Evaluation of population genetic structure in the western rock lobster

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1.0 NON TECHNICAL SUMMARY

2009/020 Evaluation of population genetic structure in the western rock lobster

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OBJECTIVES:

1. Develop additional new microsatellite markers for western rock lobster.
2. Test whether the adult population of western rock lobster is genetically homogeneous throughout its range.
3. Test whether the spatial genetic structure in the next generation of recruits (pueruli) matches the spatial genetic structure found in adults.
4. Estimate effective population size of the western rock lobster and test for severe bottlenecks in population size.

OUCOMES ACHIEVED TO DATE

The key outcomes of this project management for fishery management will be firstly, the provision of reliable data to support our understanding of the extent of genetic mixing of western rock lobsters over its range. Our results indicate that there is genetic homogeneity in the western rock lobster resource in Western Australia and thus will increase managers' confidence in the accuracy of stock assessments in the species. Secondly, our research findings on the degree of spatio-temporal pattern of the genetics mixing is also critical for validating oceanographic models of larval mixing in the fishery, as well as for providing management with advice that will assist decisions if there is a need to improve locally depleted brood stocks. Thirdly, our data indicating that the species has good genetic health and has not passed through a bottleneck are useful for fisheries management now in that it promotes significant confidence that the fishery has the genetic potential to recover from recent decreases and that loss of genetic diversity is unlikely if current management practices and breeding stock sizes are maintained. Genetic bottlenecks are early warning signs of a fishery vulnerable to reduced productivity and loss of evolutionary potential. Our analyses of genetic health may also be useful in the future since the data provide a base line for any future

comparisons. Management responses will need to be evaluated if there is evidence of loss in genetic diversity from the current point. Commercial fishers will benefit from our research through a better understanding of the extent of mixing among localities and the spatio-temporal pattern of this mixing. This will allow the development of management plans that ensure locally depleted stocks are repopulated by connected populations.

One of the assumptions underlying current management of the western rock lobster fishery is that the breeding stock is comprised of a single, genetically homogeneous population. This assumption is based on the extended pelagic larval stage of western rock lobster, which is thought to ensure high dispersal throughout the species range, and previous population genetic analyses based on allozymes. The focus of this project was to test the validity of this assumption. A secondary aim was to estimate the effective number of breeding adults, and hence determine the vulnerability of the stock to loss of genetic variation. Loss of genetic variation is a concern because it is associated with declines in reproductive capacity and the evolutionary potential of the species. Several recent studies have also shown declines in genetic diversity in commercially exploited species, even though the census population sizes (the total number of individuals) were very high. To achieve these aims we first had to develop specially designed genetic markers that would allow the measurement of genetic similarities between individuals collected from different locations within the fishery or between individuals collected at different times from the same location.

We successfully developed 18 new genetic markers for the western rock lobster. Using these markers, and four additional markers that had been developed previously, we examined 631 individuals from eight locations in a survey of adult lobsters and 367 individuals from eight locations in the survey of new recruits. Our analyses revealed there were very small genetic differences between samples collected from different locations in the adults and no genetic differences between samples collected different locations in the new recruits. These results indicate that the breeding stock is a single population. By comparing new recruits collected in 2009 with those collected in the 1990s, we were also able to show that variation in the genetic markers has not changed over this time frame. These results suggest that the western rock lobster is in no danger of losing genetic diversity if current management practices and breeding stock sizes are maintained.

KEYWORDS: microsatellites, western rock lobster, *Panulirus cygnus,* genetic variation, population structure, effective population size, mtDNA.

2.0 ACKNOWLEDGMENTS

We thank Amanda Worth and Sherralee Lukehurst for technical assistance and Mark Todd from Genetic Identification Services for his helpful advice. We also thank the Western Australian Department of Fisheries and the Department of Environment and Conservation for providing *P. cygnus* tissue samples. Tissue samples of one adult each of *P. penicillatus* and *P. versicolor* were kindly provided by M.G. Kailis Pty Ltd, Cairns. We thank Carolyn Stewardson, Mary Webberley and an anonymous reviewer for helpful comments on an earlier draft of this report. The reviewer's feedback should improve this version of the report and publications in scientific journals arising from the research reported here. We are also grateful to the Fisheries Research Development Corporation for their financial support.

3.0 BACKGROUND

The western rock lobster (*Panulirus cygnus*) fishery began in the 1940s and has since grown to become the most valuable single species caught in Australia. Over the last 20 years the annual catch has averaged approximately 10,000 tonnes, varying between 8,000 and 14,000 tonnes per year due to natural variations in the level of recruitment, with no net decline over the period of harvesting.. The catch is worth $250-350 million a year in export revenue and the market value of the fishery has been estimated to be at least $2 billion (Fletcher et al. 2005).

The sustainability of the fishery has been attributed to management principles that have been in place since the 1970s. A general theme that runs across these principles is the need to protect the breeding stock at a level that prevents recruitment overfishing and ensuring that the appropriate data and management tools are available to achieve this goal.

One of the assumptions underlying the current management system is that the breeding stock comprises of a single, demographically united population. This assumption is based on the extended pelagic larval stage of western rock lobsters which is thought to ensure high dispersal throughout the species range. Larvae hatch in spring and early summer and spend the next nine to eleven months in the plankton, with mid stages being found up to 1500 km offshore (Phillips et al. 1979). Late stage larvae metamorphose into pueruli and swim inshore to start the juvenile stage of their life-cycle (Phillips et al. 1979).

The spatial extent of genetic structures is largely dependent on the dispersal capacity of individuals (Bohonak 1999). However, the role of role of dispersal capacity in structuring marine populations is complex and can be strongly influences by other factors, with impediments to dispersal not always obvious (see Appendix 5 for discussion). For commercially exploited species failure to detect underlying genetics structure is a concern because it may result in overexploitation and depletion of localized subpopulations, with a corresponding loss of genetic variation (Carvalho and Hauser 1994).

In the case of *P. cygnus*, the belief that such a long larval stage covering so vast an area creates a mixed common larval pool is supported by genetic data. Investigations of allozyme variation (variant forms of enzymes) in the 1990s revealed there were no significant differences in larvae caught over a wide area of the fishery (Johnson and Wernham 1999). Nevertheless, while collections of the same larval cohort show no genetic differences between geographically distinct sites, in one year temporal changes in allelic frequency were observed. This temporal variation, coupled with different larval recruitment patterns, led to ephemeral genetic patchiness resulting in genetically distinct cohorts of recruits at two sites

in the same season (Johnson and Wernham 1999). This phenomenon is of special interest because with sufficient genetic markers, and assuming genetic patchiness exists in adult life stages, it may be possible to genetically track larvae from different sub-populations and hence determine the contributions of different geographical regions to the fishery and how this varies annually.

These earlier studies conducted in the 1990s, were based on analysis using relatively few loci, and may therefore have lacked the resolving power needed to detect subtle genetic differences. More recently, molecular techniques have been developed that allow much more subtle genetic differences to be detected within species. Microsatellites (small repeating units of DNA), in particular, are ideal for identifying genetic structure due to their high rate of mutation. Indeed, they have proven to be a powerful tool for detecting genetic subdivision within marine species despite high larval dispersal capabilities. For example, microsatellite analyses facilitated detection of small-scale temporal and spatial genetic structure in larval cod (*Gadus morhua*), which was undetected in earlier studies using allozymes (Ruzzante et al. 1996). Microsatellite data can also be used for determining the effective population size (the number of individuals contributing gametes to the next generation), which is usually much lower than the number of adults within a population (Allendorf and Luikart 2006), and for detecting changes in population size. Low effective population sizes and loss of genetic diversity are a major concern for exploited marine stocks because they can result in reduced adaptability, population persistence, and productivity (Hauser et al. 2003).

New genetic techniques can therefore uncover previously unknown spatial genetic structure and provide information essential for the future management of exploited marine species.

4.0 NEED

The western rock lobster fishery is considered to be sustainably managed (Phillips et al. 1979), and was the first in the world to be certified by the Marine Stewardship Council as being an environmentally sustainable fishery. Nevertheless, over the past 35 years the size at maturity has decreased, the abundance of undersized and legal-sized lobsters in deep water relative to shallow water has increased, and there have been shifts in the catch to deep water, possibly due to rising water temperatures associated with climate change (Caputi et al. 2010).

Preliminary investigations suggested that the western rock lobster breeding stock comprised of a single, demographically united and genetically homogeneous population. This is one of the key assumptions that underlie the current management system. However, this has yet to be rigorously tested with a highly sensitive genetic technique such as microsatellite DNA markers. Such an analysis may also help elucidate the reasons for the recent changes in western rock lobster demography and that would aid management.

A small number of microsatellite markers have recently been developed for paternity testing in *P. cygnus* (Groth et al. 2009). However, many additional loci are needed to allow a rigorous analysis of the spatial genetic structure of the western rock lobster fishery. To achieve a resolution beyond the previous genetic studies (Thompson et al. 1996; Johnson and Wenham 1999), we developed 18 new microsatellite markers and used 22 microsatellite loci for our study.

Evidence of spatial genetic structure would have significant implications for stock management. Temporally stable genetic differences between locations (i.e. the same spatial genetic patterns repeated across different generations) would indicate restricted mixing of adults and larvae, challenging the idea of a single population and suggesting that regional regulation would be more appropriate. Similarly, temporally unstable or ephemeral population structure, which has been found previously with allozymes (and which is the more likely scenario), also has important implications. While ephemeral patterns are consistent with extensive mixing of the larvae (because larval mixing breaks down structuring that may have established through random processes), it raises the possibility of genetic tracking of larvae from different sub-populations. These data will greatly improve our understanding of recruitment dynamics in western rock lobsters and help with future management. For example, it may be that some regions contribute disproportionally more recruits than others, in which case measures could be put in place to ensure their protection.

Lastly, relatively little is known about the genetic health and evolutionary potential of the fishery. Small effective population sizes and the associated declines in genetic diversity,

such as those recently seen in exploited fish populations (e.g. New Zealand snapper), provide early warning signals of a fishery vulnerable to reduced productivity and loss of an ability to adapt to environmental changes. The genetic data collected in this project will be used to estimate the effective population size of western rock lobsters and test for genetic bottlenecks. This will not only provide important information on the current situation, but it will also provide vital base line data for assessing the future impacts of climate change and fishing on genetic diversity and adaptation.

5.0 OBJECTIVES

1. Develop additional new microsatellite markers for western rock lobster.
2. Test whether the adult population of western rock lobster is genetically homogeneous throughout its range.
3. Test whether the spatial genetic structure in the next generation of recruits (pueruli) matches the spatial genetic structure found in adults. (If so, this suggests spatial structure is due to limited dispersal or local adaptation).
4. Estimate effective population size of the western rock lobster and test for severe bottlenecks in population size.

5.1 Reporting format

This report is structured using the standard FRDC format. The project involved tackling four different objectives (Section 5.0) and naturally fell into three fairly discrete research sub-projects. The first two sub-projects addressed objectives one and two. Objectives three and four were addressed concurrently. The same samples were used to produce the raw data for use in addressing these two objectives, with different analyses used to answer the different key questions. The methods and results and discussion for each of the three sub-projects are provided below.

When preparing the research for publication in peer-reviewed scientific journals, the results were presented in the form of three papers, each one covering one of the three sub-projects. The papers include much more background information than the main report. They are attached here as three appendices, and there are references to them at relevant points in the main report. Appendix 3 has been accepted for publication, Appendix 4 is currently in review and Appendix 5 is in the process of being submitted for publication in peer-reviewed scientific journals. The way the appendices relate to the project objectives (Section 5.0) is outlined below.

Appendix 3 is entitled 'Characterization of 18 polymorphic microsatellite loci for the western rock lobster *Panulirus cygnus*'. It fully addresses Objective 1 (to develop new microsatellite markers for western rock lobster).

Appendix 4 is entitled 'Fine-scale genetic heterogeneity, but no geographic structure in the western rock lobster (*Panulirus cygnus*)'. It fully addresses Objective 2 (to test whether the adult population of western rock lobster is genetically homogeneous throughout its range).

Appendix 5 is entitled 'Maintenance of genetic variation and panmixia in the commercially exploited western rock lobster (*Panulirus cygnus*)'. It fully addresses Objectives 3 and 4 (to test whether the spatial genetic structure in the next generation of recruits (pueruli) matches the spatial genetic structure found in adults, estimates effective population sizes and tests for severe bottlenecks in population size).

6.0 METHODS

6.1 Characterisation of 18 polymorphic microsatellite loci (Objective 1)

Microsatellites were isolated from a *P. cygnus* DNA library created by Genetic Information Services (Chatsworth, California). Methods for the DNA library construction and enrichment followed those described in Jones et al. (2002). Genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa* I, *Hae* III, *Bsr* B1, *Pvu* II, *Stu* I, *Sca* I, *Eco* RV). Fragments in the size range of 300 to 750 bp were adapted and subjected to magnetic bead capture (CPG, Inc., Lincoln Park, New Jersey), using biotinylated capture molecules. Four libraries were prepared in parallel, using Biotin-$CA_{(15)}$, Biotin-$AAC_{(12)}$, Biotin-$AAAG_{(8)}$ and Biotin-$TAGA_{(8)}$ as capture molecules in a protocol provided by the manufacturer. Captured molecules were amplified and restricted with *Hind*III to remove the adapters. The resulting fragments were ligated into the *Hind*III site of pUC19. Recombinant molecules were electroporated into *E. coli* DH5alpha. Recombinant clones were selected at random for sequencing, and enrichment levels were expressed as the fraction of sequences that contained a microsatellite. Sequences were obtained on an ABI 377, using ABI Prism *Taq* dye terminator cycle sequencing methodology. PCR primers were developed for 49 microsatellite-containing clones, using the DESIGNERPCR version 1.03 (Research Genetics, Inc.) software package.

Genomic DNA for PCR was extracted from a 5-mm × 5-mm piece of tissue from the middle lobe of the tail fan, using a QIAGEN DNeasy Blood and Tissue Kit. PCR reactions of 13µL contained 10 ng of DNA, 1× reaction buffer (Invitrogen's Platinum PCR SuperMix: 22 U/mL recombinant *Taq* DNA polymerase with Platinum *Taq* antibody, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM $MgCl_2$, 220 µM dGTP, 220 µM dATP, 220 µM dTTP, 220 µM dCTP and stabilizers) and 1.3–2.3 µM of each primer (forward primer fluorescent-labelled). PCR amplifications were carried out in an Eppendorf thermal cycler and consisted of an initial denaturation at 94 °C for 3 min, then 35 cycles of 40 s at 94 °C, 40 s at the annealing temperature (see Table 7.1) and 40 s at 72 °C, followed with a final elongation step at 72 °C for 5 min. PCR products (1.5 µL) were analyzed on an ABI 3730 Sequencer, sized using GeneScan-500 LIZ internal size standard and scored using GENEMAPPER version 3.7 (Applied Biosystems) software.

Levels of genetic diversity were assessed by genotyping 37 adult *P. cygnus* collected from Lancelin in Western Australia (30° 59.2' S, 115° 17.2' E). The online version of GENEPOP version 3.4 (Raymond and Rousset 1995) was used to calculate basic descriptive

statistics and test for significant deviations from Hardy-Weinberg expectations (HWE) and linkage disequilibrium between all pairs of loci. Markov chain parameters for both the HWE and linkage disequilibrium exact tests were: 1000 dememorization steps, 100 batches and 1000 iterations per batch. Significance levels were adjusted to the number of simultaneous tests using sequential Bonferroni correction (Rice 1989).

6.2 Tests for genetic homogeneity in adult *Panulirus cygnus* across its geographic range (Objective 2)

6.2.1 *Sample collection*

In 2009, tissue samples were collected from juvenile and adult *P. cygnus* (carapace length > 45 mm) at eight locations spanning nearly 660 km along the Western Australian coastline (Figure 6.1). A total of 631 individuals were captured using commercial lobster pots. Sample sizes at each location ranged between 21 and 348 individuals, with more intensive sampling carried out at the Houtman Abrolhos Islands to allow for the investigation of fine-scale patterns (sample sizes ranged between 40 to 68 individuals). The spatial coordinates for each individual were recorded at the time of capture.

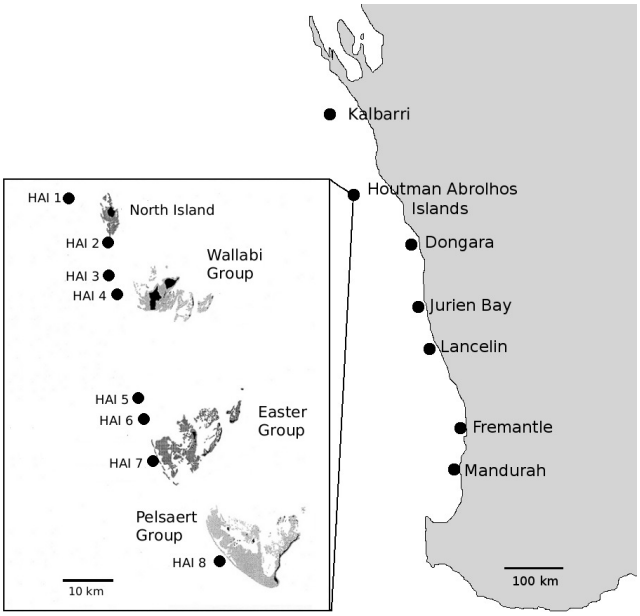


Figure 6.1 Sites where *P. cygnus* samples were collected.

6.2.2 *DNA extraction and microsatellite genotyping*

DNA was extracted from the middle lobe of the tail fan stored in 100% ethanol using a QIAGEN Dneasy Blood and Tissue kit, following the manufacturer's recommendations. After the DNA was extracted, each sample was analysed using a NanoDrop ND-1000 spectrophotometer to determine the concentration and quality of the DNA. All DNA samples were stored at –20 ºC until genotyping. Genotypes at 22 microsatellite loci (S3, S8, S28, S50, W25, *Pcyg*1 – 9 and 11– 18) were determined for each individual using primers and PCR running conditions described in Groth et al. (2009) and in section 6.1 above (published in Kennington et al. (2010), which is provided here in Appendix 3). PCR products were analyzed on an ABI 3700 sequencer using a GeneScan-500 LIZ internal size standard and scored using GENEMARKER (SoftGenetics, State College, PA, USA) software.

6.2.3 *Data analysis*

Microsatellite variation at each location was quantified by calculating allelic richness (a measure of the number of alleles independent of sample size) and gene diversity using the FSTAT version 2.9.3 software package (Goudet 2001). Tests for a deficit or excess in heterozygotes at each location were carried out using randomisation tests, and the results were characterized using the F_{IS} statistic. Significantly positive F_{IS} values indicate a deficit of heterozygotes relative to random mating or inbreeding and negative values indicate an excess of heterozygotes. Linkage disequilibrium between each pair of loci was assessed by testing the significance of association between genotypes. Genetic differentiation among sites was assessed by calculating Weir and Cockerham's (1984) estimator of F_{ST}. Microsatellite R_{ST} values (Slatkin 1995) were also calculated, but were qualitatively similar to F_{ST} values so were not reported. Tests for genetic differentiation were performed by permuting genotypes among samples. Estimates of F_{IS}, F_{ST} and tests for genetic differentiation (without assuming Hardy-Weinberg equilibrium), deficits in heterozygotes and linkage disequilibrium were calculated using the FSTAT software package. Differences in estimates of genetic variation and F_{IS} among locations were tested using either Wilcoxon's signed-rank tests (for paired comparisons between two groups) or Friedman's ANOVA (for multiple paired comparisons). To test for a relationship between genetic and geographical distance, we compared an F_{ST} /(1 – F_{ST}) matrix with a geographical distance matrix (ln km) (Rousset 1997), using a Mantel test (10 000 permutations).

Spatial genetic structure was also investigated using two Bayesian clustering methods, implemented with the software packages STRUCTURE (Pritchard et al. 2000) and GENELAND (Guillot et al. 2005). Both these programs group individuals into the most likely number of clusters (*K*) that maximizes the within cluster Hardy-Weinberg and linkage equilibria. However, GENELAND differs from STRUCTURE in that geographical information can be incorporated to produce more accurate inferences of population structure based on the spatial distribution of individuals. Analyses involving STRUCTURE were based on an ancestry model that assumed admixture and correlated allele frequencies. No prior information about the origin of the samples was used. Ten independent runs were performed for each value of *K* (1–10) with a burnin of 10 000 followed by 100 000 MCMC iterations. The most likely number of clusters was assessed by comparing the likelihood of the data for different values of *K* and using the ΔK method of Evanno et al. (2005). For the GENELAND analysis, the spatial coordinates (latitude and longitude) of each individual were used to run the spatial model. The uncertainty of coordinates was set at zero. Ten independent runs were performed for each value of *K* (1–10) using the uncorrelated and null allele models. Each run consisted of 100 000 MCMC iterations with a thinning of 100 and a burnin of 200. The most likely number of clusters was chosen as the modal *K* (from each independent run) with the highest posterior probability.

We also carried out spatial autocorrelation (SA) analysis to evaluate the genetic similarity of individuals over varying spatial scales. We used the software package GENALEX version 6 (Peakall and Smouse 2005) to calculate a spatial autocorrelation (*r*) coefficient for a range of distance classes. The results from the SA analysis were presented in two ways. Firstly, *r* was plotted as a function of distance class to produce a spatial genetic autocorrelogram. Secondly, because estimates of spatial autocorrelation are influenced by the size of distance classes (see Peakall et al. 2003), *r* was calculated for series of increasing distance class sizes. When significant positive spatial structure is present, *r* will decrease with increasing distance class sizes. The distance class where *r* no longer differs significantly from zero provides an approximation of the extent of detectable positive spatial genetic structure (Peakall et al. 2003). Tests for statistical significance were performed by random permutation and calculating the bootstrap 95% confidence limits (CL) of *r*, using 1000 replicates in each case. We also performed a two-dimensional local spatial autocorrelation analysis using GENALEX. With this analysis, the local autocorrelation (*lr*) is estimated by comparing an individual with its *n* nearest neighbours, allowing investigation of local patterns of spatial autocorrelation within the two dimensional landscape (Double et al. 2005). Calculations of *lr*

were made using the nearest five, 10, 20 and 50 individuals. As with the global autocorrelation analysis, statistical significance was determined using permutation tests.

Finally, tests for selection acting on marker loci were carried out using the F_{ST} outlier approach (Beaumont and Nichols 1996; Beaumont 2005), implemented with the LOSITAN software package (Antoa et al. 2008). The method evaluates the relationship between F_{ST} and expected heterozygosity in an island model of migration with neutral markers. This distribution is used to identify loci with excessively high or low F_{ST} values compared to neutral expectations. These loci are candidates for being subject to directional and balancing selection respectively. Simulations were run using 10 000 replications, 99% confidence intervals and the neutral and forced mean options. Analyses using both the stepwise and infinite allele mutation models were performed.

6.3 Comparing the spatial genetic structure of recruits with that of adults, estimating effective population size and testing for bottlenecks (Objectives 3 & 4)

6.3.1 *Sample collection*

Collections of *P. cygnus* pueruli were provided by the Western Australian Department of Fisheries, which monitors monthly settlement at different sites along the coast to provide a quantitative index of recruitment (Morgan et al. 1982a). Each monitoring location contains several collectors that are sampled over the full moon during the settlement season. After collection the samples were stored in 100% ethanol. Sampling locations used in this study are shown in Figure 6.2. All sampling locations except Coral Bay are within the main *P. cygnus* fishery, except for the two northerly locations of Quobba and Coral Bay.

A total of 365 puerulus were collected in 2009. Of these 130 (Coral Bay, n = 44; Port Gregory, n = 18; Rat Island, n = 18; Seven Mile Beach, n = 28; Jurien Bay, n = 16 and Alkimos, n = 6) were used for mtDNA sequencing, and 277 (Coral Bay, n = 126; Quobba, n = 40; Port Gregory, n = 24; Rat Island, n = 25; Seven Mile Beach, n = 37; Jurien Bay, n = 16; Lancelin, n =3 and Alkimos, n = 6) for microsatellite analysis. The archived material included samples of pueruli from 1995 (n = 40), 1997 (n = 40) and 1999 (n = 49). The 1995 and 1997 samples were from the Seven Mile Beach site only. The 1999 samples came from Rat Island (n = 22), Seven Mile Beach (n = 20) and Alkimos (n = 7). Tissue samples of one adult each of *P. penicillatus* and *P. versicolor* were kindly provided by M.G. Kailis Pty Ltd, Cairns. They were included in the study to quantify species level divergences.

6.3.2 *DNA extraction*

DNA was extracted from the antennae of individual pueruli (both contemporary and archived) with a QIAGEN Dneasy Blood and Tissue kit using the manufacturers protocol with the following alterations. Volumes of Buffer ATL and proteinase K were doubled, and samples incubated overnight at 56ºC. Volumes of Buffer AL and 100% ethanol were also doubled, and DNA was eluted using two aliquots of 20 µL of AE buffer heated to 56 ºC and incubated for 10 minutes at room temperature before centrifuging. After DNA was extracted, each sample was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific) and stored at –20 ºC until it was used.

