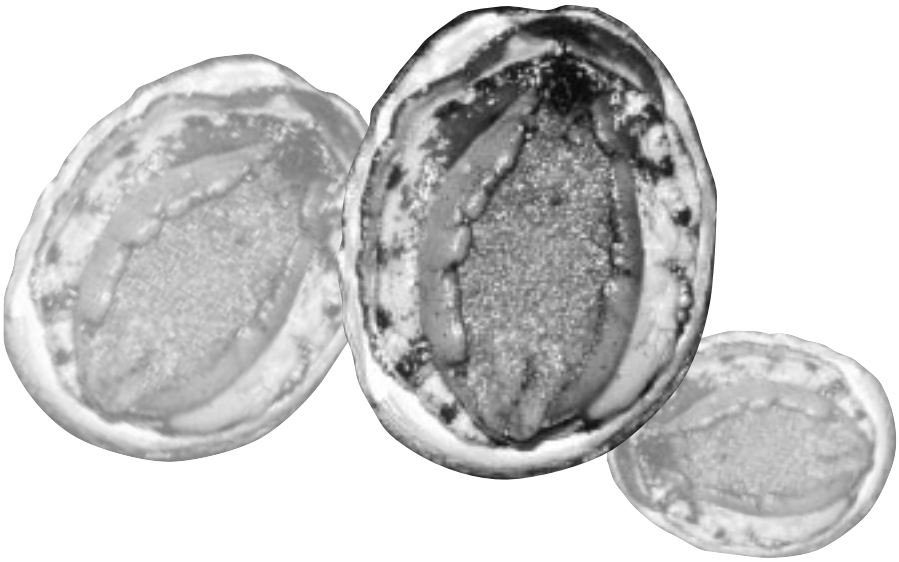
Application of molecular genetics to the Australian abalone fisheries:

Forensic protocols for species identification and blacklip stock structure

Nicholas G. Elliott, Jason Bartlett, Brad Evans, Rick Officer and Malcolm Haddon



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FRDC Project 1999/164

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1. NON-TECHNICAL SUMMARY

1999/164 Application of molecular genetics to the Australian abalone fisheries: forensic protocols for species identification and blacklip stock structure

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OBJECTIVES:

1. To refine, and where necessary establish, abalone species identification protocols to forensic standards suitable for required fisheries compliance.
2. To define the stock structure of blacklip abalone (*Haliotis rubra*) around Tasmania, using polymorphic nuclear DNA microsatellite markers.
3. To determine a suitable sampling and analysis regime for other temperate Australian abalone fisheries.
4. To determine the possible effects of harvesting on the genetic conservation of the blacklip abalone (*H. rubra*), by comparing the allozyme variation of two areas of the Tasmanian fishery with results obtained from the same areas in the late 1980s.

NON TECHNICAL SUMMARY:

- A mitochondrial DNA test has been developed that will identify 10 commercial or potentially commercial Southern Hemisphere species of abalone, including 5 Australian species. The test will allow identification from fresh, preserved, and processed tissue, and from mucous samples.
- The Western Australian brownlip abalone, *Haliotis conicopora*, could not be clearly identified as a separate species, and its status as a sub-species of the blacklip abalone, *H. rubra*, needs further consideration.
- Comparison of RFLP-mtDNA (restriction fragment length polymorphism – mitochondrial DNA) and microsatellite (nuclear DNA) analyses revealed similar levels of genetic differentiation in five blacklip abalone samples. Microsatellites were considered the most useful method for this project as they provided independent markers with a greater level of polymorphism.
- Very limited genetic heterogeneity was observed within the Tasmanian section of the blacklip abalone population following assessment of genetic diversity at 8 independent microsatellite markers (29 sites, average 106 individuals each).
- Some pairs of Tasmanian sites were significantly differentiated at individual loci, but no consistent geographic pattern was evident, except for possible differentiation of the northeast samples. Age cohort differences within the samples from each site may account for individual locus differences.
- Significant differentiation was apparent between some mainland and Tasmanian blacklip abalone samples.

- Correlation between genetic differentiation and geographic distance was observed between seven mainland collections of *H. rubra* from NSW to South Australia. The most distinct sample was from West Bay in South Australia.
- Population genetic heterogeneity was observed within the Australian greenlip abalone, *Haliotis laevigata*; in particular within Western Australia. Correlation between genetic differentiation and geographic distance was also observed.
- It is concluded that an isolation-by-distance model best fits the population structure of both *H. rubra* and *H. laevigata*. In both cases a genetic neighbourhood distance of ca. 500 to 600 km would separate sites that may be genetically distinct, but this needs to be confirmed. While local recruitment may be the norm, intermittent movement of larvae due to oceanographic conditions may result in a nearly homogeneous population. The more geographically isolated a pair of samples, the greater the genetic difference. The small but significant differentiation sometimes observed between samples over small spatial scales is suggested to be associated with age cohort differences within the samples.
- Some microsatellite loci isolated from Australian blacklip abalone, *H. rubra*, can be successfully amplified in other *Haliotis* species. A limited number of microsatellite loci are therefore available for research with other Australian commercial species; more loci, however, are required for all species.
- Commercial harvesting (over 15 years) appears to have had little measurable effect on allozyme genetic diversity in blacklip abalone sampled at two Tasmanian sites. It is recommended that monitoring of genetic diversity in settling larvae and/or known aged juveniles be commenced at selected sites using microsatellite markers. Such monitoring may provide early warnings of declines in effective (breeding) population sizes.
- Consideration needs to be given to extending the species identification test to include all species found in Australian waters (ten endemic and nine with Indo-Pacific distribution) and perhaps to all described species worldwide (56). In addition, the status of hybrids and sub-species needs further research.
- The largest and most comprehensive microsatellite DNA analysis of abalone to date has provided valuable information of abalone population structure. Some technical concerns (null alleles) exist with some microsatellites and this needs continued research. Further analyses of samples of *H. rubra* and *H. laevigata* from the mainland coast are recommended to refine the genetic heterogeneity observed in mainland populations, and provide more useful information for the ecological management of the two species.

OUTCOME ACHIEVED

A DNA based species identification test is available. Management of the Tasmanian blacklip abalone fishery can assume a single genetic stock. Management of mainland fisheries should take into account evidence of genetic population heterogeneity in the Western Australian greenlip population, and the genetic distinction of the West Bay (South Australia) blacklip sample.

KEYWORDS: Haliotidae, *Haliotis*, abalone, species identification, *H. rubra*, blacklip abalone, *H. laevigata*, population genetics, mtDNA, microsatellites

2. BACKGROUND

Of the nineteen abalone (Haliotidae) species recognised in Australian waters (Geiger 1999), ten are endemic and two dominate the commercial, recreational and illegal harvests in southern States: the blacklip abalone *Haliotis rubra* Leach and the greenlip abalone *H. laevigata* Donovan. A third species, Roe's abalone *H. roei* Gray, is a significant component of the Western Australian harvest. All three species, as well as the tropical *H. asinina* Linnaeus are also important as aquaculture species. The largest Australian (and world) fishery is in Tasmanian waters (half of Australia's harvest) and is focused predominantly on the blacklip abalone. Other species such as *H. scalaris* (Leach) and *H. conicopora* Péron have potential commercial value in temperate waters.

Internationally, overfishing, illegal fishing, pollution and recruitment failures have been implicated in the decline of many abalone fisheries (e.g. Hobday *et al.* 2001). However, the Australian fishery has had a relatively constant harvest over the past decade (1990-91 5.2 kt, 1997-98 5.2 kt, 1999/00 5.5 kt), with a total value over $230m in 1999/00 (ABARE 2001). The full extent of the illegal harvest in Australia is unknown, yet whatever the value, it is a large illegal business that may eventually impact on the commercial resource.

Although the extent of illegal fishing or poaching of abalone cannot be accurately accounted for, it has the potential to reduce or negate any significant benefits of resource management. In addition, poaching poses a serious threat to conservation and ecological sustainability as it can and does strip reefs of abalone of all sizes. Recolonisation may then occur by species other than abalone, such as sea urchins.

Poaching abalone is not just a local issue but a major concern in other abalone-producing countries such as South Africa, USA, Canada and Mexico. It is very lucrative, as the animals are ideal for black markets. Abalone are easily taken from the wild, attract a high price overseas, can be concealed and transported easily, and can be marketed as another species or from another country. Abalone poaching is more than a resource management problem; it is a social problem with highly organised and sophisticated syndicates operating. Whilst resources for policing in Australia exist, they are limited, but more importantly DNA based forensic protocols for assisting prosecution cases that go to court are non-existent; in particular it is currently difficult, even impossible, to determine the species of a poached animal and where it came from.

The survival of abalone fisheries requires improved techniques to assist the reduction of the illegal harvest (poaching) and the effective management of the legal harvest. The managing authorities need to be able to identify abalone product at the genus and species level (e.g. is it abalone and what species). They need to understand differentiation at the stock or population level within a species (for management zones; translocation policy; determination of collection sites). Molecular genetic techniques can be applied to understand the differentiation at these levels.

Forensic protocol for species identification
The molecular genetic identification of some temperate Australian and South African abalone species was initially demonstrated in a collaborative project between CSIRO Marine Research and the University of Cape Town (supported by the Tasmanian Abalone Council and Tasmanian Marine Police). The protocol was based on species differences at the lysin gene (Youn-Ho and Vacquier 1995). While the protocol was proved on a range of abalone products (e.g. fresh, canned), it required modification (for separation of additional species, e.g. *H. laevigata* and *H. scalaris*) and further assessment. One aim of this project was to produce a protocol for identification of the more common Australian species.

Population structure
The distribution of blacklip abalone (*H. rubra*) is more or less continuous along rocky coasts, except in intervening sheltered or sandy areas (Shepherd and Brown 1993). Initial genetic evidence (allozyme) suggested that the population structure of both major Australian temperate abalone species (*H. rubra* and *H. laevigata*) was best described by an "isolation-by-distance" model, with the more distantly separated populations genetically more distinct than closer populations (Brown 1991, Brown and Murray 1992). The genetic neighborhood (the distance within which inter-sample and intra-sample genetic variation is the same) for blacklip abalone was estimated to be 500 km of coastline. However, some local populations, separated by as little as 3 km of unsuitable habitat, appeared to genetically isolated from each other. Brown (1991b) suggested that this apparent contradiction was due to predominantly local recruitment, with the apparent high gene flow governed mainly by a historically large effective population size, rather than a substantial flow of gametes between populations. Larval studies support the local recruitment hypothesis (Shepherd and Brown 1993) and indicate that dispersal in blacklip abalone is very limited and may be actively avoided (Prince *et al.* 1987; McShane *et al.* 1988).

Despite the value of the world commercial harvest of abalone (ca. 10 500 mt/year, ca.$US80m, FAO 2000), there is a relatively poor understanding of the population structure of abalone species (Withler 2000). Although nearly half of the 56 recognized abalone species (Geiger 1999) are harvested, there have been few genetic differentiation studies on individual species. The genetic structure of the Californian red abalone *Haliotis rufescens* has been examined using four allozyme loci (Gaffney *et al*. 1996; Burton and Tegner 2000), sequence diversity of mitochondrial DNA (mtDNA) (Burton and Tegner 2000) and one microsatellite locus (Kirby *et al*. 1998). Overall, the data from these studies suggest Californian *H. rufescens* is undifferentiated. Results from other species from the California and Baja California coastline include allozyme differentiation in *H. corrugata* (del Río Portilla 2000), allozyme homogeneity across six loci in *H. fulgens* (Zúñiga *et al*. 2000), and population differentiation at three allozyme loci in *H. cracherodii* (Hamm and Burton 2000). Using mtDNA haplotype analyses, significant differences between populations have been reported for both Taiwanese abalone *H. diversicolor* (Jiang *et al*. 1995) and the South African species *H. midae* (Swiejd 1999). The population subdivision identified in *H. midae* using mtDNA haplotypes was in concordance with data from a recent microsatellite analysis (Evans 2002).

In Australian waters, the population structure of three abalone species has been examined with allozymes; *H. laevigata* (Brown and Murray 1992,), *H. roei* (Hancock

2000) and *H. rubra*, (Brown 1991b). In all cases an isolation-by-distance model is proposed to describe the population structure. While gene flow was found to be high, local heterogeneity was also evident. This apparent contradiction was suggested to be due to largely localized larval recruitment, with genetic differentiation being maintained by large population sizes and/or chance settlement of larvae from non-neighbouring sources.

Recent advances in molecular genetics have seen a potentially more powerful technique being used for population genetic analysis. The technique relies on differences in the lengths of short regions of nuclear DNA called microsatellites. Results of a FRDC-funded study (1995/002) demonstrated the potential value of this type of marker for blacklip abalone population studies. The results supported the likelihood of local recruitment of blacklip abalone (Huang *et al*. 2000). That study, based on few samples and loci, needed validating with a larger sampling program and more polymorphic markers.

A joint CSIRO/University of Tasmania/Aquaculture CRC project developed a further twenty-two blacklip abalone microsatellite loci for use in wild fishery and aquaculture studies (Evans *et al*. 2000). A focus of the present study was the use of a subset of these markers in an analysis of samples collected from throughtout the Tasmanian blacklip abalone fishery. The sub-set selected on their apparent level of polymorphism, gel scoring attributes and ability to be combined in a PCR multiplex.

Translocation and reseeding
The 1998 FRDC-funded review of wild abalone R&D needs in Australia established that one area of interest for many State management agencies and industries was the use of translocation or reseeding to take advantage of localised characteristics of a particular resource for stock enhancement. Without an understanding of the genetic structure of the local stocks or the genetic relationship between ‘host’ and ‘donor’ reefs, the movement of stock may have a detrimental effect on the commercial harvest or species conservation, or both. A clearer understanding of the genetic relationships among abalone stocks, as gained from the studies undertaken in this project, would be a major component of any risk assessment suggested under the draft SCFA National Translocation Policy.

Harvesting impact
The initial allozyme assessment of blacklip abalone, *H. rubra*, population structure was conducted over 15 years ago (Brown 1991a, 1991b), and the intervening time is a suitable period over which to assess the possible impact of harvesting on genetic variation. A major assumption is that any changes observed are due to harvesting alone and not to other possible environmental changes. An allozyme analysis, comparable to and with samples collected from two of the areas examined by Brown, was therefore a further focus of this project.

3. NEED

The state of molecular genetic technology in abalone is underdeveloped. There was a need for some basic research to develop molecular protocols that can be applied to various management and compliance issues; microsatellite markers are likely to have the most utility, and other abalone fisheries will benefit from Australian research (Sweijd 1997 - External review FRDC 1998/126).

Abalone populations elsewhere in the world have crashed catastrophically. Australian abalone resources are in a relatively good condition, but are under heavy pressure from many external factors, including illegal fishing, that could cause irreversible damage. In addition, a high conservation risk exists as some sections of the industry are considering translocation or reseeding for stock enhancement, but without fully understanding the genetic variability in a population. Understanding genetic variation in abalone at both the species and population level is important to the long-term sustainability of the valuable fisheries, as well as for genetic conservation.

Compliance was not only a major issue in the 1998 review of wild abalone R&D needs in Australia, but is an international issue. There are currently no suitable DNA based tools available in Australia that would assist authorities to identify abalone products and their origin.

The application of molecular genetics in this study provides managers with:
- DNA based tests for species identification, and
- estimates of gene flow between selected locations (i.e. stock identification).

Stock identification is needed not only for managing the commercial and recreational catch, but also for assessing the risks of translocation and reseeding projects, and land-based or sea-based aquaculture industries

This study built on a pilot project that developed the basis for an abalone forensic protocol, and provides a comprehensive assessment of the use of microsatellite markers for abalone stock identification. These goals are of both national and international interest.

4. OBJECTIVES

1. To refine, and where necessary establish, abalone species identification protocols to forensic standards suitable for required fisheries compliance.

2. To define the stock structure of blacklip abalone (*Haliotis rubra*) around Tasmania, using polymorphic nuclear DNA microsatellite markers.

3. To determine a suitable sampling and analysis regime for other temperate Australian abalone fisheries.

4. To determine the possible effects of harvesting on the genetic conservation of the blacklip abalone (*H. rubra*), by comparing the allozyme variation of two areas of the Tasmanian fishery with results obtained from the same areas in the late 1980s.

5. ABALONE SPECIES IDENTIFICATION

The research undertaken for Objective 1 of this project was submitted for peer review and accepted for publication in the international Journal of Shellfish Research. The paper is reproduced below (acknowledgments and references are included in Sections 14 and 15 respectively) with permission of the Journal editor.

The complete reference: Elliott, N.G., Bartlett, J., Evans, B. and Sweijd, N.A. 2002. Identification of Southern Hemisphere abalone (*Haliotis*) species by PCR-RFLP analysis of mitochondrial DNA. *J. Shellfish Res.* 21 (in press).

5.1 Abstract

Illegal fishing and species-substitution of abalone (genus *Haliotis*), a highly valuable marine gastropod, are of worldwide concern. A mitochondrial DNA PCR-RFLP analysis of fragments of the cytochrome oxidase I (mtCOI) and II (mtCOII) genes was developed for the identification of 11 Southern Hemisphere species of abalone. These included five temperate and one tropical species from Australian waters, three temperate species from New Zealand and two temperate species from South Africa. All species, with the exception of the *Haliotis rubra*/ *H. conicopora* complex, can be unequivocally identified using the combined profiles from four individual restriction enzyme digests (*DdeI*, *HhaI*, *HinFI* and *HpaII*) on a 193 bp fragment of mtCOI. Six species each displayed a unique profile for a single restriction enzyme. A 159 bp fragment of mtCOII allowed individual identification of six of the species using the combined profiles from five individual restriction enzyme digests (*DdeI*, *EcoRV*, *HhaI*, *HpaII*, and *RsaI*). These primers failed to amplify in *H. iris*. Again *H. rubra* and *H. conicopora* could not be separated, and neither could *H. australis* and *H. spadicea*. No DNA sequence variation in either fragment was observed between *H. rubra* and *H. conicopora*; the latter may be a subspecies of *H. rubra*. The use of both fragments and a minimum of two restriction enzymes is recommended for species differentiation. DNA was successfully extracted, PCR amplified and identified from canned tissue and mucous samples of *H. rubra*. A conformational mutation in the mtCOI fragment was observed in *H. midae*, but in no other species nor in the mtCOII fragment.

5.2 Introduction

Abalone, genus *Haliotis* Linnaeus, are a highly valuable commercial marine univalve mollusc. There are over 56 recognized species worldwide (Geiger 1999), of which nearly half are exploited by commercial or recreational divers. Abalone generally inhabit rocky reefs to depths of 65 m, but are more usually found in shallower waters to 30 m. The foot muscle of abalone attracts high prices in Asian markets, with species differential. Once removed from the shell and trimmed of distinguishing mantle tissue, it is very difficult to differentiate the commercial product of one species from another. The high price, market demand, ease of harvest and similarity of processed product between species makes abalone very suitable targets for illegal marketing and both highly organised and small scale poaching.

Abalone poaching and species-substitution of abalone products is of concern to many countries, including the USA (Daniels and Floren 1998), Mexico (Ponce-Díaz *et al.* 1998), South Africa (Sweijd *et al.* 1998) and Australia. The value of the illegal trade is difficult to quantify. Conservative estimates in Australia alone are over $US25M annually. The legal Australian abalone fisheries, dominated by *Haliotis rubra,* account for about half the annual world abalone harvest of ca. 10 500 mt (metric tonnes) and is worth around $US80M per year (FAO 2000). The South African abalone fishery *(H. midae* ca. 500 mt/yr) is worth approximately $US15M with legal sales of confiscated (poached) abalone from just one area fetching over $US1m (Sweijd *et al.*1998). In New Zealand the main commercial species is *H. iris* and the illegal harvest is estimated at about 33% of the annual commercial catch of 1 300 mt (Roberts *et al.* 1999). The high but unknown level of illegal harvesting of abalone creates major problems for fishery managers endeavoring to maintain viable and economic fisheries.

Whilst *H. rubra* is the dominant commercial species within temperate Australian waters, both *H. laevigata* and *H. roei* are subject to significant levels of commercial fishing under independent quota systems, and a *H. scalaris* fishery is under consideration. A problem for fisheries enforcement is the overlapping ranges of these and non-commercial species. Such species richness is common with abalone (Geiger 1999), and once the shell and mantle have been removed identification of the commercial product is obscure. The need exists for a definitive means for identifying tissue and by-products (e.g. mucous in instances of suspected poaching when tissue has been disposed) of individual abalone species.

Identification of plant and animal species when morphological characters have been removed is possible using either protein or DNA-based methods (Palumbi and Cipriano 1998, Toro 1998, Johannesson and Stenlid 1999, Hare *et al.* 2000, Sweijd *et al.* 2000). The protein based methods are very dependent on tissue quality; generally requiring fresh or frozen material. Often identification for commercial needs may require analysis of processed (dried or canned) tissue or degraded tissue. DNA-based methods are relatively independent of tissue quality, and those that rely on amplification of small DNA fragments are less likely to be affected by degradation (Mackie *et al.* 1999). A number of techniques are available for species identification including: random amplification of polymorphic DNA (RAPD) (Martinez and Malmheden Yman 1998), restriction fragment length polymorphism (RFLP) analysis (Innes *et al.* 1998, Wolf *et al.* 2000), direct DNA sequencing (Quinteiro *et al.* 1998) and single-stranded conformation polymorphism (SSCP) (Mackie *et al.* 1999) of PCR (polymerase chain reaction) amplified fragments.

A PCR-RFLP analysis of a 1,300 base-pair (bp) fragment of the nuclear lysin gene was devised for identification of two South African abalone species, *Haliotis midae* and *H. spadicea* (Sweijd *et al.* 1998). Generic PCR primers that amplify across the intron differentiated between species based on the size of the intron. Preliminary analyses found that the size of the lysin intron varied greatly between other *Haliotis* species (generally 500 to 1 100 bp), but the intron in the Australian greenlip abalone *H. laevigata* was over 4 000 bp (unpublished data). Products of such size are not ideal for species identification tests with the likelihood of unreliable PCR products due to tissue and DNA degradation.

To differentiate between the more common Southern Hemisphere abalone species a PCR-RFLP method was developed using short fragments (less than 200 bp) of the mitochondrial DNA (mtDNA) molecule. To satisfy potential legal scenarios in Australia and South Africa, 11 species were included. Within species variation and potential non-*Haliotis* amplification of our designed primers were examined in addition to testing the primers with canned abalone tissue and abalone mucous samples.

5.3 Materials and Methods

5.3.1. Sample collection and DNA extraction

Whole individuals (live or frozen) or alcohol preserved tissues were obtained for 11 purported *Haliotis* species:

Haliotis asinina Linnaeus	Queensland, Australia	(30 individuals)
Haliotis australis Gmelin	New Zealand	(10)
Haliotis conicopora Péron	Western Australia, Australia	(11)
Haliotis iris Gmelin	New Zealand	(10)
Haliotis laevigata Donovan	Tasmania & Victoria, Australia	(62)
Haliotis midae Linnaeus	South Africa	(10)
Haliotis roei Gray	Western Australia, Australia	(10)
Haliotis rubra Leach	Tasmania, Victoria & New South Wales, Australia	(50)
Haliotis scalaris (Leach)	Tasmania & Western Australia, Australia	(22)
Haliotis spadicea Donovan	South Africa	(10)
Haliotis virginea Gmelin	New Zealand	(10)

Total genomic DNA was extracted from ca. 25 mg of foot muscle or gill tissues using a modified CTAB (hexadecyltrimethlammoniumbromide) protocol (Grewe *et al.* 1993). Tissue was incubated overnight at 50°C instead of 30 to 60 min at 60°C.

To verify the use of our PCR primers on processed product, DNA was extracted from commercially canned *H. rubra*. Approximately 0.5 g tissue was digested for 30 min at 65°C in 5 mL digestion buffer (100 mM Tris, 50 mM EDTA, 400 mM NaCl, 1% SDS). 50 µL proteinase K (10 mg/mL) was then added and the solution was incubated overnight at 55°C. 150 µL NaCl (5 M) and 520 µL of 10% CTAB were added and the solution incubated at 65°C for 1 hr with regular mixing. Samples were then extracted once with equal volumes of chloroform/isoamyl alcohol (24:1) and precipitated with 2 volumes of 100% ethanol. Precipitated DNA was washed twice with 70% ethanol, once with 100% ethanol, air-dried and re-suspended in 200 µL TE.

PCR amplification was also tested using DNA extracted from *H. rubra* mucous. Two mucous samples were obtained by placing individual freshly captured *H. rubra* in separate plastic bags for approximately 2 h, removing the abalone and placing the bag and fluid contents at 4°C. Tissue samples were taken from the individual abalone as positive controls for DNA extraction. DNA was extracted from mucous swabs taken from the sides of the bags and from the control tissue samples using the modified CTAB protocol described above. In addition, a 600 µL sample of fluid (mixture of seawater and mucous) from the bottom of each plastic bag was taken, incubated overnight in 20 µL proteinase K (10 mg/mL) and 5% SDS, and then genomic DNA extracted using the same modified CTAB protocol.

Genus specificity of the PCR amplification was tested on total genomic DNA extracts (using above CTAB protocol) from a variety of marine organisms. These consisted of an alga (unidentified red alga), an anemone (unidentified), a crustacean (Antarctic krill *Euphausia superba*), molluscs (unidentified chiton and Pacific oyster *Crassostrea gigas*) and teleosts (bigeye tuna *Thunnus obesus*, southern bluefin tuna *T. maccoyii*, pink ling *Genypterus blacodes*, Patagonian toothfish *Dissostichus eleginoides*, school shark *Galeorhinus galeus* and gummy shark *Musteleus antarcticus*).

5.3.2 PCR primers and amplification

Generic PCR primers were designed for the mitochondrial cytochrome c oxidase subunit I gene (mtCOI) by alignment of either our own unpublished or published *Haliotis* sequences (Metz *et al.* 1998). DNA sequences used for the design of the mitochondrial cytochrome c oxidase subunit II gene (mtCOII) were either our own or other unpublished sequences (Sandy Degnan, University of Queensland).

The primers designed to amplify a 193 bp fragment of the mtCOI gene were designated HALCO1-NG1 (5'-CIGACATRGCITTYCCICGACT-3') and HALCO1-NG2 (5'- CCGGCTARGTGIAGIGARAAAAT-3'). Those designed for a 159 bp fragment of the mtCOII gene were designated HALCO2GENA (5'-CAATYTGAACYATTCTMCCAGC-3') and HALCO2GENB (5'-CCTTAAARTCTGAGTATTCGTAGCC-3'). (Degenerate nucleotide IUB codes: I, Inosine = A, C, G or T; M, aMino = A or C; R, puRine = A or G; Y, pyrimidine = C or T).

PCR reactions consisted of 50 to 100 ng of total genomic DNA, 2.5 mM $MgCl_2$, 200 μM each dNTP, 10 pmoles of each primer, and 0.55 U *Taq* DNA polymerase (Biotech) in a buffer supplied by the manufacturer. PCR amplifications were carried out in a 50 μL final volume using a Perkin Elmer GeneAmp® System 9600 with hotlid. The cycling parameters were as follows: denaturation at 95˚C for 3 min, 10 initial amplification cycles (94˚C for 30 s, 60–55˚C for 30 s, 72˚C for 1 min, with a decrease in the annealing temperature of 0.5˚C per cycle), a further 25 amplification cycles (94ºC for 30 s, 55ºC for 30 s, 72ºC for 1 min) and final extension at 72ºC for 5 min. Negative controls, without DNA template, were prepared for each series of amplifications to exclude the possibility that PCR reagents and buffers were contaminated with template DNA. Amplification products were examined by electrophoresis through a 2% agarose gel (GIBCOBRL) made up in 1 X TBE. Gels were stained in ethidium bromide at a concentration of 0.5 μg/mL and visualised under UV light. A 100 bp ladder (GIBCOBRL) was run concurrently to facilitate sizing of amplification products.

5.3.3. DNA Sequencing

PCR products were sequenced to confirm variation in restriction fragments and sizes, and to improve PCR primer design. PCR products were purified using Wizard™ PCR purification columns (Promega) according to manufacturers instructions, and sequenced using an ABI Prism™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). Cycle sequencing reactions were electrophoresed on an ABI377 automated DNA sequencer (Perkin Elmer) and analyzed using ABI Prism™ Sequencing Analysis Version 3.3 (Perkin Elmer).

5.3.4. RFLP analysis

For each individual of the 11 species, four separate restriction digestions of the mtCOI fragment were performed using the four enzymes *DdeI, HhaI, HinFI* and *HpaII* (New England Biolabs, Genesearch). For the mtCOII fragments five separate restriction digestions were performed for each species individual using the enzymes *DdeI, EcoRV, HhaI, HpaII* and *RsaI* (New England Biolabs, Genesearch). Restriction digestions were carried out in a 15 μL total volume consisting of 5 μL of PCR product, 1.5 μL digestion buffer supplied by the manufacturer, 0.5 μL enzyme, and 8 μL ddH_20 for all enzymes except *HhaI*. Digestions for *HhaI* were carried out in a 15 μL total volume consisting of 5 μL of PCR product, 1.5 μL digestion buffer supplied by the manufacturer, 0.5 μL enzyme, 1.5 μL 10X Bovine serum albumin (BSA) and 6.5 μL ddH_20.

Mitochondrial haplotypes were scored by electrophoresis of 10 μL of digested PCR product in a 3% agarose gel made up in 1 X TBE at 100V for 3 hr, stained in ethidium bromide (0.5μg/mL) and visualised under UV light. Electrophoresis of restriction digestions was also performed on 12% polyacrylamide (Austral Scientific) gels made up in 1 X TBE and run for 2 hr at 100 V.

5.4. Results

5.4.1. DNA extraction and PCR amplification

DNA extractions from fresh, alcohol preserved and canned tissue, resulted in high yields of high molecular weight total genomic DNA. Amplification of these extracts consistently produced high quality PCR products.

Extractions from mucous scrapings and fluid samples from plastic bags produced a small amount of high molecular weight genomic DNA. PCR amplification of these extracts failed at times to yield a product when undiluted, however when diluted 10 fold, produced a strong PCR product in all samples (Fig. 5.1**)**

PCR amplification of non-*Haliotis* DNA with the designed primers was only observed in the tuna samples. Both tuna species amplified (160 bp fragment) with the mtCOI primers. Sequencing of the tuna mtCOI products confirmed that the observed product was not contamination from abalone DNA. While nucleotide differences and RFLP cut site differences existed to separate these teleost products from abalone products, high levels of nucleotide sequence conservation suggests that the amplified product was part of the tuna COI gene.

5.4.2. Restriction digests mtCOI

The expected 193 bp fragment was generated in each abalone species following PCR amplification with the HALCO1-NG1/HALCO1-NG2 primers. Comparison of the DNA sequences indicated suitable restriction sites for discrimination between species using four restriction enzymes (Fig. 5.2).

Intraspecies restriction digest polymorphisms were observed in four species, but in each case for a single individual for only one enzyme (Table 5.1). Two of the observed polymorphisms were the result of a loss of a restriction site and two the result of a gain. All individuals showing a different restriction fragment profile for the

species were sequenced to confirm the profile. All other digestions returned a single restriction pattern in all individuals examined for each species.

At this 193 bp fragment, six of the eleven species had a unique species-specific restriction pattern for at least one enzyme, and so could be individually identified (Table 5.1). With the exception of the *H. rubra* and *H. conicopora* pairing, all species are discernible from each other using the four restriction enzymes, regardless of all but one observed polymorphism. The exception polymorphism was a single *H. rubra* individual that had gained a *DdeI* cut site, and therefore had a profile similar to *H. scalaris*. The restriction profiles for the canned tissue, mucous and fluid samples all matched that expected for *H. rubra.*

One purported *H. scalaris* individual returned a different profile at three enzymes to all other *H. scalaris* individuals. This particular individual displayed the expected cut pattern for *H. laevigata* for all four enzymes; three of which are diagnostic between the two species for all other specimens analyzed. Laboratory contamination was ruled out and this result confirmed with repeated tissue sampling, DNA extraction and PCR amplification for this one individual

The observed fragment lengths produced in this study were all examined on agarose and (non-denaturing) polyacrylamide gels and confirmed by sequence analysis. A fragment mobility change was observed in the mtCOI fragment for *H.midae* when run on a polyacrylamide gel (Fig. 5.3). This assumed conformation-induced mutation was only observed in *H. midae*.

5.4.3. Restriction digests mtCOII

The expected 159 bp fragment was generated in each abalone species following PCR amplification with the HALCO1GENA/HALCO2GENB primers, except *H. iris* that failed to amplify for all ten individuals examined. Comparison of the DNA sequences for the other species indicated suitable restriction sites for discrimination between species using five restriction enzymes (Fig. 5.4).

All restriction digestions for the five enzymes resulted in a single restriction pattern for each species, except for two enzymes for *H. rubra* (Table 5.2). The two polymorphisms were each observed in two different individuals, all were sequenced to confirm the observed RFLP. None of these four individuals were responsible for the polymorphisms observed at the mtCOI fragment, and the individual *H. rubra* with a mtCOI profile similar to *H. scalaris* was clearly identified as *H. rubra* at this fragment. The restriction profiles for the canned tissue, mucous and fluid samples all matched that expected for *H. rubra.*

As with the mtCOI RFLP analysis, an unusual species profile was observed for three enzymes with a single *H. scalaris* individual (the same individual), and again all three profiles match that recorded for *H. laevigata*. Sequence data showed a 100% similarity to *H. laevigata* across the 159 bp fragment, while three other *H. scalaris* samples each differed at 9 nucleotides from the *H. laevigata* sequence.

At this 159 bp fragment, three of the ten species (excluding *H. iris* that did not amplify) had a unique restriction pattern for the enzyme *DdeI* and so could be individually identified (Table 5.2). In addition to the *H. rubra/H. conicopora* complex

it was not possible to separate *H. australis* and *H. spadicea* using the five enzymes on this fragment. All remaining species combinations were separable from each other using one to five of the enzymes (Table 5.2).

Fragment mobilities on polyacrylamide gels were all consistent with known fragment lengths; no conformation induced mutations were observed in the mtCOII fragment.

5.5. Discussion

The ability to identify abalone species from tissue samples and/or mucous is important to the continued survival of significant abalone fisheries. The tests described in this paper will provide one more tool in the fight against illegal fishing, which has the potential, along with commercial over-fishing and environmental variables (Davis *et al.* 1998, Shepherd *et al.* 1998), to lead to the decline and collapse of fisheries. The methods are straightforward and suitable for use in any laboratory with basic DNA analytical equipment. The PCR-RFLP tests utilize short DNA fragments that can be amplified from processed products and slightly degraded material, and therefore are of potential forensic use.

Care has been taken in this study to include examination of intraspecies variation as well as possible non-*Haliotis* amplification with our PCR primers. Samples from different geographic locations were examined for the two main Australian commercial species (*H. rubra*, five locations and *H. laevigata*, three locations). While not exhaustive, the results suggest that what limited intraspecies variation exists can be accounted for using the two fragments and multiple restriction enzymes. Both PCR primer sets devised for the test are relatively degenerate and so cross genus amplification was not unexpected. However, of the groups we have examined only DNA from the tunas (*Thunnus* spp.) amplified, and it was possible to easily differentiate these from *Haliotis* species.

The restriction patterns produced by *DdeI* for the mtCOII fragment would discriminate three of the species, while five other species would be differentiated by a single restriction pattern at the mtCOI fragment. Such species-specific patterns are useful, however as rare polymorphisms may exist it would be wise to confirm identification with multiple enzymes and/or both short fragments. None of the rare polymorphisms observed occurred in more than a single individual, and no individual displayed more than one variation. With the exception of the *H. rubra/H. conicopora* pairing, all other combinations of the eleven abalone species can be differentiated from each other using two or more of the restriction profiles shown in this study. We therefore recommend using both fragments and at least two of the restriction enzymes included here to differentiate species.

The ability of our test to differentiate between species was inadvertently put to the test during the intraspecies examinations. Of 20 purported *H. scalaris* individuals, one was found to display a different restriction profile at six of the nine profiles examined. The combined profile of this individual matched completely the expected profile for *H. laevigata*; and was confirmed by DNA sequence analyses. Although occupying different microhabitats, these two species have overlapping distributions and co-occur in the same area (Shepherd 1973). Shell and mantle morphology did not separate the aberrant individual from other *H. scalaris* individuals. This individual is either a

H. laevigata and morphological characters between the two species are more plastic than currently recognized, or it is a hybrid between the two species.

Naturally occurring hybrids between abalone species with overlapping ranges, although relatively rare, have been reported (e.g. Talmadge 1977, Sasaki *et al.* 1980, Arai *et al.* 1982, Messier and Stewart 1994). The two Australian species *H. rubra* and *H. laevigata*, also show evidence of backcrossing and introgression (Brown 1995). There are no records of hybrids between *H. laevigata* and *H. scalaris*, but *H. laevigata* is more closely related to *H. scalaris* than to *H. rubra* (Brown and Murray 1992), and hybrids would not be unexpected. Allozyme analysis of the aberrant individual could not confirm nor refute its putative hybrid status as there are no known diagnostic loci between the two species (Brown 1991a).

The possible existence of hybrids, albeit at low frequencies, does not minimize the validity of our mtDNA-based test for abalone. However, the possibility of hybridization and backcrossing between species does question the legal 'species identity' of an individual. If hybrids were infertile and only F1 hybrids were possible, then a single diagnostic nuclear DNA marker would confirm the individual as a hybrid, and the mtDNA marker would confirm the maternal species. Such individuals could be legally classed as hybrids. However at least some abalone hybrids appear to be fertile and backcrossing occurs (Brown 1995), and identifying the 'nuclear lineage' of a potential backcross offspring would require multiple nuclear DNA markers. Even then it could never be proved that an individual was not the offspring of a backcrossing event, except based on probability. A suite of nuclear DNA markers could never disprove a claim of backcrossing, although making it improbable. On the other hand using a mtDNA-based test, the maternal lineage of the individual can always be validated. We suggest that for legal purposes where hybrid backcrossing may exist between abalone species that the genetic 'species identity' of an individual be classified as its maternal lineage, which can be confirmed from its mtDNA. Hybrid individuals (those with mtDNA of one species and nuclear DNA wholly or partly of another species) while biologically acknowledged should not be legally recognized as the existence of backcross hybrids can not be disproved except by probability based on a large number of diagnostic nuclear DNA markers. Mitochondrial DNA in abalone as in most organisms appears to be only maternally inherited (Conod 2000). The aberrant individual in our study therefore is classed as *H. laevigata*.

The advantage of the tests described here to previous studies (Sweijd *et al.* 1998) for abalone is the smaller size of the DNA fragment; an advantage when examining processed or slightly degraded material (Mackie *et al.* 1999). The lysin gene protocol described by Sweijd *et al.* (1998) did aim for fragments less than 300 bp, but the presence of an intron increased this at least three times, and for *H. laevigata* by about ten fold (unpublished data). The authors did however successfully use PCR primers for a smaller 146 bp fragment to discriminate between canned *H. midae* and *H. rubra* products.

PCR inhibition was observed when testing our primers on the mucous samples of *H. rubra*. Dilution (10 fold) to a lower concentration did not have the same inhibitory effect. Similar PCR inhibition due to high levels of polysaccharides is common in plant tissue extracts (Fang *et al.* 1992), and inhibition due to mucopolysaccharides in the abalone mucous may have caused the observed PCR failure.

No DNA sequence variation was observed between *H. rubra* and *H. conicopora* in either short mtDNA fragment examined in this study. In an assessment of all Recent taxa in the family Haliotidae, Geiger (1998) concluded that there was some justification for sub-species recognition of *conicopora* under *H. rubra*. Allozyme data suggested conspecifity but shell and geographic distributions indicated distinct taxa. Fifteen of 22 DNA microsatellite primers developed for use in *H. rubra* amplified a similar product in *H. conicopora* (Evans *et al.* 2001). This compares to the conservation of only 12 of the 22 markers in other temperate Australian species (*H laevigata, H.scalaris* and *H. roei*). Our short DNA sequences lend some support to the possibility of sub-species status for *conicopora*, however further research is required to resolve the issue.

The altered mobility of the *H. midae* mtCOI fragment run on polacrylamide gels is most likely due to a conformation change. Conformational mutations attributed to sequence-specific variations are restricted to polyacrylamide gels and not seen on agarose gels (Singh *et al.* 1987). The location and conservation of this conformation variant requires further investigation. Its presence, however, raises a note of caution when using RFLPs as mobility variation of fragments seen on polyacrylamide gels may be misleading as they can be length or conformation polymorphisms. It is therefore recommended that species differentiation using the RFLP tests described here be run only on agarose gels.

The test described here fulfills the aim of our study to provide a relatively straightforward and cost-effective means for identifying several abalone species of commercial importance to Australia. Costs for any DNA-based analyses are not insignificant, but the PCR-RFLP technique is generally considered more cost-effective for routine species identification than alternatives such as direct DNA sequencing of the PCR product (e.g. Asensio *et al.* 2000). The opportunity to sequence a PCR product is of course still available for differentiation of individuals if problems arise following PCR-RFLP analysis.

To increase the potential value of this study to the sustainability and protection of abalone fisheries worldwide, additional species, particularly from Northern Hemisphere waters, need to be incorporated either into this test or a modified one, so that a single test is available for discrimination of all abalone species.

Table 5.1. **Expected restriction fragment lengths for eleven *Haliotis* species for the 193 bp mtCOI fragment when cut with restriction enzymes *DdeI, HhaI, HinFI* and *HpaII*.** The number in parenthesis represents the total number of individuals examined for each species that displays the given restriction pattern. Unique restriction profiles are shown in bold. *H. laevigata* numbers include the misidentified *H. scalaris* individual.

	CO1 Restriction Digestion Patterns			
	DdeI	*HhaI*	*HinFI*	*HpaII*
H. assinina	**7,50,137** (29) ***7,187*** (1)	193 (30)	34,159 (30)	3,42,72,76 (30)
H. australis	193 (10)	**79,114** (10)	193 (10)	**3,93,97** (10)
H. conicopora	193 (11)	42,151 (11)	34,159 (11)	3,42,51,97 (11)
H. iris	193 (10)	42,151 (10)	**19,174** (10)	3,190 (10)
H. laevigata	193 (63)	193 (62) 94,99 (1)	34,159 (62) 193 (1)	3,42,148 (63)
H. midae	193 (10)	42,151 (10)	34,159 (10)	3,42,148 (10)
H. roei	29,164 (10)	193 (10)	**6,34,97** (10)	45,51,97 (10)
H. rubra	193 (49) 29,164 (1)	42,151 (50)	34,159 (49) 193 (1)	3,42,51,97 (50)
H. scalaris	29,164 (21)	42,151 (21)	34,159 (21)	3,42,51,97 (21)
H. spadicea	193 (10)	42,151 (10)	**15,19,159** (10)	3,190 (10)
H. virginea	193 (10)	**42,57,94** (9) 42,151 (1)	193 (10)	3,190 (10)

Table 5.2.Expected restriction fragment lengths for ten *Haliotis* species for the 159 bp mtCOII fragment when cut with restriction enzymes *DdeI, EcoRV, HhaI, HpaII* and *RsaI*. *H. iris* did not amplify with these primers. The number in parenthesis represents the total number of individuals examined for each species that displays the given restriction pattern. Unique restriction profiles are shown in bold. *H. laevigata* numbers include the misidentified *H. scalaris* individual.

	CO2 Restriction Digestion Patterns				
	DdeI	*EcoRV*	*HhaI*	*HpaII*	*RsaI*
H. assinina	13,38,108 (30)	71,88 (30)	159 (30)	44,115 (30)	159 (30)
H. australis	13,15,131 (10)	159 (10)	159 (10)	44,115 (10)	159 (10)
H. conicopora	13,15,131 (11)	159 (11)	58,101 (11)	159 (11)	159 (11)
H. iris	-	-	-	-	-
H. laevigata	**13,15,60,71** (63)	71,88 (63)	58,101 (63)	159 (63)	30,129 (63)
H. midae	13,15,131 (10)	159 (10)	159 (10)	159 (10)	159 (10)
H. roei	**13,146** (10)	159 (10)	159 (10)	44,115 (10)	30,129 (10)
H. rubra	13,15,131 (50)	159 (50)	58,101 (48) 159 (2)	159 (48) 44,115 (2)	159 (50)
H. scalaris	**13,71,75** (21)	159 (21)	159 (21)	159 (21)	30,129 (21)
H. spadicea	13,15,131 (10)	159 (10)	159 (10)	44,115 (10)	159 (10)
H. virginea	13,38,108 (10)	159 (10)	159 (10)	44,115 (10)	159 (10)

Figure 5.1. Electrophoretic analysis of undiluted (lanes 1 to 6) and diluted (x 10, lanes 9 to 14)) 193 bp mtCOI (upper image) and 159 bp mtCOII (lower image) PCR fragments for *H. rubra* tissue and mucous samples. M = 100 bp ladder. Samples in lanes are as follows: 1, 2, 9 & 10 muscle tissue; 3, 4, 11 & 12 fluid sample from plastic bag; 5, 6,13 & 14 mucous swab from plastic bag; 7 & 15 positive *H. rubra* DNA (x 20 dilution) control; 8 & 16 negative H_2O control.

