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Enhancement of Saucer Scallops (*Amusium balloti*) in Queensland and Western Australia — Genetic Considerations

Queensland Government
Department of Primary Industries and Fisheries

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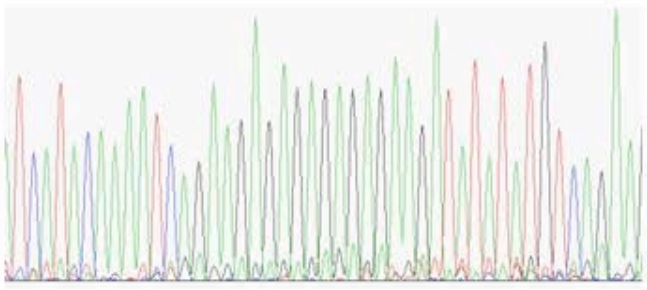

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Contents

Non-technical summary

The saucer scallop, *Amusium balloti*, is distributed along the Western and Eastern coast of Australia and supports a fishery in both Queensland and Western Australia. Two commercial companies are investing in sea-ranching operations in an attempt to stabilise and increase annual catches. These operations are reliant upon hatchery production of juveniles because of species-specific constraints on open-water harvest of spat. The mass release of scallop juveniles is expected to have an immediate effect on population abundance, but it also has the potential to alter the genetic structure of the existing saucer scallop populations. Therefore, understanding and management of the genetic diversity is needed.

Genetic diversity in a population or a species gives a range of genotypes that allows scope to adapt to environmental change, such as new diseases, new predators or competitors, or a change in climate. Loss of genetic diversity not only impacts on their capacity for adaptation but can also lead to potentially negative effects upon various performance traits such as survival and growth.

At this time, there is limited information available on the way genetic variation is spread throughout the saucer scallop populations. This project investigates the population genetic makeup of the east and west coast saucer scallops, which can give an insight into the reproductive interaction between populations and the identification of distinct stocks of scallops.

We have used microsatellites as a measure of genetic interaction between scallop locations in Queensland and Western Australia. Microsatellites are repeated sections of DNA that are spread randomly throughout the chromosomes of the scallops. They have the advantage over some other DNA markers in that they are extremely variable in the number of repeats from one scallop to the next as they are usually located in non-coding regions. These regions are less subject to selection than coding gene markers and can provide information about the levels of genetic variation within or between groups that may not be detected using other genetic markers. The locations of microsatellites are unique to each species, so they need to be developed each time a new species is investigated.

Samples of *A. balloti* DNA were screened for microsatellites and 21 types were identified. Of these, only eight microsatellite types (loci) were suitable for the study as they were shown to have between thirteen and twenty-six different repeat sizes (alleles), indicating the allelic variation between individuals was sufficient to be used to analyse genetic differences between populations.

Specimens of *A. balloti* were obtained from five Queensland and four Western Australian locations. Overall, no significant genetic difference was detected between scallops from the various Queensland locations sampled or between the Western Australian locations. There was significant genetic differentiation

between scallops from Queensland and Western Australian locations. These genetic patterns indicate that there is a high degree of gene exchange or reproductive interaction within the Queensland and Western Australian locations over time, but there appears to be reproductive isolation between the two states. We also identified significant changes in the frequencies of microsatellite alleles between two years at one location in Western Australia. This is not unexpected because of *A. balloti*'s reproductive strategy, which relies on the high production of gametes with low survival but fast growth and early age at maturity. This is a common strategy for animals existing in a variable environment.

The genetic differences between the Queensland and Western Australian populations had previously been identified through differences in protein forms (allozymes) (Dredge *et al.,* in prep). To investigate the differences further we used molecular markers that are less variable than microsatellites and therefore better in detecting long term genetic differentiation. We chose the 12S and 16S subunits of the ribosomal RNA genes on the basis of earlier taxonomic studies on other scallop species. The results support the findings from the present microsatellite and the previous allozyme analysis that the two *A. balloti* groups are reproductively isolated through geographic separation over time.

The microsatellite analysis that we have completed in this study indicate that the Queensland populations can be considered as a single genetic stock and that hatchery broodstock can be obtained from any location. The Western Australian samples do not represent the species' full distribution on the Western coastline but no genetic differences were detected between the locations sampled, which suggest a single genetic stock in that region. If hatchery broodstock are to be sourced outside of the region analysed during this study, it is recommended that the source and release sites are compared in order to determine microsatellite diversity. Where no significant differences in genetic composition are detected between the proposed source and release sites, the source site may be considered appropriate. However, it is important to understand that microsatellites are not indicative of functional differences between populations and there may be some undetected local genetic adaptation.

The ability to accurately monitor genetic diversity is essential to achieve the management objective of maintaining natural genetic variation in sea-ranching locations. The highly variable microsatellite markers developed during this study and the baseline data collected prior to significant sea-ranching activities will be a valuable tool for monitoring genetic structure in the ranched areas to ensure maintenance of genetic variation. The microsatellites can also be utilised for broodstock management in the hatchery as a means of determining the effective numbers of parental broodstock and monitoring genetic diversity through the hatchery process.

2003/033 Enhancement of saucer scallops (*Amusium balloti*) in Queensland and Western Australia — genetic considerations

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OBJECTIVES:

1. To determine the genetic population structure of wild stocks of *Amusium balloti* in Queensland and Western Australia
2. To investigate the taxonomic status of Australian *Amusium* scallops using molecular taxonomy

OUTCOMES ACHIEVED

Equipped management with a resource to make more informed decisions regarding the management of scallop sea-ranching ventures in Queensland and Western Australia.

Increased industry and management awareness and understanding of the value of genetic management in aquaculture, particularly sea-ranching ventures.

Collected baseline genetic (microsatellite) data for the *Amusium balloti* sea-ranching areas prior to significant restocking for post-stocking comparison over time.

Development of polymorphic microsatellites for *Amusium balloti* that can be used in genetic management of the hatchery.

Clarified taxonomic status of Australian *Amusium balloti*

KEYWORDS: scallops, genetics, fisheries management, restocking, aquaculture, hatchery management, microsatellites, Queensland, Western Australia.

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1 Background

The saucer scallop, *Amusium balloti*, is distributed along the Western and Eastern coast of Australia (Fig. 1) and supports valuable fisheries in both Queensland and Western Australia. Catches vary considerably from year to year in both fisheries. Queensland's annual landings have declined considerably in the past five years as a consequence of changes in the management arrangements applied to the Queensland trawl fishery, and processors seek to increase production in order to maintain economic viability. In 1998, the federal Fisheries Research and Development Corporation (FRDC) commissioned a project to investigate opportunities relating to scallop sea-ranching in Australia as a precursor to research on scallop culture. Sea-ranching has been defined as the practice of producing early life-history stages of an animal in a hatchery for eventual release into natural or modified aquatic habitats (Bartley 1999). The FRDC report (Dredge *et al*. 2002) identified *A. balloti* as the most commercially attractive of the Australian scallops for culture. However, industry, researchers and state government agencies agreed that new knowledge through research would be required to enable sustainable and profitable industry development based on *A. balloti* reseeding (Dredge *et al*. 2002).

Figure 1. *Amusium balloti* distribution along the Australian coastline highlighted in grey. Based on Kailola *et al* (1993).

Dredge *et al.* 2002 suggested that any sea-ranching operations using *A. balloti* would be reliant upon production of seed from hatcheries because the species has a very brief and transient byssal phase and is therefore not logistically feasible to harvest as spat from the wild. The mass release of hatchery-reared juveniles could be expected to have an immediate effect on stock abundance but also has the potential to alter the genetic structure of the naturally occurring saucer scallop populations. The scallop feasibility study stated that 'subject to proper genetic and disease protocols, there will be minimal risk of adverse environmental impacts from saucer scallop marine ranching or enhancement operations' (Dredge *et al*. 2002).

The use of genetic resource management has been highlighted as essential to control and optimise the results of sea-ranching or fisheries enhancement (Beaumont 2000b; Blankenship and Leber 1997). In order to manage genetic diversity in a sea-ranching or restocking venture, a measure of the existing diversity is required in order to identify discrete populations or stocks. Therefore, a genetic resource management plan should encompass genetic monitoring prior to, during and after enhancement (Blankenship and Leber 1997).

Genetic data describing population structure prior to translocations or restocking ventures are not generally available, making it impossible to conduct comparative studies on the changes brought about as a result of any population of the natural populations. Commercial scale restocking of *A. balloti* has not yet commenced in Australia and baseline genetic data of population structures are now available as a result of this study.

Understanding the interactions between populations or gene pools of saucer scallops is valuable information for managers. This may address questions about whether locally depleted populations will be replenished easily from neighbouring populations or whether new genetic input and therefore adaptive capacity of the populations is occurring or required. In the case of sea-ranching, hatchery-reared scallops from the seeded sites can contribute animals to surrounding populations. Managers may seek to understand the impact of such an event on genetic diversity within the natural population.

The differing selective pressures on a population at differing life stages, which include fertilisation, larval dispersal, recruitment, and survival to maturity mean it is difficult to identify interactions between animals from neighbouring locations and to identify demes or the smallest level of population structure. For example, larval dispersal can be affected by physical barriers or temporal barriers acting on larval transport and/or environmental factors that make habitat unsuitable for settlement, viability and reproduction (Bowen and Avise 1990; Burton 1999; Hedgecock 1986; Palumbi *et al*. 1997; Scheltema 1986). Oceanic features such as biogeographical boundaries, patterns of coastal or estuarine circulation are additional processes by which gene flow may be limited (Bilton *et al*. 2002; Wares *et al*. 2001). These features can often be difficult to detect in the marine environment.

An advantage of the genetic approach for assessing population structure is the insight we get into the history of a population. This can range from ancient interactions that identify the historical source of the populations over thousands of years (e.g. through analysis of variation in mitochondrial DNA), through to the identification of different cohorts of individuals through analysis of rapidly changing genetic markers such as microsatellites.

Genetic composition of a population or species is largely determined by the interaction of gene flow (including migration), genetic drift (random fluctuations in allele frequency due to chance, differential reproductive contributions by individuals) and adaptation (Shaklee and Bentzen 1998).

Isolation enhances genetic divergence, whether the cause of the divergence is natural selection or genetic drift (Johnson *et al*. 1986). Genetic differences could reflect variation in recruitment or post settlement selection, either of which can cause temporally unstable genetic patchiness despite extensive gene flow (Johnson and Black 1984). Conversely, substantial interbreeding between individuals or transport-induced settlement from different areas would tend to reduce genetic differences between those areas (Johnson *et al*. 1986). A major advantage of the genetic approach to population assessment is that it incorporates the migration of the individual over its entire life cycle from egg through to reproductive adult.

1.1 Genetic considerations for reseeding

There are ecological, evolutionary, economic and ethical reasons to maintain the diversity and integrity of wild (naturally reproducing) stocks of marine organisms (Shaklee and Bentzen 1998). Genetic diversity provides a competitive advantage to a population or a species because a wide range of genotypes gives increased scope to adapt to changes such as a new disease or change in climate. (Allendorf and Phelps 1980; Frankham 1994; Norris *et al*. 1999). Loss of genetic variation not only impacts on the population's ability to adapt but can also lead to potentially harmful effects upon various performance traits, such as survival and growth, due to the loss of valuable genotypes. It is conceivable that genetic diversity of a natural population or populations could be altered through translocation or introduction of genetically distinct hatchery reared spat into the natural environment.

1.1.1 Potential effects of strain translocation on genetic diversity

In a restocking or sea-ranching operation, the population structures from which broodstock are collected are important relative to those in the stocking area because of the potential for strain translocation. Genetically, translocations can be considered in two ways: 1) the introduction of an entirely new species, and 2) the introduction of new populations/strains of species already present in that locality (Nguyen and Na-Nakorn 2004). Sea-ranching or restocking can potentially result in the latter event. Such events are typically difficult to recognise due to the lack of morphological differentiation between populations/strains within species and absence of *a priori* data on population structures (Nguyen and Na-Nakorn 2004).

Direct effects on the local population from introduction of stocked animals include reduced genetic diversity and outbreeding depression by hybridisation/introgression. Indirect effects include reduced population size and changes of selective pressure or loss of genetic diversity due to ecological interactions such as competition, predation and disease transmission (Nguyen and Na-Nakorn 2004; Waples 1991).

Hybridisation between different strains is known to produce heterosis or hybrid vigour (an increase in some characteristic, e.g. growth, fecundity, in the hybrid over the parent) but is mostly likely to occur in two parental stocks that are not too genetically different.

If genetic distances between the two parental stocks increase, genetic incompatibilities becomes more likely and fitness (usually in fertility or viability) of hybrids declines (Waples 1991). This may result from the disruption of co-adapted gene complexes that result from local adaptation of a population (Nguyen and Na-Nakorn 2004).

1.1.2 Potential effects of hatchery production on genetic diversity

Genetic adaptation to the captive environment
When organisms are removed from the natural environment and placed in a cultured environment there is the likelihood of domestication effects altering the gene frequencies and reducing genetic variation (Beaumont 2000a; Nguyen and Na-Nakorn 2004) particularly in animals with high fecundity and short generation times (reviewed in Boudry *et al.* 2002; Frankham 1994).

A hatchery creates an artificial environment that causes relaxing, or the alteration of, natural selection pressures required for survival in the wild. These pressures might include the offspring's ability to avoid predators and resist pests and parasites which can negatively affect the animal on release to the wild (e.g. salmon: Einum and Flemming 1997; Flemming *et al.* 2000). Additionally, natural selection for reproductive performance will be altered towards maximising reproductive fitness and larval survival in the captive environment.

Boudry *et al* (2002) found that in the oyster *Crassostrea gigas* selection occurred in early stages of larviculture, favouring the progeny of some parents over others. A similar phenomenon was detected during the larval culture of the oyster *Ostrea edulis* (Bierne *et al.* 1998).

Reduced genetic diversity through breeding practices
Genetic diversity of hatchery seed can also be reduced through poor breeding practices such as repeat spawnings from broodstock (Nguyen and Na-Nakorn 2004), inbreeding events (Allendorf and Phelps 1980; Frankham 1994; Hedrick and Kalinowski 2000; Norris *et al*. 1999) or small numbers of parental stock contributing to the production of seed (Allendorf and Phelps 1980; Norris *et al*. 1999).

Sea-ranching or reseeding relies on the practice of supportive breeding whereby a fraction of the wild population is brought into captivity for reproduction and typically their offspring are reared in the hatchery for a period of time before they are released into the natural environment to mix with wild conspecifics (Laikre and Ryman 1996). If the same batch of broodstock are repeatedly used to seed areas then there is the chance that dilution of the wild genotypes can occur particularly with animals, like saucer scallops, which are characterised by high fecundity and rapid generation times.

Inbreeding occurs when closely related animals reproduce. This can lead to serious hereditary faults, including poor fecundity, reduced hatchery and nursery performance and loss of disease resistance. Fortunately, *A. balloti* is dioecious and hence self-fertilisation is impossible, unlike hermaphroditic scallops such as the Bay scallop (*Agropecten irradians irradians*), which can suffer high levels of inbreeding (Zheng *et al*. 2004). The effects of inbreeding in bivalves are unusually noticeable and can be expressed as a reduction in larval survival or growth rate (reviewed in Beaumont 2000a).

A number of recent case studies have shown that the number of broodstock involved in successful fertilisations (effective population size) is far smaller than the number of broodstock used in hatchery spawnings. This variance in reproductive success is proposed as the most likely explanation of loss of genetic diversity over time (Boudry *et al.* 2002; Hedgecock *et al.* 1992) and can result in a 'founder effect'. Parental contribution is uneven (Boudry *et al.* 2002) in *Crassostrea gigas*. The variation in the quality of gametes, variation in sperm mobility, sperm-egg interaction, zygotic competition and differential viability between parents are some of the reported causes (Boudry *et al.* 2002). The estimated population size of white shrimp *Litopenaeus setiferus* was orders of magnitude larger than the effective (breeding) population size (Ball and Chapman 2003), as also occurs with *Penaeus esculentus* (Ovenden *et al.* 2004).

These negative effects of captive breeding and release can be transmitted to wild stocks depending on the amount of gene flow from the captive to the wild segment (Ford 2002; Lynch and O'Hely 2001), a feature that will depend on the relative reproductive success of released captive individuals in the wild. This is particularly important for hatchery-reared saucer scallops, which may have the opportunity to reproduce in the wild prior to harvest.

1.1.3 Microsatellites—one tool in genetic management

In order to manage genetic diversity in a sea-ranching or restocking venture, a measure of the existing diversity is required in order to identify discrete populations or stocks. Some regions of DNA are shown to be variable or polymorphic between individuals. Microsatellites, which are made up of a variable number of repeated units of one to four nucleotides in length, are such an example. By combining the data from several microsatellites from different regions (or loci) in the DNA, an outline of similarity between individuals can be estimated. Microsatellite arrays are widely dispersed in the eukaryotic genomes, occur as often as once every 10kbp and hence have an overall abundance on the order of 10^4–10^5 per genome (Shaklee and Bentzen 1998). They are non-coding and therefore regarded as neutral markers and unlikely to be subject to selection.

1.1.4 Potentially different strains of Australian A. balloti.

In addition to understanding the interactions between the relationships of populations along the coastline, it is useful to understand how populations may have diverged following separation. In the case of *A. balloti* the populations in Queensland are separated from the Western Australian populations across the northern and southern limits of their distribution (Fig. 1). Previous genetic work using allozymes indicated that the two populations have been isolated from each other for a period long enough to introduce significant genetic differentiation of a magnitude normally associated with inter-specific variation (Dredge *et al.* in prep). To complement this work, we will compare the two strains using partial sequences of the mitochondrial genes for 12S rRNA and 16S rRNA to remain consistent with current molecular research in Pectinid taxonomy (Barucca *et al.* 2004; Canapa *et al.* 1999; Matsumoto and Hayami 2000; Saavedra and Peña 2004).

1.2 Project aims

This project is designed to describe the genetic structure of the native population of saucer scallops along the western and eastern coast of Australia using several microsatellite loci. This information can then be used to effectively manage broodstock collection in order to maintain high levels of genetic diversity without significantly altering the genetic makeup of the receiving population. Additionally since supported breeding inadvertently confers a level of selection in the artificial hatchery, the microsatellites identified in this study may also be used to determine the effective population size of hatchery broodstock to prevent a reduction in allelic diversity through ‘swamping’ or ‘founder effects’.

The second part of this project will determine the taxonomic status of the western and eastern Australian saucer scallops to better understand the organism and its hatchery-based production.

2 Need

The FRDC scallop feasibility report highlighted the need for proper genetic management in scallop enhancement/sea-ranching ventures (Dredge *et al*. 2002), in particular citing the key issues of genetic resource management (Blankenship and Leber 1997). This approach, and hence the need for this research, is further supported by proceedings from the second international stock enhancement and sea-ranching conference (Leber *et al.* 2002). Since domestication or selective breeding is not currently undertaken, the main genetic issue currently facing restocking saucer scallops is the potential for translocation of genetically different strains to the sea-ranching beds.

Determining the genetic structure of the wild population or populations is an essential baseline measurement in the design of an effective genetic management protocol for restocking ventures. Although previous allozyme work indicated that the Queensland stocks are a single genetic population and are a potentially different subspecies from Western Australia (Dredge *et al.,* in prep), allozyme data is not effective in discerning local genetic populations whereas microsatellites are well recognised in this application (Kolijonen *et al*. 2002; Waples 1998).

The development of appropriate genetic management protocols at an early stage of a long term proposal, such as scallop ranching in Queensland and Western Australia, is both desirable and responsible. Fisheries, conservation, public and other interests will undoubtedly critically scrutinise the progress of these operations and support the early implementation of genetic management strategies.

The development of appropriate and responsible genetic management protocols have been identified by Queensland Saucer Scallops Pty. as being a critical short-term priority which may have a significant bearing on the wider public acceptance of the operation. West Coast Scallops Pty. Ltd. has also recognised genetic management as an important factor.

3 Objectives

1. Determine the genetic population structure of the wild stocks of Queensland and Western Australian *Amusium balloti* (DNA based, e.g. microsatellites) in order to reduce impacts on the genetic diversity of natural populations from hatchery-produced stock

2. Investigate the taxonomic status of Australian *Amusium* scallops using molecular taxonomy techniques.

4 Methods

4.1 Sample collection

A total of 281 *Amusium balloti* samples were collected from ten locations: five in Queensland and five in Western Australia (Table 1). Queensland individuals were analysed from populations in Yeppoon, Bustard Head, Hervey Bay, Townsville and Noosa (Fig. 2) and from Gee Banks, Wooded Island A (collected September 2003) and Wooded Island B (collected September 2004), Pelsaert Island and Shark Bay in Western Australia (Fig. 2). Allele frequencies from the two Wooded Island samples, A and B, were analysed separately for temporal change in inter-annual genetic structure.

Table 1. Summary of *Amusium balloti* samples collected for population genetics study

Location	Adults	Location	Adults
Queensland		*Western Australia*	
Yeppoon	36	Gee Banks	18
Bustard Head	33	Wooded Island A (September 03)	34
Hervey Bay	27	Wooded Island B (September 04)	19
Townsville	28	Pelsaert Island	19
Noosa	31	Shark Bay	27

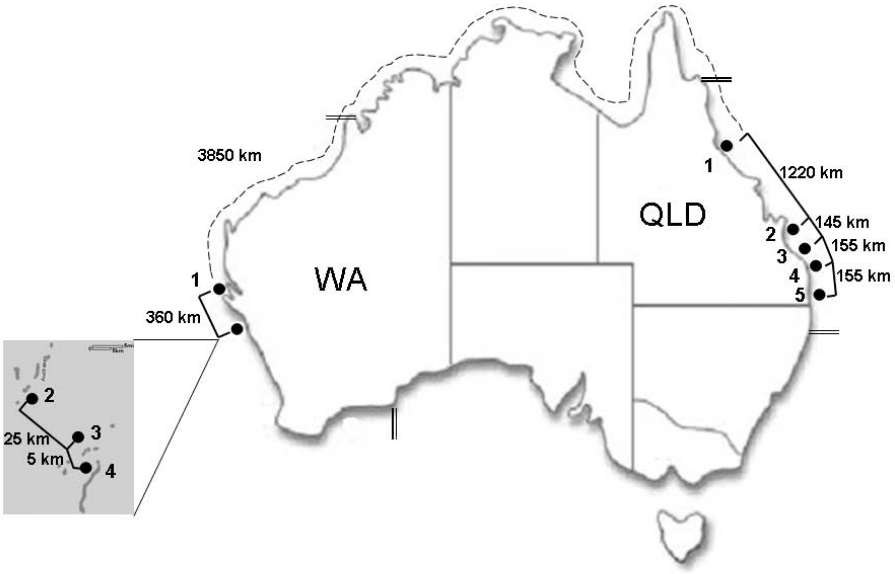


Figure 2. Approximate location of samples of *Amusium balloti* collected from Australian populations for microsatellite analysis. Queensland (QLD): 1—Townsville; 2—Yeppoon; 3—Bustard Heads; 4—Hervey Bay; 5—Noosa. Western Australia (WA): 1—Shark Bay; 2—Wooded Island; 3—Gee Bank; 4—Pelsaert Island. Approximate distances were calculated from Geoscience Australia (http://www.ga.gov.au/map/names/distance.jsp) and are indicated beside neighbouring populations. Reported species distributions are indicated by double lines. Map of the Abrolhos island group was sourced from MapQuest.com 2005.

Samples of either adductor muscle or gill filaments were collected depending on the logistical considerations. Samples were either immersed in 20% DMSO or snap frozen for air transportation or in 70% ethanol if transported by land. Regardless of the transportation method, samples were transferred to 70% ethanol and stored at –20°C following arrival at the Bribie Island Aquaculture Research Centre.

4.2 DNA extraction

Several methods of DNA extraction were used for different applications.

For the genomic library construction, DNA was extracted as follows:
Approximately 1g of muscle tissue dissected from a saucer scallop was ground on ice in 10mL of buffer containing 0.2M Tris, 0.2M EDTA, 0.2M NaCl, 2% SDS, 400 g proteinase K and then incubated at 50°C for 60 minutes. DNA was then extracted using a standard phenol/chloroform organic extraction (Sambrook *et al* 1989), washed twice in 5mL of 70% ethanol, dried and resuspended in 1mL of TE buffer.

For PCR applications, scallop DNA was extracted using Chelex resin (Biorad) at a concentration of 5%w/v. Samples were heated to 90°C in 5% w/v chelex resin for 10 minutes. Prior to PCR, DNA extracts were vortexed and spun at 600rpm in a desktop centrifuge. The supernatant was removed and added to the PCR reaction.

For highly degraded tissue samples, DNA was extracted using a DNEasy tissue kit (Qiagen) according to the manufacturer's instructions. Extractions were resuspended in 200 L ddH_2O, and stored at –20°C.

4.3 Microsatellite markers

4.3.1 Genomic library construction

A partial genomic library was constructed from DNA extracted from muscle tissue of a single saucer scallop using the above protocol. 6 g of DNA was digested with 6 units of Rsa1 enzyme and 1X Rsa1 buffer (New England Biolabs) in a final volume of 50 l at 37°C for 60 minutes. Known adaptors were then ligated to the digested DNA in a 60 l reaction containing 1 g of digested DNA, 50ng RSA21 adaptor 5'-CTC TTG CTT ACG CGT GGA CTA-3', 50ng RSA25 adaptor 5-AGT CCA CGC GTA AGC AAG AGC ACA-3, 2U T4 DNA ligase and 1X ligase buffer (Promega) for 2 hours at 37°C.

Approximately 50ng of ligated genomic/adaptor DNA was amplified in a 12.5 l PCR reaction containing 10mM Tris-HCL pH 8.8, 0.1% Triton-X, 50mM KCL, 2.5mM $MgCl_2$, 0.2mM dNTPs, 1.0 M RSA21, 0.75 units of *Taq* DNA polymerase (Invitrogen). Amplification was performed in a PCR Express (Hybaid) as follows. After an initial denaturating at 94°C there were 30 cycles of 30 seconds at 94°C, 30 seconds at 56°C and 45 seconds at 72°C followed by 10 minutes at 72°C.

4.3.2 First round enrichment for microsatellites

Amplified PCR products were enriched for microsatellite containing fragments using 5' biotin labelled microsatellite repeat probes in two pools. Pool 1 contained $(TAGA)_6$, $(GACA)_6$, and $(CCA)_7$ probes. Pool 2 contained $(AAG)_{10}$, $(TCG)_7$ and $(CTG)_7$ probes. 25 pmoles of each probe was added to 1 g of amplified genomic/adaptor product and heated to 95˚C for 3 minutes. The probe mix was incubated at 56˚C for pool 1 and 60˚C for pool 2 for 15 minutes in 6XSSC preheated to the incubation temperature. Following incubation probes were captured with streptavidin coated magnetic beads (Dynal) and washed once in 2xSSC and twice in 1XSSC.

Following elution from the magnetic beads approximately 200ng of enriched fragments were amplified in a 50 l reaction containing 10mM Tris-HCL pH 8.8, 0.1% Triton-X, 50mM KCL, 2.5mM $MgCl_2$, 0.2mM dNTPs, 1.0 M RSA21, 0.75 units of *Taq* DNA polymerase (Invitrogen). Amplification was performed in a PCR Express (Hybaid) as follows: After an initial denaturating at 94^{o}C there were 30 cycles of 30 seconds at 94^{o}C, 30 seconds at 56^{o}C and 45 seconds at 72^{o}C followed by 10 minutes at 72^{o}C.

PCR amplified fragments were ligated into the BamH1 site of the vector pGEM™-T (Promega). Ligated Plasmids were transformed into XL1–Bluescript competent cells and plated on selective media.

4.3.3 Second round enrichment for microsatellites

A second round of enrichment was conducted to increase the percentage of clones containing microsatellites. Enriched colonies were replicated to nylon filter membrane (Hybond N+, Amersham) and probed with 5' P^{32} end labelled oligonucleotide probes ($(TAGA)_6$, $(GACA)_6$, $(CCA)_7$, $(AAG)_{10}$, $(TCG)_7$, $(CTG)_7$) for 2 hours at 56^{o}C. Probed membranes were washed once with 2X SSC for 30 minutes at 56^{o}C, twice with 1X SSC for 30 minutes at 56^{o}C, and dried for 30 minutes at 80^{o}C.

Positive colonies were visualised by exposing Amersham Hyperfilm MR autoradiography film (Amersham) with an intensifying screen for 2 hours at –70^{o}C.

4.3.4 Library screening

Positive colonies were PCR amplified in a 25 l reaction containing 10mM Tris-HCL pH 8.8, 0.1% Triton-X, 50mM KCL, 2.5mM $MgCl_2$, 0.2mM dNTPs, 0.5 M T7 primer, 0.5 M SP6 primer and 0.75 units of *Taq* DNA polymerase (Invitrogen). Amplification was performed in a PCR Express (Hybaid) as follows: After an initial denaturation at 94^{o}C there were 30 cycles of 30 seconds at 94^{o}C, 30 seconds at 45^{o}C and 1 minute at 72^{o}C followed by 10 minutes at 72^{o}C. PCR products of between 500 and 1000 base pairs were cleaned via ethanol precipitation and sequenced at the Australian Genome Research Facility using big dye terminators (ABI) according to the manufacturer's instructions.

4.3.5 Identification of potential microsatellite markers

Sequenced clones were checked for the presence of microsatellite repeats and satisfactory flanking region that allowed the design of PCR primers to produce a final product between 150 base pairs and 350 base pairs. All designed PCR primers were optimised for both magnesium chloride concentration and annealing temperature via a temperature gradient PCR conducted on a Hybaid PCR express thermocycler. Each successfully optimised primer pair was used to amplify DNA from four individuals of *A. balloti* from each of three different geographic locations. These PCR products were used to assess levels of polymorphism by electrophoresis on 6% acrylamide gels and stained with ethidium bromide.

4.3.6 Optimisation of fluorescent PCR

PCR primer pairs that amplified only the target sequence and displayed polymorphism between individuals were fluorescently labelled to facilitate size separation on an ABI 377 automated sequencer. Primer pairs were designed to amplify distinct size ranges, or labelled with different fluorophores to facilitate pooling of PCR products and co-loading of up to three primer pairs in a single lane.