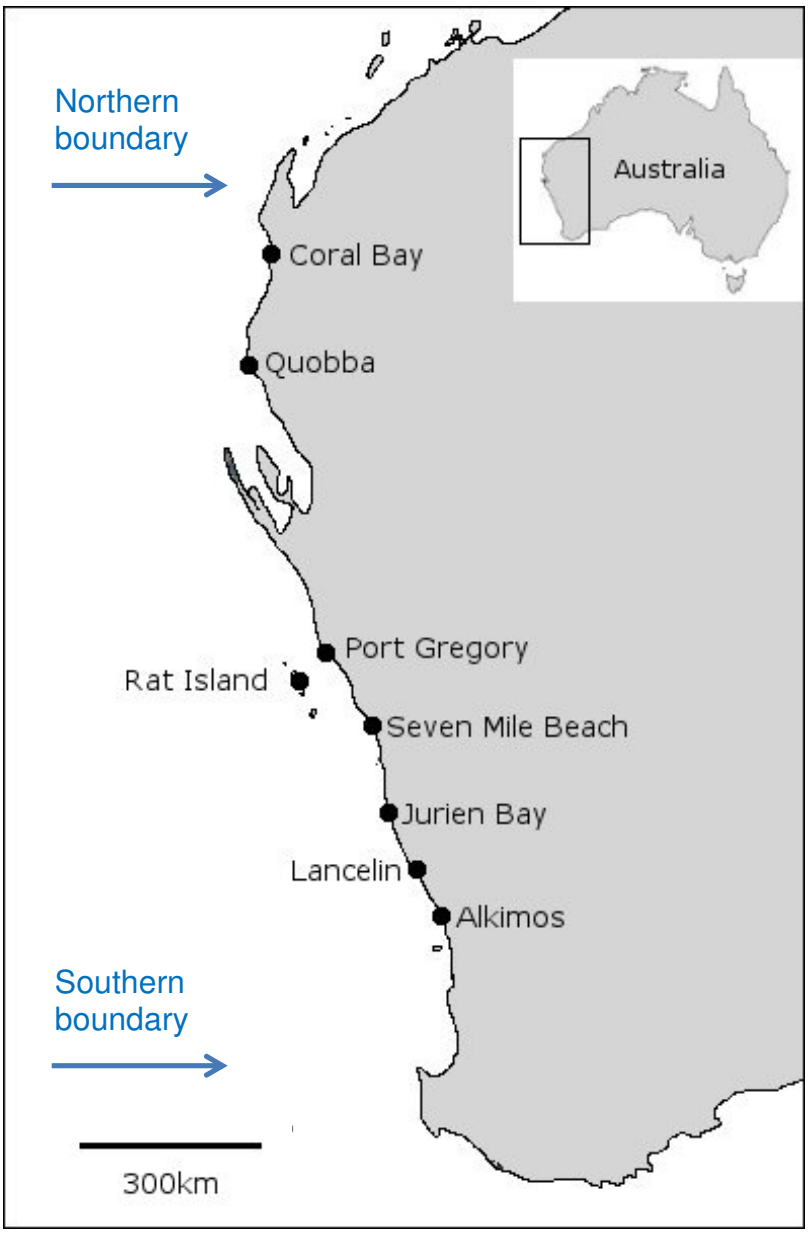


Figure 6.2 Puerulus sampling locations used in this study. Arrows mark the approximate boundaries of the main fishery.

6.3.3 *Mitochondrial DNA sequencing*

A 355-bp portion of the mitochondrial 12S rRNA gene was amplified using the primers 12S-R-J-14199 (Kambhampati and Smith 1995) and 12Sai (SR-N-14588) (Simon et al. 1994). It was chosen because it exhibits high levels of intraspecific variation and amplified consistently using DNA extracted from contemporary and archived samples. Each 25 μL PCR reaction contained 4 mM $MgCl_2$ (Fisher Biotec), 1x Reaction Buffer (Fisher Biotec), 0.2 mM dNTPs (Fisher Biotec), 1.2 μM forward primer, 1.2 μM reverse primer, 55 units of Taq (Fisher Biotec's Tth Plus DNA Polymerase) and 40 ng DNA. Cycling conditions were: an initial denaturation step of 94 ºC for 2 min, followed by 36 cycles of 94 ºC for 30 s, 40 ºC for 90 s and 72 ºC for 60 s, followed by 72 ºC for 10 min. PCR products were purified using an AxyPrep PCR clean up kit (www.axygenbio.com). PCR sequencing reactions were performed in a total volume of 10 μL, and contained 1 μL Big Dye-Terminator (Applied Biosystems), 0.75× sequencing buffer (Applied Biosystems), 0.32 pmol primer and 10–30ng of cleaned PCR product. Sequencing reactions were carried out using the following cycling conditions: 96°C for 2 min, followed by 25 cycles of 96 °C for 10 s; 55 °C for 5 s and 60 °C for 4 min. Products were sequenced on an ABI 3700 sequencer, edited using SEQUENCHER (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned with CLUSTAL W (Thompson et al. 1997) using default parameters.

6.3.4 *Microsatellite genotyping*

Genotypes at 20 microsatellite loci (S3, S8, S28, S50, W25, Pcyg1– 4, 7– 9, 11– 15 and 17–18) were determined for each individual using primers and PCR running conditions described in Groth et al. (2009) and in section 6.1 above (published in Kennington et al. (2010), which is provided here in Appendix 3). PCR products were analyzed on an ABI 3700 sequencer using a GeneScan-500 LIZ internal size standard and scored using GENEMARKER (SoftGenetics, State College, PA, USA) software. Re-amplification and scoring of 24 randomly selected individuals from the contemporary (2009) sample and 24 from the historical (1995) sample confirmed that genotype scores were highly repeatable. The mean error rate per allele (the number of allelic mismatches divided by the number of replicated alleles) was 0.037 and 0.027 in the contemporary and historical samples respectively.

6.3.5 *Data analysis*

Mitochondrial DNA (mtDNA) variation within each sample was quantified by calculating the number of haplotypes, haplotype (gene) diversity and nucleotide diversity using the ARLEQUIN version 3 software package (Excoffier et al. 2005). We also used ARLEQUIN to calculate the summary statistics D (Tajima 1989) and F_S (Fu 1997) and the distribution of pairwise nucleotide differences between sequences (mismatch distribution) to test the deviation of the observed data from neutral predictions expected in an historically constant-sized population. The dates of population expansion were estimated using the formula $T = \tau/2\mu$ (Harpending 1994), where T is the time in generations when the population expansion begins and μ is the specific fragment mutation rate. The mutation rate was calculated using a nucleotide divergence rate estimated for arthropod mitochondrial genes (2.3% per million years, Brower 1994) and allowing for an intraspecific mutation rate that is expected to be 3–10 times faster than the interspecific divergence rate (Emerson 2007).

A measure of the number of haplotypes corrected for sample size was obtained by randomly selecting the minimum sample size from each sample (with replacement) and calculating the number of haplotypes. This was repeated 1000 times for each sample, from which a mean and 95% confidence limits (CLs) were calculated. Evolutionary relationships among mtDNA haplotypes were inferred by neighbour-joining analysis of maximum composite likelihood distance estimates (Tamura et al. 2004) using MEGA version 5 software (Tamura et al. 2007). Sequences from *P. penicillatus* and *P. versicolor* were used for interspecies comparisons.

Microsatellite variation within each sample was quantified by calculating allelic richness (a measure of the number of alleles independent of sample size) and gene diversity using the FSTAT version 2.9.3 software package (Goudet 2001). The presence of null alleles was tested for each locus using MICROCHECKER (van Oosterhout et al. 2004). Tests for a deficit or excess of heterozygotes within each sample were carried out using randomisation tests, and the results were characterized using the F_{IS} statistic. Significantly positive F_{IS} values indicate a deficit of heterozygotes relative to random mating and negative values indicate an excess of heterozygotes. Linkage disequilibrium between each pair of loci was assessed by testing the significance of association between genotypes. Genetic differentiation between samples were assessed by calculating Weir and Cockerham's (1984) estimator of F_{ST}. Microsatellite R_{ST} values (Slatkin 1995) were also calculated, but were qualitatively similar to F_{ST} values so were not reported. Estimates of F_{IS}, F_{ST}, tests for deficits in heterozygotes and linkage disequilibrium were calculated using the FSTAT software package. Tests for genetic

differentiation among samples were conducted using Analysis of Molecular Variation (AMOVA) with ARLEQUIN. Differences in estimates of genetic variation and F_{IS} among samples were tested using either Wilcoxon's signed-rank tests or Friedman's ANOVAs. Regression was used to test for declines in genetic variation over time. All statistical analyses were based on samples with at least 15 individuals.

Spatial and temporal genetic structure was also investigated using the Bayesian method of Pritchard et al. (2000) and Falush et al. (2003) implemented with the program STRUCTURE. This method identifies genetically distinct clusters (K) based on allele frequencies across loci. All analyses were based on an ancestry model that assumed admixture and correlated allele frequencies. No prior information about the origin of the samples was used. Ten independent runs were performed for each value of K (1–10) with a burnin of 10 000 followed by 100 000 MCMC iterations. The most likely number of clusters was assessed by comparing the likelihood of the data for different values of K and using the ΔK method of Evanno et al. (2005). We also tested for recent reductions in effective population size (i.e. over contemporary timescales) in each sample using the software package BOTTLENECK (Piry et al. 1999). Two different methods were used. The first method was based on the principle that that the number of alleles decreases faster than expected heterozygosity after a bottleneck (Maruyama and Fuerst 1985). In this situation, expected heterozygosity should be higher than the equilibrium heterozygosity predicted in a stable population from the observed number of alleles. Following the authors' recommendation for microsatellite data, we used a two-phase model (TPM) with 95% single-step mutation and 5% multiple-step mutations (and a variance among multiple steps of 12). A Wilcoxon signed rank test was used to determine whether each sample had a significant excess of heterozygosity. The second method was a qualitative test based on allele frequency distributions. This test can discriminate between a sample exhibiting a full range of common and rare alleles (producing a typical, L-shaped distribution) and one that has lost rare alleles (producing a shifted distribution), which is indicative of a bottleneck event (Luikart et al. 1998).

Estimates of contemporary effective population size (N_e) were calculated using a single sample (Waples 2006) and a temporal method (Wang and Whitlock 2003). These analyses were carried out using the LDNE (Waples and Do 2008) and MLNE 4 (distributed by J. Wang, http://www.zoo.cam.ac.uk/ioz/software.htm#MLNE) software programs respectively. Estimates of N_e using the one sample method were performed on all samples and using three default cutoff values for minimum allele frequency: Pcrit = 0.05, 0.02 and 0.01. For the temporal method, we used the 1995, 1999 and 2009 samples in a single analysis. Based on

the age of reproductive maturity (4.9 –5.7 years, Chittleborough 1974) and the time elapsed between the temporal samples, the number of generations between the 1995 and 1999 samples was set to one and the number of generations between the 1995 and 2009 samples was set to two. The maximum N_e was set to 10 000, and a single closed population was assumed.

7.0 RESULTS/DISCUSSION

7.1 Characterisation of 18 polymorphic microsatellite loci (Objective 1)

Of the 24 microsatellite-containing clones for which PCR primers were developed, 18 produced consistent polymorphic genotypes within the expected size range. The number of alleles at these loci ranged from three to 31, and the observed and expected heterozygosities ranged between 0.056 and 0.972 and between 0.055 and 0.953, respectively (Table 7.1). Seven loci (Pcyg02, Pcyg06, Pcyg07, Pcyg10, Pcyg13, Pcyg16 and Pcyg17) deviated significantly from Hardy-Weinberg expectations after correction for multiple tests. These loci were estimated to have frequencies of null allele ranging from 0.20 (Pcyg06) to 0.49 (Pcyg13), using the CERVUS version 3.0.3 software package (Kalinowski et al. 2007). There was no evidence of linkage disequilibrium between any pair of loci.

Table 7.1 Primer sequences, repeat motifs, annealing temperatures and levels of diversity at 18 microsatellite loci in the western rock lobster *Panulirus cygnus* from Lancelin, Western Australia (n = 37). T_a, annealing temperature (°C); H_O, observed heterozygosity; H_E, expected heterozygosity; P, probability value from Hardy-Weinberg test

Locus	Primer sequence (5'-3')	Repeat motif	T_a	Number of alleles	Size range (bp)	H_O	H_E	P	GenBank Accession no.
Pcyg01	F: GCTTCTTATTGCTCCGAAATG R: GGGCTCTCTATCTTCAACTCG	$(GT)_{26}$	58	15	281-330	0.917	0.876	0.803	HM190199
Pcyg02	F: AGGTTGACAGAACACCAAGAG R: GGATAACATCAGCACCATCTC	$(GT)_{15}$	57	22	160-227	0.306	0.934	0	HM190200
Pcyg03	F: GACCCCTTCATCACACACC R: GCAGGCAGTGGGAGAAAC	$(CA)_{10}$	58	3	280-288	0.194	0.18	1	HM190201
Pcyg04	F: CTTTCCGATTGTTTGGTGAG R: GTGGTCTGGCGTAACCTCTA	$(TTG)_7$	58	14	279-325	0.800	0.885	0.158	HM190202
Pcyg05	F: AACGGTTCGTATAATTTGGAC R: CCATCACTCAAACAAAGACAG	$(TTG)_{10}$	57	8	232-259	0.696	0.758	0.176	HM190203
Pcyg06	F: GTTTTCTCTATCCGGGAACTG R: AGGTGGGAAAGCTGTTGTAGT	$(AAC)_2AGC(AAC)_3AAT(AAC)_3AATCAC(AAC)_4$	57	22	176-256	0.618	0.944	0	HM190204
Pcyg07	F: ACCTTGAGAGAGACATGAACC R: CCTTGTGTATGAGACTGGATG	$(AAC)_9(AAT)_5$	57	8	254-281	0.318	0.856	0	HM190205

Pcyg08	F: GAGTGCGATGTGTTGATGGT R: GGGAGTGTTAAACGAATGAGC	$(TTG)_7$	58	24	232-304	0.972	0.953	0.589	HM190206
Pcyg09	F: GGACCTGTTGAGGATACTGTAG R: TTGTGTTGTGAGGATGTTAGTC	$(AAC)_7$	56	4	155-164	0.364	0.418	0.653	HM190207
Pcyg10	F: TGGGACAGATCAGGCAAAC R: CCGAGTATCGAGGAGGGAG	$(TTTC)_5$	58	31	137-346	0.471	0.971	0	HM190208
Pcyg11	F: CCGTTACGAAGGTTCACTTA R: AAATGCACCTAGAGTAAAGCAG	$(TTTC)_4TCC(TTTC)_5$	56	6	164-244	0.735	0.688	0.592	HM190209
Pcyg12	F: ATTGCTTGATGAGAATGATGTG R: CATGGTTAAGGACACCTGAAA	$(AAAG)_7$	57	6	164-244	0.905	0.931	0.307	HM190210
Pcyg13	F: TTTGTGTGTCGTGATTTCATC R: ATCCCATAAAGAGAGGGTGAC	$(TAGA)_4TATT(TAGA)_7$	57	17	203-290	0.310	0.929	0	HM190211
Pcyg14	F: ACGAAGGAATGAATAGTGAATG R: TTCTGCAACATAACGAGGTC	$(ATCT)_2ATCC(ATCT)_5GTCT(ATCT)_5$	57	7	244-259	0.469	0.741	0.018	HM190212
Pcyg15	F: GTGGTTGCTTACCGCATAC R: TGTTTCCTTGTTCAGTCATTG	$(TAGA)_8$	56	3	112-124	0.676	0.607	0.796	HM190213
Pcyg16	F: GACCCCGTTCTTCGTAACT R: ACCCACCTGTCTTGCTATG	$(ATCT)_5TT(ATCT)_5AT(ATCT)_5AT(ATCT)_7$	57	14	206-301	0.333	0.903	0	HM190214
Pcyg17	F: CCCTGACGATGATACAGCC	$(TAGA)_2CAGA$	58	11	207-310	0.324	0.908	0	HM190215

	R: TGGAGTCTTGTCTTGTCTGGTC	$(TAGA)_4$							
Pcyg18	F: CGGGACCATCAAATTACC	$(ATCT)_5$	57	3	201-215	0.056	0.055	1	HM190216
	R: GCAATGGCAAATAACCATAC								

7.2 Tests for genetic homogeneity in adult *Panulirus cygnus* across its geographic range (Objective 2)

7.2.1 *Results*

High levels of genetic diversity were present at each site (collection location). Mean allelic richness ranged from 11.3 to 12.3 and gene diversity ranged from 0.75 to 0.77 (Table 7.2). There was a significant difference in allelic richness among sites ($\chi^2 = 14.62$, $P = 0.041$), but not in gene diversity ($\chi^2 = 4.30$, $P = 0.745$). Pairwise tests revealed that the significant differences in allelic richness were between Fremantle and all other sites except Kalbarri and Mandurah and between Kalbarri and the Abrolhos Islands (Wilcoxon tests, $P < 0.05$ in all cases). These differences indicate lower genetic diversity in sites at the margins of the main fishery compared to those that were more centrally located. Significant departures from Hardy-Weinberg Equilibrium (HWE) were detected in all samples, with positive F_{IS} values indicating heterozygote deficiencies (Table 7.2). The departures from HWE appear to be at least partially due to null alleles. Thirteen loci (S3, S8, S50, Pcyg02, Pcyg04, Pcyg06, Pcyg07, Pcyg09, Pcyg12, Pcyg13, Pcyg14, Pcyg16 and Pcyg17) were identified as having null alleles using MICROCHECKER. When these loci were removed from the analysis, no significant departures from HWE were found. No significant differences in F_{IS} were evident among sites ($\chi^2 = 10.19$, $P = 0.178$). There was also no significant genotypic disequilibrium between pairs of loci after adjusting for multiple comparisons.

Table 7.2 Genetic variation at each location.

Site	Mean sample size	A_R (SE)	H (SE)	F_{IS}
Kalbarri	38.1 (0.5)	11.8 (1.5)	0.75 (0.06)	0.24^{***}
Houtman Abrolhos Is.	324.1 (5.4)	12.3 (1.5)	0.76 (0.06)	0.25^{***}
Dongara	37.2 (0.5)	12.0 (1.5)	0.77 (0.06)	0.21^{***}
Jurien Bay	37.3 (0.6)	11.9 (1.5)	0.75 (0.06)	0.21^{***}
Lancelin	51.8 (0.9)	12.2 (1.5)	0.76 (0.06)	0.28^{***}
Rottnest	59.1 (1.5)	12.3 (1.5)	0.76 (0.06)	0.26^{***}
Fremantle	20.0 (0.3)	11.3 (1.4)	0.75 (0.06)	0.26^{***}
Mandurah	20.4 (0.4)	11.6 (1.4)	0.76 (0.06)	0.22^{***}

A_R: allelic richness (based on a minimum sample size of 16 individuals); H: gene diversity. Asterisks refer to the level of significance for tests of heterozygote deficiency ***, $P < 0.001$.

There was low, but significant differentiation among sites (F_{ST} = 0.001, P = 0.049). However, all tests of population differentiation between pairs of sites were non-significant (Table 7.3) and divergences between sampling locations within the Abrolhos Islands were comparable to those observed between sites at the broad geographic scale (pairwise F_{ST} ranged from –0.0029 to 0.0030). There was also no evidence for isolation-by-distance at the broad or local scales (Mantel tests, P = 0.342 and 0.400 respectively). No genetic subdivision was found using Bayesian clustering analysis. The STRUCTURE analysis revealed only slight increases in log probability estimates with increasing values of K, and there were no large fluctuations in ΔK, suggesting that the probable number of clusters was one (Figure 7.1). Further, when $K > 1$, the proportion of individuals assigned to each cluster was fairly even, and most individuals were admixed, consistent with inferred population structure not being real (Pritchard et al. 2007). The analysis involving GENELAND gave a similar result, with posterior distributions of the estimated number of populations indicating a clear mode at K = 1 in all 10 replicates.

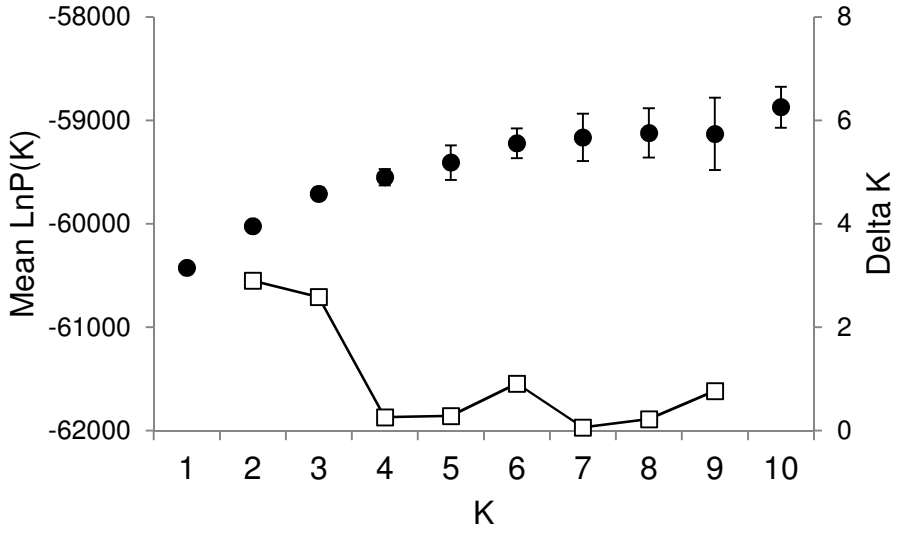


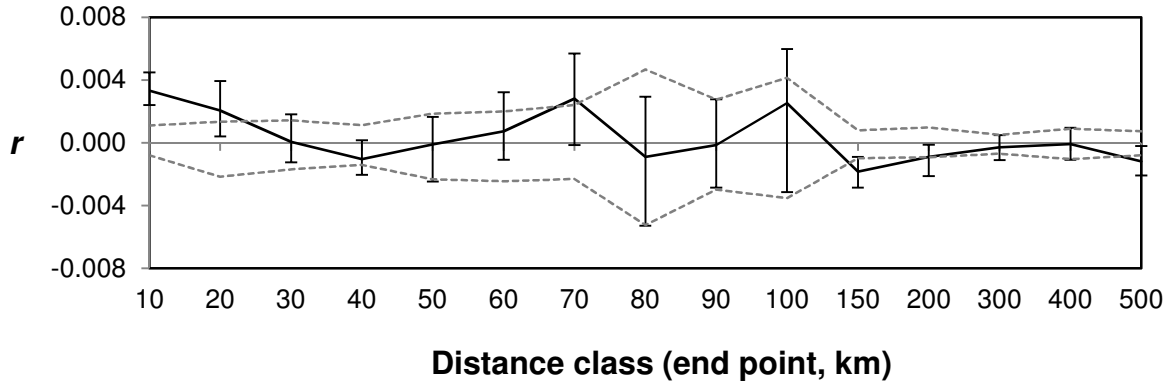
Figure 7.1 Mean estimates of the log probability (solid circles) and Delta K (open squares) for each K arising from the STRUCTURE analysis. Error bars for L(K) are standard deviations.

Table 7.3 Pairwise F_{ST} (below diagonal) and *P*-values from tests of population differentiation (above diagonal) between sites. The significance level adjusted for multiple comparisons is 0.0012.

	Kalbarri	Abrolhos	Dongara	Jurien Bay	Lancelin	Rottnest	Femantle	Mandurah
Kalbarri	–	0.0268	0.0875	0.1054	0.3982	0.1571	0.6518	0.2214
Abrolhos	0.0051	–	0.3589	0.5589	0.8429	0.0750	0.2339	0.3018
Dongara	0.0040	0.0005	–	0.1036	0.4839	0.3339	0.4750	0.6214
Jurien Bay	0.0029	0.0001	0.0011	–	0.6482	0.1625	0.4929	0.3000
Lancelin	0.0033	0.0007	0.0012	0.0007	–	0.5571	0.2929	0.4411
Rottnest	–0.0004	0.0018	–0.0015	–0.0014	0.0004	–	0.3018	0.0393
Femantle	–0.0007	0.0016	0.0001	0.0006	0.0008	–0.0008	–	0.2821
Mandurah	0.0013	–0.0009	–0.0026	–0.0049	–0.0002	–0.0013	–0.0022	–

In contrast to the Mantel tests, spatial genetic structure was detected with the SA analyses. Significantly positive *r* values were found within the first two distance classes (0–10 and 11–20 km), after which *r* decreased and did not differ significantly from zero for the remaining distance classes (Figure 7.2A). Note that the *r* value for the 70 km distance class was slightly greater than the upper 95% confidence limit determined by random permutation, but the 95% confidence limits for the estimate calculated by bootstrapping overlapped with zero. Positive spatial genetic structure at local geographical scales was confirmed when estimates of *r* were calculated with increasing distance class sizes. Figure 7.2B shows little change in *r* between 10 and 20 km, after which *r* decreased, but remained significant until 150 km. It also appears that positive genetic structure was not confined to one geographical area. Two-dimensional local spatial autocorrelation analysis revealed clusters of positive *lr* at all sampling locations (Figure 7.3). A similar number and distribution of positive *lr* values were obtained when calculations were based on sampling the nearest five, 10, 20 and 50 individuals, confirming the consistency of the result (data not shown).

A



B

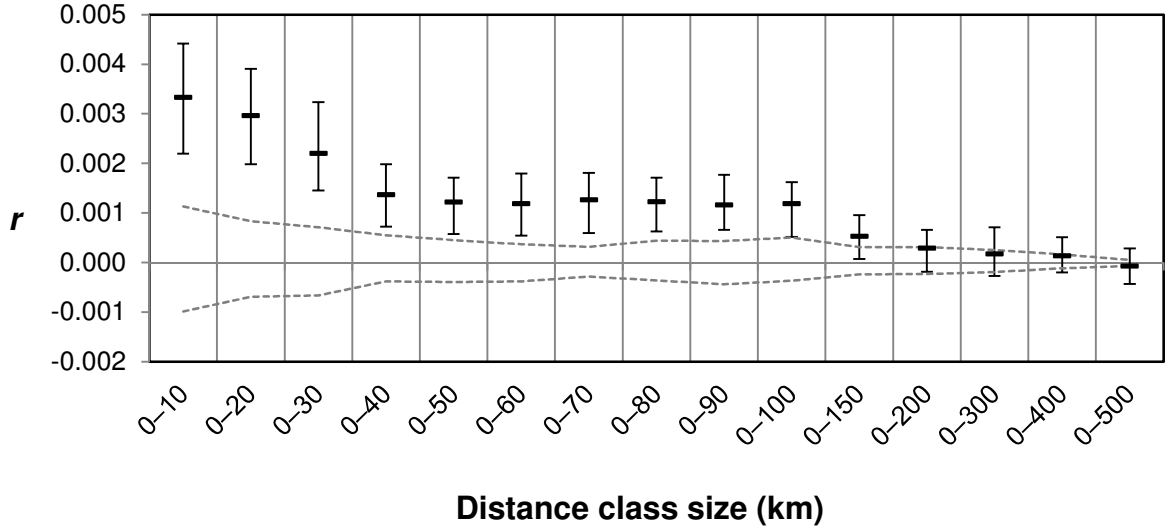


Figure 7.2 Spatial autocorrelation analyses. (A) Correlogram plot of the genetic correlation coefficient (*r*) as a function of distance. (B) Multiple distance class plot, showing the influence of different distance class sizes on genetic correlation. Permuted 95% confidence interval (dashed lines) and the bootstrap 95% confidence error bars are shown.