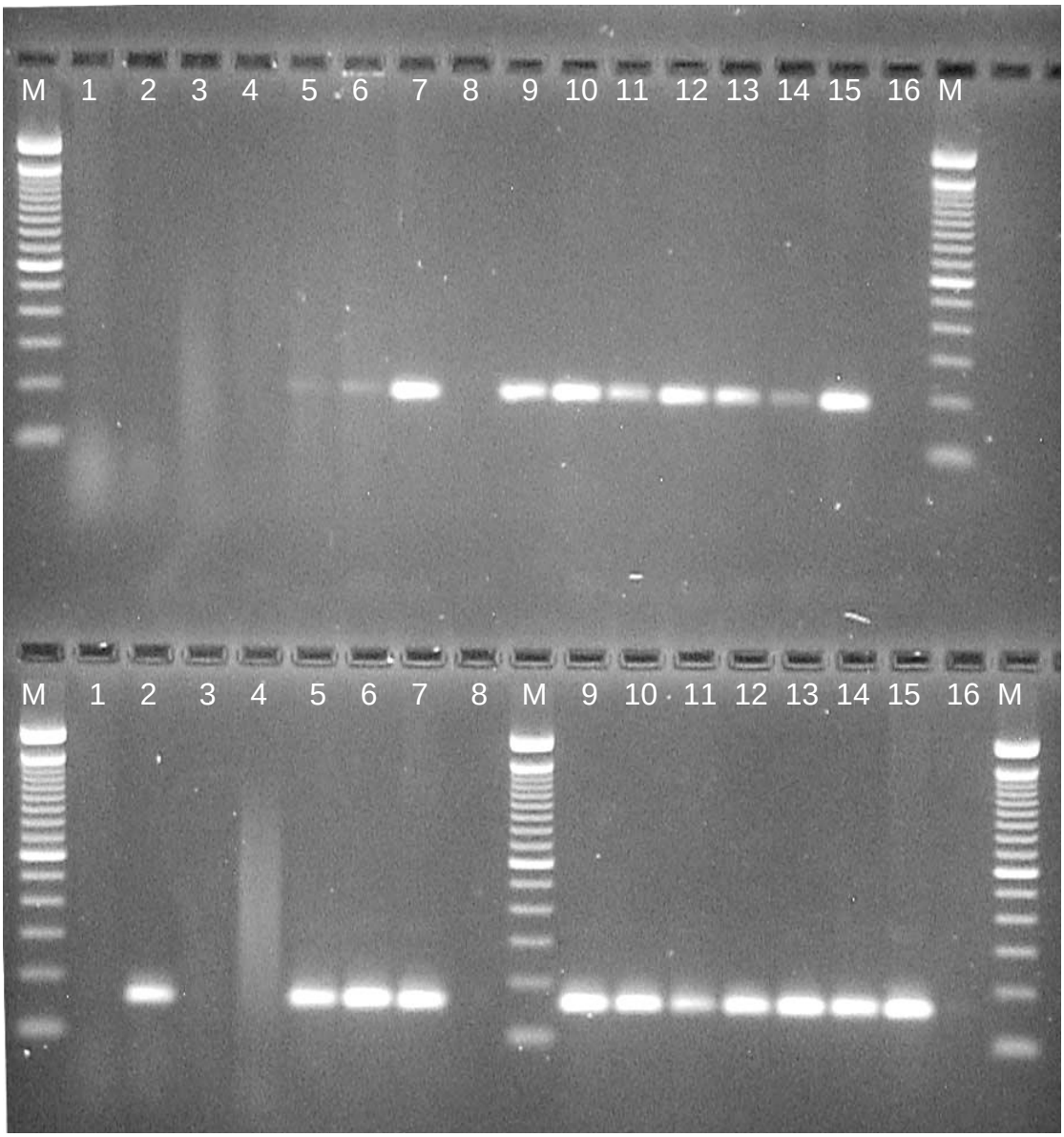


Figure 5.2 Sequence alignment of the 193 bp mtCOI fragment for ten abalone species. Primer sequences and cut sites for the four restriction enzymes *DdeI*, *HhaI*, *HinFI* and *HpaII* are included. (N = sequence data unclear whether C or T).

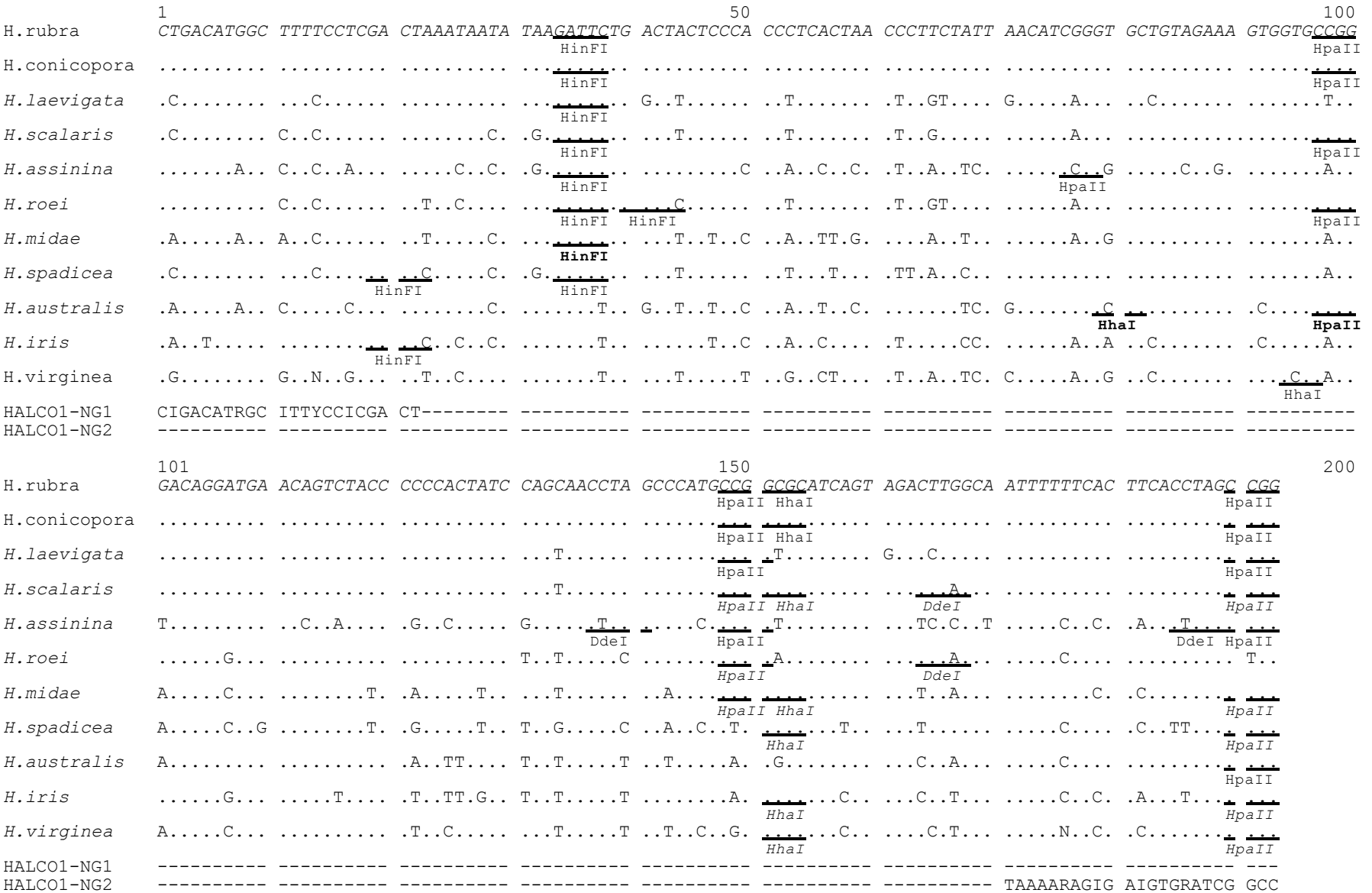


Figure 5.3. RFLP patterns on a non-denaturing polyacrylamide gel of the 193 bp mtCOI fragment for three abalone species produced with four restriction enzymes. Species 1 – *H. midae*, species 2 – *H. rubra*, species 3 – *H. laevigata*, M – 100 bp DNA ladder. Reduced mobilty in *H. midae* fragments suspected to be due to a conformational mutation**.**

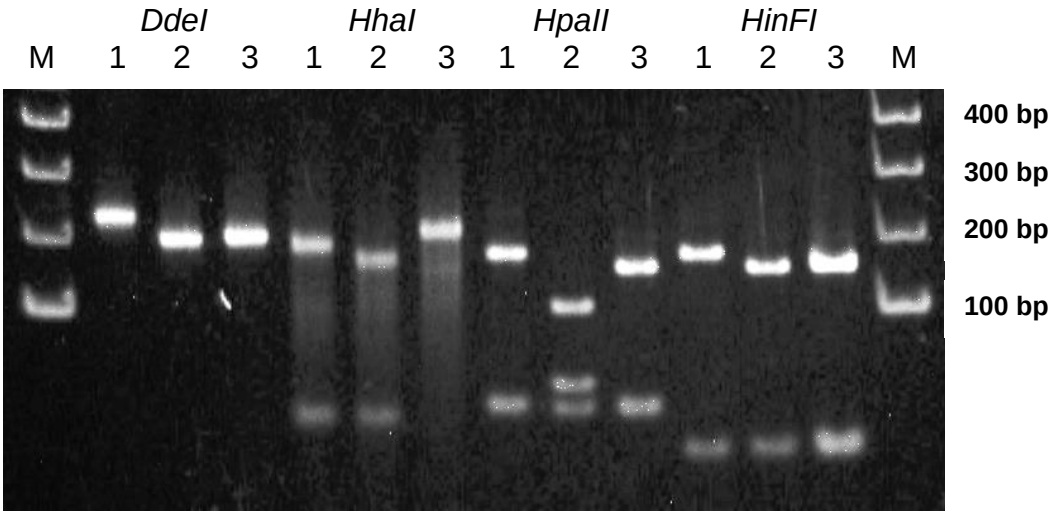


Figure 5.4. Sequence alignment of the 159 bp mtCOII fragment for ten abalone species. This fragment did not amplify for *H. iris*. Primer sequences and cut sites for the five restriction enzymes *DdeI, EcoRV, HhaI, HpaII* and *RsaI* are included. (1 = sequence data unclear whether C or T ; 2 = sequence data unclear whether C or A).

```
                1                                                  50                                                 100
H.rubra         CAATTTGAAC CATTCTACCA GCCATTATCC TTATTTTCCT CGCCCTACCA TCCTTGCGCC TCCTTTACCT ACTAGACGAA GTCGGTATAT CGTGCCTTCT
                                                                                  HhaI
H.conicopora    .......... .......... .......... .......... .......... .....CGCG. .......... .......... .......... ..........
                                                                                  HhaI
H.laevigata     .......... ......C... .......... .C..C..... .......... .....CGCG. .......... CT........ ..A..G.... .C........
                                                                                  HhaI                 DdeI          EcoRV
H.scalaris      .......... C.....C... .......... .C..C..... A.....G... .....A.... .......... CT........ ..A..A.... .C........
                                                                                                       DdeI
H.assinina      .......... T......... .....C.... .G..C..T. A.....T... ..TC.C..A. .T..A..... .......... ..T..G.... .T.....CT.
                                                    DdeI                                                              EcoRV
H.roei          .......... T.....C... .......... .C..C..... A.....C... .....A.... .T........ .......... ..A....... .C.....C..
H.midae         .......... C.....C... ..T....... .A..C..... ......C... ..TC.A..A. ....C..... C......... ..G....... .A..T..CT.
H.spadicea      .......... 1.....C... ..T....... ..G.C..... ......T... .....A..A. .......T.. ......T... ..A.....G. .A..T...T.
H.australis     .......... 1.....2... .....C..TT .A........ A.....G..G ...C.A..A. .......... C.....T... .......... .A.....CT.
H.virnginia     .......... ..C....... .....C.... .A..C..T. A.....C..C ..AC.T..A. .T........ T.....T..G .....A.... .A.....A..
                                                    DdeI
HALCO2GENA      CAATYTGAAC YATTCTMCCA GC-------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
HALCO2GENB      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

                101                                                150       159
H.rubra         AACAATCAAG GCAACTGGTA ACCAGTGATA CTGAGGCTAC GAATACTCAG ACTTTAAGG
                                                  DdeI               DdeI
H.conicopora    .......... .......... .......... .....CTCA. .....CTCA. .........
                                                  DdeI               DdeI
H.laevigata     ......T... .....G.... .T.....G.. .....CTCA. .....CTCA. .........
                                                  RsaI DdeI          DdeI
H.scalaris      ...G..... .....A.... .T..A..G.. ..CTCA..... .....CTCA. .........
                                                  RsaI               DdeI
H.assinina      .........A ....CCAA. ....A..... ...G..... .....CTCA. .........
                           HpaII                                     DdeI
H.roei          ......T... ....CCAA. .T..A..G.. ..CTCA..... .....CTCA. .........
                           HpaII               RsaI                  DdeI
H.midae         ...G..T... .......... ....A..... .....CTCA. .....CTCA. .........
                                                  DdeI               DdeI
H.spadicea      ......T..A ....CCAC. ....A..... .....CTCA. .....CTCA. .........
                           HpaII                  DdeI               DdeI
H.australis     ......T..A ....CCAA. ....A..... .....CTCA. .....CTCA. .........
                           HpaII                  DdeI               DdeI
H.virnginia     .........A ....CCAC. ....A..... T........ .....CTCA. .........
                           HpaII                                     DdeI
HALCO2GENA      ---------- ---------- ---------- ---------- ---------- ----------
HALCO2GENB      ---------- ---------- ---------- ----CCGATG CTTATGAGTC TRAAATTCC
```

6. MICROSATELLITE AND MITOCHONDRIAL DNA ANALYSES

For Objective 2 it was proposed to use nuclear DNA microsatellites to study population structure. A comparison of mitochondrial DNA and microsatellite variation was undertaken to confirm the choice of marker type. The research supported by this project formed part of the Honours Degree for Natalie Conod at the School of Zoology, University of Tasmania (Conod 2000). It was submitted for peer review and accepted for publication. The paper is reproduced (acknowledgments and references are included in Sections 14 and 15 respectively) from Marine and Freshwater Research, Volume 53 (2002) by permission of CSIRO PUBLISHING.

The complete reference: Conod, N., Bartlett, J.P., Evans, B.S. and Elliott, N.G. 2002. Comparison of mitochondrial and nuclear DNA analyses of population structuring in the blacklip abalone *Haliotis rubra* Leach. *Mar. Freshwater Res*. 53 1–8.

6.1. Abstract

Genetic variation in five geographically isolated samples of the blacklip abalone (*Haliotis rubra*) from southeast Australia was examined using two molecular techniques. An RFLP analysis using six restriction enzymes on the ND3/CO3 region of mitochondrial DNA was compared with five independent nuclear DNA microsatellite loci. The results from both techniques suggest restricted gene flow between blacklip abalone separated by Bass Strait, and homogeneity among geographically isolated samples from around the island of Tasmania. While both techniques showed similar resolving power, microsatellite DNA analysis is the preferred molecular technique for the fine scale investigation of blacklip abalone population structure, making possible the examination of numerous independent loci with potentially high levels of polymorphism. Both sample and locus specific homozygote excesses were recorded for the microsatellite loci. The most likely explanation for the locus specific deviations from Hardy Weinberg expectations is the presence of null alleles.

6.2 Introduction

Abalone are harvested by commercial and recreational divers along the coasts of many countries, with the world commercial harvest of ca. 10 500 mt/year valued at ca.$US80 m (FAO 2000). Despite the value of the wild fishery, there is a relatively poor understanding of the population structure of abalone species (Withler 2000). This is surprising considering the major decline in some abalone fisheries over the past decades (e.g. Tegner *et al*. 1992; Hamm and Burton 2000) and the efforts being placed on recovery (Nash *et al*. 1995; Sanders and Beinssen, 1998) and improved management of stocks (e.g. Dixon *et al.* 1998; Gorfine *et al.* 1998; Troynikov and Gorfine 1998). While abalone species are generally endemic, some have overlapping distributions with different microhabitat requirements and breeding seasons. Hence, what little evidence on population structure may be found for one abalone species and/or area may not be relevant to another abalone species or locality.

Although nearly half of the 56 recognized abalone species (Geiger 1999) are harvested, there have been few genetic differentiation studies on individual species. The genetic structure of the Californian red abalone *Haliotis rufescens* has been examined using four allozyme loci (Gaffney *et al*. 1996; Burton and Tegner 2000), sequence diversity of mitochondrial DNA (mtDNA) (Burton and Tegner 2000) and one microsatellite

locus (Kirby *et al*. 1998). Overall, the data from these studies suggest Californian *Haliotis rufescens* is undifferentiated. Results from other species from the California and Baja California coastline include allozyme differentiation in *H. corrugata* (del Río Portilla 2000), allozyme homogeneity across six loci in *H. fulgens* (Zúñiga *et al*. 2000), and population differentiation at three allozyme loci in *H. cracherodii* (Hamm and Burton 2000). Using mtDNA haplotype analyses, significant differences between populations have been reported for both Taiwanese abalone *H. diversicolor* (Jiang *et al*. 1995) and the South African species *H. midae* (Swiejd 1999). The population subdivision identified in *H. midae* using mtDNA haplotypes was not supported by allozyme analysis, but is in concordance with data from a recent microsatellite analysis (Evans, unpublished).

In Australian waters, the population structure of three abalone species has been examined with allozymes; *H. laevigata* (Brown and Murray 1992, F_{ST} = 0.014 across 13 loci), *H. roei* (Hancock 2000, F_{ST} = 0.009 across eight loci) and *H. rubra*, (Brown 1991; F_{ST} = 0.022 across 12 loci). In all cases an isolation-by-distance model is proposed to describe the population structure. While gene flow was found to be high, local heterogeneity was also evident. Hancock suggests this apparent contradiction is due to largely localized larvae recruitment, with low F_{ST} values being maintained by large population sizes and/or chance settlement of larvae from non-neighbouring sources. In contrast, Huang *et al*. (2000) results suggest a comparatively lower level of gene flow among *H. rubra* samples across three microsatellite loci (F_{ST} analogue, Φ_{PT} = 0.077, P < 0.001) and from a RAPD (random amplified polymorphic DNA) analysis (Φ_{PT} = 0.086, P < 0.001) despite sampling across a smaller geographical range. Their conclusions however, were based on very small sample sizes, n = 10.

The lack of uniformity in conclusions among the various genetic differentiation studies in abalone is likely to be species, sampling and analysis dependent. Studies to date have varied in the number of sites examined, the distance between sampling sites, sample sizes and numbers of markers, all of which affect the statistical power of the analyses. The different analytical techniques applied also vary the resolving power of the null hypothesis being tested. As Ward and Grewe (1994) concluded in their review of molecular genetics techniques in fisheries, recommendation of a single technique is difficult as each has advantages and disadvantages. Mitochondrial DNA analyses are generally considered more powerful than allozymes because of a smaller effective population size, as mtDNA is haploid and generally only maternally inherited (which appears to be the case for abalone, Conod 2000). However, as the mitochondrial genome is a non-recombinant single molecule it effectively acts as a single locus, whereas many independent loci are available with nuclear DNA analyses. Direct analysis of DNA, either mitochondrial or non-coding nuclear DNA, is generally considered preferable to allozymes due to faster evolutionary rates and therefore greater potential to reveal recent restriction of gene flow between populations. Despite this, a ‘cursory review’ of the literature by Bossart and Powell (1998) found little evidence to support DNA markers giving a better estimate of gene flow than allozymes. The choice of analytical method is therefore likely to be dependent on both the species and resources available for conducting the investigation.

This paper describes a comparison of a mtDNA-RFLP (restriction fragment length polymorphism) analysis and a microsatellite nuclear DNA analysis of the same individuals from five southeastern Australian samples of the blacklip abalone, *Haliotis*

rubra. The five sampling sites were chosen to provide geographic separation based on possible oceanographic barriers to gene flow between sites and also take into account the isolation-by-distance model proposed by Brown and Murray (1992). Understanding the stock structure of *H. rubra* is important for continued effective management; this species accounts for almost half the global commercial abalone harvest.

6.3. Materials and Methods

6.3.1. Sample collection and DNA isolation

Samples of *Haliotis rubra* Leach (blacklip abalone) were collected from four sites around Tasmania (Eddystone Point, Red Rocks, Curio Bay and Sundown Point) and from Point Cook, Victoria (Figure 6.1). The four Tasmanian collections (n = 100) consisted of either whole animals or viscera stored at -20° C, until gill and/or muscle tissue was dissected and stored at - 80° C. The Point Cook sample (n = 50) consisted of gill and muscle tissue preserved in 70 % ethanol. Total genomic DNA was extracted from foot muscle or gill tissues using either the CTAB (hexadecyltrimethylammonium bromide) method (Grewe *et al*. 1993) or chelex extraction (Bio-Rad chelex 100 resin). DNA was stored at -20°C.

6.3.2. Mitochondrial DNA RFLP analyses

The polymerase chain reaction (PCR) was used to amplify a target ND3/CO3 (referred to as ND3 hereafter) region of the *H. rubra* mitochondrial genome. The targeted fragment consists of a large portion of the NADH subunit 3 gene, the complete CO3 gene and an unidentified portion of the mtDNA genome (Sweijd, 1999). The primers for the fragment were,
forward P3: 5'-AAAGTGATCACAGAAATGACCCG-3', and
reverse P4: 5'-GATAAGAAGAAAGCAAAGAACCCC-3'.

PCR reactions were carried out in a total volume of 100 μL in sterile PCR tubes. Amplification reactions contained 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 67 mM Tris-HCL (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.2 mg/mL gelatin, 2 units Taq polymerase (*Biotech*), 0.2 mM of each primer, and 50-100 ng total genomic DNA. The PCR cycle conditions comprised an initial denaturation step of 2 min at 94 °C, followed by 35 cycles of 55 s at 94 °C, 1 min at 60 °C, 2 min 30 s at 72 °C, and a final 10 min extension at 72 °C. A single product of approximately 1500 bp was amplified.

Six restriction enzymes *Dde* I, *Dpn* II, *Hae* III, *Msp* I, *Rsa* I and *ScrF* I were used to digest the ND3 fragment in 40 individuals from each site. 5 to 8 μL of amplification product was digested according to the manufacture's recommendations (New England Biolabs). Restriction fragments were separated by electrophoresis on either 2 % agarose or 12 % polyacrylamide gels made up in 1 X TBE. A 100 bp ladder (Life Technologies) was used as a size standard and run on every gel. Products were visualised by staining with ethidium bromide and examined under UV illumination.

Both haplotype frequency and restriction site presence/absence data were analysed to assess genetic subdivision. Haplotypes were assigned a letter based on the existence of restriction fragment length polymorphisms (RFLPs). The letter A was used to identify the most common haplotype. Remaining haplotypes were denoted characters but not necessarily in consecutive order. A composite haplotype was composed for each individual, with each letter representing the restriction pattern produced with a

different enzyme. The relative gain or loss of restriction fragments between samples was used to determine restriction site presence or absence data. Fragments smaller than ca. 100 base pairs could not be reliably resolved and so were not always accounted for (unless by inference). Sizes are available from the authors.

Chi-square tests were performed on haplotype frequency data derived from the restriction digests of the ND3 fragment using the MONTE program in REAP, ver. 4.0 (McElroy *et al*. 1991). Monte Carlo simulation obviates the need to pool rarer haplotypes, preserving the data set in a more informative form for analysis. Nei's (1973) G_{ST} was used to quantify the extent of differentiation among samples based upon haplotype frequency data (Palumbi and Wilson 1990). The maximum likelihood method of Nei and Tajima (1983) was used to estimate the expected number of nucleotide substitutions per site based on restriction site cleavage data, providing an estimate of nucleotide diversity. Nucleotide divergence between two populations was estimated, representing the portion of the net nucleotide diversity that cannot be explained by within population diversity.

6.3.3. Microsatellite nuclear DNA analyses

Variation at five microsatellite loci, *CmrHr1.14, CmrHr 2.14, CmrHr 2.26, CmrHr2.30* (Evans *et al*. 2000) and *RUBCA1* (Huang and Hanna 1998) was investigated in 100 individuals from each of the four Tasmanian sites and 50 individuals from Point Cook, Victoria. PCR amplifications were carried out in 96-well plates in a total volume of 25 μL. All reactions were performed in a Perkin-Elmer GeneAmp® System 9600 or 9700 thermal cycler. Amplification products from the five loci were co-loaded on an ABI PRISM™ 377 DNA sequencer (Perkin-Elmer) with product sizes determined by comparison to a Genescan Tamara-500 size standard (PE Applied Biosystems) and analysed using Genotyper ver 1.1.1 software.

Genotypic linkage disequilibrium, genotype conformation to Hardy-Weinberg expectations and allele frequency differentiation were examined using GENEPOP ver. 3.2 (Raymond and Rousset 1995). An analysis of molecular variance (AMOVA) was performed using ARLEQUIN ver 2.000 (Schneider *et al*. 2000) which allows populations to be grouped and genetic structure to be examined at different hierarchical levels. R_{ST} Calc (Goodman 1997), which assumes a Step-Wise Mutation model for microsatellite evolution, was used to calculate Rho. This program provides an unbiased estimation of Slatkins (1985) R_{ST} by taking into account differences in sample sizes and loci variance. F_{ST} values, based on the Infinite Allele Model were estimated using ARLEQUIN ver 2.000. To eliminate the bias associated with performing multiple tests, the significance level of P-values was adjusted using a Bonferroni correction (Rice 1989) as required.

6.4. Results

6.4.1. Mitochondrial DNA

A total of 25 composite haplotypes were identified in the ND3 fragment of 189 individuals successfully amplified using PCR, surveyed with six restriction enzymes (Table 6.1). Two dominant haplotypes, AAAAAA and BBABBC, representing 64% of individuals, were common at all 5 sample sites, with a further 6 haplotypes present at 3 or more sites. Haplotype AAAAAA dominates Tasmanian samples, while haplotype BBABBC is the most common in the Victorian sample. All sites, except

Curio Bay, had a least 3 unique haplotypes not shared with other locations. The distribution of haplotypes between the five localities was non-random (P = 0.002 following 10 000 randomisations of a Monte Carlo chi-square analysis). Pairwise chi-square tests between the four Tasmanian sites found no genetic differentiation (P = 0.680), permitting pooling of these sites. There was significant differentiation (P = 0.0002) between the pooled Tasmanian data and the Point Cook data, indicating geographic heterogeneity. This pattern of geographic differentiation is also suggested by Nei's (1973) G_{ST}, which provides an evaluation of population structuring by calculating the proportion of total genetic diversity attributable to subpopulation differentiation. While no significant structuring was detected between Tasmanian localities (G_{ST} = 0.025, P = 0.239), the inclusion of Point Cook in the analysis gave a significant (G_{ST} = 0.054, P = 0.001) result, indicative of restricted gene flow.

Within population haplotype diversity ranged from 0.63 for Red Rocks and Curio Bay samples to 0.82 for Sundown Point, with an average of 0.72 for the five samples. Pairwise comparison of nucleotide divergence between Tasmanian samples produced estimates close to zero or with a negative value (Table 6.2). The negative values indicate there is greater genetic diversity within sites than between sites and therefore no significant geographic differentiation among the Tasmanian samples. However, when compared with the Point Cook sample, values consistently greater than zero suggest a low level of divergence. Nucleotide diversity between Point Cook and the Tasmanian samples was nearly two fold higher than between pairs of Tasmanian samples.

6.4.2. Microsatellite DNA

All five microsatellite loci were found to be polymorphic. The level of polymorphism ranged from 11 alleles at *CmrHr2.26* to 52 alleles at *RubCA1*. Individuals that failed to amplify or produced ambiguous peaks were not scored. Observed and expected heterozygosities are listed in Table 6.3. The mean heterozygosity is similar across the five samples. Sundown Point has the least variation, with a mean observed heterozygosity (mean H_o) of 0.601 while Curio Bay had the highest overall variation, mean H_o = 0.648. Despite a smaller sample size, Point Cook has a comparable heterozygosity to the Tasmanian samples, with a mean H_o of 0.609.

When tested for departure from Hardy-Weinberg (HW) expected distribution of genotypes, 9 out of 25 tests were significant (P < 0.003 following Bonferroni correction for multiple tests) (Table 6.3). In each case an excess of homozygotes was observed. This departure was not consistent across all populations, with Point Cook being in HW equilibrium at all loci and Eddystone Point being out of equilibrium at four loci. None of the Tasmanian samples were in HW equilibrium at *CmrHr2.30* while all five populations were in HW at *CmrHr2.14*. Overall, Fisher's exact test across all loci and all samples indicates that the departure from HW equilibrium is highly significant.

An analysis of molecular variance (AMOVA), comparing all five sites across five loci gave a F_{ST} of 0.0034 (P = 0.004), indicating genetic differentiation. A multilocus chi-square analysis of the distribution of microsatellite alleles across the five samples using Fisher's exact test (Raymond and Rousset, 1995) revealed significant partitioning of allele frequencies (P = 0.001). Individually, *CmrHr2.26, CmrHr2.30* and *RubCA1* indicated significant differentiation at P < 0.002. Pairwise chi-square

tests showed that the only significant comparisons, after Bonferroni correction, were between Point Cook and Tasmanian samples; no Tasmanian comparisons were significant (Table 6.4). The corresponding R_{ST} and F_{ST} values are consistently higher between Point Cook and Tasmania than between the Tasmanian comparisons. However, of the ten pair wise F_{ST} tests performed, only Point Cook x Eddystone Point (F_{ST} = 0.011) was significant after Bonferroni correction (P = 0.000). R_{ST} values were low, ranging from –0.0047 to 0.0132 with no genetic structuring identified. A number of the pairwise R_{ST} values were negative, indicative of allelic size variance being greater within the sample rather than between samples. As with the R_{ST} calculations, the values generated with the infinite alleles model analysis were low, with F_{ST} values ranging from –0.002 to 0.011. No relationship was observed between genetic difference and geographic distance.

6.5. Discussion

The sampling sites for this small study were chosen to provide representation of potentially genetically discrete populations of *H. rubra*. The Point Cook, Victoria, sample came from a population separated from the four Tasmanian samples by Bass Strait, a region previously reported as a potential barrier to gene flow (Ward and Elliott 2001 and references within). The four Tasmanian samples were well separated (> 80 km) around the island. Our RFLP-mtDNA and microsatellite results, albeit based on a limited number of samples, both demonstrate restricted gene flow of *H. rubra* across Bass Strait. This conclusion is supported by a re-analysis of the allozyme data from Brown (1991b) using combined Victorian samples and combined Tasmanian samples. The level of differentiation we observed, while statistically significant, is not large compared with the level of differentiation observed in the mtDNA genome of *H. midae* from the east and west coasts of South Africa (Sweijd 1999, Φ_{ST} = 0.406, P < 0.001). Population structure studies on flounder (van den Enden *et al*. 2000), and a venerid clam (Soh *et al.* 1998) also report limited gene flow across the Bass Strait. The geographic separation of the Point Cook sample from its nearest Tasmanian sample is less than that between some of the Tasmanian samples (Figure XX). However, unlike along the Tasmanian coast there is a lack of suitable habitat for larval settlement within the Bass Strait. Besides, there remains the possibility that the Point Cook region is unique along the Victorian coast (Huang *et al*. 2000) and it is feasible that other Victorian samples may not show the same level of differentiation.

The RFLP analysis of the mtDNA-ND3 region revealed no additional information to that from the microsatellite analyses to suggest any genetic heterogeneity within the Tasmanian blacklip abalone population. The negative nucleotide divergence figures, like the low R_{ST} and F_{ST} values, point toward larval dispersal levels that are high enough to homogenize gene pools and combat the effects of genetic drift.

Huang *et al* (2000) concluded that microsatellites were likely to be the marker of choice for population genetic studies of abalone, despite Hardy-Weinberg disequilibrium with excess homozygotes for their three loci (mean Selander's D over three microsatellite loci = -0.66, calculated from data in Huang *et al*. 2000). Discussion on this disequilibrium and the presence of null alleles at microsatellite loci is discussed in Chapter 7. Further investigation of both the heritability of microsatellite loci and presence and scoring of null alleles in abalone is necessary to confirm the resolution of these markers.

Both RFLP-mtDNA and microsatellite analysis detected low differentiation across Bass Strait, and neither detected significant differentiation among the four Tasmanian samples. While finer detailed analyses of the mtDNA genome, such as sequence analysis or SNP (single nucleotide polymorphism) technology may reveal as yet undetected heterogeneity in *H. rubra* populations, microsatellites are considered the most useful at this time. This assessment is based on a greater level of polymorphism and the ability to examine a number of independent markers.

Table 6.1: **Distribution frequency of 25 mtDNA haplotypes** among five *Haliotis rubra* sample sites generated from RFLP analysis of ND3 mtDNA fragment based on 6 restriction enzymes (*Dde* I, *Hae* III, *Rsa* I, *ScrF* I, *Dpn* II and *Msp* I). (Hap. diversity = haplotype diversity).

	Haplotype	Distribution Frequency					
		Eddystone Pt	**Red Rocks**	**Curio Bay**	**Sundown Pt**	**Point Cook**	**Total**
1	AAAAAA	16	23	21	14	12	86
2	BBABBC	5	3	4	6	17	35
3	AABAAA	4	3	4	-	1	12
4	AAAAAB	2	1	3	5	-	11
5	ADAAAB	3	1	2	2	-	8
6	AACAAA	-	1	1	3	1	6
7	AAACAA	1	3	-	1	-	5
8	BCABBC	-	1	-	1	2	4
9	CAACBC	1	-	-	1	-	2
10	AEAAAA	1	-	-	1	-	2
11	BAAABC	1	-	1	-	-	2
12	BAABBC	-	-	-	-	2	2
13	BFABBC	-	-	-	-	2	2
14	AGAAAB	-	-	-	1	-	1
15	AFAAAA	-	-	-	1	-	1
16	AADAAA	-	-	-	1	-	1
17	AAADAA	-	-	-	1	-	1
18	AAAACB	-	-	-	1	-	1
19	AAAACA	1	-	-	-	-	1
20	CAAAAA	1	-	-	-	-	1
21	ACAAAA	1	-	-	-	-	1
22	CAAAAC	-	1	-	-	-	1
23	CAACDC	-	1	-	-	-	1
24	CAAABC	-	1	-	-	-	1
25	ABABBC	-	-	-	-	1	1
	Total	**37**	**39**	**36**	**39**	**38**	**189**
Hap. diversity		**0.78**	**0.63**	**0.63**	**0.68**	**0.82**	

Table 6.2: **Nucleotide diversity (above diagonal) and divergence (below diagonal)** of the ND3 mtDNA fragment of *Haliotis rubra*, corrected for with-in population diversity.

	Eddystone Pt	**Red Rocks**	**Curio Bay**	**Sundown Pt**	**Point Cook**
Eddystone Pt		0.010060	0.009567	0.012099	0.017010
Red Rocks	-0.000153		0.008610	0.011279	0.016765
Curio Bay	-0.000187	-0.000088		0.010741	0.016339
Sundown Pt	-0.000153	0.000083	0.000004		0.017489
Point Cook	0.004732	0.005543	0.005577	0.004229	

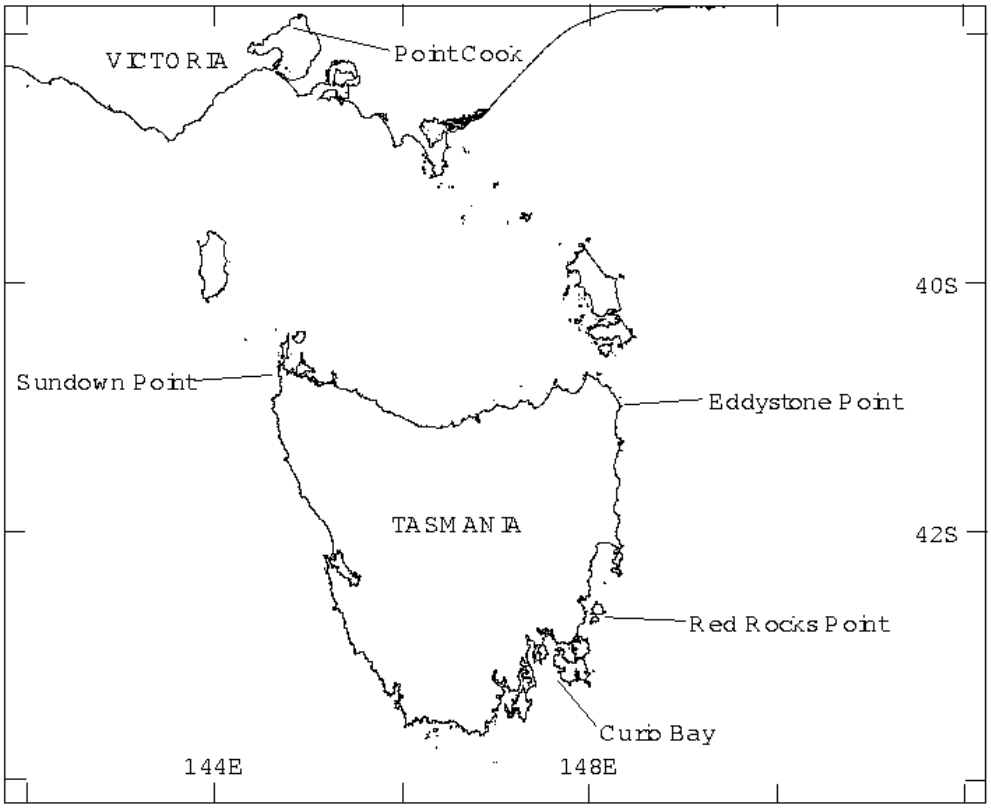
Table 6.3: **Summary statistics for five microsatellite loci** in five samples of *Haliotis rubra*, including number and size range (base pairs) of alleles observed in each sample. *n* - sample size; H_o - observed and H_E - expected heterozygosities; * - significant ($P < 0.0025$ after Bonferroni correction for 20 multiple tests) departure from Hardy-Weinberg expectations.

	Eddystone Pt	Red Rocks	Curio Bay	Sundown Pt	Point Cook
CmrHr1.14					
No. of alleles	6	8	9	9	3
Allele size	251-269	251-285	251-283	251-289	251-261
n	98	97	99	97	50
H_o	0.289*	0.268	0.255	0.268	0.220
H_E	0.415	0.319	0.301	0.280	0.250
CmrHr2.14					
No. of alleles	8	9	7	6	6
Allele size	207-237	199-237	211-241	199-237	199-237
n	86	92	87	96	50
H_o	0.770	0.735	0.841	0.698	0.673
H_E	0.756	0.763	0.773	0.802	0.691
CmrHr2.26					
No. of alleles	12	13	14	10	11
Allele size	168-296	168-232	168-256	168-216	168-220
n	96	85	79	79	47
H_o	0.489*	0.522*	0.641*	0.573	0.595
H_E	0.804	0.833	0.864	0.818	0.872
CmrHr2.30					
No. of alleles	41	44	43	40	26
Allele size	284-396	288-394	282-402	288-386	288-376
n	94	99	94	95	49
H_o	0.787*	0.847*	0.699*	0.734*	0.776
H_E	0.955	0.963	0.954	0.957	0.940
RubCA1					
No. of alleles	34	27	32	33	25
Allele size	110-196	110-188	110-208	110-208	110-192
n	99	98	99	93	50
H_o	0.707*	0.827	0.804	0.732	0.780
H_E	0.937	0.898	0.901	0.918	0.938
Mean *n*	94.6	94.2	91.6	92	49.2
Mean no. alleles	20.2	20.2	21	19.6	14.2
Mean H_o	0.608	0.640	0.648	0.601	0.609
Mean H_E	0.773	0.755	0.759	0.755	0.738

Table 6.4. **Pairwise chi-square significance value and multilocus R_{ST} and F_{ST} figures** and their corresponding significance values, calculated between five *Haliotis rubra* samples for allele frequencies at five microsatellite loci. * - significant value at $P < 0.005$ following Bonferroni correction for 10 multiple tests. (PC - Point Cook, EP - Eddystone Point, RR - Red Rocks, CP - Curio Bay, SP - Sundown Point).

	Chi-Square	**R_{ST}**		**F_{ST}**	
	P-value	**value**	***P-value***	**value**	***P-value***
PC x EP	*0.0000**	0.0132	*0.0394*	0.0111	*0.0000**
PC x RR	*0.0005**	0.0068	*0.1658*	0.0072	*0.0088*
PC x CB	*0.0029**	0.0071	*0.1744*	0.0027	*0.1279*
PC x SP	*0.0000**	0.0085	*0.0920*	0.0032	*0.1289*
EP x RR	*0.0085*	0.0032	*0.2326*	0.0012	*0.2861*
EP x CB	*0.0232*	0.0013	*0.3642*	0.0017	*0.6152*
EP x SP	*0.0053*	0.0057	*0.1002*	-0.0019	*0.9473*
RR x CP	*0.0164*	-0.0047	*0.9906*	0.0018	*0.1279*
RR x SP	*0.4433*	-0.0030	*0.8324*	-0.0009	*0.7568*
CB x SP	*0.2747*	-0.0017	*0.6858*	0.0012	*0.2529*

Figure 6.1. Location of the five collection sites for blacklip abalone, *Haliotis rubra*, in south-east Australia



7. BLACKLIP ABALONE STOCK STRUCTURE

This section reports on research undertaken towards achieving Objective 2. It will be prepared for peer review and publication in the international literature. Introduction to the subject matter is covered both in Section 2 (Background) and Section 6, and references are listed in Section 15.

7.1 Material and Methods

7.1.1. Samples

7.1.1.1. Tasmanian samples

Samples of blacklip abalone (*Haliotis rubra* Leach) were collected from 29 sites around the coast of Tasmania (Table 7.1 and Figure 7.1).

Generally 60 to 110 individuals were collected from each site. At the time of processing, sex and shell length were recorded (summarised in Table 7.2) and small pieces of foot muscle and/or gill tissue dissected and kept frozen. In all instances whole animals and tissue samples were stored at -80°C at the CSIRO laboratories. Shell length was measured at the widest part of the shell, and sex was determined by an examination of gonad colour; where this was inconclusive the animal was recorded as being immature.

Samples were collected predominantly by TAFI research divers as part of other abalone research activities. These samples consisted of whole animals returned to the TAFI laboratories where they were stored frozen at -20°C until processed.

Three sites (Lemon Bight, Long Point and Nuggets) were specifically sampled (spatial repeat) for ca. 150 individuals at each site consisting of three collections of ca. 50 individuals taken from as small an area as possible within each site. Two sites (Louisa Bay and One Tree Point) had temporal repeat samples collected (Table 7.1).

Two sites from the west (Granville Harbour) and southwest (Block 11) coasts were sampled by arrangement with commercial divers. The divers placed 100 commercial sized individuals in marked containers that were delivered to the processors as part of their quota. Following commercial processing, the shell and waste (mantle, gills and guts) of the 100 individuals per site were provided to CSIRO. In the laboratory, gill tissue was dissected and shell length and sex noted.

The Australian Maritime College collected two samples from the north of the State (Cape Portland and Low Head), each consisted of whole animals provided frozen to CSIRO. Muscle and gill tissues were dissected from these animals in the laboratory.

7.1.1.2. Mainland samples

Samples of blacklip abalone were also provided from nine sites along the mainland coast, of which seven were analysed (Table 7.3 and Figure 7.1). These seven sites consisted of two from South Australia (provided by SARDI), three from Victoria (provided by MAFRI) and two from New South Wales (provided by NSW Fisheries). Samples were processed after capture and muscle/gill tissue dissected and preserved; shell length and sex were recorded (Table 7.4). Two samples from Batemans Bay,

NSW, and one from Julia Percy Island, Victoria, were not analysed due to poor quality DNA, assumed to be due to storage problems after collection.

7.1.2. DNA extraction

Using a new sterile scalpel for each sample, approximately 20mg of foot or gill tissue were transferred to a clean, sterile 1.5mL microcentrifuge tube. DNA was extracted using either a modified CTAB (hexadecyltrimethylammonium bromide) method (Grewe *et al*. 1993) or a QUIamp DNA Mini Kit (Quiagen) according to the protocol supplied. DNA was stored at -20°C.