Pooled PCR products were sent to the Australian Genome Research Facility (AGRF) or Gribbles Molecular Science for separation on an ABI377 or Amersham Megabace respectively. Allele size was determined relative to internal size standards.

4.3.7 Allele scoring

Ten samples were amplified for all loci and sent to both AGRF and Gribbles Molecular Science to test for variance in raw length data. Any difference in raw size data between AGRF and Gribbles was corrected for prior to analysis.

Raw size data from both providers were imported into an excel spreadsheet and plotted relative to the bin score received from the provider. Allele bins were checked for accuracy and any incorrectly binned alleles were corrected prior to analysis.

4.3.8 Statistical analysis

General population diversity indices, including allele number and expected and observed heterozygosity were calculated using Genepop Version 3.4 (Raymond and Rousset 1995b). Goodness of fit to Hardy-Weinberg expectations was calculated using Markov Chain approximations in Genepop version 3.4. Genotypic disequilibrium was estimated using Fisher's method in Genepop Version 3.4 and P values were corrected for simultaneous multiple comparisons using a sequential Bonferroni correction.

An analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) was conducted in Arlequin Version 2.0 to partition the genetic variation within population components (F_{IS}) and between population components (F_{ST}).

Population pairwise F_{ST} using Slatkins (1995) linearised genetic distance were also calculated in Arlequin Version 2.0. Significance of the F_{ST} Values was tested by 10 100 permutations of the data set.

An unbiased estimate of the *P*-value for Fisher's exact test was performed in Genepop Version 3.4 as described by Raymond and Rousset (1995a).

Temporal change in allele frequency between Wooded Island A and Wooded Island B was measured by implementing Fisher's exact test in Genepop Version 3.4.

4.4 Mitochondrial markers

To align with current international research in scallop taxonomy, partial fragments of the 12S ribosomal RNA, and 16S ribosomal RNA genes were sequenced from five individuals of five species (Table 2). The genomic DNA used for PCR amplification of these genes was extracted using the same method as the genotype analysis of *A. balloti*.

Table 2. Summary of Pectinid species and collection sites. All *Amusium balloti* samples were collected in the same year, 2003.

Species	Collection location
Amusium balloti	Wooded Island, W.A.
	Townsville, Qld (Town.)
	Yeppoon, Qld (Y)
	Bustard Heads, Qld (B.H.)
	Hervey Bay, Qld (H.B.)
	Noosa, Qld (N)
Amusium pleuronectes	Gulf of Carpentaria, Qld (G.C.)
	Princess Charlotte Bay, Qld (P.C.B.)
Gloripallium pallium	New Caledonia
Belachlamys aktinos	Stradbroke Island, Qld
Pecten novazealandie	New Zealand

Fragments of the 12S and 16S rRNA genes were amplified using primers from three sources and attempts were made to amplify a section of the cytochrome oxidase subunit I (COI) gene (Table 3). Direct sequencing of the PCR product was accomplished using the forward primer of the relevant primer through a commercial sequencing facility, Macrogen Inc.

Table 3. Primer sequence used to PCR amplify 12S rRNA, 16S rRNA and COI from the scallop samples including the reference

Primer name	Primer sequence 5'-3'	Anneal. T°C	Reference
12S rRNA F	AGACATGGATTAGATACCC	52°C	Saavedra, C. *pers. comm.*
12S rRNA R	ACCCCCTACCTTGTTACGACTT		(Barucca *et al*. 2004)
16S rRNA F	CGCCTGTTTAACAAAAACAT	55°C	(Canapa *et al.* 2000)
16S rRNA R	CCGGTTTGAACTCAGATCACGT		(Canapa *et al.* 2000)
COIF	ATYGGNGGNTTYGGNAAYTG	52°C	(Matsumoto and Hayami 2000)
COIR	ATNGCRAAYTTYGGNTC		(Matsumoto and Hayami 2000)

Sequences were edited using Bioedit (Hall 1999) and aligned with sequences retrieved from NCBI (Table 4) using ClustalW (Thompson *et al.* 1994).

A distance matrix for 12S rDNA and 16S rDNA was generated using PAUP* (Swofford 1998) using an uncorrected pairwise comparison based on the percentage of nucleotide differences.

The phylogenetic trees were produced based on the methods of Barucca *et al* (2004) using Maximum Parsimony (MP) in the PAUP* program (Swofford 1998) with 1000 bootstrap replicates. Maximum parsimony trees were produced following heuristic searches with TBR (Tree bisection-replication) branch swapping and with random stepwise additions and 100 replications. Bootstrap values indicating robustness of nodes, refer to 1000 replications. *Delectopecten vitreus* from the *subf*amily Camptonectinae was used as an outgroup for the analysis of the 16S rDNA and *Spondylus gaederopus* from the Family Spondylidae was used for the 12S rDNA partial sequences as per Barucca *et al* (2004).

Table 4. Species, with associated accession numbers, used in phylogenetic analysis of scallops including species sequenced as part of the current study. —indicates no sequence data was available.

Species	Locality	Accession Numbers	
		12S	16S
Adamusium colbecki	Ross Sea (Antarctic)	AJ571589	AJ243882
Aequipecten opercularis	Mediterranean Sea (Italy)	AJ571591	AJ245397
Agropecten irradians concentricus		—	AF362384
Agropecten irradians irradians		—	AF362383
Amusium balloti	Indian Ocean (Western Australia)	**This study**	**This study**
Amusium balloti	Coral Sea (Qld. Australia)	**This study**	**This study**
Amusium pleuronectes	Gulf of Carpentaria (Australia)	**This study**	**This study**
Amusium pleuronectes	Coral Sea (Qld. Australia)	**This study**	—
Amusium pleuronectes	South China Sea (Philippines)		AJ571592
Belachlamys aktinos	Coral Sea (Qld, Australia)	**This study**	**This study**
Chlamys gabra	Mediterranean Sea (Italy)	AJ571590	AJ243574
Chlamys islandica	White Sea (Russia)	AJ571605	AJ243573
Chlamys multistrata	Mediterranean Sea (Italy)	AJ571604	AJ571617
Chlamys nobilis		—	AF362382
Chlamys farreri			AF362385
Chlamys varia	Mediterranean Sea (Italy)	AJ571593	AJ243575
Coralichlamys madreporarum	Coral Sea (Loyalty Islands)	AJ571598	AJ571608
Decatopecten plica		—	AF362388
Delectopecten vitreus	Mediterranean Sea (Italy)	—	AJ571618
Gloripallium pallium	Coral Sea (New Caledonia)	**This study**	**This study**
Gloripallium pallium	Coral Sea (Loyalty islands)	AJ571599	AJ571609
Laevichlamys cuneata	Coral Sea (Loyalty islands)	AJ571594	AJ571610
Laevichlamys wilhelminae	Coral Sea (Loyalty islands)	AJ571595	AJ571611
Mimachlamys nobilis	South China Sea (Philippines)	AJ571606	AJ571620
Mirapecten mirificus	Coral Sea (Loyalty islands)	AJ571600	AJ571612
Mirapecten rastellum	Coral Sea (Loyalty islands)	AJ571601	AJ571613
Mizuhopecten yessoensis	Japan	AB052599	AB052599
Patinopecten caurinus		AY704171	—
Patinopecten yessoensis		—	AF362386
Pecten jacobaeus	Mediterranean Sea (Italy)	AJ571596	AJ245394
Pecten maximus	Atlantic Ocean (France)	AJ571597	AJ571619
Pecten novazealandie	New Zealand	**This study**	AY650055
Semipallium amicum	Coral Sea (Loyalty islands)	AJ571602	AJ571614
Sempallium dringi	Coral Sea (Loyalty islands)	AJ571603	AJ571615
Spondylus gaederopus	Mediterranean Sea (Italy)	AJ571607	AJ571621

5 Results — Population genetics

5.1 Microsatellite isolation

Microsatellite containing clones were isolated from approximately 2000 screened colonies. Initial screening of the enriched library resulted in poor yield and a high ratio of non-target clones (with respect to the probe sequence). Fewer than 10% of sequenced clones contained the target microsatellite repeat unit. However, the second round enrichment using P^{32}-labelled microsatellite repeat probes increased the success of finding target microsatellite sequences to approximately 50% (26 target microsatellites from 49 sequenced clones). A mix of perfect and interrupted di, tri and tetra-nucleotide microsatellites and two minisatellites were identified.

Primer sets were ordered for 21 clones that contained enough satisfactory flanking region to facilitate stringent primer design that would produce a PCR product of between 150 and 350 base pairs in length. Following polymorphism testing fluorescently labelled primers were ordered for 11 loci. Three loci proved to be problematic following fluorescent labelling and were subsequently not used. One locus, Aballoti 377, was amplified for all samples but was not included in the analysis due to a high number of alleles (>50) and apparent preferential amplification of short alleles.

A total of seven loci were used for the final analysis (Table 5). Primers were labelled with one of three fluorophores enabling the co-loading of loci in a single lane for electrophoresis. Loci that were able to be co-loaded were: Aballoti 355, Aballoti 299 and Aballoti 129; Aballoti 377, Aballoti 291 and Aballoti 75; and Aballoti 341 and Aballoti 147. Table 6 contains information on the microsatellite primers that were not utilised in the current study.

Table 5. Summary of *A. balloti* microsatellite primers used in the current study.

Locus	Repeat motif of sequenced clone	Primer (5'–3')	Label	Annealing Temp	Final Primer conc.	$MgCL_2$ conc.
Aballoti 341	(CAT)11	F: GCAAATATTTACCCAGACTGTTCA R: TCTGAATTTTGTCGATTTCTTGTC	FAM	54˚C	0.2 M	1.4 mM
Aballoti 147	(GAT)14	F: CGTGTTGTATTTGTATTATTGTTTGA R: AACAATGGAGCCGTCGATAC	TET	54˚C	0.2 M	1.4 mM
Aballoti 75	(CA)24	F: TCATCAGATCCTATATCGTCATCTT R: GTCGCTCGTCCCACAGTTAC	HEX	58˚C	0.2 M	1.4 mM
Aballoti 355	(CAT)7	F: TTGCTCATAATTCAATGCCAAG R: ATTGCGACGTTGGATAGCTC	HEX	58˚C	0.2 M	1.4 mM
Aballoti 299	(GAT)8	F: TGTTTGATTATTTGTCGAAACCTT R: GCCAATGTCTCTCCTCTTGG	TET	58˚C	0.2 M	1.4 mM
Aballoti 129	(GT)12	F: CGAATCTATGGTTTCCCATGTT R: TTTTATACATCCTAGTTATTTGACGTG	FAM	54˚C	0.2 M	1.4 mM
Aballoti 291	(GAT)12	F: AACACCACCATTTACTCGAACA R: TCTCAGCTTTGAAGAATAAAGAATGA	TET	52˚C	0.2 M	1.4 mM

Table 6. Summary of *A. balloti* microsatellite primers not utilised in the current study

Locus	Repeat sequence	Primer (5'–3')	Label
Aballoti 205	(CAT)14	F: TCCCTCTCCCACTCTCCTTT R: CCATCCTCCTCATCACTACG	HEX
Aballoti 226	(GATA)5	F: TGACATGAAGAGTAACATTTTGTATG R: TTCTCAGTTAATCACACTCCATCA	FAM
Aballoti 232	GAT	F: GCAAACAATGCATTCTGGTAA R: TCTTCCTCCTCCTCATCATCA	HEX
Aballoti 377	GAT	F: GGCCATCATGTGTTGTCAAA R: TTCATGAGTTTTGAACATGAACAC	FAM
Aballoti 236	(GAT)28	F: ATGCAAAGCCCACACTAACC R: TTTGGTCGGTAGCATTGAAA	N/A
Aballoti 88	GACA	F: AACGTTGCGATATTTTACCG R: GCCAAATGCAATTTCGTATCT	N/A
Aballoti 122	(GA)22	F: TGGCAAAGGATGTGATGAAC R: CCGAAATAATGCAATGCAAA	N/A
Aballoti 152	(GA)23	F: TGATTTGTGTCATGTGGAACTG R: ATCGAGGATGTCGGATTCAA	N/A
Aballoti 253	(GA)4	F: AAAAAGGCAGCAAAAGCAAC R: AGCTCCTGATCCACCTTCCT	N/A
Aballoti 234	(CAT)14 CTT (CAT)6	F: CAGGTAATGATACACTATCCTACTCCT R: TTTGTTTTGATTCTTTGATGACG	N/A
Aballoti 254	(CAT)23	F: CCGTCATCATAAACCAGCAG R: TGGTGGAACGATAGGATGAT	N/A
Aballoti 100	GAT	F: TGCAAAAACACTTAACTGCAAG R: CAAATCATTACAGATAAACAACACTGC	N/A
Aballoti 405	CAT	F: TCATTCGTTCATTCTTATACAATGTG R: CGGTGATGACTCTATATAGATGCTG	N/A

5.2 Microsatellite characteristics

All seven microsatellites used in the present study were extremely polymorphic (Table 7). Amplification of 281 samples revealed that the number of alleles ranged from 16 to 32 and observed heterozygosities (H_o) ranged from 0.575 to 0.935. Expected heterozygosities (H_e) were consistently below those estimated under Hardy-Weinberg expectations. Five loci, Aballoti 341, Aballoti 75, Aballoti 355, Aballoti 129 and Aballoti 291 had allele frequencies that were significantly different from those expected of a population in Hardy-Weinberg equilibrium.

Table 7. Summary statistics for 7 microsatellite loci for all samples (281) combined. K is the number of alleles per locus, H_o observed heterozygosity, H_e expected heterozygosity, H_o/H_e values <1 indicate a heterozygote deficit, H_o/H_e values >1 indicate a heterozygote excess. P value is the probability that there is a deviation from Hardy-Weinberg expectations at that locus. Significant values are presented in bold.

Locus	Number of alleles (K)	Allele size range	H_o	H_e	H_o/H_e	P. value
Aballoti 341	26	130-203	0.863	0.940	0.918	**0.001**
Aballoti 147	30	166-260	0.935	0.942	0.992	0.363
Aballoti 75	32	210-287	0.745	0.954	0.781	**0.000**
Aballoti 355	23	235-299	0.575	0.769	0.747	**0.000**
Aballoti 299	29	216-306	0.876	0.940	0.931	0.121
Aballoti 129	19	195-247	0.706	0.819	0.862	**0.049**
Aballoti 291	16	166-211	0.802	0.889	0.902	**0.005**

5.3 Population structure analysis

5.3.1 Genetic diversity

A high level of genetic diversity was observed in all populations of *A. balloti.* Allele numbers ranged from 6 (Hervey Bay, locus Aballoti 355) to 29 (Pelsaert, locus Aballoti 75). Observed heterozygosities ranged from 0.393 (Townsville, locus 355) to 1.000 (Shark Bay, locus 147). There was no obvious difference in the number of alleles or heterozygosity between populations with the exception of the Hervey Bay sample for locus 299 where only nine alleles were observed, compared to the average of 18 for the other populations. This is probably the consequence of the smaller sample size from this sample site, caused by a large number of PCR failures in the sample.

5.3.2 Hardy-Weinberg Equilibrium

There was no pattern or consistency in the sample sites or loci that had allele frequencies significantly different from Hardy-Weinberg expectations (Table 8). Samples from four sites deviated significantly at locus Aballoti 75, two sites at loci Aballoti 355 and Aballoti 341, one site at Aballoti 129 and Aballoti 291, and no sites at loci Aballoti 147 and Aballoti 299. Samples from the Pelsaert site were most frequently out of Hardy Weinberg Equilibrium with five of seven loci showing significant departures from expected allele frequencies. Conversely, samples from the Yeppoon, Noosa and Shark Bay sites were in Hardy-Weinberg Equilibrium for all loci.

All significant departures from Hardy-Weinberg Equilibrium were due to heterozygote deficits. Allele number did not increase consistently with sample size, indicating that most of the available allelic variation has been investigated. Allele number and observed heterozygosity was similar for Queensland and Western Australian populations for Aballoti 341, Aballoti 129 and Aballoti 291. Western Australian populations had a slightly lower number of alleles at Aballoti 147 and Aballoti 299 (with the exception of Hervey Bay, where the low allele number observed was due to the low number of successful amplifications). However, for Aballoti 75 Queensland sites showed lower allele numbers, averaging 17 alleles per population compared with 27 alleles per population in WA. Similarly, Queensland populations averaged eight alleles per populations for Aballoti 355 compared with 16 alleles per population for WA. It is interesting to note that, despite similar allele numbers, observed, heterozygosities were lower in WA populations for Aballoti 129, probably due to the presence of a single dominant allele (frequency >0.5) in Western Australia (see figure 5.1) compared with three common alleles (frequency <0.25 each) in Queensland.

Table 8. Population statistics for seven microsatellite loci in seven *A. balloti* populations. N is the number of individual scallops sampled, K the number of alleles per locus, H_o observed heterozygosity, H_e expected heterozygosity, H_o/H_e values <1 indicate a heterozygote deficit, H_o/H_e values >1 indicate a heterozygote excess. P value is the probability that there is a deviation from Hardy-Weinberg expectations at that locus. Significant values after sequential Bonferroni correction are presented in bold.

Locus	Yeppoon	Bustard Head	Hervey Bay	Townsville	Noosa	Abrolhos Islands	Shark Bay
Aballoti 341							
N	32	27	27	22	28	54	21
K	19	21	18	17	21	19	15
Ho	0.875	0.889	0.741	0.955	0.929	0.796	0.952
He	0.937	0.946	0.941	0.937	0.943	0.925	0.921
Ho/He	0.934	0.939	0.787	1.019	0.985	0.861	1.034
P	0.111	0.034	**0.000**	0.320	0.259	**0.013**	0.319
Aballoti 147							
N	33	27	26	23	30	54	22
K	22	18	17	20	18	18	12
Ho	0.909	0.889	0.962	0.957	0.933	0.926	1.000
He	0.958	0.937	0.934	0.954	0.930	0.926	0.913
Ho/He	0.949	0.949	1.030	1.003	1.004	1.000	1.095
P	0.221	0.370	0.048	0.901	0.972	0.311	0.014
Aballoti 75							
N	29	25	27	19	31	45	26
K	21	16	18	14	16	29	25
Ho	0.793	0.640	0.593	0.824	0.710	0.756	0.923
He	0.943	0.952	0.948	0.939	0.934	0.970	0.956
Ho/He	0.841	0.672	0.625	0.877	0.760	0.779	0.966
P	0.024	**0.000**	**0.000**	0.448	**0.002**	**0.002**	0.074
Aballoti 355							
N	34	31	25	28	26	47	23
K	9	7	6	9	9	17	15
Ho	0.559	0.484	0.400	0.393	0.692	0.596	0.957
He	0.605	0.470	0.415	0.695	0.730	0.748	0.831
Ho/He	0.923	1.029	0.965	0.565	0.948	0.797	1.151
P	0.725	0.489	0.758	**0.000**	0.058	**0.000**	0.909
Aballoti 299							
N	34	31	11	26	29	45	21
K	22	20	9	20	17	16	13
Ho	0.900	0.871	0.818	0.962	0.862	0.844	0.857
He	0.953	0.932	0.909	0.950	0.919	0.926	0.923
Ho/He	0.945	0.934	0.900	1.012	0.938	0.912	0.928
P	0.050	0.131	0.314	0.889	0.868	0.074	0.006
Aballoti 129							
N	30	30	21	24	30	50	23
K	12	12	9	11	10	12	9
Ho	0.962	0.900	0.714	0.875	0.733	0.480	0.435
He	0.844	0.841	0.801	0.863	0.807	0.677	0.724
Ho/He	1.139	1.070	0.891	1.013	0.908	0.709	0.601
P	0.606	0.513	0.235	0.664	0.356	**0.000**	0.046
Aballoti 291							
N	34	29	26	27	29	46	21
K	12	12	9	13	11	12	11
Ho	0.912	0.931	0.808	0.926	0.793	0.630	0.667
He	0.874	0.903	0.839	0.892	0.836	0.893	0.912

Ho/He	1.043	1.031	0.963	1.038	0.949	0.706	0.731
P	0.317	0.339	0.862	0.685	0.173	**0.000**	0.002

5.3.3 Analysis of Molecular Variance (AMOVA)

Variance was partitioned into three hierarchical levels using AMOVA (Table 9). Approximately 93% of genetic variation was distributed within individual populations. Fixation indices for the within population (F_{ST}) and among populations within groups (F_{SC}) components were non-significant. 7.29% of variation was attributed to differences between grouped Queensland and grouped Western Australian populations. The F_{CT} value for the 'among groups' component was 0.0729 with a P-value of 0.047. AMOVA indicates a lack of structure within Queensland and within Western Australia, but significant genetic differences between the two states.

Table 9. Analysis of molecular variance (AMOVA, Excoffier et al 1992) partitioning variation into three hierarchical levels: within populations, among populations within groups and among groups. For this analysis, groups were QLD and WA.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	39.429	0.17913 Va	7.29
Among populations within groups	5	5.282	–0.01937 Vb	–0.79
Within populations	469	1077.066	2.29652 Vc	93.5
Total	475	1121.777	2.45628	

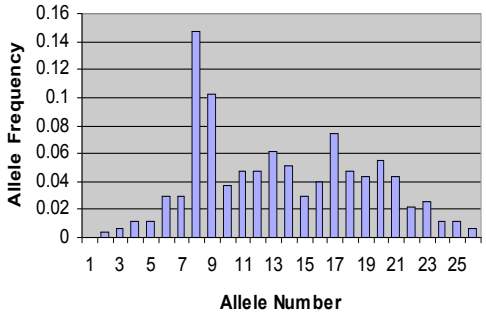
Fixation indices	Vc and F_{ST}	0.0650	P = 0.999
	Vb and F_{SC}	–0.0085	P = 0.998
	Va and F_{CT}	0.0729	P = 0.047

5.3.4 Analysis of Allele frequencies among Queensland and Western Australia

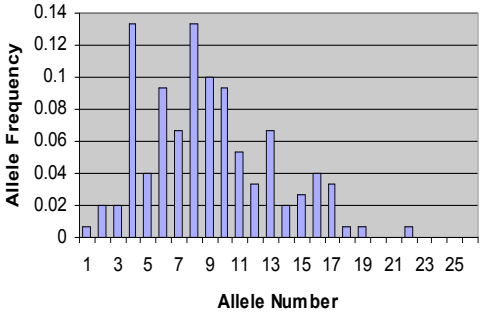
AMOVA revealed little genetic differentiation within samples from both Queensland and Western Australia sites but significant genetic differentiation between the two states. Allele frequencies were therefore pooled for all Queensland and Western Australian sites respectively. Results from the pooled data sets are presented in Figure 3. Allele frequencies between regions varied at all loci but most significantly at Aballoti 355 and Aballoti 129. At Aballoti 355, Allele 5 was at a frequency greater than 0.6 in the pooled

Queensland samples and less than 0.05 in the Western Australian samples. Conversely, Allele 6 was at a frequency less than 0.1 in Queensland samples and greater than 0.45 in Western Australian samples. At locus Aballoti 129, Alleles 7, 8 and 9 were at a frequency of approximately 0.25, 0.17 and 0.24 respectively, whilst in Western Australian samples, frequencies of Alleles 7 and 8 were below 0.05, whilst Allele 9 was at a frequency of 0.55. Other notable differences were a cluster of larger alleles at locus Aballoti 75 in Western Australian samples, but not in Queensland samples. This may have been an artifact of high allele number at this locus.

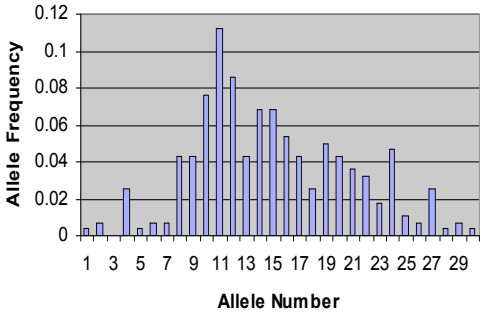
QLD Aballoti 341



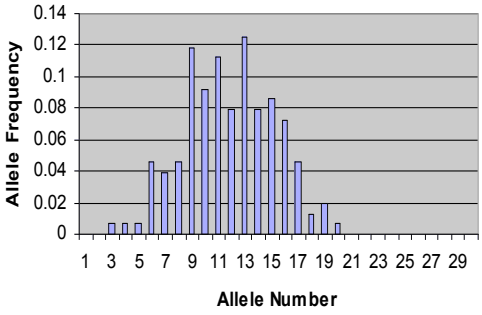
WA Aballoti 341



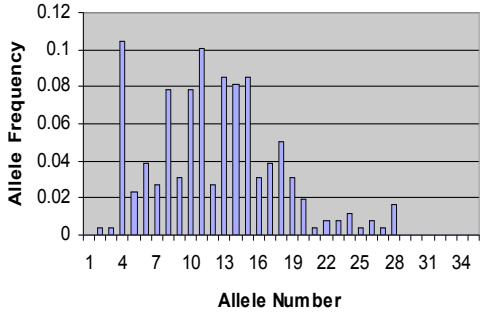
QLD Aballoti 147



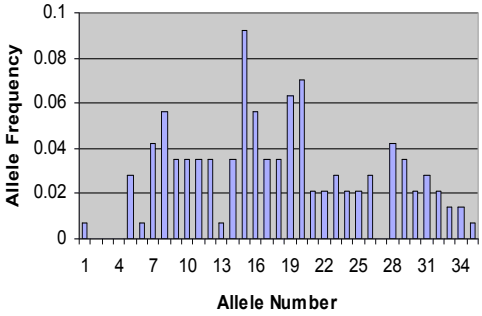
WA Aballoti 147



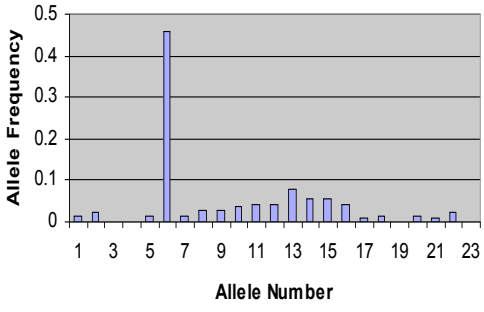
QLD Aballoti 75



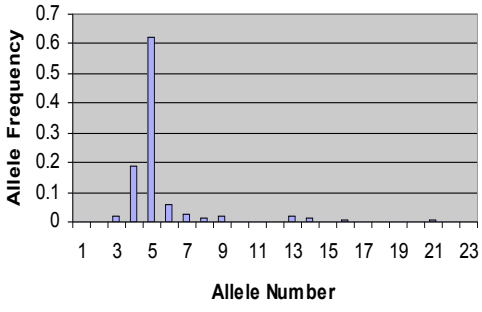
WA Aballoti 75



WA Aballoti 355



QLD Aballoti 355



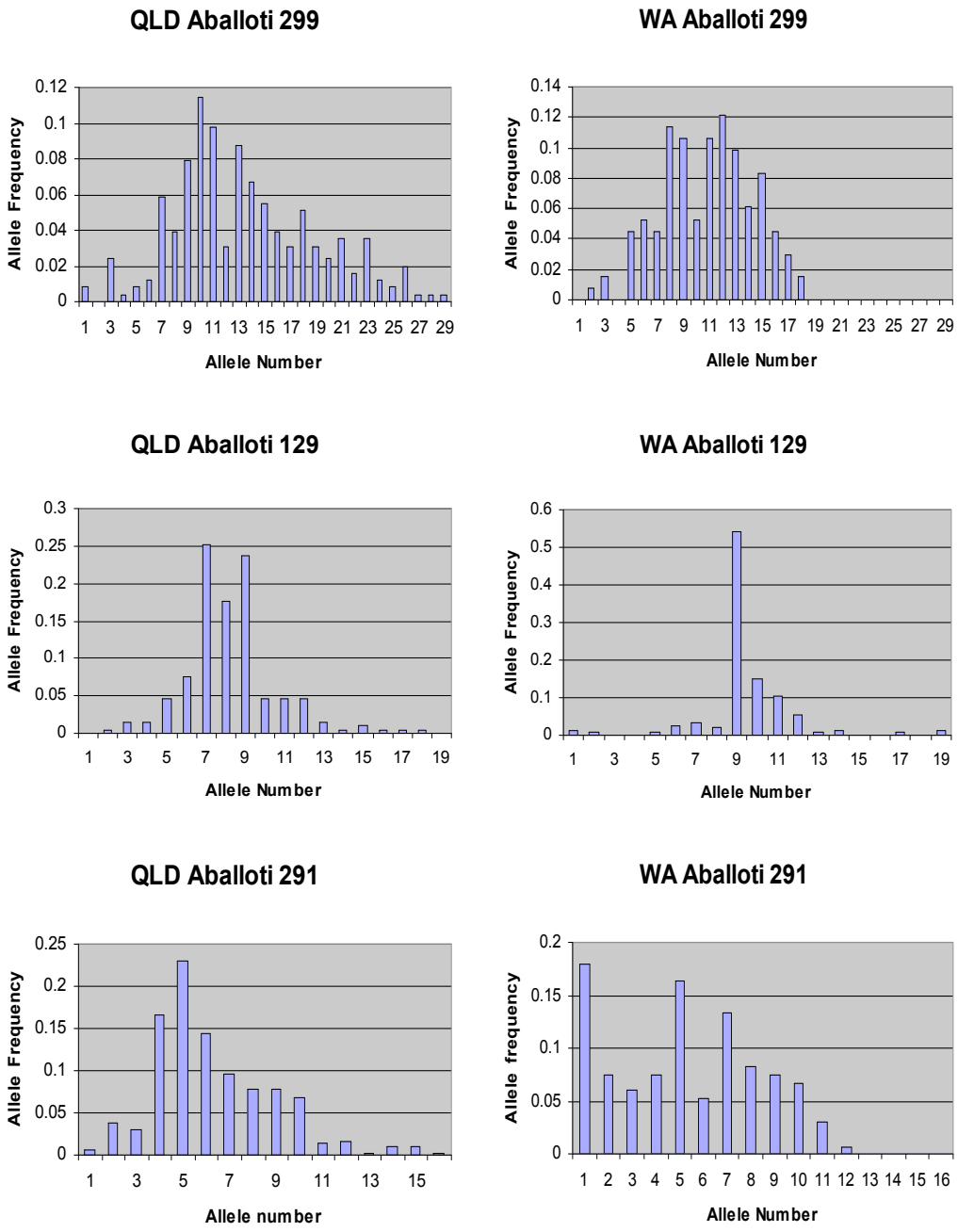


Figure 3. Allele frequency distributions for pooled Queensland Western Australian samples at equivalent loci.