The outlier analyses identified a single locus (Pcgy13) with a high F_{ST} value compared to neutral expectations (after correction for multiple comparisons) when a stepwise mutation model was used. However, none of the loci had higher than expected F_{ST} values under the infinite allele mutation model. Nor were there any loci with lower than expected F_{ST} values using either of the mutation models.

7.2.2 *Discussion*

The major finding of this study was the significant genetic heterogeneity among local populations in *P. cygnus*, without the presence of large-scale geographic variation. We found extremely low levels of differentiation among sites sampled across the species' range (F_{ST} = 0.001), consistent with extensive gene flow over large geographic distances. The lack of geographic pattern was emphasized by genetic divergences between sites separated by distances over 650 km being no larger than the divergences between sites at the Abrolhos Islands, which are separated by distances less than 85 km. We also found no evidence of isolation-by-distance at a local or broad-scale and no genetic subdivision using Bayesian clustering analysis. Our results, therefore, support the findings of previous genetic studies (Thompson et al. 1996; Johnson and Wernham 1999), which suggest that *P. cygnus* is a single, panmictic population.

Fine-scale population structure in *P. cygnus* was most clearly evident with spatial autocorrelation analysis. Significant positive genetic structure was observed when lobsters were sampled over distances up to 20 km with detectable positive spatial genetic structure extending out to 150 km when distances classes were pooled. Further, two-dimensional local SA analysis indicates that these patterns were not driven by the strong influence of one region alone, but were a common feature throughout the species' range. Microgeographic genetic patchiness indicated by these results has been demonstrated in a number of marine species with planktonic larvae (e.g., Hedgecock 1994b; Knutsen et al. 2003; Pujolar et al. 2006) including several species along the Western Australian coast (Johnson and Black 1982; Watts et al. 1990; Johnson et al. 1993; Johnson et al. 2001).

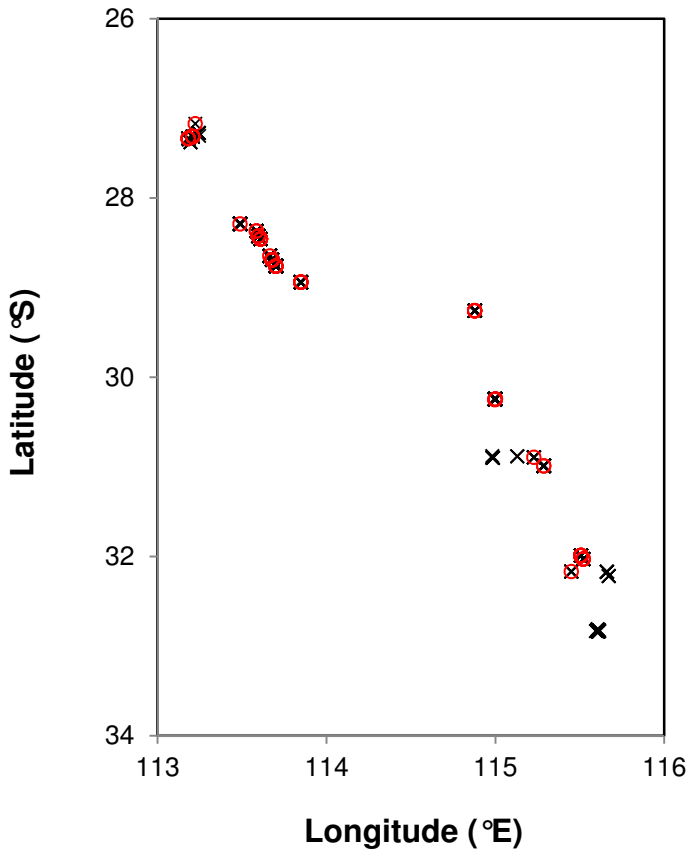


Figure 7.3 Plot of two-dimensional local spatial autocorrelation analyses. Symbols represent geographical coordinates with significantly positive (red circles) or non-significant (crosses) *lr* values. Calculations of *lr* were based on sampling the nearest 25 individuals.

There is evidence from a number of studies that suggests spatial genetic patchiness is due to temporal variation in the genetic composition of recruits (Johnson and Black 1984; Watts et al. 1990; Hedgecock 1994a; Pujolar et al. 2006). This also seems to be the case for *P. cygnus*. Johnson and Wernham (1999) were able to show that a combination of temporal variation in allele frequencies and contrasting patterns of recruitment resulted in genetically different cohorts of *P. cygnus* at two sites. Furthermore, this pattern was ephemeral, as it was not repeated in the subsequent two years. Under the 'sweepstakes reproductive success' hypothesis (Hedgecock 1994a), temporal genetic variance in recruits might be a by-product of large variance in the reproductive success of individuals, owing to chance matching of reproductive activity with oceanographic conditions conducive for larval survival. This

implies that successfully settled larvae are offspring of a few spawners. Other explanations for temporal genetic variation in *P. cygnus* recruits are (1) they originated from different source populations, (2) there was limited mixing of larvae in the plankton, or (3) natural selection was acting on larvae prior to settlement. Johnson and Wernham (1999) suggested that because larvae settling at the same time at sites 350 km apart shared the same allele frequencies, it was unlikely that related larvae stay together in the plankton. Further, given the extremely low geographic structure in *P. cygnus*, it is also unlikely that temporal genetic variation arises from different source populations.

If temporal genetic variation in recruits is responsible for the genetic patchiness observed in this study, it would require juvenile and adult *P. cygnus* to be relatively sedentary. This appears to be the case. Studies on foraging movements suggest juvenile *P. cygnus* forage over relatively small areas (~150 m radius), though the extent of movement is variable (Jernakoff et al. 1987; Jernakoff and Phillips 1988). The life-cycle of *P. cygnus* also includes a migratory phase, which occurs between four and six years of age, just after many lobsters undergo a synchronised moult that changes their normal red shell to a paler colour (Morgan et al. 1982b). During this migration, lobsters leave the coastal reefs and move into deeper water breeding grounds, where they become sedentary again on deeper reefs. Because the lobsters we collected were predominantly from shallow water sites, it is unlikely that they have undertaken these migratory movements. Nevertheless, tag and release experiments have shown that while large movements (>200 km) do occur, most lobsters (>87%) are recaptured within 10 km of their release site (Chubb et al. 1999), which is within the distance range we detected positive population structure. More recently, a study using acoustic telemetry found that only a small proportion (13.6%) of migratory phase lobsters emigrated from their resident reef, suggesting that a mass offshore migration may not hold for all inshore reefs (MacArthur et al. 2008).

Another explanation for spatial genetic patchiness is natural selection acting after settlement (Larson and Julian 1999), and that genes under selection are linked to neutral microsat markers, which show the variation among sites. Given the broad geographical range of *P. cygnus*, local populations are likely to experience highly varied environmental conditions, providing the opportunity for local adaptations to develop across populations (Kawecki and Ebert 2004). Indeed, several studies have found evidence for local adaptation in widely distributed marine fish (see Nielsen et al. 2009).While we found no clear evidence of directional selection using outlier analysis, genome scans involving a much higher numbers of neutral markers, candidates genes or population transcriptomics would be needed

to confidently exclude this possibility. A study monitoring the genetic composition of cohorts of recruits as they develop into adults would also yield valuable insights on post-settlement processes.

7.2.2.1 *Implications for fisheries management*

The implications of genetic patchiness for fisheries management has been discussed by Larson and Julian (1999). If genetic patchiness is due to selection after settlement, they suggest that the implications for fisheries management are minor, unless a fishery concentrated on one particular habitat or location, which might disproportionately affect a certain portion of the gene pool. By contrast, factors affecting the genetic composition of recruits prior to settlement may have greater consequences. The most relevant of these to *P. cygnus* is the effect of stochastic spatial variation in the sources of successful larvae (sweepstakes reproductive success). This effect implies that the sources of successful larvae vary unpredictably over time. Fisheries management should therefore ensure that both the distribution as well as the total spawning potential of the exploited population is protected. Further, spatial stochasticity of successful spawning argues for the spatial dispersion of reserves (should they become involved in managing the exploitation), increasing the chance that at least some larvae will be released into conditions favourable for their survival.

Another result with implications for management, was the lower levels of allelic richness observed in sites at the edge of the fishery compared to those that were more centrally located. Spatial variation in genetic diversity has also been found in *Jasus lalandii*, an exploited rock lobster occurring along the south and west coast of South Africa, prompting calls for the inclusion of sites with high genetic diversity within marine protected areas (Matthee et al. 2007). While the differences in *P. cygnus* were small and do not warrant special consideration, they show there may be differences in the consistency of recruitment and contributions to the larval pool among sites. However, more data are needed to determine whether this pattern is temporally stable. It should also be emphasized that with sweepstakes reproductive success, even populations at the margins of species' ranges have the potential to make important contributions to recruitment (Larson and Julian 1999).

7.3 Comparing the spatial genetic structure of recruits with that of adults, estimating effective population size and testing for bottlenecks (Objectives 3 & 4)

7.3.1 *Results*

7.3.1.1 *Genetic variation within collection locations*

Fifty three unique mtDNA haplotypes were observed among the 252 sequences examined. These haplotype sequences have been deposited in Genbank (Accession nos.: JN813475-JN813529). Overall there were 39 polymorphic sites in the 355-bp sequence. The evolutionary relationships among the haplotypes are depicted in Figure 7.4. There were no well supported clades (i.e. clades with bootstrap values greater than 50%) within *P. cygnus*, and compared to the genetic distances between species (range 0.104 to 0.152), genetic distances between haplotypes within *P. cygnus* were very low (range 0 to 0.019). Estimates of mtDNA variability within collection locations were consistently high (Table 7.4). All samples contained at least five haplotypes, with haplotype and nucleotide diversity ranging from 0.40 to 0.81 and 0.0019 to 0.0040 respectively. The most abundant haplotype (Hap1) was found in 58% of the individuals sequenced. The second most abundant haplotype (Hap8) was found in 3.1% of the individuals sequenced. After correcting for sample sizes, there was no evidence of significant differences in the number of haplotypes among collection sites in the 2009 cohort or among the historical and contemporary samples (95% CLs were overlapping in all cases). Similarly, Friedman's ANOVA revealed no significant difference in haplotype diversity among collection sites ($\chi^2 = 4.30$, $P = 0.367$) or among the historical and contemporary samples ($\chi^2 = 6.26$, $P = 0.100$).

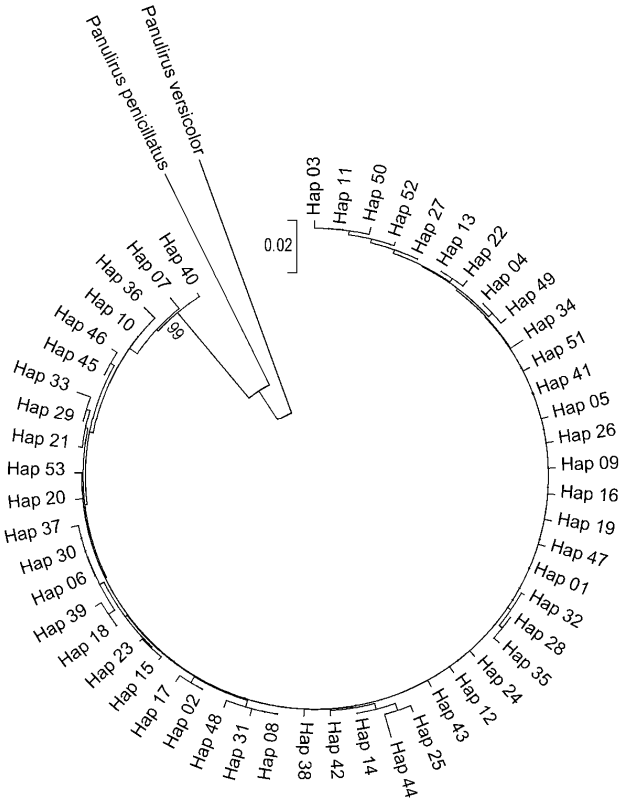


Figure 7.4. Neighbour-joining tree showing evolutionary relationships among haplotypes. Percentage consensus based on 1000 bootstrap replications of the data is shown for branches where values exceed 50%.

Table 7.4 Genetic variation within samples and results of neutrality tests based on a 355bp sequence of the 12S mitochondrial gene.

							Mismatch analysis	
Site/year	*n*	N_{Hap}	*h* (SE)	π (SE)	Fu's F_S	Tajima's *D*	SSD	RAG
2009								
Coral Bay	44	13	0.57 (0.09)	0.0024 (0.0019)	-12.0^{***}	-2.4^{***}	0.004	0.094
Port Gregory	18	5	0.40 (0.14)	0.0019 (0.0017)	-2.3^{*}	-2.0^{**}	0.010	0.178
Rat Island	18	10	0.81 (0.09)	0.0031 (0.0024)	-8.6^{***}	-2.1^{**}	0.037	0.249
Seven Mile Beach	27	11	0.66 (0.10)	0.0040 (0.0028)	-6.7^{***}	-1.7^{*}	0.014	0.064
Jurien Bay	16	8	0.70 (0.13)	0.0039 (0.0028)	-4.4^{***}	-2.0^{**}	0.000	0.023
All samples	130	34	0.62 (0.05)	0.0030 (0.0022)	-29.4^{***}	-2.3^{***}	0.003	0.060
1995	38	10	0.53 (0.10)	0.0023 (0.0019)	-7.4^{***}	-2.1^{**}	0.001	0.070
1997	40	14	0.78 (0.06)	0.0038 (0.0026)	-10.3^{***}	-2.1^{**}	0.015	0.130^{*}
1999	44	18	0.72 (0.07)	0.0034 (0.0024)	-18.8^{***}	-2.1^{**}	0.002	0.068

n, sample size; N_{Hap}, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity; SSD, sum of squared deviations; RAG, raggedness index. Asterisks refer to the level of significance of tests for departures from neutrality (Fu's F_S Tajima's *D*) or a model of population expansion (mismatch analysis) *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Genetic diversity estimates based on the microsatellite loci are given in Table 7.5. There was a significant difference in allelic richness among collection sites in the 2009 sample (χ^2 = 13.06, P = 0.023), but not in gene diversity (χ^2 = 3.04, P = 0.694). Pairwise tests revealed that the significant differences were between Jurien Bay and all other sites except Rat Island, between Rat Island and Coral Bay and between Rat Island and Quobba (Wilcoxon tests, P < 0.05 in all cases). Allelic richness also varied significantly among the historical and contemporary samples (χ^2 = 14.91, P = 0.002), with pairwise tests indicating there were significant differences between the 1995 and 2009 samples, between the 1997 and 1999 samples and between the 1999 and 2009 samples (Wilcoxon tests, P < 0.003 in all cases). However, there was no evidence of a decline in genetic variation over time. All relationships between year and genetic diversity were non-significant and positive (indicating there was a trend for increasing genetic diversity over time). There was no significant difference in gene diversity among the historical and contemporary samples (χ^2 = 6.14, P = 0.105). To test whether the larger geographical range over which the 2009 sample was collected affected the temporal comparisons, we repeated the temporal analyses using a 2009 sample comprising individuals collected from Seven Mile Beach only. The results were similar to the previous analysis, except that the difference in allelic richness among temporal samples was not significant (χ^2 = 7.47, P = 0.058) and differences in gene diversity were significant (χ^2 = 10.32, P = 0.016). Importantly, all relationships between year and genetic diversity remained non-significant and positive, confirming that the absence of a decline in genetic diversity found in the previous analysis was not an artefact of sampling intensity.

Significant departures from Hardy-Weinberg Equilibrium (HWE) were detected in all samples, with positive F_{IS} values indicating heterozygote deficiencies (Table 7.5). The departures from HWE appear to be due to null alleles. Nine loci (S3, S8, S50, *Pcyg*02, *Pcyg*07, *Pcyg*09, *Pcyg*13, *Pcyg*14 and *Pcyg*17) were identified as having null alleles using MICROCHECKER. When these loci were removed from the analysis, no significant departures from HWE were found. There were no significant differences in F_{IS} were evident among collection sites in the 2009 sample (χ^2 = 10.24, P = 0.07) or among the historical and contemporary samples (χ^2 = 3.18, P = 0.365). No significant linkage disequilibrium was detected between pairs of loci after adjusting for multiple comparisons.

Table 7.5 Genetic variation within samples based on 20 microsatellite DNA loci.

Site/year	Sample size	A_R (SE)	*H* (SE)	F_{IS}
2009				
Coral Bay	121.6 (1.4)	7.1 (0.8)	0.74 (0.06)	0.21***
Quobba	38.8 (0.5)	7.1 (0.8)	0.74 (0.06)	0.19***
Port Gregory	23.0 (0.4)	7.0 (0.7)	0.74 (0.06)	0.21***
Rat Island	23.6 (0.3)	6.7 (0.7)	0.72 (0.06)	0.23***
Seven Mile Beach	35.2 (0.5)	7.0 (0.7)	0.75 (0.06)	0.20***
Jurien Bay	13.9 (0.7)	6.5 (0.7)	0.72 (0.07)	0.28***
All samples	264.5 (3.2)	14.2 (2.0)	0.74 (0.06)	0.21***
1995	37.5 (0.9)	13.1 (1.9)	0.74 (0.06)	0.24***
1997	37.1 (0.8)	13.9 (2.0)	0.73 (0.06)	0.25***
1999	42.9 (1.2)	12.4 (1.7)	0.72 (0.06)	0.25***

A_R: allelic richness (based on a sample size of seven or 24 individuals for the sites within the 2009 sample and the combined samples for each year respectively. These values were the minimum sample sizes for each set of samples); *H*: gene diversity. Asterisks refer to the level of significance of tests for heterozygote deficiency ***, $P < 0.001$.

7.3.1.2 *Genetic differentiation among samples*

There was no evidence of spatial or temporal genetic structure in the mtDNA or microsatellite data sets. Overall Φ_{ST} values based on mtDNA data were low and nonsignificant among sites ($\Phi_{ST} = –0.002$, $P = 0.569$) and among years ($\Phi_{ST} = 0.002$, $P = 0.095$). Similar patterns were observed with F_{ST} estimates based on microsatellites ($F_{ST} = 0.003$, $P = 0.464$ and $F_{ST} = 0.003$, $P = 0.124$ for the spatial and temporal comparisons respectively). When appropriate sample sizes were available ($n > 10$ individuals), we also tested for genetic differentiation among monthly cohorts within sites. Again, these analyses failed to detect significant differentiation among samples using mtDNA (Coral Bay, $\Phi_{ST} = –0.001$, $P = 0.502$; Seven Mile Beach, $\Phi_{ST} = –0.029$, $P = 0.861$) or microsatellite data (Coral Bay, $F_{ST} = 0.001$, $P = 0.553$; Seven Mile Beach, $F_{ST} = 0.000$, $P = 0.691$).

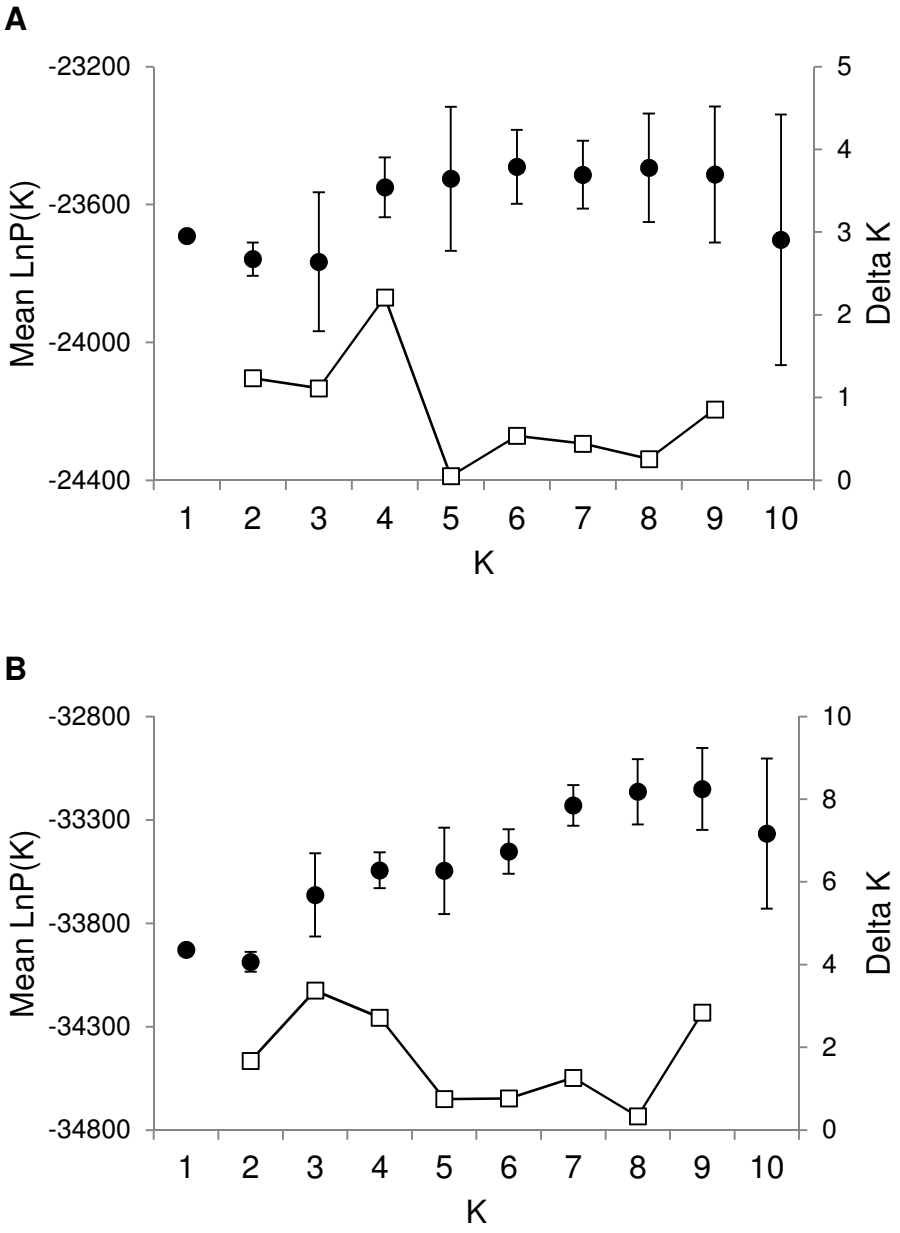


Figure 7.5 Mean estimates of the log probability (solid circles) and Delta K (open squares) for each K arising from the Bayesian clustering analysis of the (A) 2009 and (B) temporal microsatellite data. Error bars for L(K) are standard deviations.

Results from the Bayesian clustering analysis were consistent with those obtained using AMOVA. For both the spatial and temporal microsatellite data, the initial decline in log probability estimates and small fluctuations in ΔK indicated that the probable number of clusters was one (Figure 7.5). Further, when $K > 1$, we found that the proportion of

individuals assigned to each cluster was fairly even, and most individuals were admixed, as expected when the population structure is not real (Pritchard et al. 2007).

7.3.1.3 *Neutrality tests, demographic history and estimates of effective population size*

The results of Tajima's *D* test and Fu's F_S test are presented in Table 7.4. Tajima's *D* values were negative for all samples, indicating an excess of rare nucleotide site variants compared to the expectation under a neutral model of evolution. Fu's F_S tests also showed negative values for all samples, indicating an excess of rare haplotypes over what would be expected under neutrality. Both these results are consistent with rapid population growth or genetic hitch-hiking. The observed mismatch distributions also suggested there has been population growth. In all samples except 1997, SSD and raggedness indices did not differ significantly from a distribution simulated under a model of rapid population expansion (Table 7.4). Estimates of tau from the mismatch distribution ranged from 0.8 to 2.6 in the contemporary samples and 0.9 to 1.3 in the historical samples. Based on the lower mutation rate, these estimates of tau imply that the population expansion began 3 265 to 10 614 generations (~ 16 300 to 53 000 years) ago. The higher mutation rate suggests the expansion began 980 to 3 184 generations (~4 900 to 16 000 years) ago.

There was no evidence for recent bottleneck events within any sample. No significant heterozygosity excesses were detected (Wilcoxon *P*-values ranged between 0.834 and 0.999). Furthermore, all samples exhibited normal L-shaped distributions, as expected under mutation-drift equilibrium. All estimates of N_e calculated using the single sample and temporal methods were negative. This indicates that the signal caused by genetic drift was less than the noise created by sampling error.

7.3.2 *Discussion*

Using samples of pueruli collected between 1995 and 2009, we tested for a decline in genetic diversity in *P. cygnus*. Our data show there were significant differences in allelic richness at microsatellite loci between collection years, but no evidence of loss of genetic diversity or increases in the level of inbreeding. Indeed, in the few cases where significant differences were found between samples, genetic diversity was higher in the more recent samples. Variation in sample sizes and genotyping errors associated with PCR amplification and scoring were unlikely to explain these results. It therefore appears that despite heavy exploitation and recent declines in recruitment, genetic diversity is being retained in *P. cygnus*. However, it should be recognized that our study did not include samples taken before

the commencement of commercial harvesting or during the initial stages of exploitation, when loss of diversity may be more pronounced (Ryman et al. 1995), so we cannot rule out the possibility that genetic variation has been lost due to fishing.