Extraction success was confirmed by electrophoresing 5µL of each DNA extraction on a 2% agarose gel made up in 1xTBE at 140V for 1 hour. Extractions were visualised by staining in ethidium bromide at a concentration of 0.5µg per mL and viewing under ultraviolet light. Five microlitres of each sample was transferred to a new sterile eppendorf tube and diluted with 195µL of ddH_2O.

7.1.3 Microsatellite loci and PCR conditions

Genetic variation was examined at 8 microsatellite loci, *CmrHr1.14, CmrHr1.24, CmrHr1.25, CmrHr2.9, CmrHr2.14, CmrHr2.26, CmrHr2.30* (Evans *et al.* 2000) and *RubCA1* (Huang and Hanna 1998). In some instances fewer loci (and not all individuals) were scored due to poor amplification.

PCR amplifications were performed in 96-well plates (Costar) in a total volume of 25 µL. Reaction mixtures consisted of 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 2.5 µL of 10X buffer (670 mM Tris-HCL pH 8.8, 166 mM $(NH_4)_2SO_4$, 4.5% Triton X-100, 2 $mg.mL^{-1}$ gelatin), 0.66 units *Taq* DNA polymerase, 25 ng of template DNA and the reaction made up to 25 µL with sterile milli-Q water (all reagents from Fisher Biotech). Microsatellite loci were amplified in two separate multiplex reactions, with loci separated by allele size and different colour labels on the primers. Each multiplex was run on a separate gel. The first multiplex reaction (multiplex 1) comprised 3 pmoles of each *CmrHr*1.14 primer, 4 pmoles of each *CmrHr*1.24 primer, 1 pmole of each *CmrHr*2.14 primer, 4 pmoles of each *CmrHr*2.30 primer and 3 pmoles of each *RubCA1* primer. The second multiplex reaction (multiplex 2) comprised 5 pmoles of each primer pair for the three loci, *CmrHr*1.25, *CmrHr*2.9 and *CmrHr*2.26.

All PCRs were conducted in a Perkin-Elmer 9600 thermal cycler. Cycling conditions comprised an initial denaturation step of 3 min at 94˚ C and concluded with a final extension step of 10 min at 72˚ C. Amplification involved 10 cycles of: denaturation at 94˚ C for 30 s; annealing at 60-55˚ C for 30 s, dropping by 0.5˚ C per cycle; and extension at 72˚ C for 60 s. This was followed by a further 25 cycles of denaturation at 94˚ C for 30 s; annealing at 55˚ C for 30 s, and extension at 72˚ C for 60 s.

One microlitre from each amplification was diluted in 3 µL of sterile milli-Q water, and 0.7 µL of this dilution was mixed with 2 µL formamide, 0.5 µL loading dye and 0.5 µL Genescan Tamra-500 size standard (ABI), denatured for 2 min at 95˚ C, and loaded onto a 4.8% denaturing polyacrylamide gel. Samples were run on an ABI-377 DNA autosequencer and genotypes determined using Genotyper® software.

Positive and negative controls were included in all PCR reactions, and a control sample of known allele size included on each microsatellite gel run on the ABI autosequencer.

7.1.4 Statistical analysis

Genetic diversity for each locus per sample site was estimated by the number of alleles per locus and by the observed (H_o) and Hardy-Weinberg expected (H_e) heterozygosity. H_o, H_e, and tests for deviations from Hardy-Weinberg Equilibrium (HWE) within samples were estimated using GENEPOP Vers. 3.3 (Raymond and Roussett, 1995). An index of heterozygote deficiency or excess (D), where $D = [H_o - H_e] / H_e$ (Selander 1970) was also calculated from the heterozygosity estimates. Significance of departure from equilibrium levels was tested by a Markov chain procedure, with significance levels determined after 400 batches of 4000 iterations each.

Linkage disequilibrium between pairs of loci was assessed using exact tests in GENEPOP Vers. 3.3 (Raymond and Roussett 1995). Significance of departure from equilibrium levels was tested by a Markov chain procedure, as described above.

ARLEQUIN Vers. 2.000 (Schneider *et al.* 2000) was used for an analysis of variance of allele frequencies within and among populations (AMOVA), a method based on Excoffier *et al.* (1993). ARLEQUIN provided multi-locus (across loci) and individual locus estimates of Φ_{ST}, an analogue of F_{ST}, the genetic variance component attributable to population differentiation. For example, an $F_{ST} = 0.100$, indicates that 10% of the observed variation in allele frequencies is attributable to between sample variation, while 90% is attributable to within sample variation. The AMOVA method from ARLEQUIN 2000 was also used to determine F-statistics of selected groupings of samples (e.g. Tasmania compared to mainland). Significance levels in all tests were based on 10,000 steps of a Markov chain procedure.

Single locus F-statistics (Weir and Cockerham 1984) were also estimated using GENEPOP Vers. 3.3 (Raymond and Roussett 1995). In addition, this program was used to test allelic distribution by the exact test for genic differentiation for each sample pair per locus. Significance levels were determined after 400 batches of 4000 iterations of the Markov chain procedure.

Shell length and sex data was available for abalone from most samples. The data set was divided into male, female and immature groups and analysed for any sex differences in genetic variation. Male and female groups were compared using the AMOVA method of ARLEQUIN 2000 to provide an F_{ST} value. Three length groups were created, small ($\leq$ 134 mm), medium (135 - 144 mm) and large ($\geq$ 145 mm), and an AMOVA between groups performed.

Significance levels for simulation tests were adjusted in all cases with the sequential Bonferroni approach for multiple tests (Rice 1989).

7.2 Results

7.2.1. General microsatellite locus statistics

All eight microsatellite loci were highly polymorphic with between 12 and 52 alleles observed in total numbers of individual *H. rubra* scored per locus ranging from 2150 to 3328 (Appendix 4). The mean number of alleles scored per locus per sample ranged from 5.2 to 33.3 (Table 7.5), with a range of 2 to 40 alleles observed at a single locus for a single sample (Appendix 5). The mean sample size (number of individuals scored per sample) at each locus ranged from 56.6 to 76.0 (Table 7.5)

7.2.2. Microsatellite allele distribution

Only two loci had less than 20 observed alleles. *CmrHr2.14* had 12 observed alleles in 2886 scored individuals, at a mean of 7.7 alleles per sample, while *CmrHr1.24* had 14 alleles observed in 3342 individuals at a mean of 5.2 (Table 7.5). The allele distribution of the latter locus was dominated by one allele, in the middle of the observed size range, at a frequency of 0.842 in the total sample (Figure 7.2). Locus *CmrHr2.14* also had one main allele in the middle of the observed size range but at a lower frequency of 0.467 in the total sample, with two other longer (higher number of base pairs) alleles at a frequency above 0.150 (Figure 7.2).

Twenty alleles at a mean of 11.5 per sample (Table 7.5) were observed in 2554 scored individuals for locus *CmrHr2.26*; six at a frequency of about 0.100 or greater, with a slight bias to shorter alleles in the distribution (Figure 7.3). One allele at 0.803 frequency in the total sample dominated the 24 alleles observed in 3124 scored individuals for locus *CmrHr1.14*, again with a bias to the shorter length alleles in the distribution (Figure 7.3). The dominance of the one allele is reflected in a mean of only 6.7 alleles per sample (Table 7.5).

Over 40 alleles were observed in each of the other four loci. *CmrHr1.25* had 44 alleles, one at a frequency of 0.208 and four others at frequency greater than 0.075 (all at the shorter allele length of the distribution), scored in 2150 individuals (Figure 7.4). The distribution of 48 alleles (in 3328 individuals) for *RubCA1* was relatively evenly distributed within the centre of allele size distribution, with fifteen alleles at frequencies between 0.020 and 0.083 in the total sample, but there was a high frequency (0.212) of a shorter sized allele (Figure 7.4). Nineteen of 51 alleles (scored in 3122 individuals) for *CmrHr2.30* had frequencies between 0.020 and 0.080, with no dominant allele (Figure 7.5). Likewise, sixteen of 52 alleles (scored in 2822 individuals) for *CmrHr2.9* had frequencies between 0.020 and 0.050, but the allele distribution was dominated by two short alleles at frequencies of 0.145 and 0.174 (Figure 7.5). The mean number of alleles per sample for these extremely polymorphic loci ranged from 23.2 to 33.3 alleles (Table 7.5).

7.2.3. Microsatellite locus linkage

Linkage disequilibrium was assessed where possible at all locus pairs in all samples. Significant departures ($P < 0.05$) from equilibrium levels were detected in only 57 of the total 1072 tests. At least one departure from equilibrium was recorded for each of the 28 possible locus pairings of the eight loci, and in 24 of the 44 samples. The pairing of *CmrHr2.30* and *RubCA1* was observed six times and that of *CmrHr2.30* and *CmrHr2.26*, and *CmrHr1.14* and *CmrHr1.24*, four times each.

Of the 57 significant tests only 10 tests remained significant after sequential Bonferroni correction for multiple tests for individual samples. Six of these occurred for the Black Reef sample (*CmrHr1.14* and *CmrHr2.26, CmrHr1.14* and *CmrHr2.30, CmrHr1.24* and *CmrHr2.30, CmrHr2.14* and *CmrHr2.30, CmrHr2.14* and *RubCA1, CmrHr2.30* and *RubCA1),* and one each for Kiama (*CmrHr2.14* and *CmrHr2.9*), Smiths Gulch (*CmrHr1.14* and *CmrHr2.9*), Mount Cameron (*CmrHr1.24* and *CmrHr2.30*) and Sandblow Point (*CmrHr1.25* and *CmrHr2.26*).

No two loci were shown to be linked in all samples at the sample sizes we examined.

7.2.4. Sex and size

The sex ratio of 3251 mature abalone used in this study was 1 female to 0.95 males. No significant allele differences at the eight loci were observed between male and female groups ($F_{ST} < 0.001$; $P = 0.663$) or between the three length classes examined ($F_{ST} = 0.002$; $P = 0.131$) for a subset of the samples. Given this result the full data set was not examined further for either sex or size differences.

7.2.5 Microsatellite locus heterozygosities

Generally there was a deficiency in the number of heterozygotes scored in relation to the number expected under Hardy-Weinberg Equilibrium (HWE) for populations of randomly mating individuals (Appendix 5, Tables 7.5 and 7.6). Five of the eight loci (*CmrHr2.30, RubCA1, CmrHr1.25, CmrHr2.26* and *CmrHr2.9*) had high numbers of samples with significant deficiencies of scored heterozygotes, while the other three loci were generally in equilibrium (Table 7.5 and Appendix 5). The largest deviation from equilibrium was for *CmrHr1.25* with a mean D value of –0.603. The deviation from equilibrium is very much a locus rather than sample effect as no sample was out of equlibrium for all eight loci (Table 7.6 and Appendix 5). This suggests a technical (null or non-amplifying alleles) rather than biological issue. The loci with greater deviation from HWE tended to have a predominance of shorter alleles and a non-uniform allele distribution (Figures 7.2 to 7.5). The two loci with fewer alleles (*CmrHr1.24* and *CmrHr2.14*) had better fits to HWE (Table 7.5).

7.2.6 Sample differentiation

Each of the eight microsatellite loci were scored and analysed in all but eight of the 44 samples (Tables 7.5 and 7.6, and Appendices 4 and 5). Five samples were not examined for the three loci showing highly significant departures from HWE (*CmrHr1.25, CmrHr2.26* and *CmrHr2.9*) and three samples have one or two loci omitted from the analyses due to poor PCR amplification for some individuals at those loci.

Mean sample sizes (number of scored individuals per sample) per locus ranged from 45.9 to 97.4; this is excluding the nine small spatial samples (Long Point, Nuggets and Lemon Bight) that ranged from 14.6 to 47.1 individuals (Table 7.6). The mean number of alleles per locus ranged from 13.0 to 21.6 for the samples examined for all eight loci; the lowest number being for the West Bay sample (Table 7.6). Compared to other samples of similar sample size (30 to 50 individuals), the West Bay sample had a relatively low number of alleles (13.0 compared with mean of 16.5), particularly at both *CmrHr2.30* and *CmrHr2.9* (Appendix A5). The mean number of alleles over the eight loci for four NSW and Victorian samples (20.0) was similar to that for the 21 Tasmanian samples (19.6) of similar sample sizes (>60).

The three loci *CmrHr1.25, CmrHr2.26* and *CmrHr2.9* were excluded from some of the following analyses, and the results are focussed on the other five loci. We have erred on the side of caution due to the uncertainty associated with the highly significant departures from HWE in these three loci (Table 7.5 and Appendix 5). This is also shown by the high F_{IS} values for these loci in Table 7.10 (with random mating natural populations and loci without null alleles the F_{IS} value is typically close to zero). While we acknowledge the presence of null alleles in these loci we have yet to elucidate the molecular basis for all of them, and so consider it wise to exclude them.

7.2.6.1 Temporal and Spatial Differentiation

Genetic differentiation between the two temporally separated samples collected from Louisa Bay and One Tree Point were examined across eight (seven for Louisa Bay) and five loci (multi-locus analyses) and by individual locus (Table 7.7). No evidence of differentiation was seen between the two One Tree Point samples, except at the allelic exact test at locus *CmrHr1.25,* reflected in the 8 locus test. Exact tests indicated significant differentiation between the two Louisa Bay samples (Table 7.7), but this was not reflected by the F_{ST} tests. The exact tests may be more sensitive than F_{ST} tests to the presence of rare alleles. While the exact tests are significant, the F_{ST} values are very low suggesting that the difference between the two samples is overall very small.

Genetic differentiation between three small spatially separated collections of individuals from the one site was examined for three sites, Long Point, Nuggets and Lemon Bight (Table 7.8). No evidence of differentiation (AMOVA results) was apparent in either the Long Point or Lemon Bight sites. Overall there was no differentiation between the Nuggets samples, but significant allele frequency differences were present at locus *CmrHr2.30* (Table 7.8, Appendix 4). Pairwise exact tests suggest this difference is predominantly between Nuggets #4 Gap and Nuggets #4 North Face samples, although Nuggets #2 and Nuggets #4 North Face also differed at *CmrHr2.30* (Table 7.9). This suggests the Nuggets #4 North Face sample is the cause of the heterogeneity. Exact tests also showed some differentiation between Long Point samples and Lemon Bight samples, but none was significant with Bonferroni correction.

Given the lack of consistency from these repeat samples, they are maintained as separate samples and not combined by site in the following geographic differentiation analyses.

7.2.6.2 Geographic Differentiation

There was no suggestion of any genetic differentiation among the 44 samples from multi-locus analyses across eight or five loci, with F_{ST} values of –0.0755 for eight loci and –0.0249 for five loci (Table 7.10). The AMOVA result across the five loci (*CmrHr1.14, CmrHr1.24, CmrHr2.14, CmrHr2.30* and *RubCA1*) for only the 37 Tasmanian samples also revealed no differentiation (F_{ST} = –0.0296), but for the seven mainland samples significant differentiation was apparent (F_{ST} = 0.0081, $P < 0.001$). Inclusion of the other three loci resulted in negative non-significant F_{ST} values (–0.0832 and –0.0322 respectively). A five locus analysis grouping the Tasmanian samples and the mainland samples was however not significant (F_{ST} = –0.0021).

Analysis by individual locus, however, returned low but highly significant F_{ST} values (Table 7.10). The F_{ST} values were ca. 0.010 or less, suggesting that only 1% or less of the observed variation in allele frequencies was due to between sample variation, compared with 99% or more for within sample variation. Yet each locus alone indicates some genetic heterogeneity exists within the population of *H. rubra* analysed (Tables 7.11).

The AMOVA results show that the more loci that are included in an analysis the lower the F_{ST} value and the results are often non-significant. For individual loci F_{ST} values are positive and in many instances significant, while across multiple loci the F_{ST} values are lower and more often negative. As will be described below, the differentiation being shown by each individual locus is not the same; i.e. different samples are being highlighted as statistically different at different loci. Therefore when multiple loci are combined these individual locus differences are lost in the overall level of differentiation within and between samples.

Comparisons of allele frequencies for each sample with every other sample by pairwise exact tests, both across multi-loci (8 and 5) and by individual locus, showed highly significant differentiation between many pairs of samples (Tables 7.12 and 7.13). Despite negative and non-significant F_{ST} values when examining all samples across multi-loci, 86% of the 946 pairwise exact tests of allele frequencies between pairs of samples were significant at the 0.05 level across the eight loci, and 68% across the five more reliable loci (Table 7.12). After Bonferroni correction (for total number of tests) the number remaining significant were still high at 56% and 31% respectively.

The West Bay (South Aust.), Black Reef (SWTas.) and Cape Portland (NETas.) samples were significantly different to all other samples at the 0.05 level across all eight loci, while 16 other samples were significantly different to 40 or more of the 43 samples (Table 7.12). West Bay and Cape Portland remained significantly different to all other samples after Bonferroni correction, while High Rocky Point (WTas.) and Low Head (NETas.) were highly significantly different to all but two other samples after correction (Table 7.12). The two samples not significantly different to High Rocky Point were Curio Bay (STas.)and Nuggets #4 North Face (ETas.), while for Low Head they were Nuggets #2 and Point Cook (Vic.). Analysis with only the five more reliable loci had the same samples standing out from the others, although Cape Portland not as strongly, and some of the others such as Jervis Bay (NSW), Sandblow Point (NWTas.), Black Reef and Waterwitch Reef (NWTas.) were also less significantly different.

On a locus by locus basis, West Bay stands out from the other samples with significant differentiation (without Bonferroni correction) from more than half the samples at all eight loci (Table 7.13). Low Head was significantly differentiated from many other samples at three loci, Cape Portland over two loci, High Rocky Point at only one.

For locus *CmrHr1.14*, 307 of the tests were significant at the 0.05 level, of which 56 were significant after Bonferroni correction for the total number of tests at the locus (Table 7.13a). Of these 56 highly significant cases only two did not involve either West Bay (in 26 cases) or Low Head (28); both of those involved the Hunter Island

(NWTas.) sample. West Bay was significantly differentiated from its closest sample of Port MacDonell (South Aust.), but the Low Head and its nearest neighbor sample of Cape Portland were not differentiated.

Only four pairwise tests for *CmrHr1.24* remained significant after Bonferroni correction; 26 % were significant at the 0.05 level. While only 19% of the tests for *CmrHr2.14* were significant at 0.05 level, 48 of these remained significant after multiple test correction (Table 7.13a). The West Bay sample was again dominant in these tests appearing in 31 cases. For locus *CmrHr2.30,* the High Rocky Point sample dominated the significant cases, although Nuggets #4 North Face, Black Reef, Sandblow Point and Cape Portland also has a high number of significant cases (Table 7.13a). West Bay was again dominant for *RubCA1*, *CmrHr1.25* and *CmrHr2.9* (Table 7.13b). The Low Head, Cape Portland and Point Cook samples were also differentiated at most samples at *RubCA1*.

As discussed above examination of the seven mainland samples across eight loci suggested homogeneity, but highly significant heterogeneity was evident at the five more reliable loci. Across multi-loci there was no evidence of differentiation within the 37 Tasmanian samples, but each individual locus value was highly significant. Comparison of Tasmanian and mainland samples again resulted in no differentiation from a multi-locus analysis but significant differentiation at some individual loci (Table 7.11). An AMOVA of all samples (5 loci) with West Bay identified as a separate group resulted in a significant F_{CT} value (differentiation between groups) of 0.0371 (P = 0.0224).

Grouping samples into various geographic groupings for analysis resulted in non-significant results across multi-loci, but invariably significant results at individual loci. Interpretation of any heterogeneity is difficult, as the differences were not consistent between loci, except for the separation of West Bay.

7.3 Discussion

This project represents the largest undertaken on abalone genetic population structure in terms of sample sites and number of microsatellite loci (Table 7.14). The size and extent of the study has raised a number of issues that centre on the efficacy of some microsatellite loci for population genetics analyses in abalone and the interpretation of the results. Important issues include: excess homozygotes, differentiation between temporal and small-scale spatial samples, and lack of heterogeneity across multi-loci but significant differentiation at individual loci.

7.3.1. Excess homozygosity

The efficiency of some of the microsatellite loci for population structure analyses of blacklip abalone appears low. Of major concern is the correct scoring of individuals, highlighted by the very significant homozygote excess observed. Homozygote excesses are not uncommon in abalone population studies (allozymes e.g. Brown and Murray 1992; Smith and Conroy 1992; Gonzalez *et al*. 2000; and microsatellites Huang et al 2000), but no consensus has been reached in regard to a reason.

In our study the excess in homozygotes is locus specific, with no sample showing significant excess at all eight loci, and some loci showing no evidence of homozygote excess. Therefore, localized inbreeding or genetic substructure within a sample are unlikely explanations. Other potential explanations for the deviations include sex linkage, pre-settlement selection (David *et al*. 1997), gel mis-scoring and null alleles. No sex-linkage was evident for any of the loci. Pre-settlement selection is unlikely as microsatellites are considered to be neutral, Mendelian markers (Jarne and Lagoda 1996). Linkage to genes undergoing selection however cannot be ruled out, nor possible age class differences with some environmental selection between years. Gel mis-scoring is considered unlikely as peak calling and gel images were all rechecked. Null or non-amplifying alleles due to mutations in the primer region and/or preferential PCR amplification are considered the most likely explanations.

The term ‘null allele’ is used to describe alleles that cannot be visualised on a gel, and their occurrence is a potential problem in population genetic studies. Depending on the null allele frequency they may go undetected in some loci. If detected or assumed to exist, a single ‘null allele’ may be called where in fact a number of different null alleles may exist and may provide potential variation between populations. The reported frequency of microsatellite loci containing null alleles varies between organisms (e.g. 30% in humans, 25% in swallows and 16% in rainbow trout, references in Holm et al 2001). Of the eight loci examined in this study it is likely that at least five possess null alleles, but these have been confirmed so far in only two loci (Evans and Elliott unpublished).

It is generally assumed that null alleles are the result of a mutation in the flanking sequence that is complementary to the PCR primers (e.g. Eggleston-Stott et al 1997, Uzunova and Ecke 1999, Holm *et al.* 2001). In addition, preferential PCR amplification of one allele in a heterozygote may result in a null allele. This occurs when there is a large size difference between a pair of alleles, with the likelihood of PCR failure reported to be greater for the larger allele (O’Connell and Wright 1997).

Locus *CmrHr1.25* has a large homozygote excess in all samples and also an appreciable number of failed PCR amplifications. The amplification failure was not consistent in other loci for an individual, nor dependent on whether the locus was amplified in a single reaction or in a multiplex, therefore ruling out the possibility of non-specific PCR inhibitors or poor quality template DNA. It is assumed many of the failed PCR amplifications were null homozygotes. The presence of null alleles, and null homozygotes, at this locus was confirmed with new PCR primer pairs designed external to the original set, but the cause of the non-amplifications could not be determined (data not presented, Evans and Elliott unpublished). There were no mutations in the original primer regions and the size range of null alleles when determined were within the normal distribution of observed alleles.

Six of the eight loci had allele size distributions skewed to the small size, with size ranges in some instances of 100 base pairs between the more common size observed and the largest allele observed (e.g. Fig 7.4). Locus *CmrHr2.30*, for example, had a wide range of allele sizes from 268 to 394 base pairs. Some homozygote individuals for the original primers for this locus proved to be heterozygous when amplified and sequenced with new, external primers (data not presented, Evans and Elliott unpublished). For some of these individuals a primer mutation was identified, yet in one individual (of four examined) this was not apparent. In this individual it appears that the larger allele may have been preferentially amplified; which may explain the distribution skew in Figure 7.4 to the larger alleles, with smaller alleles not amplifying and being detected.

The consistent observation of homozygous excess at other loci (e.g.*CmrHr2.26, CmrHr2.9* and *RubCA1*) is considered to be also due to the presence of null alleles. The high level of homozygote excess observed in *CmrHr1.25, CmrHr2.26* and *CmrHr2.9,* and the confirmed presence of null homozygotes in *CmrHr1.25* raises some doubt about their usefulness in determining population structure. Therefore in many instances analyses were conducted only on the other five loci.

7.3.2. Small scale spatial and temporal variation

Significant differences in allele frequencies were observed between geographically close samples (e.g. Nuggets samples, or Black Reef with Actaeon, George III Reef and Sterile Island) and temporally separated samples (e.g. Louisa Bay). The latter raises some doubt about the ability of microsatellites to show population differences in abalone. However, it is suggested that the observed differences (that are very small $F_{ST} < 1\%$) may be a reflection of examining year-class variation within the samples. The differences observed have little geographic or biological meaning, and do not appear to be sex or size related, but may be age or recruitment cohort related. At this time it is not possible to age individuals and separate the samples by year-class to confirm this.

7.3.3. Multi-locus vs individual locus results

When our large data set was examined across multiple loci, there was little evidence of any heterogeneity, yet significant differences existed at individual loci. Because the significant differences at individual loci are not consistent between samples, the multi-locus analyses are unable to determine any overall heterogeneity. This raises the possible need for caution in interpretation of population heterogeneity from microsatellite analysis studies consisting of small numbers of either loci, sampled

sites or individuals per sample. For example, *H. rubra* from Point Cook (Vic.) when compared with just four samples from Tasmania at five loci showed significant differentiation from the homogeneous Tasmanian samples (Conod *et al.* 2002 and Chapter 6), yet if other sites or loci are included the differentiation is less obvious.

This rather confusing result of no overall differentiation seen across multi-loci but significant differentiation at individual loci, with little geographical or biological meaning could be interpreted to imply that microsatellite loci are too variable and/or have too high a mutation rate to be of use in abalone population studies. Yet there is similarity in conclusion between our study and an earlier allozyme study (Brown 1991b), suggesting that the hypervariable microsatellites are informative, despite technical problems (null alleles) with some loci.

7.3.4. Population structure

Previous research into the genetic structure of abalone populations has yielded variable results (Table 7.14). Strong genetic structuring has been reported in *H. cracherodii* along the central Californian coast (Hamm and Burton 2000; allozymes), and in *H. rubra* around southern Australia (Huang *et al.* 2000; microsatellites and minisatellites). Weaker structure was detected in allozyme studies of *H. roei, H. rubra* and *H. laevigata* (Hancock 2000; Brown and Murray 1992) populations in Australia. Studies of *H. fulgens* in Baja California and *H. rufescens* populations along the Californian coast revealed no evidence of genetic (allozyme) structuring (Zúñiga *et al.* 2000; Burton and Tegner 2000).

The analysis of all 44 samples across multiple (eight or five) independent microsatellite loci suggests homogeneity and a panmictic model to the *H. rubra* population. Yet analyses of individual loci indicate some heterogeneity within the population. With a few exceptions however, the differentiation is not consistent and difficult to interpret.

On the broad scale, the West Bay sample from South Australia is significantly different to all other samples when examined across all loci by the exact test and from a majority of samples when examined at individual loci. This sample is the most western analysed within the mainland population, and the difference may reflect isolation-by-distance. A correlation analysis of estimated geographic distance and genetic distance (represented by F_{ST}) between the seven mainland samples does lend some support to this (Figure 7.5). Interestingly, the intercept of a possible line of best fit for an F_{ST} of zero is between 500 and 600 km. Brown (1991b) suggested a similar distance from his allozyme study for a ‘neighbourhood size’. He suggested this could be the size for the conservation of regional gene pools. No similar correlation plot was attempted with the Tasmanian data as samples are essentially evenly distributed around the island, and distance between two samples may be in either direction about the island. Yet a neighbourhood distance of 500 to 600 km does reduce the likelihood for finding genetic differences around Tasmania.

Significant differences do exist however within the Tasmanian samples. For example, Low Head and Cape Portland show differentiation from other samples at a number of loci. Other samples (High Rocky Point, Nuggets #4 North Face, Passage Island, Waterwitch Reef and Sandblow Point) also showed high numbers of significant pairwise differences but at a single locus, e.g. High Rocky Point and Nuggets #4

North Face at *CmrHr2.30*, and the differences were not consistent geographically. Black Reef, for example stood out as being significantly differentiated from other samples at 0.05 level but not after Bonferroni correction (Table 7.12). It was one of four southern Tasmanian samples collected from within a small geographic area (< 10 km between collection sites), the others being George III Reef, Actaeon, and Sterile Island. Multi-locus analysis indicated no differentiation between these four samples, yet at locus *RubCA1* there was significant differentiation (F_{ST} = 0.011). Black Reef was also significantly differentiated from the other three samples at this locus and at *CmrHr2.30*. Local recruitment at Black Reef, but not at the other three sites could account for this difference, but seems unlikely. Sampling of different year-classes and/or genetic variation in recruits being unequal between the sites may be plausible explanations.

From his allozyme study on *H. rubra*, Brown (1991b) suggested a broad scale isolation-by-distance model for the population, but with some geographically proximate samples being relatively genetically discrete. Our microsatellite study would support that conclusion, with evidence of broad scale differences, correlation of genetic distance with geographic distance on the mainland, and some geographically close samples showing differentiation. Brown (1991b) suggests that the most plausible explanation for this model is a climate of predominantly local recruitment (McShane *et al.* 1988 and Prince *et al.* 1987; 1988), with high gene flow estimates governed by large local effective population sizes, rather than high migration. This may be true and needs to be examined, but we would also suggest that year-class differences might also play a major role in the small-scale differentiation observed in both studies.

The possible genetic neighbourhood size from both studies (500 to 600 km) restricts the likelihood of any differentiation being observed around Tasmania. Yet small differences are apparent between individual samples at some loci, even those from the same site. It is suggested that while local recruitment may be dominant in the population, in some years some larvae are subjected to particular environmental conditions such that they are transported and settle on other reefs. These influxes of recruits result in two aspects relating to the interpretation of genetic heterogeneity. Firstly, the influx of any gametes from outside will create an overall homogenous population, and genetic differences will be difficult to measure. Secondly, the potential exists to be sampling predominantly different year-classes from different reefs at the various sampling locations and this prevents a true examination of the genetic variation within and between different sections of the population. Recruitment from outside if sporadic and dependent on particular environmental conditions may consist of larvae from a relatively small effective population size. Such recruits, if a large proportion of a sample, could strongly influence the allelic frequencies.

Both the present study and that of Huang *et al.* (2000) examined genetic variation at the *RubCA1* locus. Expected heterozygosity at this locus was similar in both studies (this study = 0.918; Huang *et al.* 2000 = 0.955), but observed heterozygosities varied greatly (this study = 0.770; Huang *et al.* 2000 = 0.380). The large difference in observed heterozygosities between the two studies is difficult to explain in a biological sense. Huang *et al.* (2000) considered the possibility of null alleles and the mis-typing of results, but concluded that neither was likely. They argue that the dramatic homozygote excess seen at all three microsatellite loci in their study was an

indication of inbreeding in abalone populations. Predominantly local recruitment may lead to some inbreeding and contribute to a general trend of excess homozygosity at most loci. It is however unlikely that such large deviations from expected heterozygosity can be explained in this way. The levels of inbreeding required to produce such large homozygote excesses at microsatellite loci would also be expected to lead to an excess at other loci. Such an excess was not present at the two minisatellite markers examined by Huang *et al.* (2000), in fact they report a slight heterozygote excess across those two loci ($D = 0.06$). It would therefore seem more likely that the large homozygote excess reported by Huang *et al.* (2000) is due to some technical problem, such as the presence of null alleles, preferential amplification of alleles or perhaps the inability of their analysis to detect and score weakly amplified alleles. Our larger data set also revealed very limited differentiation between samples along the NSW and Victorian coasts, in contrast to the results presented by Huang *et al.* (2000).

In summary, the data generated by genetic studies of the Australian *H. rubra* population suggest that some structure does exist on a broad scale. Differentiation has been detected by allozymes (Brown 1991b), mitochondrial DNA (Conod *et al* 2002 and Chapter 6), RAPDs (Huang *et al.* 2000) and microsatellites. Whether any significant fine scale heterogeneity within the Tasmanian or mainland populations exists remains unclear. This lack of clarity may reflect infrequent and unpredictable larval transport and settlement events, analysis of mixed age cohorts or a lack of sensitivity of the markers.

Table 7.1. Details of Tasmanian *H. rubra* samples. Includes site name in alphabetic order, approximate latitude and longitude of site location (decimal degrees), date of collection, number of individuals collected, and site reference number for the map in Figure 7.1. All samples collected by TAFI research staff, except those marked by *, that were collected by commercial divers or the Australian Maritime College staff, see text for details.

Collection Site (alphabetic order)	Location		Date	No.	Map No.
Actaeon Island	43.53°S	147.00°E	28/3/00	91	18
Black Reef	43.54°S	146.97°E	29/3/00	93	19
Block 11*	43.10°S	145.65°E	9/11/99	100	23
Bluff Point	40.76°S	144.69°E	18/11/99	100	29
Cape Portland*	40.78°S	147.98°E	7/9/00	100	35
Cat Island	39.95°S	148.35°E	26/9/00	100	8
Church Rocks	41.04°S	144.63°E	5/4/00	100	27
Curio Bay	43.19°S	147.71°E	25/2/99	100	16
Georges III Reef	43.51°S	146.98°E	29/2/00	68	17
George Rocks	40.93°S	148.33°E	3/6/99	100	10
Granville Harbour*	41.81°S	145.04°E	18/8/00	60	25
High Rocky Point	42.77°S	145.39°E	4/2/00	100	24
Hunter Island	40.43°S	144.79°E	17/6/99	100	32
Lemon Bight (east) *(spatial 1)*	42.18°S	148.35°E	29/8/00	46	13
Lemon Bight (west) *(spatial 2)*	42.18°S	148.34°E	29/8/00	50	13
Lemon Rock *(spatial 3)*	42.18°S	148.35°E	29/8/00	49	13
Little Trefoil Island	40.65°S	144.70°E	17/11/99	100	31
Long Point - site 1 *(spatial 1)*	41.74°S	148.30°E	30/8/00	43	11
Long Point - site 2 *(spatial 2)*	41.74°S	148.30°E	30/8/00	50	11
Long Point Reef *(spatial 3)*	41.74°S	148.30°E	30/8/00	50	11
Louisa Bay	43.52°S	146.34°E	10/2/00	95	21
Louisa Bay *(temporal)*	43.52°S	146.34°E	31/1/01	93	21
Low Head*	41.06°S	146.79°E	6/9/00	96	36
Mount Cameron	40.86°S	144.70°E	20/5/99	100	28
Nuggets No. 2 *(spatial 1)*	42.12°S	148.35°E	29/8/00	50	12
Nuggets No. 4 -North face *(spatial 2)*	42.12°S	148.35°E	29/8/00	50	12
Nuggets No. 4 -The Gap *(spatial 3)*	42.12°S	148.35°E	29/8/00	50	12
One Tree Point	43.11°S	147.38°E	25/9/00	92	15
One Tree Point *(temporal)*	43.11°S	147.38°E	29/5/01	64	15
Passage Island	40.51°S	148.35°E	21/9/99	100	9
Sandblow Point	40.08°S	144.05°E	8/10/99	100	33
Smiths Gulch	41.31°S	144.74°E	19/5/99	100	26
Sterile Island	43.55°S	146.99°E	28/3/00	93	20
Suicide Bay	40.68°S	144.69°E	18/11/00	100	30
Trumpeter Corner	42.73°S	148.04°E	5/5/99	100	14
Waterwitch Reef	39.90°S	143.80°E	6/10/99	100	34
Whalers Point	43.30°S	145.92°E	2/5/2000	100	22
Total Tasmanian Samples				**3083**	

Table 7.2. Morphological details of Tasmanian *H. rubra* samples. Includes sex ratio, shell size (mm) range and mean. All samples collect by TAFI research staff, except those marked by *, that were collected by commercial divers or the Australian Maritime College staff, see text for details.

Collection Site (alphabetic order)	No.	Sex ratio (F/M)	Size range	Size mean
Actaeon Island	91	26/36	74-172	133.63
Black Reef	93	70/14	102-155	131.99
Block 11 Flat Island *	100	57/43	142-184	160.34
Bluff Point	100	44/50	101-154	125.95
Cape Portland*	100	46/51	75-144	112.58
Cat Island	100	37/36	37-151	116.64
Church Rocks	100	38/49	89-151	128.04
Curio Bay	100	34/54	95-158	127.97
Georges III Reef	68	34/32	129-164	147.16
Georges Rocks	100	33/39	72-149	118.77
Granville Harbour*	60	29/31	139-180	152.14
High Rocky Point	100	48/48	125-181	158.75
Hunter Island	100	43/50	60-145	112.36
Lemon Bight (east) *(fine scale 1)*	46	15/14	98-161	131.42
Lemon Bight (west) *(fine scale 2)*	50	22/24	106-155	130.42
Lemon Rock *(fine scale 3)*	49	23/22	110-166	134.82
Little Trefoil Island	100	49/50	109-164	132.11
Long Point - site 1 *(fine scale 1)*	43	17/14	95-153	132.02
Long Point - site 2 *(fine scale 2)*	50	12/22	98-150	125.48
Long Point Reef *(fine scale 3)*	50	19/18	103-166	134.52
Louisa Bay	95	33/57	99-175	149.68
Louisa Bay *(temporal) sample*	93	33/55	117-173	149.95
Low Head*	96	39/52	80-133	108.76
Mount Cameron	100	53/38	61-149	122.03
Nuggets No. 2 *(fine scale 1)*	50	26/16	105-167	139.14
Nuggets No. 4 -North face *(fine scale 2)*	50	20/10	97-154	128.02
Nuggets No. 4 -The Gap *(fine scale 3)*	50	17/31	100-157	125.40
One Tree Point	92	46/41	116-166	138.60
One Tree Point *(temporal sample)*	64	27/34	103-164	131.94
Passage Island	100	40/47	68-138	108.07
Sandblow Point	100	57/40	109-148	128.54
Smiths Gulch	100	44/53	104-156	134.94
Sterile Island	93	38/43	109-156	132.44
Suicide Bay	100	46/43	77-168	128.22
Trumpeter Corner	100	46/53	117-173	142.79
Waterwitch Reef	100	29/39	101-150	127.77
Whalers Point, Port Davey	100	50/36	87-185	144.45
Total Tasmanian Samples	**3083**			

Table 7.3. Details of mainland *H. rubra* samples. Includes site name in alphabetic order by State, approximate latitude and longitude of site location (decimal degrees), date of collection, number of individuals collected, and site reference number for the map in Figure 7.1. Samples from South Australia were provide by SARDI, Victoria by MAFRI and New South Wales by NSW Fisheries.

Collection Site (alphabetic order)	Location		Date	No.	Map No.
South Australia					
Port MacDonnell	38.05°S	140.70°E	2/10/01	60	6
West Bay	35.89°S	136.54°E	2/7/01	64	7
Victoria					
Cape Conran	37.82°S	148.73°E	3/12/99	93	4
Pearl Point	37.79°S	148.88°E	9/12/99	100	5
Point Cook	37.92°S	144.80°E	8/9/99	50	3
New South Wales					
Jervis Bay	35.07°S	150.74°E	17/4/01	100	2
Kiama	34.67°S	150.85°E	17/4/01	93	1
Total Mainland Samples				**560**	

Table 7.4. Morphological details of mainland *H. rubra* samples. Includes sex ratio, shell size (mm) range and mean. Samples from South Australia were provide by SARDI, Victoria by MAFRI and New South Wales by NSW Fisheries.

Collection Site (alphabetic order)	No.	Sex Ratio (F/M)	Size range	Size Mean
South Australia				
Port MacDonell	60	37/23	111-139	122.15
West Bay	64	24/28	90-174	131.16
Victoria				
Cape Conran	93	34/58	117-156	135.30
Pearl Point	100	40/60	120-161	135.31
Point Cook	50	20/9	93-123	105.40
New South Wales				
Jervis Bay	100	47/53	95-151	118.33
Kiama	93	42/51	72-129	107.41

Table 7.5. Mean general genetic statistics for eight microsatellite loci. n = mean number of individuals scored per sample, A = number of alleles observed, Total and Mean per sample, Ho = mean observed heterozygosity, He = mean Hardy-Weinberg Equilibrium expected heterozygosity per sample, D = Selander's D value, a measure of heterozygote equilibrium with a negative value indicating a heterozygote deficiency, Sign HW = number of samples with Ho significantly different to He. (Full details for each locus and each sample provided in Appendix 5)

	CmrHr 1.14	***CmrHr 1.24***	***CmrHr 2.14***	***CmrHr 2.30***	***RubCA1***	***CmrHr 1.25***	***CmrHr 2.26***	***CmrHr 2.9***
Samples	44	44	43	43	44	38	39	38
n	71.0	76.0	67.1	72.6	75.6	56.6	65.5	74.3
Total A	24	14	12	51	48	44	20	52
Mean A	6.7	5.2	7.7	33.3	29.1	23.2	11.5	30.9
Ho	0.276	0.267	0.643	0.791	0.770	0.360	0.573	0.622
He	0.340	0.269	0.692	0.954	0.918	0.906	0.851	0.917
D	-0.177	-0.008	-0.070	-0.171	-0.158	-0.603	-0.318	-0.319
Sign. HW	7	0	2	27	14	38	37	38

Table 7.6. Mean general genetic statistics for each collection site. n = mean number of individuals scored per locus, Alleles = mean number of alleles observed per locus, Ho = mean observed heterozygosity, He = mean Hardy-Weinberg Equilibrium expected heterozygosity, Sign HW = number of sites with Ho significantly different to He. (Full details for each locus and each collection site provided in Appendix 5)

	Loci	n	Allele	Ho	He	Sign HW
Kiama	8	86.6	20.6	0.5415	0.7349	3
Jervis Bay	8	93.0	19.8	0.5036	0.7163	5
Pearl Point	8	82.0	20.0	0.5163	0.7115	5
Cape Conran	8	66.1	19.4	0.5041	0.7208	5
Point Cook	6	29.3	10.2	0.4701	0.6022	2
Port Macdonell	8	47.3	15.1	0.4821	0.7229	4
West Bay	8	45.9	13.0	0.4346	0.6550	4
Cat Island	5	69.8	16.8	0.5378	0.6440	1
Passage Island	8	73.0	17.1	0.4515	0.7338	6
Georges Rock	8	96.9	21.5	0.5526	0.7467	4
Long Point Reef	8	40.0	16.5	0.5278	0.7096	3
Long Point Site 2	8	45.1	17.4	0.5820	0.7274	3
Long Point Site 1	8	31.0	15.9	0.5030	0.7601	4
Nuggets 4 gap	5	43.6	14.8	0.5007	0.6441	3
Nuggets 2	5	14.6	9.0	0.6379	0.6232	0
Nuggets 4 North Face	5	44.0	14.4	0.4546	0.6425	3
Lemon Rock	8	40.5	16.3	0.6472	0.7458	2
Lemon Bight East	8	33.0	15.6	0.5398	0.7168	4
Lemon Bight West	8	47.1	18.4	0.5736	0.7290	3
Trumpeter Corner	8	97.4	21.1	0.5882	0.7543	4
One Tree Point	8	83.6	19.8	0.5562	0.7187	4
One Tree Point Temporal	8	48.4	16.4	0.5884	0.7168	2
Curio Bay	7	83.7	17.7	0.5341	0.7093	3
Georges III Reef	8	59.0	17.5	0.5429	0.7382	4
Louisa Bay	7	89.1	22.0	0.5439	0.7401	4
Louisa Bay Temporal	8	84.5	19.3	0.5815	0.7209	3
Acteon Island	8	82.5	20.4	0.5023	0.7578	6
Black Reef	8	86.4	18.3	0.5616	0.7210	5
Sterile Island	8	81.5	19.3	0.5689	0.7426	4
Whalers Point	8	89.8	21.6	0.5692	0.7284	4
Block 11	8	68.1	17.3	0.5068	0.7410	5
High Rocky Point	8	85.4	20.6	0.5118	0.7434	5
Granville Harbour	8	52.0	17.1	0.5873	0.7277	3
Hunter Island	8	96.8	21.6	0.5606	0.7266	4
Smiths Gulch	8	96.5	20.3	0.5807	0.7536	3
Mount Cameron	8	97.1	20.6	0.5699	0.7410	4
Little Trefoil Island	8	83.0	21.0	0.4924	0.7342	5
Bluff Point	5	95.4	16.4	0.6189	0.6460	1
Suicide Bay	8	87.6	21.0	0.5048	0.7527	5
Church Rocks	8	92.4	20.9	0.6106	0.7536	4
Sandblow Point	8	68.6	18.4	0.5598	0.7299	4
Waterwitch reef	8	63.1	17.1	0.5415	0.7432	4
Cape Portland	8	63.9	17.8	0.4864	0.7367	4
Low Head	8	80.6	17.5	0.4478	0.7223	4

Table 7.7. Temporal genetic variation analysis. AMOVA and exact test results for analysis of temporally separated samples from Louisa Bay and One Tree Point. F-statistic values presented from distance method of pairwise differences for all loci and individual loci. Five loci analysis is for *CmrHr1.14*, *CmrHr1.24*, *CmrHr2.14*, *CmrHr2.30* and *RubCA1*. Probability value of differentiation presented for exact tests. Values significant with Bonferroni correction are in bold. Data for *CmrHr2.14* in Louisa Bay is missing due to poor amplification in some samples.