5.3.5 Population pairwise F_{ST} (Slatkins distance)

Slatkin's pairwise F_{ST} values across all loci revealed significant genetic differentiation between Bustard Head and Noosa within Queensland (Table 10). All other pairwise population comparisons within Queensland and within Western Australian samples were non-significant. All population pairwise comparisons between Queensland and Western Australian populations were significant. The significant F_{ST} value between Bustard Head and Noosa is likely driven by significant allele frequency differences at Loci

129 (Fig. 4). Estimates of migration of individuals between populations ranged from 5.1 between the Abrolhos Islands and Bustard Head to infinity.

Table 10. Below diagonal—matrix of estimates of Slatkin's (1995) linearised F_{ST} values over all loci. Significant F_{ST} values are presented in bold. Above diagonal—estimated number of migrants between populations pere generation based on Slatkin's genetic distance.

	Yeppoon	Bustard Head	Hervey Bay	Townsville	Noosa	Abrolhos	Shark Bay
Yeppoon	~	719.4805	infinity	infinity	infinity	7.6361	7.3473
Bustard Head	0.0007	~	infinity	232.0333	39.2839	5.1160	5.3076
Hervey Bay	–0.0185	–0.0420	~	infinity	infinity	7.0869	7.2655
Townsville	–0.0011	0.0022	–0.0383	~	infinity	7.2363	7.6557
Noosa	–0.0008	**0.0126**	–0.0079	–0.0097	~	10.1184	9.9023
Abrolhos	**0.0615**	**0.0890**	**0.0659**	**0.0646**	**0.0471**	~	infinity
Shark Bay	**0.0637**	**0.0861**	**0.0644**	**0.0613**	**0.0481**	–0.0091	~

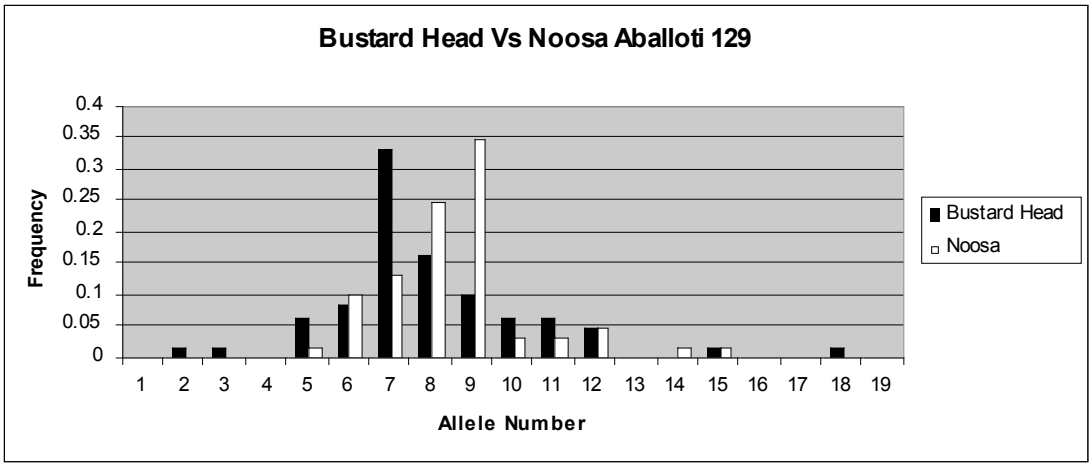


Figure 4. Allele frequencies for Aballoti 129 for Bustard Head and Noosa.

5.3.6 Fisher's exact test of allele frequencies

Estimates of Fisher's exact test for all loci were tested for significance by Markov chain randomisation. Significant exact test values are presented in Table 11. Within Queensland, 7 of 10 pairwise comparisons were not significantly different at any loci. Samples from Noosa were significantly different to Bustard Head for Aballoti 129, Hervey Bay for Aballoti 355 and Townsville for Aballoti 147, Aballoti 355 and Aballoti 299. No loci were significantly different between Shark Bay and the Abrolhos Islands. A high number of loci were significantly different from samples from both the

Abrolhos Islands and Shark Bay and all Queensland populations. Fisher's Exact test was significant for three loci, Aballoti 147, Aballoti 355 and Aballoti 129 for all pairwise comparisons of Queensland locations/sites against Western Australian locations/sites.

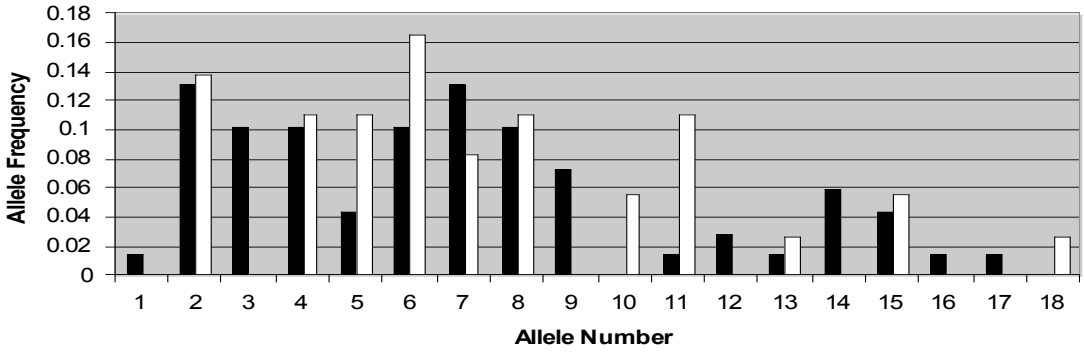
Table 11. Number of loci (above diagonal) and locus name (below diagonal) that are significant for a pairwise unbiased estimate of Fisher's Exact test (Raymond and Rousset 1995).

	Yeppoon	Bustard Head	Hervey Bay	Townsville	Noosa	Abrolhos	Shark Bay
Yeppoon	—	0	0	0	0	6	5
Bustard Head		—	0	0	1	5	3
Hervey Bay			—	0	1	5	4
Townsville				—	3	6	3
Noosa		129	355	147, 355, 299	—	7	5
Abrolhos	341, 147, 75, 355, 299, 129	147, 75, 355, 299, 129,	341, 147, 75, 355, 129,	341, 147, 355, 299, 129, 291	341, 147, 75, 355, 299, 129, 291	—	0
Shark Bay	341, 147, 75, 355, 129	147, 355, 129	341, 147, 355, 129	147, 355, 129	341, 147, 75, 355, 129		—

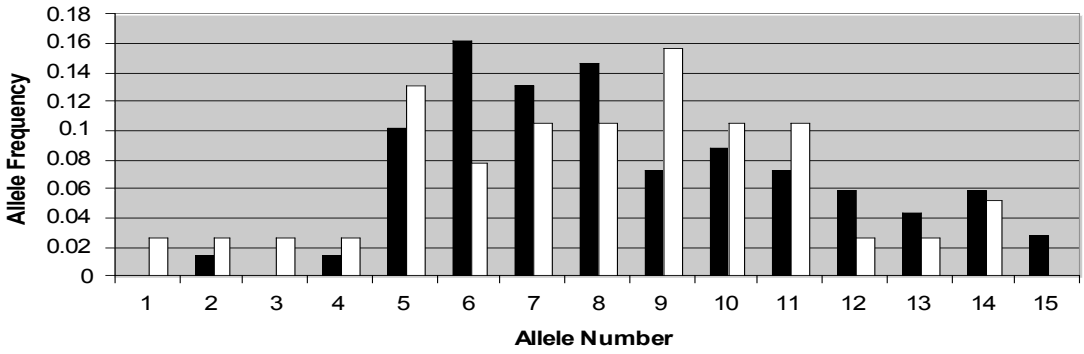
5.4 Analysis of temporal variation within Wooded Island

Fisher's Exact test revealed significant change in allele frequencies between Wooded Island A (collected September 2003) and Wooded Island B (collected September 2004) at loci Aballoti 355 (P = 0.000) and Aballoti 129 (P = 0.000). Allele frequencies at all other loci were not significantly different between the temporally-spaced samples. Allele frequencies for Wooded Island A and Wooded Island B are presented in Figure 5. The significant P value is likely to be driven by a shift in the common allele at both loci between years. At locus Aballoti 355, Allele 2 is at a frequency of 0.32 in Wooded Island A and completely absent in Wooded Island B. Allele 3 is absent in Wooded Island A and at a frequency of 0.37 in Wooded Island B. Similarly, at locus Aballoti 129, Allele 4 is at a frequency of 0.6 in Wooded Island B and completely absent from Wooded Island A. Conversely, Allele 5 is at a frequency of 0.5 in Wooded Island A and 0.1 in Wooded Island B.

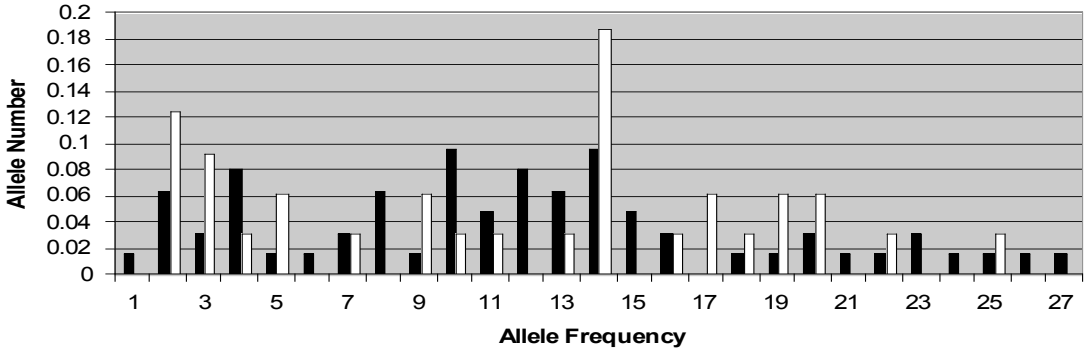
Aballoti 341



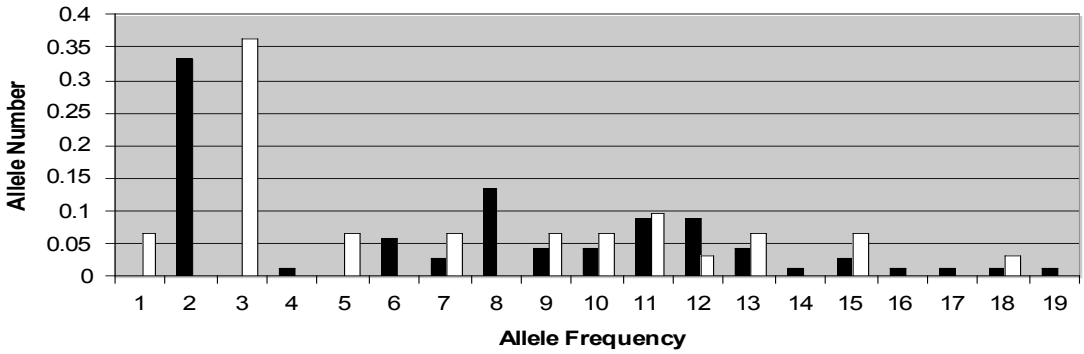
Aballoti 147



Aballoti 75



Aballoti 355



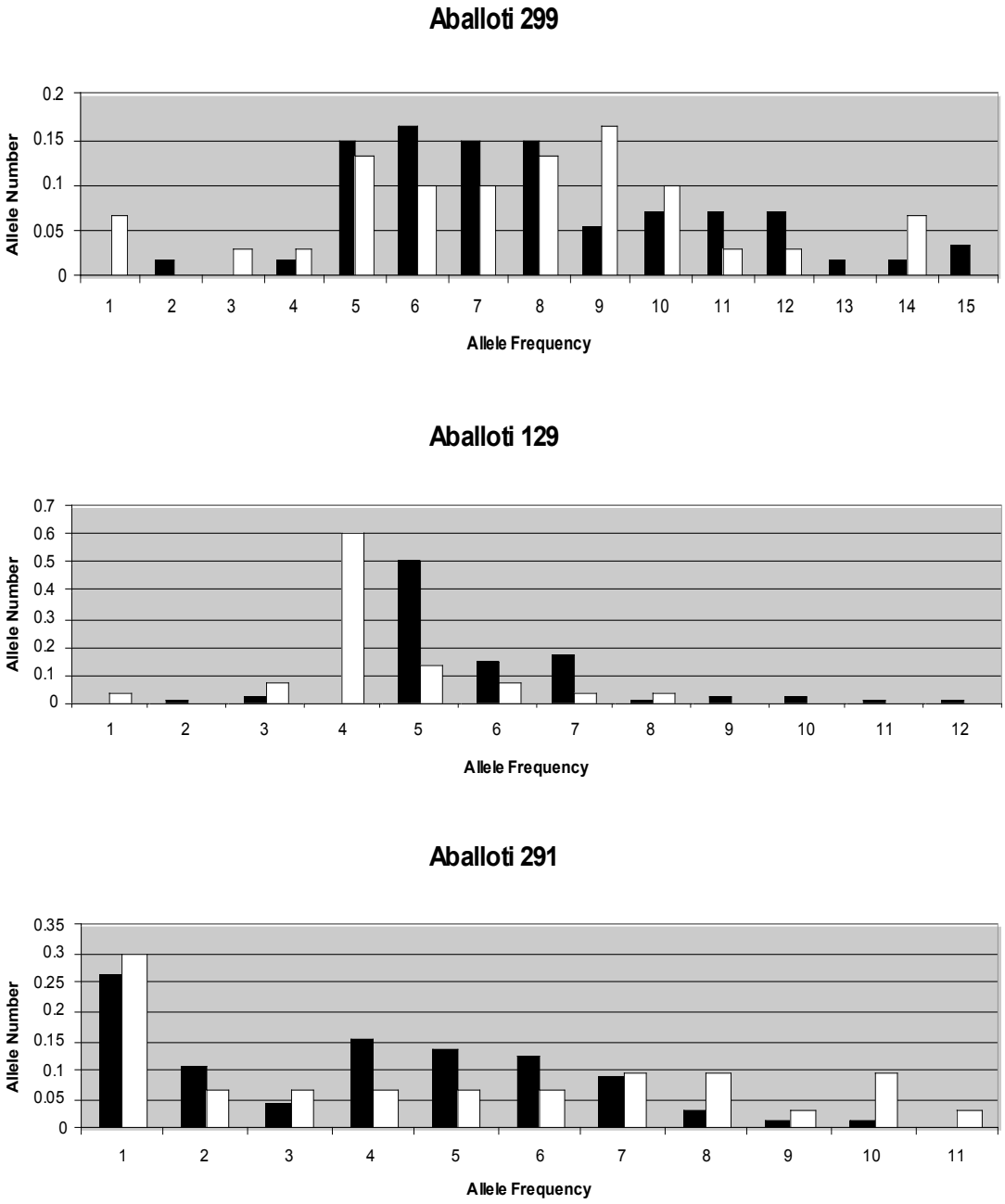


Figure 5. Temporal changes in Wooded Island group *Amusium balloti* allele frequencies at equivalent loci. Allele frequencies for Wooded Island A (Black) and Wooded Island B (White) samples collected in 2003 and 2004 respectively.

6 Discussion — Population genetics

6.1 *Amusium balloti* microsatellites

A major component of the study was to develop the molecular tools to investigate the recent population structure of *A. balloti*. The genomic DNA of *A. balloti* was screened for useable microsatellite loci because of their high variability and the supposed neutral status of the microsatellite repeat regions. During this process we found 21 microsatellites with useable flanking regions. Eight of these loci were found to be suitable for amplification with reasonable polymorphism. The difficulties encountered in isolating these microsatellites were similar to those experienced by Benzie and Smith in pearl oysters (2002). For example, in a number of instances the microsatellite sequence was directly adjacent to the end of the insert and lacked a suitable flanking region for primer design. Failure to PCR using some primer sets and non-polymorphic microsatellite loci also reduced the number of useful loci.

Our study was designed to identify relatively fine scale population structure so we aimed to sample within a putative deme (i.e. subpopulation) rather than including samples derived from a number of distinct demes. Population structuring within samples will lead to an underestimation of between-sample structuring. This approach also accounts for special conditions that might favour isolation of populations within an otherwise widely connected distribution (Johnson and Black 1998) as barriers to gene flow are fairly difficult to discern (Briggs 1974) and rarely absolute (Perrin *et al*. 2004). For example, island archipelagos have been associated with higher levels of genetic subdivision (e.g. Johnson *et al*. 1994). Allozyme analysis of *A. constricta* populations in Western Australia suggested that there were strong barriers to gene flow within the Abrolhos Group due to complex reef systems and shore habitats, as well as substantial blockages caused by local bathymetry and tides (Parsons 1996).

Because we were unsure of the degree of structuring between samples collected from the Abrolhos group (e.g. Wooded, Gee, Pelsaert) the samples were cumulatively pooled during analyses to estimate the size of the deme or breeding unit, as recommended by Goudet *et al.* (1994). This was done to prevent the underestimation of between sample structuring (Balloux and Lugon-Moulin 2002). We found no structure through comparison of the three samples. This indicated that the samples belonged to the same deme (Balloux and Lugon-Moulin 2002), so these three data sets were pooled for greater statistical power.

The population samples were mostly found to be in Hardy-Weinberg Equilibrium, which indicates that the genotype frequencies we observed were consistent with random mating. There were some instances of heterozygote deficiencies which may be due to null alleles (failure to PCR amplify an allele). It may also be due to the Wahlund effect, which assumes that species exist as a collection of subpopulations and that matings occur randomly within sub-populations, but matings between sub-populations do not occur or are severely restricted (Beaumont in press).

The resulting offspring may, however, disperse and be mixed, so that upon settlement, the sampled population will not consist of individuals resulting from one but from several random mating pools of individuals (Beaumont in press). In general, heterozygote deficiency appears to be common in marine molluscs (Singh and Green 1984; Zouros and Foltz 1984).

Heterozygote deficiencies were detected in three out of 25 allozyme loci in the pearl oyster, *Pinctada maxima* (Johnson and Joll 1993). Controlled multifactorial crosses undertaken with *Crassostrea gigas* showed that early selective effects during the larval cycle, which favoured the progeny of some parents over others, created an apparent non-Mendelian segregation (Boudry *et al.* 2002). This is a phenomenon often reported in oysters for both allozymes and microsatellite markers (McGoldrick and Hedgecock 1997; McGoldrick *et al.* 2000).

Deficiencies of heterozygous genotypes have also been widely detected in cephalopod populations (Garoia et al. 2004; Pérez-Losada et al. 2002; Shaw and Pérez-Losada 2000). The presence of null alleles may be one explanation for the heterozygote deficiencies in the Adriatic stock of *Loligo vulgaris*, but the admixture of different cohorts was also suggested as a contribution to the observed departures from Hardy-Weinberg Equilibrium (Garoia et al. 2004). Significant deviations from Hardy-Weinberg expectations were also observed in both wild and hatchery populations of *Haliotis discus hannai* (Li *et al*. 2004). Li *et al* (2004) suggested that null alleles and homoplasy, frequently found in microsatellite loci (Callen *et al*. 1993; Estoup *et al*. 1995), were the likely causes for the Hardy-Weinberg disequilibrium. However, heterozygote deficiency and homozygote excess were found in all allozyme genotype classes in *Haliotis rubra* and no null homozygotes were found (Brown 1991).

6.2 *Amusium balloti* shows genetic homogeneity of microsatellites over large distances

Population genetic structure was investigated in five Queensland and two Western Australian populations of *Amusium balloti*. An initial hierarchical analysis of population differentiation by AMOVA did not reveal any significant genetic heterogeneity from within Queensland or within Western Australian sites. However, comparative analysis of all Queensland samples against all Western Australian samples revealed significant ($P = 0.047$) genetic differentiation between Queensland and Western Australian with approximately 7% of genetic variation attributed to site differences between the pooled sample areas.

To further investigate the distribution of genetic variation within the populations of *A. balloti* we estimated Slatkin's (1995) linearised FST and Fisher's exact test of allele frequencies differences for population pairwise comparisons. Both tests revealed the presence of subtle genetic subdivision within Queensland that was not detected with AMOVA. However, this finding appeared to be locus specific and not consistent across all loci investigated.

There was also no pattern to describe the observed heterogeneity within Queensland. For example, when estimating Slatkin's FST over all loci combined, 1 out of 10 location pairwise comparisons within Queensland (Noosa vs Bustard Head) gave a significant FST. It should be noted that the FST value was 0.0126, indicating that less than 2% of the available genetic variation was observed between populations, and that the two samples had 98% of common genetic material. It has been suggested (Hartl and Clark 1997; Wright and Bentzen 1994), that an FST value lying in the range of 0–0.05 indicates little genetic variation; between 0.05–0.15, moderate differentiation; 0.15–0.25 great differentiation; 0.25 and greater, very great genetic differentiation. All pairwise comparisons of Queensland samples against those from (pooled) Western Australian samples gave a significant FST values. These ranged from 0.0471 for Noosa versus Pelseart, to 0.0890 for Bustard Head versus Pelsaert. The average difference was 0.065. This indicates that somewhere between 4.7% and 8.9% of variation, depending on the locus, is distributed between Queensland and Western Australian samples. This agrees well with the estimate of approximately 7.3% by AMOVA.

Fisher's exact test uses a contingency table of allele frequencies to estimate genetic differences. Fisher's exact test (as calculated in Genepop V3.4) also indicated that there was significant heterogeneity within Queensland (see Table 5.7) although again, not consistently across populations or loci.

Out of 70 possible pairwise comparisons within Queensland (10 pairwise comparisons for seven loci each) five were significant. Samples from Bustard Head and Noosa were again significantly different, but only for locus Aballoti 129 and not the remaining six loci. Hervey Bay and Noosa samples had significantly different allele frequencies at locus Aballoti 355 only, and Townsville and Noosa were significantly different at three loci (Aballoti 147, Aballoti 355 and Aballoti 299). When comparing Queensland populations with Western Australian populations, 49 of a possible 70 comparisons (10 pairwise comparisons for seven loci) were significantly different.

When considering the overall AMOVA, the single significant Slatkin's FST value and the inconsistent (with respect to loci) Fisher's exact test results, all suggest a high amount of gene flow between populations within Queensland and population homogeneity.

Neither Slatkin's FST or Fisher's exact test revealed any significant genetic differentiation between the two Western Australian sample sites, supporting the AMOVA data. These data are indicative of high levels of gene flow between Shark Bay and Pelsaert Island, and consequent population homogeneity.

It is important to consider and complement the statistical results with the biology of the animal (Waples 1998). Most marine species have lifecycles that alternate a relatively sedentary stage with an ecologically distinct pelagic larval phase capable of potentially great dispersal (Gosling 1994; Hilbish 1996; Nielsen 1995; Strathman 1985; Thompson *et al.* 1996).

Distance dispersed is partially correlated with time spent in the planktonic phase (Crisp 1976) and this in turn may strongly influence geographical range and genetic structure of populations (Crisp 1978; Jackson 1986; Scheltema 1989) with potentially profound consequences for phylogeny (Taylor 1988). Generally, this dispersal results in extensive gene exchange among distant groups and high levels of genetic homogeneity over large distances (Arnaud *et al.* 2003; Hellberg 1996; Hilbish 1996; Hunt 1993; Johnson 2000; Palumbi 1992). In addition, planktotrophic species tend to show greater genetic homogeneity among populations than lecithotrophic species (Doherty *et al*. 1995; Duffy 1993; Edmunds and Potts 1997; Hoskin 1997; Hunt 1993; Russo *et al*. 1994).

Planktotrophic development does not necessarily preclude genetic differentiation among populations (Todd *et al*. 1998). For example, it is possible for selection within a single generation to produce differences between areas or cohorts within an area (Johnson *et al*. 1986). Localised genetic structuring might occur as a result of larval behaviour (Burton 1983; Burton 1997; Doherty *et al*. 1995; Todd *et al*. 1998) and/or local hydrography (Fevolden 1992; Helleberg 1996; Palumbi *et al.* 1997; Roger *et al.* 1998).

The genetic homogeneity of *A. balloti* populations in Queensland and Western Australian waters that was detected during this study was not unexpected, given the biology of the animal. This species is a highly fecund broadcast spawner releasing up to six million eggs per female per spawning (Rose *et al.* 1988). The planktonic larvae take between 18 days (Queensland; Wang pers. comm.) and 10 days (Western Australia; McGowan pers. comm.) in a hatchery environment to become competent to settle out of the plankton. In the natural environment the larvae are susceptible to oceanic and wind-driven currents, including the East Australian eddy system in Queensland and the Leeuwin current in Western Australia, during this larval period. They also have a relatively limited ability to attach to the substrate (Wang *et al.* 2002) and newly metamorphosed spat have been reported to crawl actively and detach and swim in the water column (Cropp 1993), which may indicate the potential for additional short range dispersal following settlement. Adults are reported to be relatively sedentary, although moving less than 10km (Williams and Dredge 1981) in the post-settlement phase of their lives, which suggests that the major dispersal phase occurs during larval development. Functional reproductive status is reportedly reached within one year (Dredge 1981) and the animal is thought to live a maximum three to four years (Gwyther *et al.* 1991; Heald 1978).

Extensive gene flow, as evidenced by very low levels of genetic subdivision, is common among marine species along the Western Australian coast (Johnson 2000) particularly in animals with a relatively long planktonic phase (e.g. Table 12). This is thought to be facilitated by the Leeuwin current which flows southward transporting warm, clear low-nutrient waters along the edge of the continental shelf of the Western Australian coast and eastward into the Great Australian Bight (Cresswell and Golding 1980). This current is thought to play a major role in the diversity and abundance of biota in the area (Caputi *et al.* 1996; Hutchins and Pearce 1994; Joll and Caputi 1995; Pearce 1997).

Hutchins and Pearce (1994) calculated that planktonic larvae could travel from the Abrolhos Islands (29°S) to Rottnest Island (32°S) in 10 to 30 days on the basis of measured velocity of the Leeuwin current. However, current patterns are variable in space and time and may lead to gene flow at some times but not others (Palumbi *et al*. 1997). The Western Australian populations (Exmouth and Cape Bossut) of *Pinctada maxima* that were investigated using allozymes seem to be highly connected over distances of at least 800km (Johnson and Joll 1993).

Table 12. Summary of some marine invertebrate species comparing length of planktonic phase with detection of genetic homogeneity or panmictia.
1. R. Scoones, pers. comm. 2. S. Wang pers. comm. 3. Braine et al (1979).

Species	Planktonic phase	Panmictic?	Reference
*A. ballo*ti — WA	10 days	✓	This study
A. balloti — Qld	18 days	✓	This study
Panulirus cygnusi — Western rock lobster	9–11 months[2]	✓	(Thompson *et al*. 1996)
Bemmbicium vittatum — marine snail	Direct developer	x	(Parsons 1996)
Austrocochlea constricta — marine snail	Short planktonic	x	(Parsons 1996)
Acanthaster planci — crown of thorns starfish	14 days	✓	(Benzie 1992)
Pinctada maxima		✓	(Johnson and Joll 1993)
Haliotis rubra	3 days	✓	(Elliot *et al*. 2002)

Variable settlement of *A. balloti* larvae in Queensland appears to be related to a complex array of variable environmental conditions particularly wind, current, temperature and winter rainfall (Campbell 1981). It has been found that species from the Great Barrier Reef with relatively long larval phases (7–28 days) have the potential for dispersal over several hundred kilometres within the reef system, which is reflected in low levels of genetic divergence (Benzie 1994). For example, the outbreak populations of *Acanthaster planci* (Crown of Thorns starfish) on the Great Barrier Reef are suggested to be derived by dispersal from a single source (Benzie 1992).

It is therefore important to understand that this level of mixing does not preclude genetic management of any saucer scallop sea-ranching program, as hatchery-reared animals may have the opportunity to reproduce prior to harvest, thereby contributing to the overall genetic makeup of *A. balloti* in the area.

6.2.1 Possible temporal variation in A. balloti *genotypes*

For organisms with very high fecundity and high mortality in early life stages (type III survivorship curves), a small minority of individuals can replace the entire population in each generation by a sweepstakes-chance matching of reproductive activity with opportune environmental conditions conducive to spawning, fertilisation, larval survival and successful recruitment (Hedgecock 1994).

Variation in larval mortality among years typically results in density-independent relationships between breeding population size and strength of recruitment for marine animals (reviewed in Hedgecock 1994). Variation in recruitment between years is caused chiefly by variation in climate either though indirect effects on food availability or direct effects via physical

transport of larvae away from nursery areas or suitable settlement sites (Hedgecock 1994). Oceanographic processes and conditions that affect the reproduction of marine animal life vary not only among years but also within and among seasons and over mesoscale distances (tens to hundreds of kilometres) (Caputi *et al.* 1996; Hedgecock 1994).

Although not part of the original project objective we had the opportunity to analyse two temporally separate samples from the same geographic location (Wooded Island, Abrolhos group, WA—in 2003 and 2004). The analyses detected significant genetic differentiation in allele frequencies between the two years which, given the biology of the animal and the variability of its environment, was no surprise.

Genetic differentiation on a local scale requires either differential survival of genotypes after recruitment or temporal variation in the genetic composition of recruits (Hedgecock 1994). The Leeuwin current plays a major role in determining the diversity and distribution of the biota at the Houtman Abrolhos islands (Pearce 1997). However, there is a strong seasonality of the current flow and indications of substantial interannual variability (Caputi *et al*. 1996). The Leeuwin current frequently flows adjacent to the island chain, but on occasions larger meanders carry the warm waters offshore (Pearce 1997).

These complex hydrographic patterns coupled with the 'sweepstakes' method of reproduction for *A. balloti* create an environment in which variable recruitment and survival in space and time is likely. This, in turn is likely to cause variation in allele frequencies from year to year.