The maintenance of genetic diversity in *P. cygnus* is most likely a consequence of large effective population sizes. No estimates of N_e were obtained in this study because the signal caused by genetic drift was less than the noise created by sampling error. However, we did not detect any significant temporal variation in haplotype or allele frequencies, suggesting that effective population sizes were sufficiently large to ensure genetic drift was negligible. This contrasts with estimates of N_e of less than 200 in a population of New Zealand snapper, which showed significant declines in genetic diversity and temporal fluctuations in allele frequencies (Hauser et al. 2003). In northern pike, declines in heterozygosity were reported in a population with estimates of N_e ranging from 35 to 72 (Miller and Kapuscinski 1997), and significant declines in the number of alleles across loci were detected in a population of North Sea cod over time intervals similar to this study, with estimates of N_e ranging from 69 to 121 (Hutchinson et al. 2003). Nevertheless, given the large variance in individual reproductive success expected with broadcast spawning (Hedgecock 1994a), N_e for *P. cygnus* is likely to be much lower than the census population size. It is also worth noting that several marine species have undergone heavy exploitation and retained genetic diversity despite low N_e/N ratios (Consuegra et al. 2005; Hoarau et al. 2005; Riccioni et al. 2010; Cuveliers et al. 2011). Riccioni et al (2010) suggested that gene flow, overlapping generations and high mutation rates may explain the maintenance of variation in Atlantic Bluefin tuna. Some of these factors may also be contributing to the maintenance of genetic variation in *P. cygnus*.

Despite a severe downturn in *P. cygnus* recruitment particularly between the 2007/08 to 2009/10 seasons, we found no evidence of recent severe reductions in effective population size using genetic bottleneck tests. No correlations between recent years of exploitation could be made because contemporary N_e values were negative. Over much longer, historical timescales the demographic history inferred from mtDNA sequences suggests *P. cygnus* has undergone a population expansion. This is a consistent pattern observed in a range of spiny lobster species from both the northern and southern hemispheres (Tolley et al. 2005; Gopal et al. 2006; García-Rodrguez and Perez-Enriquez 2008; Paleroa et al. 2008; Naro-Maciel et al. 2011). Expansion events in these species have been attributed to sea level and temperature changes at the end of the last glacial maximum that led to increases in suitable shallow water habitats (Naro-Maciel et al. 2011). Our data suggest the population expansion in *P. cygnus*

began 4,900 to 53,000 years ago, which is also consistent with a Holocene population expansion.

The absence of spatial genetic structure in *P. cygnus* puerulus is consistent with larval mixing during the extended planktonic stage (Phillips et al. 1979). Lack of population structure has been reported in other spiny lobsters (Ovenden et al. 1992; Tolley et al. 2005; García-Rodrguez and Perez-Enriquez 2008; Naro-Maciel et al. 2011), but there are exceptions (Perez-Enriquez et al. 2001; Gopal et al. 2006; Paleroa et al. 2008). In the exceptional species, geographic barriers and oceanic currents may be restricting gene flow. For example, Gopal et al.(2006) found genetic differences among northern and southern populations of *Palinurus delagoae* along the southeastern African coast and between coastal populations and those occurring on the African continental shelf, which they attributed to oceanic currents forming a barrier to gene flow and possible reproductive barriers. In *Palinurus elephas,* genetic differentiation was found between Atlantic and Mediterranean populations, which was attributed to restricted gene flow through the Straits of Gibraltar (Paleroa et al. 2008). Genetic differences between continental Europe and Irish-Scottish populations and among Mediterranean populations were also found and were attributed to the Gulf Stream and mesoscale processes resulting from incoming Atlantic waters respectively (Paleroa et al. 2008). In contrast to these studies, the boundary current off the Western Australian coast is relatively uninterrupted by complex coastal topography, though there are some high larval retention areas associated with coastal geomorphic features (Feng et al. 2010).

Consistent with our findings, allozyme studies on *P. cygnus* found high genetic similarity among populations over large spatial scales (Thompson et al. 1996; Johnson and Wernham 1999). However, those studies also reported minor genetic differences among local populations that were ephemeral. Such patterns can result from temporal genetic differences in the larval pool combined with patchy settlement (Johnson and Black 1984; Johnson and Wernham 1999). Low samples sizes prevented comparisons between monthly cohorts at most sites in our study. Nevertheless, in the few tests we performed, no significant differences in allele or haplotype frequencies were found, although we did detect heterozygote deficiencies in all samples, which appears to be due to null alleles. It may be that greater sample sizes and more extensive sampling are needed to detect temporal genetic variation amongst monthly cohorts. Alternatively, it may be that differences between cohorts are more likely found with allozymes than microsatellites and mtDNA. This might be expected if temporal genetic variation of recruits results from natural selection.

In conclusion, mtDNA and microsatellite markers show high levels of genetic diversity in contemporary and historical samples of *P. cygnus*. The maintenance of genetic variation over time and lack of significant temporal variation in haplotype and allele frequencies suggest large effective population sizes in *P. cygnus,* though historical samples spanning a larger number of generations is needed to make a more robust test of a decline in genetic diversity. Our analyses also support the view that *P. cygnus* is single, panmictic population. While further research is needed to ascertain the basis and long-term consequences of recent reductions in recruitment, loss of genetic diversity appears unlikely if current management practices and breeding stock sizes are maintained.

8.0 BENEFITS

The benefits of this research lay mainly for the administrators of fisheries management, but also for scientists, the fishing community and groups concerned about marine conservation.

When funds were granted to this project it was unclear whether the western rock lobster was comprised of a single population or multiple genetically distinct local populations. This was a concern because failure to detect underlying population structure may lead to overexploitation and depletion of localized subpopulations, with a subsequent loss of genetic diversity within the species. This project has shown that, not only is the western rock lobster likely to consist of a single population, it is unlikely to lose neutral genetic variation if current management practices and breeding stock sizes are maintained. The benefit to fishers, administrators and researchers, is that assumptions about population structure in the western rock lobster can now be made with far more certainty than prior to this work being undertaken. This should aid in decision making about fishery management policy. The benefit to commercial and recreational fishing communities and conservation groups is greater confidence in the management and sustainability of the western rock lobster fishery. The results have been communicated on local radio in WA in non-technical terms and hence, should have reached at least some members of the fishing and conservation communities.

The benefits and beneficiaries of these results are largely in line with those identified in the original application. It was assumed that the research would aid fisheries management in the design of policy and that there would be flow in effects to the fishing industry and the recreational fishing community. The benefits are largely 'positive' in that they indicate little the current model for the fishery does not need to be modulated on genetic grounds. This could not have been be anticipated prior to the research being undertaken, indeed the large drop in catch numbers in recent years strongly indicated that genetic effects and bottlenecks may have occurred. The research was crucial to maintenance of confidence in the current panmictic model of the fishery when designing management schemes. As such 'adoption' of the research may actually be through inaction or maintenance of the current management plan..

This project has also provided insight into the importance of a broad spatial distribution of spawning potential and the amount of movement occurring after settlement of the pueruli. Both these factors will need to be considered when designing coastal no-take zones, should they become part of the stock management for the species. Hence, this aspect of the research may benefit fishery management teams in the future by providing them with a scientific basis for their designs. This benefit was also anticipated in the original application.

The research output is also of benefit to the scientific community. The *P. cygnus* microsatellites developed may be useful in other investigations on this and related species. In addition, the results on spatial genetic structure aid our general understanding of the population genetics of marine invertebrates. This benefit was also anticipated in the original application.

9.0 FURTHER DEVELOPMENT

There are two areas where the results of our research may be developed further, firstly to investigate the cause of ephemeral genetic patchiness in adults and secondly to investigate the adaptive genetic variation in the western rock lobster.

One of the most interesting results coming out of this project was the detection of low levels of genetic differentiation among local populations in the survey of adults. Local scale genetic patchiness has been observed in other broadcast spawners, including several species from Western Australia. While several mechanisms have been proposed to account for these patterns, including natural selection or chance acting before settlement, or to natural selection acting after settlement, the basis of genetic patchiness has rarely been resolved. One way this could be done in the western rock lobster would be use the markers we developed in this project to track changes in the genetic composition of cohorts across different life-history stages (e.g., phyllosoma to adults). Evidence of genetic changes occurring within a cohort over time would suggest natural selection contributed to the genetic patchiness, while no genetic changes would suggest random sampling acting before settlement was responsible. However, a challenge for this proposed study would be grouping juvenile and adult stages into the same age cohorts.

Further development could also be achieved by using the samples collected in this project to investigate adaptive genetic variation in the western rock lobster. While neutral DNA markers such as those used in this project provide information about demographic parameters such as migration rates and effective population sizes, population genomic approaches that involve high numbers of neutral markers, candidate genes or population transcriptomics can provide insights into local adaptation and evolutionary consequences of selective harvesting. The information gained from these studies is essential for understanding natural and human-induced evolutionary processes and to make predictions about future changes to the stock and defining management units.

The research could be disseminated further by publishing the scientific papers presented in the appendices. Appendix 5 has been published in a peer reviewed scientific journal and a

media broadcast has already been undertaken. Appendix 6 is currently under review in a scientific journal. The data will be managed by data storage at The University of Western Australia.

10.0 PLANNED OUTCOMES

This projects main output was to provide reliable data on the extent of genetic mixing of western rock lobsters over its distributional range. Our analyses showed that despite low levels of fine-scale genetic heterogeneity, there was no population structure over broader scales (>20 km), consistent with the fishery being comprised of a single, well-mixed population. This output will contribute to the planned outcome of providing fisheries with knowledge and data to make scientifically informed decisions on western rock lobster management. The data in spatial genetic structure is critical for validating oceanographic models of larval mixing in the fishery, as well as for providing management with information on stock structure.

A second output of this project was analyses indicating that the fishery has a large effective population size and is not currently or has not recently passed through a genetic bottleneck. Such bottlenecks are early warning signs of a fishery vulnerable to reduced productivity and loss of evolutionary potential. This output has contributed to the planned outcome of providing information about the genetic health and evolutionary potential of the fishery.

These outputs (our research results on spatial genetic structure and large effective population size) were communicated to the Western Australian Department of Fisheries department though interim progress reports and meetings. They will also be communicated by this report. The project outputs have also been communicated to the fishing community though a meeting and a radio broadcast. This should aid with boosting this groups confidence in the health of the *P. cygnus* population.

Finally, the research findings have or are the process of being published in scientific per reviewed journals. This will aid in the planned outcome of increasing knowledge of the biology of *P. cygnus* amongst the scientific community.

11.0 CONCLUSION

The key objective of this project was to provide reliable data on the extent of genetic mixing of western rock lobsters over its distributional range. This information is critical for validating oceanographic models of larval mixing in the fishery, as well as for providing management with information on stock structure. Another aim was to test for evidence of genetic bottlenecks, which are early warning signs of a fishery vulnerable to reduced productivity and loss of evolutionary potential. Both of these objectives were successfully achieved. Our analyses showed that despite low levels of fine-scale genetic heterogeneity, there was no population structure over broader scales (>20 km), consistent with the fishery being comprised of a single, well-mixed population. They also suggest that genetic diversity will be maintained if current management practices and breeding stock sizes are maintained

REFERENCES

Addison JA, Hart MW (2005) Spawning, copulation and inbreeding coefficients in marine invertebrates. Biol Lett 1: 450-453

Allendorf FW, England PR, Luikart G, Ritchie PA, Ryman N (2008) Genetic effects of harvest on wild animal populations. Trends Ecol Evol 23: 327-337

Allendorf FW, Luikart G (2006) Conservation and the genetics of populations. Blackwell Publishing, Malden, MA

Antoa T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: A workbench to detect molecular adaptation based on a F_{st}-outlier method. BMC Bioinformatics 9: 323

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54: 1590-1605

Beaumont MA (2005) Adaptation and speciation: what can F_{st} tell us? Trends Ecol Evol 20: 435-440

Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. Proc R Soc Lond 363: 1619-1626

Begg GA, Friedland KD, Pearce JB (1999) Stock identification and its role in stock assessment and fisheries management: an overview. Fish Res 43: 1-8

Benzie JH, Stoddart JA (1992) Genetic structure of outbreaking and non-outbreaking crown-of-thorns starfish (*Acanthaster planci*) populations on the Great Barrier Reef Mar Biol 112: 119-130

Bohonak AJ (1999) Dispersal, gene flow and population structure. Q Rev Biol 74: 21-45

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc Natl Acad Sci USA 91: 6491-6495

Brown R (2009) Western rock lobster low puerulus settlement risk assessment workshop. In: Fisheries WADo (ed), Perth, Western Australia.

Caputi N (2008) Impact of the Leeuwin Current on the spatial distribution of the puerulus settlement of the western rock lobster (*Panulirus cygnus*) and implications for the fishery of Western Australia. Fish Oceanogr 17: 147-152

Caputi N, Chubb C, Melville-Smith R, Pearce A, Griffin D (2003) Review of relationships between life histrory stages of the western rock lobster, *Panularis cygnus*, in Western Australia. Fish Res 65: 47-61

Caputi N, Melville-Smith R, de Lestang S, Pearce A, Feng M (2010) The effect of climate change on the western rock lobster (*Panulirus cygnus*) fishery of Western Australia. Can J Fish Aquat Sci 67: 85-96

Carvalho GR, Hauser L (1994) Molecular genetics and the stock concept in fisheries. Rev Fish Biol Fisher 4: 326-350

Chittleborough RG (1974) Western rock lobster reared to maturity. Aust J Mar Fresh Res 25: 221-225

Chubb CF, Rossbach M, Melville-Smith R, Cheng YW (1999) Mortality, growth and movement of the western rock lobster (*Panulirus cygnus*). Final Report FRDC Project No. 95/020

Conover DO, Munch SB (2002) Sustaining fisheries yields over evolutionary time scales. Science 297: 94-96

Consuegra S, Verspoor E, Knox D, García de Leániz C (2005) Asymmetric gene flow and the evolutionary maintenance of genetic diversity in small, peripheral Atlantic salmon populations. Conserv Genet 6: 823-842 doi 10.1007/s10592-005-9042-4

Cuveliers EL, Volckaert FAM, Rijnsdorp AD, Larmuseau MHD, Maes GE (2011) Temporal genetic stability and high effective population size despite fisheries-induced life-history trait evolution in the North Sea sole. Mol Ecol 20: 3555-3568

Double MC, Peakall R, Beck NR, Cockburn A (2005) Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). Evolution 59: 625-635

Emerson BC (2007) Alarm bells for the molecular clock? No support for Ho et al.'s model of time-dependent molecular rate estimates. Syst Biol 56: 337-345

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-2620

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis Evol Bioinform Online 1: 47-50

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567-1587

Feng M, Slawinski D, Beckley LE, Keesing JK (2010) Retention and dispersal of shelf waters influenced by interactions of ocean boundary current and coastal geography. Mar Freshwater Res 61: 1259-1267

Fletcher W, Chubb C, McCrea J, Caputi N, Webster F, Gould R, Bray T (2005) ESD Report Series No. 4 – Western Rock Lobster Fishery, Perth, WA

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and backgroud selection. Genetics 147: 915-925

García-Rodrguez FJ, Perez-Enriquez R (2008) Lack of genetic differentiation of blue spiny lobster *Panulirus inflatus* along the Pacific coast of Mexico inferred from mtDNA sequences. Mar Ecol Prog Ser 361: 203-212 doi 10.3354/meps07381

Gopal K, Tolley KA, Groeneveld JC, Matthee CA (2006) Mitochondrial DNA variation in spiny lobster *Palinurus delagoae* suggests genetically structured populations in the south-western Indian Ocean. Mar Ecol Prog Ser 319: 191-198

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3), pp Available from <http://www.unil.ch/izea/softwares/fstat.html>

Groth DM, Lim F, de Lestang SN, Beale N, Melville-Smith R (2009) Characterization of polymorphic microsatellite loci in the western rock lobster (*Panulirus cygnus*). Conserv Genet Resour 1: 163-166

Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape genetics. Mol Ecol Notes 5: 712-715

Harpending H (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Hum Biol 66: 591-600

Hauser L, Adcock GJ, Smith PJ, Ramirez JHB, Carvalho GR (2003) Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proc Natl Acad Sci USA 99: 11742-11747

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 (1994a) Does variance in reproductive success limit effective population sizes of marine organisms. In: Beaumont AR (ed) Genetics and evolution of aquatic organisms. Chapman and Hall, London, pp 122-134

Hedgecock D (1994b) Spatial and temporal genetic structure of marine animal populations in the California current. CalCOFI Rep 35: 73-81

Hoarau G, Boon E, Jongma DN, Ferber S, Palsson J, Van der Veer HW, Rijnsdorp AD, Stam WT, Olsen JL (2005) Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). Proc R Soc Lond 272: 497-503

Hutchinson JA, Reynolds JD (2004) Marine fish population collapses: consequences for recovery and extinction risk. Bioscience 54: 297-309

Hutchinson WF, van Oosterhout C, Rogers SI, Carvalho GR (2003) Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). Proc R Soc Lond 270: 2125-2132

Jernakoff P, Phillips BF (1988) Effect of a baited trap on the foraging movements of juvenile western rock lobsters, *Panulirus cygnus* George. Aust J Mar Fresh Res 39: 185-192

Jernakoff P, Phillips BF, Maller RA (1987) A quantitative study of nocturnal foraging distances of the western rock lobster *Panulirus cygnus* George. J Exp Mar Biol Ecol 113: 9-21

Johnson MS, Bentley SL, Ford SS, Ladyman MT, Lambert GJ (2001) Effects of a complex archipelago on genetic subdivision of the intertidal limpet *Siphonaria kurracheensis*. Mar Biol 139: 1087-1094

Johnson MS, Black R (1982) Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. Mar Biol 70: 157-164

Johnson MS, Black R (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38: 1371-1383

Johnson MS, Black R (2006a) Effects of mode of reproduction on genetic divergence over large spatial and temporal scales in intertidal snails of the genus *Bembicium* Philippi (Gastropoda: Littorinidae). Biol J Linn Soc 89: 689-704

Johnson MS, Black R (2006b) Islands increase genetic subdivision and disrupt patterns of connectivity of intertidal snails in a complex archipelago. Evolution 60: 2498-2506

Johnson MS, Holborn K, Black R (1993) Fine-scale patchiness and genetic heterogeneity of recruits of the corallivorous gastropod *Drupella cornus*. Mar Biol 117: 91-96

Johnson MS, Wernham J (1999) Temporal variation of recruits as a basis of ephemeral genetic heterogeneity in the western rock lobster *Panulirus cygnus*. Mar Biol 135: 133-139

Jones KC, Levine KF, Banks JD (2002) Characterization of 11 polymorphic tetranucleotide microsatellites for forensic applications in California elk (*Cervus elaphus canadensis*). Molecular Ecology Notes 2: 425-427

Jørgensen C, Enberg K, Dunlop ES, Arlinghaus R, Boukal DS, Brander K, Ernande B, Goerdmark A, Johnston F, Matsumura S, Pardoe H, Raab K, Silva A, Vainikka A, Dieckmann U, Heino M, Rijnsdorp AD (2007) Managing evolving fish stocks. Science 318: 1247-1248

Jørgensen HBH, Hansen MM, Bekkevold D, Ruzzante DE, Loeschcke V (2005) Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. Mol Ecol 14: 3219-3234

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16: 1099-1006

Kambhampati S, Smith PT (1995) PCR primers for the amplification of four insect mitochondrial gene fragments. Insect Mol Biol 4: 233-236

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Lett 7: 1225-1241

Kennington WJ, Levy E, Berry O, Groth DM, Waite AM, Johnson MS, Melville-Smith R (2010) Characterization of 18 polymorphic microsatellite loci for the western rock lobster *Panulirus cygnus*. Conserv Genet Resour 2: 389-391

Knutsen H, Jorde PE, André C, Stenseth NC (2003) Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. Mol Ecol 12: 385-394

Kuparinen A, Merilä J (2007) Detecting and managing fisheries-induced evolution. Trends Ecol Evol 22: 652-659

Larson RJ, Julian RM (1999) Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. CalCOFI Rep 40: 94-99

Lee HJE, Boulding EG (2009) Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on variation at one mitochondrial and two nuclear DNA markers. Mol Ecol 18: 2165-2184

Luikart G, Allendorf FW, Sherwin B, Cornuet J-M (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 12: 238-247

MacArthur LD, Babcock RC, Hyndes GA (2008) Movements of the western rock lobster (*Panulirus cygnus*) within shallow coastal waters using acoustic telemetry. Marine and Freshwater Research 59: 603-613

Maruyama T, Fuerst PA (1985) Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 111: 675-689

Matthee CA, Cockcroft AC, Gopal K, von der Heyden S (2007) Mitochondrial DNA variation of the west-coast rock lobster, *Jasus lalandii*: marked genetic diversity differences among sampling sites. Mar Freshwater Res 58: 1130-1135

Melville-Smith R, de Lestang S (2006) Spatial and temporal variation in the size at maturity of the western rock lobster *Panulirus cygnus* George. Mar Biol 150: 183-195

Miller LM, Kapuscinski AR (1997) Historical analysis of genetic variation reveals low effective population size ina northern pike (*Esox lucius*) population. Genetics 147: 1249-1258

Morgan GR, Phillips B, Joll L (1982a) Stock and recruitment relationships in *Panulirus cygnus*, the commercial rock (Spiny) lobster of Western Australia. Fish Bull 80: 475-486

Morgan GR, Phillips BF, Joll LM (1982b) Stock recruitment relationships in *Panulirus cygnus* the commercial rock (spiny) lobster of Western Australia. Fish Bull 80: 475-486

Naro-Maciel E, Reid B, Holmes K, Brumbaugh D, Martin M, DeSalle R (2011) Mitochondrial DNA sequence variation in spiny lobsters: population expansion, panmixia, and divergence. Mar Biol 158: 2027-2041

Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D (2009) Population genomics of marine fishes: identifying adaptive variation in space and time. Mol Ecol 18: 3128-3150

Olsen EM, Heino M, Lilly GR, Morgan MJ, Brattey J, Ernande B, Dieckmann U (2004) Maturation trends indicative of rapid evolution preceded the collapse of northern cod. Nature 428: 932-935

Ovenden JR, Brasher DJ, White RWG (1992) Mitochondrial DNA analyses of the red rock lobster J*asus edwardsii* supports an apparent absence of population subdivision throughout Australasia. Mar Biol 112: 319-326

Palero F, Abellób P, Macpherson E, Gristinad M, Pascual M (2008) Phylogeography of the European spiny lobster (*Palinurus elephas*): Influence of current oceanographical features and historical processes Mol Phylo Evol 48: 708-717

Paleroa F, Abellób P, Macpherson E, Gristinad M, Pascual M (2008) Phylogeography of the European spiny lobster (*Palinurus elephas*): Influence of current oceanographical features and historical processes Mol Phylo Evol 48: 708-717

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. Annu Rev Ecol Syst 25: 547-572

Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. Evolution 57: 1182-1195

Peakall R, Smouse PE (2005) GenAlEx V6: Genetic analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra. Available via http://www.anu.edu.au/BoZo/GenAlEx

Perez-Enriquez R, Vega A, Avila S, Sandoval JL (2001) Population genetics of red spiny lobster (*Panulirus interruptus*) along the Baja California Peninsula, Mexico. Mar Freshwater Res 52: 1541-1549

Phillips BF (1986) Prediction of commercial catches of the western rock lobster *Panulirus cygnus* George Can J Fish Aquat Sci 43: 2126-2130

Phillips BF, Brown PA, Rimmev DW, Reid DD (1979) Distribution and dispersal of the phyllosoma larvae of the western rock lobster, *Panulivus cygnus*, in the south-eastern Indian Ocean. Aust J Mar Fresh Res 30: 773-783

Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity 90: 502-503

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

Pritchard JK, Wen X, Falush D (2007) Documentation for structure software: version 2.2. http://pritbsduchicagoedu/software/structure22/readmepdf

Pujolar JM, Maes GE, Volckaert FAM (2006) Genetic patchiness among recruits in the European eel *Anguilla anguilla*. Mar Ecol Prog Ser 307: 209-217

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicisms. Journal of Heredity 86: 249-249

Riccioni G, Landi M, Ferrara G, Milano I, Cariani A, Zane L, Sella M, Barbujani G, Tinti F (2010) Spatio-temporal population structuring and genetic diversity retention in depleted Atlantic Bluefin tuna of the Mediterranean Sea. Proc Natl Acad Sci USA 107: 2102-2107 doi 10.1073/pnas.0908281107

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223-225

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145: 1219-1228

Ruzzante DE, Taggart CT, Cook D, Goddard S (1996) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Can J Fish Aquat Sci 53: 634-645

Ryman N, Utter F, Laikre L (1995) Protection of intraspecific biodiversity of exploited fishes. Rev Fish Biol Fisher 5: 417-446

Silberman JD, Sarver SK, Walsh PJ (1994) Mitochondrial DNA variation and population structure in the spiny lobster *Panulirus argus*. Mar Biol 120: 601-608

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87: 651-701

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457-462

Smith PJ, Francis RICC, McVeagh M (1991) Loss of genetic diversity due to fishing pressure. Fish Res 10: 309-316

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585-595

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24: 1596-1599

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA 101: 11030-11035

Thompson AP, Hanley JR, Johnson MS (1996) Genetic structure of the western rock lobster, *Panulirus cygnus*, with the benefit of hindsight. Mar Freshwater Res: 889-896

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876-4882

Tolley KA, Groeneveld JC, Gopal K, Matthee CA (2005) Mitochondrial DNA panmixia in spiny lobster *Palinurus gilchristi* suggests a population expansion. Mar Ecol Prog Ser 297: 225-231

Turner TF, Wares JP, Gold JR (2002) Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics 162: 1329-1339

van Oosterhout C, Hutchinson, W. F. , Wills DPM, Shipley P (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4: 535-538

Wang J, Whitlock MC (2003) Estimating effective population size and migration rates from genetic samples over space and time. Genetics 163: 429-446

Waples RS (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41: 385-400

Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv Genet 7: 167-184

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Res 8: 753-756

Watts RJ, Johnson MS, Black R (1990) Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in Western Australia. Mar Biol 105: 145-151

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358-1370

White TA, Stamford J, Hoelzel AR (2010) Local selection and population structure in a deep-sea fish, the roundnose grenadier (*Coryphaenoides rupestris*). Mol Ecol 19: 216-226

Wright I, Caputi N, Penn J (2006) Depletion-based population estimates for western rock lobster (*Panulirus cygnus*) fishery in Western Australia. New Zeal J Mar Fresh 40: 107-122

APPENDIX 1:

Intellectual property

There is no intellectual property associated with this research project.