	Louisa Bay			**One Tree Point**		
	F_{ST}	**P**	**Exact test P**	**F_{ST}**	**P**	**Exact test P**
Across all loci	-0.0830	1.000	**<0.001**	-0.0785	1.000	**<0.001**
Across 5 loci	-0.1372	1.000	<0.001	-0.0185	1.000	0.842
CmrHr1.14	0.0024	0.228	0.053	-0.0033	0.588	0.294
CmrHr1.24	0.0170	0.022	**0.004**	0.0002	0.289	0.471
CmrHr2.14				-0.0050	0.867	0.517
CmrHr2.30	0.0084	**<0.001**	**<0.001**	-0.0033	0.987	0.969
RubCA1	0.0043	0.054	**<0.001**	-0.0018	0.709	0.824
CmrHr1.25	0.0074	0.147	**0.002**	0.0274	0.018	**<0.001**
CmrHr2.26	0.0012	0.472	0.069	0.0155	0.165	0.012
CmrHr2.9	0.0010	0.620	0.038	0.0081	0.079	0.092

Table 7.8. Spatial genetic variation analysis. AMOVA results for analysis of spatially separated samples from Long Point, Nuggets and Lemon Bight. F-statistic values presented from distance method of pairwise differences for all loci and individual loci. Five loci analysis is for *CmrHr1.14, CmrHr1.24, CmrHr2.14, CmrHr2.30* and *RubCA1*. Values significant with Bonferroni correction are in bold. Nuggets samples were not examined for three loci.

	Long Point		Nuggets		Lemon Bight	
	F_{ST}	P	F_{ST}	P	F_{ST}	P
Across 8 loci	-0.0301	1.000			-0.0606	1.000
Across 5 loci	-0.0575	1.000	0.0012	0.590	-0.0360	1.000
CmrHr1.14	0.0094	0.205	0.0154	0.236	-0.0009	0.522
CmrHr1.24	0.0074	0.159	-0.0016	0.488	0.0251	0.020
CmrHr2.14	-0.0104	0.632	-0.0126	0.996	-0.0041	0.653
CmrHr2.30	0.0013	0.349	0.0185	**0.002**	-0.0013	0.766
RubCA1	0.0043	0.246	-0.0005	0.812	-0.0035	0.939
CmrHr1.25	0.0029	0.716			0.0037	0.769
CmrHr2.26	0.0047	0.368			0.0119	0.250
CmrHr2.9	0.0027	0.715			0.0015	0.600

Table 7.9. Spatial genetic variation analysis. Genic differentiation pairwise exact tests results (probability values) for analysis of spatially separated samples from Long Point, Nuggets and Lemon Bight. Values significant with Bonferroni correction are in bold. Nuggets samples were not examined for three loci. Loci 1 to 8 are *CmrHr1.14, CmrHr1.24, CmrHr2.14, CmrHr2.30, RubCA1, CmrHr1.25, CmrHr2.26* and *CmrHr2.9***.** Samples abbreviations Rf = Reef, 4G = #4 Gap, 4N = #4 North face, Rk = Rock, E = East and W = West.

	Across		Locus							
	8 loci	**5 loci**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Long Point										
Rf/I	0.017	0.072	0.152	0.007	0.961	0.701	0.244	0.106	0.208	0.070
Rf/II	0.063	0.126	0.291	0.047	0.816	0.006	0.685	0.154	0.503	0.076
I/II	**0.015**	0.316	0.190	0.849	0.569	0.208	0.151	0.013	0.035	0.172
Nuggets										
4G/2		0.407	0.018	0.614	0.860	0.904	0.574	-	-	-
4G/4N		**<0.001**	**0.001**	0.471	0.968	**<0.001**	0.270	-	-	-
2/4N		0.018	0.286	0.409	0.862	**<0.001**	0.491	-	-	-
Lemon Bight										
Rk/E	0.024	0.076	0.137	0.024	0.482	0.458	0.323	0.137	0.027	0.580
Rk/W	0.073	0.594	0.846	0.112	0.530	0.430	0.651	0.007	0.166	0.250
E/W	0.132	0.634	0.173	0.799	0.299	0.456	0.962	0.013	0.505	0.1140

Table 7.10. Overall genetic variation analysis. Results from analyses of all samples. F_{ST} values presented from distance method of pairwise differences using the Arlequin program and F-statistics from Genepop correlation using allele frequencies. (F_{IS} – measure of non-random mating or reduction in heterozygosity, F_{ST} – measure of population subdivision, F_{IT} – measure of inbreeding). Five loci analysis is for *CmrHr1.14, CmrHr1.24, CmrHr2.14, CmrHr2.30* and *RubCA1*. Genepop does not provide multi-locus estimates, only average over multiple loci.

	Samples	Arlequin		Genepop		
		F_{ST}	**P**	**F_{IS}**	**F_{ST}**	**F_{IT}**
Eight loci	44	-0.0755	1.000			
Five loci	44	-0.0249	1.000			
CmrHr1.14	44	0.0064	**<0.001**	0.1592	0.0053	0.1637
CmrHr1.24	44	0.0081	**<0.001**	0.0046	0.0081	0.0126
CmrHr2.14	43	0.0070	**<0.001**	0.0762	0.0064	0.0821
CmrHr2.30	43	0.0041	**<0.001**	0.1715	0.0029	0.1739
RubCA1	44	0.0106	**<0.001**	0.1597	0.0095	0.1677
CmrHr1.25	38	0.0099	**<0.001**	0.5898	0.0046	0.5917
CmrHr2.26	39	0.0094	**<0.001**	0.3189	0.0069	0.3236
CmrHr2.9	38	0.0129	**<0.001**	0.3293	0.0107	0.3365

Table 7.11. Spatial genetic variation analysis. AMOVA results for analysis of all 37 Tasmanian samples, all 7 Mainland samples and comparison of the two groups. F-statistic values presented from distance method of pairwise differences for all loci and individual loci. F_{CT} represents amount of variation between the two groups. Five loci analysis is for *CmrHr1.14, CmrHr1.24, CmrHr2.14, CmrHr2.30* and *RubCA1*. Values significant with Bonferroni correction are in bold.

	Tasmanian		Mainland		Tasmania vs mainland	
	F_{ST}	**P**	**F_{ST}**	**P**	**F_{CT}**	**P**
Across 8 loci	-0.0832	1.000	-0.0322	1.000		
Across 5 loci	-0.0296	1.000	0.0081	**<0.001**	-0.0021	0.825
CmrHr1.14	0.0047	**0.002**	0.0127	0.013	0.0037	**<0.001**
CmrHr1.24	0.0061	**<0.001**	0.0101	**0.002**	0.0062	**<0.001**
CmrHr2.14	0.0031	0.021	0.0293	**<0.001**	-0.0007	0.736
CmrHr2.30	0.0037	**<0.001**	0.0036	0.020	0.0013	0.009
RubCA1	0.0091	**<0.001**	0.0140	**<0.001**	0.0031	**<0.001**
CmrHr1.25	0.0076	0.011	0.0130	0.021	0.0070	**<0.001**
CmrHr2.26	0.0087	**<0.001**	0.0154	**0.002**	-0.0004	0.542
CmrHr2.9	0.0050	**<0.001**	0.0600	**<0.001**	-0.0011	0.302

Table 7.12. Pairwise sample differentiation. For each sample the number of significant pairwise exact tests with other samples across eight and five loci. Presented are the numbers significant at the 0.05 level and then remaining significant after Bonferroni correction for the total number of pairwise tests (946). Instances of >30 (of 43) significant cases after Bonferroni correction shown in bold. The five loci analysis is for *CmrHr1.14, CmrHr1.24, CmrHr2.14, CmrHr2.30* and *RubCA1*. * = not all loci analysed

Sample	**Across 8 loci**		**Across 5 loci**	
	0.05	**Bonf.**	**0.05**	**Bonf.**
Kiama	40	29	30	9
Jervis Bay	41	**36**	41	17
Pearl Point	40	26	33	9
Cape Conran	42	**32**	39	15
Point Cook*	41	26	41	20
Port Macdonell	40	20	30	8
West Bay	43	**43**	43	**43**
Cat Island*	24	7	24	5
Passage Island	42	30	39	26
Georges Rock	39	24	26	14
Long Point Reef	30	10	26	7
Long Point Site 2	34	18	12	4
Long Point Site 1	38	9	27	3
Nuggets 4 gap*	24	11	24	7
Nuggets 2*	6	3	7	2
Nuggets 4 North Face*	42	**32**	42	26
Lemon Rock	31	9	15	5
Lemon Bight East	37	11	35	5
Lemon Bight West	36	30	7	4
Trumpeter Corner	38	23	23	7
One Tree Point	40	**31**	22	7
One Tree Point Temporal	34	18	16	7
Curio Bay*	39	17	26	8
Georges III Reef	32	12	24	4
Louisa Bay*	41	29	37	17
Louisa Bay Temporal	41	26	33	16
Acteon Island	38	28	33	18
Black Reef	43	**33**	42	18
Sterile Island	35	14	21	8
Whalers Point	35	20	28	12
Block 11	38	17	31	10
High Rocky Point	41	**41**	41	**41**
Granville Harbour	38	17	19	5
Hunter Island	39	25	26	8
Smiths Gulch	34	20	19	8
Mount Cameron	35	23	19	7
Little Trefoil Island	41	28	37	11
Bluff Point*	27	15	27	12
Suicide Bay	39	27	26	8
Church Rocks	37	25	30	14
Sandblow Point	42	**37**	40	20
Waterwitch reef	42	**36**	36	7
Cape Portland	43	**43**	43	**36**
Low Head	42	**41**	42	**40**
Total No. pairwise cases	812	526	641	289

Table 7.13a. Pairwise sample differentiation. For each sample the number of pairwise exact tests significant at 0.05 and after Bonferroni correction (*Bnf*) for total number of tests per locus. Instances of >35 case (of 43) significant (0.05) and >30 with Bonferroni correction are shown in bold. Loci *RubCA1, CmrHr1.25, CmrHr2.26* and *CmrHr2.9* are presented in Table 7.13b. * = not all loci analysed, - = not analysed

	Locus							
	CmrHr1.14		***CmrHr1.24***		***CmrHr2.14***		***CmrHr2.30***	
Sample	**0.05**	***Bnf***	**0.05**	***Bnf***	**0.05**	***Bnf***	**0.05**	***Bnf***
Kiama	12	*2*	2	*0*	5	*1*	24	*5*
Jervis Bay	22	*2*	15	*0*	9	*4*	30	*6*
Pearl Point	17	*2*	10	*0*	5	*1*	17	*3*
Cape Conran	5	*0*	22	*0*	11	*3*	12	*2*
Point Cook*	7	*0*	10	*0*	1	*1*	14	*2*
Port Macdonell	9	*2*	21	*0*	7	*1*	6	*2*
West Bay	**40**	26	30	*3*	**42**	***31***	34	*6*
Cat Island*	19	*1*	5	*0*	9	*1*	15	*2*
Passage Island	14	*0*	29	*0*	22	*3*	15	*2*
Georges Rock	10	*1*	8	*0*	8	*4*	22	*5*
Long Point Reef	5	*0*	18	*2*	1	*0*	18	*2*
Long Point Site 2	2	*0*	3	*0*	1	*0*	9	*2*
Long Point Site 1	5	*0*	16	*1*	1	*0*	9	*2*
Nuggets 4 gap*	22	*3*	2	*0*	1	*1*	9	*1*
Nuggets 2*	5	*0*	0	*0*	1	*0*	7	*2*
Nuggets 4 *North Face	29	*0*	6	*0*	2	*0*	**41**	28
Lemon Rock	11	*2*	12	*0*	14	*0*	8	*1*
Lemon Bight East	21	*0*	10	*0*	3	*0*	15	*2*
Lemon Bight West	11	*2*	2	*0*	1	*1*	6	*1*
Trumpeter Corner	16	*2*	9	*0*	5	*1*	11	*2*
One Tree Point	17	*2*	6	*0*	8	*2*	15	*3*
One Tree Point Temporal	5	*2*	8	*0*	11	*2*	5	*2*
Curio Bay*	4	*2*	17	*0*	13	*2*	-	-
Georges III Reef	11	*2*	4	*0*	5	*1*	13	*2*
Louisa Bay*	13	*2*	16	*0*	-	-	22	*6*
Louisa Bay Temporal	8	*2*	13	*0*	4	*1*	20	*4*
Acteon Island	26	*2*	10	*0*	6	*2*	10	*3*
Black Reef	7	*0*	8	*0*	13	*4*	**37**	*10*
Sterile Island	8	*2*	4	*0*	6	*1*	26	*5*
Whalers Point	9	*1*	15	*0*	11	*0*	14	*3*
Block 11	5	*2*	13	*0*	4	*1*	6	*3*
High Rocky Point	16	*3*	13	*0*	2	*1*	**41**	***41***
Granville Harbour	6	*1*	6	*0*	3	*1*	9	*1*
Hunter Island	26	*4*	4	*0*	6	*2*	8	*2*
Smiths Gulch	11	*2*	13	*0*	3	*1*	10	*3*
Mount Cameron	9	*2*	8	*0*	3	*1*	9	*2*
Little Trefoil Island	13	*2*	15	*0*	4	*1*	11	*2*
Bluff Point*	14	*2*	15	*1*	4	*1*	5	*2*
Suicide Bay	8	*1*	4	*0*	9	*2*	11	*3*
Church Rocks	17	*2*	15	*0*	5	*1*	14	*5*
Sandblow Point	16	*1*	12	*0*	6	*1*	**39**	*15*
Waterwitch reef	21	*0*	3	*0*	14	*1*	16	*1*
Cape Portland	23	*0*	23	*0*	28	*4*	**38**	*11*
Low Head	**39**	28	23	*1*	29	*10*	9	*1*
No. pairwise tests for locus	946		946		903		903	
No. cases	307	*56*	249	*4*	173	*48*	355	*104*

Table 7.13b. Pairwise sample differentiation. For each sample the number of pairwise exact tests significant at 0.05 and after Bonferroni correction (*Bnf*) for total number of tests per locus. Instances of >35 case (of 43) significant (0.05) and >30 with Bonferroni correction are shown in bold. Loci *CmrHr1.14, CmrHr1.24, CmrHr2.14, CmrHr2.30* presented in Table 7.13a. * = not all loci analysed, - = not analysed

	Locus							
	RubCA1		***CmrHr1.25***		***CmrHr2.26***		***CmrHr2.9***	
Sample	**0.05**	***Bnf***	**0.05**	***Bnf***	**0.05**	***Bnf***	**0.05**	***Bnf***
Kiama	30	*5*	33	*20*	19	*3*	12	*3*
Jervis Bay	22	*6*	31	*17*	20	*1*	28	*10*
Pearl Point	23	*6*	33	*10*	13	*2*	26	*6*
Cape Conran	**36**	*10*	31	*14*	30	*2*	17	*4*
Point Cook*	**40**	*19*	-	-	15	*0*	-	-
Port Macdonell	31	*3*	25	*2*	26	*1*	14	*1*
West Bay	**43**	***42***	36	*34*	27	*3*	**37**	***37***
Cat Island*	6	*3*	-	-	-	-	-	-
Passage Island	34	*10*	9	*1*	22	*4*	25	*5*
Georges Rock	22	*12*	26	*9*	18	*0*	15	*4*
Long Point Reef	14	*3*	11	*3*	7	*0*	5	*2*
Long Point Site 2	17	*4*	31	*13*	10	*0*	12	*1*
Long Point Site 1	23	*2*	15	*2*	18	*4*	10	*2*
Nuggets 4 gap*	20	*4*	-	-	-	-	-	-
Nuggets 2*	3	*1*	-	-	-	-	-	-
Nuggets 4 *North Face	20	*3*	-	-	-	-	-	-
Lemon Rock	7	*3*	23	*3*	20	*0*	7	*1*
Lemon Bight East	17	*3*	6	*1*	7	*0*	10	*3*
Lemon Bight West	9	*2*	**37**	30	9	*1*	15	*1*
Trumpeter Corner	19	*5*	30	*15*	20	*0*	15	*2*
One Tree Point	14	*3*	34	*23*	25	*2*	11	*2*
One Tree Point Temporal	16	*4*	25	*8*	25	*1*	14	*3*
Curio Bay*	22	*6*	25	*7*	10	*0*	25	*5*
Georges III Reef	14	*2*	23	*3*	19	*0*	6	*1*
Louisa Bay*	34	*7*	29	*11*	17	*0*	16	*1*
Louisa Bay Temporal	29	*13*	25	*9*	14	*1*	20	*2*
Acteon Island	32	*12*	22	*7*	15	*1*	10	*4*
Black Reef	**37**	*15*	24	*11*	28	*5*	16	*3*
Sterile Island	15	*4*	23	*5*	22	*4*	13	*3*
Whalers Point	23	*10*	14	*4*	16	*0*	15	*4*
Block 11	32	*14*	4	*2*	19	*2*	9	*2*
High Rocky Point	11	*4*	20	*7*	18	0	13	*3*
Granville Harbour	19	*3*	30	*9*	24	*4*	14	*3*
Hunter Island	19	*8*	31	*14*	20	*0*	11	*2*
Smiths Gulch	15	*3*	32	*9*	25	*0*	10	*3*
Mount Cameron	20	*8*	34	*19*	18	*2*	9	*3*
Little Trefoil Island	**36**	*11*	28	*5*	14	*3*	25	*4*
Bluff Point*	26	*11*	-	-	-	-	-	-
Suicide Bay	26	*12*	26	*10*	22	*3*	29	*8*
Church Rocks	23	*12*	25	*11*	30	*6*	13	*4*
Sandblow Point	14	*2*	**37**	*16*	35	*9*	6	*1*
Waterwitch reef	33	*3*	33	*10*	30	*7*	35	22
Cape Portland	**41**	27	29	*11*	31	*13*	34	21
Low Head	**43**	***40***	**36**	*25*	32	*16*	32	14
No. pairwise tests for locus	946		703		741		703	
No. cases	515	*190*	493	*205*	395	*508*	317	*100*

Table 7.14 Examples of genetic variation among abalone populations. Average number of individuals sampled at each location is presented as mean N. F_{ST} is the degree of population differentiation and *P* is the probability of significant departure from panmixia, as reported in each study. NS = not significant. np = not provided

Marker/Author	Species	Loci	Locations	mean N	F_{ST}	*P*
Allozymes						
Brown 1991	*H. rubra*	12	17	90.3	0.022	np
Brown & Murray 1992	*H. laevigata*	13	8	72.4	0.014	np
Burton & Tegner 2000	*H. rufescens*	4	3	45.0	0.012	NS
Hamm & Burton 2000	*H. cracherodii*	3	7	61.0	0.039	< 0.001
Hancock 2000	*H. roei*	8	10	62.4	0.009	< 0.001
Zúñiga *et al.* 2000	*H. fulgens*	7	5	20.4	0.036	NS
Microsatellites						
Huang *et al.* 2000	*H. rubra*	3	10	10.0	0.067	< 0.001
Conod et al 2002	*H. rubra*	5	5	84.3	0.0034	0.004
Elliott *et al.* (this study)	*H. rubra*	8	36	70.1	-0.075	1.000
Elliott *et al.* (this study)	*H. rubra*	Single	32 to 36	56.6 to 76.0	0.013 to 0.004	< 0.001
Minisatellites						
Huang *et al.* 2000	*H. rubra*	84	10	10.0	0.074	< 0.001
Mitochondrial DNA						
Conod et al 2002	*H. rubra*		5	37.8	0.054	0.001
RAPDs						
Huang *et al.* 2000	*H. rubra*	2	10	10.0	0.001	NS

Figure 7.1. Map of southeastern Australia. Approximate locations of collection sites for blacklip abalone. Numbers refer to site names in Tables 7.1 and 7.3. (SA – South Australia, Vic – Victoria, NSW – New South Wales, Tas – Tasmania)

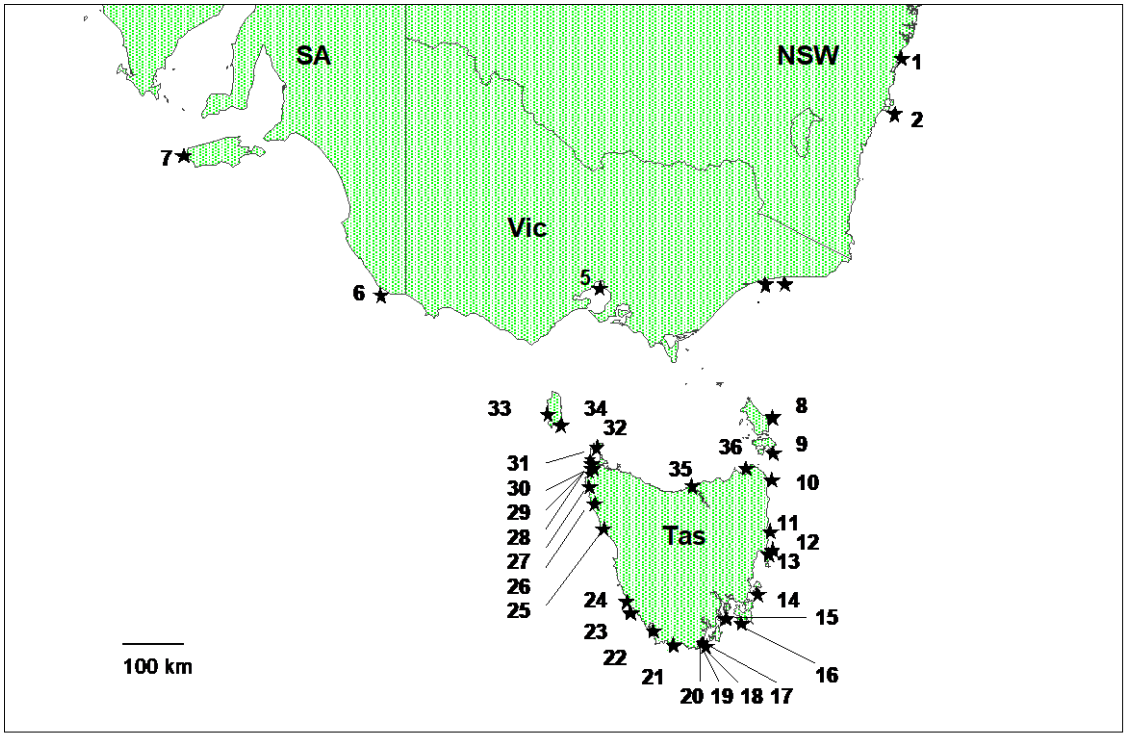


Figure 7.2. Microsatellite allele frequency histograms for total number of *H.rubra* individuals scored at *CmrHr2.14* and *CmrHr1.24*. Total and by collection site allele frequencies are presented in Appendix 4.

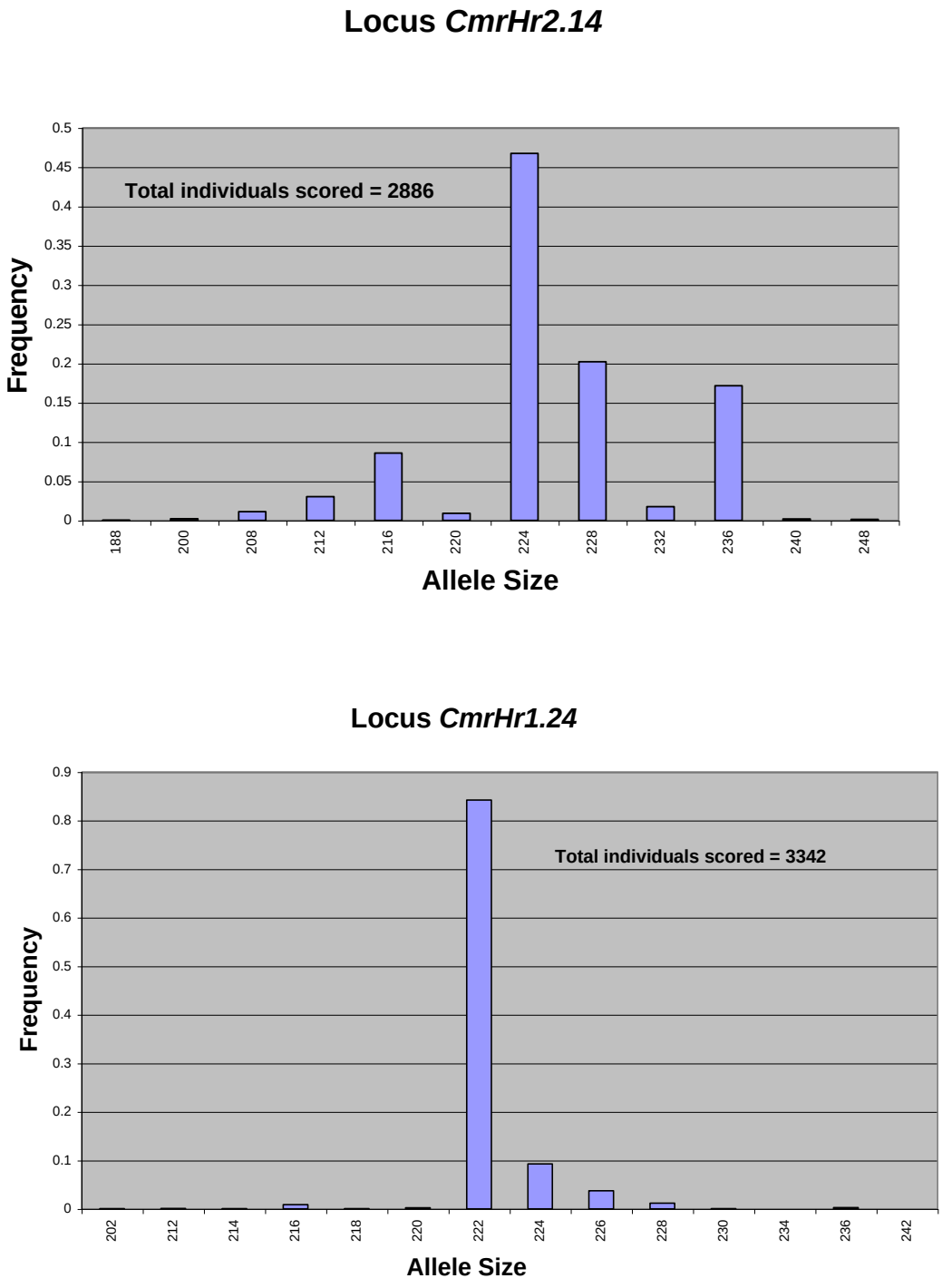


Figure 7.3. Microsatellite allele frequency histograms for total number of *H.rubra* individuals scored at *CmrHr2.26* and *CmrHr1.14*. Total and by collection site allele frequencies are presented in Appendix 4.

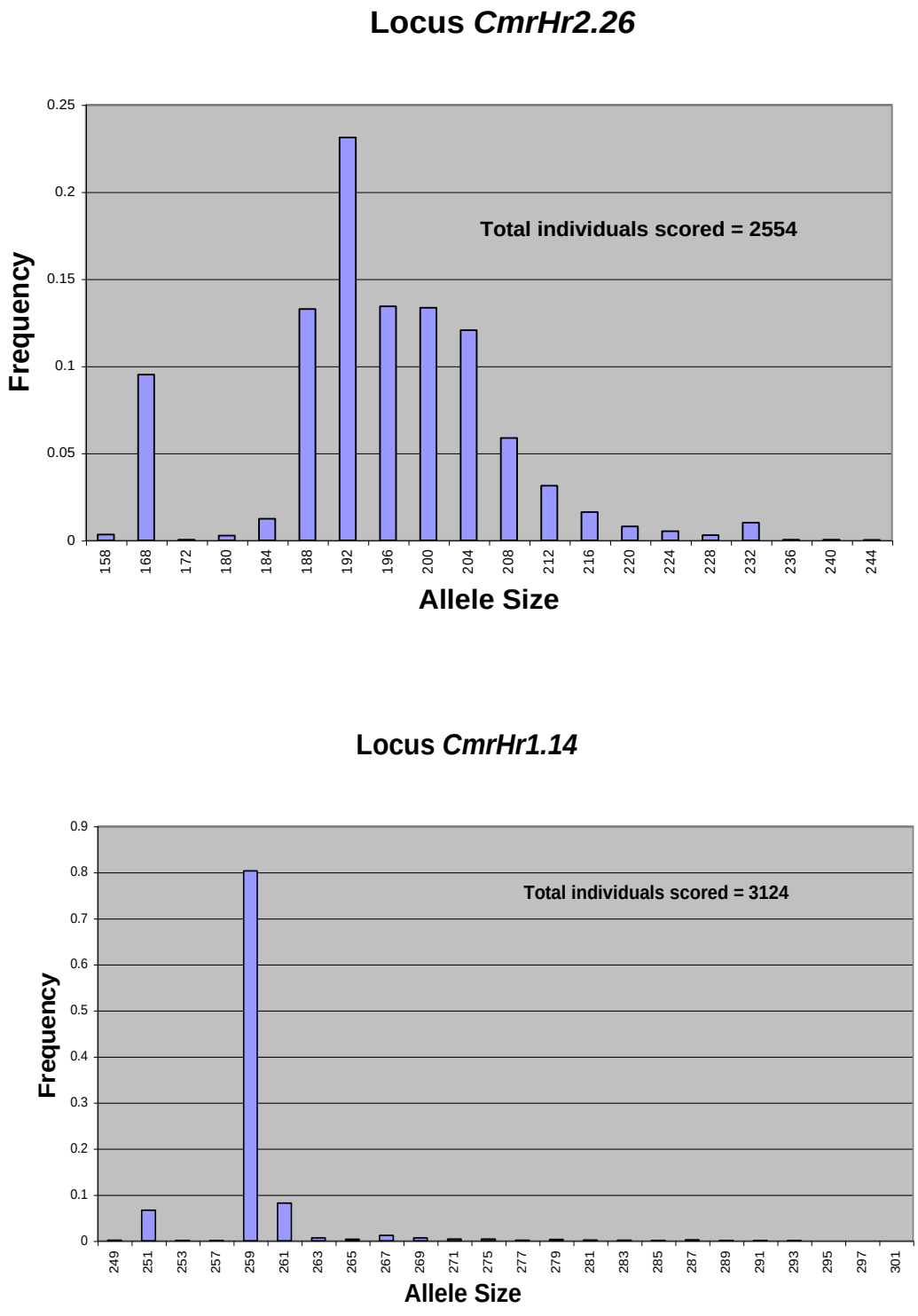
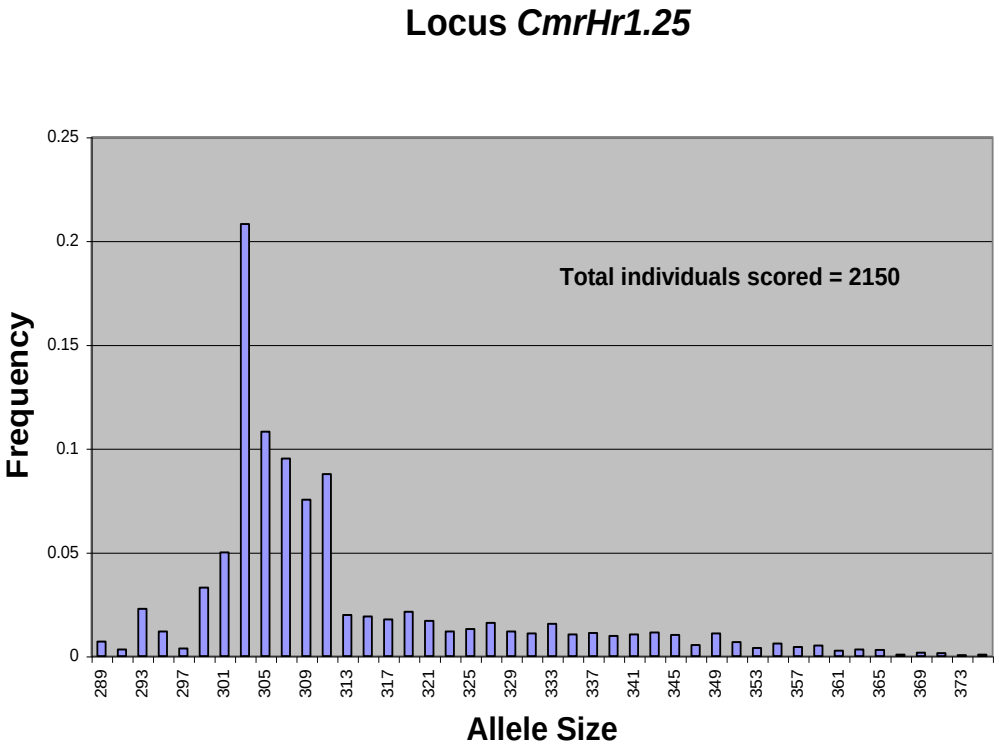


Figure 7.4. Microsatellite allele frequency histograms for total number of *H.rubra* individuals scored at *CmrHr1.25* and *RubCA1*. Total and by collection site allele frequencies are presented in Appendix 4.



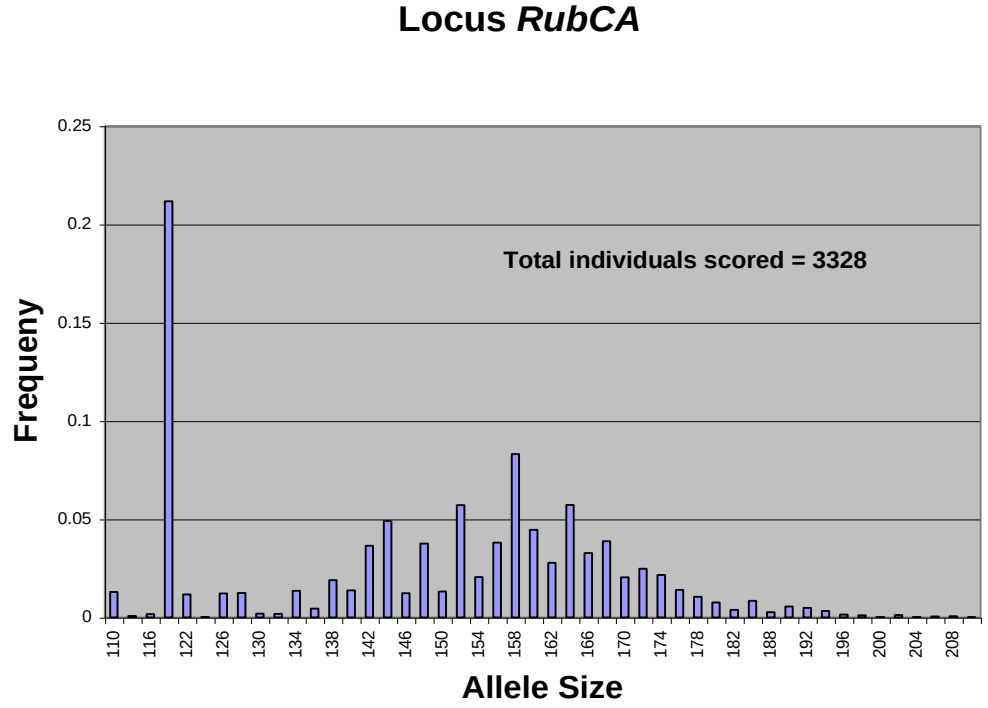
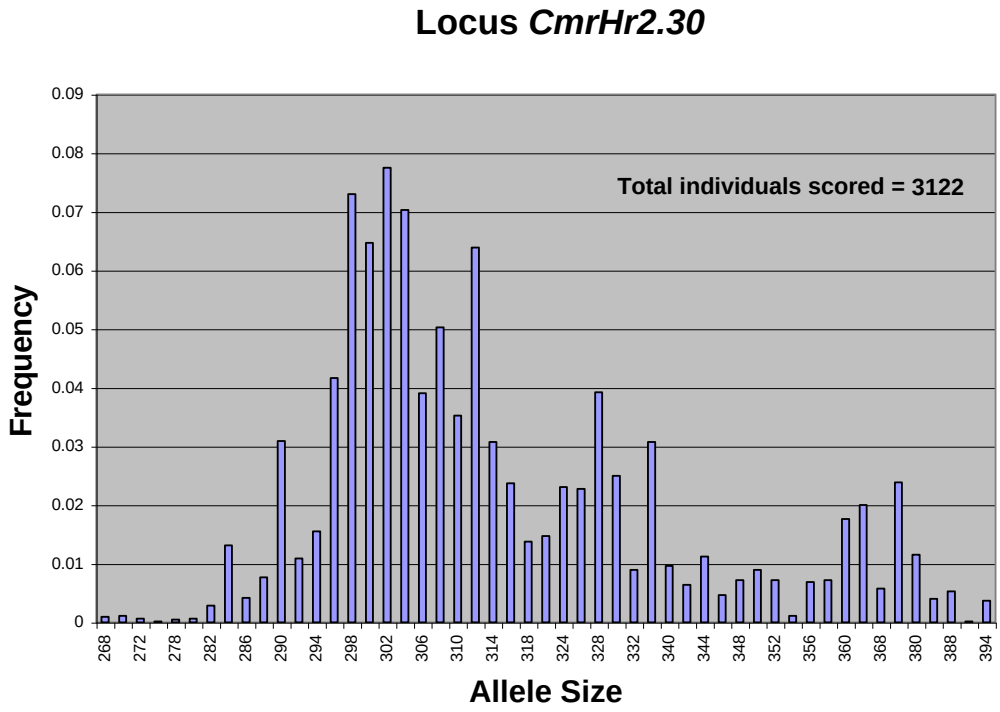
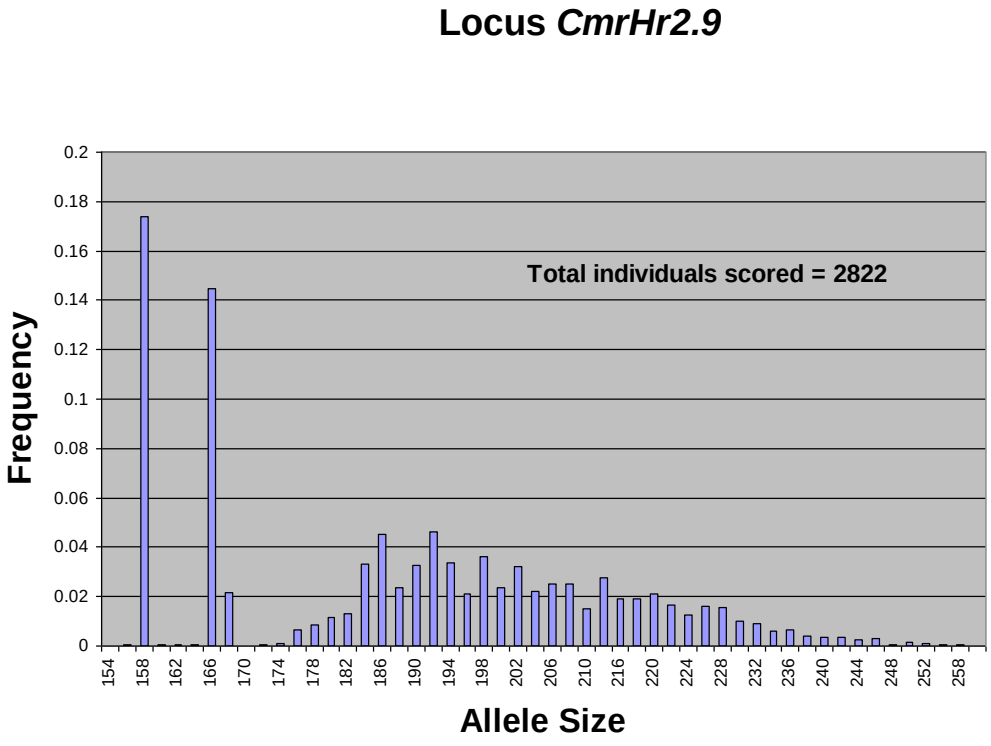


Figure 7.5. Microsatellite allele frequency histograms for total number of *H.rubra* individuals scored at *CmrHr2.30* and *CmrHr2.9*. Total and by collection site allele frequencies are presented in Appendix 4.





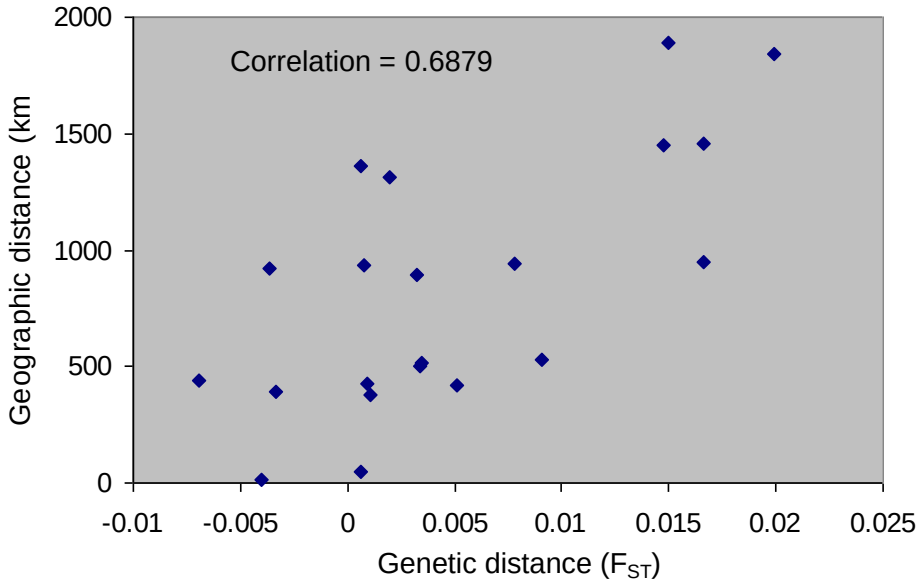


Figure 7.6 Geographic and genetic distance correlation. Plot of approximate geographic distance (km) between pairs of mainland samples and genetic distance represented by F_{ST} value across five microsatellite loci.

8. *HALIOTIS RUBRA* MICROSATELLITES IN OTHER SPECIES.

The research below and in Section 9 was undertaken towards achieving Objective 3.

The evaluation of *Haliotis rubra* microsatellites in a larger number of species than reported here has been peer reviewed and accepted for publication in the international Journal of Shellfish Research. The complete reference is Evans B, Conod, N and Elliott N. G. (2001). Evaluation of microsatellite primer conservation in abalone. *J. Shellfish Res.* 20, 1065-1070. This section is reproduced with permission of the Journal editor and reports on the research on Australian species that was supported by this project.

8.1 Introduction

Molecular genetic markers are widely used in many seafood industries for both wild and aquaculture needs; for example, in salmonids (O'Reilly *et al.* 1998) and oysters (McGoldrick *et al*. 2000). They can be used for applications as diverse as tracking the biological history of populations (Chambers and MacAvoy 2000), or as specific as determining the parentage of individuals in culture (O'Reilly *et al.* 1998). Microsatellite DNA is one such marker that has been applied in other genera (Dallimer 1999; Wu *et al.* 1999; Nesje *et al.* 2000) but has only recently become popular in abalone research. Microsatellite markers consist of a nucleotide sequence of between 2 and 6 base pairs repeated in series at a set point on a chromosome (locus). The number of times that sequence is repeated at a single locus varies within (heterozygous individuals) and between individuals (intra-specific variation), and where that same locus is conserved across species, the number of repeats may vary widely (inter-specific variation) (Wright and Bentzen 1994).

Microsatellite markers have been developed from partial genomic libraries of five *Haliotis* species: *H. asinina* (Selvamani *et al.* 2000); *H. discus discus* (Sekino *et al.* 1999); *H. kamtschatkana* (Miller *et al.* 2000); *H. rubra* (Huang and Hanna 1998; Evans *et al.* 2000); and *H. rufescens* Swainson 1822 (Kirby *et al.* 1998). The development of microsatellite DNA markers, as described in each of these papers, is a time consuming and expensive process (Wright and Bentzen 1994). For these reasons, the efficacy of markers between species within the same genus or family have been examined in both plant and animal groups with variable results (Rosetto *et al.* 2000; Huang and Hanna 1998).

Huang and Hanna (1998) considered the cross-species amplification of their three *H. rubra* microsatellite loci in species from USA (2 species), South Africa (2 species), South Korea (6 species) and Australia (5 species). Of the 10 species tested from outside of Australia, only two of the South Korean species produced any amplification product. Within Australian species the markers were more conserved, with at least two of the three loci producing an amplification product in all Australian species tested, except for *Haliotis laevigata*, the greenlip abalone, which failed to amplify a product at any of the three loci. As *H. rubra* and *H. laevigata* are known to produce hybrids in the wild (Brown 1995), this latter result is unexpected.