Temporal genetic differences are not uncommon in marine species (e.g. Carvalho *et al.* 1992; Chapman *et al.* 2002; Fillatre *et al.* 2003; Garoia *et al.* 2004; Lundy *et al.* 2000; Olsen *et al.* 2002b; Palm *et al.* 2003; Ruzzante *et al.* 1998; Thompson *et al.* 1996). The high variability in reproductive success in oysters, coupled with high fecundity in a variable environment and planktonic larvae (Hedgecock 1994) may lead to temporal genetic differentiation (Olsen *et al.* 2002a). Strong genetic differentiation between samples collected over two seasons (June 2001, October 2002) indicate that Adriatic cuttlefish undergo temporal genetic changes (Garoia *et al.* 2004). Significant temporal variation in microsatellite allele frequency was also observed in captive bred and wild populations of sea trout (*Salmo trutta*) (Palm *et al.* 2003), sockeye salmon (*Oncorhynchus nerka*) (Fillatre *et al.* 2003), Bornholm brown trout (*Salmo trutta*) (Østergaard *et al.* 2003) and the red drum (*Sciaenops ocellatus*) (Chapman *et al.* 2002).

Temporal differences in allele frequencies within subpopulations were also observed in the shortfin squid (Carvalho *et al.* 1992), the Atlantic cod (Ruzzante *et al.* 1998) and hake (Lundy *et al.* 2000), suggesting that genetically different schools may migrate to spawning grounds at different times within a given area (Garoia *et al.* 2004). In a number of instances effective populations sizes are small and individual populations may occasionally become extinct. This phenomenon is counterbalanced by strong gene flow among populations (Østergaard *et al.* 2003).

7 Results — Taxonomy

Partial sequences of 12S and 16S rRNA genes were successfully amplified and sequenced from *A. balloti*, *A. pleuronectes, G. pallium, B. aktinos* and *P. novaezelandiae* (alignments presented in Appendix 5). Unsuccessful attempts were made to amplify a region of the cytochrome oxidase subunit I (COI) gene from *A. balloti*.

Comparison of the genetic distance between *A. balloti* 12S rDNA sequences indicated some genetic differentiation between the Western Australian and Queensland samples (Table 13). This difference was even more apparent when 16S sequences were compared. The genetic distance between Queensland and Western Australian *A. balloti* ranged from 0.0028 to 0.03 (Table 8). This is greater than the distance observed between *P. maximus* and *P. jacobaeus* and *P. novaezelandiae* (0.002 and 0.014 respectively; Table 8), and *L. wilhelminae* and *C. madreporarum* (0.000; Table 8), which have historically been classified as separate species. *Argopecten irradians irradians* and *A. i. concentricus*, currently classified as subspecies of *A. irradians*, have a genetic distance of 0.013, which is also smaller than that estimated between the *A. balloti* strains. On this basis, Queensland and Western Australian *A. balloti* would be considered to be at least subspecific status.

The difference is also illustrated in the 16S rDNA and 12S rDNA MP trees as the Western Australian *A. balloti* sits outside of the Queensland clade in 99% of the bootstrap replications (Fig. 6 and 7). The results of the MP trees are consistent with those presented by Barucca *et al* (2004), Canapa *et al* (2000) and Saavedra and Peña. (2004).

Table 13. (Following page). Pairwise sequence divergence for the 12S rDNA (above the diagonal) and 16S rDNA (below the diagonal) gene fragments. Cells highlighted in bold indicate the estimated divergence between the *A. balloti* specimens sequenced during this study. Cells highlighted in grey indicate a divergence less than those estimated between the Western Australian and Queensland *A. balloti* individuals. Note: although all species listed in Table 4 were included in the analysis a number were omitted from this table to exhibit the data in a presentable format (i.e. on a single page).
The full results are available in Appendix 5.

	P. maximus	*P. jacobaeus*	*P. novaezealandiae*	*A. pleuronectes* (Phil.)	*A. pleuronectes* (G.C.)	*A. pleuronectes (Town.)*	*G. pallium*	*B. aktinos*	***A. balloti (P.C.B.)***	***A. balloti (Yep.)***	***A. balloti (B.H.)***	***A. balloti (H. Bay)***	***A. balloti (Noosa)***	***A. balloti (W.A.)***	*A. i. irradians*	*A. i.concentricus*	*C. varia*	*M. nobilis*	*S. dringi*	*L. wilhelminae*	*C. multistrata*	*C. madreporarum*
P. maximus		0.014	0.027	0.073	0.081	0.081	0.177	0.166	0.136	—	—	—	0.136	0.141	—	—	0.187	0.183	0.193	0.177	0.211	0.173
P. jacobaeus	0.002		0.035	0.076	0.090	0.090	0.178	0.169	0.136	—	—	—	0.136	0.142	—	—	0.193	0.194	0.194	0.183	0.217	0.179
P. novaezelandiae	0.014	0.012		0.070	0.084	0.084	0.171	0.160	0.135	—	—	—	0.135	0.141	—	—	0.174	0.180	0.195	0.171	0.211	0.168
A. pleuronectes (Philippines)	0.121	0.121	0.116		0.030	0.030	0.193	0.170	0.149	—	—	—	0.149	0.155	—	—	0.181	0.190	0.173	0.176	0.213	0.173
A. pleuronectes (G.C.)	0.131	0.131	0.128	0.045		0.000	0.201	0.173	0.157	—	—	—	0.157	0.163	—	—	0.192	0.198	0.192	0.187	0.224	0.189
A. pleuronectes (Town.)	—	—	—	—	—		0.201	0.173	0.157	—	—	—	0.157	0.163	—	—	0.192	0.198	0.192	0.187	0.224	0.189
G. pallium	0.184	0.186	0.184	0.175	0.169	—		0.214	0.114	—	—	—	0.114	0.109	—	—	0.251	0.230	0.214	0.212	0.252	0.208
B. aktinos	0.214	0.213	0.213	0.189	0.192	—	0.249		0.159	—	—	—	0.159	0.165	—	—	0.207	0.189	0.147	0.112	0.236	0.115
A. balloti (P.C.B.)	0.217	0.219	0.214	0.202	0.205	—	0.191	0.204		—	—	—	**0.000**	**0.008**	—	—	0.207	0.194	0.183	0.156	0.224	0.159
A. balloti (Yep.)	0.217	0.219	0.214	0.202	0.205	—	0.191	0.204	**0.000**		—	—	—	—	—	—	—	—	—	—	—	—
A. balloti (B.H.)	0.217	0.219	0.214	0.202	0.205	—	0.191	0.204	**0.000**	**0.000**		—	—	—	—	—	—	—	—	—	—	—
A. balloti (H.B.)	0.217	0.219	0.214	0.200	0.203	—	0.189	0.204	**0.000**	**0.000**	**0.000**		—	—	—	—	—	—	—	—	—	—
A. balloti (Noosa)	0.217	0.219	0.214	0.202	0.205	—	0.191	0.204	**0.000**	**0.000**	**0.000**	**0.000**		**0.008**	—	—	0.207	0.194	0.183	0.156	0.224	0.159
A. balloti (W.A.)	0.210	0.212	0.210	0.199	0.203	—	0.190	0.204	**0.030**	**0.030**	**0.030**	**0.028**	**0.030**		—	—	0.207	0.186	0.184	0.162	0.222	0.165
A. i. irradians	0.614	0.612	0.611	0.617	0.607	—	0.639	0.627	0.618	0.618	0.618	0.619	0.618	0.620		—	—	—	—	—	—	—
A. i.concentricus	0.619	0.616	0.616	0.619	0.609	—	0.641	0.629	0.622	0.622	0.622	0.623	0.622	0.624	0.013		—	—	—	—	—	—
C. varia	0.234	0.232	0.234	0.229	0.218	—	0.237	0.191	0.240	0.240	0.240	0.240	0.240	0.243	0.623	0.627		0.160	0.216	0.208	0.149	0.202
M. nobilis	0.247	0.245	0.250	0.201	0.213	—	0.230	0.208	0.229	0.229	0.229	0.227	0.229	0.226	0.603	0.610	0.243		0.168	0.166	0.181	0.168
S. dringi	0.282	0.284	0.284	0.250	0.253	—	0.283	0.137	0.285	0.285	0.285	0.285	0.285	0.274	0.622	0.624	0.280	0.232		0.115	0.223	0.112
L. wilhelminae	0.262	0.265	0.267	0.217	0.224	—	0.260	0.174	0.262	0.262	0.262	0.262	0.262	0.268	0.640	0.645	0.249	0.237	0.191		0.240	0.003
C. multistrata	0.196	0.193	0.193	0.203	0.204	—	0.197	0.205	0.181	0.181	0.181	0.181	0.181	0.185	0.625	0.630	0.130	0.227	0.248	0.190		0.237
C. madreporarum	0.262	0.265	0.267	0.217	0.224	—	0.260	0.174	0.262	0.262	0.262	0.262	0.262	0.268	0.640	0.645	0.249	0.237	0.191	0.000	0.190	

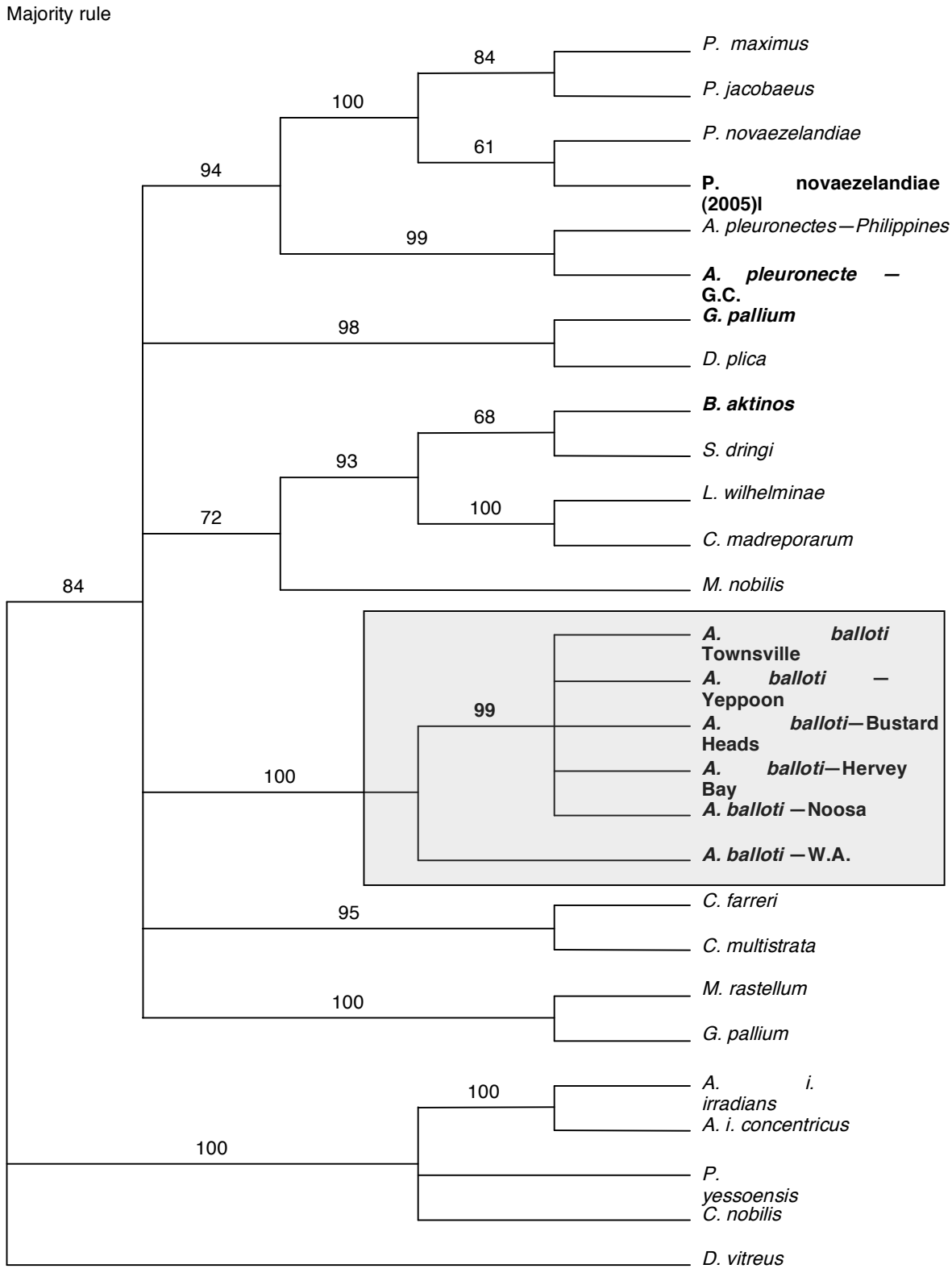


Figure 6. Phylogenetic tree obtained through maximum parsimony analysis using 16S rDNA data available a range of scallop species. Numbers on the braches indicate 1000 bootstrap replicates—greater than 50 indicates strong support for that clade, values less than 50 have been deleted. *A. balloti* samples are highlighted in bold and boxed in grey

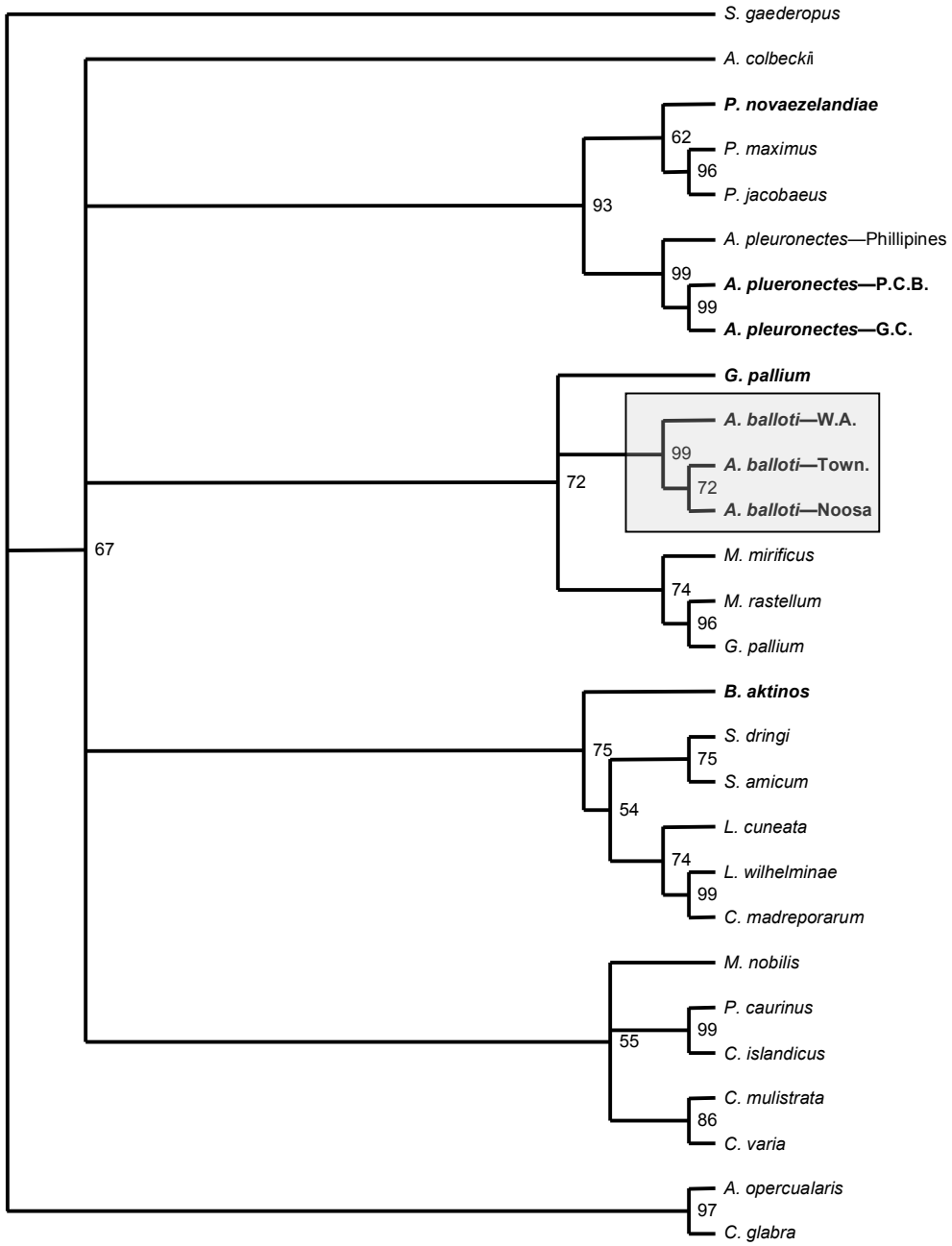


Figure 7. Phylogenetic tree obtained through maximum parsimony analysis using 12S rDNA data available a range of scallop species. Numbers on the braches indicate 1000 bootstrap replicates — greater than 50 indicates strong support for that clade, values less than 50 have been deleted. *A. balloti* samples are highlighted in bold and boxed in grey.

8 Discussion—Taxonomy

If populations are separated by a physical barrier to dispersal, speciation may follow. The acquisition of intrinsic reproductive isolation is then an incidental consequence of the accumulation of genetic differentiation (Mayr 1963). It is now quite generally accepted that geographic or allopatric speciation is the almost exclusive mode of speciation among animals (Gosling 1994). The West and East coast populations of *A. balloti* are geographically separated and allozyme analysis has indicated that the two populations may have been isolated for so long that they may be considered as separate species or at least subspecies (Dredge *et al*. in prep). This may have occurred during the last ice ages which occurred 350 000, 150 000 and 17 000 years before present {Keenan, 2000 #1755}.

To investigate the classification question further we analysed sequence data from the mitochondrial genome of *A. balloti* to determine the level of genetic differentiation between the Western Australian and Queensland strains. Both the 12S and 16S rRNA genes have sequence differences. The level of divergence is greater than that detected between different species (*P. maximus, P. jacobaeus* and *P. novaezelandiae; L. wilhelminae* and *C. madreporarum*) and different subspecies (*A. i. irradians* and *A. i. concentricus*). However, the species status of *P. maximus* and *P. jacobaeus* is under dispute. Based on genetic data, the two may be regarded as sub-populations of the same species or as sub-species (Beaumont 1991; Canapa *et al.* 2000; Wilding *et al.* 1999), but this is not supported by morphological studies which separate the species even in regions where populations co-exist (Waller, in press) .

It is interesting to note that the superficially similar species, *A. balloti* and *A. pleuronectes* are separated into different clades based on both 16S and 12S rDNA sequence data. In the present study and in a study by Barucca *et al.* (2004), *A. pleuronectes* appear closely related to *P. maximus* and *P. jacobaeus* based on the 12S and 16S datasets. This may indicate that the apparent morphological similarity of the species (Appendix 4) occurred through convergent evolution. Habe (Habe 1964) separates the two species due to the differences in internal rib of the right valve, 22–24 in *A. pleuronectes* and 50 in *A. balloti*. *A. pleuronectes* also employs a different reproductive strategy to *A. balloti* in that it is a hermaphrodite as is *P. maximus* and *P. jacobaeus* {Slack-Smith, 1998 #1756}. Similar separation of species based on 16s rDNA sequence has also occurred with the *Chlamys* group implying that not all *Chlamys* spp. can be assigned to a single sub-family (Canapa *et al.* 2000).

The differences detected between these two *A. balloti* populations is likely to result from historical geographic separation and may lead to significant differences over time. Previous studies have also identified genetic separation between Western and Eastern Australian populations of shellfish.

Johnson and Joll (1993) have shown that *Pinctada maxima* populations in Western Australia are genetically different from other populations in Australia. Genetic differentiation and differences in genetic diversity were also detected among *Penaeus monodon* populations from eastern and western Australia (Brooker *et al.* 2000). Much of the differentiation in *P. monodon* has been attributed to major historical biogeographical barriers, or to founder events of the recent ice ages; these patterns of differentiation have remained despite any potential for present-day gene flow (Benzie 2000). However, due to the variation in rate of evolution of different genes in different organisms there is no prescribed genetic distance that would equate to definition of a species and it is recommended that delineation of a species should be based on more than just one approach.

Elizabeth Gosling (1994) presents a succinct review of the four major concepts for delineation of species but concludes that no one species concept is inherently superior to another. She did support White's (1978) view that the delineation of a species should adopt a multidisciplinary approach, ideally including data on:

1. geographic distribution, present and past
2. morphology
3. ecology, e.g. habitat, food, temperature, salinity, substrate preference
4. physiology and biochemistry
5. reproductive cycle, behavioural or chemical isolating mechanisms
6. genetics (allozymic, nuclear, mitochondrial, cytogenetics, immunology and hybridisation).

Gosling (1994) also pointed out that there are few, if any, cases where all of this information has been collated. In general there is a good fit between the morphological and genetic classification of scallops (Waller, in press).

Due to their geographic isolation it is predicted that the two Australian strains or forms of *A. balloti* will continue to diverge. The two strains are geographically isolated but morphologically similar. The genetic data indicates that the strains be considered at least as separate sub-species, requiring further information from other disciplines, as proposed by Gosling, to definitively assign species or subspecies status to the two strains.

9 Benefits and adoption

The potential beneficiaries of this project are fisheries and aquaculture managers, commercial operators (specifically saucer scallop sea-ranching ventures), and fisheries and aquaculture scientists. The benefits relate to the environmental acceptability of scallop sea-ranching ventures through responsible genetic management and maintenance of natural genetic diversity in existing populations.

It is suggested that the results of this study are adopted in the following way:

1. The data suggest that there is relatively uniform reproductive interaction between scallops located along both the Queensland and Western Australian coastlines in the range of areas studied. This implies scallops occurring on each coast can be managed as single genetic stocks.

2. Hatchery operations that involve release of hatchery-spawned scallops should be managed to maximise genetic diversity, through single use of broodstock for spawnings and by ensuring that the effective broodstock number is sufficient to maintain genetic diversity as hatchery-produced scallops are likely to contribute gametes and/or larvae to the wild populations. The capture of hatchery-reared scallops should be managed to ensure the bulk of these animals are captured before they spawn when possible.

3. DNA samples of the broodstock and larvae from each successful larval run to be released should be collected and stored in order to monitor the affect on genetic diversity on the wild populations if required (see Appendix 5 for storage protocols). It is recommended that at least 50 individuals per sample area are collected to facilitate accurate interpretation of the results.

4. Clear records of spawnings including parental source location, should be maintained for effective hatchery management.

5. Although not currently proposed or recommended, if broodstock are to be reused for multiple spawnings it is important to monitor the effective breeding population in the hatchery to reduce the likelihood of inbreeding as well as monitoring the potential resultant change in genetic structure in the reseeding zone. Hatcheries can identify which brood members are producing offspring through parentage analysis using the microsatellites analysed during this study.

10 Further development

This project provides the initial baseline data required to monitor the genetic structure of saucer scallop populations following introduction of hatchery produced seed. However, the observed seasonal differences in allele frequencies in wild populations highlights the need for longer term monitoring to capture short term fluctuations in allele frequencies from temporally variable recruitment of wild seed. This will facilitate a valid interpretation of the genetic impacts of sea-ranching inputs over time. Long term monitoring of the wild stock following sea-ranching should also occur to ensure high levels of genetic diversity and hence population fitness are maintained,

The micosatellites identified and used in this study are predicted to be very valuable in genetic management of the hatcheries, These loci can be used as identifiers of parentage due to their high levels of polymorphism (variability). Hence, they can be used to address questions of mating success and therefore effective population size (i.e. the number of parents successfully contributing to the offspring). These markers can also be used to document the spontaneous selection of genotypes (domestication) that is occurring during the hatchery process and the potential reduction in genetic diversity.

11 Planned outcomes

The outputs of this project were:

1. *Microsatellite markers for Amusium balloti*—Eight polymorphic microsatellite loci have been optimised for amplification from *Amusium balloti* DNA.

2. *Baseline data of A. balloti microsatellite allele frequencies*—Microsatellite allele frequencies for five Queensland and two Western Australian populations have been analysed for samples collected in 2003 and 2004 respectively. This can be used as baseline data prior to commercial sea-ranching operations to monitor microsatellite diversity over time.

3. *New scientific knowledge*—The value and results of this project has been presented at the Australasian Aquaculture Conference 2004 and the 15th International Pectinid Workshop 2005 to a mixed audience of scientists, managers and commercial operators. A scientific publication based on the results of the project is being prepared for submission to a peer-reviewed scientific journal.

4. *Final report*—The FRDC final report is available in print and on CD.

Outcomes of this project were relevant to the Australian fishing industry and fisheries managers. Specifically they were:

1. *Communicated value of genetic management.* Through the course of the project and interactions with the commercial companies the concept of genetic management was formally introduced. Through the conference presentations and the availability of the final report a range of interested parties including operators, managers and scientists from other fields were educated about microsatellites and the value of genetic management.

2. *Management tool for responsible sea-ranching*. The population genetic analysis indicates that the populations along the coasts of Australia are intermixing, which suggests they can be managed as a single genetic stock. Through provision of baseline data and molecular tools for ongoing monitoring, genetic diversity can be managed in the sea-ranching and adjoining areas.

3. *Hatchery management tool.* The microsatellites developed during this project can also be used to better understand and manage the reproductive and developmental dynamics occurring during the hatchery phase (e.g. determining effective broodstock size for maintaining genetic diversity, determining the effect of unnatural selection during the hatchery phase).

4. *Additional information generated from the genetic analyses*. The opportunity to analyse temporally disconnected samples from the same location indicated that there is significant changes in microsatellite allele frequencies between years.

12 Conclusion

This study provided the opportunity to educate, through example, the value of genetic management in aquaculture and sea-ranching ventures and the opportunity to present the concepts and results at national and international meetings facilitated this.

During the course of the project we successfully developed eight polymorphic loci of which seven were used to analyse the genetic structure of *A. balloti* populations in Queensland and Western Australia. No significant genetic differences were detected between the five locations from Queensland based on the microsatellite allele frequencies, indicating intermixing along the coast. The three Abrolhos group sample sites from Western Australia were pooled and comparative analysis with the Shark Bay samples could not detect any significant genetic differentiation, indicating that these populations are interbreeding. These results indicate that the populations on either coast can be managed as single genetic stocks, although there is potentially undetected local adaptation.

We also took the opportunity to analyse temporally-separated samples from the same location and found that there can be significant differences in allele frequencies between years. This is expected based on the 'sweepstake' nature of the reproductive strategy of this species and the variable environmental conditions they experience during their lifecycle.

When comparing the eastern and western populations, significant differences in microsatellite allele frequencies and mitochondrial DNA sequence was detected. This indicates that the populations have been reproductively isolated over time and have evolved into distinct strains. This data supports the previous work of Dredge *et al* (in prep) that recommended taxonomic reclassification of the two strains. However, this should only be considered if complemented with information from other criteria as outlined by Gosling (1994).

As the hatchery-produced seed will be released to natural scallop beds there is the opportunity for the scallops to breed and enhance the numbers of saucer scallops in adjoining populations. Genetic diversity should be managed at the hatchery stage and it is important to consider the effects of using a small effective breeding population whereby only a limited number of individuals used in a spawning run successfully contribute to the spat released. This is particularly important with the saucer scallop because the high fecundity of the species allows the use of only small numbers of broodstock to produce commercial numbers of offspring. It should be noted that this can also occur in natural spawning events in the wild due to the sweepstakes reproductive strategy of the species. Furthermore, genetic diversity can be reduced through unintentional selection of genotypes during the hatchery process.

The microsatellite markers identified during this study will be a valuable tool in understanding the reproductive dynamics of saucer scallops during culture. These microsatellites will also be valuable in determining any potential hatchery effects on genetic diversity.

It is also recommended that the genetic diversity of scallop populations at the seeding site and surrounding areas are monitored over time taking into account the temporal differences expected between samples.

13 References

Abercrombie, M., Hickman and M.L. Johnson. 1985. *Dictionary of Biology.* Penguin Books Ltd., Middlesex.

Allendorf, F.W. and S.R. Phelps. 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. *Trans. Am. Fish. Soc.* **109**: 537–543.

Arnaud, -.H., S., V. Vonau, F. Bonhomme, P. Boudry, J. Prou, T. Seaman, M. Veyret and E. Goyard. 2003. Spat collection of the pearl oyster (*Pinctada margaritifera cumingii*) in French Polynesia: an evaluation of the potential impact on genetic variability of wild and farmed populations after 20 years of commercial exploitation. *Aquaculture* **219**: 181–192.

Ball, A.O. and R.W. Chapman. 2003. Population genetic analysis of white shrimp, *Litopenaeus setiferus*, using microsatellite genetic markers. *Mol. Ecol.* **12**: 2319–2330.

Balloux, F. and N. Lugon-Moulin. 2002. The estimation of population differentiation with microsatellite markers. *Mol. Ecol.* **11**: 155–165.

Bartley, D.M. 1999. Marine ranching: a global perspective. In *Stock Enhancement and Sea Ranching.* (ed. B.R. Howell, E. Moksness and T. Svasand), pp. 79–91. Fishing News Books, Oxford.

Barucca, M., E. Olmo, S. Schiaparelli. and A. Canapa. 2004. Molecular phylogeny of the family Pectinidae (Mollusc: Bivalvia) based on mitochondrial 16S and 12S rRNA genes. *Mol. Phylo. Evol.* **31**: 89–95.

Beaumont, A.R. 1991. Genetic distances between some scallop species. In *An international Compedium of Scallop Biology and Culture* (ed. S.E. Shumway and P.A. Sandifer), pp. 151–155. World Aquaculture Society, Baton Rouge.

Beaumont, A.R. 2000a. Genetic considerations in hatchery culture of bivalve shellfish. In *Recent advances in marine biotechnology. Aquaculture: Seaweeds and invertebrates* (ed. M. Fingerman and R. Nagabhushanam), pp. 87–109. Science Publishers, Inc.

Beaumont, A.R. 2000b. Genetic considerations in transfers and introductions of scallops. *Aqua. Inter.* **8**: 493–512.

Beaumont, A.R. In press. Genetics. In *Scallops Biology, Ecology and Aquaculture.* (ed. S.A. Shumway and G.J. Parsons). Elsevier.

Benzie, J.A.H. 1992. Review of the genetics, dispersal and recruitment of crown-of-thorns starfish (*Acanthaster planci*). *Aust. J. Mar. Freshwat. Res.* **43**: 597–610.

Benzie, J.A.H. 1994. Patterns of gene flow in the Great Barrier Reed and Coral Sea. In *Genetics and evolution of aquatic organisms.* (ed. A.R. Beaumont), pp. 67–79. Chapman and Hall, London.