APPENDIX 2:

Staff

The following academics staff worked on this research project.

Dr Jason Kennington

Dr Oliver Berry

Professor Michael Johnson

Dr Roy Melville-Smith

Associate Professor David Groth

The following technical staff and research assistants were engaged on the project.

Ms Sherralee Lukehurst

Ms Amanda Worth

APPENDIX 3:

Characterization of 18 polymorphic microsatellite loci for the western rock lobster *Panulirus cygnus*

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Characterization of 18 polymorphic microsatellite loci for the western rock lobster *Panulirus cygnus*

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Abstract

We describe the isolation and development of 18 polymorphic microsatellite loci for the western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae). The loci were tested in 37 individuals from a single population situated near the centre of the species' distribution. No evidence of linkage disequilibrium was detected between any pair of loci. However, seven loci showed significant departures from Hardy-Weinberg expectations. The number of alleles per locus ranged from three to 31.

Keywords: microsatellites, western rock lobster, *Panulirus cygnus*

Introduction

The western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae) is confined to the west coast of Australia, from Cape Naturaliste in the south to North West Cape in the north (Phillips et al. 1979). *P. cygnus* supports Australia's most valuable wild-caught single-species fishery with an average annual catch of approximately 10,000 tonnes that is worth $250-350 million a year in export revenue (Fletcher et al. 2005). The fishery is considered to be sustainably managed (Phillips et al. 1979), and was the first in the world to be certified by the Marine Stewardship Council as being an environmentally sustainable fishery. Nevertheless, over the past 35 years the size at maturity has decreased, the abundance of undersized and legal-sized lobsters in deep water relative to shallow water has increased, and there have been shifts in the catch to deep water, possibly due to rising water temperatures associated with climate change (Caputi et al. 2010).

One of the assumptions that underlie the current management system is that the breeding stock comprises a single, demographically united population. This assumption is based on the extended pelagic larval stage of western rock lobsters, which is thought to ensure high dispersal throughout the species range (Phillips et al. 1979). The available genetic data support this idea. Investigations of allozyme variation within *P. cygnus* reveal no significant differences between sites in adults or larvae caught over a wide area of the fishery (Thompson et al. 1996; Johnson and Wernham 1999). However, these analyses were based on relatively few loci, and may therefore lack the resolving power needed to detect subtle genetic differences. Here, we present 18 new microsatellite loci for *P. cygnus* for evaluating population structure within this commercially important species. These markers add to nine recently published microsatellite loci of *P. cygnus* that were developed for paternity testing (Groth et al. 2009), but are too few for a definitive evaluation of genetic subdivision.

Methods

Microsatellites were isolated from a *Panulirus cygnus* DNA library created by Genetic Information Services (Chatsworth, California). Methods for the DNA library construction and enrichment followed those described in Jones et al. (2002). Genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa* I, *Hae* III, *Bsr* B1, *Pvu* II, *Stu* I, *Sca* I, *Eco* RV). Fragments in the size range of 300 to 750 bp were adapted and subjected to magnetic bead capture (CPG, Inc., Lincoln Park, New Jersey), using biotinylated capture molecules. Four libraries were prepared in parallel, using Biotin-$CA_{(15)}$, Biotin-$AAC_{(12)}$, Biotin-$AAAG_{(8)}$ and Biotin-$TAGA_{(8)}$ as capture molecules in a protocol provided by the manufacturer. Captured molecules were amplified and restricted with *Hind*III to remove the adapters. The resulting fragments were ligated into the *Hind*III site of pUC19. Recombinant molecules were electroporated into *E. coli* DH5alpha. Recombinant clones were selected at random for sequencing, and enrichment levels were expressed as the fraction of sequences that contained a microsatellite. Sequences were obtained on an ABI 377, using ABI Prism *Taq* dye terminator cycle sequencing methodology. PCR primers were developed for 49 microsatellite-containing clones, using the DESIGNERPCR version 1.03 (Research Genetics, Inc.) software package.

Genomic DNA for PCR was extracted from a 5-mm × 5-mm piece of tissue from the middle lobe of the tail fan, using a QIAGEN DNeasy Blood and Tissue Kit. PCR reactions of 13µL contained 10 ng of DNA, 1× reaction buffer (Invitrogen's Platinum PCR SuperMix: 22 U/mL complexed recombinant *Taq* DNA polymerase with Platinum *Taq* antibody, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM $MgCl_2$, 220 µM dGTP, 220 µM dATP, 220 µM dTTP, 220 µM dCTP and stabilizers) and 1.3–2.3 µM of each primer (forward primer fluorescent-labelled). PCR amplifications were carried out in an Eppendorf thermal cycler and consisted of an initial denaturation at 94 °C for 3 min, then 35 cycles of 40 s at 94 °C, 40 s at the annealing temperature (see Table 3.1) and 40 s at 72 °C, followed with a final elongation step at 72 °C for 5 min. PCR products (1.5 µL) were analyzed on an ABI 3730 Sequencer, sized using GeneScan-500 LIZ internal size standard and scored using GENEMAPPER version 3.7 (Applied Biosystems) software. Levels of genetic diversity were assessed by genotyping 37 adult *Panulirus cygnus* collected from Lancelin in Western Australia (30° 59.2' S, 115° 17.2' E). The online version of GENEPOP version 3.4 (Raymond and Rousset 1995) was used to calculate basic descriptive statistics and test for significant deviations from Hardy-Weinberg expectations (HWE) and linkage disequilibrium between all pairs of loci. Markov chain parameters for both the HWE and linkage

disequilibrium exact tests were: 1000 dememorization steps, 100 batches and 1000 iterations per batch. Significance levels were adjusted to the number of simultaneous tests using sequential Bonferroni correction (Rice 1989).

Results and Discussion

Of the 24 microsatellite-containing clones for which PCR primers were developed, 18 produced consistent polymorphic genotypes within the expected size range. The number of alleles at these loci ranged from three to 31, and the observed and expected heterozygosities ranged between 0.056 and 0.972 and between 0.055 and 0.953, respectively (Table 1). Seven loci (Pcyg02, Pcyg06, Pcyg07, Pcyg10, Pcyg13, Pcyg16 and Pcyg17) deviated significantly from Hardy-Weinberg expectations after correction for multiple tests. These loci were estimated to have frequencies of null allele ranging from 0.20 (Pcyg06) to 0.49 (Pcyg13), using the CERVUS version 3.0.3 software package (Kalinowski et al. 2007). There was no evidence of linkage disequilibrium between any pair of loci.

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References

Caputi N, Melville-Smith R, de Lestang S, Pearce A, Feng M (2010) The effect of climate change on the western rock lobster (*Panulirus cygnus*) fishery of Western Australia. Can J Fish Aquat Sci 67: 85-96

Fletcher W, Chubb C, McCrea J, Caputi N, Webster F, Gould R, Bray T (2005) ESD Report Series No. 4 – Western Rock Lobster Fishery, Perth, WA

Groth DM, Lim F, de Lestang SN, Beale N, Melville-Smith R (2009) Characterization of polymorphic microsatellite loci in the western rock lobster (*Panulirus cygnus*). Conserv Genet Resour 1: 163-166

Johnson MS, Wernham J (1999) Temporal variation of recruits as a basis of ephemeral genetic heterogeneity in the western rock lobster *Panulirus cygnus*. Mar Biol 135: 133-139

Jones KC, Levine KF, Banks JD (2002) Characterization of 11 polymorphic tetranucleotide microsatellites for forensic applications in California elk (*Cervus elaphus canadensis*). Molecular Ecology Notes 2: 425-427

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16: 1099-1006

Phillips BF, Brown PA, Rimmev DW, Reid DD (1979) Distribution and dispersal of the phyllosoma larvae of the western rock lobster, *Panulivus cygnus*, in the south-eastern Indian Ocean. Aust J Mar Fresh Res 30: 773-783

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicisms. Journal of Heredity 86: 249-249

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223-225

Thompson AP, Hanley JR, Johnson MS (1996) Genetic structure of the western rock lobster, *Panulirus cygnus*, with the benefit of hindsight. Mar Freshwater Res: 889-896

Table 1 Primer sequences, repeat motifs, annealing temperatures and levels of diversity at 18 microsatellite loci in the western rock lobster *Panulirus cygnus* from Lancelin, Western Australia (n = 37). T_a, annealing temperature (°C); H_O, observed heterozygosity; H_E, expected heterozygosity; P, probability value from Hardy-Weinberg test

Locus	Primer sequence (5'-3')	Repeat motif	T_a	Number of alleles	Size range (bp)	H_O	H_E	P	GenBank Accession no.
Pcyg01	F: GCTTCTTATTGCTCCGAAATG R: GGGCTCTCTATCTTCAACTCG	$(GT)_{26}$	58	15	281-330	0.917	0.876	0.803	HM190199
Pcyg02	F: AGGTTGACAGAACACCAAGAG R: GGATAACATCAGCACCATCTC	$(GT)_{15}$	57	22	160-227	0.306	0.934	0	HM190200
Pcyg03	F: GACCCCTTCATCACACACC R: GCAGGCAGTGGGAGAAAC	$(CA)_{10}$	58	3	280-288	0.194	0.18	1	HM190201
Pcyg04	F: CTTTCCGATTGTTTGGTGAG R: GTGGTCTGGCGTAACCTCTA	$(TTG)_7$	58	14	279-325	0.800	0.885	0.158	HM190202
Pcyg05	F: AACGGTTCGTATAATTTGGAC R: CCATCACTCAAACAAAGACAG	$(TTG)_{10}$	57	8	232-259	0.696	0.758	0.176	HM190203
Pcyg06	F: GTTTTCTCTATCCGGGAACTG R: AGGTGGGAAAGCTGTTGTAGT	$(AAC)_2AGC(AAC)_3AAT(AAC)_3AATCAC(AAC)_4$	57	22	176-256	0.618	0.944	0	HM190204
Pcyg07	F: ACCTTGAGAGAGACATGAACC R: CCTTGTGTATGAGACTGGATG	$(AAC)_9(AAT)_5$	57	8	254-281	0.318	0.856	0	HM190205

Pcyg08	F: GAGTGCGATGTGTTGATGGT R: GGGAGTGTTAAACGAATGAGC	$(TTG)_7$	58	24	232-304	0.972	0.953	0.589	HM190206
Pcyg09	F: GGACCTGTTGAGGATACTGTAG R: TTGTGTTGTGAGGATGTTAGTC	$(AAC)_7$	56	4	155-164	0.364	0.418	0.653	HM190207
Pcyg10	F: TGGGACAGATCAGGCAAAC R: CCGAGTATCGAGGAGGGAG	$(TTTC)_5$	58	31	137-346	0.471	0.971	0	HM190208
Pcyg11	F: CCGTTACGAAGGTTCACTTA R: AAATGCACCTAGAGTAAAGCAG	$(TTTC)_4TCC(TTTC)_5$	56	6	164-244	0.735	0.688	0.592	HM190209
Pcyg12	F: ATTGCTTGATGAGAATGATGTG R: CATGGTTAAGGACACCTGAAA	$(AAAG)_7$	57	6	164-244	0.905	0.931	0.307	HM190210
Pcyg13	F: TTTGTGTGTCGTGATTTCATC R: ATCCCATAAAGAGAGGGTGAC	$(TAGA)_4TATT(TAGA)_7$	57	17	203-290	0.310	0.929	0	HM190211
Pcyg14	F: ACGAAGGAATGAATAGTGAATG R: TTCTGCAACATAACGAGGTC	$(ATCT)_2ATCC(ATCT)_5GTCT(ATCT)_5$	57	7	244-259	0.469	0.741	0.018	HM190212
Pcyg15	F: GTGGTTGCTTACCGCATAC R: TGTTTCCTTGTTCAGTCATTG	$(TAGA)_8$	56	3	112-124	0.676	0.607	0.796	HM190213
Pcyg16	F: GACCCCGTTCTTCGTAACT R: ACCCACCTGTCTTGCTATG	$(ATCT)_5TT(ATCT)_5AT(ATCT)_5AT(ATCT)_7$	57	14	206-301	0.333	0.903	0	HM190214
Pcyg17	F: CCCTGACGATGATACAGCC	$(TAGA)_2CAGA$	58	11	207-310	0.324	0.908	0	HM190215

	R: TGGAGTCTTGTCTTGTCTGGTC	$(TAGA)_4$							
Pcyg18	F: CGGGACCATCAAATTACC	$(ATCT)_5$	57	3	201-215	0.056	0.055	1	HM190216
	R: GCAATGGCAAATAACCATAC								

APPENDIX 4:

Fine-scale genetic heterogeneity, but no geographic structure in the western rock lobster (*Panulirus cygnus*)

Fine-scale genetic heterogeneity, but no geographic structure in the western rock lobster (*Panulirus cygnus*)

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Abstract

The western rock lobster (*Panulirus cygnus*) is found in shallow and deep water reef habitats along the Western Australia coastline, where it supports a large commercial fishery. To investigate population structure, microsatellite DNA variation at 22 loci was examined in 631 individuals collected from eight locations across the species' range. Consistent with expectations of extensive larval mixing during an extended planktonic stage, there was low (F_{ST} = 0.001), but significant genetic differentiation among locations. While there was no evidence of isolation-by-distance or genetic subdivision using Bayesian clustering analysis, significant positive spatial autocorrelation (SA) was detected over distances up to 20 km. Further, a two-dimensional local SA analysis revealed that fine-scale genetic heterogeneity was common throughout the species' range. Such patterns could result from natural selection acting before or after settlement or temporal variation in the genetic composition of recruits arising from large variance in the reproductive success among individuals. Our results support previous evidence that *P. cygnus* is a single, panmictic population. However, more work is needed to determine the roles of local selection and sweepstakes reproductive success in creating fine-scale genetic patchiness in this species.

Key words: western rock lobster, genetic variation, microsatellites, population structure

Introduction

The spatial extent of genetic structure is largely dependent on the dispersal capacity of individuals (Bohonak 1999). This is especially apparent in the marine environment, where species with planktotrophic larvae that spend months in the water column tend to maintain low levels of genetic structure across large geographic scales, while those with short planktotrophic larval phases or direct developers usually have much higher levels of subdivision (Waples 1987; Palumbi 1994; Johnson and Black 2006a; Lee and Boulding 2009). However, the role of dispersal capacity in structuring marine populations is complex, and can be strongly influenced by other factors. For example, in spiny lobsters, most species are characterized by a lack of genetic differentiation among localities, consistent with their high dispersal capability during an extended planktonic larval stage (e.g., Ovenden et al. 1992; Silberman et al. 1994; Tolley et al. 2005; García-Rodrguez and Perez-Enriquez 2008; Naro-Maciel et al. 2011), but in some species, barriers to dispersal created by topographic or oceanographic features can lead to moderate to high levels of population structure (Perez-Enriquez et al. 2001; Gopal et al. 2006; Palero et al. 2008).

Impediments to dispersal are not always obvious. For example, Johnson and Black (2006b) showed that over short distances (< 2 km) genetic subdivision increased fivefold between populations on different islands compared to different populations on the same island in both a direct developing snail and a planktonic disperser. Such genetic heterogeneity at local scales can occur even when there is little genetic subdivision over large distances (e.g., Hedgecock 1986; Benzie and Stoddart 1992; Johnson et al. 1993; Ayre and Hughes 2000). Adaptation to local environments (e.g. low salinity or temperature) can also lead to genetic differentiation at selected and linked neutral loci, despite high levels of gene flow (see Nielsen et al. 2009). The extent of population structure can therefore vary considerably among species and is not always determined by life-history characteristics alone. For commercially exploited species, failure to detect underlying population structure is a concern, because it may result in overexploitation and depletion of localized subpopulations, with a corresponding loss of genetic variation (Carvalho and Hauser 1994; Begg et al. 1999).

The western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae) is found in shallow and deep water reef habitats along the Western Australia coastline, from Cape Leeuwin (34° 22' S) to North West Cape (21° 45' S). It supports one of the most economically important single species fisheries in Australia, with until recently, an annual commercial catch of between 8 000 and 14 500 *t* (Fletcher et al. 2005). A key assumption underlying the management of *P. cygnus* is that the breeding stock comprises a single, demographically united population. This assumption is based on the extended pelagic larval stage of *P. cygnus*, which is thought to ensure high dispersal throughout the species' range. Larvae hatch in spring and early summer, and spend the next nine to eleven months in the plankton, with mid stages being found up to 1500 km offshore. The late stage larvae metamorphose into pueruli and swim inshore to start the juvenile stage of their life-cycle (Phillips et al. 1979). Allozyme studies suggest *P. cygnus* is a single panmictic population, with ephemeral genetic patchiness (small-scale genetic heterogeneity among local populations) caused by temporal variation in allele frequencies of recruits (Thompson et al. 1996; Johnson and Wernham 1999).

The aim of this study was to investigate population structure in adult *P. cygnus* across the main geographic distribution of the species. We also examine the spatial scale over which genetic patchiness occurs, and discuss the management implications of such patterns. To achieve a resolution beyond previous genetic studies, we used 22 microsatellite loci for our study. Microsatellites have proven to be a powerful tool for detecting genetic subdivision within marine species with high larval dispersal capabilities (e.g., Knutsen et al. 2003;

Riccioni et al. 2010; White et al. 2010) and have revealed spatial genetic structure on finer scales than found with allozymes and mtDNA (e.g., Ruzzante et al. 1996; Jørgensen et al. 2005).

Methods

Sample collection

In 2009, tissue samples were collected from juvenile and adult *P. cygnus* (carapace length > 45 mm) at eight locations spanning nearly 660 km along the Western Australian coastline (Fig. 1). A total of 631 individuals were captured using commercial lobster pots. Sample sizes at each location ranged between 21 and 348 individuals, with more intensive sampling carried out at the Houtman Abrolhos Islands to allow investigation of fine-scale patterns. Within the Abrolhos Islands, samples were collected from eight sites between four and 82 km apart (sample sizes ranged between 40 and 68 individuals). The spatial coordinates for each individual were recorded at the time of capture.

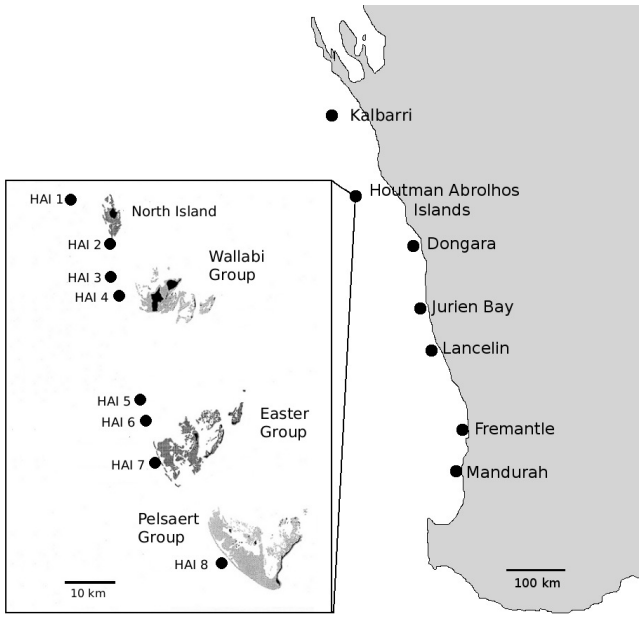

Figure 1 Sites where *P. cygnus* samples were collected.

DNA extraction and microsatellite genotyping

DNA was extracted from the middle lobe of the tail fan stored in 100% ethanol, using a QIAGEN Dneasy Blood and Tissue kit, following the manufacturer's recommendations.

After the DNA was extracted, each sample was analysed using a NanoDrop ND-1000 spectrophotometer to determine the concentration and quality of the DNA. All DNA samples were stored at –20 ºC until genotyping. Genotypes at 22 microsatellite loci (S3, S8, S28, S50, W25, Pcyg1 – 9 and 11– 18) were determined for each individual using primers and PCR running conditions described in Groth et al. (2009) and Kennington et al. (2010). PCR products were analyzed on an ABI 3700 sequencer using a GeneScan-500 LIZ internal size standard and scored using GENEMARKER (SoftGenetics, State College, PA, USA) software.

Data analysis

Microsatellite variation at each location was quantified by calculating allelic richness (a measure of the number of alleles independent of sample size) and gene diversity using the FSTAT version 2.9.3 software package (Goudet 2001). The presence of null alleles was tested for each locus using MICROCHECKER (van Oosterhout et al. 2004). Tests for a deficit or excess in heterozygotes at each location were carried out using randomisation tests, and the results were characterized using the F_{IS} statistic. Significantly positive F_{IS} values indicate a deficit of heterozygotes relative to random mating, and negative values indicate an excess of heterozygotes. Linkage disequilibrium between each pair of loci was assessed by testing the significance of association between genotypes. Corrections for multiple comparisons were carried out using the Sequential Bonferroni method (Rice 1989).

Genetic differentiation among locations was assessed by calculating Weir and Cockerham's (1984) estimator of F_{ST}. Microsatellite R_{ST} values (Slatkin 1995) were also calculated, but were qualitatively similar to F_{ST} values so are not reported. Tests for genetic differentiation were performed by permuting genotypes among samples. Estimates of F_{IS}, F_{ST} and tests for genetic differentiation, deficits in heterozygotes and linkage disequilibrium were calculated using the FSTAT software package. Differences in estimates of genetic variation and F_{IS} among locations were tested using either Wilcoxon's signed-rank tests (for paired comparisons between two groups) or Friedman's ANOVA (for multiple paired comparisons). To test for a relationship between genetic and geographical distance, we compared a matrix of F_{ST} /(1 – F_{ST}) with a matrix of geographical distance (ln km) (Rousset 1997), using a Mantel test with 10 000 permutations.

Spatial genetic structure was also investigated using two Bayesian clustering methods, implemented with the software packages STRUCTURE (Pritchard et al. 2000) and GENELAND (Guillot et al. 2005). Both these programs group individuals into the most likely number of clusters (K) that maximizes the within cluster Hardy-Weinberg and linkage equilibria.

However, GENELAND differs from STRUCTURE in that geographical information can be incorporated to produce more accurate inferences of population structure based on the spatial distribution of individuals. Analyses involving STRUCTURE were based on an ancestry model that assumed admixture and correlated allele frequencies. No prior information about the origin of the samples was used. Ten independent runs were performed for each value of K (1–10), with a burnin of 10 000 followed by 100 000 MCMC iterations. The most likely number of clusters was assessed by comparing the likelihood of the data for different values of K and using the ΔK method of Evanno et al. (2005). For the GENELAND analysis, the spatial coordinates (latitude and longitude) of each individual were used to run the spatial model. The uncertainty of coordinates was set at zero. Ten independent runs were performed for each value of K (1–10) using the uncorrelated and null allele models. Each run consisted of 100 000 MCMC iterations with a thinning of 100 and a burnin of 200. The most likely number of clusters was chosen as the modal K (from each independent run) with the highest posterior probability.

We also carried out spatial autocorrelation (SA) analysis to evaluate the genetic similarity of individuals over varying spatial scales. We used the software package GENALEX version 6 (Peakall and Smouse 2005) to calculate a spatial autocorrelation (r) coefficient for a range of distance classes. The results from the SA analysis were presented in two ways. Firstly, r was plotted as a function of distance class to produce a spatial genetic autocorrelogram. Secondly, because estimates of spatial autocorrelation are influenced by the size of distance classes (see Peakall et al. 2003) , r was calculated for series of increasing distance class sizes. When significant positive spatial structure is present, r will decrease with increasing distance class sizes. The distance class where r no longer differs significantly from zero provides an approximation of the extent of detectable positive spatial genetic structure (Peakall et al. 2003). Tests for statistical significance were performed by random permutation and calculating the bootstrap 95% confidence limits (CL) of r, using 1000 replicates in each case. We also performed a two-dimensional local spatial autocorrelation analysis using GENALEX. With this analysis, the local autocorrelation (lr) is estimated by comparing an individual with its n nearest neighbours, allowing investigation of local patterns of spatial autocorrelation within the two dimensional landscape (Double et al. 2005). Calculations of lr were made using the nearest five, 10, 20 and 50 individuals. As with the global autocorrelation analysis, statistical significance was determined using permutation tests.

Finally, tests for selection acting on marker loci were carried out using the F_{ST} outlier approach (Beaumont and Nichols 1996; Beaumont 2005), implemented with the LOSITAN

software package (Antoa et al. 2008). The method evaluates the relationship between F_{ST} and expected heterozygosity in an island model of migration with neutral markers. This distribution is used to identify loci with excessively high or low F_{ST} values compared to neutral expectations. These loci are candidates for being subject to directional and balancing selection respectively. Simulations were run using 10 000 replications, 99% confidence intervals and the neutral and forced mean options. For this analysis, individuals were grouped by location and both the stepwise and infinite allele mutation models were performed.

