RESEARCH ARTICLE

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 and 14,500 tons (∼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.

Keywords 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 (Hauser et al. 2003; Hutchinson et al. 2003; Smith et al. 1991). These changes can occur rapidly and are ongoing. For example, in orange roughy (*Hoplostethus atlanticus*), significant declines in genetic diversity were observed over a 6 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

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millions of individuals, well in excess of the population sizes considered vulnerable to genetic drift. Such declines in genetic diversity are a concern because they potentially result 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 1994). 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}–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 1,500 km offshore by wind-driven surface currents, where they spend between 9 and 11 months at sea before returning to the coast. After 1 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 3 and 4 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 (Allendorf et al. 2008; Melville-Smith and de Lestang

2006). 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.

Materials and 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. 1982). 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 Fig. 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 56 °C. Volumes of

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

Buffer AL and 100 % ethanol were also doubled, and DNA was eluted using two aliquots of 20 μL of AE buffer heated to 56 °C and incubated for 10 min 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 °C until it was used.

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). We chose this marker because it consistently provided high quality DNA sequences for analysis and exhibits high levels of variation. Each 25 μL PCR reaction contained 4 mM MgCl$_2$ (Fisher Biotec), 1× reaction buffer (Fisher Biotec), 0.2 mM dNTPs (Fisher Biotec), 1.2 μM forward primer, 1.2 μM reverse primer, 55 U of Taq (Fisher Biotec's Tth Plus DNA Polymerase) and 40 ng DNA. Cycling conditions were: an initial denaturation step of 94 °C for 2 min, followed by 36 cycles of 94 °C for 30 s, 40 °C for 90 s and 72 °C for 60 s, followed by 72 °C 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 10–30 ng 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). We also used the divergence rate of 0.88–2.2 % per million years, which was estimated for the mitochondrial 16S rRNA gene in marine crustaceans (Cunningham et al. 1992; Schubart et al. 1998).

Following Palero et al. (2008), intraspecific mutation rates were assumed to be 3–10 times faster than the interspecific 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 1,000 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 genotypic 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 Friedman's ANOVAs. 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 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 38,000, and a single closed population was assumed.

Results

Genetic diversity and departures from Hardy–Weinberg equilibrium (HWE)

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 Fig. 2. There were no well supported clades within *P. cygnus*, and compared to the genetic distances between species (range 0.104–0.152), genetic distances between haplotypes within *P. cygnus* were very low (range 0–0.019). 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 ($\chi^2 = 4.30$, $P = 0.367$) or among the historical and contemporary samples ($\chi^2 = 6.26$, $P = 0.100$).

Nine microsatellite loci (S3, S8, S50, Pcyg02, Pcyg07, Pcyg09, Pcyg13, Pcyg14 and Pcyg17) were found to have null alleles using MICROCHECKER and were therefore excluded from further analysis. Genetic diversity estimates for the remaining loci are given in Table 2. There were no significant differences in allelic richness ($\chi^2 = 5.90$, $P = 0.316$) or gene diversity ($\chi^2 = 1.87$, $P = 0.867$) among collection sites in the 2009 sample. There were also

no significant differences in allelic richness ($\chi^2 = 4.05$, $P = 0.257$) or gene diversity ($\chi^2 = 3.08$, $P = 0.379$) among the temporal samples. For the temporal comparisons all samples within each year were pooled and allelic richness was calculated using the minimum sample size in an individual sampling year (24 individuals). 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. For this analysis we re-calculated the allelic richness for the 2009 Seven Mile Beach sample using the same minimum sample size used to calculate allelic richness in the temporal samples ($n = 24$). The results were similar to the previous analysis, with no significant differences in allelic richness ($\chi^2 = 4.76$, $P = 0.190$) or gene diversity ($\chi^2 = 5.25$, $P = 0.154$) found among temporal samples. Tests using all 20 microsatellite loci (i.e. including those with null alleles) also failed to detect any evidence of a decline in allelic richness or gene diversity over time (data not shown).

Most samples were in HWE. The only exception was the 1997 temporal sample, which had a significantly positive overall F_{IS} value, indicating a deficit of heterozygotes (Table 2). There were no significant differences in F_{IS} among collection sites in the 2009 sample ($\chi^2 = 7.06$, $P = 0.216$) or among the temporal samples ($\chi^2 = 1.36$, $P = 0.714$). There was also no significant genotypic disequilibrium between pairs of loci after adjusting for multiple comparisons.