Benzie, J.A.H. 2000. Population genetic structure in penaeid prawns. *Aqua Research* **31**: 95–119.

Benzie, J.A.H. and C. Smith. 2002. Pearl Oyster genetics. Fisheries Research and Development Corporation. *FRDC 97/344*. pp. 87.

Bierne, N., S. Launey, G.Y. Naciri and F. Bonhomme. 1998. Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. **148**: 1893–1906.

Bilton, D.T., J. Paula and J.D.D. Bishop. 2002. Dispersal, genetic differentiation and speciation in estuarine organisms. *Est. Coast. Shelf Sci.* **55**: 937–952.

Blankenship, M.W. and K.M. Leber. 1997. A responsible approach to marine stock enhancement. In *Developing and sustaining world fisheries resources. Proceedings of the 2nd World Fisheries Congress.* (ed. D.A. Hancock, D.C. Smith, A. Grant and J.P. Beumer). CSIRO, Australia.

Boudry, P., B. Collet, F. Cornette, V. Hervouet and F. Bonhome. 2002. High variance in reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by microsatellite-based parentage analysis of multifactorial crosses. *Aquaculture* **204**: 283–296.

Bowen, B.W. and J.C. Avise. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon — influence of zoogeographic factors and life history patterns. *Mar. Biol.* **107**: 371–381.

Braine, S.J., D.W. Rimmer and B.F. Phillips. 1979. An illustrated key and notes on the phyllosoma and stages of the western rock lobster *Panulirus cygnus* George.CSIRO, Division of Fisheries and Oceanography.*102*. pp. 13.

Briggs, J.C. 1974. *Marine Zoogeography*. McGraw-Hill, New York.

Brooker, A.L., J.A.H. Benzie, D. Blair and J.-J. Versini. 2000. Population structure of the giant tiger prawn *Penaeus monodon* in Australian waters, determined using microsatellite markers. *Mar. Bio.* **136**: 149–157.

Brown, L.D. 1991. Genetic variation and population structure in the Blacklip abalone, *Haliotis rubra. Aust. J. Mar. Freshwat. Res.* **42**: 77–90.

Burton, R.S. 1983. Protein polymorphisms and genetic differentiation in marine invertebrate populations. *Mar. Biol. Lett.* **4**: 193–206.

Burton, R.S. 1997. Genetic evidence for long-term persistence of marine invertebrate populations in an ephemeral environment. *Evolution* **51**: 993–998.

Burton, R.S. 1999. Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evol.* **52**: 734–745.

Callen, D.F., A.D. Thompson, Y. Shen, H.A. Phillips, R.I. Richards, J.C. Mulley and G.R. Sutherland. 1993. Incidence and origin of 'null' alleles in the (AC)n microsatellite markers. *Am. J. Hum. Genet.* **52**: 922–927.

Campbell, G. 1981. A final report to the fishing industry research committee on the recruitment into commercial stocks of the saucer scallop *Amusium japonicum balloti.* DPI.

Canapa, A., M. Barucca, A. Marinelli and E. Olmo. 1999. A molecular approach to the systematics of Antarctic scallop *Adamussium colbecki. Ital. J. Zool.* **66**: 379–382.

Canapa, A., M. Barucca, A. Marinelli and E. Olmo. 2000. Molecular data from the 16S rRNA gene for the phylogeny of Pectinidae (Mollusca: Bivalvia). *J. Mol. Evol.* **50**: 93–97.

Caputi, N., W.J. Fletcher, A. Pearce and C.F. Chubb. 1996. Effect of the Leeuwin Current on the recruitment of fish and invertebrates along the Western Australian Coast. *Mar. Freshwater Res.* **47**: 147–155.

Carvalho, G.R., A. Thompson and A.L. Stoner. 1992. Genetic diversity and population differentiation of the shortfin squid *Illex argentinus* in the south-west Atlantic. *J. Exp. Mar. Biol. Ecol.* **158**: 105–121.

Chapman, R.W., A.O. Ball and L.R. Mash. 2002. Spatial homogeneity and temporal heterogeneity of Red Drum (*Sciaenops ocellatus*) microsatellites: Effective population sizes and management implications. *Mar. Biotech.* **4**: 589–603.

Cresswell, G.R. and T.J. Golding. 1980. Observations of a south-flowing current in the southeastern Indian Ocean. *Deep Sea Research* **27**: 449–466.

Crisp, D.J. 1976. The role of pelagic larvae. In *Perspectives in experimental Zoology.* (ed. P. Spencer-Davies), pp. 145–155. Pergamon, Oxford.

Crisp, D.J. 1978. Genetic consequences of different reproductive strategies in marine invertebrates. In *Marine organisms: genetics, ecology and evolution.* (ed. B. Battaglia and J.A. Beardmore), pp. 257–273. Plenum, New York.

Cropp, D.A. 1993. Development of large scale hatchery production techniques for *Amusium balloti* (Bernardi 1861) in Western Australia. *Aquaculture* **115**: 285–296.

Delbrudge, A., J.R.L. Bernard, D. Blair, P. Peters and S. Butler. 1995. *The Macquarie Dictionary*. The Macquarie Library, Pty, Ltd. Vol. pp. 2050.

Doherty, P.J., S. Planes and P. Mather. 1995. Gene flow and larval dispersal in seven species of fish from the Great Barrier Reef. *Ecology* **76**: 2373–2391.

Dredge, M., P. Duncan, M. Heasman, B. Johnston, L. Joll, J. Mercer, D. Souter and T. Whittingham. 2002. Feasibility of scallop enhancement and culture in Australian waters.*FRDC Project 190/2000*. pp. 124.

Dredge, M., C. Keenan, D. Mayer and M. Cosgrove. In prep. Growth, colour and allozyme variation: Stock structure in the Queensland population of saucer scallops (*Amusium japonicum balloti*, Bernardi).

Dredge, M.C.I. 1981. Reproductive biology of the saucer scallop *Amusium japonicum balloti* (Bernardi) in central Queensland waters. *Aust. J. Mar. Freshwat. Res.* **32**: 775–787.

Duffy, J.E. 1993. Genetic population structure in two tropical sponge-dwelling shrimps that differ in dispersal potential. *Mar. Biol.* **127**: 485–498.

Edmunds, S. and D.C. Potts. 1997. Population genetic structure in brooding sea anemones (*Epiactis* spp.) with contrasting reproductive modes. *Mar. Biol.* **127**: 485–498.

Einum, S. and I.A. Flemming. 1997. Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. *J. Fish. Biol.* **50**: 634–651.

Elliot, N.G., J. Bartlett, B. Evans, R. Officer and M. Haddon. 2002. Application of molecular genetics to the Australian abalone fisheries: Forensic protocols for species identification and blacklip stock structure.CSIRO.*FRDC 1999/164*. pp. 132.

Estoup, A., C. Talliez, J. Cornuet and M. Solignac. 1995. Size homoplasy and mutational processes of interupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestis* (Apidae). *Mol. Biol. Evol.* **12**: 1074–1084.

Excoffier, L., P. Smouse and J. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.

Fevolden, S.E. 1992. Allozymic variability in the Icelandic scallop *Chlamys icelandica*: geographic variation and lack of growth heterozygosity correlations. *Mar. Ecol. Prog. Ser.* **85**: 259–268.

Fillatre, E.K., P. Etherton and D.D. Heath. 2003. Bimodal run distribution in a northern population of sockeye salmon (*Oncorhynchus nerka*): life history and genetic analysis on a temporal scale. *Mol. Ecol.* **12**: 1793–1805.

Fleming, I.A., K. Hindar, I.B. Mjolnerod, B. Jonsson, T. Balstad and A. Lamberg. 2000. Lifetime success and interactions of farm salmon invading a native population. *Proc. R. Soc. Lond. B.* **267**: 1517–1523.

Ford, M.J. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. *Cons. Biol.* **16**: 815–825.

Frankham, R. 1994. Genetic management of captive populations for reintroduction. In *Reintroduction biology of Australian and New Zealand fauna.* (ed. M. Serena), pp. 31–34. Surrey Beatty and Sons, Sydney.

Garoia, F., I. Guarniero, A. Ramšak, N. Ungaro, M. Landi, C. Piccinetti, P. Mannini and F. Tinti. 2004. Microsatellite DNA variation reveals high gene flow and panmictic populations in the Adriatic shared stocks of the European squid and cuttlefish (Cephalopoda). *Heredity* **93**: 166–174.

Gosling, E.M. 1994. Speciation and species concepts in the marine environment. In *Genetics and evolution of aquatic organisms.* (ed. A.R. Beaumont). Chapman and Hall, London.

Goudet, J., T. De Meeus, A.J. Day and C.J. Gliddon. 1994. The different levels of population structuring of the dogwhelk *Nucella lapillus*, along the south Devon coast. In *Genetics and Evolution of Aquatic Organisms* (ed. A.R. Beaumont), pp. 81–95. Chapman and Hall, London.

Gwyther, D., D.A. Cropp, L.M. Joll and M.C.L. Dredge. 1991. Australia. In *Scallops: Biology, Ecology and Aquaculture.* (ed. S.A. Shumway), pp. 835–851. Elsevier, Amsterdam.

Habe, T. 1964. Notes on the species of the Genus *Amusium* (Mollusca). *Bull. Nat. Sci. Mus. Tokyo* **7**: 1–5.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser* **41**: 95-98.

Harrison, R.G. 1993. *Hybrid zones and the evolutionary process.* New York.

Hartl, D. 1998. *Genetics*. Jones and Bartlett Publishers, London.

Hartl, D.L. and A.G. Clark. 1997. *Principles of population genetics.* Sinauer Associates, Sunderland.

Heald, D. 1978. A successful marking method for saucer scallop *Amusium balloti* (Bernardi). *Aust. J. Mar. Freshwat. Res.* **29**: 845–851.

Hedgecock, D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bull. Mar. Sci.* **39**: 550–564.

Hedgecock, D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In *Genetics and evolution of aquatic organisms.* (ed. A.R. Beaumont), pp. 123–134. Chapman and Hall, London.

Hedgecock, D., V. Chow and R.E. Waples. 1992. Effective population numbers of shellfish broodstocks estimated from temporal variance in allelic frequencies. *Aquaculture* **108**: 215–232.

Hedrick, P.W. and S.T. Kalinowski. 2000. Inbreeding depression in conservation biology. *Ann. Rev. Ecol. Syst.* **31**: 139–162.

Hellberg, M.E. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution* **50**: 1167–1175.

Helleberg, M.E. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution* **50**: 1167–1175.

Hilbish, T.J. 1996. Population genetics of marine species: the interaction of natural selection and historically differentiation populations. *J. Exp. Mar. Biol. Ecol.* **200**: 67–83.

Hoskin, M.G. 1997. Effects of contrasting modes of larval development on the genetic structures of populations of three species of Prosobranch Gastropods. *Mar. Biol.* **127**: 647–656.

Hunt, A. 1993. Effects of contrasting patterns of larval dispersal on the genetic connectedness of two intertidal starfish *Patiriella clacar* and *P. exigua. Mar. Ecol. Prog. Ser.* **92**: 172–186.

Hutchins, J.B. and A.F. Pearce. 1994. Influence of the Leeuwin current on recruitment of tropical reef fishes at Rottnest island, Western Australia. *Bull. Mar. Sci.* **54**: 245–255.

Jackson, J.B.C. 1986. Modes of dispersal of clonal benthic invertebrates: consequences for species distributions and genetic structure of local populations. *Ann. Rev. Ecol. Syst.* **39**: 588–606.

Johnson, M.S. 2000. Measuring and interpreting genetic structure to minimise the genetic risks of translocations. *Aqua Research* **31**: 133–143.

Johnson, M.S. and R. Black. 1984. Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution* **38**: 1371–1383.

Johnson, M.S. and R. Black. 1998. Effects of isolation by distance and geographical discontinuity on genetic subdivision of *Littorria cingulata. Mar. Biol.* **132**: 295–303.

Johnson, M.S., S. Creagh and M.J. Moran. 1986. Genetic subdivision of stocks of snapper, *Chrysophrys unicolor*, in Shark Bay, Western Australia. *Aust. J. Mar. Freshwat. Res.* **37**: 337–345.

Johnson, M.S. and L.M. Joll. 1993. Genetic subdivision of the pearl oyster *Pinctada maxima* (Jameson, 1901) (Molluscs: Pteridae) in Northern Australia. *Aust. J. Mar. Freshwat. Res.* **44**: 519–526.

Johnson , M.S., R.J. Watts and R. Black. 1994. High levels of genetic subdivision in peripherally isolation populations of the antherinid fish *Craterocephalus capreoli* in the Houtman Abrohlos Island, Western Australia. *Mar. Biol.* **119**: 179–184.

Joll, L.M. and N. Caputi. 1995. Environmental influences on recruitment of the saucer scallop (*Amusium balloti*) fishery of Shark Bay, Western Australia. *ICES Mar. Sci. Sym.* **199**.

Kailola, P.J., M.J. Williams, P.C. Stewart, R.E. Reichelt, A. McNee and C. Grieve. 1993. *Australian Fisheries Resources.* Bureau of Resource Sciences, Canberra.

Kolijonen, M.L., J. Tahitinen, M. Saisa and J. Koskiniemi. 2002. Maintenance of genetic diversity of Atlantic salmon (*Salmo salar*) by captive breeding programmes and the geographic distribution of microsatellite variation. *Aquaculture* **212**: 69–92.

Laikre, L. and N. Ryman. 1996. Effects on intraspecific biodiversity from harvesting and enhancing natural populations. *AMBIO* **25**: 504–509.

Leber, K.M., S. Kitada, H.L. Blankenship and T. Svaasand. 2002. Proceedings from the Second International Symposium on Stock enhancement and Sea Ranching. In Kobe, Japan.

Li, Q., C. Park, T. Endo and A. Kijima. 2004. Loss of genetic variation at microsatellite loci in hatchery strains of the Pacific abalone (*Haliotis discus hannai*). *Aquaculture* **235**: 207–222.

Lundy, C.J., C. Ricco and G.M. Hewitt. 2000. Temporal and spatial genetic variation in spawning grounds of European hake (*Merluccius merluccius*) in the Bay of Biscay. *Mol. Ecol.* **9**: 2067–2079.

Lynch, M. and M. O'Hely. 2001. Captive breeding and the genetic fitness of natural populations. *Cons. Genet.* **2**: 363–378.

Matsumoto, M. and I. Hayami. 2000. Phylogenetic analysis of the family Pectinidae (Bivalvia) based on mitochondrial cytochrome C oxidase subunit I. *J. Moll. Stud.* **66**: 477–488.

Mayr, E. 1963. *Animal Species and Evolution*. The Bellknap Press of Harvard University Press., Cambridge, Massachusetts.

McGoldrick, D.J. and D. Hedgecock. 1997. Fixation, segregation and linkage of allozyme loci in inbred families of the Pacific oyster *Crassostrea gigas* (Thunberg): implications for the causes of inbreeding depression. *Genetics* **146**: 321–344.

McGoldrick, D.J., D. Hedgecock, L.J. English, P. Baoprasertkul and R.D. Ward. 2000. The transmission of microsatellite alleles in Australian and north American stocks of the Pacific oyster (*Crassostrea gigas*): selection and null alleles. *J. Shell. Res* **19**: 779–788.

Nguyen, T.T.T. and U. Na-Nakorn. 2004. Genes and Fish. *Aquaculture Asia* **IX**: 4–7.

Nielsen, C. 1995. *Animal Evolution. Interrelationships of the living phyla.* Oxford University Press, Oxford.

Norris, A.T., D.G. Bradley and E.P. Cunningham. 1999. Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. *Aquaculture* **180**: 247–264.

Olsen, J.B., S.E. Merkouris and J.E. Seeb. 2002a. An examination of spatial and temporal genetic variation in walleye pollock (*Theragra chalcogramma*) using allozyme, mitochondrial DNA and microsatellite data. *Fishery Bulletin* **100**: 752–764.

Olsen, J.B., S.E. Merkouris and J.E. Seeb. 2002b. An examination of spatial and temporal genetic variation in walleye pollock (Theragra chalcogramma) using allozyme, mitochondrial DNA, and microsatellite data. **100**: 752–764.

Østergaard, S., M.M. Hansen, V. Loeschcke and E.E. Nielsen. 2003. Long-term temporal changes of genetic composition in brown trout (*Salmo tutta* L.) populations inhabiting an unstable environment. *Mol. Ecol.* **12**: 3123–3135.

Ovenden, J. R., R. Street, D. Peel, S. Peel, T. Courtney, H. Podlich, K. Basford and C. Dichmont. 2004. Development of a genetic method to estimate effective spawner numbers in the NPF and Queensland tiger prawn fisheries.FRDC*.2001/018*. pp. 206.

Palm, S., J. Dannewitz, T. Järvi, E. Petersson, T. Prestegaard and N. Ryman. 2003. Lack of molecular genetic divergence between sea-ranched and wild sea trout (*Salmo trutta*). *Mol. Ecol.* **12**: 2057–2071.

Palumbi, S.R. 1992. Marine speciation on a small planet. *Trends Ecol. Evol.* **7**: 114–118.

Palumbi, S.R., G. Grabowsky, T. Duda, L. Geyer and N. Tachino. 1997. Speciation and population genetic structure in tropical Pacific sea urchins. *Evol.* **51**: 1506–1517.

Parsons, K.E. 1996. The genetic effects of larval dispersal depend on spatial scale and habitat characteristics. *Mar. Biol.* **126**: 403–414.

Pearce, A. 1997. The Leeuwin current and the Houtman Abrolhos Islands. In *The marine flora and fauna of the Houtman Abrolhos Islands, Western Australia.* (ed. F.E. Wells), pp. 11–46. Western Australian Museum, Perth.

Pérez-Losada, M., A. Guerra, G.R. Carvalho, A. Sanjuan and P.W. Shaw. 2002. Extensive population subdivision of the cuttlefish *Sepia officinalis* (Cephalopoda) around the Iberian Penninsula indicated by microsatellite DNA variation. *Heredity* **89**: 417–424.

Perrin, C., S.R. Wing and M.S. Roy. 2004. Effects of hydrographic barriers on population genetic structure of the sea star *Coscinasterias muricat*a (Echinodermata, Asteroidea) in the New Zealand fiords. *Mol. Ecol.* **13**: 2183–2195.

Raymond, M. and F. Rousset. 1995a. An exact test for population differentiation. *Evolution* **49**: 1280–1283.

Raymond, M. and F. Rousset. 1995b. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Heredity* **86**: 248–249.

Roger, A.D., A. Clarke and L.S. Peck. 1998. Population genetics of the Antarctic heteronermetean *Parbolasia corrugatus* from the South Orkney Islands. *Mar. Biol.* **131**: 1–13.

Rose, R.A., G.R. Campbell and S.G. Sanders. 1988. Larval development of the saucer scallop *Amusium balloti* (Bernardi) (Molusca:Pectinidae). *Aust. J. Mar. Freshwat. Res.* **39**: 153–60.

Russo, C.A.M., A.M. Sole-Cava and J.P. Thorpe. 1994. Population structure and genetic variation in two tropical sea anemones (Cnidaria, Actinidae) with different reproductive strategies. *Mar. Biol.* **119**: 267–276.

Ruzzante, D.E., C.T. Taggart and D. Cook. 1998. A nuclear DNA basis for shelf- and bank-scale population structure in north-west Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. *Mol. Ecol.* **7**: 1663–1680.

Saavedra, C. and J.B. Peña. 2004. Phylogenetic relationships of commercial European and Australasian king scallops (*Pecten* spp.) based on partial 16S ribosomal RNA gene sequences. *Aquaculture* **235**: 153–166.

Scheltema, R.S. 1986. On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. *Bull. Mar. Sci.* **39**: 290–322.

Scheltema, R.S. 1989. Planktonic and non-planktonic development among Prosobranch gastropods and its relationship to the geographic range of the species. In *Reproduction, genetics and distributions of marine organisms.* (ed. J.S. Ryland and P.A. Tyler), pp. 183–188. Olsen and Olsen. Fredensborg, Denmark.

Shaklee, J.B. and P. Bentzen. 1998. Genetic identification of stocks of marine fish and shellfish. *Bull. Mar. Sci.* **62**: 589–621.

Shaw, P.W. and M. Pérez-Losada. 2000. Polymorphic microsatellites in the common cuttlefish *Sepia officinalis* (Cephalopoda). *Mol. Ecol.* **9**: 327–238.

Singh, S.M. and R.H. Green. 1984. Excess of allozyme homozygosity in marine molluscs and its possible biological significance. *Malacologia* **25**: 569–581.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**: 457–462.

Strathman, R.R. 1985. Feeding and non-feeding larval development and life history evolution in marine invertebrates. *Ann. Rev. Ecol. Syst.* **16**: 339–361.

Swofford, D.L. 1998 PAUP*. Phylogenetic analysis using parsimony (* and other methods) V. 4 Sinauer and Associates. Sunderland.

Taylor, P.D. 1988. Major radiation of cheilostome bryozoans triggered by the evolution of a new larval type? *J. Hist. Biol.* **1**: 45–64.

Thompson, A.P., J.R. Hanley and M.S. Johnson. 1996. Genetic structure of the Western Rock Lobster, *Panulirus cygnus*, with the benefit of hindsight. *Mar. Freshwater Res.* **47**: 889–896.

Thompson, J.D., D.G. Higgins and T.J. Gibson. 1994. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* **22**: 4673–4680.

Todd, C.D., W.J. Lambert and J.P. Thorpe. 1998. The genetic structure of intertidal populations of two species of nudibranch molluscs with planktrophic and pelagic lecithotropic larval stages: Are pelagic larvae 'for' dispersal? **228**: 1–28.

Waller, T.R. In press. New Phylogenies of the Pectinidae (Mollusca: Bivalvia): Reconciling morphological and molecular approaches. In *Scallop Biology, Ecology and Aquaculture.* (ed. S.E. Shumway and G.J. Parsons), pp. 1–44. Elsevier.

Wang, S., P. Duncan, W. Knibb, and B.M. Degnan. 2002. Byssal attachment and movement of Amusium balloti (Bivalvia: Pectinidae) spat. *J. Shell. Res.* **21**: 563–569.

Waples, R.S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences* **48**: 124–133.

Waples, R.S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *J. Heredity* **89**: 438–450.

Wares, J.P., S.D. Gaines and C.W. Cunningham. 2001. A comparative study of asymmetric migration events across a marine biogeographic boundary. *Evol.* **55**: 295–306.

White, M.J.D. 1978. *Modes of speciation.* Freeman, W.H. San Francisco.

Wilding, C.M., A.R. Beaumont and J.W. Latchford. 1999. Are *Pecten maximus* and *Pecten jacobaeus* different species? *J. Mar. Biol. Ass. UK.* **79**: 949-952.

Williams, M.J. and M.C.L. Dredge. 1981. Growth of the saucer scallop *Amusium japonicum balloti* Habe in Central Eastern Queensland. *Aust. J. Mar. Freshwat. Res.* **32**: 657–666.

Wright, J.M. and P. Bentzen. 1994. Microsatellites: genetic markers for the future. *Rev. Fish. Biol. Fish.* **4**: 384–388.

Zheng, H., G. Zhang, X. Liu, F. Zhang and X. Guo. 2004. Different responses to selection in two stocks of the bay scallop, *Agropecten irradians irradians* Lamarck (1819). *J. Exp. Mar. Biol. Ecol.* **313**: 213–223.

Zouros, E. and D.W. Foltz. 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia* **25**: 583–591.

Appendix 1: Intellectual property

Eight microsatellite loci were developed during the project that can be used to monitor the genetic impact of restocking as well as having potential use for broodstock and larval management in the hatchery.

Appendix 2: Staff

Dr Elizabeth O'Brien	DPI&F	Principal Investigator
Jason Bartlett	DPI&F	Senior Research Assistant
Dr Bryony Dixon	DPI&F	Research Assistant
Provan Crump	DPI&F	Research Assistant

Appendix 3: Glossary

(Abercrombie *et al.* 1985; Delbrudge *et al.* 1995; Hartl 1998)

allele: different forms of a gene at equivalent loci
allele frequency: the frequency at which at specific allele is present in the population
deme: smallest level of population structure
fitness: a measure of the average ability of organisms with a given genotype to survive and reproduce
FST: provides a rough estimate of the genetically effective number of migrants per generation into the area
genetic drift: changes in the genetic makeup of a population occurring as a result of chance
Hardy-Weinberg Equilibrium: the genotype frequencies expected with random mating
heterosis: the increase in growth, size, fecundity, function, yield or other characteristics in hybrids over those of the parents (Delbrudge *et al*. 1995)
heterozygote: having two different alleles of a gene in the two corresponding loci of a pair of chromosomes
homozygote: having the same allele of a gene in the two corresponding loci of a pair of chromosomes
hybridisation: interbreeding of individuals from genetically distinct populations (Harrison 1993)
inbreeding: reproduction of closely related individuals
introgression: gene flow between populations when hybrids backcross with one or both parental populations
loci: the location or position of a particular gene on a chromosome
microsatellite: a short sequence of repeated nucleotides in a genome
panmictic population: a single stock
PCR: polymerase chain reaction
polymorphism: the presence of genetic variation within a population or locus

Appendix 4: *Amusium balloti* and *Amusium pleuronectes*—examples of shells

From left to right: *Amusium pleuronectes* (Gulf of Carpentaria), *Amusium balloti* (Abrolhos Islands, Western Australia) and *Amusium balloti* (Hervey Bay, Queensland).

Appendix 5: Methodology for storing genetic samples

Broodstock samples

1. Following a spawning, take a ~ 50mm^3 section of muscle from each of the broodstock used in the fertilisation.
2. Add the muscle sample to a 2ml screw cap tube filled with 90% ethanol.
3. Submerge the tissue sample in the ethanol and top up with 90% ethanol if required, then seal the tube.
4. Label the tube using a permanent waterproof marker, with a unique number that also relates to the hatchery records (including broodstock source) for that spawning. For example: the fifth males used in the third spawning of the season in 2005 may be labelled as 2005-3-M5.
5. Store the samples in the freezer at –20^{o}C or lower.

Offspring samples

1. Sample around 100 one month-old spat from each spawning run/batch.
2. Add to 2ml screw cap and remove as much seawater as possible.
3. Fill with 90% ethanol, seal and gently invert the tube to mix the spat with the ethanol.
4. Label the tube using a permanent waterproof marker, with a unique number that also relates to the hatchery records (including broodstock source) for that spawning. For example: spat resulting from the third spawning of the season in 2005 may be labelled as 2005-3-S.
5. Store the samples in the freezer at –20^{o}C or lower.