In this Section we describe the cross-species amplification in four temperate Australian abalone species species (*H. conicopora, H. laevigata, H. roei* and *H. scalaris*) of 21

microsatellite loci (22 primer pairs) developed for use in the blacklip abalone, *Haliotis rubra* (Evans 2002).

To bridge the gap between simply identifying the presence of a locus (or DNA sequence) in a related species, and the use of that marker for further research, we undertook a pilot population structure study on *H. laevigata*, which is presented in Section 9.

8.2 Materials and methods

8.2.1 Samples and DNA extraction.

Tissue samples were obtained for each of *H. conicopora, H. laevigata, H. roei* and *H. scalaris* from a minimum of two sites (Table 8.1). DNA was extracted using either a modified CTAB (hexadecyltrimethylammonium bromide) method (Grewe *et al*. 1993) or a QUIamp DNA Mini Kit (Quiagen) according to the protocol supplied. DNA was stored at -20°C.

8.2.2 Microsatellite amplification.

Cross-species amplification was examined for 22 microsatellite primer pairs developed for *Haliotis rubra* (Table 8.2, Evans *et al* 2000; Evans *et al* 2001). Their potential amplification in the four temperate Australian species was tested under standard PCR conditions. All loci were tested on a minimum of 10 individuals per species.

PCR reactions were performed in a volume of 25 μL consisting of 67 mM TrisHCl, pH 8.8; 16.6 mM $(NH_4)_2SO_4$; 0.45% Triton X-100; 0.2 mg.mL^{-1} gelatin; 2.5 mM $MgCl_2$; 10 pmoles of each primer; 200 μM dNTPs; 0.5 U *Taq* F1 polymerase (Fisher Biotech); and ~ 20 ng genomic DNA template. Amplification was in a Perkin Elmer 9600 thermocycler with one cycle of 94˚C for 1 min, 50˚C for 15 s and 72˚C for 1 min, followed by 30 cycles of 94˚C for 15 s, 50˚C for 15 s and 72˚C for 1 min. These cycles were followed by a final extension step of 72˚C for 10 min. Amplification products were separated on 2% TBE agarose gels, and visualised under UV illumination after Ethidium Bromide staining.

Amplification was scored as present when a band of between 75 and 450 bp was detected. Any amplification products above this size range, although possibly containing the microsatellite repeat unit, can not be reliably scored on the ABI377 using the size standards commonly available. It is possible to score larger alleles with different systems, but such large products increase the possibility of mutation in the regions flanking the microsatellite rather than within the repeat unit. In such cases it would be better to design new primers closer to the repeat unit. Amplification products less than 75 base pairs in size are difficult to score reliably due to their proximity to such PCR artefacts as primer-dimer, and unincorporated dyes.

8.2.3 *H. laevigata* optimisation.

All loci that produced an amplification product in this species under standard amplification conditions were subjected to further testing for optimisation. This included a range of annealing temperatures from 48˚C to 58˚C, and "touchdown-PCR" to improve primer specificity. (Touchdown-PCR is where the annealing temperature at the beginning of the cycling program was high and then lowered by either 0.5 or 1.0˚C each cycle until the lowest selected annealing temperature was reached). In addition, DNA

template concentrations tested ranged from 1 ng.μL^{-1} to 30 ng.μL^{-1}, and $MgCl_2$ concentrations tested ranged from 1 mM to 5 mM. All loci were tested on at least 20 *H. laevigata* individuals.

8.3 Results

8.3.1 Microsatellite amplification

Eighteen of the 22 *H. rubra* primer-pairs successfully amplified a product of the expected size in at least one of the four species (Table 8.3). Not surprisingly, the species that appears to have retained the most loci, at 15, is *H. conicopora*, a species that may be a sub-species of *H. rubra* (Geiger 1999). The three other species produced an amplification product from 12 of the 22 primer pairs. Some primer pairs produced an amplification product that was dramatically different in size to that expected in *H. rubra*. Where that product was greater than 450 or less than 75 base pairs the marker was denoted by an "a" for altered product size. Although these altered products may contain the same microsatellite as other amplification products they are of little use, as they can't be scored reliably. Further sequencing and the design of new PCR primers may prove useful in those instances.

8.3.3 *H. laevigata* optimisation

Of the 12 loci identified as being conserved in *H. laevigata* (Table 8.3), only five proved to be reliable for further studies after evaluation in 20 greenlip individuals. Two of these loci, *CmrHr 1.6* and *CmrHr 1.24* were monomorphic at 81 bp and 228 bp respectively in the 20 individuals examined, while the remaining three loci were variable with 7, 6 and 7 alleles detected for *CmrHr 2.14*, *CmrHr 2.23* and *CmrHr 2.30* respectively. These three loci were used in a pilot population structure study of *H. leavigata* (Chapter 9).

The other 7 loci were excluded due to either non-specific or unreliable amplification (Table 8.4). Touchdown PCR cycles failed to clean up the gel profiles of locus *CmrHr 2.22*.

8.4 Discussion

The development of microsatellite markers is known to be both expensive and time consuming (Wright and Bentzen 1994). Many researchers that have produced microsatellite markers for their species have therefore examined the applicability of those markers to similar questions in related species.

White and Powell (1997) tested 11 microsatellite markers developed for the hardwood, *Swietenia humilus* for conservation within 11 members of the Meliaceae family, representing 7 genera. They detailed 4 species-specific, 1 genus-specific and 3 family-wide markers. This trend of good marker conservation within plant families is supported by other studies such as that by Thomas and Scott (1993) who found that primer sequence conservation existed among grapevine species, and more recently by Rosetto *et al.* (2000) who showed similar sequence conservation among members of the Myrtaceae family.

Conservation of 11 microsatellite loci developed for the walleye, *Stizostedion vitreum* in four species representing two genera of the Percidae family was examined by Wirth *et al.* (1999) . Three of the markers were conserved in all species tested, two were found to be specific to *Stizostedion* genus, four produced amplification from both genera, but

not all species within them, and the remaining two markers amplified only one other *Stizostedion* species. Primer sequences have also shown some conservation across 10 species of 4 genera of Lemur, endemic to Madagascar (Jekielek and Strobek 1999).

The use of agarose gel detection of PCR products has been utilized for the estimation of microsatellite loci conservation across species by researchers of other taxa (White and Powell 1997; Isagi *et al.* 1999). Wirth *et al.* (1999) and Rossetto *et al.* (2000) examined the products further by denaturing polyacrylamide gel electrophoresis (PAGE) methods which reveal allele sizes and genotypes. Others have sequenced the markers in the new species to ensure that the locus being amplified does indeed match that expected (Ezenwa *et al.* 1998).

Whilst the screening of microsatellite markers in related species by sequencing techniques is obviously the most thorough method to determine marker conservation, it is also expensive and time consuming. In instances where large numbers of microsatellite markers are being screened across many related species for which markers are not immediately required, this process may be considered to be excessive. Likewise, the optimization of primer pairs for genotyping through either radioactive labels or fluorescence primed, automated detection techniques is also time consuming and expensive. For this reason we took the simplest approach to determining marker conservation within local abalone species.

The research shows that a simplistic approach such as the initial screening can lead to a misleadingly high estimate of useful markers for a related species. Not all markers that are conserved in a related species appear to be able to be optimised satisfactorily. We had a 42% (5 from 12) success rate in the optimisation of *H. rubra* markers for *H. laevigata*. Evans *et al.* (2001) report a 60% (6 from 10) success for *H. rubra* markers with the South African species *H. midae*, and 0% (0 from 3) with the North American species *H. fulgens*. Whilst the testing of molecular markers in related species is an important component in the sharing of information, it should be noted that simply determining that a product of similar size can be amplified in another species does not suggest that marker will be useful for that species.

It should also be noted that the optimal conditions for PCR amplification can vary dramatically with different thermal cyclers. This was exemplified in the transfer of 6 *H. rubra* microsatellite loci that produced clean PCR products in *H. midae* in research in Australia, but required extensive re-optimisation when used in a genetic variation study in Cape Town, South Africa (Evans unpublished). The most obvious reason for this discrepancy was the large variation in ramp times between the respective PCR machines. For this reason any attempt to transfer molecular marker technology between laboratories using different equipment, and particularly between species will require additional PCR optimisation. The substitution of specified reagents with those that are cheaper or more readily available may also affect amplification.

One thing that is often overlooked when testing microsatellite primers is the design of the primer sites. The failure of a particular locus to amplify in another species may not mean that the microsatellite repeat is not present in that species, but that one or both of the primer sites have not been completely conserved. As the majority of microsatellite primers are published as part of a larger sequence on the GenBank (NCBI) database, the option of primer re-design is available. It could be argued that the examination of

published sequences, and if necessary the re-design of primer sites, could be a more affordable solution to marker development than the creation of a new microsatellite library.

Our research (here and in Evans *et al.* (2001) shows that some microsatellite loci isolated from Australian blacklip abalone, *H. rubra*, can be amplified in related *Haliotis* species. Rosetto *et al.* (2000) suggest that the selection of a single species from a large genera for microsatellite locus development will result in a suite of markers for most taxa in that genus. They detail only minimal PCR optimisation for the transfer of markers between species of the *Melaleuca* genus. Scribner *et al.* (1996) provide examples of high levels of marker conservation in species ranging from whales to rodents to support their results in salmon and trout from North America and the UK. However, with abalone there appears to be a much lower rate of marker conservation between species (Huang and Hanna 1998; Evans *et al.* 2001). This, together with very high levels of polymorphism encountered in most abalone species (*H. midae* – Evans 2002; *H. asinina* - Selvamani *et al.* 2000; *H. rubra* - Evans *et al.* 2000 and Chapter 7), may point towards a more rapid mutation rate of microsatellite repeats and flanking sequence in abalone than that seen in some other organisms.

Table 8.1. Sample details of four abalone species tested. Includes site name in alphabetic order by State and approximate latitude and longitude of site location.

Sample	State	Approximate location
H. conicopora		
North Twin Peak Island	Western Australia	34° 00′ S 122° 50′ E
Sandy Island	Western Australia	34° 51′ S 116° 03′ E
H. laevigata		
Christmas Island	Tasmania	39° 41′ S 143° 50′ E
Councillor Island	Tasmania	39° 50′ S 144° 10′ E
Little Green Island	Tasmania	40° 13′ S 148° 15′ E
Augusta	Western Australia	34° 19′ S 115° 10′ E
Dukes, Esperence	Western Australia	34° 05′ S 122° 12′ E
Twilight Cove	Western Australia	32° 17′ S 126° 02′ E
H. roei		
Albany Boat Harbour	Western Australia	35° 00′ S 117° 52′ E
Watermans Reef	Western Australia	31° 50′ S 115° 45′ E
H. scalaris		
North Pascoe Island	Tasmania	39° 55′ S 147° 40′ E
Woolnorth Point	Tasmania	40° 38′ S 144° 43′ E

Table 8.2. Details of the 22 microsatellite primer pairs tested for cross-species amplification. Citation is given for PCR primer details. GeneBank Accession number (No.) is provided for each locus, as well as approximate microsatellite size expected in *H. rubra*. The two primer pairs marked by the "***** " both amplify the same locus, with *CmrH*r 2.9 being internal to *CmrHr* 2.15.

Locus	Repeat Sequence	Reference	No.	Size (bp)
CmrHr 1.5	$(CAGA)_5$	Evans *et al*. 2001	AF 302824	126
CmrHr 1.6	$(CA)_4..(CA)_3$	Evans *et al*. 2001	AF 302825	89
CmrHr 1.11	$(AC)_{15}$	Evans *et al*. 2000	AF 194951	172-176
CmrHr 1.14	$(GT)_{13}TT(GT)_2GA\ (GT)_3$	Evans *et al*. 2000	AF 195952	252-262
CmrHr 1.23	$(AC)_{32}$	Evans *et al*. 2001	AF 302826	122
CmrHr 1.24	$(AT)_8$	Evans *et al*. 2000	AF 195953	216-236
CmrHr 1.25	$(CA)_{25}(AT)_6TT(AT)_5(TG)_3$	Evans *et al*. 2000	AF 195954	291-309
CmrHr 2.3	$(GT)_{14}TT(TG)_3$	Evans *et al*. 2001	AF 302827	100
CmrHr 2.5	$(GT)_{21}$	Evans *et al*. 2001	AF 194955	283-299
*CmrHr 2.9**	$(GT)_{27}$	Evans *et al*. 2000	AF 195956	156-202
CmrHr 2.14	$(GAGT)_{8...}(GAGT)_5$	Evans *et al*. 2000	AF 195957	209-235
*CmrHr 2.15**	$(CA)_{27}$	Evans *et al*. 2001	AF 195956	288
CmrHr 2.17	$(GT)_{38}$	Evans *et al*. 2001	AF 302828	226
CmrHr 2.18	$(GAGT)_3$	Evans *et al*. 2001	AF 302829	134
CmrHr 2.20	$(AC)_{23}(GCAC)_{18}$	Evans *et al*. 2001	AF 302830	186
CmrHr 2.22	$(CA)_{22}$	Evans *et al*. 2001	AF 302831	117-193
CmrHr 2.23	$(AC)_{16}$	Evans *et al*. 2001	AF 302832	258-266
CmrHr 2.26a	$(ATTC)_5T_4C\ (ATTC)_2$	Evans *et al*. 2000	AF 195958	190-212
CmrHr 2.27	$(GT)_{17}(GCGT)_{23}(GT)_2$	Evans *et al*. 2001	AF 302833	347
CmrHr 2.29	$(CA)_{58}$	Evans *et al*. 2001	AF 302834	321
CmrHr 2.30	$(GT)_6..(GT)_{13}\,(TG)_{12}(AG)_5.(TG)_3.(TG)_{16}$	Evans *et al*. 2000	AF 195959	284-328
CmrHr 2.36	$(AC)_{21}$	Evans *et al*. 2000	AF 195960	83-121

Table 8.3 Cross-species amplification using primers designed for *Haliotis rubra*. Assays producing a PCR product of expected size are indicated by +, those producing multiple bands or no product as -, and those producing bands of an altered size to that expected are represented by "a".

Locus	*H. conicopora*	*H. laevigata*	*H. roei*	*H. scalaris*
CmrHr 1.5	+	-	+	-
CmrHr 1.6	+	+	-	+
CmrHr 1.11	+	-	-	-
CmrHr 1.14	+	+	+	-
CmrHr 1.23	-	-	-	+
CmrHr 1.24	+	+	+	+
CmrHr 1.25	-	a	-	-
CmrHr 2.3	+	+	+	+
CmrHr 2.5	+	a	a	+
*CmrHr 2.9**	+	-	+	-
CmrHr 2.14	+	+	+	+
*CmrHr 2.15**	+	-	+	-
CmrHr 2.17	-	+	-	+
CmrHr 2.18	-	a	-	a
CmrHr 2.20	+	+	+	+
CmrHr 2.22	a	+	a	-
CmrHr 2.23	+	+	+	+
CmrHr 2.26	-	-	a	-
CmrHr 2.27	+	+	+	+
CmrHr 2.29	+	+	+	+
CmrHr 2.30	+	+	+	+
CmrHr 2.36	-	-	-	-
Total positives	**15**	**12**	**12**	**12**

Table 8.4. Evaluation of microsatellite loci initially identified as conserved in H. laevigata. Sample size was n = 20.

Locus	Result
CmrHr 1.6	Monomorphic
CmrHr 1.14	Non-specific amplification
CmrHr 1.24	Monomorphic
CmrHr 2.3	Non-specific amplification
CmrHr 2.14	Suitable for research
CmrHr 2.17	Non-Specific amplification
CmrHr 2.20	Unreliable amplification
CmrHr 2.22	Unscoreable
CmrHr 2.23	Suitable for research
CmrHr 2.27	Unreliable amplification
CmrHr 2.29	Unreliable amplification
CmrHr 2.30	Suitable for research

9. GREENLIP ABALONE STOCK STRUCTURE

The research below and in Section 8 was undertaken towards achieving Objective 3.

A study was undertaken to assess the effectiveness of microsatellite DNA markers developed for blacklip abalone (*Haliotis rubra*) for examining genetic differentiation in greenlip abalone (*Haliotis laevigata*). Variation was examined at eight microsatellite markers in samples from six selected sites. The samples examined were from three Western Australian sites and three sites on the Bass Strait islands north of Tasmania.

Fisheries WA supported this section of the project and requested that additional samples to those in the orignal proposal be analysed. A report was submitted to Fisheries WA and was included in the Proceedings of the 8th Annual Abalone Aquaculture Workshop held in Fremantle WA in July 2001.

9.1. Materials and Methods

Gill tissue samples of *H. laevigata* from three sites along the Western Australian coast and three sites from the Bass Strait islands north of Tasmania were examined (Table 8.1). Thirty individuals from each site were randomly chosen for examination.

DNA extraction and purification from all samples used QIAamp DNA Mini Kits (*Qiagen*) according to the protocol supplied by the manufacturer. Extraction success was confirmed by electrophoresis of each DNA extract on agarose gels. Bands were visualized by staining in ethidium bromide and were viewed under UV light.

An initial examination of PCR (polymerase chain reaction) amplification success and polymorphism was made of eight microsatellite loci using 60 greenlip abalone (10 from each sampling site). Each of the loci was originally developed for use with blacklip abalone (*H. rubra*) DNA. The eight loci were *cmrHr1.24, cmrHr2.14* and *cmrHr2.30* (Evans *et al*. 2000), *cmrHr1.6* and *cmrHr2.23* (Evans *et al*. 2001) and *RubCA1, RubGACA* and *RubGT* (Huang and Hanna 1998).

The genetic analysis programs GENEPOP v 3.2 and ARLEQUIN 2000 were used to analyze the data for agreement to Hardy-Weinberg equilibrium and to determine the extent of genetic differentiation among the six sampled sites.

9.2. Results

All eight microsatellite loci were successfully PCR amplified from greenlip abalone DNA. Four loci (*cmrHr1.6*, *cmrHr1.24, RubCA1* and *RubGACA*) were monomorphic for a single allele in each locus across the individuals examined, and were therefore of little use in a genetic differentiation study. The locus *cmrHr2.30* proved to be problematic with inconsistent amplification (although subsequent tests rectified this problem, and the locus appears to be polymorphic in greenlip abalone).

The three polymorphic loci *cmrHr2.14, cmrHr2.23* and *RubGT* were PCR amplified for the 30 individuals from each site. Non-amplification or poor resolution of allele peaks on the gel resulted in some individuals not being scored for locus *cmrHr2.23*. However, DNA from all individuals was successfully amplified and reliably scored for the other two loci (Table 9.1).

Only locus *cmrHr2.14* showed a significant deviation in observed genotypes to that expected under Hardy-Weinberg equilibrium (Table 9.2). This was in four of the six samples and after standard correction for multiple tests. In all instances a deficiency of heterozygotes was observed.

There was highly significant ($P < 0.001$) genetic differentiation between the six sites analyzed. The overall AMOVA estimated that 5.65% ($F_{ST} = 0.0565$) of the observed variance in allele frequencies at the three loci was attributable to variation between the six sites (Table 9.3). Grouping the sites by area, i.e Western Australia and Tasmania, resulted in significant ($P = 0.007$) evidence of differentiation between the two groups, with 64% of the among site differentiation being due to differences between the two areas ($F_{CT} = 0.0369$; Table 9.3).

Site pairwise analyses of F_{ST} showed significant differences between the three Western Australian sites and the three Tasmanian sites (Table 9.4). Significant differences were also observed between the three Western Australian sites. Some of the *P* values were not significant when corrected for table-wide multiple tests, but are highly significant when taken individually or by smaller groups (e.g. three tests for three Western Australian sample comparisons). There was no evidence of allele frequency differences between the three Tasmanian sites.

When the pairwise genetic differentiation was examined by individual locus, the same pattern of differentiation was evident, although most of the differentiation occurred at locus *RubGT* (Table 9.5). This was also reflected in the locus by locus AMOVA results where the level of differentiation between sites (F_{ST}) for *cmrHr2.14* at 1.52% was not significant ($P = 0.071$), while that for the other two loci (*cmrHr2.23* 6.51% and *RubGT* 9.54%) was highly significant ($P = 0.004$ and <0.001, respectively).

9.3 Discussion

A scorable microsatellite product was amplified in greenlip abalone using the three loci *RubCA*, *RubGACA* and *RubGT*. Huang and Hanna (1998) reported that all three did not amplify in this species. This adds further to the comments in Chapter 8 that inter-laboratory differences can affect the ability of PCR primers to amplify a suitable product.

The locus *cmrHr2.30* proved to be problematic with inconsistent amplification during this study despite earlier results (Chapter 8) indicating that this locus was reliable in the greenlip abalone. The problem was finally traced to the source of the PCR primers. Whilst this locus was subsequently shown to be polymorphic in greenlip abalone, the problem was only rectified after the laboratory phase of this small study was completed. Locus *cmrHr2.30* was therefore not included in this study, but should be considered for future studies with greenlip abalone.

The deficiency in heterozygotes at locus *cmrHr2.14* may be a technical problem, an artefact of the small sample sizes, or biological and indicating admixture of populations. Scoring errors are dismissed as a probable cause as this locus, a tetra-nucleotide microsatellite repeat, produced clean peaks that were reliably and consistently scored. If a technical problem, it is more likely a consequence of non-amplifying (or null) alleles. As no null homozygotes (complete lack of amplification at this locus) were observed,

preferential amplification of smaller sized alleles in heterozygotes may be an explanation. If this were the case, a heterozygote for two widely different sized alleles could be incorrectly scored as a homozygote for the smaller allele. Modification of the PCR primers originally designed for blacklip abalone may resolve this problem, although the issue of non-amplifying microsatellite alleles appears to be relatively common in marine molluscs. A biological explanation (population admixture) is considered less likely as the deviation observed was locus specific, not site specific, and this locus showed the least differentiation between the six samples.

The analyses conducted on the six samples of greenlip abalone indicate significant genetic differentiation between the three individual Western Australian sites (Augusta, Dukes and Twilight), and between these three and the three Tasmanian sites (Christmas Island, Councillor Island and Little Green Island). The null hypothesis that the six samples of greenlip abalone all came from the same genetic population can be refuted, and there is good evidence of structuring in the Australian population. There was, however, no evidence of structuring between the three Tasmanian sites.

The results from this pilot study support the conclusion reached by Brown and Murray (1992) from their allozyme study, that distinct greenlip abalone populations may exist. Both studies however suffer from incomplete sampling in the species range. Like the allozyme study, our results indicate an isolation-by-distance model, with strong correlation (0.84) observed between the pairwise F_{ST} estimates of genetic distance and the approximate geographic distance between samples (Figure 9.1).

No significant genetic differentiation was detected between the three Tasmanian sites. This may reflect the relatively close geographic position of these sites, the particular oceanographic environment associated with the Bass Strait islands that may encourage transport of larvae, or the small sample sizes examined. The shorter geographic distance may not be a legitimate explanation as the separation of the Dukes and Twilight samples in Western Australia is not that much greater (ca. 433 km vs ca. 382 between Christmas Island and Little Green island). A comparison of the extent and size of the greenlip abalone population along the two different coasts and an interpretation of coastal oceanographic features may help to resolve this difference. As well a more comprehensive sampling program, consisting of larger and more samples across a wider geographic range, would be advised.

The results from this study have implications for the management of the wild fisheries for greenlip abalone, location and control of aquaculture ventures with this species, and future reseeding or translocation projects. Evidence has been provided that genetically discrete stocks of greenlip abalone, *Haliotis laevigata*, exist and the conservation of these stocks need to be considered when making decisions in relation to zoning for wild capture, aquaculture or reseeding.

9.4 Conclusion

This study has demonstrated the effectiveness of blacklip abalone microsatellite loci in the analysis of population structure of the greenlip abalone. Genetic variation was examined using three polymorpohic microsatellite loci in samples from three sites along the southern coast of Western Australia and three Tasmanian sites from the Bass Strait islands. Five additional loci were tested and four proved to be monomorphic for a single allele, and a further locus proved problematic during the study but subsequent

development indicates that it would be useful for future studies. Despite small sample sizes (30 individuals per site) there was strong evidence of genetic differences between the three Western Australian sites, and between these and the three Tasmanian sites. There was also a significant correlation between genetic differentiation and geographic distance between pairs of samples. No differentiation was observed between the three Tasmanian sites. The results have implications for the management of greenlip abalone harvesting, location and control of aquaculture ventures with this species, and future reseeding or translocation projects. Further research is encouraged (1) to increase the number and improve the reliability of microsatellite loci for the greenlip abalone, and (2) to conduct a more intensive population survey of this species with larger sample sizes and more samples.

Table 9.1. **Microsatellite allele frequencies within each sampled site for three loci**. *n*, number of individuals scored per locus. -, allele not detected. Alleles are presented as size in base pairs.

	Western Australia			Northern Tasmania		
Locus/Allele	**Augusta**	**Dukes**	**Twilight**	**Christmas I.**	**Councillor I.**	**Little Green I.**
cmrHr2.14						
246	0.017	-	0.017	-	-	-
252	-	-	-	0.017	0.017	0.050
266	-	-	-	-	-	0.033
270	0.017	-	0.017	0.017	0.033	-
274	0.050	0.067	0.017	0.150	0.083	0.100
278	0.283	0.133	0.233	0.200	0.283	0.150
282	0.400	0.283	0.367	0.300	0.283	0.250
286	0.033	0.100	0.150	0.100	0.083	0.285
290	0.033	0.133	0.083	0.033	0.100	0.017
294	0.000	0.117	0.017	0.033	0.067	0.067
298	0.150	0.050	0.033	0.150	0.050	0.050
302	0.017	0.083	0.017	-	-	-
306	-	0.033	0.017	-	-	-
310	-	-	0.033	-	-	-
n	*30*	*30*	*30*	*30*	*30*	*30*
cmrHr2.23						
255	-	-	0.019	-	-	-
259	0.833	0.839	0.750	0.660	0.741	0.552
265	0.167	0.125	0.231	0.320	0.241	0.431
267	-	-	-	0.020	0.019	0.017
269	-	0.018	-	-	-	-
277	-	0.018	-	-	-	-
n	*30*	*28*	*26*	*25*	*27*	*29*
RubGT						
149	0.033	0.017	0.017	-	-	-
151	0.033	0.017	0.033	0.017	-	0.017
153	0.583	0.333	0.367	0.317	0.333	0.350
155	0.200	0.100	0.067	0.333	0.450	0.450
157	0.017	0.133	0.050	0.250	0.117	0.150
159	0.017	-	0.033	0.050	0.033	0.017
161	0.050	0.017	0.217	0.033	0.017	-
163	0.050	0.267	0.067	-	0.033	0.017
165	0.017	0.067	0.150	-	0.017	-
167	-	0.017	-	-	-	-
175	-	0.017	-	-	-	-
177	-	0.017	-	-	-	-
n	*30*	*30*	*30*	*30*	*30*	*30*

Table 9.2: Genotype statistics. The observed number of heterozygotes, *Ho*; expected number of heterozygotes under Hardy-Weinberg Equilibrium, *He;* the F-statistic *Fis*; *P* probability of agreement with Hardy-Weinberg Equilibrium with * indicating sites not in agreement at $P < 0.05$ following Bonnferroni correction for multiple test; *n* = number of individuals scored.

	Western Australia			Northern Tasmania		
Locus	**Augusta**	**Dukes**	**Twilight**	**Christmas I.**	**Councillor I.**	**Little Green I**
cmrHr2.14						
Ho	0.600	0.500	0.633	0.367	0.500	0.600
He	0.761	0.875	0.792	0.841	0.823	0.834
Fis	0.196	0.423	0.202	0.560	0.395	0.279
P	0.086	<0.001*	0.261	<0.001*	0.002*	<0.001*
n	*30*	*30*	*30*	*30*	*30*	*30*
cmrHr2.23						
Ho	0.333	0.286	0.346	0.360	0.516	0.414
He	0.337	0.314	0.420	0.471	0.400	0.518
Fis	-0.184	-0.005	0.118	0.137	-0.302	0.205
P	0.563	0.037	0.688	0.448	0.356	0.269
n	*30*	*28*	*26*	*25*	*27*	*29*
RubGT						
Ho	0.667	0.733	0.833	0.600	0.700	0.500
He	0.659	0.797	0.795	0.744	0.681	0.688
Fis	-0.073	0.081	-0.048	0.186	-0.028	0.249
P	0.504	0.251	0.440	0.062	0.580	0.030
n	*30*	*30*	*30*	*30*	*30*	*30*

Table 9.3. **AMOVA and F-statistics -** for all three loci the significance levels following AMOVA with six sites grouped by area (Western Australia and Tasmania)

Source of variation	Fixation index	F-statistic	Significance test
Among groups	F_{CT}	0.0369	$P = 0.007$
Among samples within groups	F_{SC}	0.0203	$P = 0.002$
Among samples	F_{ST}	0.0565	$P < 0.001$

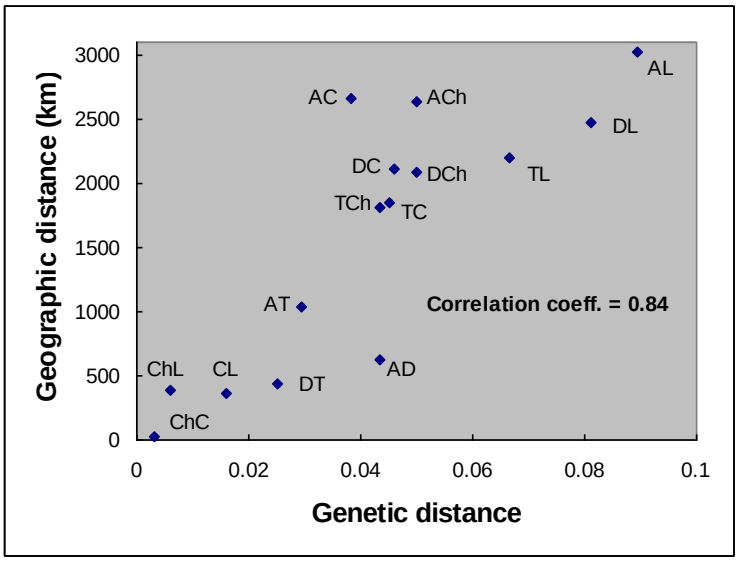
Table 9.4. **Pairwise comparison of the six greenlip abalone sites at the three microsatellite loci**. Below diagonal = F_{ST} values using the distance method; above diagonal = *P* values, with those significant at $P < 0.05$ in bold, and those still significant after a table-wide Bonferroni adjustment for multiple tests marked by *.

	Western Australia			Northern Tasmania		
	Augusta	**Dukes**	**Twilight**	**Christmas I.**	**Councillor I.**	**Little Green I**
Augusta		**0.003**	**0.005**	**0.005**	**0.004**	**<0.001***
Dukes	0.0433		**0.012**	**<0.001***	**0.001***	**<0.001***
Twilight	0.0294	0.0251		**0.002***	**<0.001***	**<0.001***
Christmas I.	0.0500	0.0500	0.0433		0.449	0.355
Councillor I.	0.0384	0.0459	0.0451	0.0031		0.086
Little Green I	0.0894	0.0813	0.0667	0.0059	0.0161	

Table 9.5. **Genetic differentiation (*P* value) of allele frequencies for each site pair per locus.** Values significant at 0.05 are in bold and those still significant after Bonferroni adjustment for multiple tests marked by *. Augusta, Dukes and Twilight are Western Australian sites, and Christmas I., Councillor I. and Little Green I. are northern Tasmanina sites.

Sample pair	*cmrHr2.14*	*cmrHr2.23*	*RubGT*
Augusta/Dukes	**0.001***	0.475	**<0.001***
Augusta/Twilight	0.080	0.405	**0.002***
Augusta/Christmas I.	0.258	0.055	**<0.001***
Augusta/Councillor I.	0.088	0.296	**0.002***
Augusta/Little Green I.	**<0.001***	**0.002***	**<0.001***
Dukes/Twilight	0.083	0.195	**<0.001***
Dukes/Christmas I.	**0.013**	**0.018**	**<0.001***
Dukes/Councillor I.	0.142	0.142	**<0.001***
Dukes/Little Green I.	**0.004**	**<0.001***	**<0.001***
Twilight/Christmas I.	**0.028**	0.345	**<0.001***
Twilight/Councillor I.	0.369	1.000	**<0.001***
Twilight/Little Green I.	**0.013**	**0.034**	**<0.001***
Christmas I./Councillor I.	0.405	0.692	0.262
Christmas I/Little Green I.	0.081	0.571	0.345
Councillor I./Little Green I.	**0.028**	0.058	0.964

Figure 9.1. **Relationship between estimated genetic distance and geographic distance between pairs of sample sites**. Site pairs are indicated as A – Augusta, D – Dukes and T – Twilight all from Western Australia, and Ch – Christmas Island, C – Councillor Island and L – Little Green Island from northern Tasmania.



10. HARVESTING IMPACT ON ALLOZYME VARIATION

The research below was undertaken towards achieving Objective 4.

10.1 Introduction

The population sizes of marine organisms are known to vary between years as a consequence of natural fluctuations in recruitment or subsequent survival. Reductions in population size and particularly effective population size (actual breeding numbers) would be expected to result in a decrease in genetic variation within the population. Such short-term reductions are likely to have less of an effect than long-term effects due to sustained harvesting (Ward 2002). For ecological sustainability of a population, large and continuous decreases in genetic variation either naturally or due to harvesting would be undesirable.

While there is little direct evidence to date of the negative effect of harvesting of marine organisms on population genetic diversity (Ward 2002), this may be due to the limited number of studies conducted. There are however many reports over the past decades of collapsed fisheries, including abalone fisheries (e.g. Tegner *et al*. 1992; Hamm and Burton 2000; Hobday *et al.* 2001), that will have shown reductions in effective population sizes. Monitoring population genetic diversity may help as an early warning of over harvesting, but by the time it is measurable it may be too late, as significant reductions in genetic diversity in a population are irreversible.

Measure of change in genetic variation can be through loss of alleles and loss of diversity (or heterozygosity). The loss of rare allozyme alleles in hatchery produced abalone populations has been reported for *H. iris* (Smith and Conroy 1992), and *H. tuberculata* (Mgaya *et al.* 1995), with a significant reduction in heterozygosity also reported in the former study. More recently, Evans (2002) reported losses of microsatellite alleles and changes in allele frequencies in first generation hatchery populations of both *H. midae* and *H. rubra,* but no evidence of a reduction in heterozygosity compared to wild samples.

The first investigation of population genetic diversity in the blacklip abalone (*H. rubra*) was an allozyme study of samples collected between 1985 and 1989 (Brown 1991b). Since then the Tasmanian fishery has remained the world's largest and possibly most stable, with annual catches averaging over 2,200t. The aim of this part of the project was to compare present day allozyme variation (number of alleles and overall heterozygosity) in samples from two locations with that reported by Brown (1991b) for samples from similar locations 10 to 15 years ago.

10.2 Materials and Methods

Frozen muscle tissue of *H. rubra* was collected from two sites, Cat Island off Flinders Island, and One Tree Point on Bruny Island (see Table 7.1 and Figure 7.1). Genetic variation at Cat Island (part of Furneaux Islands group) was compared with that reported by Brown (1991b) for his sample Furneaux Island. The One Tree Point sample was compared with Brown's two southeast samples, Ninepin Point and South Arm. Exact locations of Brown's sampling were not available, and we consider the comparisons the most suitable given logistical constraints for sample collection.

Small pieces of muscle tissue were placed in 1.5 mL Eppendorf tubes, homogenised manually with 1 to 4 drops of distilled water, and spun at 11,000 *g* in a microcentrifuge at 4˚C for 5 min. The supernatant was drawn off for electrophoresis.

In the original study (Brown 1991b), 15 allozyme loci encoding 12 enzymes were examined on cellulose acetate gels (Cellogel). Of these only seven loci showed sufficient variation (i.e. the frequency of the most common allele was less than 0.95 in the Tasmanian samples) to warrant investigation in this study. Extracts from muscle tissue were examined for these loci using Helena Titan III cellulose acetate gels and two buffer systems, TC (continuous tris-citrate buffer system 75 mM tris, 25 mM citric acid, pH 7.0) and TG (continuous tris-glycine buffer system 0.02 M tris, 0.192 M glycine, pH 8.5; Hebert and Beaton, 1989). Five of these enzymes were found to produce a reliable and scorable product suitable for analysis:

Aspartate aminotransferase (AAT, EC No. 2.6.1.1; buffer TC)
Glucose phosphate isomerase (GPI, EC No. 5.3.1.9; buffer TG)
Isocitrate dehydrogenase (IDHP, EC No. 1.1.1.42; buffer TC)
Malate dehydrogenase (MDH, EC No. 1.1.1.37; buffer TC)
Phosphoglucomutase (PGM, EC No. 5.4.2.2; buffer TG)

Histochemical stains were taken from Hebert and Beaton (1989) or Richardson *et al.* (1986).

Loci and alleles were designated by the nomenclature used by Brown (1991b). Alleles within each locus were allocated a letter based on the code in Brown assuming letter ‘a’ designates fastest migrating allele and the most common allele in both studies was the same allele. Conformance of genotype frequencies to Hardy-Weinberg equilibrium and chi-square tests for allele frequency homogeneity between the samples were tested by the Monte Carlo approach (5000 replicates) of Zaykin and Pudovkin (1993). Hardy-Weinberg expected heterozygosities (He) were determined for each locus from allele frequencies ($He = 1-\sum(a_n^2)$, where a_n is allele frequency of nth allele).

10.3 Results

Overall there was no evidence of a major loss of alleles in the samples analysed (Table 10.1). In fact at all but the *PGM* locus, rare alleles previously unrecorded from these regions were detected. Alleles not observed in our samples were previously observed only at low frequencies (< 0.017).

Genotype frequencies at the four polymorphic loci (frequency of most common allele < 0.95; *AAT-2*, *GPI*, *IDH-1* and *PGM*) in the two samples analysed were all in agreement with Hardy-Weinberg equilibrium, as were these loci in Brown’s (1991) original study.

No consistent loss in Hardy-Weinberg expected heterozygosities (He) was observed between values reported by Brown (1991b) and our study, both for individual loci and mean values (Table 10.2).

The most common allele per locus had similar frequencies between all five samples (Table 10.1), except for *AAT-2*, where in our two samples allele *AAT-2b* was the most common of the two main alleles, compared with *AAT-2e* in Brown’s samples. This shift in common allele at *AAT-2* was also apparent in 6 of the 17 samples analysed by Brown. Chi-square tests of allele frequency homogeneity between the five samples for the four

polymorphic loci revealed no significant differences, after Bonferroni corrections for multiple tests (*AAT-2* P = 0.077, *GPI* P = 0.025, *IDH-1* P = 0.069 and *PGM* P = 0.116). Pairwise chi-square tests did reveal some significant differences, although only that between Cat Island and Ninepin Point for *GPI* was significant after correction (Tables 10.3 and 10.4). Our One Tree Point sample was marginally different to both Ninepin Point and South Arm at the *PGM* locus, and Cat Island was marginally different to Brown's three samples at *AAT-2*.

10.4 Discussion

The gel systems used in our study were not identical to those used by Brown (1991b), and this may account for the observation of additional alleles in the Tasmanian samples at *GPI* and *IDH-1* loci. In general, however, there appears to be good agreement in regards to alleles observed between the two sets of data.

There is no evidence from this study of a decrease in either the number of allozyme alleles or allozyme heterozygosity in blacklip abalone over the intervening 15 years between the two analyses. Therefore there is no evidence that commercial harvesting of blacklip abalone at around 2,000t a year has had any detectable impact on allozyme genetic diversity in the population.

The result of the microsatellite study (Chapter 7) suggests sufficient gene flow among blacklip abalone around Tasmania to preclude any major population genetic heterogeneity. This combined with a relatively large population both pre-harvest and current may reduce the likelihood of detecting any measurable decrease in heterozygosity, based on the sample sizes employed in the studies.

The polymorphic allozyme loci had from three to eight alleles per locus, which compared with microsatellites (12 to 52 alleles per locus, Chapter 7) is relatively low. Likewise mean expected heterozygosity per locus ranged from 0.013 to 0.515 for the allozyme loci compared to 0.269 to 0.954 for microsatellite loci. Allozymes may therefore not be the most effective marker to assess genetic changes over time. The more polymorphic microsatellites should be more useful markers for future studies.

However, waiting a further 10 or more years to see a signal may be too late. An abalone population reduced to low effective breeding numbers may become commercially unsustainable and result in a decline of the species (e.g. *H. sorenseni*; Hobday *et al*. 2001) from which recovery may be slow (Hamm and Burton 2000). A more useful method of monitoring harvest impact may be to routinely examine effective population sizes through an assessment of genetic diversity in settling larvae (recruitment) at selected sites and times.

Table 10.1. Allele frequencies at seven allozyme loci. Two sites scored in this study and three corresponding sites (*) investigated by Brown (1991b)**.** For allele designation see text; *n*, number of individuals scored. - = allele not observed.

Locus/allele	Cat Island	Furneaux Isl.*	One Tree Pt	Ninepin Pt*	South Arm*
AAT-1					
c	0.006	-	0.010	0.018	0.009
e	0.994	1.000	0.990	0.974	0.982
f	-	-	-	0.009	0.009
n	*89*	*30*	*97*	*114*	*110*
AAT-2					
a	0.052	-	0.024	0.009	0.023
b	0.579	0.450	0.595	0.474	0.464
e	0.360	0.550	0.381	0.504	0.509
g	0.009	-	-	0.009	-
h	-	-	-	0.004	0.009
n	*57*	*30*	*42*	*114*	*110*
GPI					
a, b	-	-	0.020	-	-
c	0.085	0.150	0.135	0.158	0.095
d, e, f	0.005	-	0.010	-	-
g	0.795	0.700	0.635	0.636	0.714
j	0.010	-	0.015	-	0.005
o	-	-	-	-	0.005
p	0.105	0.150	0.170	0.193	0.177
q, r	-	-	0.005	-	-
s	-	-	0.005	0.013	0.005
n	*100*	*30*	*100*	*114*	*110*
IDH-1					
'new'	-	-	0.005	-	-
a	0.103	0.167	0.076	0.079	0.095
c	-	0.017	-	-	-
d	0.892	0.817	0.909	0.917	0.905
e	0.005	-	0.010	0.004	-
n	*97*	*30*	*99*	*114*	*110*
MDH-1					
e	-	-	0.005	-	-
f	1.000	0.983	0.990	1.000	0.995
g	-	0.017	0.005	-	0.005
n	*100*	*30*	*100*	*114*	*110*
MDH-2					
b	0.994	1.000	1.000	1.000	1.000
c	0.006	-	-	-	-
n	*78*	*30*	*100*	*114*	*110*
PGM					
a	-	-	-	-	0.005
b	0.076	0.017	0.075	0.026	0.023
d	0.904	0.950	0.875	0.943	0.936
e	-	-	-	-	0.005
g	-	-	-	-	0.005
h	0.015	0.017	0.050	0.018	0.023
k	0.005	0.017	-	0.009	0.005
n	*99*	*30*	*100*	*114*	*110*
Total no. alleles	**21**	**16**	**24**	**21**	**25**

Table 10.2. Hardy-Weinberg expected heterozygosities (He). Individual locus values and the mean value over the 7 loci scored and the mean value over 4 polymorphic loci (*AAT-2*, *GPI*, *IDH-1*, *PGM*) are presented for two sites scored in this study and three corresponding sites (*) investigated by Brown (1991b)**. - =** no heterozygotes observed.

Locus	Cat Island	Furneaux Isl.*	One Tree Pt	Ninepin Pt*	South Arm*
AAT-1	0.0119	-	0.0198	0.0509	0.0355
AAT-2	0.5324	0.4950	0.5002	0.5211	0.5250
GPI	0.3496	0.4650	0.5489	0.5331	0.4498
IDH-1	0.1937	0.3043	0.1678	0.1529	0.1720
MDH-1	-	0.0334	0.0199	-	0.0100
MDH-2	0.0119	-	-	-	-
PGM	0.1768	0.0966	0.2263	0.1097	0.1227
Mean 7 loci	**0.1823**	**0.1992**	**0.2118**	**0.1954**	**0.1878**
Mean 4 loci	0.3131	0.3402	0.3608	0.3292	0.3174

Table 10.3. Chi-square test probabilities of allele homogeneity between pairs of samples for loci *AAT-2* (above diagonal) and *GPI* (below diagonal). Values significant at 0.05 are in bold, and * indicates those still significant after Bonferroni correction for multiple tests.

	Cat Island	Furneaux Isl.	One Tree Pt	Ninepin Pt	South Arm
Cat Island		**0.027**	0.693	**0.009**	**0.016**
Furneaux Isl.	0.416		0.069	0.902	0.581
One Tree Pt	**0.010**	0.824		0.182	0.176
Ninepin Pt	**<0.001***	0.660	**0.048**		0.502
South Arm	0.152	0.746	0.089	0.128	

Table 10.4. Chi-square test probabilities of allele homogeneity between pairs of samples for loci *IDH-1* (above diagonal) and *PGM* (below diagonal). Values significant at 0.05 are in bold, and * indicates those still significant after Bonferroni correction for multiple tests.