Results

High levels of genetic diversity were present at each location. Mean allelic richness ranged from 11.3 to 12.3, and gene diversity ranged from 0.75 to 0.77 (Table 1). There was a significant difference in allelic richness among locations (χ^2 = 14.62, P = 0.041), but not in gene diversity (χ^2 = 4. 30, P = 0.745). Pairwise tests revealed that the significant differences were between Fremantle and all other locations except Kalbarri and Mandurah and between Kalbarri and the Abrolhos Islands (Wilcoxon tests, $P < 0.05$ in all cases). Significant departures from Hardy-Weinberg Equilibrium (HWE) were detected in all samples, with positive F_{IS} values indicating heterozygote deficiencies (Table 1). The departures from HWE appear to be at least partially due to null alleles. Thirteen loci (S3, S8, S50, Pcyg02, Pcyg04, Pcyg06, Pcyg07, Pcyg09, Pcyg12, Pcyg13, Pcyg14, Pcyg16 and Pcyg17) were identified as having null alleles using MICROCHECKER. When these loci were removed from the analysis, no significant departures from HWE were found. No significant differences in F_{IS} were evident among locations (χ^2 = 10.19, P = 0.178). There was also no significant genotypic disequilibrium between pairs of loci after adjusting for multiple comparisons.

There was low, but significant differentiation among locations (F_{ST} = 0.001, P = 0.049). However, all tests of population differentiation between pairs of locations were non-significant (Table 2), and divergences between sampling sites within the Abrolhos Islands were comparable to those observed between locations at the broad geographic scale (pairwise F_{ST} ranged from –0.0029 to 0.0030). There was also no evidence for isolation-by-distance using pairwise F_{ST} values calculated between locations (broad-scale) or between sampling sites within the Abrolhos Islands (local-scale) (Mantel tests, P = 0.342 and 0.400 respectively). No genetic subdivision was found using Bayesian clustering analysis. The STRUCTURE analysis revealed only slight increases in log probability estimates with increasing values of K, and there were no large fluctuations in ΔK, suggesting that the probable number of clusters was one (Fig. 2). Further, when $K > 1$, the proportion of

individuals assigned to each cluster was fairly even (mean alpha ranged from 0.059 to 0.086), and most individuals were admixed, consistent with inferred population structure not being real (Pritchard et al. 2007). The analysis involving GENELAND gave a similar result, with posterior distributions of the estimated number of populations indicating a clear mode at K = 1 in all 10 replicates.

Table 1 Genetic variation at each location.

Site	Mean sample size	A_R (SE)	H (SE)	F_{IS}
Kalbarri	38.1 (0.5)	11.8 (1.5)	0.75 (0.06)	0.24***
Houtman Abrolhos Is.	324.1 (5.4)	12.3 (1.5)	0.76 (0.06)	0.25***
Dongara	37.2 (0.5)	12.0 (1.5)	0.77 (0.06)	0.21***
Jurien Bay	37.3 (0.6)	11.9 (1.5)	0.75 (0.06)	0.21***
Lancelin	51.8 (0.9)	12.2 (1.5)	0.76 (0.06)	0.28***
Rottnest	59.1 (1.5)	12.3 (1.5)	0.76 (0.06)	0.26***
Fremantle	20.0 (0.3)	11.3 (1.4)	0.75 (0.06)	0.26***
Mandurah	20.4 (0.4)	11.6 (1.4)	0.76 (0.06)	0.22***

A_R: allelic richness (based on a sample size of seven or 16 individuals); H: gene diversity. Asterisks refer to the level of significance for tests of heterozygote deficiency ***, $P < 0.001$.

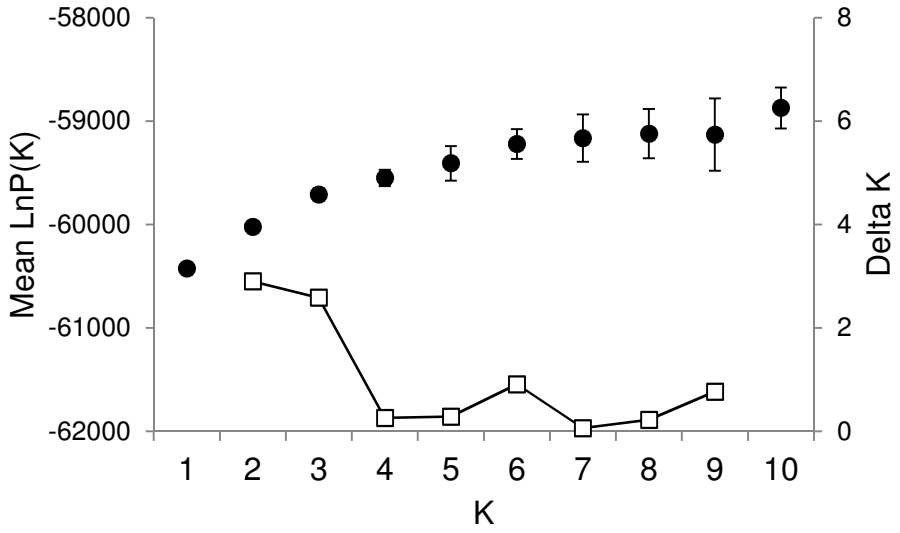

Figure 2 Mean estimates of the log probability (solid circles) and Delta K (open squares) for each K arising from the STRUCTURE analysis. Error bars for L(K) are standard deviations.

Table 2 Pairwise F_{ST} (below diagonal) and *P*-values from tests of population differentiation (above diagonal) between sites. The significance level adjusted for multiple comparisons is 0.0012.

	Kalbarri	Abrolhos	Dongara	Jurien Bay	Lancelin	Rottnest	Femantle	Mandurah
Kalbarri	–	0.0268	0.0875	0.1054	0.3982	0.1571	0.6518	0.2214
Abrolhos	0.0051	–	0.3589	0.5589	0.8429	0.0750	0.2339	0.3018
Dongara	0.0040	0.0005	–	0.1036	0.4839	0.3339	0.4750	0.6214
Jurien Bay	0.0029	0.0001	0.0011	–	0.6482	0.1625	0.4929	0.3000
Lancelin	0.0033	0.0007	0.0012	0.0007	–	0.5571	0.2929	0.4411
Rottnest	–0.0004	0.0018	–0.0015	–0.0014	0.0004	–	0.3018	0.0393
Femantle	–0.0007	0.0016	0.0001	0.0006	0.0008	–0.0008	–	0.2821
Mandurah	0.0013	–0.0009	–0.0026	–0.0049	–0.0002	–0.0013	–0.0022	–

In contrast to the Mantel tests, spatial genetic structure was detected with the SA analyses. Significantly positive *r* values were found within the first two distance classes (0–10 and 11–20 km), after which *r* decreased and did not differ significantly from zero for the remaining distance classes (Fig. 3A). Positive spatial genetic structure at local geographical scales was confirmed when estimates of *r* were calculated with increasing distance class sizes. Figure 3B shows little change in *r* between 10 and 20 km, after which *r* decreased, but remained significant until 150 km. It also appears that positive genetic structure was not confined to one geographical area. Two-dimensional local spatial autocorrelation analysis revealed clusters of positive *lr* at all sampling locations (Fig. 4). A similar number and distribution of positive *lr* values were obtained when calculations were based on sampling the nearest five, 10, 20 and 50 individuals, confirming the consistency of the result (data not shown).

The outlier analyses identified a single locus (Pcgy13) with a high F_{ST} value compared to neutral expectations (after correction for multiple comparisons) when a stepwise mutation model was used. However, none of the loci had higher than expected F_{ST} values under the infinite allele mutation model. Nor were there any loci with lower than expected F_{ST} values using either of the mutation models.

Discussion

The major finding of this study was the significant genetic heterogeneity among local populations in *P. cygnus*, without the presence of large-scale geographic structure. We found extremely low levels of differentiation among locations sampled across the species' range (F_{ST} = 0.001), consistent with extensive gene flow over large geographic distances. The lack of geographic pattern was emphasized by genetic divergences between locations separated by distances over 650 km being no larger than the divergences between sites at the Abrolhos Islands, which are separated by distances less than 85 km. We also found no evidence of isolation-by-distance using pairwise F_{ST} estimates and no genetic subdivision using Bayesian clustering analysis. Our results, therefore, support the findings of previous genetic studies (Thompson et al. 1996; Johnson and Wernham 1999), which suggest that *P. cygnus* is a single, panmictic population.

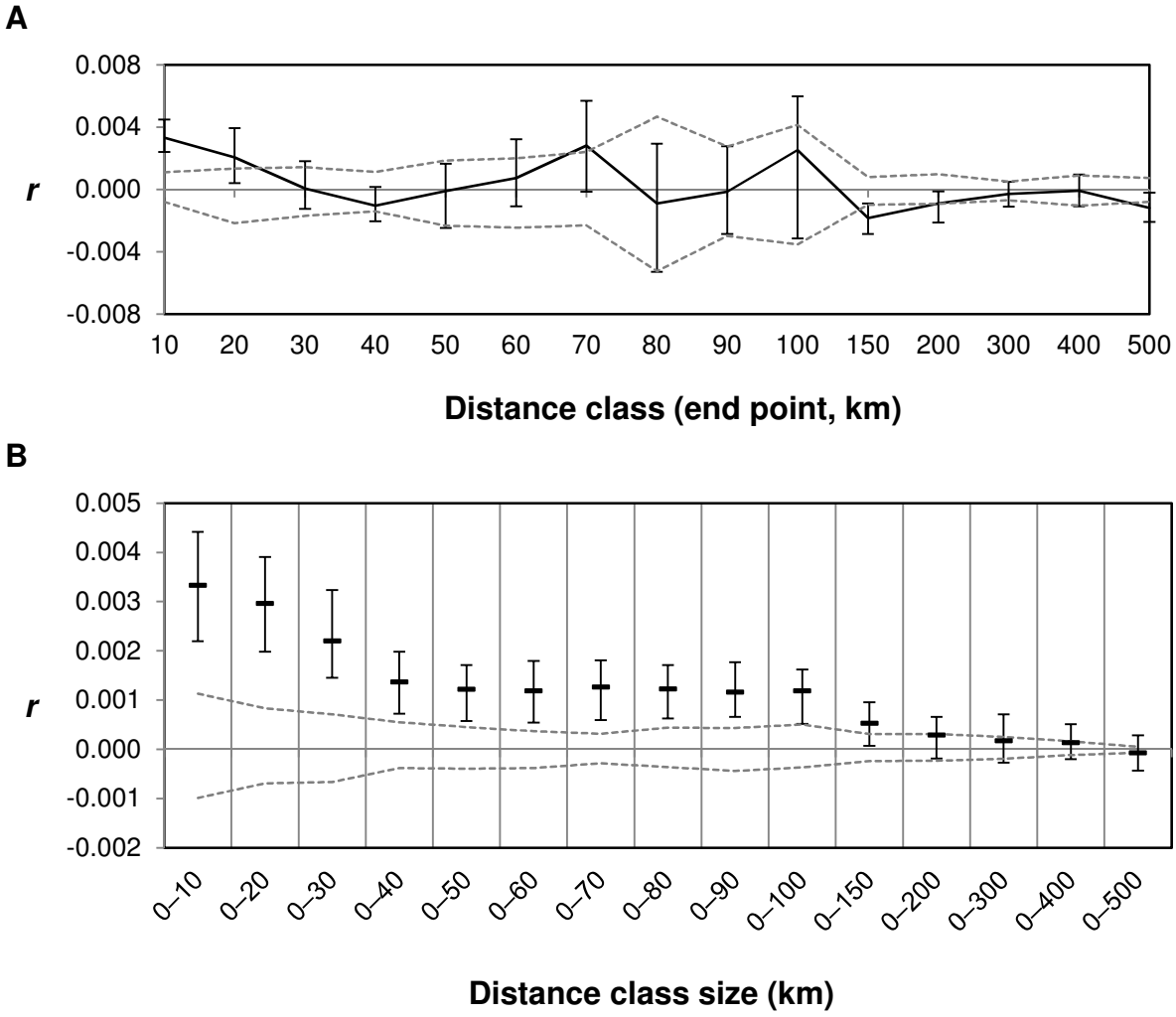

Figure 3 Spatial autocorrelation analyses. (A) Correlogram plot of the genetic correlation coefficient (*r*) as a function of distance. (B) Multiple distance class plot, showing the influence of different distance class sizes on genetic correlation. Permuted 95% confidence interval (dashed lines) and the bootstrap 95% confidence error bars are shown.

Fine-scale population structure in *P. cygnus* was most clearly evident with spatial autocorrelation analysis. Significant genetic structure was observed when lobsters were sampled over distances up to 20 km, with detectable positive spatial genetic structure extending out to 150 km when distance classes were pooled. Further, two-dimensional local SA analysis indicates that these patterns were not driven by the strong influence of one region alone, but were a common feature throughout the species' range. Such microgeographic genetic patchiness has been demonstrated in many marine species with planktonic larvae (e.g., Hedgecock 1994b; Knutsen et al. 2003; Pujolar et al. 2006), including several species

along the Western Australian coast (Johnson and Black 1982; Watts et al. 1990; Johnson et al. 1993; Johnson et al. 2001). Genetic patchiness has also been observed in *P. cygnus* using allozymes (Johnson and Wernham 1999), but the scale of the genetic heterogeneity reported here is much smaller than shown previously. This likely reflects the increased genetic sensitivity and fine-scale geographic information of this study.

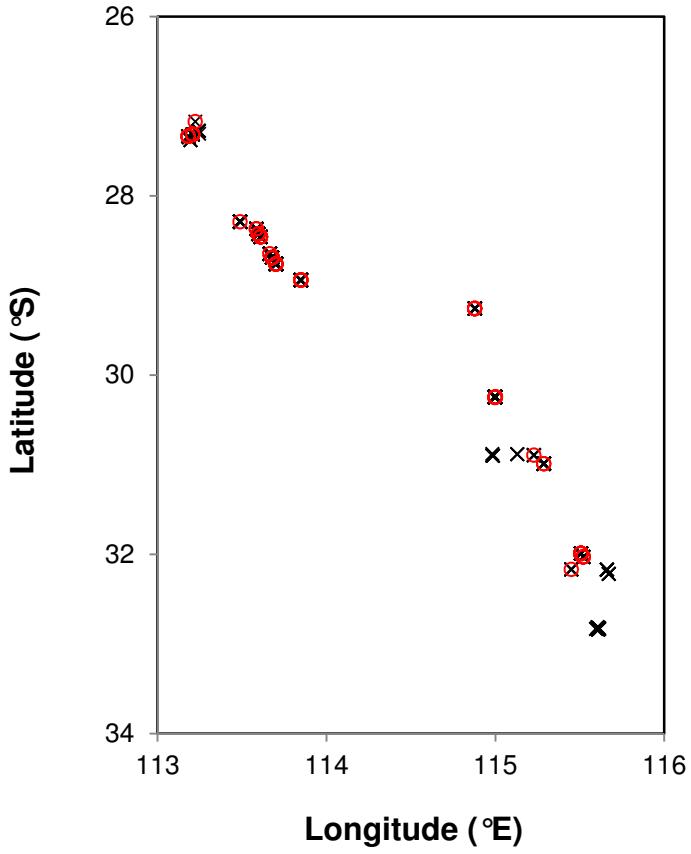

Figure 4 Plot of two-dimensional local spatial autocorrelation analyses. Symbols represent geographical coordinates with significantly positive (red circles) or non-significant (crosses) *lr* values. Calculations of *lr* were based on sampling the nearest 25 individuals.

Spatial genetic patchiness in some species is due to temporal variation in the genetic composition of recruits (Johnson and Black 1984; Watts et al. 1990; Hedgecock 1994a; Pujolar et al. 2006). This also seems to be the case for *P. cygnus*, in which a combination of temporal variation in allele frequencies and contrasting patterns of recruitment resulted in genetically different cohorts of *P. cygnus* at two sites (Johnson and Wernham 1999). Furthermore, this pattern was ephemeral, as it was not repeated in the subsequent two years.

Under the 'sweepstakes reproductive success' hypothesis (Hedgecock 1994a), temporal genetic variance in recruits might be a by-product of large variance in the reproductive success of individuals, owing to chance matching of reproductive activity with oceanographic conditions conducive for larval survival. Other possible explanations for temporal genetic variation in *P. cygnus* recruits are (1) origin from different source populations, (2) limited mixing of larvae in the plankton, or (3) natural selection on larvae prior to settlement. Given the low geographic structure in *P. cygnus*, it is unlikely that temporal genetic variation arises from different source populations. The finding that *P. cygnus* larvae settling at the same time at locations 350 km apart shared the same allele frequencies (Johnson and Wernham 1999) also argues against temporal genetic variation being due to the cohesion of larvae in the plankton, though this result was based on only three allozyme loci.

If a combination of temporal variation in allele frequencies and contrasting patterns of recruitment is responsible for the genetic patchiness observed in this study, it would require juvenile *P. cygnus* to be relatively sedentary. This appears to be the case. Studies on foraging movements suggest juvenile *P. cygnus* forage over relatively small areas (~150 m radius), though the extent of movement is variable (Jernakoff et al. 1987; Jernakoff and Phillips 1988). The life-cycle of *P. cygnus* also includes a migratory phase, which occurs between four and six years of age, just after many lobsters undergo a synchronised moult that changes their normal red shell to a paler colour (Morgan et al. 1982b). During this migration, lobsters leave the coastal reefs and move into deeper water breeding grounds, where they become sedentary again on deeper reefs. Because the lobsters we collected were predominantly from shallow water locations, it is unlikely that they had undertaken these migratory movements. Nevertheless, tag and release experiments have shown that while large movements (>200 km) do occur, most lobsters (>87%) are recaptured within 10 km of their release site (Chubb et al. 1999), which is within the distance range we detected positive population structure. More recently, a study using acoustic telemetry found that only a small proportion (13.6%) of migratory phase lobsters emigrated from their resident reef, suggesting that a mass offshore migration may not hold for all inshore reefs (MacArthur et al. 2008).

Another explanation for spatial genetic patchiness is natural selection acting after settlement (Larson and Julian 1999). Given the broad latitudinal range of *P. cygnus* (> 1200 km), local populations are likely to experience highly varied environmental conditions, providing the opportunity for local adaptations to develop across populations (Kawecki and Ebert 2004). Indeed, several studies have found evidence for local adaptation in widely distributed marine fish (see Nielsen et al. 2009). While we found no clear evidence of

directional selection using outlier analysis, genome scans involving many more neutral markers, candidate genes or population transcriptomics would be needed to exclude confidently this possibility. A study monitoring the genetic composition of cohorts of recruits as they develop into adults would also yield valuable insights on post-settlement processes.

Implications for fisheries management

The implications of genetic patchiness for fisheries management have been discussed by Larson and Julian (1999). If genetic patchiness is due to selection after settlement, they suggest that the implications for fisheries management are minor, unless a fishery is concentrated on one particular habitat or location, which might disproportionately affect a certain portion of the gene pool. By contrast, factors affecting the genetic composition of recruits prior to settlement may have greater consequences. The most relevant of these to *P. cygnus* is the effect of stochastic spatial variation in the sources of successful larvae (sweepstakes reproductive success). This effect implies that the sources of successful larvae vary unpredictably over time. Fisheries management should therefore ensure that both the distribution as well as the total spawning potential of the exploited population is protected. Further, spatial stochasticity of successful spawning argues for either the spatial dispersion of reserves (if they are involved in managing the exploitation), or suitably low exploitation rates across the fishery, thereby increasing the chance that at least some larvae will be released into conditions favourable for their survival. Local genetic patchiness also suggests juvenile and adult lobsters are comparatively sedentary, so they may be more susceptible to environmental/anthropogenic impacts at a finer scale than previously thought.

Another result with implications for management was the lower levels of allelic richness observed in locations at the edge of the fishery compared to those that were more centrally located. Spatial variation in genetic diversity has also been found in *Jasus lalandii*, an exploited rock lobster occurring along the south and west coast of South Africa, prompting calls for the inclusion of sites with high genetic diversity within marine protected areas (Matthee et al. 2007). While the differences in *P. cygnus* were small and do not warrant special consideration, they show there may be differences in the consistency of recruitment and contributions to the larval pool among locations. Such differences may result from the Leeuwin Current. In years when the southward-flowing Leeuwin Current is very strong, peak puerulus settlement occurs further south by up to 2° latitude compared with years of weak Leeuwin Current (Caputi 2008). However, more data are needed to determine whether differences in

genetic diversity among locations are temporally stable. It should also be emphasized that with sweepstakes reproductive success, even populations at the margins of species' ranges can make important contributions to recruitment (Larson and Julian 1999).

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References

Antoa T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: A workbench to detect molecular adaptation based on a F_{st}-outlier method. BMC Bioinformatics 9: 323

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54: 1590-1605

Beaumont MA (2005) Adaptation and speciation: what can F_{st} tell us? Trends Ecol Evol 20: 435-440

Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. Proc R Soc Lond 363: 1619-1626

Begg GA, Friedland KD, Pearce JB (1999) Stock identification and its role in stock assessment and fisheries management: an overview. Fish Res 43: 1-8

Benzie JH, Stoddart JA (1992) Genetic structure of outbreaking and non-outbreaking crown-of-thorns starfish (*Acanthaster planci*) populations on the Great Barrier Reef. Mar Biol 112: 119-130

Bohonak AJ (1999) Dispersal, gene flow and population structure. Q Rev Biol 74: 21-45

Caputi N (2008) Impact of the Leeuwin Current on the spatial distribution of the puerulus settlement of the western rock lobster (*Panulirus cygnus*) and implications for the fishery of Western Australia. Fish Oceanogr 17: 147-152

Carvalho GR, Hauser L (1994) Molecular genetics and the stock concept in fisheries. Rev Fish Biol Fisher 4: 326-350

Chubb CF, Rossbach M, Melville-Smith R, Cheng YW (1999) Mortality, growth and movement of the western rock lobster (*Panulirus cygnus*). Final Report FRDC Project No. 95/020

Double MC, Peakall R, Beck NR, Cockburn A (2005) Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). Evolution 59: 625-635

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-2620

Fletcher W, Chubb C, McCrea J, Caputi N, Webster F, Gould R, Bray T (2005) ESD Report Series No. 4 – Western Rock Lobster Fishery, Perth, WA

García-Rodrguez FJ, Perez-Enriquez R (2008) Lack of genetic differentiation of blue spiny lobster *Panulirus inflatus* along the Pacific coast of Mexico inferred from mtDNA sequences. Mar Ecol Prog Ser 361: 203-212

Gopal K, Tolley KA, Groeneveld JC, Matthee CA (2006) Mitochondrial DNA variation in spiny lobster *Palinurus delagoae* suggests genetically structured populations in the south-western Indian Ocean. Mar Ecol Prog Ser 319: 191-198

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3), pp Available from <http://www.unil.ch/izea/softwares/fstat.html>

Groth DM, Lim F, de Lestang SN, Beale N, Melville-Smith R (2009) Characterization of polymorphic microsatellite loci in the western rock lobster (*Panulirus cygnus*). Conserv Genet Resour 1: 163-166

Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape genetics. Mol Ecol Notes 5: 712-715

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 (1994a) Does variance in reproductive success limit effective population sizes of marine organisms. In: Beaumont AR (ed) Genetics and evolution of aquatic organisms. Chapman and Hall, London, pp 122-134

Hedgecock D (1994b) Spatial and temporal genetic structure of marine animal populations in the California current. CalCOFI Rep 35: 73-81

Jernakoff P, Phillips BF (1988) Effect of a baited trap on the foraging movements of juvenile western rock lobsters, *Panulirus cygnus* George. Aust J Mar Fresh Res 39: 185-192

Jernakoff P, Phillips BF, Maller RA (1987) A quantitative study of nocturnal foraging distances of the western rock lobster *Panulirus cygnus* George. J Exp Mar Biol Ecol 113: 9-21

Johnson MS, Bentley SL, Ford SS, Ladyman MT, Lambert GJ (2001) Effects of a complex archipelago on genetic subdivision of the intertidal limpet *Siphonaria kurracheensis*. Mar Biol 139: 1087-1094

Johnson MS, Black R (1982) Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. Mar Biol 70: 157-164

Johnson MS, Black R (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38: 1371-1383

Johnson MS, Black R (2006a) Effects of mode of reproduction on genetic divergence over large spatial and temporal scales in intertidal snails of the genus *Bembicium* Philippi (Gastropoda: Littorinidae). Biol J Linn Soc 89: 689-704

Johnson MS, Black R (2006b) Islands increase genetic subdivision and disrupt patterns of connectivity of intertidal snails in a complex archipelago. Evolution 60: 2498-2506

Johnson MS, Holborn K, Black R (1993) Fine-scale patchiness and genetic heterogeneity of recruits of the corallivorous gastropod *Drupella cornus*. Mar Biol 117: 91-96

Johnson MS, Wernham J (1999) Temporal variation of recruits as a basis of ephemeral genetic heterogeneity in the western rock lobster *Panulirus cygnus*. Mar Biol 135: 133-139

Jørgensen HBH, Hansen MM, Bekkevold D, Ruzzante DE, Loeschcke V (2005) Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. Mol Ecol 14: 3219-3234

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Lett 7: 1225-1241

Kennington WJ, Levy E, Berry O, Groth DM, Waite AM, Johnson MS, Melville-Smith R (2010) Characterization of 18 polymorphic microsatellite loci for the western rock lobster *Panulirus cygnus*. Conserv Genet Resour 2: 389-391

Knutsen H, Jorde PE, André C, Stenseth NC (2003) Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. Mol Ecol 12: 385-394

Larson RJ, Julian RM (1999) Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. CalCOFI Rep 40: 94-99

Lee HJE, Boulding EG (2009) Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on variation at one mitochondrial and two nuclear DNA markers. Mol Ecol 18: 2165-2184

MacArthur LD, Babcock RC, Hyndes GA (2008) Movements of the western rock lobster (*Panulirus cygnus*) within shallow coastal waters using acoustic telemetry. Mar Freshwater Res 59: 603-613