Appendix 6: Raw data

Alignment of 12S rDNA fragments from scallops. Sequence generated during this study highlighted in bold.

```
                                10        20        30        40        50
                          ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)            TCAAAGAACTTGGCGGCTTGTTAACTACCTAGGGGAATATGTGCCTTAAT
A. balloti (N)            ..................................................
A. balloti (W.A.)         ..................................................
A. pleuronectes (Phil)    ..........................................C.......
A. pleuronectes (Qld)     ..........................................C.......
A. pleuronectes (G.C.)    ..........................................C.......
P. maximus                ..........................................C.......
P. jacobaeus              ..........................................C.......
P. novaezelandiae         ..........................................C.......
C. pallium                ..................................................
B. aktinos                ........T.........C.......................C.......
M. rastellum              ....................C.....T..C...........C.......

                                60        70        80        90       100
                          ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)            CCGATGATCCCCGTCGCATCTTACTAGGCCTTGAAAAAGTGCAGCTGGTG
A. balloti (N)            ..................................................
A. balloti (W.A.)         ..................................................
A. pleuronectes (Phil)    ..............TAT........GA.T.....................
A. pleuronectes (Qld)     ..............A.AT........GA.T.....................
A. pleuronectes (G.C.)    ..............A.AT........GA.T.....................
P. maximus                ..............AT........GA.T.....................
P. jacobaeus              ..............AT........GA.T.....................
P. novaezelandiae         ..............TAT........GA......................
C. pallium                ......C.......A.A........GT.........G...C....C....
B. aktinos                ........A.....T.T.........C............AA.........
M. rastellum              ..............A........GT.........G.............

                               110       120       130       140       150
                          ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)            TATTGCCGTTGTCAGCTTGTTGTTCGAGCAAGGAGTAACAGGCTTAATGG
A. balloti (N)            ..................................................
A. balloti (W.A.)         ..................................................C.....
A. pleuronectes (Phil)    ............................GA...A..................
A. pleuronectes (Qld)     ............................GA...A.........G....
A. pleuronectes (G.C.)    ............................GA...A.........G....
P. maximus                .............................A...A..................
P. jacobaeus              .............................A--G..................
P. novaezelandiae         .............................A...A..................
C. pallium                .......................A..............GA...C.....
B. aktinos                .....................-GA..T..AA.....................
M. rastellum              .....................T......A............C.....

                               160       170       180       190       200
                          ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)            AGCCGTAGCGATTAT-ATCGTTGGTGTCCGTGAATTCAGGTCGTAATACC
A. balloti (N)            ..............-...................................
A. balloti (W.A.)         ..............-...................................
A. pleuronectes (Phil)    TTTTTATTT...A.GTT..A.AAAAA...A...................T
A. pleuronectes (Qld)     TTTTTATTT...A.GTT..A.AAAAAG..A...................T
A. pleuronectes (G.C.)    TTTTTATTT...A.GTT..A.AAAAAG..A...................T
P. maximus                .T..CGGT-...AG.CT..ACG..AA...A...................T
P. jacobaeus              .T..CGGT-...A..CT..ACG..AA...A...................T
P. novaezelandiae         .T..TGGT-.....CTT..ACG..AA...A...................T
C. pallium                .....-........G-T.............CN...........A....T.
B. aktinos                ..TT--.CTA...T--T.TAG..TA.C..A.............A...G.T
M. rastellum              .....-GT......G-T...C.........C............A...G..
```

```
                                 210       220       230       240       250
                          ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)            GCCTACGGCCTAAGGGAGTAGGTGTTACAATTT-AGATTTAAAAATACGG
A. balloti (N)            ................................-................
A. balloti (W.A.)          ...C............................-.........-.....
A. pleuronectes (Phil)    .....T...TC.......CG.............-.T.C...G..T.....
A. pleuronectes (Qld)     .....T...TC.......CG............C-GT.....G..TC....
A. pleuronectes (G.C.)    .....T...TC.......CG............C-GT.....G..TC....
P. maximus                .....T...TC.......CG............C-.T....GG..T.....
P. jacobaeus              .....T...TC.......CG............C-.T.C..GG..T.....
P. novaezelandiae         .....T...TC.......CG............C-.T....GG..T.....
C. pallium                ...A.T...TC.......................-TT....G...T.....
B. aktinos                ..T..T..TG.........G.............TGA.G..GG..T.....
M. rastellum              ...G.T..TA.........G...A.....G...-.T.....G..T.....

                                 260       270       280       290       300
                          ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)            ATCTTTA-TTTTGAAATATTAGGGTGAAGGTGAACTTAGGAGTAAGGGGG
A. balloti (N)            .......-.........................................
A. balloti (W.A.)         .......-.........................................
A. pleuronectes (Phil)    .......-..G..G...C...AA.........................A
A. pleuronectes (Qld)     .......-..G......C...AA.......................A..
A. pleuronectes (G.C.)    .......-..G......C...AA.......................A..
P. maximus                ...C.C.-..G..G...C..G.........................A.A
P. jacobaeus              ...C.C.C..G..G...C..G.........................A.A
P. novaezelandiae         ...C.C.-.CG..G...C..G.........................ATA
C. pallium                ..TC...-.............A.A......A..............A.A
B. aktinos                ....CA.T..GG...T.C..T.A.........G............AA.
M. rastellum              ......T-C...........GA..............G.A..........

                                 310       320       330       340       350
                          ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)            AATTAATATGTTTCCCTGAACATGAATCTGGCTTGTGTACAAACTGCCCG
A. balloti (N)            .................................................
A. balloti (W.A.)         ......................G..........................
A. pleuronectes (Phil)    G.........C..T...............AA..C...............
A. pleuronectes (Qld)     G.........C..T...............AA..C...............
A. pleuronectes (G.C.)    G.........C..T...............AA..C...............
P. maximus                G......G..C..T...............AA..C...............
P. jacobaeus              G......G..C..T...............AA..C...............
P. novaezelandiae         G.........C..T...............AA..C...............
C. pallium                ......C.......T.......TGTGC.....................
B. aktinos                G............T...............AA.............C.....
M. rastellum              ............C...............A...C.A.............

                                 360       370
                          ....|....|....|....|...
A. balloti (T)            TCGCTCTCGCCGAGAGGTGAGAT
A. balloti (N)            .......................
A. balloti (W.A.)         .......................
A. pleuronectes (Phil)    .........GT....AC......
A. pleuronectes (Qld)     .........GT....AC......
A. pleuronectes (G.C.)    .........GT....AC......
P. maximus                .........G......C......
P. jacobaeus              .........G......C......
P. novaezelandiae         .........G......C......
C. pallium                ....C.......T........G.
B. aktinos                ............GA.........
M. rastellum              ............T..........
```

Alignment of 16S rDNA fragments from scallops. Sequence generated during this study highlighted in bold.

```
                              10        20        30        40        50
                             ....|....|....|....|....|....|....|....|....|....|
***A. balloti (T)***          AGGTAGCTAAATTATGGCCTATTAATTGTAGGTCCTGTGAATGGTTTGAC
***A. balloti (Y)***          ..................................................
***A. balloti (B.H.)***       ..................................................
***A. balloti (H.B.)***       ..................................................
***A. balloti (N)***          ..................................................
***A. balloti (W.A.)***       ..................................................
A. pleuronectes (Phil)       ..................................................
***A. pleuronectes (G.C.)***  ..................................................
P. maximus                   ..................................................
P. jacobaeus                 ..................................................
***P. novaezelandiae***       ..................................................
***C. pallium***              ..............C...................................
***B. aktinos***              ..............C.................C.................
M. rastellum                 ..............C.....G.......C.....................

                                       60        70        80        90       100
                             ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)               GAGTCTTTAACTGTCTCTAGTTTGTTTTGGTGAAATTGAATTGGATGTGC
A. balloti (Y)               ..................................................
A. balloti (B.H.)            ..................................................
A. balloti (H.B.)            ..................................................
A. balloti (N)               ..................................................
A. balloti (W.A.)            ..................................................
A. pleuronectes (Phil)       ....T.CC...........A...T.........C................
A. pleuronectes (G.C.)       ....T.CC...........A...T.........C................
P. maximus                   ....T.CC.............T.........C................
P. jacobaeus                 ....T.CC.............T.........C................
P. novaezelandiae            ....T.CC.............T.........C................
C. pallium                   ...C..CCG.........A.C...........C................
B. aktinos                   .........T.......G..G..A.............T....AA......
M. rastellum                 ...C..CC.............A.........C................

                                      110       120       130       140       150
                             ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)               AAATGCTTCCATAGGTGAGAAAGACGAGAAGACCCCGTGAAGTTAGAAAT
A. balloti (Y)               ..................................................
A. balloti (B.H.)            ..................................................
A. balloti (H.B.)            ..................................................
A. balloti (N)               ..................................................
A. balloti (W.A.)            ..................................................
A. pleuronectes (Phil)       ............G...A.................................
A. pleuronectes (G.C.)       ............G...A.................................
P. maximus                   ............G...A.................................
P. jacobaeus                 ............G...A.................................
P. novaezelandiae            ............G...A.................................
C. pallium                   ..........T.GAAAA.........................C.......
B. aktinos                   ......A.TT.....GA............................A...C
M. rastellum                 ............G.............................C.......
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                                160       170       180       190       200
                               ....|....|....|....|....|....|....|....|....|
A. balloti (T)                 TTAAGTTGTAAGCGTTATTCCTCTTAGTTTTATACTGTTTCCTAGAATAG
A. balloti (Y)                 ...................................................
A. balloti (B.H.)              ...................................................
A. balloti (H.B.)              ..................................................R
A. balloti (N)                 ...................................................
A. balloti (W.A.)              ......................C.........-.........T.......A
A. pleuronectes (Phil)         ..T..C.A.T---.AG---------GCC..----T.AAC.ATG.A---GT
A. pleuronectes (G.C.)         ..T.AC.ACT---.AG---------GCC.CA---T.ARC.GTA.A.--GT
P. maximus                     .......A.T---.GGGAGTTC.AAG.C.A----T.TG.GG.CGA.TCGT
P. jacobaeus                   .......A.T---.GG.AGTTC.AAG.C.A----T.TG.GG.CGA.TCGT
P. novaezelandiae              .......A.T---.GG.AGTTC.AAG.C.AA---T.TG.GG.CGA.TCGT
C. pallium                     .....C..C.G......AGTTCTCAG.CCC---------------.TGG.
B. aktinos                     ..TTA..A..----....CGAC..--------------------------
M. rastellum                   ..T......GT.G....GG..C.CAG.CCA---------------..GGT

                                210       220       230       240       250
                               ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)                 CAGGTTGATTTGAGGCTTCATGTTAGTTCAAGCAGAGGGGTGCAGCGGC-
A. balloti (Y)                 ...................................................-
A. balloti (B.H.)              ...................................................-
A. balloti (H.B.)              ...................................................-
A. balloti (N)                 ...................................................-
A. balloti (W.A.)              ......A....A..TT..A......A......T......A.........-
A. pleuronectes (Phil)         T.A.---TG..A.A.-G.T..AC.TT----GTT..--.T.AAT..T.A.-
A. pleuronectes (G.C.)         T.A.---TG..A.AA.AAT.....TT----GTG..--.T.G.T..T.A.-
P. maximus                     TGA.GA.CAC.AGAA...AG....TC----..A..G..T.AAT.ATA..-
P. jacobaeus                   TGA.GA.CAC.AGAA...AG....TC----..A..G..T.AAT.ATA..-
P. novaezelandiae              TGA.GA.CAC.ATAATC.AG....TT----..A..G..T.AAT.ATA..-
C. pallium                     -GTCGATT.....A.T----A...T--CAG..TGAGTCAA..TG.T.AT-
B. aktinos                     -....C...----------------------------AA.AT.ATAAA-
M. rastellum                   TG.C.AA.AC....CTGATGA...TAGCTG.ATG.G...A-....T.ATA

                                260       270       280       290       300
                               ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)                 AGTTTTGGCTGGGGCAGCAAAGGAGCAAGACTAGACTCCTATTATTT-AA
A. balloti (Y)                 ...................................................-..
A. balloti (B.H.)              ...................................................-..
A. balloti (H.B.)              ...................................................-..
A. balloti (N)                 ...................................................-..
A. balloti (W.A.)              ...................................................-..
A. pleuronectes (Phil)         ......................A......AGT.......T.T.AG...T.T
A. pleuronectes (G.C.)         ......................A......A.GC......T.T.AG...T.T
P. maximus                     ............................AG..........T.AGGG.-GT
P. jacobaeus                   ............................AG..........T.AGGG.-GT
P. novaezelandiae              ............................AG..........T.AGGG.-.T
C. pallium                     G.....................A.......GTA......T.-...GGA-..
B. aktinos                     ......T..............AAG....A.......-.T.T......-GG
M. rastellum                   G..................G..A......A.........T.-...G.A-G.

                                310       320       330       340       350
                               ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)                 AGTGCGGGTGCGCTACGACCCACAGAGGAATATAT-TTGTGATTAGCAGA
A. balloti (Y)                 ..................................-..............
A. balloti (B.H.)              ..................................-..............
A. balloti (H.B.)              ..................................-..............
A. balloti (N)                 ..................................-..............
A. balloti (W.A.)              ............................T..G...-..............
A. pleuronectes (Phil)         GTGA.T....T.T...........A.TTTTAGGG.---.......T....
A. pleuronectes (G.C.)         GTGA.T....T.T...........A.TTTTAGGGC---.............
P. maximus                     .AAA.A.A..T.T...........A.CATTA.GG.---.......T....
P. jacobaeus                   .AAA.A.A..T.T...........A.CATTA.GG.---.......T....
P. novaezelandiae              .AAA.A.A..T.T...........A.CATTA.GG.---.......T....
C. pallium                     .AA....A..........Y.....ATTTT..TGG.C..............
B. aktinos                     TTAAT....ATAT........T..C.AT..GGA.A-G..A.T........
M. rastellum                   .AG.........TG..........A..TT-..AT.T.........T....
```

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                                 360       370       380       390       400
                          ....|....|....|....|....|....|....|....|....|....|
A. balloti (T)            AGAAGTTACTCCGGGGATAACAGCGTTATCCGCCCTGATAGTTCTTATAG
A. balloti (Y)            .................................................
A. balloti (B.H.)         .................................................
A. balloti (H.B.)         .................................................
A. balloti (N)            .................................................
A. balloti (W.A.)         .................................................
A. pleuronectes (Phil)    ........................A...T.T.....C...........
A. pleuronectes (G.C.)    ........................A...T.T.....C...........
P. maximus                ........................A...T.T.T...............
P. jacobaeus              ........................A...T.T.T...............
P. novaezelandiae         ........................A...T.T.T...............
C. pallium                .A.............................T...............
B. aktinos                ..............................T...............
M. rastellum              ................................C..C...C.......