	Cat Island	Furneaux Isl.	One Tree Pt	Ninepin Pt	South Arm
Cat Island		0.173	0.533	0.704	0.733
Furneaux Isl.	0.239		0.055	0.038	0.051
One Tree Pt	0.168	0.056		0.719	0.262
Ninepin Pt	0.124	1.000	**0.006**		0.608
South Arm	0.088	0.960	**0.016**	0.886	

11. BENEFITS

The commercial abalone fishers are the ultimate beneficiaries of the research undertaken during this project. This benefit arises through the provision of improved technology and information to the compliance authorities and fishery managers to assist the sustainable management of the fisheries.

The research has provided:

1. a DNA-based protocol for the identification of Australian abalone products;
2. evidence of limited genetic heterogeneity in the Tasmanian blacklip abalone population;
3. evidence of some genetic differentiation between Tasmanian and mainland blacklip abalone;
4. evidence of some genetic variation in the mainland blacklip abalone population;
5. evidence of genetic heterogeneity in the greenlip abalone population in Western Australia;
6. evidence of some conservation of microsatellite loci between abalone species;
7. no evidence of a measurable impact on allozyme variation in harvested populations of the blacklip abalone.

The annual cost to FRDC of the two-year project was less than 0.1% of the current annual value of the industry (over $200m). Therefore, if the outcome of this research assists in either the prevention of a 0.1% decline in value of the fishery, an equivalent increase in value, or equivalent reduction in illegal catch, the research will have been cost effective.

The growing aquaculture industry and future stock enhancement or translocation programs benefit from the improved understanding of natural genetic diversity. In addition, future abalone research programs intending to utilise microsatellite DNA technology will benefit from the increased understanding of this type of molecular marker in abalone.

12. FURTHER DEVELOPMENT

This project provides a definitive species identification test for ten Southern Hemisphere abalone species, a comprehensive analysis of the genetic diversity of the Tasmanian blacklip abalone population, and a preliminary analysis of genetic variation in greenlip abalone in Western Australia and Tasmania. Continued abalone genetic research to further the activities and findings of this project is required and recommended.

The need for continued research relates to aspects of ecological sustainable management, compliance (illegal activities), stock enhancement/ranching and aquaculture.

In particular further investigation is required into the genetic diversity of blacklip and greenlip abalone populations along the mainland coast including examination of diversity in settling larvae at individual reefs, and improving the number and quality of available genetic markers for both species. Additional research is also recommended on species identification.

12.1 Species Identification – other species

Worldwide there are 56 'currently described' abalone species (Geiger 1999), with 19 from Australian waters (10 endemic and nine of Indo-Pacific distribution). The species identification test developed in this project (Chapter 5) covers only ten species of which five are from Australian waters. The species included are the main commercial species in the Southern Hemisphere. For completeness and to assist authorities to curtail the international illegal abalone industries, a DNA species identification test is recommended that would include all recognised species (and sub-species).

12.2 Species Identification – sub-species

Our test was unable to differentiate the two local species *H. rubra* and *H. conicopora* (Chapter 5). It has been suggested that the latter is a sub-species of *H. rubra* (Geiger 1998). The species status of *H. conicopora* would be best resolved using further genetic markers. Likewise, in Australian waters, the status of *H. emmae* as a species or sub-species of *H. scalaris* requires resolution. Many other instances of possible sub-species status require examination, as do distributions of some species, such as the Japanese *H. diversicolor* that has a reported distribution into northern Australia (Geiger 1999).

12.3 Species Identification – Hybrids and "when is a species a species"

The species identification test is based on small regions of mitochondrial DNA (mtDNA). Unlike nuclear DNA that is inherited from both parents, mtDNA is inherited only from the maternal side. Therefore our test (Chapter 5) identifies the maternal line of inheritance of an individual.

Naturally occurring hybrids between *H. laevigata* and *H. rubra* are known to exist, as well as evidence of local introgression (back-crossing) of *H. rubra* genes into *H. laevigata* and vice versa in sympatric populations (Brown 1995). We are able to differentiate a suspected hybrid between these two species from either pure species based on species specific size differences in nuclear DNA microsatellite markers; in addition to identifying by our mtDNA test the maternal species line. In the course of our research it became apparent that natural hybrids may also exist (based on morphology

and the mtDNA test) between other pairs of species, e.g. *H. laevigata* and *H. scalaris* (Chapter 5). Our mtDNA test can identify the maternal line of such hybrids but nuclear DNA markers are not available to differentiate the two species. A nuclear DNA test covering all species of interest is therefore also required to complement the mtDNA test and cover the possibility of hybrids.

A complicating issue associated with abalone hybrids is their ability to cross back with either of the parental species (introgression of one species nuclear genes into another species). This raises the question as to which species to call an individual that is the result of a hybrid crossing back with a pure species. Should it be classified based on its mtDNA (maternal inheritance), based on only some nuclear DNA regions and morphology, or called a hybrid based on a set number of nuclear DNA regions? Resolution of this issue is recommended.

12.4 Genetic diversity H. rubra *and* H. laevigata – *sampling range*

An extensive analysis of genetic diversity within the *H. rubra* population around Tasmania has been achieved in this project through the analysis of a large number of samples. A similar level of analysis is recommended for the mainland populations of both species, with sampling to cover the range of both species.

This sampling expansion is important given the suggested 'isolation-by-distance' model for the population structure, as the size of the 'genetic neighbourhoods' needs to be refined. The sampling for *H. rubra* should include further samples within the NSW, Victoria and South Australia coasts, with sampling extending into Western Australia. The latter samples would help to determine whether *H. conicopora* is a separate species or a sub-species of *H. rubra*. The West Bay sample from South Australia stands out in the current analysis (Chapter 7) and may be showing a general cline in gene diversity from east to west.

Further sampling and analysis of *H. laevigata* is recommended to clarify the separation of the three genetic regions already identified in Western Australia (Chapter 8). Additional samples are required from between the previous three samples to refine the isolation-by-distance model for this region, and samples are required to be analysed from South Australian and Victorian waters to further refine the model and determine the genetic heterogeneity in these regions.

12.5 Genetic diversity H. rubra *and* H. laevigata – *genetic markers*

Technical problems (null alleles and excessive number of alleles) were found to exist with some of the microsatellite markers used in this project. These are issues that only become apparent after examining a large data set. Development and testing of new, more reliable microsatellite markers for *H. rubra* is recommended prior to further genetic diversity studies. Likewise additional microsatellite markers for *H. laevigata* are also recommended to strengthen the analyses. However, should resources not be available for development and testing of new markers, those used in this project are adequate with due consideration given to the observed problems.

Comparison of RFLP-mtDNA (restriction fragment length polymorphism – mitochondrial DNA) and microsatellite (nuclear DNA) analyses revealed similar levels of differentiation of population differentiation in five blacklip abalone samples (Chapter 6). Microsatellites were considered the most useful method at this time, as they provide

a number of independent markers with greater levels of polymorphism. Other techniques, e.g. sequence analysis or SNPs (single nucleotide polymorphisms) or different regions of either the nuclear DNA or mtDNA genomes, may reveal as yet undetected population heterogeneity and pilot studies of these are recommended.

12.6 Effects of harvesting on genetic diversity

Commercial harvesting (over 15 years) appears to have had little effect on allozyme genetic diversity in blacklip abalone sampled at two Tasmanian sites (Chapter 10). It is recommended that consideration be given to long-term monitoring of genetic diversity at a number of commercial sites using the more polymorphic microsatellite markers that are now available and for which there is a baseline data base. Such monitoring should take into account where possible age cohort differences.

Small changes in genetic diversity (loss of alleles or heterozygosity) would reflect a decrease in effective (breeding) population size, and provide an early warning of population decline. Such changes are likely to be more apparent at the more variable microsatellite loci compared with allozyme loci. It is further recommended that routine sampling and analysis of settling larvae and/or juveniles of known age at selected sites commence to monitor effective population sizes through changes in genetic diversity. Such information will be invaluable to the ecologically sustainable management of the fisheries.

13. CONCLUSIONS

13.1 Objective 1.

To refine, and where necessary establish, abalone species identification protocols to forensic standards suitable for required fisheries compliance.

- A mitochondrial DNA based test was developed and tested that allows for the identification of 10 commercial or potentially commercial species Southern Hemisphere species of abalone. The species covered include five (*H.conicopora* classed as sub-species of *H. rubra*) from Australian waters, three from New Zealand and two from South Africa. The protocol has been peer reviewed and accepted for publication in the international scientific literature. The test allows for identifcation of fresh, preserved and canned product as well as mucous samples.
- Further research is recommended to strengthen the test by inclusion of additional species (Australian and/or worldwide), resolution of sub-species and identification and nomenclature of hybrids.

13.2 Objective 2.

To define the stock structure of blacklip abalone (Haliotis rubra) around Tasmania, using polymorphic nuclear DNA microsatellite markers.

- Very limited evidence of genetic heterogeneity was observed in the Tasmanian blacklip abalone population following examination of genetic diversity at 8 microsatellite markers in samples collected from 28 sites around Tasmania.
- Significant genetic differentiation was observed between some Tasmanian samples and ones from the mainland, indicating limited gene flow across Bass Strait.
- The Tasmanian population of blacklip abalone based on the evidence available from this study should be managed as one genetic stock.
- The results should be treated with some caution due to technical issues associated with some of the DNA markers and lack of age cohort information for the samples analysed, both of which should be clarified with further research.
- From the limited number of samples analysed from the mainland coast, an isolation-by-distance model may best fit the blacklip abalone population. This result should be examined in greater detail with more samples across the species range, including Western Australia and the sub-species status of *H. conicopora*.
- RFLP-mtDNA and microsatellite analyses detected similar levels of differentiation. While finer detailed analyses of the mtDNA genome, such as sequence analysis or SNP (single nucleotide polymorphism) technology may reveal as yet undetected population heterogeneity, nuclear DNA microsatellites are considered the most useful at this time. This assessment is based on a greater level of polymorphism and the ability to examine a number of independent markers. However, further investigation of mtDNA variation is recommended.

13.3 Objective 3.

To determine a suitable sampling and analysis regime for other temperate Australian abalone fisheries.

- There is limited conservation of microsatellite PCR primers between abalone species. However, optimising PCR conditions and minor modification of primer sequences does allow some markers developed for one species to be used for another species without the necessity of developing new genomic libraries for each species.
- Markers that will amplify for other potential Australian commercial species are available, but further species specific markers need to be developed and/or PCR conditions optimised further for the blacklip derived markers. Overall, more molecular markers are required for abalone.
- Significant population differentiation in greenlip abalone using four microsatellite markers (originally developed for blacklip abalone) was observed between three sites along the Western Australian coast. These sites were also significantly differentiated from three Tasmanian sites, which were not significantly different to each other.
- The heterogeneity within the Western Australian greenlip population requires further examination involving more sample sites and additional markers.
- The results of the greenlip study, like the blacklip study, suggest an isolation-by-distance model may best fit the population. This result should be examined further with more samples along the mainland coast.

13.4 Objective 4.

To determine the possible effects of harvesting on the genetic conservation of the blacklip abalone (H. rubra), by comparing the allozyme variation of two areas of the Tasmanian fishery with results obtained from the same areas in the late 1980s.

- No evidence was observed that current harvesting levels of blacklip abalone at two regions of the Tasmanian fishery have had a measureable negative impact on allozyme genetic diversity.
- The microsatellite loci (higher number of alleles and higher heterozygosity) now available will be more useful markers for assessing temporal genetic changes. A good baseline has now been established.
- However, waiting to detect any measureable change in genetic diversity may be too late for the long-term survival of a population. A more useful method of monitoring harvest impact may be to routinely examine effective (breeding) population sizes through annual analysis of genetic diversity in settling larvae at selected sites.

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APPENDIX 1: Intellectual Property

The intellectual property and valuable information arising from this research are:

1. DNA sequences for the COI, COII and lysin genes of various species.

2. PCR amplification primers for the species identification protocol, and species specific restriction fragments (published in Journal of Shellfish Research).

3. Copyright in this report

APPENDIX 2: Staff

Staff engaged on the project:

Principal Investigator	
Dr Rickard Officer (up to November 2000)	Tasmanina Aquaculture and Fisheries Institute
Assoc.Prof. Malcolm Haddon (from November 2000)	Tasmanina Aquaculture and Fisheries Institute
Co Investigator	
Dr Nicholas Elliott	CSIRO Marine Research
Research Assistants	
Mr Jason Bartlett	CSIRO Marine Research
Ms Natalie Conod	CSIRO Marine Research
Dr Brad Evans	CSIRO Marine Research
Collaborative researcher	
Dr Neville Sweijd	Univeristy of Cape Town, South Africa

APPENDIX 3: Presentations and Publications

1. Conference and workshop presentations made in relation to research arising from this project (first author presenting)

Elliott, N. and Officer R. 1999. Application of molecular genetics to Australian abalone fisheries: Forensic protocol for species ID and blacklip stock structure. Invited paper to the National Abalone Compliance Workshop, Queenscliff, Victoria (May 1999).

Elliott, N.G., Evans, B., Conod, N., Bartlett, J. Officer, R. and Sweijd, N. 2000. Application of molecular genetics to the understanding of abalone population structure – Australian and South African case studies. Invited paper at the 4th International Abalone Symposium, Cape Town, South Africa (February 2000).

Sweijd, N. A., Evans, B., Elliott, N.G. and Cook, P.A. 2000. Molecular tools for compliance enforcement – the identification of southern hemisphere abalone species from abalone products. 4th International Abalone Symposium, Cape Town, South Africa (February 2000).

Bartlett, J., Evans, B., Conod, N., Sweijd, N., Elliott, N., and Officer, R. 2000. Molecular tools for abalone research: stock structure and species identification. Australian Marine Sciences Association Annual Conference, Sydney, New South Wales (July 2000).

Sweijd, N.A., Evans B.S., Elliott, N.G., Bartlett, J. and Cook, P. 2000. DNA markers for the delineation of Southern Hemisphere abalone (*Haliotis*) species for forensic applications. (Poster) International Congress on Authenticity of Species in Meat and Seafood Products Vigo, Spain (September 2000)

Bartlett, J., Elliott, N. G., Evans, B. S., Sweijd, N. A., Cook, P. 2001. DNA markers for the delineation of southern hemisphere abalone (*Haliotis*) species for forensic applications. (Poster) 48th Annual Meeting of the Genetics Society of Australia. Adelaide, South Australia (July 2001).

Elliott, N.G. 2001. Genetic discrimination of abalone – genetic markers for species to individuals. 8th Abalone Aquaculture Workshop. Perth, Western Australia (July 2001)

Elliott, N.G. 2001.Genetics and abalone stock enhancement. Abalone Stock Enhancement Workshop, Cronulla, New South Wales (August 2001).

Elliott, N. G. 2001. Genetic diversity in Australian abalone populations. Invited paper at the Inaugural National Abalone Convention, Adelaide, South Australia. (August 2001).

Elliott, N. 2001. Genetic discrimination. Invited paper at the National Abalone Compliance Workshop, Hobart, Tasmania (October 2001).

In addition, presentations were made at the Tasmanian Abalone Research Advisory Group meetings.

2. Publications in relation to research arising from this project

a. Peer-reviewed scientific literature

Evans B, Conod N, Elliott N.G. 2001 Evaluation of microsatellite primer conservation in abalone. *Journal of Shellfish Research* 20: 1065-1070

Elliott, N. G., Bartlett, J., Evans, B., and Sweijd, N.A. 2002. Identification of Southern Hemisphere abalone (*Haliotis*) species by PCR-RFLP analysis of mitochondrial DNA. *Journal of Shellfish Research* 21 (in press)

Conod, N., Bartlett, J.P., Evans, B.S. and Elliott, N.G. 2002. Comparison of mitochondrial and nuclear DNA analyses of population structuring in the blacklip abalone *Haliotis rubra* Leach. *Marine and Freshwater Research* 53: 1–8

Other articles planned for the international scientific literature are in progress at the time of publication.

b. Reports and other publications

Officer, R and Elliott, N. 1999. New Research project: Using molecular genetics to develop forensic protocols for species ID and stock structure in Australian abalone fisheries. TAC News June 1999

Elliott, N. and Bartlett, J. 2000. DNA-based species identification of confiscated abalone. Report to Marine and Freshwater Resources Institute, Victoria. June 2000.

Elliott, N., Bartlett, J., Mooney, B. and Nichols, P. 2000. Analyses of discoloured blacklip abalone. Report to Tasmanian Aquaculture and Fisheries Institute. July 2000

Elliott, N. 2001. Application of Molecular Genetics to Australian Abalone Fisheries FRDC Project 1999/164. Protocol for Species ID. Progress Report to National Compliance Committee.

Elliott, N. and Conod, N. 2001. Microsatellite Analysis of Western Australian and Tasmanian Greenlip Abalone (*Haliotis laevigata)*. Report to Fisheries Western Australia.

Elliott, N., Conod, N., Maguire, G., Evans, B., Bartlett, J. 2001. Preliminary genetic comparison of Western Australian and Tasmanian greenlip abalone (*Haliotis laevigata*). *In* Proceedings of the 8th Abalone Aquaculture Workshop. Fremantle, Western Australia. July 2001

Elliott, N. 2001.Genetics and abalone stock enhancement. *In* Proceedings of Abalone Stock Enhancement Workshop, Cronulla, New South Wales. August 2001.

Elliott, N., Evans, B., Bartlett, J. and Conod, N. 2001. Genetic diversity in Australian abalone populations. *In* Proceedings of Inaugural National Abalone Convention, Adelaide, South Australia. August 2001.

APPENDIX 4: Microsatellite allele frequency tables

Tables of allele frequencies for each of the eight microsatellite loci for each *Haliotis rubra* collection analysed.

In all tables: 'Allele' represents the allele size in base pairs, and '*N'* is the number of individuals scored at that locus for the respective site.

Sites analysed were:

Abbreviation	Site name	Abbreviation	Site name
KIA	Kiama	CUB	Curio Bay
JEB	Jervis Bay	GTR	Georges III Reef
PEP	Pearl Point	LBA	Louisa Bay
CAP	Cape Conran	LBAT	Louisa Bay – temporal sample
PC	Point Cook	ACT	Actaeon Island
PMC	Port MacDonell	BLR	Black Reef
WEB	West Bay	STI	Sterile Island
CAT	Cat Island	WHA	Whalers Point
PAS	Passage Island	BLO	Block 11
GRK	Georges Rocks	HRP	High Rocky Point
LOPR	Long Point Reef	GRA	Granville Harbour
LOPII	Long Point Site 2	HI	Hunter Island
LOPI	Long Point Site 1	SMG	Smiths Gulch
NUIVG	Nuggets No. 4 - The Gap	MCA	Mount Cameron
NUGII	Nuggets No. 2	LTI	Little Trefoil Island
NUIVN	Nuggets No. 4 - North Face	BLP	Bluff Point
LBR	Lemon Rock	SUB	Suicide Bay
LBE	Lemon Bight (east)	CR	Church Rocks
LBW	Lemon Bight (west)	SBP	Sandblow Point
TRC	Trumpeter Corner	WWR	Waterwitch Reef
OTP	One Tree Point	CAP	Cape Portland
OTPT	One Tree Point -temporal sample	LOH	Low Head

Table A4.1. *Haliotis rubra* allele frequencies for locus *CmrHr*1.14.

***CmrHr*1.14**													
Allele	**KIA**	**JEB**	**PEP**	**CAP**	**PC**	**PMC**	**WEB**	**CAT**	**PAS**	**GRK**	**LOPR**	**LOPII**	**LOPI**
249	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
251	0.045	0.034	0.067	0.058	0.000	0.078	0.010	0.100	0.100	0.060	0.063	0.064	0.300
253	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
257	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000
259	0.826	0.879	0.872	0.895	0.900	0.775	0.792	0.822	0.758	0.760	0.850	0.830	0.700
261	0.112	0.080	0.049	0.047	0.100	0.137	0.010	0.033	0.050	0.095	0.050	0.074	0.000
263	0.006	0.000	0.000	0.000	0.000	0.000	0.010	0.033	0.017	0.020	0.000	0.000	0.000
265	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.011	0.000	0.000	0.000	0.000	0.000
267	0.006	0.000	0.006	0.000	0.000	0.000	0.073	0.000	0.025	0.030	0.000	0.021	0.000
269	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.010	0.038	0.000	0.000
271	0.000	0.006	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.005	0.000	0.011	0.000
275	0.000	0.000	0.000	0.000	0.000	0.010	0.063	0.000	0.008	0.010	0.000	0.000	0.000
277	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
279	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000
281	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
283	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
285	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
287	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
289	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000
291	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
293	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
297	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
301	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*89*	*87*	*82*	*43*	*30*	*51*	*48*	*45*	*60*	*100*	*40*	*47*	*5*

***CmrHr*1.14 cont.**											
Allele	**NUIVG**	**NUGII**	**NUIVN**	**LBR**	**LBE**	**LBW**	**TRC**	**OTP**	**OTPT**	**CUB**	**GTR**
249	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
251	0.179	0.033	0.024	0.083	0.000	0.070	0.091	0.043	0.078	0.060	0.113
253	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
257	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
259	0.769	0.800	0.810	0.740	0.833	0.750	0.768	0.864	0.845	0.832	0.766
261	0.051	0.067	0.060	0.135	0.117	0.130	0.116	0.076	0.069	0.076	0.073
263	0.000	0.033	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.011	0.000
265	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.008
267	0.000	0.000	0.024	0.021	0.017	0.000	0.005	0.016	0.000	0.011	0.016
269	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
271	0.000	0.000	0.024	0.010	0.033	0.010	0.000	0.000	0.009	0.000	0.024
275	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
277	0.000	0.000	0.000	0.010	0.000	0.000	0.010	0.000	0.000	0.000	0.000
279	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
281	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
283	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
285	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
287	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
289	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
291	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
293	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
297	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
301	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*39*	*15*	*42*	*48*	*30*	*50*	*99*	*92*	*58*	*92*	*62*

***CmrHr*1.14 cont.**

Allele	LBA	LBAT	ACT	BLR	STI	WHA	BLO	HRP	GRA	HI	SMG
249	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000
251	0.068	0.077	0.067	0.048	0.083	0.042	0.068	0.112	0.074	0.015	0.065
253	0.000	0.000	0.011	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
257	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
259	0.835	0.797	0.742	0.851	0.800	0.844	0.779	0.719	0.824	0.871	0.785
261	0.045	0.099	0.101	0.065	0.089	0.068	0.105	0.107	0.074	0.077	0.120
263	0.011	0.000	0.011	0.012	0.006	0.010	0.011	0.005	0.000	0.005	0.000
265	0.000	0.000	0.000	0.006	0.000	0.000	0.005	0.000	0.009	0.000	0.005
267	0.017	0.005	0.000	0.012	0.011	0.010	0.005	0.010	0.000	0.015	0.010
269	0.000	0.016	0.039	0.000	0.006	0.000	0.011	0.020	0.000	0.000	0.010
271	0.000	0.000	0.022	0.000	0.000	0.005	0.000	0.005	0.000	0.000	0.005
275	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.005	0.000	0.005	0.000
277	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000
279	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
281	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.005	0.000
283	0.000	0.000	0.000	0.000	0.000	0.005	0.005	0.000	0.000	0.000	0.000
285	0.000	0.000	0.000	0.000	0.000	0.005	0.005	0.000	0.000	0.005	0.000
287	0.000	0.005	0.000	0.000	0.000	0.000	0.005	0.000	0.019	0.000	0.000
289	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
291	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
293	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
297	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
301	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*88*	*91*	*89*	*84*	*90*	*96*	*95*	*98*	*54*	*97*	*100*

***CmrHr*1.14 cont.**

Allele	MCA	LTI	BLP	SUB	CR	SBP	WWR	CAP	LOH	Total
249	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
251	0.076	0.103	0.076	0.086	0.091	0.082	0.023	0.034	0.016	0.066
253	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
257	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
259	0.783	0.799	0.842	0.763	0.753	0.755	0.788	0.814	0.780	0.803
261	0.101	0.067	0.076	0.108	0.121	0.054	0.098	0.051	0.005	0.082
263	0.000	0.000	0.000	0.005	0.000	0.011	0.015	0.008	0.027	0.006
265	0.000	0.005	0.000	0.000	0.000	0.005	0.015	0.008	0.033	0.003
267	0.010	0.005	0.000	0.016	0.010	0.016	0.015	0.025	0.038	0.012
269	0.005	0.015	0.000	0.000	0.015	0.011	0.008	0.000	0.005	0.006
271	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.004
275	0.005	0.005	0.000	0.005	0.000	0.016	0.000	0.000	0.016	0.004
277	0.000	0.000	0.000	0.005	0.000	0.011	0.000	0.000	0.000	0.001
279	0.000	0.000	0.000	0.000	0.000	0.011	0.023	0.025	0.038	0.003
281	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.017	0.011	0.002
283	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.016	0.001
285	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.001
287	0.000	0.000	0.000	0.005	0.000	0.027	0.008	0.000	0.000	0.002
289	0.005	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.001
291	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
293	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
297	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
301	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*99*	*97*	*92*	*93*	*99*	*92*	*66*	*59*	*91*	*3124*

Table A4.2. *Haliotis rubra* allele frequencies for locus *CmrHr*1.24.

***CmrHr*1.24**													
Allele	**KIA**	**JEB**	**PEP**	**CAP**	**PC**	**PMC**	**WEB**	**CAT**	**PAS**	**GRK**	**LOPR**	**LOPII**	**LOPI**
202	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
214	0.005	0.000	0.000	0.000	0.000	0.010	0.000	0.006	0.000	0.000	0.000	0.000	0.000
216	0.022	0.000	0.005	0.007	0.000	0.000	0.000	0.006	0.000	0.005	0.000	0.010	0.000
218	0.000	0.000	0.000	0.013	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000
220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
222	0.846	0.855	0.896	0.860	0.967	0.913	0.968	0.825	0.938	0.810	0.802	0.837	0.793
224	0.088	0.075	0.049	0.087	0.017	0.029	0.021	0.123	0.040	0.095	0.177	0.071	0.073
226	0.027	0.065	0.038	0.007	0.017	0.048	0.000	0.026	0.023	0.060	0.021	0.051	0.098
228	0.011	0.005	0.005	0.000	0.000	0.000	0.000	0.013	0.000	0.025	0.000	0.020	0.024
230	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
234	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
236	0.000	0.000	0.005	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.012
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*91*	*100*	*91*	*75*	*30*	*52*	*47*	*77*	*88*	*100*	*48*	*49*	*41*

***CmrHr*1.24 cont.**											
Allele	**NUIVG**	**NUGII**	**NUIVN**	**LBR**	**LBE**	**LBW**	**TRC**	**OTP**	**OTPT**	**CUB**	**GTR**
202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
216	0.000	0.033	0.000	0.000	0.000	0.000	0.010	0.011	0.018	0.027	0.000
218	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
220	0.011	0.000	0.000	0.000	0.013	0.011	0.005	0.000	0.000	0.000	0.008
222	0.851	0.900	0.815	0.793	0.934	0.904	0.780	0.797	0.851	0.824	0.894
224	0.064	0.067	0.130	0.141	0.053	0.064	0.115	0.110	0.114	0.115	0.061
226	0.043	0.000	0.033	0.033	0.000	0.021	0.065	0.044	0.009	0.011	0.030
228	0.021	0.000	0.011	0.022	0.000	0.000	0.020	0.027	0.009	0.022	0.008
230	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
234	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
236	0.000	0.000	0.011	0.011	0.000	0.000	0.005	0.005	0.000	0.000	0.000
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*47*	*15*	*46*	*46*	*38*	*47*	*100*	*91*	*57*	*91*	*66*

***CmrHr*1.24 cont.**											
Allele	**LBA**	**LBAT**	**ACT**	**BLR**	**STI**	**WHA**	**BLO**	**HRP**	**GRA**	**HI**	**SMG**
202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
212	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
216	0.000	0.017	0.000	0.011	0.011	0.000	0.005	0.010	0.000	0.010	0.020
218	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
220	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.005	0.000
222	0.812	0.878	0.818	0.837	0.826	0.806	0.896	0.811	0.827	0.820	0.785
224	0.145	0.061	0.131	0.073	0.103	0.102	0.047	0.133	0.112	0.110	0.115
226	0.022	0.044	0.028	0.067	0.043	0.066	0.031	0.026	0.020	0.030	0.060
228	0.011	0.000	0.011	0.006	0.005	0.010	0.021	0.020	0.020	0.020	0.020
230	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
234	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
236	0.000	0.000	0.000	0.006	0.011	0.000	0.000	0.000	0.020	0.005	0.000
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*93*	*90*	*88*	*89*	*92*	*98*	*96*	*98*	*49*	*100*	*100*

***CmrHr*1.24 cont.**										
Allele	**MCA**	**LTI**	**BLP**	**SUB**	**CR**	**SBP**	**WWR**	**CAP**	**LOH**	**Total**
202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
212	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
216	0.025	0.020	0.005	0.010	0.015	0.011	0.007	0.008	0.011	0.009
218	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
220	0.000	0.010	0.005	0.005	0.000	0.000	0.000	0.016	0.000	0.002
222	0.800	0.869	0.773	0.835	0.790	0.895	0.833	0.847	0.905	0.842
224	0.110	0.071	0.146	0.108	0.145	0.079	0.087	0.040	0.032	0.093
226	0.040	0.020	0.061	0.031	0.045	0.011	0.051	0.040	0.042	0.037
228	0.015	0.000	0.010	0.010	0.005	0.005	0.022	0.048	0.005	0.012
230	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
234	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
236	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
N	*100*	*99*	*99*	*97*	*100*	*95*	*69*	*62*	*95*	*3342*

Table A4.3. *Haliotis rubra* allele frequencies for locus *CmrHr*2.14.

*CmrHr*2.14 Allele	KIA	JEB	PEP	CAP	PC	PMC	WEB	CAT	PAS	GRK	LOPR	LOPII	LOPI
188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
200	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
208	0.011	0.005	0.011	0.011	0.000	0.017	0.000	0.007	0.021	0.016	0.000	0.000	0.042
212	0.028	0.026	0.029	0.017	0.000	0.008	0.008	0.020	0.032	0.032	0.000	0.026	0.000
216	0.133	0.107	0.115	0.124	0.050	0.059	0.008	0.092	0.027	0.112	0.100	0.105	0.083
220	0.011	0.005	0.011	0.034	0.000	0.000	0.008	0.000	0.011	0.000	0.000	0.013	0.042
224	0.444	0.561	0.506	0.489	0.567	0.449	0.238	0.421	0.410	0.537	0.700	0.447	0.542
228	0.161	0.117	0.195	0.135	0.217	0.263	0.369	0.250	0.207	0.165	0.100	0.224	0.208
232	0.011	0.020	0.011	0.039	0.000	0.025	0.025	0.000	0.048	0.011	0.000	0.013	0.000
236	0.194	0.148	0.115	0.152	0.167	0.178	0.344	0.204	0.234	0.128	0.100	0.171	0.083
240	0.006	0.005	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000
248	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*90*	*98*	*87*	*89*	*30*	*59*	*61*	*76*	*94*	*94*	*5*	*38*	*12*

*CmrHr*2.14 cont. Allele	NUIVG	NUGII	NUIVN	LBR	LBE	LBW	TRC	OTP	OTPT	CUB	GTR
188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
200	0.000	0.000	0.000	0.010	0.029	0.000	0.000	0.000	0.000	0.000	0.016
208	0.010	0.033	0.011	0.031	0.000	0.021	0.005	0.007	0.008	0.000	0.016
212	0.031	0.000	0.022	0.042	0.059	0.021	0.025	0.048	0.085	0.056	0.016
216	0.052	0.067	0.033	0.042	0.088	0.083	0.095	0.089	0.068	0.099	0.081
220	0.000	0.000	0.000	0.021	0.000	0.000	0.010	0.000	0.025	0.037	0.016
224	0.500	0.500	0.500	0.427	0.441	0.469	0.475	0.527	0.508	0.420	0.419
228	0.240	0.200	0.217	0.198	0.294	0.188	0.185	0.151	0.144	0.247	0.274
232	0.021	0.033	0.022	0.063	0.000	0.021	0.010	0.007	0.008	0.019	0.016
236	0.146	0.167	0.196	0.167	0.088	0.198	0.195	0.171	0.144	0.123	0.145
240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000
248	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*48*	*15*	*46*	*48*	*17*	*48*	*100*	*73*	*59*	*81*	*62*

*CmrHr*2.14 cont. Allele	LBAT	ACT	BLR	STI	WHA	BLO	HRP	GRA	HI	SMG	MCA
188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
200	0.000	0.006	0.000	0.000	0.008	0.000	0.000	0.000	0.005	0.005	0.005
208	0.005	0.006	0.000	0.006	0.008	0.017	0.023	0.009	0.025	0.015	0.015
212	0.033	0.071	0.045	0.056	0.008	0.039	0.023	0.036	0.040	0.035	0.030
216	0.071	0.058	0.119	0.113	0.038	0.084	0.045	0.080	0.136	0.085	0.071
220	0.022	0.013	0.000	0.006	0.008	0.011	0.000	0.000	0.005	0.005	0.005
224	0.484	0.519	0.494	0.438	0.508	0.444	0.591	0.491	0.455	0.475	0.475
228	0.212	0.162	0.205	0.206	0.192	0.157	0.227	0.223	0.182	0.205	0.197
232	0.011	0.019	0.000	0.025	0.023	0.022	0.000	0.009	0.010	0.015	0.015
236	0.147	0.143	0.131	0.138	0.200	0.219	0.091	0.152	0.136	0.160	0.187
240	0.016	0.000	0.006	0.000	0.008	0.006	0.000	0.000	0.005	0.000	0.000
248	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*92*	*77*	*88*	*80*	*65*	*89*	*22*	*56*	*99*	*100*	*99*

*CmrHr*2.14 cont. Allele	LTI	BLP	SUB	CR	SBP	WWR	CAP	LOH	Total
188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
200	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.002
208	0.027	0.005	0.021	0.008	0.012	0.015	0.000	0.011	0.011
212	0.027	0.033	0.062	0.008	0.012	0.000	0.016	0.005	0.030
216	0.091	0.098	0.116	0.082	0.100	0.110	0.066	0.053	0.086
220	0.018	0.000	0.000	0.008	0.006	0.022	0.008	0.000	0.009
224	0.536	0.429	0.493	0.508	0.506	0.449	0.320	0.367	0.467
228	0.136	0.212	0.158	0.156	0.200	0.169	0.311	0.330	0.202
232	0.009	0.022	0.007	0.025	0.012	0.037	0.025	0.011	0.017
236	0.155	0.201	0.137	0.205	0.153	0.199	0.221	0.223	0.172
240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
248	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.001
N	*55*	*92*	*73*	*61*	*85*	*68*	*61*	*94*	*2886*

Table A4.4. *Haliotis rubra* allele frequencies for locus *CmrHr*2.30.

***CmrHr*2.30**													
Allele	**KIA**	**JEB**	**PEP**	**CAP**	**PC**	**PMC**	**WEB**	**CAT**	**PAS**	**GRK**	**LOPR**	**LOPII**	**LOPI**
268	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
270	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
272	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
274	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
278	0.000	0.000	0.006	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
280	0.005	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
282	0.016	0.005	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
284	0.005	0.020	0.000	0.006	0.000	0.009	0.000	0.020	0.011	0.015	0.010	0.010	0.012
286	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.020	0.000	0.012
288	0.000	0.000	0.011	0.006	0.000	0.000	0.000	0.000	0.027	0.000	0.010	0.010	0.012
290	0.016	0.005	0.034	0.018	0.000	0.009	0.026	0.059	0.032	0.045	0.010	0.042	0.012
292	0.011	0.000	0.011	0.000	0.000	0.009	0.000	0.007	0.000	0.030	0.000	0.031	0.012
294	0.022	0.015	0.011	0.018	0.000	0.000	0.060	0.026	0.022	0.060	0.020	0.000	0.000
296	0.044	0.066	0.073	0.059	0.017	0.082	0.043	0.059	0.038	0.020	0.000	0.052	0.024
298	0.132	0.136	0.056	0.059	0.067	0.064	0.034	0.099	0.054	0.095	0.050	0.031	0.049
300	0.055	0.076	0.039	0.041	0.017	0.073	0.017	0.020	0.048	0.060	0.100	0.042	0.110
302	0.132	0.126	0.084	0.065	0.167	0.100	0.078	0.066	0.059	0.075	0.070	0.135	0.098
304	0.137	0.111	0.096	0.065	0.067	0.082	0.095	0.079	0.118	0.020	0.070	0.042	0.049
306	0.033	0.025	0.056	0.041	0.017	0.027	0.026	0.039	0.048	0.030	0.010	0.063	0.037
308	0.027	0.061	0.073	0.071	0.100	0.045	0.034	0.072	0.038	0.045	0.070	0.052	0.024
310	0.027	0.035	0.022	0.024	0.050	0.018	0.095	0.026	0.022	0.035	0.050	0.042	0.012
312	0.071	0.051	0.062	0.053	0.100	0.073	0.095	0.046	0.108	0.025	0.110	0.073	0.061
314	0.022	0.020	0.017	0.006	0.000	0.036	0.034	0.026	0.032	0.035	0.030	0.000	0.049
316	0.011	0.015	0.011	0.006	0.033	0.027	0.060	0.007	0.032	0.020	0.030	0.010	0.049
318	0.022	0.020	0.000	0.018	0.067	0.018	0.017	0.039	0.032	0.010	0.000	0.021	0.012
320	0.005	0.025	0.000	0.006	0.033	0.009	0.043	0.007	0.005	0.015	0.000	0.000	0.012
324	0.005	0.010	0.011	0.012	0.000	0.027	0.026	0.013	0.027	0.040	0.010	0.052	0.000
326	0.016	0.025	0.011	0.047	0.033	0.027	0.034	0.007	0.032	0.030	0.000	0.010	0.061
328	0.022	0.025	0.051	0.059	0.017	0.082	0.026	0.020	0.048	0.020	0.030	0.042	0.037
330	0.027	0.005	0.022	0.059	0.050	0.018	0.017	0.026	0.011	0.040	0.020	0.031	0.012
332	0.005	0.010	0.006	0.018	0.017	0.009	0.009	0.000	0.011	0.010	0.010	0.000	0.000
338	0.005	0.010	0.022	0.018	0.050	0.027	0.060	0.046	0.005	0.020	0.020	0.031	0.012
340	0.016	0.010	0.000	0.012	0.000	0.000	0.000	0.020	0.011	0.010	0.000	0.000	0.012
342	0.000	0.005	0.011	0.006	0.000	0.000	0.000	0.000	0.005	0.000	0.010	0.021	0.000
344	0.000	0.005	0.000	0.012	0.000	0.018	0.017	0.020	0.022	0.015	0.020	0.010	0.012
346	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.005	0.005	0.020	0.010	0.000
348	0.005	0.000	0.017	0.012	0.000	0.000	0.017	0.007	0.005	0.010	0.020	0.010	0.024
350	0.016	0.010	0.006	0.018	0.000	0.000	0.000	0.007	0.011	0.005	0.010	0.010	0.000
352	0.005	0.015	0.006	0.024	0.000	0.018	0.000	0.000	0.000	0.010	0.000	0.010	0.000
354	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
356	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.013	0.005	0.005	0.020	0.000	0.024
358	0.011	0.000	0.022	0.012	0.033	0.000	0.009	0.007	0.000	0.005	0.010	0.000	0.012
360	0.011	0.015	0.045	0.035	0.017	0.027	0.000	0.007	0.027	0.015	0.030	0.031	0.049
364	0.011	0.020	0.028	0.018	0.000	0.009	0.000	0.020	0.011	0.025	0.040	0.010	0.049
368	0.005	0.005	0.011	0.012	0.000	0.009	0.000	0.007	0.000	0.010	0.000	0.000	0.012
372	0.016	0.010	0.022	0.018	0.050	0.018	0.017	0.026	0.011	0.045	0.060	0.021	0.024
380	0.005	0.000	0.011	0.012	0.000	0.000	0.000	0.026	0.011	0.025	0.000	0.010	0.012
384	0.011	0.000	0.017	0.012	0.000	0.009	0.000	0.013	0.005	0.005	0.000	0.010	0.000
388	0.000	0.000	0.000	0.012	0.000	0.009	0.009	0.000	0.000	0.000	0.000	0.010	0.000
392	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000
394	0.000	0.000	0.006	0.000	0.000	0.009	0.000	0.007	0.000	0.000	0.000	0.010	0.000
N	*91*	*99*	*89*	*85*	*30*	*55*	*58*	*76*	*93*	*100*	*50*	*48*	*41*

***CmrHr*2.30 cont.**												
Allele	**NUIVG**	**NUGII**	**NUIVN**	**LBR**	**LBE**	**LBW**	**TRC**	**OTP**	**OTPT**	**GTR**	**LBA**	**LBAT**
268	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
270	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
272	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
274	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
278	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
280	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
282	0.000	0.000	0.000	0.021	0.000	0.000	0.005	0.007	0.000	0.000	0.000	0.000
284	0.026	0.000	0.000	0.042	0.012	0.020	0.015	0.021	0.017	0.017	0.005	0.005
286	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
288	0.000	0.000	0.000	0.010	0.000	0.040	0.015	0.007	0.000	0.000	0.016	0.005
290	0.066	0.077	0.036	0.031	0.024	0.030	0.051	0.035	0.033	0.034	0.038	0.044
292	0.000	0.000	0.036	0.010	0.024	0.010	0.005	0.000	0.000	0.034	0.005	0.016
294	0.000	0.000	0.000	0.021	0.024	0.020	0.015	0.000	0.008	0.017	0.005	0.011
296	0.000	0.000	0.000	0.031	0.036	0.070	0.031	0.070	0.050	0.026	0.033	0.044
298	0.026	0.000	0.060	0.083	0.071	0.060	0.061	0.056	0.092	0.078	0.049	0.115
300	0.053	0.115	0.012	0.104	0.095	0.070	0.077	0.021	0.042	0.103	0.016	0.104
302	0.066	0.192	0.012	0.042	0.036	0.070	0.061	0.113	0.092	0.112	0.071	0.071
304	0.053	0.154	0.060	0.052	0.071	0.070	0.066	0.070	0.083	0.060	0.044	0.016
306	0.039	0.038	0.048	0.052	0.000	0.030	0.031	0.042	0.033	0.009	0.055	0.066
308	0.053	0.077	0.024	0.031	0.024	0.040	0.051	0.070	0.067	0.060	0.077	0.022
310	0.039	0.000	0.095	0.031	0.024	0.050	0.051	0.014	0.025	0.026	0.055	0.027
312	0.053	0.038	0.048	0.063	0.024	0.070	0.087	0.085	0.050	0.060	0.077	0.049
314	0.026	0.000	0.024	0.042	0.048	0.030	0.036	0.028	0.008	0.026	0.038	0.022
316	0.013	0.000	0.036	0.031	0.048	0.020	0.010	0.021	0.025	0.026	0.016	0.033
318	0.013	0.000	0.012	0.000	0.000	0.010	0.036	0.007	0.008	0.000	0.011	0.005
320	0.000	0.000	0.071	0.010	0.012	0.010	0.010	0.007	0.008	0.000	0.038	0.011
324	0.013	0.000	0.071	0.010	0.024	0.020	0.026	0.014	0.008	0.000	0.027	0.005
326	0.053	0.000	0.000	0.021	0.036	0.040	0.005	0.042	0.033	0.026	0.000	0.033
328	0.066	0.000	0.000	0.042	0.036	0.000	0.026	0.014	0.050	0.052	0.022	0.049
330	0.066	0.038	0.024	0.052	0.083	0.020	0.020	0.028	0.033	0.026	0.022	0.027
332	0.000	0.000	0.012	0.000	0.012	0.020	0.010	0.007	0.008	0.009	0.022	0.005
338	0.013	0.000	0.167	0.021	0.060	0.030	0.000	0.021	0.042	0.009	0.044	0.027
340	0.013	0.000	0.000	0.000	0.048	0.010	0.020	0.007	0.008	0.000	0.000	0.005
342	0.026	0.000	0.000	0.000	0.000	0.010	0.010	0.014	0.000	0.009	0.011	0.000
344	0.013	0.000	0.024	0.010	0.012	0.010	0.010	0.000	0.008	0.000	0.011	0.011
346	0.000	0.000	0.000	0.052	0.000	0.000	0.005	0.007	0.017	0.000	0.022	0.005
348	0.000	0.000	0.000	0.000	0.000	0.010	0.005	0.000	0.000	0.000	0.033	0.005
350	0.026	0.000	0.000	0.000	0.024	0.000	0.026	0.007	0.017	0.009	0.011	0.005
352	0.026	0.000	0.000	0.000	0.000	0.020	0.005	0.014	0.000	0.009	0.011	0.005
354	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000
356	0.013	0.000	0.000	0.000	0.012	0.010	0.020	0.000	0.008	0.000	0.005	0.000
358	0.013	0.000	0.012	0.010	0.000	0.000	0.005	0.007	0.025	0.017	0.005	0.005
360	0.026	0.038	0.036	0.021	0.000	0.000	0.010	0.014	0.025	0.026	0.016	0.005
364	0.039	0.077	0.036	0.021	0.036	0.000	0.020	0.014	0.000	0.017	0.016	0.038
368	0.000	0.000	0.012	0.000	0.012	0.010	0.005	0.007	0.000	0.017	0.005	0.011
372	0.039	0.038	0.012	0.021	0.012	0.020	0.020	0.070	0.058	0.017	0.011	0.055
380	0.013	0.038	0.000	0.000	0.024	0.030	0.020	0.014	0.008	0.017	0.033	0.005
384	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.007	0.000	0.000	0.005	0.000
388	0.000	0.038	0.024	0.010	0.000	0.010	0.010	0.000	0.000	0.026	0.005	0.005
392	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
394	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.026	0.005	0.011
N	*38*	*13*	*42*	*48*	*42*	*50*	*98*	*71*	*60*	*58*	*91*	*91*