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 ($F_{ST} = -0.002$, $P = 0.997$ and $F_{ST} = 0.001$, $P = 0.093$ 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, $F_{ST} = -0.001$, $P = 0.715$; Seven Mile Beach, $F_{ST} = -0.003$, $P = 0.581$).

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
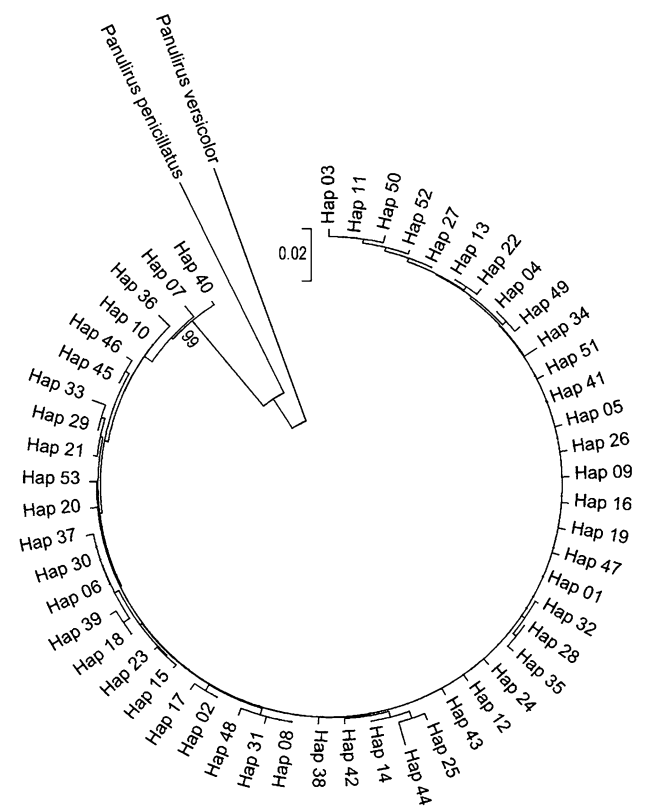
Fig. 2 Neighbour-joining tree showing evolutionary relationships among haplotypes. Percentage consensus based on 1,000 bootstrap replications of the data is shown for branches where values exceed 50 %

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

Site/year	n	N_{Hap}	h (SE)	π (SE)	Fu's F_S	Tajima's D	Mismatch analysis	
							SSD	RAG
Spatial variation (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
Temporal variation								
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
2009 (all sites)	130	34	0.62 (0.05)	0.0030 (0.0022)	−29.4***	−2.3***	0.003	0.060

Sites correspond to those on Fig. 1. 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) *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

n sample size, N_{Hap} number of haplotypes, h haplotype diversity, π nucleotide diversity, SSD sum of squared deviations, RAG raggedness index

Table 2 Genetic variation within *P. cygnus* samples based on 11 microsatellite DNA loci

Site/year	Sample size	A_R	H	F
Spatial variation (2009)				
Coral Bay	124.8 (0.3)	6.9 (1.3)	0.67 (0.09)	0.01
Quobba	39.9 (0.1)	6.9 (1.3)	0.67 (0.10)	−0.01
Port Gregory	23.8 (0.1)	6.8 (1.3)	0.66 (0.09)	−0.03
Rat Island	24.3 (0.3)	6.7 (1.3)	0.65 (0.10)	0.02
Seven Mile Beach	36.3 (0.2)	7.0 (1.4)	0.67 (0.10)	−0.01
Jurien Bay	14.4 (0.8)	6.8 (1.3)	0.66 (0.10)	0.01
Temporal variation				
1995	38.0 (1.3)	12.2 (3.1)	0.67 (0.09)	0.04
1997	37.9 (1.0)	12.7 (3.1)	0.66 (0.10)	0.05*
1999	43.5 (2.0)	11.6 (2.7)	0.64 (0.10)	0.02
2009 (all sites)	272.1 (0.9)	12.6 (3.1)	0.67 (0.09)	0.00
2009 (Seven Mile Beach only)	36.3 (0.2)	12.8 (3.3)	0.67 (0.10)	−0.01

Standard errors are in parentheses. Note that the 2009 temporal sample was calculated using data from all sites and Seven Mile Beach only. Asterisks refer to the level of significance of tests for heterozygote deficiency *$P < 0.05$