                                 410       420       430
                          ....|....|....|....|....|....|.
A. balloti (T)            ATGGGCGGGTTTGCGACCTCGATGTTGGCTC
A. balloti (Y)            ...............................
A. balloti (B.H.)         ...............................
A. balloti (H.B.)         ...............................
A. balloti (N)            ...............................
A. balloti (W.A.)         ...............................
A. pleuronectes (Phil)    ...............................
A. pleuronectes (G.C.)    ...............................
P. maximus                ...............................
P. jacobaeus              ...............................
P. novaezelandiae         ..................S............
C. pallium                .C.............................
B. aktinos                ...............................
M. rastellum              .C.............................
```

16 S

	P. maximus	*P. jacobaeus*	*P. novazealandiae*	*A. pleuronectes (Phil.)*	*A. pleuronectes (N.T.)*	*C. pallium*	*B. aktinos*	***A. balloti (Town.)***	***A. balloti (Yep.)***	***A. balloti (B.H.)***	***A. balloti (H. Bay)***	***A. balloti (Noosa)***	***A. balloti (W.A.)***	*A. i. irradians*	*A. i.concentricus*	*C. varia*	*P. yessoensis*	*M. nobilis*	*S. dringi*	*M. rastellum*	*L. wilhelminae*	*G. pallium*	*D. vitreus*	*C. multistrata*	*C. madreporarum*	*D. plica*
P. maximus																										
P. jacobaeus	0.002																									
P. novazealandiae	0.014	0.012																								
A. pleuronectes (Phil.)	0.121	0.121	0.116																							
A. pleuronectes (N.T.)	0.131	0.131	0.128	0.045																						
C. pallium	0.184	0.186	0.184	0.175	0.169																					
B. aktinos	0.214	0.213	0.213	0.189	0.192	0.249																				
A. balloti (Town.)	0.217	0.219	0.214	0.202	0.205	0.191	0.204																			
A. balloti (Yep.)	0.217	0.219	0.214	0.202	0.205	0.191	0.204	**0.000**																		
A. balloti (B.H.)	0.217	0.219	0.214	0.202	0.205	0.191	0.204	**0.000**	**0.000**																	
A. balloti (H. Bay)	0.217	0.219	0.214	0.200	0.203	0.189	0.204	**0.000**	**0.000**	**0.000**																
A. balloti (Noosa)	0.217	0.219	0.214	0.202	0.205	0.191	0.204	**0.000**	**0.000**	**0.000**	**0.000**															
A. balloti (W.A.)	0.210	0.212	0.210	0.199	0.203	0.190	0.204	**0.030**	**0.030**	**0.030**	**0.028**	**0.030**														
A. i. irradians	0.614	0.612	0.611	0.617	0.607	0.639	0.627	0.618	0.618	0.618	0.619	0.618	0.620													
A. i.concentricus	0.619	0.616	0.616	0.619	0.609	0.641	0.629	0.622	0.622	0.622	0.623	0.622	0.624	0.013												
C. varia	0.234	0.232	0.234	0.229	0.218	0.237	0.191	0.240	0.240	0.240	0.240	0.240	0.243	0.623	0.627											
P. yessoensis	0.606	0.604	0.595	0.609	0.602	0.601	0.613	0.609	0.609	0.609	0.609	0.609	0.620	0.205	0.208	0.625										
M. nobilis	0.247	0.245	0.250	0.201	0.213	0.230	0.208	0.229	0.229	0.229	0.227	0.229	0.226	0.603	0.610	0.243	0.597									
S. dringi	0.282	0.284	0.284	0.250	0.253	0.283	0.137	0.285	0.285	0.285	0.285	0.285	0.274	0.622	0.624	0.280	0.615	0.232								
M. rastellum	0.197	0.199	0.199	0.175	0.181	0.175	0.214	0.203	0.203	0.203	0.202	0.203	0.196	0.639	0.641	0.262	0.625	0.249	0.275							
L. wilhelminae	0.262	0.265	0.267	0.217	0.224	0.260	0.174	0.262	0.262	0.262	0.262	0.262	0.268	0.640	0.645	0.249	0.631	0.237	0.191	0.255						
G. pallium	0.187	0.190	0.192	0.169	0.175	0.158	0.202	0.195	0.195	0.195	0.195	0.195	0.191	0.639	0.640	0.240	0.630	0.236	0.275	0.075	0.255					
D. vitreus	0.224	0.224	0.224	0.237	0.231	0.248	0.267	0.247	0.247	0.247	0.247	0.247	0.247	0.604	0.608	0.233	0.598	0.269	0.287	0.262	0.271	0.233				
C. multistrata	0.196	0.193	0.193	0.203	0.204	0.197	0.205	0.181	0.181	0.181	0.181	0.181	0.185	0.625	0.630	0.130	0.609	0.227	0.248	0.214	0.190	0.209	0.226			
C. madreporarum	0.262	0.265	0.267	0.217	0.224	0.260	0.174	0.262	0.262	0.262	0.262	0.262	0.268	0.640	0.645	0.249	0.631	0.237	0.191	0.255	0.000	0.255	0.271	0.190		
D. plica	0.217	0.219	0.212	0.198	0.198	0.119	0.232	0.218	0.218	0.218	0.219	0.218	0.216	0.640	0.641	0.264	0.625	0.263	0.301	0.192	0.249	0.185	0.254	0.193	0.249	
C. nobilis	0.625	0.622	0.621	0.611	0.619	0.628	0.616	0.635	0.635	0.635	0.635	0.635	0.636	0.204	0.206	0.625	0.197	0.610	0.615	0.643	0.636	0.642	0.616	0.615	0.636	0.637

12 S

	P. maximus	*P. jacobaeus*	*P. novazealandiae*	*A. pleuronectes (Phil.)*	*A. pleuronectes (Qld.)*	*A. pleuronectes (N.T.)*	*C. pallium*	*B. aktinos*	***A. balloti (Town.)***	***A. balloti (Noosa)***	***A. balloti (W.A.)***	*P. caurinus*	*S. gaederopus*	*M. nobilis*	*S. dringi*	*S. amicum*	*M. rastellum*	*M. mirficus*	*G. pallium*	*L. wilhelminae*	*L. cuneata*	*C. islandica*	*C. mulistrata*	*C. madreporarum*	*C. varia*	*A. opercularis*	*C. glabra*
P. maximus																											
P. jacobaeus	0.014																										
P. novazealandiae	0.027	0.035																									
A. pleuronectes (Phil.)	0.073	0.076	0.070																								
A. pleuronectes (Qld.)	0.081	0.090	0.084	0.030																							
A. pleuronectes (N.T.)	0.081	0.090	0.084	0.030	0.000																						
C. pallium	0.177	0.178	0.171	0.193	0.201	0.201																					
B. aktinos	0.166	0.169	0.160	0.170	0.173	0.173	0.214																				
A. balloti (Town.)	0.136	0.136	0.135	0.149	0.157	0.157	0.114	0.159																			
A. balloti (Noosa)	0.136	0.136	0.135	0.149	0.157	0.157	0.114	0.159	**0.000**																		
A. balloti (W.A.)	0.141	0.142	0.141	0.155	0.163	0.163	0.109	0.165	**0.008**	**0.008**																	
P. caurinus	0.246	0.261	0.244	0.254	0.259	0.259	0.299	0.265	0.249	0.249	0.247																
S. gaederopus	0.332	0.343	0.329	0.344	0.336	0.336	0.338	0.321	0.326	0.326	0.333	0.368															
M. nobilis	0.183	0.194	0.180	0.190	0.198	0.198	0.230	0.189	0.194	0.194	0.186	0.213	0.325														
S. dringi	0.193	0.194	0.195	0.173	0.192	0.192	0.214	0.147	0.183	0.183	0.184	0.273	0.334	0.168													
S. amicum	0.193	0.196	0.195	0.193	0.190	0.190	0.209	0.145	0.189	0.189	0.184	0.257	0.323	0.160	0.109												
M. rastellum	0.152	0.158	0.149	0.168	0.176	0.176	0.139	0.189	0.108	0.108	0.106	0.249	0.316	0.186	0.189	0.179											
M. mirficus	0.149	0.148	0.149	0.171	0.182	0.182	0.133	0.195	0.111	0.111	0.106	0.282	0.337	0.224	0.195	0.190	0.095										
G. pallium	0.147	0.148	0.150	0.153	0.166	0.166	0.131	0.195	0.109	0.109	0.106	0.264	0.319	0.183	0.184	0.196	0.043	0.090									
L. wilhelminae	0.177	0.183	0.171	0.176	0.187	0.187	0.212	0.112	0.156	0.156	0.162	0.246	0.312	0.166	0.115	0.110	0.168	0.184	0.182								
L. cuneata	0.181	0.184	0.170	0.183	0.194	0.194	0.200	0.123	0.153	0.153	0.158	0.233	0.317	0.173	0.123	0.123	0.164	0.188	0.175	0.054							
C. islandica	0.162	0.171	0.160	0.168	0.181	0.181	0.212	0.180	0.168	0.168	0.166	0.119	0.313	0.115	0.172	0.172	0.176	0.201	0.177	0.157	0.153						
C. mulistrata	0.211	0.217	0.211	0.213	0.224	0.224	0.252	0.236	0.224	0.224	0.222	0.265	0.325	0.181	0.223	0.220	0.210	0.240	0.216	0.240	0.232	0.171					
C. madreporarum	0.173	0.179	0.168	0.173	0.189	0.189	0.208	0.115	0.159	0.159	0.165	0.248	0.308	0.168	0.112	0.112	0.170	0.181	0.184	0.003	0.057	0.152	0.237				
C. varia	0.187	0.193	0.174	0.181	0.192	0.192	0.251	0.207	0.207	0.207	0.207	0.233	0.314	0.160	0.216	0.203	0.201	0.234	0.210	0.208	0.204	0.131	0.149	0.202			
A. opercularis	0.283	0.290	0.289	0.315	0.310	0.310	0.275	0.311	0.267	0.267	0.265	0.373	0.352	0.315	0.322	0.293	0.283	0.280	0.290	0.293	0.292	0.313	0.338	0.295	0.348		
C. glabra	0.277	0.289	0.288	0.291	0.293	0.293	0.280	0.292	0.254	0.254	0.255	0.354	0.355	0.307	0.297	0.289	0.257	0.285	0.269	0.292	0.291	0.299	0.325	0.295	0.331	0.242	
A. colbecki	0.166	0.181	0.158	0.159	0.182	0.182	0.222	0.188	0.187	0.187	0.184	0.270	0.330	0.203	0.195	0.197	0.181	0.184	0.184	0.170	0.172	0.187	0.232	0.167	0.212	0.293	0.305

Raw allele Sizes for all loci. YEP—Yeppoon, BUS—Bustard Head, HER— Hervey Bay, TOW—Townsville, NOO—Noosa, PEL—Pelseart, SHA—Shark Bay.
N is the number of individuals scored at that locus.

Locus 341								
Allele Number	Allele Size	YEP	BUS	HER	TOW	NOO	PEL	SHA
1	128	0.000	0.000	0.000	0.000	0.000	0.009	0.000
2	131	0.000	0.019	0.000	0.000	0.000	0.019	0.024
3	134	0.000	0.000	0.000	0.000	0.036	0.019	0.024
4	137	0.000	0.000	0.037	0.023	0.000	0.120	0.167
5	140	0.000	0.019	0.019	0.000	0.018	0.037	0.048
6	143	0.000	0.074	0.019	0.045	0.018	0.093	0.095
7	146	0.031	0.019	0.000	0.068	0.036	0.083	0.024
8	149	0.172	0.111	0.111	0.159	0.179	0.148	0.095
9	152	0.062	0.167	0.093	0.136	0.071	0.074	0.167
10	155	0.078	0.037	0.019	0.000	0.036	0.102	0.071
11	158	0.047	0.056	0.019	0.045	0.071	0.037	0.095
12	161	0.031	0.037	0.056	0.023	0.089	0.046	0.000
13	164	0.047	0.056	0.111	0.045	0.054	0.074	0.048
14	167	0.016	0.056	0.111	0.045	0.036	0.019	0.024
15	170	0.031	0.056	0.019	0.045	0.000	0.009	0.071
16	173	0.094	0.019	0.000	0.045	0.036	0.046	0.024
17	176	0.047	0.093	0.111	0.068	0.054	0.046	0.000
18	179	0.094	0.056	0.056	0.000	0.018	0.000	0.024
19	182	0.078	0.019	0.056	0.045	0.018	0.009	0.000
20	185	0.016	0.037	0.074	0.114	0.054	0.000	0.000
21	188	0.047	0.019	0.056	0.045	0.054	0.000	0.000
22	191	0.031	0.000	0.019	0.023	0.036	0.009	0.000
23	194	0.031	0.019	0.000	0.023	0.054	0.000	0.000
24	197	0.000	0.019	0.019	0.000	0.018	0.000	0.000
25	200	0.016	0.019	0.000	0.000	0.018	0.000	0.000
26	203	0.031	0.000	0.000	0.000	0.000	0.000	0.000
	N	32	27	27	22	28	54	21

Table 14 cont.

Locus 147								
Allele Number	Allele Size	YEP	BUS	HER	TOW	NOO	PEL	SHA
1	167	0.000	0.000	0.019	0.000	0.000	0.000	0.000
2	170	0.015	0.000	0.000	0.000	0.017	0.000	0.000
3	173	0.000	0.000	0.000	0.000	0.000	0.009	0.000
4	176	0.045	0.019	0.019	0.022	0.017	0.009	0.000
5	182	0.000	0.000	0.000	0.022	0.000	0.009	0.000
6	185	0.000	0.000	0.000	0.022	0.017	0.019	0.114
7	188	0.015	0.000	0.000	0.022	0.000	0.037	0.045
8	191	0.076	0.000	0.000	0.022	0.100	0.056	0.023
9	194	0.061	0.037	0.058	0.065	0.000	0.120	0.114
10	197	0.076	0.093	0.096	0.065	0.050	0.083	0.114
11	200	0.061	0.167	0.135	0.065	0.133	0.093	0.159
12	203	0.091	0.074	0.058	0.022	0.167	0.083	0.068
13	206	0.030	0.019	0.096	0.000	0.067	0.120	0.136
14	209	0.061	0.093	0.058	0.065	0.067	0.102	0.023
15	212	0.015	0.056	0.154	0.065	0.067	0.083	0.091
16	215	0.061	0.093	0.038	0.065	0.017	0.065	0.091
17	218	0.030	0.019	0.000	0.152	0.033	0.056	0.023
18	221	0.030	0.000	0.000	0.043	0.050	0.019	0.000
19	224	0.076	0.037	0.058	0.087	0.000	0.028	0.000
20	227	0.091	0.056	0.019	0.000	0.033	0.009	0.000
21	230	0.000	0.056	0.038	0.043	0.050	0.000	0.000
22	233	0.061	0.037	0.019	0.000	0.033	0.000	0.000
23	236	0.000	0.019	0.038	0.043	0.000	0.000	0.000
24	239	0.030	0.037	0.058	0.043	0.067	0.000	0.000
25	242	0.015	0.019	0.000	0.022	0.000	0.000	0.000
26	245	0.030	0.000	0.000	0.000	0.000	0.000	0.000
27	248	0.015	0.074	0.038	0.000	0.000	0.000	0.000
28	251	0.015	0.000	0.000	0.000	0.000	0.000	0.000
29	254	0.000	0.000	0.000	0.043	0.000	0.000	0.000
30	260	0.000	0.000	0.000	0.000	0.017	0.000	0.000
	N	33	27	26	23	30	54	22

Table 14 cont.

Locus 75								
Allele Number	Allele Size	YEP	BUS	HER	TOW	NOO	PEL	SHA
1	210	0.000	0.000	0.000	0.000	0.000	0.000	0.019
2	214	0.017	0.000	0.000	0.000	0.000	0.000	0.000
3	220	0.017	0.000	0.000	0.000	0.000	0.000	0.000
4	222	0.069	0.100	0.167	0.088	0.097	0.000	0.000
5	224	0.017	0.000	0.074	0.029	0.000	0.011	0.058
6	226	0.034	0.020	0.037	0.029	0.065	0.000	0.019
7	228	0.000	0.000	0.000	0.088	0.065	0.044	0.038
8	230	0.017	0.060	0.056	0.176	0.113	0.067	0.038
9	232	0.034	0.060	0.019	0.029	0.016	0.044	0.019
10	234	0.155	0.060	0.074	0.000	0.065	0.056	0.000
11	236	0.121	0.080	0.111	0.059	0.113	0.033	0.038
12	238	0.000	0.100	0.019	0.000	0.016	0.033	0.038
13	240	0.086	0.060	0.056	0.118	0.113	0.011	0.000
14	242	0.052	0.100	0.019	0.118	0.129	0.033	0.038
15	244	0.052	0.100	0.148	0.059	0.065	0.056	0.154
16	246	0.017	0.060	0.000	0.000	0.065	0.044	0.077
17	248	0.069	0.040	0.037	0.029	0.016	0.033	0.038
18	250	0.052	0.100	0.019	0.088	0.016	0.022	0.058
19	252	0.069	0.000	0.037	0.059	0.000	0.089	0.019
20	254	0.000	0.000	0.074	0.000	0.016	0.056	0.096
21	256	0.017	0.000	0.000	0.000	0.000	0.011	0.038
22	258	0.000	0.020	0.019	0.000	0.000	0.022	0.019
23	260	0.017	0.000	0.019	0.000	0.000	0.033	0.019
24	262	0.052	0.000	0.000	0.000	0.000	0.022	0.019
25	264	0.017	0.000	0.000	0.000	0.000	0.022	0.019
26	266	0.017	0.000	0.019	0.000	0.000	0.022	0.038
27	268	0.000	0.020	0.000	0.000	0.000	0.000	0.000
28	270	0.000	0.020	0.000	0.029	0.032	0.067	0.000
29	272	0.000	0.000	0.000	0.000	0.000	0.033	0.038
30	274	0.000	0.000	0.000	0.000	0.000	0.022	0.019
31	276	0.000	0.000	0.000	0.000	0.000	0.033	0.019
32	278	0.000	0.000	0.000	0.000	0.000	0.033	0.000
33	280	0.000	0.000	0.000	0.000	0.000	0.011	0.019
34	288	0.000	0.000	0.000	0.000	0.000	0.022	0.000
35	302	0.000	0.000	0.000	0.000	0.000	0.011	0.000
	N	29	25	27	17	31	45	26

Table 14 cont.

Locus 355								
Allele Number	Allele Size	YEP	BUS	HER	TOW	NOO	PEL	SHA
1	214	0.000	0.000	0.000	0.000	0.000	0.000	0.043
2	235	0.000	0.000	0.000	0.000	0.000	0.021	0.022
3	238	0.000	0.016	0.020	0.036	0.019	0.000	0.000
4	241	0.176	0.177	0.100	0.214	0.269	0.000	0.000
5	244	0.618	0.726	0.760	0.536	0.462	0.021	0.000
6	247	0.074	0.032	0.060	0.018	0.096	0.489	0.391
7	250	0.029	0.016	0.000	0.036	0.058	0.021	0.000
8	253	0.029	0.000	0.000	0.018	0.000	0.032	0.022
9	256	0.000	0.000	0.000	0.054	0.038	0.032	0.022
10	259	0.000	0.000	0.000	0.000	0.000	0.021	0.065
11	262	0.000	0.016	0.000	0.000	0.000	0.032	0.065
12	265	0.000	0.000	0.000	0.000	0.000	0.053	0.022
13	268	0.015	0.000	0.000	0.071	0.000	0.074	0.087
14	271	0.015	0.000	0.040	0.000	0.000	0.064	0.043
15	274	0.000	0.000	0.000	0.000	0.000	0.043	0.087
16	277	0.000	0.016	0.000	0.000	0.019	0.043	0.043
17	280	0.000	0.000	0.000	0.000	0.000	0.000	0.022
18	283	0.015	0.000	0.000	0.000	0.000	0.021	0.000
19	286	0.000	0.000	0.000	0.000	0.019	0.000	0.000
20	289	0.000	0.000	0.020	0.000	0.000	0.011	0.022
21	295	0.029	0.000	0.000	0.000	0.000	0.011	0.000
22	298	0.000	0.000	0.000	0.018	0.000	0.011	0.043
23	301	0.000	0.000	0.000	0.000	0.019	0.000	0.000
	N	34	31	25	28	26	47	23

Table 14 cont.

Locus 299								
Allele Number	Allele Size	YEP	BUS	HER	TOW	NOO	PEL	SHA
1	216	0.017	0.000	0.000	0.000	0.017	0.000	0.000
2	219	0.000	0.000	0.000	0.000	0.000	0.011	0.000
3	222	0.033	0.016	0.000	0.038	0.017	0.022	0.000
4	228	0.000	0.000	0.000	0.019	0.000	0.000	0.000
5	231	0.000	0.000	0.000	0.019	0.017	0.022	0.095
6	234	0.017	0.016	0.000	0.019	0.000	0.044	0.071
7	237	0.083	0.032	0.000	0.019	0.121	0.056	0.024
8	240	0.067	0.016	0.091	0.058	0.000	0.122	0.095
9	243	0.067	0.113	0.136	0.058	0.052	0.078	0.167
10	246	0.083	0.177	0.091	0.058	0.138	0.056	0.048
11	249	0.117	0.048	0.091	0.038	0.190	0.111	0.095
12	252	0.017	0.016	0.136	0.000	0.052	0.122	0.119
13	255	0.067	0.097	0.045	0.115	0.086	0.122	0.048
14	258	0.017	0.065	0.227	0.058	0.069	0.067	0.048
15	261	0.067	0.097	0.000	0.077	0.000	0.067	0.119
16	264	0.017	0.000	0.000	0.135	0.034	0.044	0.048
17	267	0.033	0.032	0.000	0.038	0.034	0.033	0.024
18	270	0.067	0.032	0.136	0.077	0.000	0.022	0.000
19	273	0.050	0.048	0.000	0.000	0.034	0.000	0.000
20	276	0.000	0.016	0.000	0.058	0.034	0.000	0.000
21	279	0.067	0.032	0.045	0.000	0.034	0.000	0.000
22	282	0.000	0.032	0.000	0.038	0.000	0.000	0.000
23	285	0.033	0.032	0.000	0.038	0.052	0.000	0.000
24	288	0.033	0.000	0.000	0.019	0.000	0.000	0.000
25	291	0.017	0.016	0.000	0.000	0.000	0.000	0.000
26	294	0.017	0.065	0.000	0.000	0.000	0.000	0.000
27	297	0.017	0.000	0.000	0.000	0.000	0.000	0.000
28	300	0.000	0.000	0.000	0.019	0.000	0.000	0.000
29	306	0.000	0.000	0.000	0.000	0.017	0.000	0.000
	N	30	31	11	26	29	45	21

Table 14 cont.

Locus 129								
Allele Number	Allele Size	YEP	BUS	HER	TOW	NOO	PEL	SHA
1	194	0.000	0.000	0.000	0.000	0.000	0.000	0.043
2	212	0.000	0.017	0.000	0.000	0.000	0.010	0.000
3	214	0.058	0.017	0.000	0.000	0.000	0.000	0.000
4	216	0.077	0.000	0.000	0.000	0.000	0.000	0.000
5	218	0.038	0.067	0.095	0.021	0.017	0.010	0.000
6	220	0.077	0.083	0.071	0.042	0.100	0.030	0.022
7	222	0.212	0.333	0.333	0.271	0.133	0.020	0.065
8	224	0.115	0.167	0.071	0.250	0.250	0.030	0.000
9	226	0.308	0.100	0.286	0.146	0.350	0.540	0.543
10	228	0.019	0.067	0.048	0.062	0.033	0.130	0.196
11	230	0.038	0.067	0.048	0.042	0.033	0.120	0.065
12	232	0.019	0.050	0.024	0.083	0.050	0.070	0.022
13	234	0.019	0.000	0.024	0.042	0.000	0.010	0.000
14	236	0.000	0.000	0.000	0.000	0.017	0.010	0.022
15	238	0.000	0.017	0.000	0.021	0.017	0.000	0.000
16	240	0.019	0.000	0.000	0.000	0.000	0.000	0.000
17	242	0.000	0.000	0.000	0.021	0.000	0.000	0.022
18	244	0.000	0.017	0.000	0.000	0.000	0.000	0.000
19	246	0.000	0.000	0.000	0.000	0.000	0.020	0.000
	N	26	30	21	24	30	50	23

Locus 291								
Allele Number	Allele Size	YEP	BUS	HER	TOW	NOO	PEL	SHA
1	166	0.015	0.017	0.000	0.000	0.000	0.174	0.190
2	169	0.059	0.034	0.019	0.056	0.017	0.065	0.095
3	172	0.015	0.069	0.058	0.019	0.000	0.054	0.071
4	175	0.176	0.103	0.173	0.204	0.172	0.065	0.095
5	178	0.250	0.190	0.327	0.130	0.259	0.185	0.119
6	181	0.088	0.121	0.077	0.185	0.259	0.054	0.048
7	184	0.088	0.138	0.115	0.074	0.069	0.120	0.167
8	187	0.103	0.086	0.077	0.074	0.052	0.098	0.048
9	190	0.103	0.069	0.077	0.111	0.034	0.098	0.024
10	193	0.059	0.103	0.077	0.037	0.069	0.065	0.071
11	196	0.000	0.017	0.000	0.037	0.017	0.011	0.071
12	199	0.000	0.052	0.000	0.000	0.034	0.011	0.000