Matthee CA, Cockcroft AC, Gopal K, von der Heyden S (2007) Mitochondrial DNA variation of the west-coast rock lobster, *Jasus lalandii*: marked genetic diversity differences among sampling sites. Mar Freshwater Res 58: 1130-1135

Morgan GR, Phillips BF, Joll LM (1982) Stock recruitment relationships in *Panulirus cygnus* the commercial rock (spiny) lobster of Western Australia. Fish Bull 80: 475-486

Naro-Maciel E, Reid B, Holmes K, Brumbaugh D, Martin M, DeSalle R (2011) Mitochondrial DNA sequence variation in spiny lobsters: population expansion, panmixia, and divergence. Mar Biol 158: 2027-2041

Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D (2009) Population genomics of marine fishes: identifying adaptive variation in space and time. Mol Ecol 18: 3128-3150

Ovenden JR, Brasher DJ, White RWG (1992) Mitochondrial DNA analyses of the red rock lobster J*asus edwardsii* supports an apparent absence of population subdivision throughout Australasia. Mar Biol 112: 319-326

Palero F, Abellób P, Macpherson E, Gristinad M, Pascual M (2008) Phylogeography of the European spiny lobster (*Palinurus elephas*): Influence of current oceanographical features and historical processes Mol Phylo Evol 48: 708-717

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. Annu Rev Ecol Syst 25: 547-572

Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. Evolution 57: 1182-1195

Peakall R, Smouse PE (2005) GenAlEx V6: Genetic analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra. Available via http://www.anu.edu.au/BoZo/GenAlEx

Perez-Enriquez R, Vega A, Avila S, Sandoval JL (2001) Population genetics of red spiny lobster (*Panulirus interruptus*) along the Baja California Peninsula, Mexico. Mar Freshwater Res 52: 1541-1549

Phillips BF, Brown PA, Rimmev DW, Reid DD (1979) Distribution and dispersal of the phyllosoma larvae of the western rock lobster, *Panulivus cygnus*, in the south-eastern Indian Ocean. Aust J Mar Fresh Res 30: 773-783

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

Pritchard JK, Wen X, Falush D (2007) Documentation for structure software: version 2.2. http://pritbsduchicagoedu/software/structure22/readmepdf

Pujolar JM, Maes GE, Volckaert FAM (2006) Genetic patchiness among recruits in the European eel *Anguilla anguilla*. Mar Ecol Prog Ser 307: 209-217

Riccioni G, Landi M, Ferrara G, Milano I, Cariani A, Zane L, Sella M, Barbujani G, Tinti F (2010) Spatio-temporal population structuring and genetic diversity retention in depleted Atlantic Bluefin tuna of the Mediterranean Sea. Proc Natl Acad Sci USA 107: 2102-2107

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223-225

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145: 1219-1228

Ruzzante DE, Taggart CT, Cook D, Goddard S (1996) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Can J Fish Aquat Sci 53: 634-645

Silberman JD, Sarver SK, Walsh PJ (1994) Mitochondrial DNA variation and population structure in the spiny lobster *Panulirus argus*. Mar Biol 120: 601-608

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457-462

Thompson AP, Hanley JR, Johnson MS (1996) Genetic structure of the western rock lobster, *Panulirus cygnus*, with the benefit of hindsight. Mar Freshwater Res: 889-896

Tolley KA, Groeneveld JC, Gopal K, Matthee CA (2005) Mitochondrial DNA panmixia in spiny lobster *Palinurus gilchristi* suggests a population expansion. Mar Ecol Prog Ser 297: 225-231

van Oosterhout C, Hutchinson, W. F. , Wills DPM, Shipley P (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4: 535-538

Waples RS (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41: 385-400

Watts RJ, Johnson MS, Black R (1990) Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in Western Australia. Mar Biol 105: 145-151

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358-1370

White TA, Stamford J, Hoelzel AR (2010) Local selection and population structure in a deep-sea fish, the roundnose grenadier (*Coryphaenoides rupestris*). Mol Ecol 19: 216-226

APPENDIX 5:

Maintenance of genetic variation and panmixia in the commercially exploited western rock lobster (*Panulirus cygnus*)

(Submitted for publication in *Conservation Genetics*. Currently in review.)

Maintenance of genetic variation and panmixia in the commercially exploited western rock lobster (*Panulirus cygnus*)

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Abstract

Marine species with high fecundities and mortalities in the early life stages can have low effective population sizes, making them vulnerable to declines in genetic diversity when they are commercially harvested. Here, we compare levels of microsatellite and mitochondrial sequence variation in the western rock lobster (*Panulirus cygnus*) over a 14-year period to test whether genetic variation is being maintained. *Panulirus cygnus* is a strong candidate for loss of genetic variation because it is a highly fecund species that is likely to experience high variance in reproductive success due to an extended larval planktonic stage. It also supports one of the largest and most economically important fisheries in Australia, with landings of between 8 000 to 14 500 tonnes (~70% of the total legal-sized biomass) being harvested in some years. We found remarkably high levels of genetic variation in all samples and no evidence of a decline in genetic diversity over the time interval we studied. Furthermore, there was no evidence of a recent genetic bottleneck, and effective population size estimates based on single sample and temporal methods were infinitely large. Analysis of molecular variance indicated no significant population structure along 960 km of coastline or genetic differentiation among temporal samples. Our results support the view that *P. cygnus* is a single, panmictic population, and suggest genetic drift is not strong enough to reduce neutral genetic diversity in this species if current management practices and breeding stock sizes are maintained.

Key words: western rock lobster, genetic variation, effective population size, microsatellites, mtDNA

Introduction

Over-fishing can cause sharp declines in the abundance of target species, and may also affect ecological processes, such as population growth and the structure of food webs (Hutchinson and Reynolds 2004). In addition to adverse ecological consequences, intensive fishing can lead to evolutionary changes over short time scales (e.g., Conover and Munch 2002; Olsen et al. 2004). Several commercially exploited marine species have also shown declines in genetic diversity (Smith et al. 1991; Hauser et al. 2003; Hutchinson et al. 2003). These changes can occur rapidly and are ongoing. For example, in orange roughy (*Hoplostethus atlanticus*), significant declines in genetic diversity were observed over a six year period, and were evident in samples collected after commercial harvesting had begun (Smith et al. 1991). While life-history theory predicts evolutionary changes will take place in harvested fish

species (Jørgensen et al. 2007; Kuparinen and Merilä 2007), reports of declines in genetic diversity were unexpected because even collapsed stocks consist of many millions of individuals, well in excess of the population sizes considered vulnerable to genetic drift. Such declines in genetic diversity are a concern because it potentially results in reduced adaptability, population persistence and productivity (Hauser et al. 2003).

Exploited marine species with large census population sizes (N) are susceptible to loss of genetic diversity because genetic effective population sizes (N_e) can be much lower than the number of individuals in the population (Hutchinson and Reynolds 2004). In fish and shellfish with broadcast spawning, low N_e /N ratios may result from both very high fecundities and mortalities in the early life stages causing high variance in reproductive success, which in turn may result in a low N_e (Hedgecock 1994a). Empirical estimates of N_e suggest the difference between N_e and N can be very large. For example, Hauser et al. (2003) used the decline in heterozygosity and temporal fluctuations in allele frequency to estimate N_e in an exploited population of New Zealand snapper, and found it was five orders of magnitude smaller than N. Ratios of N_e /N in the range of 10^{-5} to 10^{-3} have also been reported in North Sea cod (Hutchinson et al. 2003), red drum (Turner et al. 2002) and plaice (Hoarau et al. 2005).

The western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae) is found along the middle and lower western coast of Australia in shallow and deep water reef habitats (Phillips et al. 1979). It supports one of the most economically important single species fisheries in Australia, with until recently, an annual commercial catch of between 8 000 and 14 500 *t* (Fletcher et al. 2005). This represents estimated exploitation rates greater than 70% of the legal-sized biomass in some years (Wright et al. 2006). *Panulirus cygnus* has a complex life-cycle with an extended larval planktonic stage that promotes mixing across the species' range. After hatching the planktonic phyllosoma larvae are transported more than 1500 km offshore by wind-driven surface currents, where they spend between nine and eleven months at sea before returning to the coast. After one year as planktonic larvae they metamorphose into pueruli, which resemble small lobsters. The pueruli actively migrate inshore to settle on shallow coastal reefs with peak settlement occurring between August and January (Phillips et al. 1979).

Management of the *P. cygnus* fishery is aided by measurements of annual puerulus settlement, which provide a good predictor of commercial catches three and four years later, when they reach legal size (Phillips 1986). Various management strategies have also been introduced to protect the brood stock and sustain egg production (Caputi et al. 2003).

Nevertheless, over the past 35 years the size at maturity has decreased, possibly due to fisheries induced evolution or rising water temperatures associated with climate change (Melville-Smith and de Lestang 2006; Allendorf et al. 2008). The last few years have also seen some of the lowest puerulus settlement abundances on record. It is unknown whether the recent below average and low puerulus settlements are due to long- or short-term environmental changes or depletion of breeding stocks in the north of the fishery (Brown 2009).

The aim of this study is to test whether genetic variation in *P. cygnus* is being maintained. To achieve this objective we used DNA extracted from archived samples of puerulus collected between 1995 and 1999 and a contemporary sample collected in 2009. We also used these samples to estimate the effective population size in *P. cygnus* and test for temporal and spatial genetic differentiation.

Methods

Sample collection

Collections of *P. cygnus* pueruli were provided by the Western Australian Department of Fisheries, which monitors monthly settlement at different sites along the coast to provide a quantitative index of recruitment (Morgan et al. 1982a). Each monitoring location contains several collectors that are sampled over the full moon during the settlement season. After collection the samples were stored in 100% ethanol. Sampling locations used in this study are shown in Figure 1. All sampling locations are within the main *P. cygnus* fishery, except for the two northerly locations of Quobba and Coral Bay.

A total of 365 puerulus were collected in 2009. Of these 130 (Coral Bay, n = 44; Port Gregory, n = 18; Rat Island, n = 18; Seven Mile Beach, n = 28; Jurien Bay, n = 16 and Alkimos, n = 6) were used for mtDNA sequencing, and 277 (Coral Bay, n = 126; Quobba, n = 40; Port Gregory, n = 24; Rat Island, n = 25; Seven Mile Beach, n = 37; Jurien Bay, n = 16; Lancelin, n =3 and Alkimos, n = 6) for microsatellite analysis. The archived material included samples of pueruli from 1995 (n = 40), 1997 (n = 40) and 1999 (n = 49). The 1995 and 1997 samples were from the Seven Mile Beach site only. The 1999 samples came from Rat Island (n = 22), Seven Mile Beach (n = 20) and Alkimos (n = 7). Tissue samples of one adult each of *P. penicillatus* and *P. versicolor* were kindly provided by M.G. Kailis Pty Ltd, Cairns.

DNA extraction

DNA was extracted from the antennae of individual pueruli with a QIAGEN Dneasy Blood and Tissue kit using the manufacturers protocol with the following alterations. Volumes of Buffer ATL and proteinase K were doubled, and samples incubated overnight at 56ºC. Volumes of Buffer AL and 100% ethanol were also doubled, and DNA was eluted using two aliquots of 20 µL of AE buffer heated to 56ºC and incubated for 10 minutes at room temperature before centrifuging. After DNA was extracted, each sample was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific) and stored at –20 ºC until it was used.

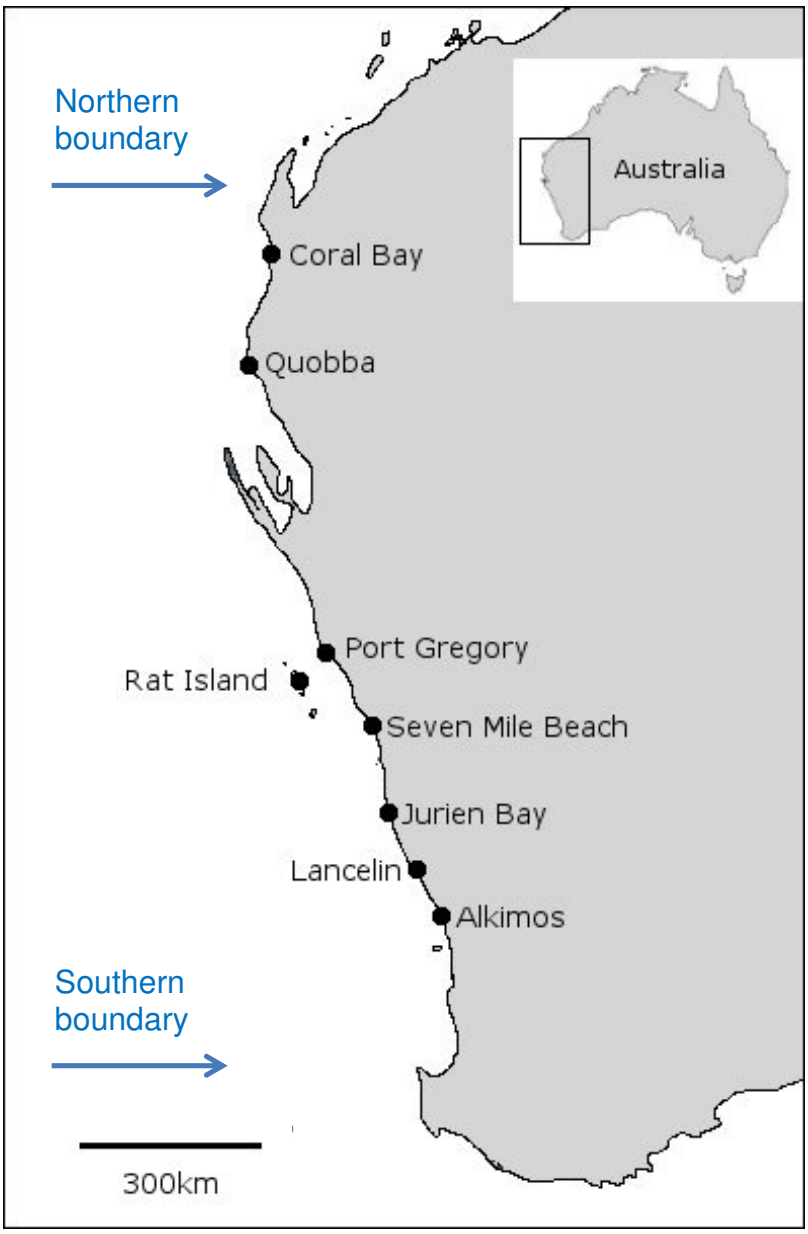

Figure 1 Puerulus sampling locations used in this study. Arrows mark the approximate boundaries of the main fishery.

Mitochondrial DNA sequencing

A 355-bp portion of the mitochondrial 12S rRNA gene was amplified using the primers 12S-R-J-14199 (Kambhampati and Smith 1995) and 12Sai (SR-N-14588) (Simon et al. 1994). Each 25 μL PCR reaction contained 4 mM $MgCl_2$ (Fisher Biotec), 1x Reaction Buffer (Fisher Biotec), 0.2 mM dNTPs (Fisher Biotec), 1.2 μM forward primer, 1.2 μM reverse primer, 55 units of Taq (Fisher Biotec's Tth Plus DNA Polymerase) and 40 ng DNA. Cycling conditions were: an initial denaturation step of 94 ºC for 2 min, followed by 36 cycles of 94 ºC for 30 s, 40 ºC for 90 s and 72 ºC for 60 s, followed by 72 ºC for 10 min. PCR products were purified using an AxyPrep PCR clean up kit (www.axygenbio.com). PCR sequencing reactions were performed in a total volume of 10 μL, and contained 1 μL Big Dye-Terminator (Applied Biosystems), 0.75× sequencing buffer (Applied Biosystems), 0.32 pmol primer and 10–30ng of cleaned PCR product. Sequencing reactions were carried out using the following cycling conditions: 96°C for 2 min, followed by 25 cycles of 96 °C for 10 s; 55 °C for 5 s and 60 °C for 4 min. Products were sequenced on an ABI 3700 sequencer, edited using SEQUENCHER (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned with CLUSTAL W (Thompson et al. 1997) using default parameters.

Microsatellite genotyping

Genotypes at 20 microsatellite loci (S3, S8, S28, S50, W25, Pcyg1– 4, 7– 9, 11– 15 and 17–18) were determined for each individual using primers and PCR running conditions described in Groth *et al.* (2009) and Kennington *et al.* (2010). PCR products were analyzed on an ABI 3700 sequencer using a GeneScan-500 LIZ internal size standard and scored using GENEMARKER (SoftGenetics, State College, PA, USA) software. Re-amplification and scoring of 24 randomly selected individuals from the contemporary (2009) sample and 24 from the historical (1995) sample confirmed that genotype scores were highly repeatable. The mean error rate per allele (the number of allelic mismatches divided by the number of replicated alleles) was 0.037 and 0.027 in the contemporary and historical samples respectively.

Data analysis

Mitochondrial DNA (mtDNA) variation within each sample was quantified by calculating the number of haplotypes, haplotype (gene) diversity and nucleotide diversity using the ARLEQUIN version 3 software package (Excoffier et al. 2005). We also used ARLEQUIN to

calculate the summary statistics *D* (Tajima 1989) and F_S (Fu 1997) to test the deviation of the observed data from neutral predictions expected in a constant-sized population. The possibility of demographic change was also investigated using mismatch distributions (pairwise nucleotide differences between sequences). The dates of putative population expansions were estimated using the formula $T = \tau/2\mu$ (Harpending 1994), where T is the time in generations when the population expansion begins and μ is the specific fragment mutation rate. The mutation rate was calculated using a nucleotide divergence rate estimated for arthropod mitochondrial genes (2.3% per million years, Brower 1994) and allowing for an intraspecific mutation rate that is expected to be 3–10 times faster than the interspecific divergence rate (Emerson 2007).

A measure of the number of haplotypes corrected for sample size was obtained by randomly selecting the smallest number of individuals in any one sample (with replacement) and calculating the number of haplotypes. This was repeated 1000 times for each sample, from which a mean and 95% confidence limits (CLs) were calculated. Evolutionary relationships among mtDNA haplotypes were inferred by neighbour-joining analysis of maximum composite likelihood distance estimates (Tamura et al. 2004) and were conducted using MEGA version 5 software (Tamura et al. 2007). Sequences from *P. penicillatus* and *P. versicolor* were used for interspecies comparisons.

Microsatellite variation within each sample was quantified by calculating allelic richness (a measure of the number of alleles independent of sample size) and gene diversity using the FSTAT version 2.9.3 software package (Goudet 2001). The presence of null alleles was tested for each locus using MICROCHECKER (van Oosterhout et al. 2004). Tests for a deficit or excess of heterozygotes within each sample were carried out using randomisation tests, and the results were characterized using the F_{IS} statistic. Significantly positive F_{IS} values indicate a deficit of heterozygotes relative to random mating and negative values indicate an excess of heterozygotes. Linkage disequilibrium between each pair of loci was assessed by testing the significance of association between genotypes. Genetic differentiation between samples were assessed by calculating Weir and Cockerham's (1984) estimator of F_{ST}. Microsatellite R_{ST} values (Slatkin 1995) were also calculated, but were qualitatively similar to F_{ST} values so are not reported. Estimates of F_{IS}, F_{ST}, tests for deficits in heterozygotes and linkage disequilibrium were calculated using the FSTAT software package. Tests for genetic differentiation among samples were conducted using Analysis of Molecular Variation (AMOVA) with ARLEQUIN. Differences in estimates of genetic variation and F_{IS} among samples were tested using either Wilcoxon's signed-rank tests or Friedman's ANOVAs.

Regression was used to test for declines in genetic variation over time. All statistical analyses were based on samples with at least 15 individuals.

Spatial and temporal genetic structure was also investigated using the Bayesian method of Pritchard *et al.* (2000) and Falush *et al.* (2003) implemented with the program STRUCTURE. This method identifies genetically distinct clusters (*K*) based on allele frequencies across loci. All analyses were based on an ancestry model that assumed admixture and correlated allele frequencies. No prior information about the origin of the samples was used. Ten independent runs were performed for each value of *K* (1–10) with a burnin of 10 000 followed by 100 000 MCMC iterations. The most likely number of clusters was assessed by comparing the likelihood of the data for different values of *K* and using the ΔK method of Evanno *et al.* (2005). We also tested for recent reductions in effective population size in each sample using the software package BOTTLENECK (Piry et al. 1999). Two different methods were used. The first method was based on the principle that that the number of alleles decreases faster than expected heterozygosity after a bottleneck (Maruyama and Fuerst 1985). In this situation, expected heterozygosity should be higher than the equilibrium heterozygosity predicted in a stable population from the observed number of alleles. Following the authors' recommendation for microsatellite data, we used a two-phase model (TPM) with 95% single-step mutation and 5% multiple-step mutations (and a variance among multiple steps of 12). A Wilcoxon signed rank test was used to determine whether each sample had a significant excess of heterozygosity. The second method was a qualitative test based on allele frequency distributions. This test can discriminate between a sample exhibiting a full range of common and rare alleles (producing a typical, L-shaped distribution) and one that has lost rare alleles (producing a shifted distribution), which is indicative of a bottleneck event (Luikart et al. 1998).

Estimates of contemporary effective population size (N_e) were calculated using a single sample (Waples 2006) and a temporal method (Wang and Whitlock 2003). These analyses were carried out using the LDNE (Waples and Do 2008) and MLNE 4 (distributed by J. Wang, http://www.zoo.cam.ac.uk/ioz/ software.htm#MLNE) software programs respectively. Estimates of N_e using the one sample method were performed on all samples and using three critical values of minimum allele frequency (0.01, 0.02 and 0.05). For the temporal method, we used the 1995, 1999 and 2009 samples. Based on the age of reproductive maturity (4.9 – 5.7 years, Chittleborough 1974) and the time elapsed between the temporal samples, the number of generations between samples was set to one and two. The maximum N_e was set to 10 000, and a single closed population was assumed.

Results

Genetic diversity and departures from Hardy-Weinberg equilibrium

Fifty-three unique mtDNA haplotypes were observed among the 252 sequences examined. These haplotype sequences have been deposited in Genbank (Accession nos.: JN813475-JN813529). Overall there were 39 polymorphic sites. The evolutionary relationships among the haplotypes are depicted in Figure 2. There were no well supported clades within *P. cygnus*, and compared to the genetic distances between species (range 0.104 to 0.152), genetic distances between haplotypes within *P. cygnus* were very low (range 0 to 0.019). Estimates of mtDNA variability within samples were consistently high (Table 1). All samples contained at least five haplotypes, with haplotype and nucleotide diversity ranging from 0.40 to 0.81 and 0.0019 to 0.0040 respectively. The most abundant haplotype (Hap1) was found in 58% of the individuals sequenced. The second most abundant haplotype (Hap8) was found in 3.1% of the individuals sequenced. After correcting for sample sizes, there was no evidence of significant differences in the number of haplotypes among collection sites in the 2009 cohort or among the historical and contemporary samples (95% CLs were overlapping in all cases). Similarly, Friedman's ANOVA revealed no significant difference in haplotype diversity among collection sites (χ^2 = 4.30, P = 0.367) or among the historical and contemporary samples (χ^2 = 6.26, P = 0.100).

Genetic diversity estimates based on the microsatellite loci are given in Table 2. There was a significant difference in allelic richness among collection sites in the 2009 sample (χ^2 = 13.06, P = 0.023), but not in gene diversity (χ^2 = 3.04, P = 0.694). Pairwise tests revealed that the significant differences were between Jurien Bay and all other sites except Rat Island, between Rat Island and Coral Bay and between Rat Island and Quobba (Wilcoxon tests, $P <$ 0.05 in all cases). Allelic richness also varied significantly among the historical and contemporary samples (χ^2 = 14.91, P = 0.002), with pairwise tests indicating there were significant differences between the 1995 and 2009 samples, between the 1997 and 1999 samples and between the 1999 and 2009 samples (Wilcoxon tests, $P <$ 0.003 in all cases). However, there was no evidence of a decline in genetic variation over time. All relationships between year and genetic diversity were non-significant and positive (indicating increasing genetic diversity over time). There was no significant difference in gene diversity among the historical and contemporary samples (χ^2 = 6.14, P = 0.105). To test whether the larger geographical range over which the 2009 sample was collected affected the temporal comparisons, we repeated the temporal analyses using a 2009 sample comprising individuals collected from Seven Mile Beach only. The results were similar to the previous analysis,

except that the difference in allelic richness among temporal samples was not significant (χ^2 = 7.47, P = 0.058) and differences in gene diversity were significant (χ^2 = 10.32, P = 0.016). Importantly, all relationships between year and genetic diversity remained non-significant and positive, confirming that the absence of a decline in genetic diversity found in the previous analysis was not an artifact of sampling intensity.

Significant departures from Hardy-Weinberg Equilibrium (HWE) were detected in all samples, with positive F_{IS} values indicating heterozygote deficiencies (Table 2). The departures from HWE appear to be due to null alleles. Nine loci (S3, S8, S50, Pcyg02, Pcyg07, Pcyg09, Pcyg13, Pcyg14 and Pcyg17) were identified as having null alleles using MICROCHECKER. When these loci were removed from the analysis, no significant departures from HWE were found. There were no significant differences in F_{IS} among collection sites in the 2009 sample (χ^2 = 10.24, P = 0.07) or among the historical and contemporary samples (χ^2 = 3.18, P = 0.365). There was also no significant linkage disequilibrium between pairs of loci after adjusting for multiple comparisons.