H gene diversity, A_R allelic richness (based on a sample size of 8 or 24 individuals for the spatial and temporal samples respectively)

ΔK indicated that the probable number of clusters was one (Fig. 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 hitchhiking. 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
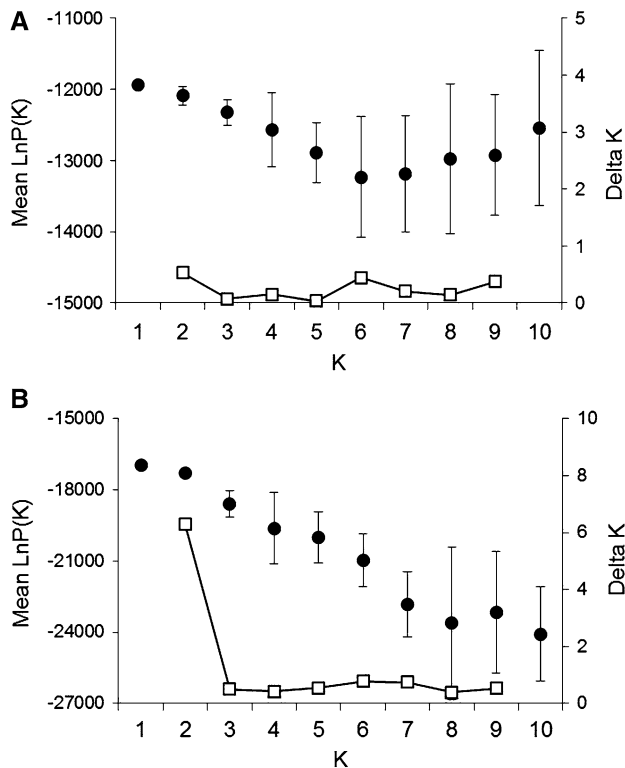
Fig. 3 Mean estimates of the log probability (*solid circles*) and Delta K (*open squares*) for each K arising from the Bayesian clustering analysis of the **a** 2009 and **b** temporal microsatellite data. *Error bars* for L(K) are standard deviations

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 lowest mutation rate, these estimates of tau imply that the population expansion began 27,742–8,536 generations ($\sim$139,000–43,000 years) ago. The highest mutation rate suggests the expansion began 1,024–3,329 generations ($\sim$17,000–5,000 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 or infinite. This indicates that the signal caused by genetic drift was less than the noise created by the sampling error.

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 no evidence of loss of genetic diversity or increases in the

level of inbreeding. 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. 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. Large effective population sizes have also been reported for European spiny lobster (*Palinurus elephas*) (Palero et al. 2011). 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 1994), 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; Cuveliers et al. 2011; Hoarau et al. 2005; Riccioni et al. 2010). Riccioni et al. (2010) suggested that gene flow, overlapping generations and high mutation rates may explain the maintenance of variation in Atlantic Bluefin tuna. A long generation time and directional human impact may also mask the genetic consequences of overfishing. Palero et al. (2011) have suggested that in *P. elephas*, exploitation of mainly large individuals may have led to a reduction in the number of large females, reducing the variance of reproductive success. As a result, more individuals contribute to the next generation and the effective population size would appear to increase despite a decrease in census size. 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 and 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 (García-Rodrguez and Perez-Enriquez 2008; Gopal et al. 2006; Naro-Maciel et al. 2011; Palero et al. 2008; Tolley et al. 2005). 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 139,000–5,000 years ago, which partly overlaps with a postglacial population expansion dates.

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 (García-Rodrguez and Perez-Enriquez 2008; Naro-Maciel et al. 2011; Ovenden et al. 1992; Tolley et al. 2005), but there are exceptions (Gopal et al. 2006; Palero et al. 2008; Perez-Enriquez et al. 2001). 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 *P. elephas*, genetic differentiation was found between Atlantic and Mediterranean populations, which was attributed to restricted gene flow through the Straits of Gibraltar (Palero 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 (Palero 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 (Johnson and Wernham 1999; Thompson et al. 1996). 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. 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, though the likelihood of large effective population sizes means that restrictions to gene flow cannot be ruled out (Waples 1998). It is also possible that post-settlement selection effects lead to different population structuring in the adult spawning 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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