***CmrHr*2.30 cont.**											
Allele	**ACT**	**BLR**	**STI**	**WHA**	**BLO**	**HRP**	**GRA**	**HI**	**SMG**	**MCA**	**LTI**
268	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
270	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.005	0.000
272	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.006
274	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
278	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
280	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
282	0.000	0.006	0.019	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.006
284	0.011	0.000	0.039	0.031	0.017	0.000	0.009	0.015	0.010	0.010	0.023
286	0.000	0.024	0.000	0.005	0.000	0.000	0.000	0.005	0.005	0.000	0.000
288	0.006	0.006	0.000	0.020	0.011	0.000	0.000	0.005	0.015	0.015	0.006
290	0.056	0.035	0.045	0.026	0.022	0.000	0.028	0.031	0.025	0.046	0.040
292	0.000	0.000	0.013	0.005	0.006	0.021	0.019	0.005	0.010	0.010	0.006
294	0.011	0.000	0.000	0.005	0.022	0.000	0.009	0.015	0.020	0.026	0.029
296	0.061	0.041	0.039	0.026	0.051	0.016	0.056	0.052	0.020	0.031	0.052
298	0.056	0.088	0.091	0.061	0.067	0.085	0.083	0.077	0.080	0.082	0.052
300	0.078	0.100	0.058	0.071	0.079	0.027	0.065	0.067	0.075	0.093	0.069
302	0.061	0.065	0.071	0.077	0.073	0.000	0.065	0.057	0.100	0.072	0.115
304	0.061	0.047	0.078	0.066	0.079	0.048	0.074	0.052	0.100	0.072	0.034
306	0.039	0.065	0.032	0.041	0.045	0.032	0.009	0.026	0.025	0.041	0.057
308	0.028	0.024	0.013	0.061	0.079	0.064	0.037	0.057	0.035	0.082	0.040
310	0.061	0.024	0.026	0.036	0.039	0.059	0.046	0.057	0.035	0.015	0.011
312	0.061	0.071	0.026	0.071	0.056	0.064	0.074	0.052	0.070	0.052	0.063
314	0.039	0.041	0.039	0.031	0.045	0.027	0.056	0.036	0.035	0.015	0.052
316	0.006	0.053	0.013	0.020	0.017	0.021	0.019	0.036	0.045	0.026	0.029
318	0.006	0.000	0.006	0.000	0.022	0.032	0.000	0.015	0.005	0.005	0.011
320	0.006	0.012	0.006	0.005	0.006	0.064	0.028	0.005	0.015	0.021	0.017
324	0.017	0.029	0.065	0.031	0.022	0.043	0.019	0.014	0.008	0.000	0.027
326	0.011	0.006	0.013	0.015	0.006	0.000	0.028	0.042	0.033	0.026	0.000
328	0.039	0.041	0.084	0.061	0.051	0.021	0.009	0.014	0.050	0.052	0.022
330	0.044	0.041	0.026	0.026	0.022	0.005	0.056	0.028	0.033	0.026	0.022
332	0.017	0.000	0.006	0.000	0.000	0.005	0.009	0.007	0.008	0.009	0.022
338	0.022	0.000	0.019	0.026	0.011	0.165	0.056	0.021	0.042	0.009	0.044
340	0.011	0.024	0.006	0.010	0.022	0.000	0.009	0.007	0.008	0.000	0.000
342	0.011	0.012	0.000	0.026	0.000	0.000	0.009	0.014	0.000	0.009	0.011
344	0.033	0.012	0.039	0.000	0.011	0.032	0.000	0.000	0.008	0.000	0.011
346	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.017	0.000	0.022
348	0.006	0.000	0.006	0.005	0.011	0.011	0.019	0.000	0.000	0.000	0.033
350	0.006	0.000	0.013	0.000	0.006	0.005	0.000	0.007	0.017	0.009	0.011
352	0.017	0.006	0.006	0.005	0.000	0.000	0.000	0.014	0.000	0.009	0.011
354	0.000	0.000	0.006	0.000	0.000	0.011	0.009	0.014	0.000	0.000	0.000
356	0.006	0.012	0.006	0.020	0.006	0.016	0.000	0.000	0.008	0.000	0.005
358	0.000	0.018	0.000	0.005	0.000	0.005	0.000	0.007	0.025	0.017	0.005
360	0.017	0.024	0.032	0.031	0.011	0.027	0.000	0.014	0.025	0.026	0.016
364	0.028	0.000	0.019	0.020	0.017	0.011	0.009	0.014	0.000	0.017	0.016
368	0.000	0.012	0.000	0.005	0.011	0.005	0.019	0.007	0.000	0.017	0.005
372	0.050	0.024	0.006	0.026	0.028	0.011	0.037	0.070	0.058	0.017	0.011
380	0.017	0.000	0.013	0.020	0.011	0.021	0.000	0.014	0.008	0.017	0.033
384	0.000	0.012	0.006	0.010	0.000	0.000	0.000	0.007	0.000	0.000	0.005
388	0.000	0.000	0.000	0.000	0.011	0.027	0.000	0.000	0.000	0.026	0.005
392	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
394	0.000	0.029	0.006	0.000	0.000	0.021	0.000	0.000	0.008	0.026	0.005
N	*90*	*85*	*77*	*98*	*89*	*94*	*54*	*97*	*100*	*97*	*87*

***CmrHr*2.30 cont.**

Allele	BLP	SUB	CR	SBP	WWR	CAP	LOH	Total
268	0.005	0.000	0.005	0.000	0.000	0.000	0.000	0.001
270	0.010	0.006	0.000	0.000	0.000	0.000	0.000	0.001
272	0.000	0.000	0.005	0.006	0.000	0.000	0.000	0.001
274	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
278	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000
280	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
282	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.003
284	0.015	0.006	0.015	0.017	0.008	0.012	0.019	0.013
286	0.005	0.006	0.020	0.000	0.008	0.012	0.028	0.004
288	0.005	0.006	0.015	0.000	0.008	0.000	0.019	0.008
290	0.036	0.028	0.015	0.034	0.015	0.024	0.028	0.031
292	0.026	0.017	0.015	0.028	0.008	0.012	0.019	0.011
294	0.021	0.017	0.010	0.017	0.015	0.024	0.000	0.016
296	0.041	0.033	0.040	0.063	0.068	0.012	0.028	0.042
298	0.062	0.056	0.070	0.017	0.061	0.226	0.065	0.073
300	0.067	0.044	0.115	0.102	0.038	0.060	0.065	0.065
302	0.098	0.072	0.075	0.057	0.091	0.036	0.065	0.078
304	0.077	0.072	0.065	0.080	0.061	0.024	0.139	0.070
306	0.036	0.039	0.055	0.068	0.068	0.012	0.019	0.039
308	0.046	0.061	0.030	0.080	0.038	0.024	0.046	0.050
310	0.021	0.022	0.040	0.051	0.030	0.000	0.037	0.035
312	0.067	0.094	0.045	0.063	0.045	0.083	0.074	0.064
314	0.031	0.044	0.010	0.040	0.068	0.024	0.019	0.031
316	0.010	0.011	0.030	0.034	0.045	0.024	0.009	0.024
318	0.005	0.017	0.000	0.034	0.030	0.000	0.019	0.014
320	0.021	0.000	0.000	0.034	0.023	0.000	0.037	0.015
324	0.031	0.028	0.015	0.000	0.023	0.048	0.028	0.023
326	0.021	0.044	0.025	0.028	0.023	0.060	0.019	0.023
328	0.041	0.061	0.040	0.023	0.023	0.071	0.028	0.039
330	0.021	0.028	0.020	0.000	0.008	0.012	0.019	0.025
332	0.000	0.022	0.000	0.034	0.023	0.000	0.019	0.009
338	0.010	0.039	0.020	0.028	0.076	0.048	0.019	0.031
340	0.005	0.000	0.015	0.006	0.015	0.000	0.000	0.010
342	0.000	0.022	0.015	0.000	0.008	0.000	0.000	0.006
344	0.000	0.006	0.015	0.000	0.008	0.000	0.000	0.011
346	0.000	0.006	0.005	0.011	0.000	0.000	0.000	0.005
348	0.010	0.000	0.005	0.000	0.008	0.000	0.009	0.007
350	0.015	0.011	0.015	0.006	0.000	0.000	0.000	0.009
352	0.015	0.006	0.000	0.006	0.015	0.000	0.000	0.007
354	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
356	0.015	0.011	0.010	0.006	0.000	0.024	0.000	0.007
358	0.015	0.000	0.005	0.011	0.008	0.012	0.000	0.007
360	0.005	0.011	0.020	0.006	0.008	0.000	0.028	0.018
364	0.021	0.022	0.045	0.006	0.023	0.000	0.037	0.020
368	0.005	0.000	0.005	0.000	0.000	0.000	0.019	0.006
372	0.021	0.006	0.035	0.000	0.000	0.060	0.009	0.024
380	0.026	0.006	0.005	0.000	0.008	0.060	0.019	0.012
384	0.005	0.000	0.005	0.006	0.000	0.000	0.000	0.004
388	0.005	0.011	0.005	0.000	0.000	0.000	0.000	0.005
392	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
394	0.005	0.006	0.000	0.000	0.000	0.000	0.000	0.004
N	*97*	*90*	*100*	*88*	*66*	*42*	*54*	*3122*

Table A4.5. *Haliotis rubra* allele frequencies for locus *rubCA*.

rubCA													
Allele	**KIA**	**JEB**	**PEP**	**CAP**	**PC**	**PMC**	**WEB**	**CAT**	**PAS**	**GRK**	**LOPR**	**LOPII**	**LOPI**
110	0.000	0.005	0.000	0.011	0.000	0.008	0.000	0.027	0.000	0.015	0.020	0.000	0.000
114	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
116	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
118	0.267	0.137	0.167	0.149	0.033	0.153	0.000	0.220	0.086	0.305	0.270	0.290	0.154
122	0.011	0.011	0.012	0.000	0.000	0.017	0.000	0.007	0.000	0.020	0.020	0.030	0.000
124	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
126	0.006	0.011	0.012	0.000	0.000	0.000	0.000	0.013	0.006	0.020	0.020	0.020	0.013
128	0.011	0.011	0.030	0.023	0.033	0.000	0.000	0.007	0.006	0.005	0.020	0.010	0.013
130	0.000	0.000	0.006	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
132	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.010	0.000
134	0.017	0.022	0.012	0.023	0.017	0.000	0.000	0.000	0.029	0.015	0.040	0.020	0.013
136	0.000	0.000	0.000	0.006	0.033	0.000	0.000	0.000	0.000	0.005	0.020	0.000	0.026
138	0.000	0.011	0.012	0.006	0.017	0.034	0.017	0.020	0.023	0.015	0.010	0.030	0.026
140	0.006	0.038	0.030	0.023	0.000	0.008	0.076	0.007	0.023	0.000	0.010	0.040	0.013
142	0.033	0.022	0.012	0.000	0.100	0.051	0.076	0.020	0.034	0.045	0.070	0.060	0.064
144	0.017	0.038	0.030	0.029	0.050	0.042	0.076	0.053	0.080	0.065	0.020	0.060	0.013
146	0.000	0.038	0.018	0.017	0.000	0.008	0.008	0.013	0.011	0.010	0.020	0.000	0.013
148	0.039	0.033	0.077	0.011	0.100	0.042	0.025	0.027	0.063	0.020	0.040	0.010	0.000
150	0.011	0.016	0.006	0.023	0.017	0.034	0.017	0.013	0.006	0.015	0.010	0.000	0.013
152	0.067	0.077	0.065	0.132	0.100	0.042	0.034	0.073	0.046	0.020	0.050	0.030	0.026
154	0.050	0.033	0.012	0.023	0.000	0.051	0.000	0.040	0.011	0.005	0.020	0.020	0.026
156	0.061	0.016	0.065	0.029	0.033	0.051	0.059	0.053	0.063	0.030	0.010	0.030	0.026
158	0.094	0.115	0.101	0.132	0.033	0.034	0.000	0.067	0.075	0.105	0.100	0.040	0.115
160	0.022	0.049	0.071	0.046	0.083	0.034	0.017	0.033	0.057	0.035	0.030	0.050	0.077
162	0.028	0.027	0.018	0.034	0.000	0.025	0.017	0.027	0.034	0.010	0.030	0.020	0.026
164	0.083	0.055	0.065	0.069	0.050	0.085	0.068	0.060	0.092	0.035	0.030	0.040	0.077
166	0.017	0.011	0.036	0.023	0.017	0.051	0.102	0.033	0.034	0.025	0.010	0.030	0.077
168	0.044	0.038	0.024	0.052	0.083	0.076	0.136	0.047	0.075	0.035	0.010	0.020	0.064
170	0.017	0.016	0.018	0.034	0.017	0.034	0.076	0.033	0.011	0.020	0.010	0.020	0.000
172	0.033	0.049	0.006	0.011	0.100	0.059	0.076	0.033	0.017	0.005	0.020	0.010	0.038
174	0.011	0.027	0.000	0.011	0.000	0.025	0.034	0.013	0.011	0.025	0.010	0.020	0.013
176	0.017	0.011	0.024	0.017	0.000	0.000	0.017	0.020	0.029	0.020	0.000	0.030	0.000
178	0.000	0.022	0.018	0.017	0.000	0.000	0.025	0.007	0.017	0.005	0.010	0.010	0.000
180	0.000	0.016	0.024	0.011	0.033	0.017	0.008	0.000	0.000	0.000	0.020	0.020	0.013
182	0.000	0.000	0.000	0.000	0.033	0.017	0.008	0.000	0.011	0.010	0.000	0.000	0.000
186	0.011	0.027	0.012	0.011	0.000	0.000	0.000	0.013	0.011	0.005	0.040	0.010	0.013
188	0.011	0.000	0.006	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
190	0.006	0.005	0.000	0.000	0.017	0.000	0.000	0.000	0.017	0.020	0.000	0.000	0.038
192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.017	0.015	0.000	0.010	0.000
194	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
196	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.007	0.000	0.010	0.010	0.010	0.000
198	0.006	0.000	0.006	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000
200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
206	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
208	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*90*	*91*	*84*	*87*	*30*	*59*	*59*	*75*	*87*	*100*	*50*	*50*	*39*

***rubCA* cont.**											
Allele	**NUIVG**	**NUGII**	**NUIVN**	**LBR**	**LBE**	**LBW**	**TRC**	**OTP**	**OTPT**	**CUB**	**GTR**
110	0.011	0.033	0.011	0.011	0.026	0.010	0.010	0.011	0.017	0.033	0.008
114	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.006	0.000	0.000	0.000
116	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
118	0.163	0.233	0.159	0.277	0.218	0.250	0.240	0.282	0.308	0.255	0.164
122	0.011	0.000	0.000	0.021	0.000	0.000	0.010	0.006	0.033	0.022	0.000
124	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
126	0.000	0.000	0.023	0.032	0.013	0.020	0.040	0.006	0.008	0.016	0.008
128	0.000	0.000	0.045	0.011	0.000	0.000	0.025	0.000	0.000	0.000	0.016
130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000
132	0.000	0.000	0.000	0.000	0.000	0.010	0.005	0.000	0.000	0.005	0.000
134	0.000	0.033	0.011	0.000	0.013	0.030	0.015	0.011	0.000	0.011	0.016
136	0.000	0.033	0.000	0.000	0.013	0.000	0.010	0.000	0.000	0.005	0.000
138	0.033	0.000	0.034	0.032	0.013	0.010	0.005	0.017	0.008	0.005	0.025
140	0.000	0.000	0.011	0.011	0.000	0.000	0.005	0.000	0.000	0.016	0.008
142	0.011	0.067	0.023	0.032	0.051	0.040	0.020	0.034	0.025	0.011	0.049
144	0.033	0.067	0.034	0.064	0.051	0.050	0.040	0.040	0.075	0.022	0.082
146	0.011	0.000	0.034	0.011	0.013	0.020	0.005	0.017	0.017	0.011	0.008
148	0.065	0.033	0.011	0.032	0.064	0.040	0.020	0.023	0.025	0.054	0.025
150	0.022	0.033	0.023	0.000	0.000	0.010	0.005	0.017	0.008	0.005	0.016
152	0.054	0.100	0.080	0.053	0.064	0.060	0.050	0.046	0.033	0.082	0.074
154	0.054	0.000	0.034	0.032	0.026	0.010	0.020	0.017	0.008	0.011	0.025
156	0.022	0.000	0.045	0.032	0.051	0.060	0.055	0.029	0.025	0.038	0.049
158	0.152	0.067	0.148	0.085	0.013	0.070	0.100	0.092	0.092	0.098	0.057
160	0.120	0.067	0.034	0.032	0.038	0.030	0.050	0.040	0.075	0.033	0.090
162	0.033	0.067	0.023	0.032	0.013	0.000	0.030	0.046	0.017	0.033	0.057
164	0.033	0.033	0.034	0.064	0.115	0.070	0.040	0.069	0.025	0.098	0.041
166	0.022	0.067	0.011	0.043	0.026	0.020	0.015	0.034	0.042	0.022	0.049
168	0.000	0.000	0.057	0.032	0.000	0.040	0.020	0.052	0.025	0.027	0.033
170	0.033	0.000	0.000	0.021	0.013	0.020	0.040	0.017	0.017	0.011	0.008
172	0.022	0.000	0.011	0.000	0.038	0.040	0.005	0.011	0.025	0.016	0.025
174	0.011	0.000	0.023	0.000	0.038	0.030	0.035	0.006	0.017	0.016	0.016
176	0.011	0.000	0.000	0.011	0.013	0.010	0.015	0.011	0.025	0.011	0.008
178	0.011	0.033	0.011	0.000	0.013	0.000	0.010	0.006	0.017	0.016	0.008
180	0.011	0.000	0.023	0.021	0.013	0.010	0.015	0.006	0.008	0.000	0.000
182	0.000	0.000	0.000	0.000	0.013	0.010	0.015	0.000	0.000	0.005	0.000
186	0.011	0.000	0.011	0.000	0.013	0.020	0.010	0.000	0.008	0.000	0.000
188	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000
190	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.006	0.008	0.000	0.000
192	0.011	0.000	0.000	0.000	0.026	0.000	0.005	0.000	0.000	0.005	0.016
194	0.011	0.000	0.011	0.011	0.000	0.000	0.005	0.017	0.000	0.000	0.016
196	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
198	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000
200	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000
204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
206	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000
208	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	46	15	44	47	39	50	100	87	60	92	61

***rubCA* cont.**											
Allele	**LBA**	**LBAT**	**ACT**	**BLR**	**STI**	**WHA**	**BLO**	**HRP**	**GRA**	**HI**	**SMG**
110	0.016	0.033	0.006	0.011	0.000	0.020	0.005	0.010	0.009	0.010	0.030
114	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
116	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.015	0.000
118	0.204	0.280	0.089	0.247	0.293	0.338	0.099	0.273	0.259	0.316	0.230
122	0.011	0.033	0.006	0.017	0.017	0.005	0.010	0.010	0.009	0.026	0.010
124	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
126	0.000	0.016	0.006	0.028	0.000	0.010	0.005	0.015	0.000	0.015	0.010
128	0.022	0.000	0.030	0.051	0.011	0.010	0.016	0.021	0.026	0.005	0.015
130	0.000	0.011	0.006	0.000	0.000	0.005	0.000	0.000	0.000	0.005	0.005
132	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
134	0.000	0.005	0.018	0.000	0.023	0.015	0.000	0.015	0.009	0.005	0.005
136	0.000	0.000	0.006	0.000	0.011	0.005	0.000	0.005	0.000	0.005	0.010
138	0.027	0.000	0.030	0.022	0.011	0.020	0.010	0.031	0.043	0.010	0.015
140	0.022	0.000	0.018	0.017	0.017	0.015	0.031	0.015	0.000	0.000	0.005
142	0.065	0.033	0.036	0.028	0.034	0.020	0.005	0.031	0.017	0.026	0.050
144	0.070	0.071	0.042	0.011	0.034	0.061	0.104	0.041	0.052	0.026	0.045
146	0.000	0.011	0.000	0.006	0.006	0.015	0.005	0.015	0.026	0.020	0.015
148	0.032	0.022	0.042	0.067	0.040	0.025	0.026	0.021	0.043	0.056	0.040
150	0.011	0.011	0.012	0.028	0.017	0.000	0.042	0.015	0.009	0.005	0.005
152	0.027	0.077	0.071	0.051	0.052	0.045	0.057	0.052	0.060	0.061	0.030
154	0.005	0.011	0.012	0.045	0.011	0.010	0.026	0.015	0.017	0.020	0.030
156	0.043	0.011	0.036	0.056	0.046	0.040	0.057	0.036	0.000	0.031	0.030
158	0.134	0.121	0.077	0.039	0.080	0.061	0.109	0.067	0.078	0.082	0.090
160	0.016	0.027	0.060	0.045	0.040	0.025	0.052	0.041	0.052	0.031	0.070
162	0.027	0.027	0.054	0.006	0.029	0.005	0.047	0.041	0.034	0.031	0.020
164	0.075	0.055	0.030	0.090	0.046	0.096	0.068	0.072	0.034	0.046	0.055
166	0.027	0.038	0.095	0.028	0.011	0.020	0.021	0.031	0.026	0.005	0.020
168	0.043	0.005	0.036	0.039	0.057	0.025	0.052	0.010	0.034	0.031	0.035
170	0.011	0.016	0.012	0.000	0.011	0.005	0.016	0.005	0.000	0.005	0.010
172	0.005	0.011	0.036	0.006	0.006	0.025	0.010	0.010	0.034	0.026	0.020
174	0.022	0.011	0.042	0.022	0.023	0.015	0.021	0.036	0.034	0.026	0.040
176	0.032	0.011	0.006	0.011	0.023	0.020	0.021	0.005	0.000	0.020	0.025
178	0.000	0.005	0.024	0.011	0.006	0.000	0.010	0.010	0.026	0.015	0.005
180	0.011	0.005	0.006	0.000	0.011	0.000	0.016	0.005	0.009	0.010	0.000
182	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.005	0.005
186	0.005	0.000	0.018	0.017	0.011	0.005	0.010	0.005	0.000	0.005	0.000
188	0.000	0.000	0.000	0.000	0.006	0.000	0.005	0.000	0.026	0.000	0.005
190	0.000	0.016	0.012	0.000	0.000	0.005	0.010	0.010	0.026	0.005	0.000
192	0.000	0.005	0.000	0.000	0.011	0.010	0.005	0.005	0.009	0.000	0.005
194	0.005	0.000	0.018	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000
196	0.005	0.000	0.006	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
198	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.005
200	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
202	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
206	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
208	0.000	0.000	0.006	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*93*	*91*	*84*	*89*	*87*	*99*	*96*	*97*	*58*	*98*	*100*

rubCA cont.

Allele	MCA	LTI	BLP	SUB	CR	SBP	WWR	CAP	LOH	Total
110	0.025	0.005	0.026	0.005	0.035	0.011	0.008	0.027	0.000	0.013
114	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
116	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.002
118	0.303	0.145	0.299	0.112	0.280	0.213	0.212	0.080	0.031	0.212
122	0.010	0.000	0.036	0.000	0.005	0.017	0.030	0.009	0.000	0.012
124	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
126	0.025	0.000	0.015	0.000	0.035	0.006	0.008	0.018	0.016	0.012
128	0.000	0.011	0.000	0.020	0.020	0.000	0.015	0.000	0.005	0.012
130	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.018	0.005	0.002
132	0.000	0.005	0.000	0.000	0.005	0.006	0.000	0.000	0.000	0.002
134	0.005	0.016	0.000	0.010	0.020	0.011	0.023	0.045	0.047	0.014
136	0.005	0.000	0.000	0.000	0.000	0.022	0.008	0.000	0.016	0.005
138	0.015	0.043	0.036	0.041	0.005	0.028	0.038	0.027	0.000	0.019
140	0.015	0.000	0.010	0.015	0.000	0.017	0.061	0.009	0.005	0.014
142	0.056	0.032	0.026	0.036	0.020	0.051	0.045	0.107	0.073	0.037
144	0.061	0.016	0.052	0.071	0.055	0.045	0.038	0.116	0.036	0.049
146	0.015	0.005	0.000	0.020	0.010	0.039	0.000	0.000	0.016	0.012
148	0.025	0.081	0.010	0.020	0.035	0.028	0.061	0.071	0.063	0.038
150	0.015	0.000	0.026	0.005	0.025	0.017	0.015	0.000	0.010	0.013
152	0.030	0.081	0.062	0.056	0.045	0.084	0.061	0.036	0.057	0.057
154	0.020	0.022	0.031	0.041	0.000	0.017	0.008	0.027	0.010	0.021
156	0.035	0.027	0.031	0.066	0.025	0.051	0.023	0.009	0.026	0.038
158	0.086	0.140	0.077	0.082	0.055	0.051	0.068	0.027	0.047	0.083
160	0.040	0.059	0.046	0.051	0.045	0.034	0.000	0.018	0.063	0.045
162	0.030	0.032	0.015	0.046	0.045	0.022	0.023	0.018	0.016	0.028
164	0.045	0.065	0.041	0.046	0.070	0.034	0.038	0.027	0.016	0.057
166	0.010	0.022	0.021	0.051	0.020	0.034	0.015	0.071	0.125	0.033
168	0.025	0.027	0.052	0.041	0.035	0.028	0.053	0.063	0.042	0.039
170	0.020	0.048	0.000	0.020	0.015	0.006	0.038	0.071	0.094	0.020
172	0.015	0.022	0.021	0.031	0.010	0.022	0.030	0.063	0.109	0.025
174	0.010	0.016	0.026	0.046	0.030	0.022	0.030	0.000	0.031	0.022
176	0.015	0.016	0.010	0.010	0.005	0.006	0.015	0.000	0.010	0.014
178	0.015	0.000	0.021	0.000	0.020	0.017	0.000	0.009	0.016	0.011
180	0.000	0.000	0.000	0.005	0.000	0.017	0.000	0.018	0.005	0.008
182	0.010	0.011	0.000	0.010	0.000	0.000	0.008	0.009	0.000	0.004
186	0.005	0.016	0.005	0.010	0.010	0.011	0.000	0.000	0.005	0.008
188	0.000	0.000	0.000	0.010	0.000	0.000	0.015	0.000	0.005	0.003
190	0.000	0.005	0.000	0.010	0.005	0.006	0.008	0.009	0.000	0.006
192	0.005	0.011	0.005	0.000	0.005	0.011	0.008	0.000	0.000	0.005
194	0.000	0.011	0.000	0.005	0.000	0.011	0.000	0.000	0.000	0.003
196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
198	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
202	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.001
204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
206	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
208	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
212	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000
N	*99*	*93*	*97*	*98*	*100*	*89*	*66*	*56*	*96*	*3328*

Table A4.6. *Haliotis rubra* allele frequencies for locus *CmrHr*1.25.

***CmrHr*1.25**														
Allele	**KIA**	**JEB**	**PEP**	**CAP**	**PMC**	**WEB**	**PAS**	**GRK**	**LOPR**	**LOPII**	**LOPI**	**LBR**	**LBE**	**LBW**
289	0.037	0.000	0.009	0.000	0.033	0.000	0.000	0.006	0.000	0.000	0.030	0.000	0.000	0.000
291	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
293	0.000	0.013	0.009	0.000	0.000	0.000	0.000	0.022	0.024	0.070	0.045	0.000	0.052	0.000
295	0.007	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.012	0.035	0.000	0.000	0.000	0.000
297	0.007	0.033	0.036	0.011	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.013	0.000	0.000
299	0.051	0.039	0.000	0.033	0.017	0.033	0.143	0.051	0.037	0.012	0.030	0.013	0.052	0.013
301	0.066	0.039	0.064	0.056	0.033	0.033	0.143	0.062	0.037	0.023	0.091	0.051	0.086	0.079
303	0.088	0.053	0.209	0.133	0.217	0.067	0.143	0.180	0.268	0.267	0.182	0.308	0.224	0.329
305	0.103	0.105	0.100	0.044	0.183	0.000	0.143	0.112	0.110	0.198	0.152	0.141	0.155	0.105
307	0.074	0.026	0.109	0.111	0.083	0.000	0.107	0.135	0.122	0.093	0.076	0.038	0.069	0.000
309	0.059	0.072	0.027	0.022	0.117	0.267	0.036	0.051	0.098	0.012	0.045	0.064	0.034	0.013
311	0.066	0.066	0.073	0.044	0.033	0.000	0.143	0.118	0.061	0.047	0.061	0.064	0.086	0.000
313	0.074	0.046	0.064	0.011	0.000	0.000	0.000	0.011	0.024	0.023	0.000	0.000	0.017	0.039
315	0.015	0.026	0.018	0.067	0.000	0.000	0.000	0.011	0.024	0.000	0.015	0.013	0.000	0.066
317	0.029	0.033	0.000	0.011	0.000	0.033	0.036	0.000	0.000	0.000	0.045	0.000	0.034	0.039
319	0.044	0.039	0.045	0.056	0.033	0.000	0.036	0.006	0.000	0.012	0.045	0.000	0.052	0.026
321	0.000	0.026	0.027	0.033	0.017	0.133	0.000	0.039	0.000	0.023	0.000	0.013	0.034	0.000
323	0.037	0.026	0.045	0.022	0.000	0.067	0.000	0.011	0.000	0.000	0.015	0.013	0.000	0.000
325	0.007	0.046	0.000	0.056	0.000	0.033	0.000	0.017	0.012	0.000	0.045	0.013	0.000	0.000
327	0.015	0.026	0.000	0.000	0.033	0.067	0.000	0.000	0.000	0.000	0.000	0.013	0.017	0.000
329	0.000	0.046	0.027	0.022	0.000	0.133	0.000	0.000	0.000	0.000	0.015	0.038	0.000	0.013
331	0.022	0.013	0.000	0.022	0.017	0.067	0.000	0.006	0.000	0.000	0.030	0.013	0.017	0.053
333	0.015	0.020	0.018	0.011	0.000	0.000	0.000	0.022	0.024	0.047	0.015	0.038	0.017	0.013
335	0.029	0.007	0.009	0.000	0.017	0.000	0.000	0.006	0.012	0.012	0.000	0.000	0.000	0.013
337	0.037	0.026	0.000	0.022	0.000	0.000	0.000	0.006	0.037	0.000	0.000	0.026	0.000	0.013
339	0.037	0.007	0.000	0.022	0.033	0.000	0.000	0.006	0.000	0.000	0.015	0.013	0.000	0.000
341	0.000	0.020	0.000	0.022	0.000	0.000	0.000	0.000	0.012	0.023	0.000	0.026	0.000	0.000
343	0.015	0.026	0.036	0.033	0.000	0.000	0.000	0.028	0.024	0.023	0.000	0.026	0.000	0.026
345	0.015	0.026	0.009	0.044	0.000	0.033	0.000	0.006	0.000	0.023	0.000	0.000	0.000	0.000
347	0.015	0.020	0.000	0.022	0.017	0.000	0.000	0.000	0.012	0.000	0.000	0.013	0.000	0.000
349	0.000	0.007	0.018	0.033	0.033	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.034	0.053
351	0.000	0.020	0.009	0.022	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.026
353	0.015	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.015	0.000	0.000	0.000
355	0.007	0.013	0.000	0.011	0.000	0.000	0.071	0.011	0.012	0.000	0.000	0.000	0.017	0.013
357	0.000	0.013	0.018	0.000	0.000	0.033	0.000	0.022	0.012	0.000	0.000	0.000	0.000	0.000
359	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.013
361	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.012	0.000	0.000	0.000	0.000
363	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.023	0.000	0.026	0.000	0.000
365	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.012	0.000	0.030	0.000	0.000	0.026
367	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000
369	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
371	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.026
373	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
379	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*68*	*76*	*55*	*45*	*30*	*15*	*14*	*89*	*41*	*43*	*33*	*39*	*29*	*38*

***CmrHr*1.25 cont.**

Allele	TRC	OTP	OTPT	CUB	GTR	LBA	LBAT	ACT	BLR	STI	WHA	BLO	HRP
289	0.000	0.000	0.000	0.030	0.000	0.012	0.000	0.000	0.000	0.008	0.013	0.000	0.006
291	0.000	0.000	0.014	0.000	0.024	0.006	0.000	0.008	0.000	0.000	0.000	0.000	0.006
293	0.006	0.013	0.108	0.023	0.012	0.030	0.045	0.069	0.038	0.000	0.013	0.000	0.049
295	0.041	0.013	0.041	0.008	0.012	0.012	0.006	0.015	0.013	0.000	0.013	0.000	0.019
297	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.024	0.000
299	0.047	0.038	0.054	0.015	0.000	0.042	0.032	0.054	0.051	0.032	0.033	0.048	0.031
301	0.106	0.051	0.041	0.030	0.048	0.012	0.064	0.062	0.025	0.040	0.020	0.048	0.031
303	0.229	0.288	0.149	0.182	0.202	0.274	0.269	0.208	0.209	0.230	0.213	0.190	0.179
305	0.053	0.160	0.122	0.106	0.107	0.054	0.115	0.100	0.146	0.095	0.093	0.143	0.117
307	0.100	0.038	0.095	0.174	0.131	0.173	0.064	0.115	0.089	0.143	0.127	0.143	0.117
309	0.124	0.147	0.054	0.076	0.155	0.065	0.083	0.085	0.063	0.071	0.033	0.119	0.080
311	0.082	0.032	0.162	0.091	0.071	0.095	0.135	0.100	0.051	0.087	0.107	0.167	0.111
313	0.018	0.013	0.000	0.061	0.048	0.018	0.000	0.008	0.006	0.040	0.013	0.000	0.037
315	0.006	0.051	0.000	0.023	0.024	0.012	0.000	0.023	0.013	0.016	0.033	0.000	0.025
317	0.029	0.013	0.027	0.000	0.000	0.018	0.019	0.000	0.006	0.008	0.007	0.048	0.031
319	0.018	0.006	0.000	0.023	0.012	0.024	0.019	0.015	0.032	0.024	0.013	0.024	0.006
321	0.012	0.000	0.041	0.000	0.012	0.024	0.026	0.008	0.013	0.008	0.027	0.024	0.025
323	0.018	0.013	0.014	0.008	0.012	0.000	0.019	0.000	0.013	0.008	0.007	0.000	0.000
325	0.012	0.000	0.027	0.008	0.000	0.000	0.006	0.015	0.019	0.032	0.020	0.000	0.006
327	0.012	0.026	0.027	0.023	0.024	0.006	0.000	0.000	0.019	0.000	0.020	0.024	0.000
329	0.000	0.000	0.000	0.015	0.000	0.006	0.019	0.023	0.013	0.008	0.027	0.000	0.006
331	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.032	0.024	0.000	0.000	0.012
333	0.012	0.000	0.000	0.008	0.024	0.018	0.006	0.000	0.044	0.000	0.027	0.000	0.025
335	0.012	0.000	0.000	0.015	0.000	0.006	0.006	0.000	0.038	0.016	0.000	0.000	0.025
337	0.012	0.013	0.000	0.008	0.000	0.000	0.013	0.008	0.000	0.024	0.013	0.000	0.006
339	0.018	0.019	0.000	0.000	0.024	0.000	0.006	0.015	0.000	0.032	0.013	0.000	0.006
341	0.000	0.006	0.014	0.015	0.000	0.006	0.006	0.008	0.006	0.016	0.013	0.000	0.019
343	0.012	0.006	0.000	0.000	0.000	0.000	0.013	0.000	0.006	0.000	0.007	0.000	0.012
345	0.006	0.006	0.000	0.008	0.024	0.018	0.000	0.015	0.000	0.016	0.007	0.000	0.000
347	0.000	0.000	0.014	0.000	0.000	0.000	0.006	0.023	0.000	0.000	0.000	0.000	0.000
349	0.006	0.013	0.000	0.008	0.000	0.006	0.013	0.015	0.006	0.000	0.013	0.000	0.000
351	0.000	0.013	0.000	0.008	0.000	0.018	0.000	0.000	0.000	0.000	0.007	0.000	0.000
353	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.006
355	0.000	0.000	0.000	0.023	0.012	0.000	0.000	0.008	0.019	0.000	0.000	0.000	0.000
357	0.000	0.000	0.000	0.000	0.000	0.024	0.006	0.000	0.006	0.008	0.013	0.000	0.000
359	0.012	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000
361	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000
363	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.008	0.000	0.000	0.000
365	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
367	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000
369	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.013	0.000	0.000
371	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
373	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006
379	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*85*	*78*	*37*	*66*	*42*	*84*	*78*	*65*	*79*	*63*	*75*	*21*	*81*

***CmrHr*1.25 cont.**

Allele	GRA	HI	SMG	MCA	LTI	SUB	CR	SBP	WWR	CAP	LOH	Total
289	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.045	0.043	0.000	0.000	0.007
291	0.021	0.006	0.007	0.000	0.000	0.014	0.000	0.000	0.000	0.018	0.000	0.003
293	0.021	0.011	0.026	0.011	0.044	0.021	0.018	0.030	0.000	0.018	0.000	0.023
295	0.000	0.006	0.000	0.000	0.026	0.007	0.018	0.000	0.000	0.027	0.056	0.012
297	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004
299	0.010	0.006	0.013	0.011	0.044	0.079	0.018	0.000	0.033	0.027	0.083	0.033
301	0.073	0.040	0.039	0.057	0.053	0.100	0.006	0.030	0.141	0.018	0.000	0.050
303	0.208	0.256	0.211	0.339	0.193	0.164	0.214	0.167	0.141	0.179	0.097	0.208
305	0.094	0.148	0.099	0.057	0.105	0.107	0.131	0.061	0.087	0.089	0.139	0.108
307	0.104	0.136	0.099	0.057	0.088	0.050	0.107	0.015	0.098	0.098	0.028	0.095
309	0.115	0.063	0.053	0.046	0.070	0.114	0.065	0.106	0.120	0.054	0.139	0.075
311	0.125	0.051	0.132	0.103	0.035	0.086	0.089	0.106	0.087	0.143	0.222	0.088
313	0.021	0.011	0.000	0.029	0.000	0.007	0.006	0.030	0.000	0.018	0.000	0.020
315	0.000	0.006	0.033	0.052	0.009	0.000	0.024	0.061	0.011	0.000	0.000	0.019
317	0.042	0.011	0.026	0.000	0.053	0.021	0.012	0.045	0.033	0.000	0.056	0.018
319	0.010	0.011	0.007	0.029	0.026	0.000	0.030	0.000	0.000	0.080	0.028	0.021
321	0.021	0.023	0.013	0.017	0.009	0.029	0.006	0.000	0.000	0.000	0.028	0.017
323	0.000	0.017	0.007	0.006	0.009	0.021	0.006	0.030	0.011	0.009	0.000	0.012
325	0.000	0.017	0.000	0.006	0.026	0.007	0.024	0.000	0.000	0.027	0.000	0.013
327	0.000	0.017	0.046	0.023	0.053	0.007	0.048	0.030	0.022	0.027	0.000	0.016
329	0.010	0.000	0.033	0.000	0.000	0.014	0.006	0.000	0.022	0.018	0.000	0.012
331	0.000	0.006	0.000	0.000	0.009	0.000	0.018	0.000	0.043	0.000	0.069	0.011
333	0.000	0.000	0.000	0.029	0.009	0.007	0.024	0.015	0.011	0.054	0.000	0.016
335	0.010	0.000	0.026	0.017	0.000	0.021	0.006	0.030	0.022	0.000	0.000	0.010
337	0.000	0.023	0.013	0.017	0.009	0.021	0.000	0.000	0.000	0.027	0.000	0.011
339	0.000	0.006	0.000	0.023	0.000	0.021	0.018	0.000	0.000	0.000	0.000	0.010
341	0.000	0.006	0.000	0.000	0.000	0.014	0.018	0.061	0.043	0.018	0.056	0.010
343	0.000	0.006	0.026	0.000	0.018	0.007	0.006	0.061	0.011	0.000	0.000	0.011
345	0.063	0.040	0.000	0.011	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.010
347	0.000	0.000	0.013	0.011	0.009	0.014	0.000	0.015	0.000	0.000	0.000	0.005
349	0.000	0.051	0.013	0.011	0.026	0.000	0.000	0.030	0.000	0.018	0.000	0.011
351	0.000	0.011	0.013	0.017	0.000	0.007	0.006	0.000	0.011	0.009	0.000	0.007
353	0.010	0.000	0.007	0.000	0.000	0.007	0.012	0.015	0.000	0.009	0.000	0.004
355	0.021	0.006	0.020	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006
357	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004
359	0.000	0.000	0.020	0.000	0.018	0.007	0.030	0.015	0.000	0.000	0.000	0.005
361	0.000	0.006	0.000	0.011	0.018	0.014	0.000	0.000	0.000	0.000	0.000	0.003
363	0.021	0.000	0.000	0.000	0.009	0.000	0.006	0.000	0.000	0.000	0.000	0.003
365	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.011	0.009	0.000	0.003
367	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.001
369	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.002
371	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.001
373	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000
379	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
N	*48*	*88*	*76*	*87*	*57*	*70*	*84*	*33*	*46*	*56*	*36*	*2150*

Table A4.7. *Haliotis rubra* allele frequencies for locus *CmrHr*2.26.