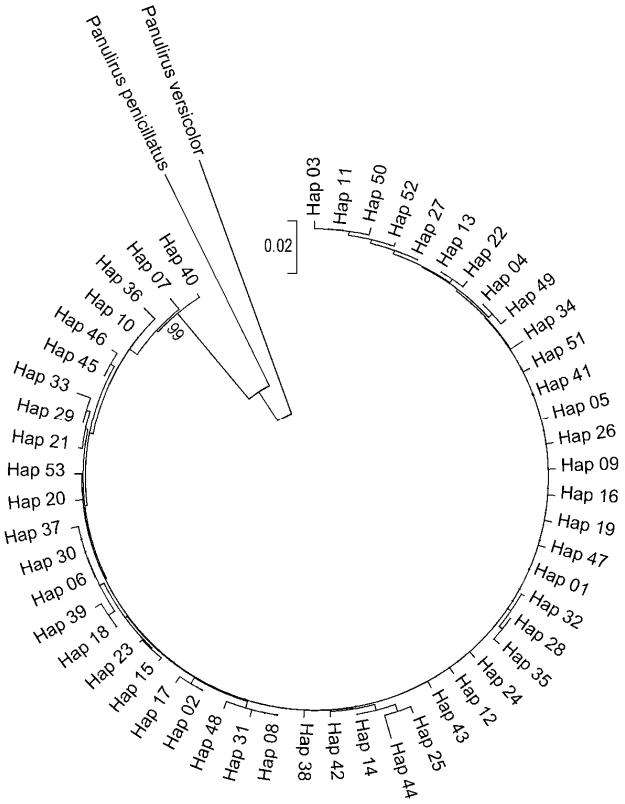

Figure 2. Neighbour-joining tree showing evolutionary relationships among haplotypes. Percentage consensus based on 1000 bootstrap replications of the data is shown for branches where values exceed 50%.

Table 1. Genetic variation within samples and results of neutrality tests based on a 355bp sequence of the 12S mitochondrial gene.

							Mismatch analysis	
Site/year	*n*	N_{Hap}	*h* (SE)	π (SE)	Fu's F_S	Tajima's *D*	SSD	RAG
2009								
Coral Bay	44	13	0.57 (0.09)	0.0024 (0.0019)	-12.0^{***}	-2.4^{***}	0.004	0.094
Port Gregory	18	5	0.40 (0.14)	0.0019 (0.0017)	-2.3^{*}	-2.0^{**}	0.010	0.178
Rat Island	18	10	0.81 (0.09)	0.0031 (0.0024)	-8.6^{***}	-2.1^{**}	0.037	0.249
Seven Mile Beach	27	11	0.66 (0.10)	0.0040 (0.0028)	-6.7^{***}	-1.7^{*}	0.014	0.064
Jurien Bay	16	8	0.70 (0.13)	0.0039 (0.0028)	-4.4^{***}	-2.0^{**}	0.000	0.023
All samples	130	34	0.62 (0.05)	0.0030 (0.0022)	-29.4^{***}	-2.3^{***}	0.003	0.060
1995	38	10	0.53 (0.10)	0.0023 (0.0019)	-7.4^{***}	-2.1^{**}	0.001	0.070
1997	40	14	0.78 (0.06)	0.0038 (0.0026)	-10.3^{***}	-2.1^{**}	0.015	0.130^{*}
1999	44	18	0.72 (0.07)	0.0034 (0.0024)	-18.8^{***}	-2.1^{**}	0.002	0.068

n, sample size; N_{Hap}, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity; SSD, sum of squared deviations; RAG, raggedness index. Asterisks refer to the level of significance of tests for departures from neutrality (Fu's F_S Tajima's *D*) or a model of population expansion (mismatch analysis) *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 2 Genetic variation within samples based on 20 microsatellite DNA loci.

Site/year	Sample size	A_R (SE)	H (SE)	F_{IS}
2009				
Coral Bay	121.6 (1.4)	7.1 (0.8)	0.74 (0.06)	0.21***
Quobba	38.8 (0.5)	7.1 (0.8)	0.74 (0.06)	0.19***
Port Gregory	23.0 (0.4)	7.0 (0.7)	0.74 (0.06)	0.21***
Rat Island	23.6 (0.3)	6.7 (0.7)	0.72 (0.06)	0.23***
Seven Mile Beach	35.2 (0.5)	7.0 (0.7)	0.75 (0.06)	0.20***
Jurien Bay	13.9 (0.7)	6.5 (0.7)	0.72 (0.07)	0.28***
All samples	264.5 (3.2)	14.2 (2.0)	0.74 (0.06)	0.21***
1995	37.5 (0.9)	13.1 (1.9)	0.74 (0.06)	0.24***
1997	37.1 (0.8)	13.9 (2.0)	0.73 (0.06)	0.25***
1999	42.9 (1.2)	12.4 (1.7)	0.72 (0.06)	0.25***

A_R: allelic richness (based on a sample size of seven or 24 individuals for the sites within the 2009 sample and the combined samples for each year respectively); H: gene diversity. Asterisks refer to the level of significance of tests for heterozygote deficiency ***, $P < 0.001$.

Genetic differentiation among samples

There was no evidence of spatial or temporal genetic structure in the mtDNA or microsatellite data sets. Overall Φ_{ST} values based on mtDNA data were low and nonsignificant among sites (Φ_{ST} = –0.002, P = 0.569) and among years (Φ_{ST} = 0.002, P = 0.095). Similar patterns were observed with F_{ST} estimates based on microsatellites (F_{ST} = 0.003, P = 0.464 and F_{ST} = 0.003, P = 0.124 for the spatial and temporal comparisons respectively). When appropriate sample sizes were available (n > 10 individuals), we also tested for genetic differentiation among monthly cohorts within sites. Again, these analyses failed to detect significant differentiation among samples using mtDNA (Coral Bay, Φ_{ST} = –0.001, P = 0.502; Seven Mile Beach, Φ_{ST} = –0.029, P = 0.861) or microsatellite data (Coral Bay, F_{ST} = 0.001, P = 0.553; Seven Mile Beach, F_{ST} = 0.000, P = 0.691).

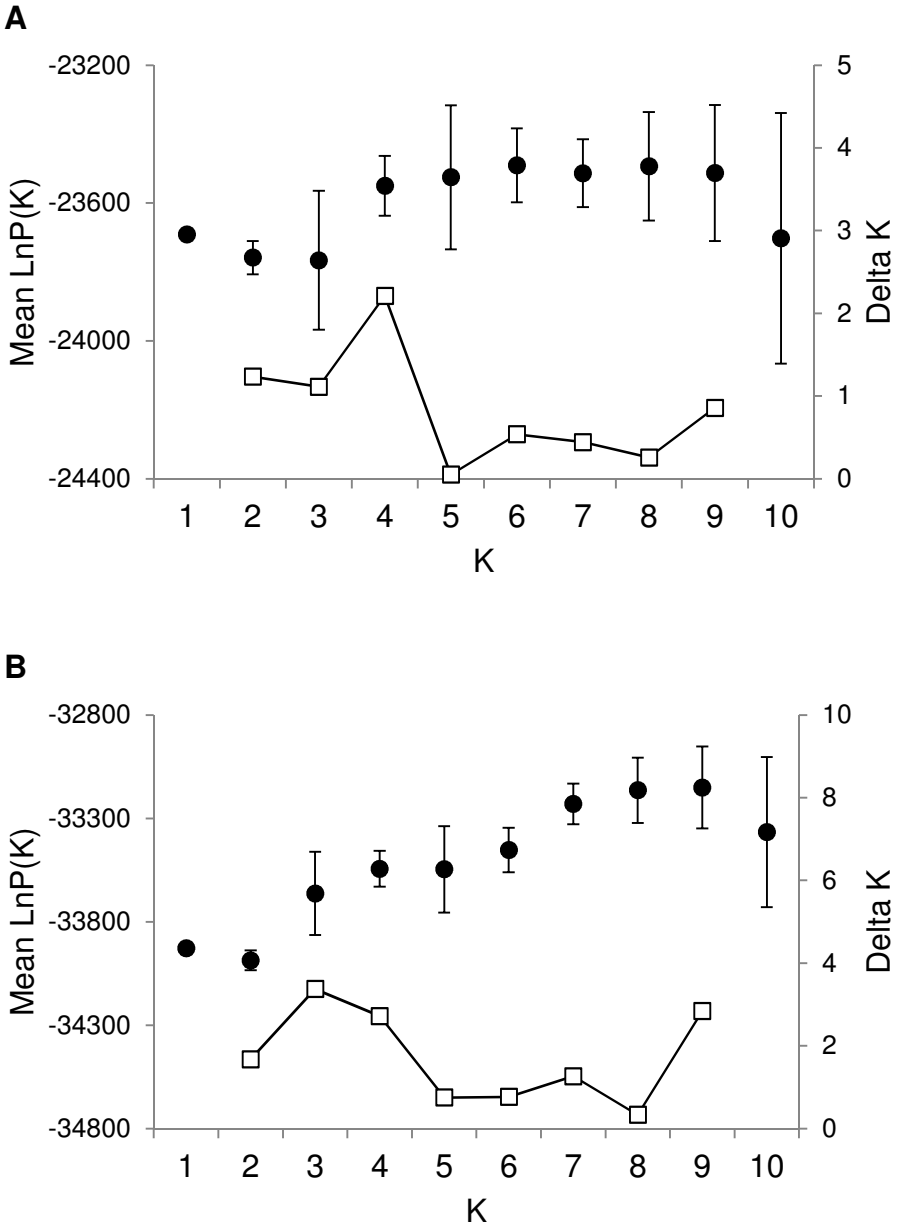

Figure 3 Mean estimates of the log probability (solid circles) and Delta K (open squares) for each K arising from the Bayesian clustering analysis of the (A) 2009 and (B) temporal microsatellite data. Error bars for L(K) are standard deviations.

Results from the Bayesian clustering analysis were consistent with those obtained using AMOVA. For both the spatial and temporal microsatellite data, the initial decline in log probability estimates and small fluctuations in ΔK indicated that the probable number of clusters was one (Figure 3). Further, when $K > 1$, we found that the proportion of individuals assigned to each cluster was fairly even, and most individuals were admixed, as expected when the population structure is not real (Pritchard et al. 2007).

Neutrality tests, demographic history and estimates of effective population size

The results of Tajima's *D* test and Fu's F_S test are presented in Table 1. Tajima's *D* values were negative for all samples, indicating an excess of rare nucleotide site variants compared to the expectation under a neutral model of evolution. Fu's F_S tests also showed negative values for all samples, indicating an excess of rare haplotypes over what would be expected under neutrality. Both these results are consistent with rapid population growth or genetic hitch-hiking. The observed mismatch distributions also suggested there has been population growth. In all samples except 1997, SSD and raggedness indices did not differ significantly from a distribution simulated under a model of rapid population expansion (Table 1). Estimates of tau from the mismatch distribution ranged from 0.8 to 2.6 in the contemporary samples and 0.9 to 1.3 in the historical samples. Based on the lower mutation rate, these estimates of tau imply that the population expansion began 3 265 to 10 614 generations (~ 16 300 to 53 000 years) ago. The higher mutation rate suggests the expansion began 980 to 3 184 generations (~4 900 to 16 000 years) ago.

There was no evidence for recent bottleneck events within any sample. No significant heterozygosity excesses were detected (Wilcoxon *P*-values ranged between 0.834 and 0.999). Furthermore, all samples exhibited normal L-shaped distributions, as expected under mutation-drift equilibrium. All estimates of N_e calculated using the single sample and temporal methods were negative, indicating they were infinitely large.

Discussion

This study shows that *P. cygnus* retains high levels of neutral genetic diversity despite being heavily exploited and having recent declines in recruitment. Using samples of pueruli collected between 1995 and 2009, we found differences in allelic richness between collection years, but no evidence of loss of genetic diversity or increases in the level of inbreeding. Indeed, in the few cases where significant differences were found between samples, genetic diversity was higher in the more recently collected samples. However, it should be recognized that our study did not include samples taken before the commencement of commercial harvesting or during the initial stages of exploitation, when loss of diversity may be more pronounced (Ryman et al. 1995). Hence we cannot rule out the possibility that some genetic variation has been lost due to fishing.

The maintenance of genetic diversity in *P. cygnus* most likely reflects very large effective population sizes. Estimates of N_e obtained in this study were infinite. We also did not detect any significant temporal variation in haplotype or allele frequencies, indicating

genetic drift was negligible. This contrasts with estimates of N_e of less than 200 in a population of New Zealand snapper, which showed significant declines in genetic diversity and temporal fluctuations in allele frequencies (Hauser et al. 2003). In northern pike, declines in heterozygosity were reported in a population with estimates of N_e ranging from 35 to 72 (Miller and Kapuscinski 1997), and significant declines in the number of alleles across loci were detected in a population of North Sea cod over time intervals similar to this study, with estimates of N_e ranging from 69 to 121 (Hutchinson et al. 2003). Nevertheless, given the large variance in individual reproductive success expected with broadcast spawning (Hedgecock 1994a), N_e for *P. cygnus* is likely to be much lower than the census population size. It is also worth noting that several marine species have undergone heavy exploitation and retained genetic diversity despite low N_e/N ratios (Consuegra et al. 2005; Hoarau et al. 2005; Riccioni et al. 2010; Cuveliers et al. 2011). Riccioni *et al.* (2010) suggested that gene flow, overlapping generations and high mutation rates may explain the maintenance of variation in Atlantic Bluefin tuna. Some of these factors may also be contributing to the maintenance of genetic variation in *P. cygnus*.

Despite a severe downturn in *P. cygnus* recruitment particularly between the 2007/08 to 2009/10 seasons, we found no evidence of recent severe reductions in effective population size. Indeed, the demographic history inferred from mtDNA sequences suggests *P. cygnus* has undergone a population expansion. This is a consistent pattern observed in a range of spiny lobster species from both the northern and southern hemispheres (Tolley et al. 2005; Gopal et al. 2006; García-Rodrguez and Perez-Enriquez 2008; Paleroa et al. 2008; Naro-Maciel et al. 2011). Expansion events in these species have been attributed to sea level and temperature changes at the end of the last glacial maximum that led to increases in suitable shallow water habitats (Naro-Maciel et al. 2011). Our data suggest the population expansion in *P. cygnus* began 4,900 to 53,000 years ago, which is also consistent with a postglacial population expansion.

The absence of spatial genetic structure in *P. cygnus* puerulus is consistent with larval mixing during the extended planktonic stage (Phillips et al. 1979). Lack of population structure has been reported in other spiny lobsters (Ovenden et al. 1992; Tolley et al. 2005; García-Rodrguez and Perez-Enriquez 2008; Naro-Maciel et al. 2011), but there are exceptions (Perez-Enriquez et al. 2001; Gopal et al. 2006; Paleroa et al. 2008). In the exceptional species, geographic barriers and oceanic currents may be restricting gene flow. For example, Gopal et al.(2006) found genetic differences among northern and southern populations of *Palinurus delagoae* along the southeastern African coast and between coastal

populations and those occurring on the African continental shelf, which they attributed to oceanic currents forming a barrier to gene flow and possible reproductive barriers. In *Palinurus elephas,* genetic differentiation was found between Atlantic and Mediterranean populations, which was attributed to restricted gene flow through the Straits of Gibraltar (Paleroa et al. 2008). Genetic differences between continental Europe and Irish-Scottish populations and among Mediterranean populations were also found and were attributed to the Gulf Stream and mesoscale processes resulting from incoming Atlantic waters respectively (Paleroa et al. 2008). In contrast to these studies, the boundary current off the Western Australian coast is relatively uninterrupted by complex coastal topography, though there are some high larval retention areas associated with coastal geomorphic features (Feng et al. 2010).

Consistent with our findings, allozyme studies on *P. cygnus* found high genetic similarity among populations over large spatial scales (Thompson et al. 1996; Johnson and Wernham 1999). However, those studies also reported minor genetic differences among local populations that were ephemeral. Such patterns can result from temporal genetic differences in the larval pool combined with patchy settlement (Johnson and Black 1984; Johnson and Wernham 1999). Low samples sizes prevented comparisons between monthly cohorts at most sites in our study. Nevertheless, in the few tests we performed, no significant differences in allele or haplotype frequencies were found, although we did detect heterozygote deficiencies in all samples, which may have resulted from spatial or temporal structure within samples (Addison and Hart 2005). It may be that greater sample sizes and more extensive sampling are needed to detect temporal genetic variation amongst monthly cohorts. Alternatively, it may be that differences between cohorts are more likely found with allozymes than microsatellites and mtDNA. This might be expected if temporal genetic variation of recruits results from natural selection.

In conclusion, mtDNA and microsatellite markers show high levels of genetic diversity in contemporary and historical samples of *P. cygnus*. The maintenance of genetic variation over time and lack of significant temporal variation in haplotype and allele frequencies suggest large effective population sizes in *P. cygnus.* Our analyses also support the view that *P. cygnus* is single, panmictic population. While further research is needed to ascertain the basis and long-term consequences of recent reductions in recruitment, loss of neutral genetic variation appears unlikely if current management practices and breeding stock sizes are maintained.

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References

Antoa T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: A workbench to detect molecular adaptation based on a F_{st}-outlier method. BMC Bioinformatics 9: 323

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54: 1590-1605

Beaumont MA (2005) Adaptation and speciation: what can F_{st} tell us? Trends Ecol Evol 20: 435-440

Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. Proc R Soc Lond 363: 1619-1626

Begg GA, Friedland KD, Pearce JB (1999) Stock identification and its role in stock assessment and fisheries management: an overview. Fish Res 43: 1-8

Benzie JH, Stoddart JA (1992) Genetic structure of outbreaking and non-outbreaking crown-of-thorns starfish (*Acanthaster planci*) populations on the Great Barrier Reef. Mar Biol 112: 119-130

Bohonak AJ (1999) Dispersal, gene flow and population structure. Q Rev Biol 74: 21-45

Caputi N (2008) Impact of the Leeuwin Current on the spatial distribution of the puerulus settlement of the western rock lobster (*Panulirus cygnus*) and implications for the fishery of Western Australia. Fish Oceanogr 17: 147-152

Carvalho GR, Hauser L (1994) Molecular genetics and the stock concept in fisheries. Rev Fish Biol Fisher 4: 326-350

Chubb CF, Rossbach M, Melville-Smith R, Cheng YW (1999) Mortality, growth and movement of the western rock lobster (*Panulirus cygnus*). Final Report FRDC Project No. 95/020

Double MC, Peakall R, Beck NR, Cockburn A (2005) Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). Evolution 59: 625-635

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-2620

Fletcher W, Chubb C, McCrea J, Caputi N, Webster F, Gould R, Bray T (2005) ESD Report Series No. 4 – Western Rock Lobster Fishery, Perth, WA

García-Rodrguez FJ, Perez-Enriquez R (2008) Lack of genetic differentiation of blue spiny lobster *Panulirus inflatus* along the Pacific coast of Mexico inferred from mtDNA sequences. Mar Ecol Prog Ser 361: 203-212

Gopal K, Tolley KA, Groeneveld JC, Matthee CA (2006) Mitochondrial DNA variation in spiny lobster *Palinurus delagoae* suggests genetically structured populations in the south-western Indian Ocean. Mar Ecol Prog Ser 319: 191-198

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3), pp Available from <http://www.unil.ch/izea/softwares/fstat.html>

Groth DM, Lim F, de Lestang SN, Beale N, Melville-Smith R (2009) Characterization of polymorphic microsatellite loci in the western rock lobster (*Panulirus cygnus*). Conserv Genet Resour 1: 163-166

Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape genetics. Mol Ecol Notes 5: 712-715

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 (1994a) Does variance in reproductive success limit effective population sizes of marine organisms. In: Beaumont AR (ed) Genetics and evolution of aquatic organisms. Chapman and Hall, London, pp 122-134

Hedgecock D (1994b) Spatial and temporal genetic structure of marine animal populations in the California current. CalCOFI Rep 35: 73-81

Jernakoff P, Phillips BF (1988) Effect of a baited trap on the foraging movements of juvenile western rock lobsters, *Panulirus cygnus* George. Aust J Mar Fresh Res 39: 185-192

Jernakoff P, Phillips BF, Maller RA (1987) A quantitative study of nocturnal foraging distances of the western rock lobster *Panulirus cygnus* George. J Exp Mar Biol Ecol 113: 9-21

Johnson MS, Bentley SL, Ford SS, Ladyman MT, Lambert GJ (2001) Effects of a complex archipelago on genetic subdivision of the intertidal limpet *Siphonaria kurracheensis*. Mar Biol 139: 1087-1094

Johnson MS, Black R (1982) Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. Mar Biol 70: 157-164

Johnson MS, Black R (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38: 1371-1383

Johnson MS, Black R (2006a) Effects of mode of reproduction on genetic divergence over large spatial and temporal scales in intertidal snails of the genus *Bembicium* Philippi (Gastropoda: Littorinidae). Biol J Linn Soc 89: 689-704

Johnson MS, Black R (2006b) Islands increase genetic subdivision and disrupt patterns of connectivity of intertidal snails in a complex archipelago. Evolution 60: 2498-2506

Johnson MS, Holborn K, Black R (1993) Fine-scale patchiness and genetic heterogeneity of recruits of the corallivorous gastropod *Drupella cornus*. Mar Biol 117: 91-96

Johnson MS, Wernham J (1999) Temporal variation of recruits as a basis of ephemeral genetic heterogeneity in the western rock lobster *Panulirus cygnus*. Mar Biol 135: 133-139

Jørgensen HBH, Hansen MM, Bekkevold D, Ruzzante DE, Loeschcke V (2005) Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. Mol Ecol 14: 3219-3234

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Lett 7: 1225-1241

Kennington WJ, Levy E, Berry O, Groth DM, Waite AM, Johnson MS, Melville-Smith R (2010) Characterization of 18 polymorphic microsatellite loci for the western rock lobster *Panulirus cygnus*. Conserv Genet Resour 2: 389-391

Knutsen H, Jorde PE, André C, Stenseth NC (2003) Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. Mol Ecol 12: 385-394

Larson RJ, Julian RM (1999) Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. CalCOFI Rep 40: 94-99

Lee HJE, Boulding EG (2009) Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on variation at one mitochondrial and two nuclear DNA markers. Mol Ecol 18: 2165-2184

MacArthur LD, Babcock RC, Hyndes GA (2008) Movements of the western rock lobster (*Panulirus cygnus*) within shallow coastal waters using acoustic telemetry. Mar Freshwater Res 59: 603-613

Matthee CA, Cockcroft AC, Gopal K, von der Heyden S (2007) Mitochondrial DNA variation of the west-coast rock lobster, *Jasus lalandii*: marked genetic diversity differences among sampling sites. Mar Freshwater Res 58: 1130-1135

Morgan GR, Phillips BF, Joll LM (1982) Stock recruitment relationships in *Panulirus cygnus* the commercial rock (spiny) lobster of Western Australia. Fish Bull 80: 475-486

Naro-Maciel E, Reid B, Holmes K, Brumbaugh D, Martin M, DeSalle R (2011) Mitochondrial DNA sequence variation in spiny lobsters: population expansion, panmixia, and divergence. Mar Biol 158: 2027-2041

Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D (2009) Population genomics of marine fishes: identifying adaptive variation in space and time. Mol Ecol 18: 3128-3150

Ovenden JR, Brasher DJ, White RWG (1992) Mitochondrial DNA analyses of the red rock lobster J*asus edwardsii* supports an apparent absence of population subdivision throughout Australasia. Mar Biol 112: 319-326

Palero F, Abellób P, Macpherson E, Gristinad M, Pascual M (2008) Phylogeography of the European spiny lobster (*Palinurus elephas*): Influence of current oceanographical features and historical processes Mol Phylo Evol 48: 708-717

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. Annu Rev Ecol Syst 25: 547-572

Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. Evolution 57: 1182-1195

Peakall R, Smouse PE (2005) GenAlEx V6: Genetic analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra. Available via http://www.anu.edu.au/BoZo/GenAlEx

Perez-Enriquez R, Vega A, Avila S, Sandoval JL (2001) Population genetics of red spiny lobster (*Panulirus interruptus*) along the Baja California Peninsula, Mexico. Mar Freshwater Res 52: 1541-1549

Phillips BF, Brown PA, Rimmev DW, Reid DD (1979) Distribution and dispersal of the phyllosoma larvae of the western rock lobster, *Panulivus cygnus*, in the south-eastern Indian Ocean. Aust J Mar Fresh Res 30: 773-783

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

Pritchard JK, Wen X, Falush D (2007) Documentation for structure software: version 2.2. http://pritbsduchicagoedu/software/structure22/readmepdf

Pujolar JM, Maes GE, Volckaert FAM (2006) Genetic patchiness among recruits in the European eel *Anguilla anguilla*. Mar Ecol Prog Ser 307: 209-217

Riccioni G, Landi M, Ferrara G, Milano I, Cariani A, Zane L, Sella M, Barbujani G, Tinti F (2010) Spatio-temporal population structuring and genetic diversity retention in depleted Atlantic Bluefin tuna of the Mediterranean Sea. Proc Natl Acad Sci USA 107: 2102-2107

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223-225

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145: 1219-1228

Ruzzante DE, Taggart CT, Cook D, Goddard S (1996) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Can J Fish Aquat Sci 53: 634-645

Silberman JD, Sarver SK, Walsh PJ (1994) Mitochondrial DNA variation and population structure in the spiny lobster *Panulirus argus*. Mar Biol 120: 601-608

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457-462

Thompson AP, Hanley JR, Johnson MS (1996) Genetic structure of the western rock lobster, *Panulirus cygnus*, with the benefit of hindsight. Mar Freshwater Res: 889-896

Tolley KA, Groeneveld JC, Gopal K, Matthee CA (2005) Mitochondrial DNA panmixia in spiny lobster *Palinurus gilchristi* suggests a population expansion. Mar Ecol Prog Ser 297: 225-231

van Oosterhout C, Hutchinson, W. F. , Wills DPM, Shipley P (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4: 535-538

Waples RS (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41: 385-400

Watts RJ, Johnson MS, Black R (1990) Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in Western Australia. Mar Biol 105: 145-151

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358-1370

White TA, Stamford J, Hoelzel AR (2010) Local selection and population structure in a deep-sea fish, the roundnose grenadier (*Coryphaenoides rupestris*). Mol Ecol 19: 216-226.