13	202	0.000	0.000	0.000	0.019	0.000	0.000	0.000
14	205	0.015	0.000	0.000	0.037	0.000	0.000	0.000
15	211	0.029	0.000	0.000	0.019	0.000	0.000	0.000
16	223	0.000	0.000	0.000	0.000	0.017	0.000	0.000
	N	34	29	26	27	29	46	21

Appendix 7: 15th International Pectinid Workshop Presentation – Presented by Dr Liz O'Brien

Australian Saucer scallops — sea-ranching genetics

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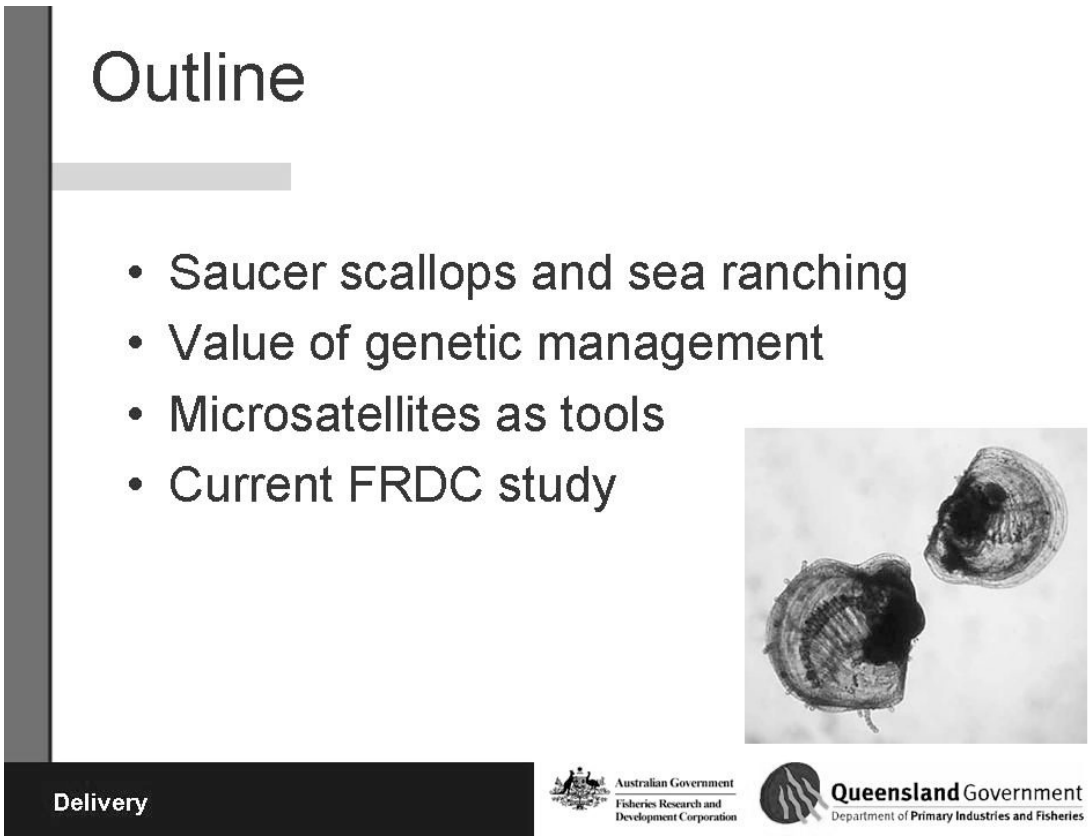
The saucer scallop, *Amusium balloti*, is distributed along the Western and Eastern coast of Australia and supports a fishery in both states. To stabilise the annual catch rates private companies from both states are investing in sea-ranching or reseeding operations. Due to biological constraints on wild harvest of spat, the enhancement operations are reliant upon production of seed from hatcheries. The mass release of scallop juveniles is expected to have an immediate effect on stock abundance but it also has the potential to alter the genetic structure of the wild saucer scallop populations. To minimise the environmental impacts of reseeding, genetic resource management is recommended and both companies are supportive of understanding the genetic structure of the native populations.

To facilitate this, the genetic population structure of saucer scallops along the Western and Eastern Australian coastline have been analysed using eight microsatellite loci and the results will be presented during this presentation.

Previous allozyme work compared the Queensland and Western Australian forms of *A. balloti* and results indicated that they might be classed as different species (Dredge et al, in prep.). We have used 16S and 12S rRNA sequence fragments to verify these findings and present the results here.

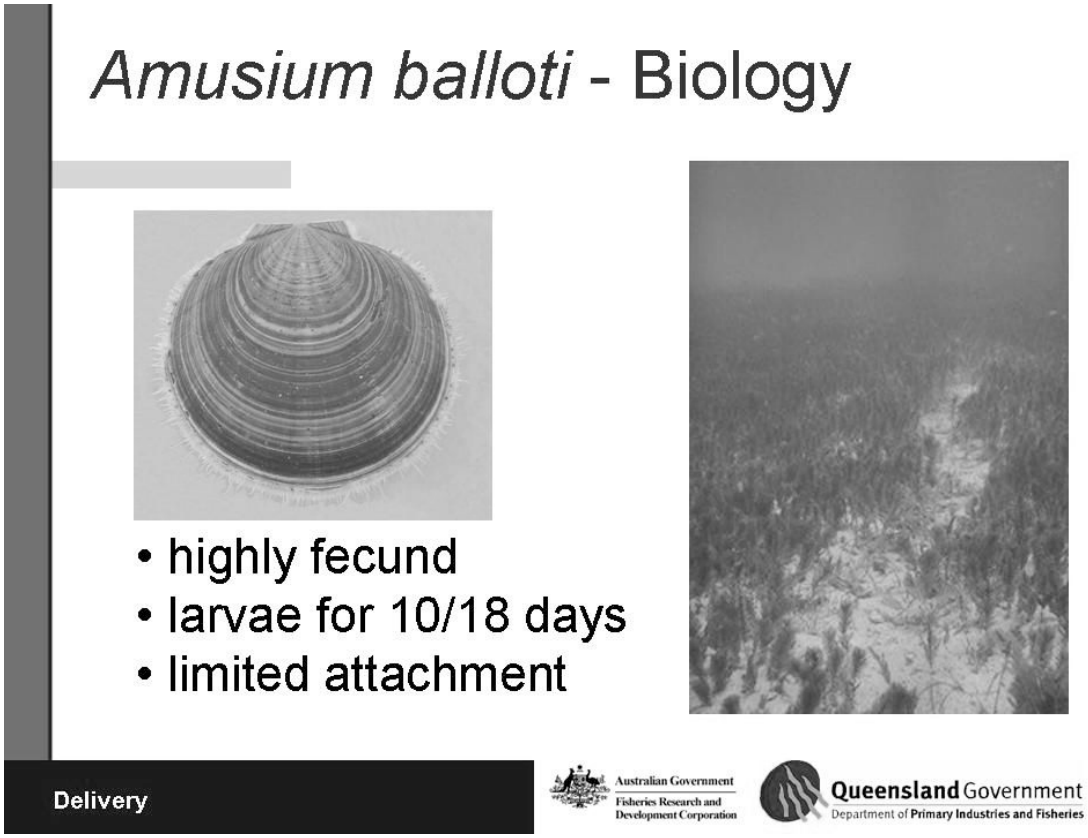
Finally we will present a suggested genetic management strategy for Australian saucer scallop ranching based on these findings.

Today, I am presenting the background to a project which is producing baseline datafor genetic management of saucer scallop sea-ranching in Australia. The project involved several people: Jason Bartlett and Bryony Dixon from the Queensland Department of Primary Industries and Fisheries and Peter Duncan from the University of the Sunshine Coast.



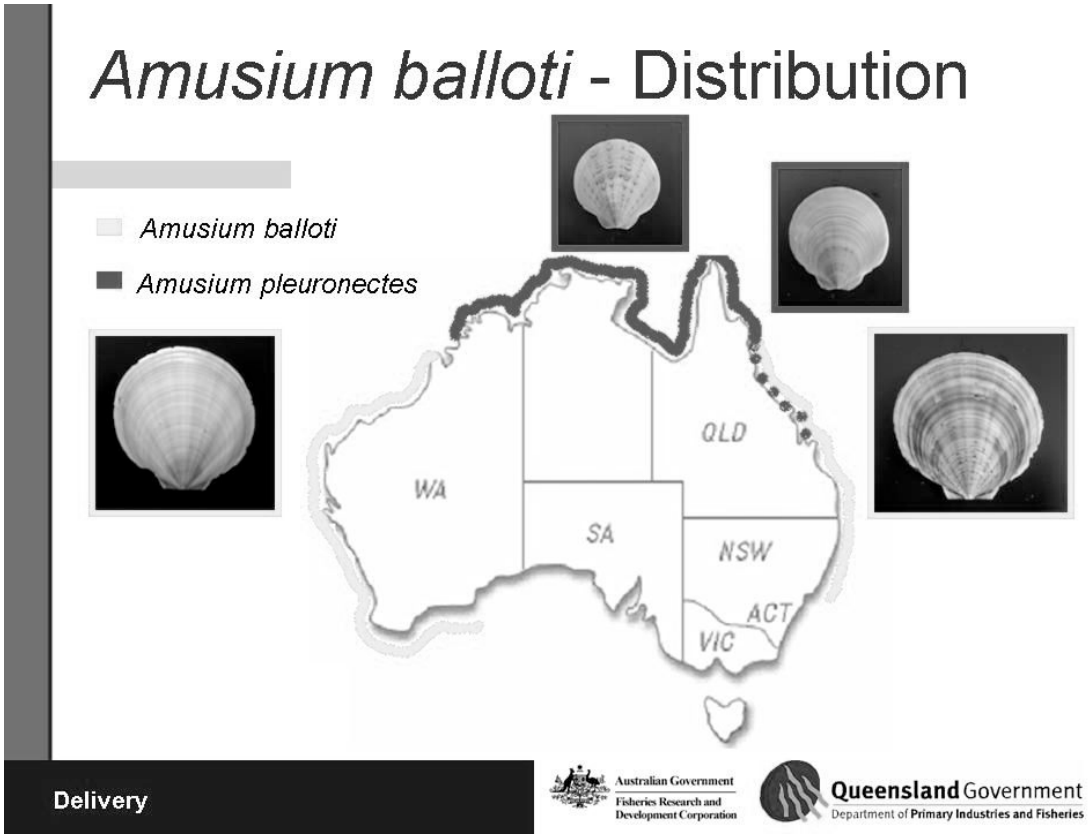
2. During the presentation I will give you some information on the biology of the saucer scallop, as well as the commercial importance of the species. Then I will talk about why there is value in managing the genetic make-up of a sea-ranching venture, followed by a description of one of the tools that can be used—microsatellites.

Then I will talk about the details and results of our current study, which is funded by the Australian Government through the Fisheries Research and Development Corporation.



The saucer scallop *Amusium balloti* has smooth shell valves with red-brown banding on the left valve and a pale creamy white right valve. They sit slightly recessed in the substrate, which ranges from sand to slightly muddy. Example: **habitat** Hervey Bay which has supported balloti populations. The animals are of light weight and are a very effective swimmers. They are found in water 10 to 75m deep, on sandy or slightly muddy substrate, and their distribution can range from one per square meter to one per 150 square metres. They are trawled, not dredged, and the product is sold roe-off.

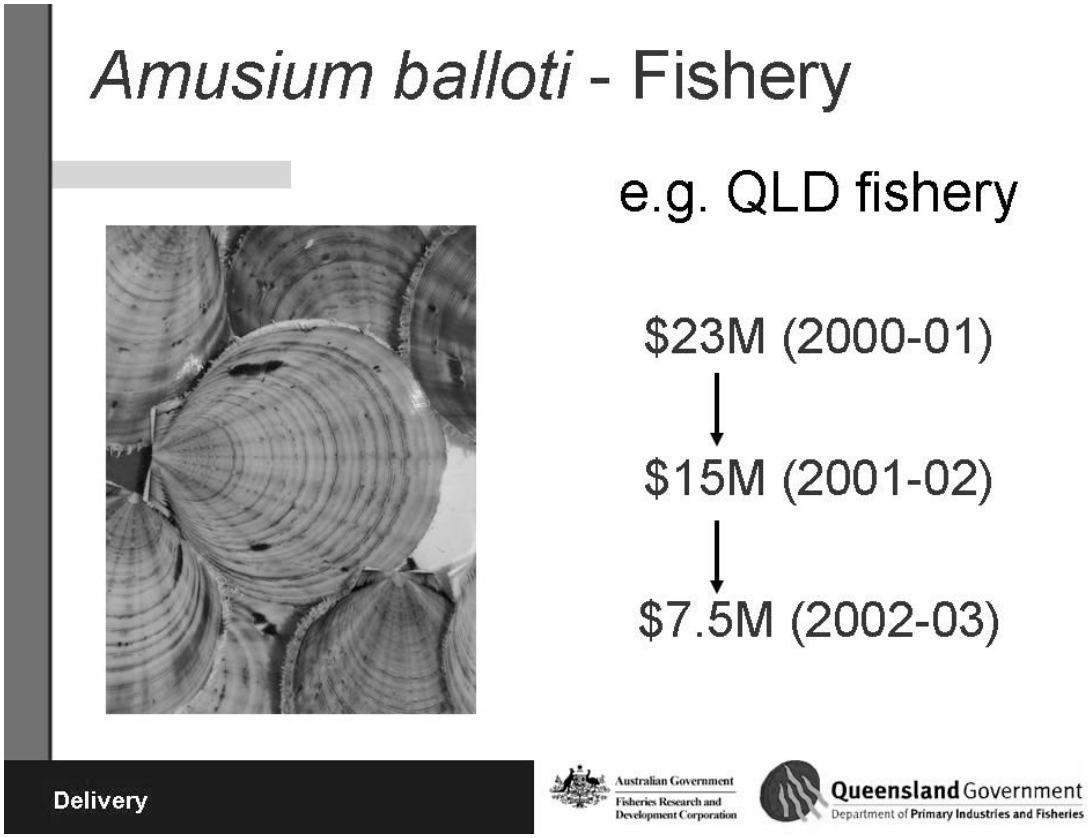
They are estimated to live up to three years and are sexually mature within the first year. The sexes are separate and are highly fecund broadcast spawners, with females spawning between 2 to 10 million eggs per spawn. Larval development to settlement varies from 10 days in Western Australia to 18 days in Queensland. Unlike other scallop species, juvenile *balloti* have a transient and weak attachment phase



The distribution occurs along the Western Australian and Queensland coastline, which are highlighted here in Light Grey, and the populations in the two states are separated by a different species, *Amusium pleuronectes*, shown here in Black.

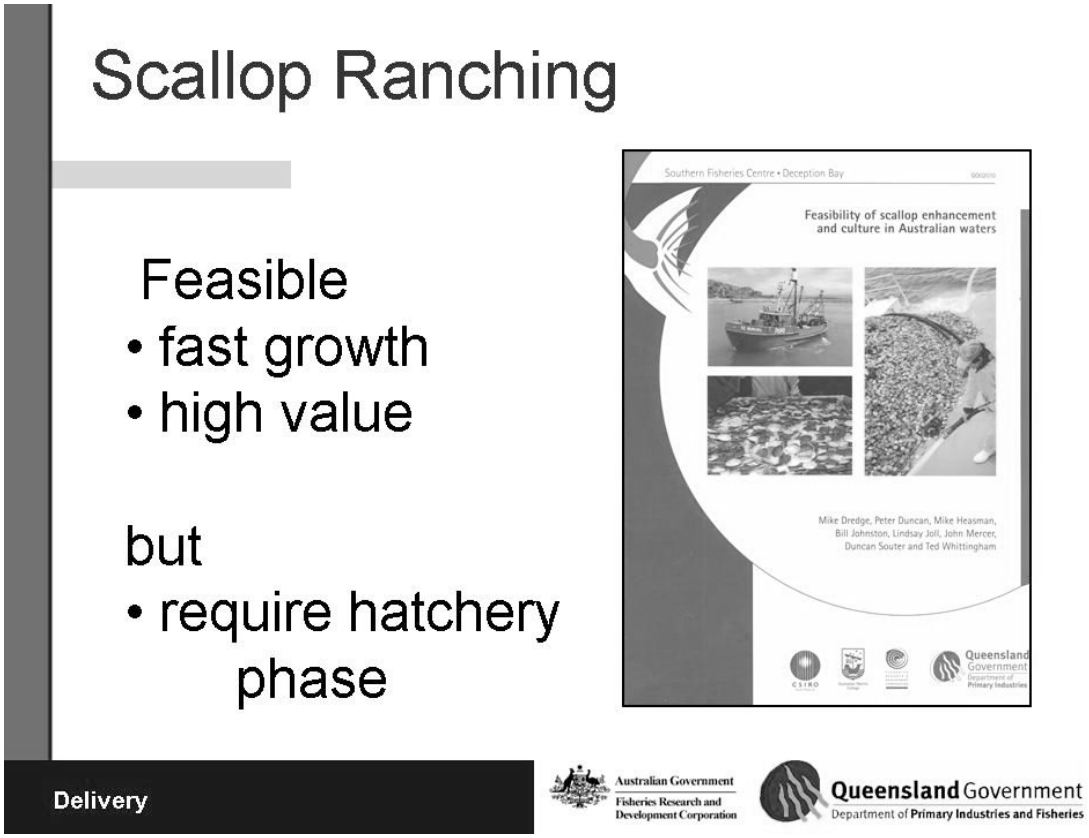
Numbers of adult *balloti* are abundant enough to support commercial fisheries in both states and because they are active swimmers they are trawled rather than dredged. Processing scallops involves 'shucking' (removing the animal from the shell) and trimming back to the white 'meat' by removing the roe sac. Small quantities of scallops may be left 'roe-on' to supply the gourmet seafood market.

However, catches in the fisheries can vary widely between years.



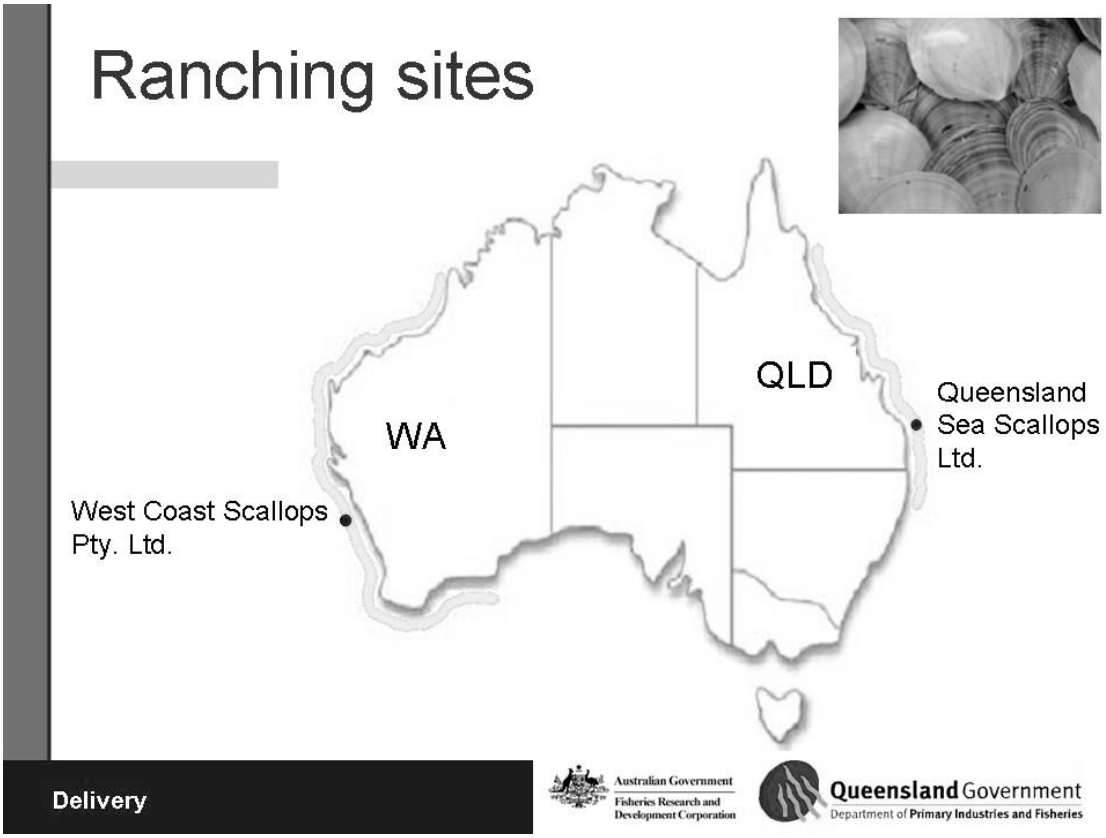
In Queensland, the establishment of the fishery took place in the 1950s; catches increased in the 1970s, but declined markedly in the 1990s and management strategies were implemented to address this. However, the recruitment success can still vary.

In common with Queensland, fluctuations in scallop catches have been experienced overseas. **Restocking** depleted fisheries has been shown to be beneficial and a very cost-effective means of improving catches.



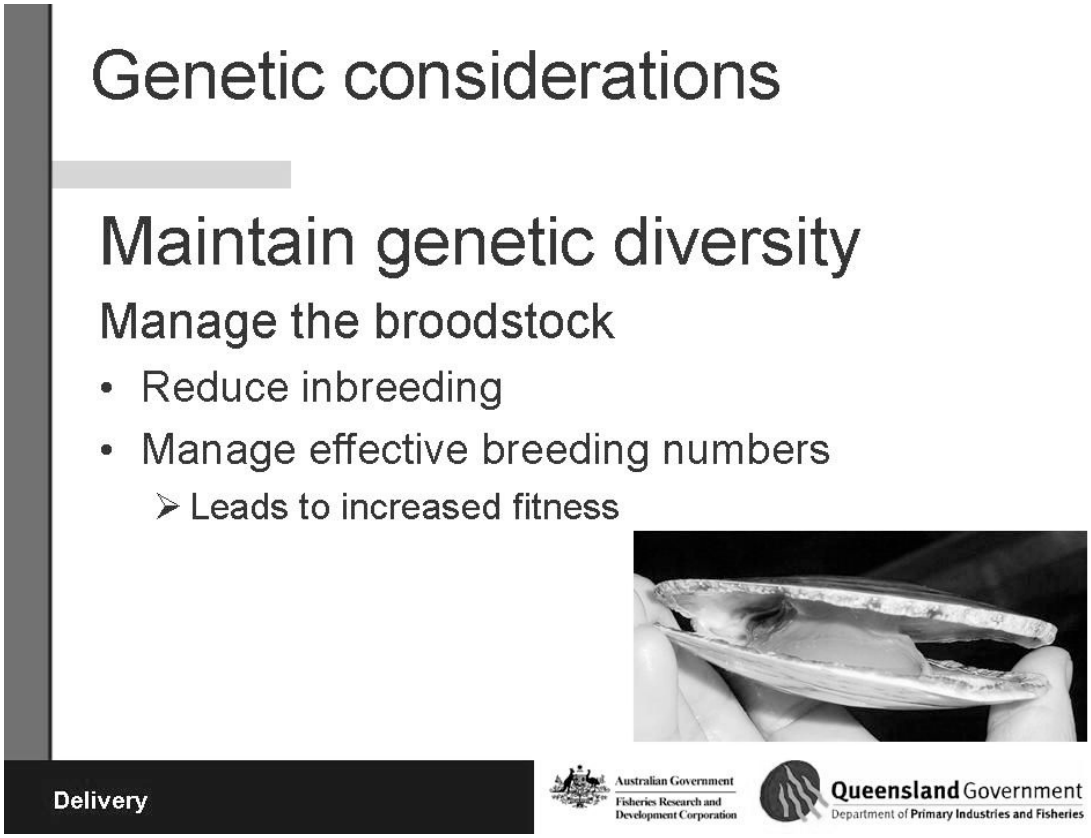
The feasibility of enhancement of the saucer scallop fishery in Australia was assessed in an FRDC project led by Mike Dredge. The study included biological, economic and social analysis and concluded that it was feasible. In particular the rapid growth rate and high value of the product was an advantage.

There were, however, specific deficits in information and technology that needed to be addressed. A major point was that due to the weak ability to attach to substrates the seed for the enhancement had to be produced in a hatchery and could not be collected from the wild.



There are currently two companies in Australia investing in saucer scallop sea-ranching: West Coast Scallops in Geraldton and Queensland Sea Scallops in Hervey Bay. The two operations collect broodstock from the wild, spawn them in the hatchery and release them back to the sea. It is valuable to manage the hatchery phase to ensure genetic diversity is maintained.

With genetic diversity a population or a species have a range of genotypes that can give them scope to adapt to change, such as a new disease or in climate. Loss of genetic variation not only impacts on their ability to adapt, but can also lead to potential harmful effects upon various performance traits such as survival and growth. Therefore, managing the broodstock is important in the hatchery.



One way that genetic diversity can be reduced is through inbreeding, where closely related animals reproduce. This can lead to hereditary faults that can be very detrimental, such as poor fecundity, reduced hatchery and nursery performance and loss of disease resistance.

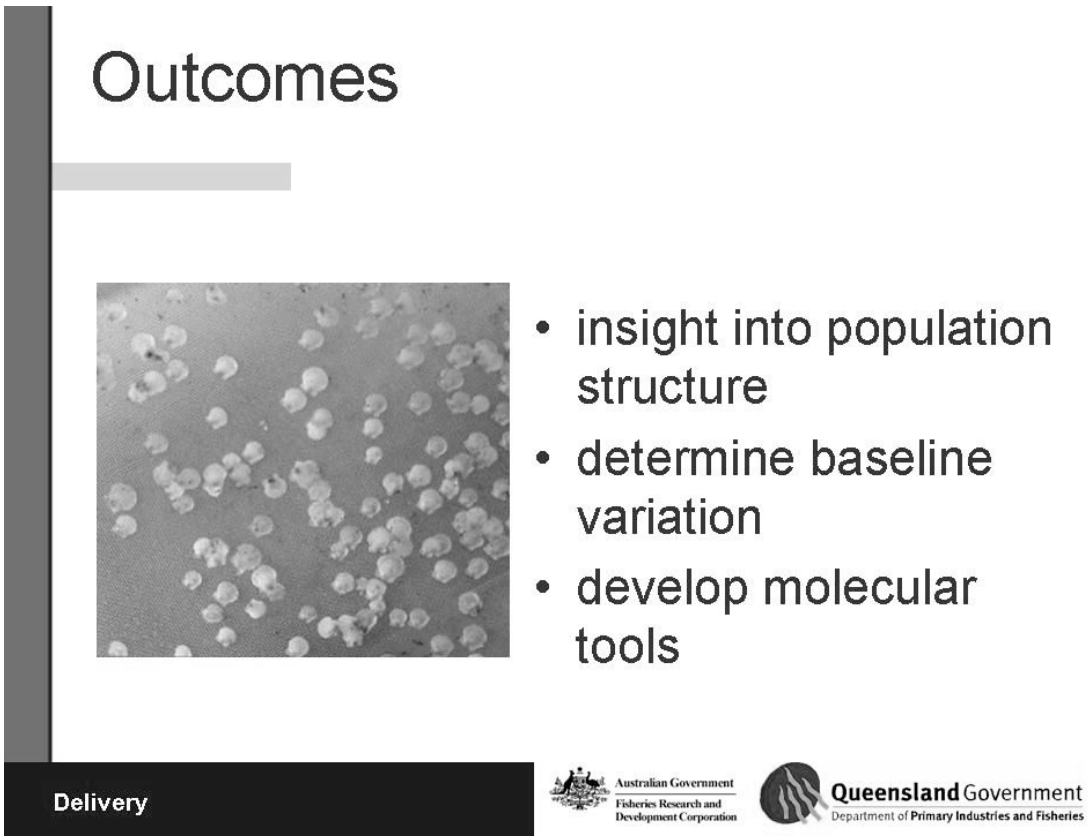
Genetic diversity can also be reduced when there is an unequal contribution of broodstock to the next generation. The use of many individuals as spawners for the production of shellfish larvae does not guarantee that all the parents will make an equal contribution to the offspring. It is very common that the true number of breeding individuals is lower than the actual broodstock number used in a spawning.

By managing the broodstock it is important that fitness or the ability of the animals to survive and reproduce is protected.

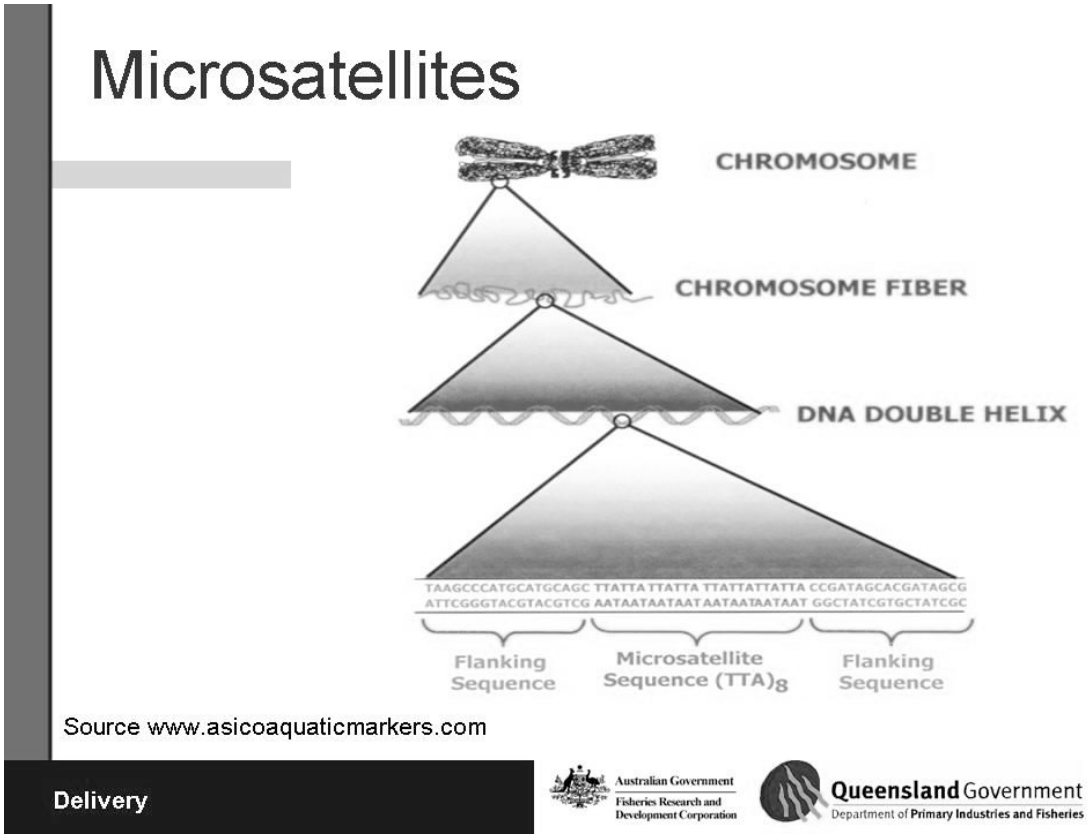
Currently there is limited information available on the way that genetic variation is spread throughout the saucer scallop populations. We wanted to investigate the population genetic makeup of the east and west coast saucer scallop population to see if there is much gene flow up and down the coast.

Many studies of marine invertebrates have shown a high level of genetic similarity over large distances due to genetic exchange between populations. You can predict how far larvae may be able to disperse and hence breed with other populations, but detecting barriers to larval dispersal — such as currents and thermoclines—can be difficult. Numerous other issues will contribute to that larvae surviving to reproduce. The genetic population structure can give an insight into the reproductive interaction between populations.

We have decided to use DNA markers called microsatellites to answer this question.



The results of this study will contribute to our understanding of the wild saucer scallop populations as well as providing a baseline of genetic variation in the microsatellites we have chosen to examine. The microsatellites used in this study may also be used as molecular tools in other studies, such as investigating the effective breeding number in the hatchery.

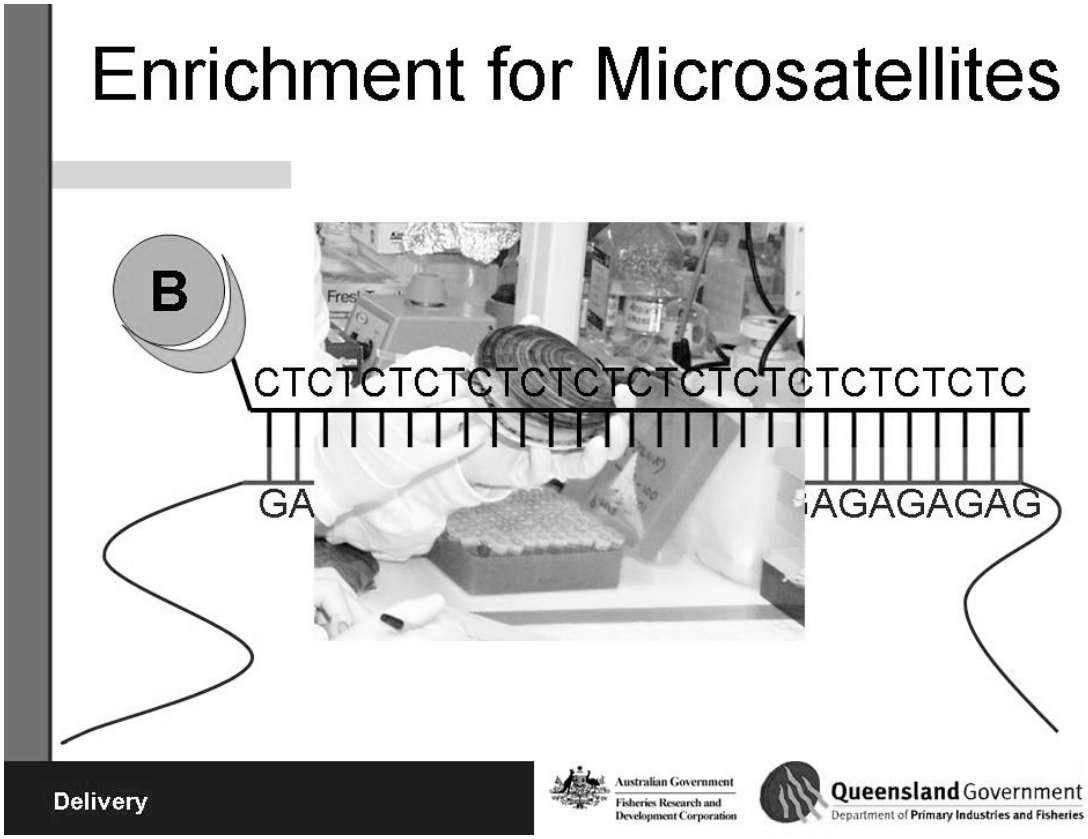


For those who are not familiar with microsatellites, I am going to give a quick description of what they are and how we isolated them.

Microsatellites are repeated bits of DNA that are spread randomly throughout the chromosomes of the scallops. So the example you see here is a tri-nucleotide repeat, where the three nucleotides TTA are repeated eight times within this stretch of DNA. Within the same region of the chromosome the number of repeats can vary between individuals and populations.

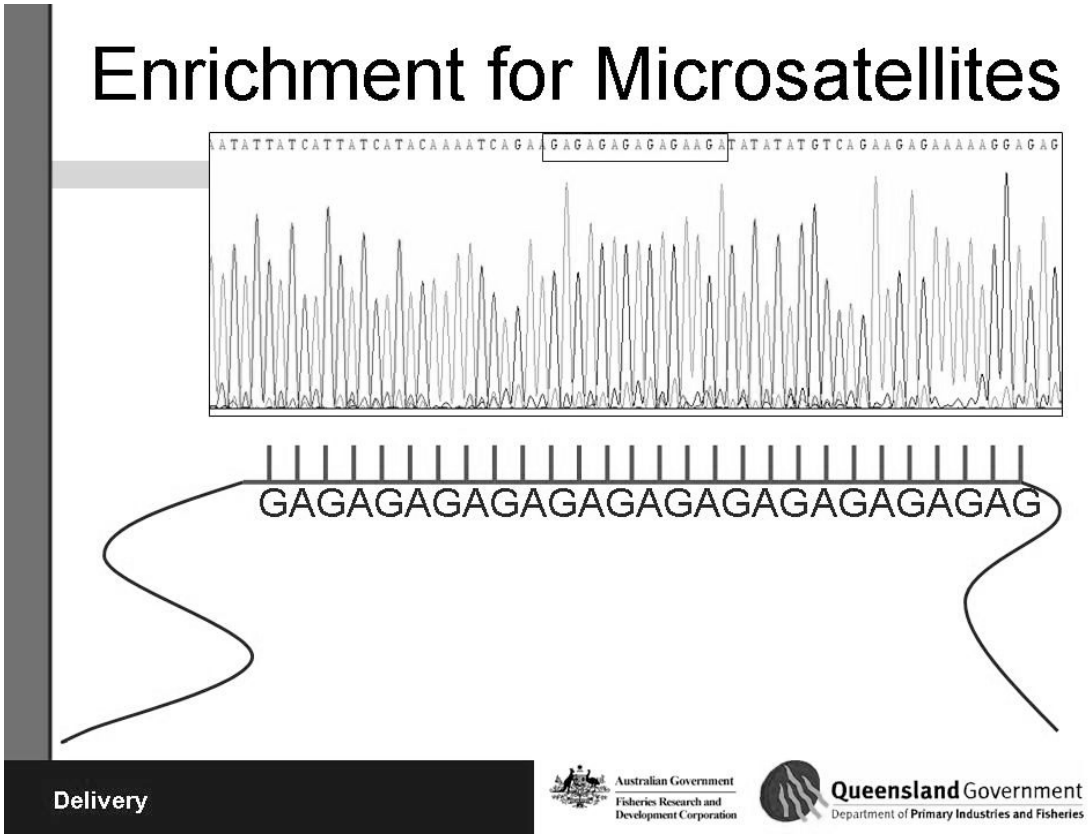
Microsatellites have an advantage over some other DNA markers; they are extremely variable in the number of repeats from one scallop to the next as they are usually located in non-coding regions which are less subject to selection than are functional markers, and hence can vary a lot. This lets us pick up a great deal of information about the levels of genetic variation within or between groups that may not be detected using other genetic markers.

Microsatellites are unique to each species, so they need to be developed each time a new species is being worked on. A large part of the FRDC grant was the initial isolation of the microsatellite markers.



Because microsatellites are intermittently spread throughout the scallops' DNA, we use methods that increase our chance of locating them. Therefore, we extract DNA from the scallop. We synthesise a short piece of DNA that is the complementary match of the microsatellite that we are trying to extract. The short piece of DNA is then bound to a small biotin-coated magnetic bead that can be retrieved with a magnet. That tagged DNA will bind to microsatellites with the complementary code. We then separate the probe DNA from the target scallop DNA and sequence the fragment.

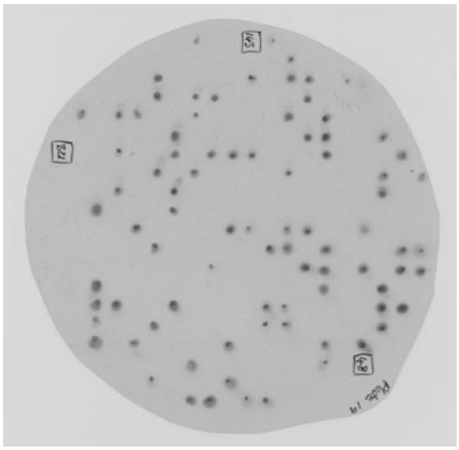
After our first round of enrichment we sequenced a large number of DNA fragments and found that a lot did not have microsatellites in them. We found that as few as 1 in every 20 fragments had a microsatellite in it that was usable. To overcome this problem we did a second round of selection.



We patched our enriched clones in a grid so we could identify them later on. We then used a radioactively labelled probe to bind to those clones that contained the microsatellites. In this way we were able to cut the costs of developing the microsatellites significantly. We have only sequenced a small proportion of the clones and have found 12 di repeats or repeats of two nucleotides such as GA. Of those positive clones that we have sequenced 79% have contained microsatellites.

Secondary Selection

- Secondary selection of clones
- 14 tri
- 12 di
- 1 tetra
- 79% +ve clones for msats
- 21 primer sets designed



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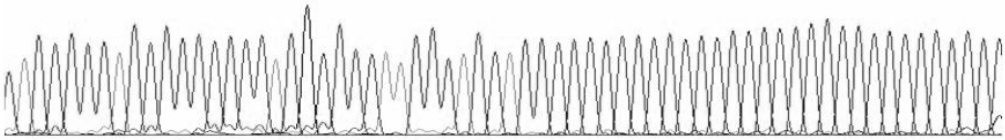
Saucer scallop microsatellites

Locus	Repeat	# alleles
341	GAT	24
355	GAT	17
299	GAT	24
147	CAT	26
291	CAT	14
129	GT	13



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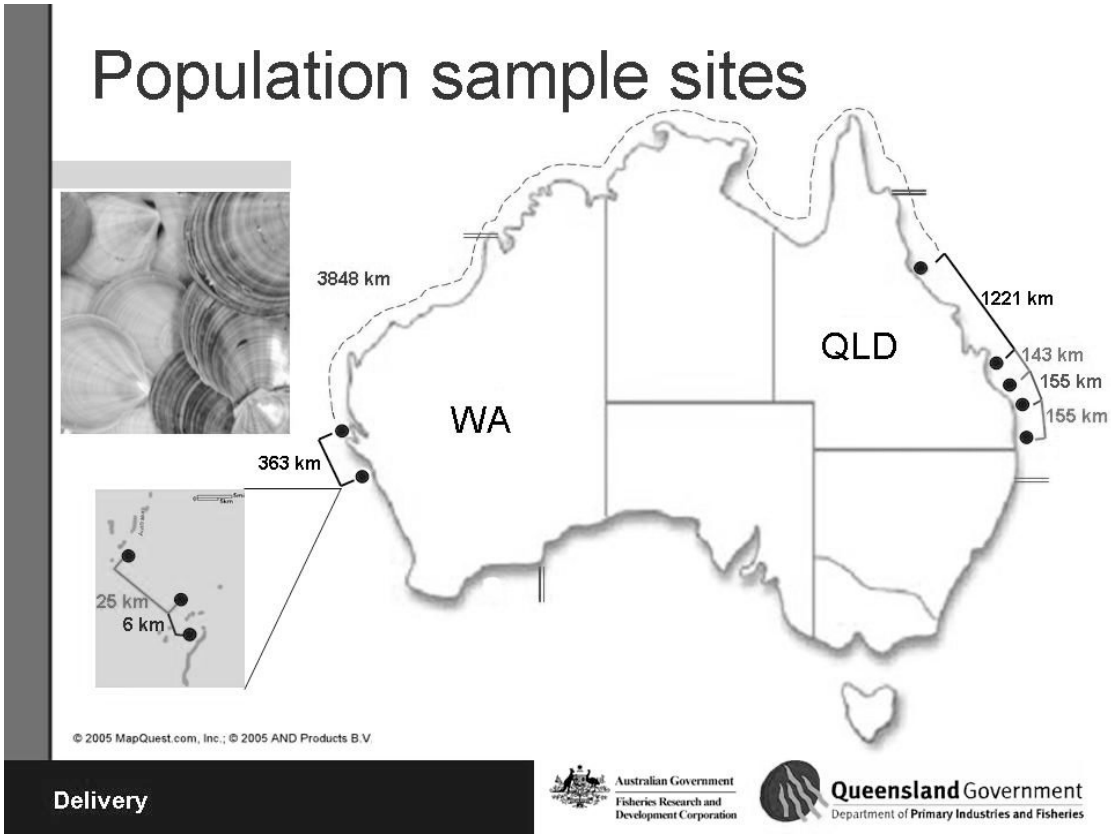

We have samples from four populations in Western Australia, one in Shark Bay and three from the Abrolhos Island group where a majority of the commercial fishery is based. Unfortunately, due to the difficulties in obtaining samples from Western Australia, we only had 20 individuals per site in the Abrolhos so the data was pooled leaving only two populations analysed in Western Australia. We were more fortunate in Queensland and have samples from five populations of 30 individuals from Noosa, Hervey Bay, Yeppoon, Bustard Heads and Townsville. I have included an estimate of the distance separating the populations as well as the red double bars indicating the natural distribution of the species.

We used two types of statistical analysis to look for differences in the genetic makeup of the individual populations.

Queensland - AMOVA

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Between populations	4	5.401	-0.01164 Va	-0.57
Within populations	299	614.691	2.05582 Vb	100.57
Total	303	620.092	2.04419	

Fixation Index FST : -0.00569 P = 0.96990

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The first one I will present is an Analysis of Molecular Variance, which takes into account heterozygosity (levels of variation within individuals—two state—homo and hetero) as well as allele frequency (the number of types of microsatellites/DNA variants) and compares all the populations at once.

The results show that in Queensland there is no detectable variation between populations in the microsatellites that we have analysed here, suggesting that from Townsville to Noosa the saucer scallop is freely interbreeding (gene flow between populations is high).

Queensland - AMOVA

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Between populations	4	5.401	-0.01164 Va	-0.57
Within populations	299	614.691	2.05582 Vb	100.57
Total	303	620.092	2.04419	

Fixation Index FST : -0.00569 P = 0.96990

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We did the same with WA and cannot detect any significant genetic differences indicating gene flow between the populations.

Population structure

	Yep	Bust	Herv	Town	Noosa	Abrol
Yeppoon						
Bustard Head	×					
Hervey Bay	×	×				
Townsville	×	×	×			
Noosa	×	×	×	×		
Abrolhos	✓	✓	✓	✓	✓	
Shark Bay	✓	✓	✓	✓	✓	×

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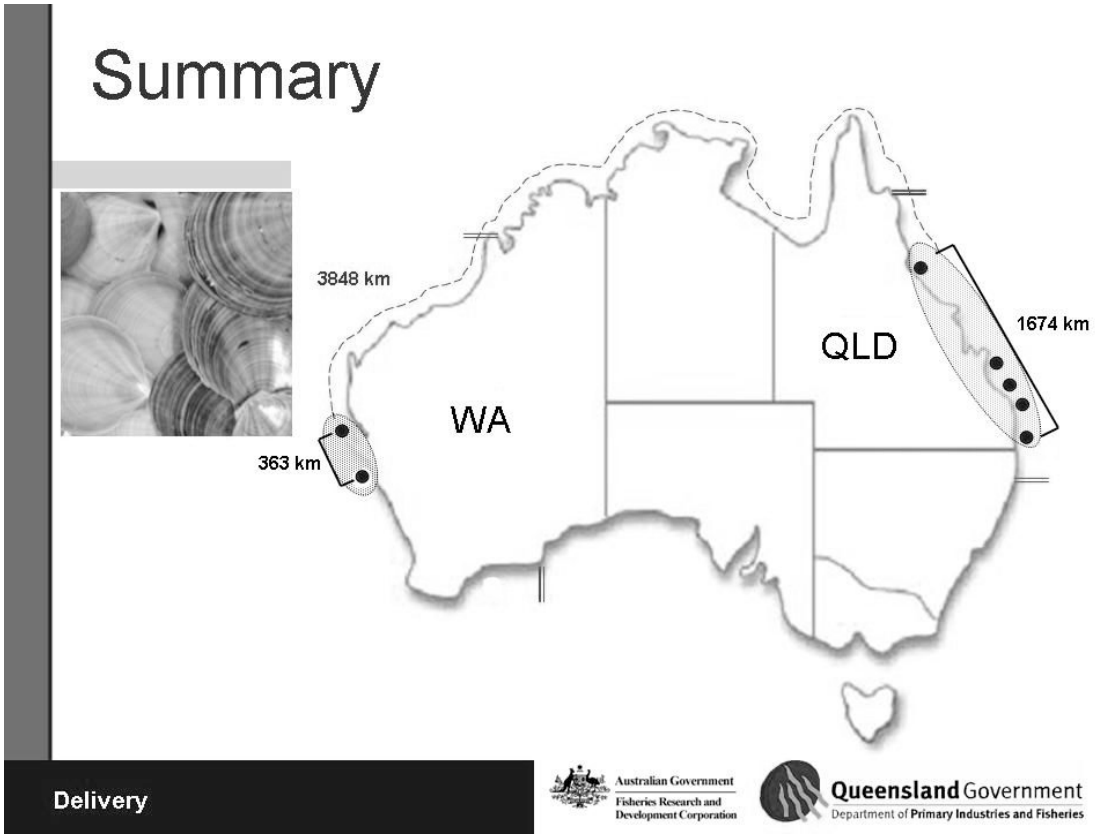
With the second type of statistical test, we looked at each population separately and compared them with the others based on the allele frequency in these populations.

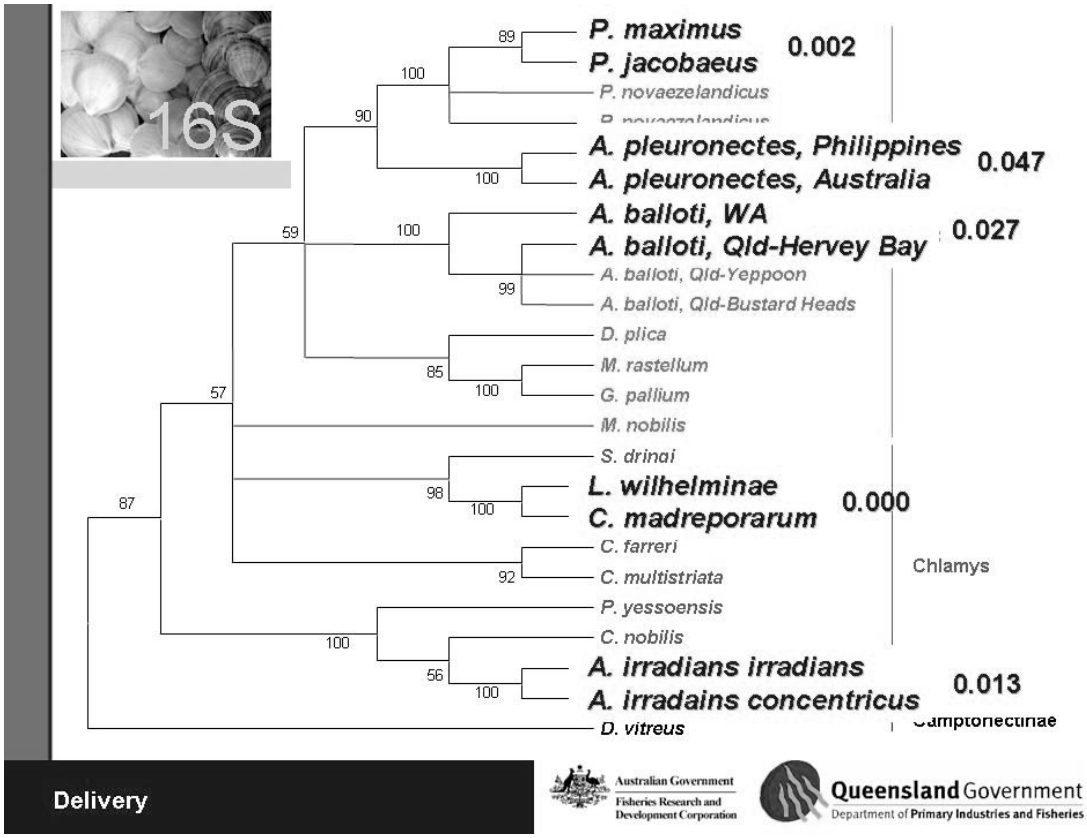
From this analysis it verifies that the populations in both Queensland and WA are interbreeding, but that when populations from the different states are compared they show significant genetic differences



So, between the populations we have surveyed using the microsatellites we have isolated we cannot detect any genetic difference between the populations in each state. This is not unexpected due to the long larval times and the current systems that operate in these states. Other studies looking at starfish, corals, pearl oysters and fish also show genetic mixing between populations over relatively large distances.

The differences that indicates separation of the populations of the two states was also not unexpected. The natural distribution is marked by the red bars and the populations do not show any overlap in distribution. They are also separated by a reproductively separate species, *Amusium pleuronectes*.

When we commenced this study, a previously unpublished study by Clive Keenan showed genetic separation of the two states and indicated that they may even have separated into different species. In a subsection of our study we used additional genetic markers to investigate this further.


16S
P. maximus
P. jacobaeus
0.002
P. novaezelandicus
A. pleuronectes, Philippines
A. pleuronectes, Australia
0.047
A. balloti, WA
A. balloti, Qld-Hervey Bay
0.027
A. balloti, Qld-Yeppoon
A. balloti, Qld-Bustard Heads
D. plica
M. rastellum
G. pallium
M. nobilis
S. drinai
L. wilhelminae
C. madreporarum
0.000
C. farreri
C. multistriata
Chlamys
P. yessoensis
C. nobilis
A. irradians irradians
A. irradains concentricus
0.013
D. vitreus
89
100
90
100
59
100
99
85
100
57
98
100
87
92
100
56
100
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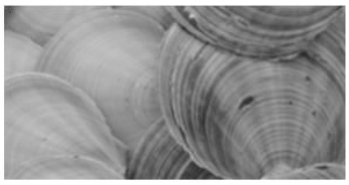
Recommendations



- Mixing along the Queensland and Western Australian coast
  - Genetic management of the hatchery
    - Broodstock source
    - Successful numbers of broodstock
  - Genetic management of reseeding
    - Monitor change in genetic variation

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Thank you

- **Queensland Sea Scallops Ltd.**
- **West Coast Scallops Pty. Ltd.**
- **Farwest Scallops Industries Pty. Ltd.**
- **Rick Scoones, Western Australia**
- **Carlos Saavedra, IATS, Spain**
- **James Williams, U. Aukland**
- **Emmanuel Goyard, IFREMER, New Cal.**
- **Clive Turnbull, DPI&F, Cairns**
- **Provan Crump, Jenny Ovendon, Damian Broderick, Martin Elphinstone, Dan Heath**
- **Alan Pearce, CSIRO**

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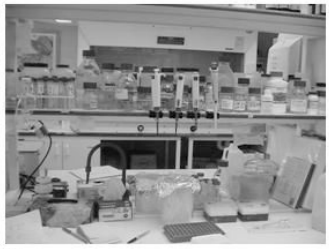
University of the Sunshine Coast Queensland, Australia



Bribie Island Aquaculture Research Centre

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