***CmrHr*2.26**												
Allele	**KIA**	**JEB**	**PEP**	**CAP**	**PC**	**PMC**	**WEB**	**PAS**	**GRK**	**LOPR**	**LOPII**	**LOPI**
158	0.006	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
168	0.041	0.072	0.038	0.021	0.058	0.048	0.267	0.016	0.126	0.047	0.076	0.013
172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
180	0.012	0.005	0.006	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000
184	0.012	0.010	0.025	0.000	0.038	0.000	0.000	0.008	0.011	0.012	0.043	0.013
188	0.140	0.119	0.131	0.167	0.231	0.143	0.033	0.145	0.105	0.093	0.130	0.179
192	0.203	0.273	0.275	0.083	0.212	0.310	0.133	0.242	0.216	0.267	0.261	0.244
196	0.180	0.139	0.150	0.333	0.058	0.000	0.067	0.113	0.126	0.105	0.109	0.205
200	0.140	0.113	0.144	0.167	0.077	0.190	0.367	0.145	0.116	0.186	0.196	0.077
204	0.169	0.165	0.088	0.104	0.115	0.119	0.100	0.145	0.100	0.163	0.065	0.141
208	0.041	0.072	0.038	0.042	0.154	0.048	0.033	0.097	0.105	0.047	0.043	0.064
212	0.041	0.005	0.025	0.083	0.019	0.048	0.000	0.048	0.021	0.023	0.043	0.038
216	0.006	0.015	0.019	0.000	0.019	0.024	0.000	0.000	0.037	0.012	0.022	0.000
220	0.012	0.010	0.013	0.000	0.019	0.071	0.000	0.000	0.000	0.023	0.000	0.000
224	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.008	0.021	0.012	0.000	0.000
228	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.012	0.000	0.013
232	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.011	0.000
236	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013
240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
244	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*86*	*97*	*80*	*24*	*26*	*21*	*15*	*62*	*95*	*43*	*46*	*39*

***CmrHr*2.26 cont.**											
Allele	**LBR**	**LBE**	**LBW**	**TRC**	**OTP**	**OTPT**	**CUB**	**GTR**	**LBA**	**LBAT**	**ACT**
158	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000
168	0.000	0.047	0.032	0.170	0.117	0.000	0.090	0.103	0.089	0.040	0.064
172	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000
180	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000
184	0.071	0.016	0.011	0.010	0.011	0.000	0.007	0.000	0.028	0.024	0.006
188	0.000	0.141	0.191	0.165	0.106	0.071	0.160	0.151	0.122	0.135	0.099
192	0.429	0.234	0.245	0.195	0.283	0.321	0.278	0.262	0.239	0.270	0.262
196	0.000	0.219	0.096	0.115	0.083	0.179	0.097	0.095	0.150	0.143	0.157
200	0.286	0.109	0.128	0.105	0.078	0.214	0.111	0.079	0.133	0.190	0.128
204	0.071	0.156	0.117	0.110	0.111	0.000	0.111	0.183	0.117	0.079	0.134
208	0.000	0.047	0.064	0.060	0.089	0.071	0.049	0.079	0.022	0.079	0.052
212	0.143	0.016	0.032	0.035	0.039	0.000	0.049	0.024	0.028	0.016	0.029
216	0.000	0.000	0.043	0.015	0.011	0.036	0.014	0.016	0.011	0.016	0.023
220	0.000	0.000	0.021	0.010	0.028	0.000	0.000	0.000	0.017	0.000	0.000
224	0.000	0.000	0.011	0.005	0.000	0.036	0.007	0.000	0.000	0.008	0.000
228	0.000	0.000	0.011	0.005	0.017	0.000	0.000	0.008	0.000	0.000	0.000
232	0.000	0.000	0.000	0.000	0.028	0.036	0.014	0.000	0.039	0.000	0.041
236	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006
244	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*7*	*32*	*47*	*100*	*90*	*14*	*72*	*63*	*90*	*63*	*86*

***CmrHr*2.26 cont.**

Allele	BLR	STI	WHA	BLO	HRP	GRA	HI	SMG	MCA	LTI	SUB
158	0.000	0.000	0.000	0.018	0.016	0.000	0.005	0.005	0.000	0.006	0.000
168	0.067	0.056	0.112	0.143	0.105	0.024	0.177	0.126	0.061	0.025	0.030
172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
180	0.000	0.000	0.000	0.000	0.000	0.012	0.010	0.010	0.000	0.000	0.006
184	0.000	0.043	0.011	0.036	0.016	0.000	0.005	0.000	0.010	0.012	0.000
188	0.096	0.130	0.160	0.161	0.168	0.195	0.152	0.096	0.148	0.167	0.133
192	0.326	0.160	0.245	0.071	0.279	0.317	0.182	0.202	0.179	0.259	0.217
196	0.124	0.179	0.090	0.161	0.121	0.171	0.116	0.152	0.122	0.130	0.205
200	0.169	0.160	0.176	0.196	0.121	0.098	0.126	0.106	0.184	0.130	0.145
204	0.107	0.130	0.101	0.089	0.063	0.061	0.101	0.162	0.133	0.123	0.145
208	0.090	0.093	0.048	0.054	0.042	0.037	0.051	0.056	0.051	0.080	0.060
212	0.017	0.019	0.027	0.036	0.042	0.000	0.040	0.030	0.041	0.025	0.024
216	0.006	0.012	0.016	0.036	0.011	0.012	0.010	0.010	0.026	0.019	0.024
220	0.000	0.000	0.016	0.000	0.000	0.024	0.010	0.000	0.026	0.006	0.006
224	0.000	0.019	0.000	0.000	0.005	0.012	0.000	0.005	0.000	0.012	0.006
228	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
232	0.000	0.000	0.000	0.000	0.011	0.037	0.015	0.020	0.020	0.000	0.000
236	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
244	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000
N	*89*	*81*	*94*	*28*	*95*	*41*	*99*	*99*	*98*	*81*	*83*

***CmrHr*2.26 cont.**

Allele	CR	SBP	WWR	CAP	LOH	Total
158	0.005	0.000	0.008	0.000	0.024	0.003
168	0.086	0.059	0.219	0.262	0.241	0.095
172	0.000	0.000	0.000	0.000	0.000	0.000
180	0.000	0.000	0.000	0.000	0.000	0.003
184	0.005	0.015	0.016	0.017	0.006	0.012
188	0.157	0.044	0.078	0.093	0.118	0.133
192	0.152	0.309	0.234	0.174	0.171	0.231
196	0.101	0.103	0.164	0.145	0.159	0.134
200	0.131	0.059	0.148	0.116	0.088	0.134
204	0.197	0.221	0.055	0.093	0.100	0.121
208	0.045	0.015	0.023	0.041	0.047	0.059
212	0.066	0.059	0.031	0.017	0.029	0.031
216	0.040	0.000	0.016	0.012	0.018	0.016
220	0.000	0.015	0.008	0.006	0.000	0.008
224	0.005	0.000	0.000	0.012	0.000	0.005
228	0.010	0.000	0.000	0.000	0.000	0.003
232	0.000	0.103	0.000	0.012	0.000	0.010
236	0.000	0.000	0.000	0.000	0.000	0.000
240	0.000	0.000	0.000	0.000	0.000	0.000
244	0.000	0.000	0.000	0.000	0.000	0.000
N	*99*	*34*	*64*	*86*	*85*	*2554*

Table A4.8. *Haliotis rubra* allele frequencies for locus *CmrHr*2.9.

***CmrHr*2.9**														
Allele	**KIA**	**JEB**	**PEP**	**CAP**	**PMC**	**WEB**	**PAS**	**GRK**	**LOPR**	**LOPII**	**LOPI**	**LBR**	**LBE**	**LBW**
154	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
156	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
158	0.108	0.078	0.091	0.086	0.235	0.672	0.151	0.155	0.116	0.163	0.158	0.195	0.135	0.191
160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000
162	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
164	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
166	0.199	0.224	0.250	0.160	0.157	0.000	0.163	0.144	0.163	0.238	0.237	0.122	0.108	0.202
168	0.011	0.010	0.011	0.025	0.020	0.000	0.041	0.026	0.035	0.000	0.000	0.012	0.027	0.011
170	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
172	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
174	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000
176	0.017	0.010	0.006	0.000	0.000	0.000	0.012	0.000	0.012	0.000	0.026	0.000	0.041	0.000
178	0.006	0.010	0.006	0.000	0.000	0.008	0.000	0.010	0.000	0.000	0.000	0.012	0.000	0.011
180	0.023	0.016	0.011	0.006	0.010	0.000	0.006	0.021	0.000	0.013	0.026	0.012	0.014	0.011
182	0.017	0.026	0.028	0.012	0.029	0.023	0.000	0.005	0.023	0.013	0.000	0.000	0.014	0.021
184	0.023	0.057	0.028	0.062	0.039	0.008	0.029	0.052	0.047	0.000	0.053	0.037	0.000	0.011
186	0.028	0.063	0.017	0.068	0.029	0.008	0.052	0.057	0.035	0.025	0.079	0.024	0.027	0.032
188	0.045	0.057	0.017	0.025	0.020	0.000	0.017	0.000	0.012	0.050	0.026	0.049	0.027	0.021
190	0.080	0.057	0.040	0.019	0.029	0.031	0.012	0.031	0.035	0.063	0.013	0.049	0.068	0.011
192	0.034	0.031	0.034	0.043	0.049	0.016	0.047	0.062	0.047	0.025	0.013	0.037	0.068	0.043
194	0.045	0.036	0.051	0.062	0.029	0.016	0.017	0.010	0.047	0.038	0.026	0.037	0.014	0.011
196	0.034	0.016	0.011	0.012	0.039	0.047	0.029	0.010	0.000	0.025	0.013	0.012	0.054	0.032
198	0.017	0.005	0.023	0.037	0.010	0.023	0.058	0.046	0.105	0.000	0.026	0.037	0.014	0.021
200	0.028	0.000	0.057	0.037	0.039	0.016	0.017	0.021	0.000	0.013	0.026	0.012	0.014	0.011
202	0.023	0.016	0.000	0.037	0.088	0.039	0.041	0.062	0.023	0.038	0.013	0.012	0.054	0.011
204	0.017	0.047	0.017	0.043	0.010	0.000	0.029	0.021	0.035	0.025	0.026	0.037	0.027	0.011
206	0.017	0.031	0.017	0.012	0.020	0.023	0.006	0.021	0.035	0.038	0.053	0.049	0.027	0.043
208	0.017	0.036	0.040	0.049	0.000	0.008	0.047	0.021	0.023	0.038	0.013	0.012	0.014	0.011
210	0.023	0.026	0.017	0.019	0.010	0.031	0.012	0.015	0.023	0.013	0.000	0.000	0.014	0.011
212	0.023	0.016	0.023	0.019	0.020	0.023	0.076	0.021	0.058	0.013	0.013	0.037	0.027	0.064
216	0.017	0.010	0.011	0.012	0.010	0.000	0.000	0.021	0.012	0.013	0.026	0.037	0.054	0.000
218	0.017	0.016	0.006	0.019	0.029	0.008	0.023	0.026	0.023	0.013	0.000	0.012	0.027	0.021
220	0.017	0.031	0.040	0.037	0.010	0.000	0.023	0.046	0.035	0.013	0.013	0.000	0.000	0.011
222	0.006	0.010	0.023	0.012	0.049	0.000	0.006	0.000	0.023	0.025	0.000	0.061	0.014	0.000
224	0.006	0.010	0.023	0.006	0.010	0.000	0.029	0.010	0.000	0.000	0.013	0.000	0.000	0.011
226	0.017	0.021	0.023	0.012	0.000	0.000	0.029	0.015	0.000	0.038	0.000	0.012	0.000	0.032
228	0.017	0.005	0.028	0.006	0.000	0.000	0.017	0.021	0.000	0.000	0.053	0.024	0.054	0.011
230	0.006	0.000	0.006	0.012	0.000	0.000	0.000	0.015	0.000	0.025	0.000	0.000	0.014	0.032
232	0.006	0.016	0.006	0.025	0.000	0.000	0.006	0.010	0.000	0.000	0.026	0.012	0.014	0.000
234	0.017	0.005	0.000	0.000	0.000	0.000	0.006	0.000	0.012	0.013	0.000	0.000	0.000	0.000
236	0.011	0.000	0.006	0.000	0.010	0.000	0.000	0.005	0.000	0.000	0.000	0.037	0.000	0.011
238	0.006	0.000	0.006	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.013	0.000	0.014	0.000
240	0.006	0.005	0.011	0.006	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.014	0.000
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.013	0.000	0.012	0.000	0.000
244	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.043
246	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.012	0.013	0.000	0.000	0.000	0.011
248	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000
252	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
254	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
258	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
262	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*88*	*96*	*88*	*81*	*51*	*64*	*86*	*97*	*43*	*40*	*38*	*41*	*37*	*47*

***CmrHr*2.9 cont.**

Allele	TRC	OTP	OTPT	CUB	GTR	LBA	LBAT	ACT	BLR	STI	WHA	BLO	HRP
154	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000
158	0.165	0.213	0.107	0.120	0.147	0.176	0.188	0.105	0.210	0.195	0.124	0.161	0.112
160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
162	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
164	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.005
166	0.175	0.184	0.238	0.098	0.155	0.129	0.113	0.130	0.125	0.122	0.134	0.097	0.133
168	0.010	0.023	0.012	0.033	0.034	0.024	0.038	0.012	0.000	0.024	0.027	0.016	0.015
170	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
174	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.010
176	0.000	0.000	0.000	0.033	0.000	0.012	0.006	0.000	0.017	0.000	0.011	0.000	0.000
178	0.036	0.029	0.000	0.000	0.009	0.024	0.000	0.012	0.000	0.006	0.000	0.000	0.005
180	0.021	0.011	0.012	0.016	0.000	0.018	0.006	0.006	0.017	0.012	0.011	0.000	0.015
182	0.010	0.000	0.000	0.027	0.017	0.006	0.000	0.000	0.017	0.012	0.011	0.016	0.020
184	0.026	0.023	0.060	0.027	0.026	0.018	0.044	0.012	0.011	0.043	0.059	0.065	0.031
186	0.046	0.052	0.071	0.060	0.052	0.018	0.031	0.062	0.068	0.055	0.027	0.016	0.082
188	0.005	0.011	0.024	0.016	0.017	0.059	0.006	0.037	0.034	0.000	0.038	0.032	0.036
190	0.052	0.023	0.024	0.011	0.017	0.018	0.031	0.019	0.045	0.024	0.038	0.032	0.036
192	0.046	0.029	0.119	0.027	0.052	0.059	0.088	0.056	0.074	0.079	0.016	0.065	0.056
194	0.026	0.023	0.012	0.065	0.017	0.018	0.031	0.025	0.034	0.018	0.016	0.032	0.031
196	0.031	0.023	0.012	0.005	0.052	0.006	0.013	0.043	0.011	0.024	0.032	0.000	0.015
198	0.026	0.029	0.024	0.060	0.034	0.029	0.025	0.049	0.028	0.067	0.070	0.032	0.015
200	0.026	0.040	0.000	0.033	0.017	0.035	0.006	0.025	0.034	0.018	0.048	0.032	0.031
202	0.021	0.017	0.012	0.027	0.060	0.047	0.025	0.006	0.023	0.012	0.022	0.048	0.031
204	0.010	0.023	0.024	0.005	0.009	0.035	0.056	0.031	0.034	0.030	0.005	0.000	0.020
206	0.052	0.023	0.024	0.038	0.034	0.024	0.044	0.043	0.006	0.018	0.038	0.000	0.020
208	0.021	0.011	0.012	0.011	0.017	0.006	0.038	0.043	0.006	0.037	0.027	0.016	0.026
210	0.005	0.017	0.000	0.016	0.009	0.012	0.013	0.012	0.017	0.012	0.038	0.000	0.041
212	0.010	0.011	0.024	0.049	0.043	0.024	0.038	0.019	0.040	0.018	0.016	0.048	0.046
216	0.031	0.023	0.012	0.038	0.017	0.029	0.025	0.025	0.023	0.049	0.027	0.000	0.010
218	0.031	0.006	0.012	0.022	0.026	0.018	0.013	0.019	0.023	0.024	0.022	0.032	0.010
220	0.021	0.011	0.000	0.038	0.009	0.018	0.006	0.056	0.017	0.012	0.016	0.081	0.015
222	0.026	0.023	0.024	0.005	0.009	0.018	0.006	0.019	0.006	0.024	0.016	0.048	0.005
224	0.000	0.023	0.000	0.027	0.026	0.018	0.025	0.025	0.017	0.006	0.022	0.000	0.010
226	0.021	0.023	0.024	0.022	0.017	0.024	0.006	0.019	0.017	0.018	0.011	0.016	0.026
228	0.010	0.011	0.012	0.027	0.017	0.000	0.044	0.012	0.011	0.000	0.005	0.032	0.000
230	0.010	0.011	0.024	0.005	0.026	0.000	0.013	0.019	0.000	0.012	0.016	0.016	0.031
232	0.000	0.000	0.036	0.005	0.009	0.012	0.006	0.012	0.011	0.006	0.000	0.000	0.000
234	0.010	0.011	0.000	0.011	0.000	0.018	0.013	0.006	0.006	0.000	0.005	0.000	0.005
236	0.005	0.000	0.012	0.005	0.009	0.024	0.000	0.000	0.000	0.006	0.016	0.000	0.010
238	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.000	0.000	0.015
240	0.005	0.006	0.000	0.005	0.017	0.006	0.000	0.006	0.006	0.000	0.005	0.000	0.005
242	0.005	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.016	0.000	0.005
244	0.000	0.006	0.000	0.000	0.000	0.006	0.000	0.012	0.000	0.000	0.011	0.000	0.000
246	0.000	0.006	0.012	0.011	0.000	0.006	0.006	0.006	0.000	0.006	0.000	0.000	0.005
248	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.016	0.010
252	0.005	0.000	0.024	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.005
254	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
258	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000
262	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*97*	*87*	*42*	*92*	*58*	*85*	*80*	*81*	*88*	*82*	*93*	*31*	*98*

***CmrHr*2.9 cont.**

Allele	GRA	HI	SMG	MCA	LTI	SUB	CR	SBP	WWR	CAP	LOH	Total
154	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000
156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
158	0.241	0.156	0.149	0.138	0.126	0.155	0.135	0.303	0.225	0.275	0.340	0.174
160	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000
162	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.015	0.000	0.000	0.000	0.000
164	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
166	0.089	0.125	0.155	0.168	0.121	0.093	0.109	0.091	0.133	0.140	0.112	0.145
168	0.027	0.042	0.021	0.010	0.016	0.041	0.021	0.045	0.042	0.039	0.016	0.022
170	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
172	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.001
174	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
176	0.009	0.005	0.005	0.015	0.005	0.010	0.000	0.000	0.000	0.006	0.000	0.007
178	0.018	0.010	0.000	0.000	0.011	0.005	0.000	0.030	0.000	0.039	0.016	0.009
180	0.000	0.016	0.005	0.005	0.026	0.005	0.005	0.015	0.000	0.011	0.016	0.012
182	0.009	0.005	0.015	0.020	0.037	0.005	0.010	0.030	0.000	0.006	0.005	0.013
184	0.000	0.036	0.036	0.056	0.047	0.052	0.047	0.000	0.000	0.011	0.027	0.033
186	0.080	0.042	0.082	0.051	0.026	0.041	0.078	0.030	0.000	0.011	0.032	0.045
188	0.045	0.016	0.031	0.010	0.026	0.031	0.021	0.015	0.000	0.011	0.027	0.024
190	0.045	0.021	0.046	0.041	0.011	0.046	0.021	0.030	0.025	0.006	0.048	0.033
192	0.045	0.047	0.021	0.046	0.053	0.026	0.068	0.015	0.125	0.017	0.027	0.046
194	0.018	0.042	0.021	0.051	0.084	0.052	0.010	0.015	0.058	0.051	0.037	0.034
196	0.036	0.031	0.031	0.020	0.016	0.015	0.005	0.015	0.042	0.011	0.005	0.021
198	0.018	0.057	0.021	0.036	0.047	0.046	0.026	0.015	0.000	0.084	0.048	0.036
200	0.009	0.016	0.036	0.020	0.005	0.026	0.021	0.015	0.025	0.028	0.016	0.024
202	0.027	0.073	0.026	0.026	0.058	0.067	0.052	0.030	0.025	0.028	0.000	0.032
204	0.018	0.005	0.021	0.026	0.016	0.010	0.016	0.030	0.050	0.017	0.011	0.022
206	0.018	0.021	0.015	0.015	0.021	0.010	0.016	0.030	0.067	0.017	0.021	0.025
208	0.009	0.042	0.031	0.046	0.011	0.000	0.026	0.045	0.033	0.039	0.037	0.025
210	0.018	0.021	0.010	0.010	0.016	0.005	0.026	0.015	0.000	0.006	0.005	0.015
212	0.018	0.021	0.031	0.005	0.011	0.057	0.031	0.045	0.025	0.006	0.021	0.027
216	0.009	0.016	0.031	0.005	0.011	0.005	0.042	0.000	0.017	0.022	0.011	0.019
218	0.027	0.021	0.041	0.010	0.005	0.005	0.026	0.015	0.017	0.017	0.043	0.019
220	0.027	0.021	0.026	0.020	0.021	0.026	0.021	0.030	0.000	0.000	0.016	0.021
222	0.045	0.026	0.015	0.020	0.021	0.031	0.016	0.000	0.017	0.011	0.021	0.017
224	0.009	0.000	0.005	0.015	0.037	0.010	0.016	0.000	0.000	0.006	0.005	0.012
226	0.009	0.005	0.015	0.010	0.016	0.010	0.026	0.000	0.000	0.045	0.005	0.016
228	0.009	0.021	0.010	0.026	0.016	0.021	0.042	0.030	0.025	0.006	0.005	0.016
230	0.027	0.005	0.015	0.020	0.005	0.005	0.010	0.000	0.008	0.000	0.000	0.010
232	0.000	0.005	0.015	0.020	0.037	0.000	0.005	0.015	0.033	0.000	0.000	0.009
234	0.027	0.000	0.000	0.010	0.000	0.015	0.016	0.000	0.000	0.006	0.005	0.006
236	0.000	0.000	0.005	0.010	0.005	0.036	0.005	0.030	0.000	0.000	0.005	0.007
238	0.009	0.005	0.000	0.000	0.005	0.015	0.016	0.000	0.000	0.011	0.011	0.004
240	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.011	0.005	0.004
242	0.009	0.010	0.010	0.005	0.000	0.010	0.005	0.000	0.000	0.000	0.000	0.003
244	0.000	0.005	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.006	0.000	0.002
246	0.000	0.005	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.003
248	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
250	0.000	0.000	0.000	0.010	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.001
252	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
254	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.001
258	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
262	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	*56*	*96*	*97*	*98*	*95*	*97*	*96*	*33*	*60*	*89*	*94*	*2822*

APPENDIX 5. General genetic statistics.

For each microsatellite locus at each collection site: n = number of individuals scored, Alleles = number of alleles observed, Ho = observed heterozygosity, He = Hardy-Weinberg Equilibrium expected heterozygosity, P = probability of agreement between Ho and He with values in bold significantly different following sequential Bonferroni correction for multiple tests, D = Selander's D value, a measure of heterozygote equilibrium (a negative value indicates heterozygote deficiency in the sample).

	Kiama	Jervis Bay	Pearl Point	Cape Conran	Point Cook	Port Macdonell	West Bay	Cat Island	Passage Island
CmrHr1.14									
n	89	87	82	43	30	51	48	45	60
Alleles	6	4	5	3	2	4	9	5	9
Ho	0.2584	0.1724	0.2195	0.1628	0.2000	0.2941	0.2708	0.0889	0.2667
He	0.3050	0.2204	0.2342	0.1951	0.1831	0.3788	0.3669	0.3151	0.4143
P	0.2326	0.0534	0.0868	0.1030	1.0000	0.0355	0.0288	**0.0000**	**0.0002**
D	-0.1526	-0.2177	-0.0626	-0.1655	0.0926	-0.2235	-0.2618	-0.7179	-0.3563
CmrHr1.24									
n	91	100	91	75	30	52	47	77	88
Alleles	6	4	6	7	3	4	3	6	3
Ho	0.2967	0.2800	0.1758	0.2533	0.0667	0.1538	0.0638	0.2727	0.1023
He	0.2764	0.2604	0.1949	0.2540	0.0661	0.1639	0.0629	0.3057	0.1197
P	0.8363	1.0000	0.1978	0.1367	1.0000	0.3144	1.0000	0.5503	0.0577
D	0.0734	0.0753	-0.0981	-0.0025	0.0085	-0.0615	0.0145	-0.1080	-0.1454
CmrHr2.14									
n	90	98	87	89	30	59	61	76	94
Alleles	9	10	9	8	4	7	7	7	10
Ho	0.6444	0.6429	0.6782	0.6742	0.6333	0.6441	0.6557	0.6316	0.7340
He	0.7238	0.6400	0.6823	0.7055	0.6119	0.6990	0.6938	0.7144	0.7337
P	0.2925	0.7476	0.0808	0.5124	0.2145	0.2560	0.8831	0.2110	0.1403
D	-0.1096	0.0045	-0.0060	-0.0444	0.0351	-0.0785	-0.0549	-0.1159	0.0005
CmrHr2.30									
n	91	99	89	85	30	55	58	76	93
Alleles	37	33	36	40	20	31	26	36	36
Ho	0.8022	0.7980	0.8427	0.8588	0.8000	0.8909	0.6034	0.8289	0.6882
He	0.9335	0.9344	0.9556	0.9637	0.9384	0.9533	0.9511	0.9588	0.9533
P	0.0296	**0.0000**	**0.0021**	**0.0027**	0.0131	0.2806	**0.0000**	0.1010	**0.0000**
D	-0.1406	-0.1460	-0.1181	-0.1088	-0.1475	-0.0654	-0.3655	-0.1354	-0.2781
RubCA1									
n	90	91	84	87	30	59	59	75	87
Alleles	28	31	31	30	21	24	22	30	29
Ho	0.7222	0.5824	0.6190	0.6897	0.4667	0.6610	0.9322	0.8667	0.5287
He	0.8981	0.9455	0.9355	0.9294	0.9475	0.9444	0.9361	0.9259	0.9502
P	0.0019	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.1701	0.0973	**0.0000**
D	-0.1958	-0.3840	-0.3383	-0.2579	-0.5075	-0.3000	-0.0042	-0.0640	-0.4435
CmrHr1.25									
n	68	76	55	45	-	30	15	-	14
Alleles	28	33	23	27	-	17	13	-	10
Ho	0.3088	0.2895	0.3636	0.4000	-	0.3000	0.2000	-	0.2857
He	0.9514	0.9616	0.9183	0.9513	-	0.8977	0.8989	-	0.9101
P	**0.0000**	**0.0000**	**0.0000**	**0.0000**	-	**0.0000**	**0.0000**	-	**0.0000**
D	-0.6754	-0.6990	-0.6040	-0.5795	-	-0.6658	-0.7775	-	-0.6860
CmrHr2.26									
n	86	97	80	24	26	21	15	-	62
Alleles	13	12	15	8	11	9	7	-	12
Ho	0.6512	0.6804	0.6750	0.5000	0.6538	0.2857	0.2667	-	0.5645
He	0.8583	0.8453	0.8561	0.8236	0.8665	0.8409	0.7862	-	0.8597
P	**0.0001**	**0.0160**	**0.0000**	**0.0004**	**0.0018**	**0.0000**	**0.0000**	-	**0.0000**
D	-0.2413	-0.1951	-0.2116	-0.3929	-0.2454	-0.6602	-0.6608	-	-0.3433
CmrHr2.9									
n	88	96	88	81	-	51	64	-	86
Alleles	38	31	35	32	-	25	17	-	28
Ho	0.6477	0.5833	0.5568	0.4938	-	0.6275	0.4844	-	0.4419
He	0.9331	0.9230	0.9152	0.9441	-	0.9051	0.5439	-	0.9295
P	**0.0000**	**0.0000**	**0.0000**	**0.0000**	-	**0.0000**	**0.0242**	-	**0.0000**
D	-0.3058	-0.3680	-0.3916	-0.4769	-	-0.3067	-0.1095	-	-0.5246

Table A5 cont. General genetic statistics

	Georges Rock	Long Point Reef	Long Point Site 2	Long Point Site 1	Nuggets 4 gap	Nuggets 2	Nuggets 4 North Face	Lemon Rock	Lemon Bight East
CmrHr1.14									
n	100	40	47	5	39	15	42	48	30
Alleles	9	4	5	2	3	6	7	6	4
Ho	0.3600	0.2250	0.3404	0.2000	0.1795	0.3333	0.1429	0.4167	0.1000
He	0.4102	0.2731	0.3045	0.4667	0.3783	0.3632	0.3417	0.4316	0.2955
P	0.0045	0.0701	1.0000	0.3333	**0.0006**	0.4630	**0.0000**	0.8386	**0.0001**
D	-0.1224	-0.1761	0.1180	-0.5714	-0.5255	-0.0823	-0.5819	-0.0346	-0.6616
CmrHr1.24									
n	100	48	49	41	47	15	46	46	38
Alleles	6	3	6	5	6	3	5	5	3
Ho	0.3600	0.3125	0.2653	0.3902	0.2553	0.2000	0.2826	0.3478	0.1316
He	0.3323	0.3283	0.2946	0.3604	0.2720	0.1908	0.3206	0.3526	0.1260
P	0.9298	0.7557	0.0926	0.8780	0.5683	1.0000	0.0939	0.3195	1.0000
D	0.0835	-0.0481	-0.0993	0.0827	-0.0614	0.0482	-0.1185	-0.0136	0.0446
CmrHr2.14									
n	94	5	38	12	48	15	46	48	17
Alleles	7	4	7	6	7	6	7	9	6
Ho	0.5851	0.6000	0.7368	0.5833	0.5833	0.7333	0.5435	0.7708	0.7059
He	0.6575	0.5333	0.7179	0.6739	0.6741	0.6989	0.6696	0.7496	0.7201
P	0.1060	1.0000	0.4482	0.7671	0.1883	0.8258	0.1092	0.7260	0.8251
D	-0.1101	0.1250	0.0264	-0.1344	-0.1347	0.0493	-0.1884	0.0284	-0.0198
CmrHr2.30									
n	100	50	48	41	38	13	42	48	42
Alleles	40	31	33	32	30	14	25	30	29
Ho	0.8500	0.8400	0.9375	0.9268	0.7895	0.9231	0.6905	0.9583	0.9524
He	0.9630	0.9548	0.9579	0.9606	0.9688	0.9323	0.9423	0.9618	0.9624
P	**0.0000**	0.0122	0.0765	0.3205	**0.0000**	0.8390	**0.0000**	0.5486	0.1930
D	-0.1173	-0.1202	-0.0213	-0.0351	-0.1851	-0.0099	-0.2673	-0.0036	-0.0104
RubCA1									
n	100	50	50	39	46	15	44	47	39
Alleles	34	30	29	26	28	16	28	24	27
Ho	0.7900	0.7800	0.8400	0.4872	0.6957	1.0000	0.6136	0.8511	0.7949
He	0.8838	0.9063	0.9010	0.9404	0.9271	0.9310	0.9381	0.9019	0.9257
P	0.3023	0.0731	0.0603	**0.0000**	**0.0000**	1.0000	**0.0000**	0.3143	0.0053
D	-0.1061	-0.1393	-0.0677	-0.4819	-0.2497	0.0741	-0.3459	-0.0563	-0.1414
CmrHr1.25									
n	89	41	43	33	-	-	-	39	29
Alleles	30	22	21	20	-	-	-	24	17
Ho	0.3820	0.4878	0.3023	0.4242	-	-	-	0.4359	0.3103
He	0.9136	0.8904	0.8758	0.9249	-	-	-	0.8768	0.9074
P	**0.0000**	**0.0000**	**0.0000**	**0.0000**	-	-	-	**0.0000**	**0.0000**
D	-0.5819	-0.4521	-0.6548	-0.5413	-	-	-	-0.5028	-0.6580
CmrHr2.26									
n	95	43	46	39	-	-	-	7	32
Alleles	13	13	11	11	-	-	-	5	10
Ho	0.5368	0.5814	0.6087	0.5385	-	-	-	0.7143	0.5938
He	0.8781	0.8517	0.8579	0.8452	-	-	-	0.7582	0.8442
P	**0.0000**	**0.0000**	**0.0001**	**0.0000**	-	-	-	0.3632	**0.0001**
D	-0.3886	-0.3174	-0.2904	-0.3629	-	-	-	-0.0580	-03008
CmrHr2.9									
n	97	43	40	38	-	-	-	41	37
Alleles	33	25	27	25	-	-	-	27	29
Ho	0.5567	0.3953	0.6250	0.4737	-	-	-	0.6829	0.7297
He	0.9352	0.9387	0.9095	0.9084	-	-	-	0.9341	0.9530
P	**0.0000**	**0.0000**	**0.0000**	**0.0000**	-	-	-	**0.0000**	**0.0000**
D	-0.4047	-0.5788	-0.3128	-0.4786	-	-	-	-0.2689	-0.2343

Table A5 cont. General genetic statistics

	Lemon Bight West	Trumpeter Corner	One Tree Point	One Tree Point Temporal	Curio Bay	Georges III Reef	Loiusa Bay	Louisa Bay Temporal	Acteon Island
CmrHr1.14									
n	50	99	92	58	92	62	88	91	89
Alleles	8	7	4	4	7	6	8	6	8
Ho	0.3600	0.3333	0.2609	0.2759	0.2717	0.3226	0.2955	0.3187	0.3258
He	0.4194	0.3907	0.2467	0.2778	0.3005	0.3973	0.2968	0.3512	0.4354
P	0.5732	0.0188	0.5524	0.6060	0.0024	0.0714	0.6528	0.0665	**0.0001**
D	-0.1416	-0.1469	0.0575	-0.0070	-0.0958	-0.1881	-0.0044	-0.0925	-0.2516
CmrHr1.24									
n	47	100	91	57	91	66	93	90	88
Alleles	4	7	7	5	5	5	5	4	6
Ho	0.1915	0.3600	0.3846	0.2982	0.2747	0.1970	0.3226	0.2222	0.3182
He	0.1796	0.3755	0.3523	0.2649	0.3078	0.1977	0.3209	0.2248	0.3143
P	1.0000	0.8337	0.6837	1.0000	0.2941	0.5344	0.1452	0.4251	0.1432
D	0.0662	-0.0412	0.0919	0.1260	-0.1073	-0.0035	0.0053	-0.0113	0.0124
CmrHr2.14									
n	48	100	73	59	81	62	-	92	77
Alleles	7	8	7	9	7	9	-	9	9
Ho	0.6875	0.6300	0.5616	0.6271	0.8272	0.5968	-	0.5870	0.5584
He	0.7050	0.6957	0.6641	0.6932	0.7376	0.7259	-	0.6964	0.6786
P	0.4110	0.2066	0.3627	0.8175	0.0247	0.0066	-	0.1666	0.0046
D	-0.0249	-0.0945	-0.1542	-0.0953	0.1214	-0.1779	-	-0.1572	-0.1771
CmrHr2.30									
n	50	98	71	60	-	58	91	91	90
Alleles	34	40	36	32	-	31	39	39	35
Ho	0.9000	0.8265	0.6338	0.8333	-	0.6724	0.7912	0.9121	0.7667
He	0.9655	0.9613	0.9534	0.9569	-	0.9544	0.9633	0.9513	0.9600
P	0.3249	**0.0000**	**0.0000**	0.0033	-	**0.0000**	**0.0000**	0.4558	**0.0000**
D	-0.0678	-0.1402	-0.3352	-0.1291	-	-0.2955	-0.1786	-0.0412	-0.2014
RubCA1									
n	50	100	87	60	92	61	93	91	84
Alleles	27	36	33	28	31	28	28	30	34
Ho	0.9200	0.9100	0.8736	0.9167	0.8478	0.7869	0.8172	0.9231	0.4524
He	0.9152	0.9163	0.8961	0.8821	0.9019	0.9394	0.9185	0.8889	0.9554
P	0.7387	0.3051	0.2770	0.8778	0.1191	0.0019	0.0097	0.5579	**0.0000**
D	0.0053	-0.0069	-0.0251	0.0392	-0.0599	-0.1624	-0.1103	0.0385	-0.5265
CmrHr1.25									
n	38	85	78	37	66	42	84	78	65
Alleles	21	25	24	17	27	20	26	24	23
Ho	0.2105	0.3765	0.3333	0.3514	0.3333	0.3571	0.4048	0.4359	0.4462
He	0.8681	0.8993	0.8629	0.9148	0.9086	0.9028	0.8770	0.8806	0.9085
P	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
D	-0.7575	-0.5814	-0.6137	-0.6159	-0.6331	-0.6044	-0.5384	-0.5050	-0.5089
CmrHr2.26									
n	47	100	90	14	72	63	90	63	86
Alleles	13	13	13	9	13	10	13	11	12
Ho	0.6596	0.6400	0.6778	0.7143	0.6944	0.6349	0.6111	0.6032	0.5581
He	0.8648	0.8685	0.8629	0.8333	0.8556	0.8489	0.8670	0.8437	0.8579
P	**0.0016**	**0.0000**	**0.0001**	0.2636	**0.0006**	**0.0018**	**0.0000**	**0.0001**	**0.0000**
D	-0.2373	-0.2631	-0.2146	-0.1429	-0.1883	-0.2521	-0.2952	-0.2851	-0.3494
CmrHr2.9									
n	47	97	87	42	92	58	85	80	81
Alleles	33	33	34	27	34	31	35	31	36
Ho	0.6596	0.6289	0.7241	0.6905	0.4891	0.7759	0.5647	0.6500	0.5926
He	0.9147	0.9267	0.9115	0.9114	0.9531	0.9393	0.9371	0.9307	0.9518
P	**0.0000**	**0.0000**	**0.0037**	**0.0053**	**0.0000**	**0.0041**	**0.0000**	**0.0000**	**0.0000**
D	-0.2789	-0.3214	-0.2056	-0.2424	-0.4868	-0.1740	-0.3974	-0.3016	-0.3774

Table A5 cont. General genetic statistics

	Black Reef	Sterile Island	Whalers Point	Block 11	High Rocky Point	Granville Harbour	Hunter Island	Smiths Gulch	Mount Cameron
CmrHr1.14									
n	84	90	96	95	98	54	97	100	99
Alleles	7	7	10	10	10	5	8	7	9
Ho	0.2381	0.3000	0.2396	0.3368	0.2653	0.2963	0.2062	0.3300	0.3636
He	0.2702	0.3469	0.2829	0.3791	0.4600	0.3124	0.2358	0.3667	0.3728
P	0.0142	0.1406	0.3808	0.0447	**0.0000**	0.0874	0.2051	0.3768	0.2168
D	-0.1187	-0.1351	-0.1531	-0.1115	-0.4233	-0.0515	-0.1255	-0.1002	-0.0246
CmrHr1.24									
n	89	92	98	96	98	49	100	100	100
Alleles	6	6	5	5	5	5	7	5	6
Ho	0.3034	0.2935	0.3469	0.1667	0.3265	0.3061	0.3400	0.3600	0.3600
He	0.2909	0.3064	0.3367	0.1949	0.3248	0.3061	0.3156	0.3680	0.3471
P	0.7635	0.7781	0.0081	0.0432	0.4481	0.8183	0.6955	0.4428	0.3693
D	0.0430	-0.0423	0.0303	-0.1447	0.0053	0.0000	0.0772	-0.0217	0.0372
CmrHr2.14									
n	88	80	65	89	22	56	99	100	99
Alleles	6	9	10	9	6	7	10	9	9
Ho	0.6705	0.6625	0.5385	0.6517	0.5455	0.6071	0.5657	0.6800	0.6667
He	0.6843	0.7351	0.6681	0.7248	0.6015	0.6842	0.7244	0.7013	0.6980
P	0.0580	0.1655	0.1180	0.4171	0.1909	0.2224	**0.0000**	0.7050	0.2720
D	-0.0202	-0.0987	-0.1940	-0.1008	-0.0931	-0.1126	-0.2191	-0.0304	-0.0449
CmrHr2.30									
n	85	77	98	89	94	54	97	100	97
Alleles	31	36	35	35	31	32	37	35	39
Ho	0.6706	0.7273	0.8367	0.8539	0.5319	0.8889	0.8763	0.8800	0.7526
He	0.9551	0.9571	0.9599	0.9572	0.9416	0.9600	0.9618	0.9531	0.9549
P	**0.0000**	**0.0000**	**0.0000**	**0.0002**	**0.0000**	0.1544	0.0534	0.0054	**0.0000**
D	-0.2979	-0.2402	-0.1283	-0.1079	-0.4351	-0.0741	-0.0889	-0.0767	-0.2118
RubCA1									
n	89	87	99	96	97	58	98	100	99
Alleles	26	30	34	33	35	26	33	34	31
Ho	0.7191	0.8276	0.8788	0.5417	0.8247	0.8966	0.8571	0.8900	0.8485
He	0.9104	0.8923	0.8634	0.9462	0.9039	0.9112	0.8792	0.9199	0.8862
P	**0.0000**	0.0220	0.9836	**0.0000**	0.0923	0.5361	0.3196	0.1721	0.0925
D	-0.2101	-0.0725	0.0178	-0.4275	-0.0875	-0.0161	-0.0251	-0.0325	-0.0426
CmrHr1.25									
n	79	63	75	21	81	48	88	76	87
Alleles	29	25	33	12	27	19	29	26	25
Ho	0.5570	0.3175	0.4933	0.3333	0.3580	0.4167	0.3977	0.3421	0.3333
He	0.9141	0.9026	0.9152	0.8931	0.9169	0.9035	0.8840	0.9115	0.8589
P	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
D	-0.3907	-0.6483	-0.4609	-0.6268	-0.6095	-0.5388	-0.5501	-0.6247	-0.6119
CmrHr2.26									
n	89	81	94	28	95	41	99	99	98
Alleles	9	11	11	11	13	12	14	14	12
Ho	0.6180	0.6790	0.5957	0.4286	0.5789	0.5366	0.5859	0.5657	0.5408
He	0.8213	0.8739	0.8539	0.8851	0.8496	0.8248	0.8727	0.8730	0.8745
P	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
D	-0.2476	-0.2230	-0.3023	-0.5158	-0.3186	-0.3494	-0.3287	-0.3521	-0.3816
CmrHr2.9									
n	88	82	93	31	98	56	96	97	98
Alleles	32	31	35	24	38	31	35	32	34
Ho	0.7159	0.7439	0.6237	0.7419	0.6633	0.7500	0.6563	0.5979	0.6939
He	0.9219	0.9267	0.9472	0.9476	0.9490	0.9191	0.9396	0.9352	0.9354
P	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
D	-0.2234	-0.1972	-0.3416	-0.2171	-0.3011	-0.1840	-0.3016	-0.3606	-0.2582

Table A5 cont. General genetic statistics

	Little Trefoil Island	Bluff Point	Suicide Bay	Church Rocks	Sandblow Point	Waterwitch reef	Cape Portland	Low Head
CmrHr1.14								
n	97	92	93	99	92	66	59	91
Alleles	7	4	9	7	11	10	9	13
Ho	0.2990	0.2935	0.3548	0.4040	0.3043	0.2576	0.3051	0.3626
He	0.3480	0.2803	0.3999	0.4124	0.4202	0.3705	0.3352	0.3875
P	0.0632	0.6117	0.0424	0.3455	0.0015	0.0047	0.1146	0.1355
D	-0.1409	0.0470	-0.1128	-0.0204	-0.2758	-0.3047	-0.0899	-0.0642
CmrHr1.24								
n	99	99	97	100	95	69	62	95
Alleles	6	6	6	5	5	5	6	6
Ho	0.2222	0.3939	0.2887	0.3400	0.2000	0.2899	0.2419	0.1895
He	0.2406	0.3795	0.2913	0.3544	0.1940	0.2970	0.2793	0.1785
P	0.0064	0.6801	0.6337	0.2447	0.4358	0.7159	0.1199	1.0000
D	-0.0763	0.0380	-0.0090	-0.0406	0.0310	-0.0242	-0.1338	0.0615
CmrHr2.14								
n	55	92	73	61	85	68	61	94
Alleles	8	7	8	8	8	7	8	7
Ho	0.5636	0.6957	0.6301	0.6885	0.6471	0.6618	0.6066	0.7021
He	0.6657	0.7231	0.7001	0.6735	0.6742	0.7219	0.7517	0.7073
P	0.0206	0.6347	0.3458	0.8537	0.1336	0.0187	**0.0001**	0.7688
D	-0.1533	-0.0379	-0.1000	0.0223	-0.0403	-0.0833	-0.1930	-0.0073
CmrHr2.30								
n	87	97	90	100	88	66	42	54
Alleles	38	40	38	39	31	34	24	31
Ho	0.8161	0.8454	0.8222	0.8200	0.7955	0.8939	0.3095	0.3889
He	0.9581	0.9603	0.9593	0.9571	0.9523	0.9585	0.9228	0.9559
P	**0.0000**	**0.0000**	**0.0013**	**0.0000**	**0.0003**	**0.0008**	**0.0000**	**0.0000**
D	-0.1482	-0.1197	-0.1429	-0.1433	-0.1647	-0.0673	-0.6646	-0.5932
RubCA1								
n	93	97	98	100	89	66	56	96
Alleles	30	25	31	30	34	29	26	29
Ho	0.5269	0.8660	0.5306	0.8700	0.9101	0.8636	0.8571	0.8125
He	0.9327	0.8869	0.9515	0.8992	0.9310	0.9299	0.9450	0.9401
P	**0.0000**	0.4631	**0.0000**	0.8015	0.5320	0.2313	0.0344	0.0116
D	-0.4351	-0.0236	-0.4424	-0.0325	-0.0224	-0.0713	-0.0930	-0.1357
CmrHr1.25								
n	57	-	70	84	33	46	56	36
Alleles	28	-	29	30	21	20	24	12
Ho	0.4737	-	0.3857	0.4286	0.3939	0.3478	0.3393	0.0278
He	0.9290	-	0.9239	0.9098	0.9375	0.9212	0.9199	0.8916
P	**0.0000**	-	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
D	-0.4901	-	-0.5825	-0.5289	-0.5798	-0.6224	-0.6312	-0.9688
CmrHr2.26								
n	81	-	83	99	34	64	86	85
Alleles	14	-	12	13	11	12	13	11
Ho	0.4691	-	0.4699	0.6465	0.5000	0.4844	0.5349	0.4824
He	0.8530	-	0.8510	0.8751	0.8341	0.8436	0.8514	0.8569
P	**0.0000**	-	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
D	-0.4500	-	-0.4478	-0.2613	-0.4005	-0.4258	-0.3718	-0.4371
CmrHr2.9								
n	95	-	97	96	33	60	89	94
Alleles	37	-	35	35	26	20	32	31
Ho	0.5684	-	0.5567	0.6875	0.7273	0.5333	0.6966	0.6170
He	0.9467	-	0.9444	0.9470	0.8956	0.9034	0.8887	0.8607
P	**0.0000**	-	**0.0000**	**0.0000**	**0.0079**	**0.0000**	**0.0000**	**0.0000**
D	-0.3996	-	-0.4105	-0.2741	-0.1879	-0.4096	-0.2161	-0.2831