

Genetic (microsatellite) determination of the stock structure of the blue swimmer crab in Australia

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FRDC Project 98/118

Fisheries Research and Development Corporation Report
FRDC Project No. 98/118

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of the blue swimmer crab in Australia**

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March 2001

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Acknowledgements

We gratefully acknowledge the support of (i) Wayne Sumpton, Southern Fisheries Centre, Queensland, (ii) Sue Murray-Jones, SARDI, and (iii) Simon de Lestang, Centre for Fish and Fisheries Research, Murdoch University, who provided most of the samples of *Portunus pelagicus*. We thank Dr Peter Spencer, Perth Zoo, who helped with the isolation and characterisation of the microsatellite markers in *Portunus pelagicus*. We also thank various people from the Centre for Fish and Fisheries Research, including Ass. Prof. Norman Hall who provided professional advice and Simon de Lestang, David Fairclough, and Alex Hesp who assisted with the preparation of the report. Finally, we gratefully acknowledge the support provided by the FRDC, without which this project would not have been possible.

Background

The blue swimmer crab *Portunus pelagicus* has a wide Indo-Pacific distribution (Kailola *et al.*, 1993). In Australia, this species is found in coastal waters ranging northwards from Cape Naturaliste (33°37'S) on the west coast of Australia, across northern Australia and then down the east coast to Eden (37°04'S) in southern New South Wales (Kailola *et al.*, 1993). However, the southern limit of its range in either eastern or western Australia has not been firmly established and could change over time. Certainly there are, for example, reports of *P. pelagicus* occurring in Victoria (*e.g.* Stephenson, 1962), while juveniles/adults of *P. pelagicus* have been recorded in the Nornalup-Walpole Estuary on the south coast of Western Australia (Hodgkin & Clark, 1988). Regardless, assemblages of *P. pelagicus* are known to occur in Gulf Saint Vincent, Spencer Gulf and the west coast region in South Australia (Kailola *et al.*, 1993; Bryars & Adams, 1999).

Portunus pelagicus supports important commercial fisheries in Queensland, New South Wales, South Australia and Western Australia and, at least in the latter two states, the fisheries have the potential for substantial expansion (see Kumar, 1997). In addition, the blue swimmer crab is an important component of both the incidental catch of inshore fisheries and the by-catch of the Northern Prawn Fishery (Calogeros, 1997). Moreover, this species is a common target of recreational fishers in most states, although the exact magnitude of these catches is generally unknown (see Kumar, 1997).

Appropriate management and development of the blue swimmer crab fisheries in Australia requires information about the identity and characteristics of the stocks of this species. Nevertheless, although there have been a few detailed studies of the biology of *Portunus pelagicus* (e.g. Meagher, 1970; Potter, I. *et al.*, 1983; Edgar, 1990; Potter, M. *et al.*, 1991; Sumpton *et al.*, 1994; Bryars, 1997; Potter & de Lestang, 2000), there has been only one study of its population genetic structure and that study focussed mainly on assemblages in South Australia (Bryars & Adams, 1999).

The identification and characterisation of stocks depends, to a certain extent, on the particular definition of a stock that is used (Carvalho & Hauser, 1995). From a biological perspective, a stock is generally regarded as "a group of conspecific individuals whose abundance is mainly determined by self-recruitment and mortality rather than by immigration or emigration" (see Carvalho & Hauser, 1995). Since genetic markers can unambiguously reveal the presence and identity of genetically differentiated groups within a species, they can play an important role in identifying these self-perpetuating units and in defining their temporal and spatial boundaries (see below, this section).

The degree to which the spatially-isolated assemblages of a species evolve independently of each other will depend largely on the extent of gene flow among

them (Slatkin, 1985). In theory, disjunct assemblages of a species that are genetically isolated (*i.e.* not connected by gene flow) will diverge via a combination of mutation, genetic drift and spatially variable patterns of selection (Wright, 1978; Slatkin 1985). On the other hand, gene flow will tend to prevent or retard any such divergence (Wright, 1978; Slatkin, 1985). Thus, given certain assumptions, including the selective neutrality of markers, the genetic relatedness of the assemblages of a species will be directly related to the extent of gene exchange that occurs among them. In particular, differences at selectively neutral loci are only likely to arise between assemblages that are isolated or have very weak genetic connections.

Since *Portunus pelagicus* has both a relatively mobile adult stage (Edgar, 1970; Potter, M. *et al.*, 1991) and a planktonic larval phase that lasts for at least two weeks (see below, this section), the individuals of this species have the potential to disperse from their natal assemblage. However, the amount and spatial extent of gene flow in a species will be determined by a combination of factors, in addition to its intrinsic potential for dispersal. These additional factors include the strength and direction of current movements, the presence of physical barriers to dispersal and the degree to which spatially-isolated assemblages have become adapted to local environmental conditions. Clearly, from a fisheries management point of view, there is a need for studies of the population genetic structure of *P. pelagicus* to determine the extent to which spatially-isolated assemblages are connected via gene flow.

Bryars & Adams (1999) have used patterns of allozyme variation to investigate the population genetic structure of the blue swimmer crab in Australia. The main findings of their study are as follows.

(1) Two genetically and morphologically distinct ‘forms’ of blue swimmer crab occur in waters off Darwin. One of these ‘forms’ is widely distributed in

Australia and is termed *Portunus pelagicus*. The distribution and taxonomic status of the other 'form', termed *Portunus* sp., is uncertain, but it appears to be a separate species that does not occur in southern Australia. One putative individual hybrid of *P. pelagicus* and *Portunus* sp. was also found.

(2) Assemblages of adult *Portunus pelagicus* in Spencer Gulf, Gulf Saint Vincent and the west coast region of South Australia are genetically distinct from each other. Hence, despite its apparent vagility, genetic exchange in this species between even the neighbouring Spencer Gulf and Gulf Saint Vincent appears to be restricted.

(3) Although local fishers have speculated about the existence of inshore and offshore stocks of *Portunus pelagicus* in Spencer Gulf, the allozyme data provided no evidence of genetic sub-division within the assemblages in Spencer Gulf, Gulf Saint Vincent or the west coast region in South Australia.

(4) No significant differences were found among assemblages of *Portunus pelagicus* from the Northern Territory, Queensland and Western Australia, although the South Australian assemblages formed a distinctive group relative to these others. This finding has prompted a suggestion that the assemblages of this species that occur in Australian waters outside of South Australia represent a single stock (but see below, this section).

The implications of the findings of Bryars & Adams (1999) for the population genetic structure of *Portunus pelagicus* in general are not clear. Since the assemblages in Spencer Gulf, Gulf Saint Vincent and the west coast region in South Australia appear to be remnants of a once widespread southern population, that has persisted only in isolated pockets of warmer water since temperatures declined and sea-levels fell about 6,000 years ago, one would not necessarily expect to find genetic isolation over such relatively fine spatial scales in other parts of Australia. On the other hand, the sample sizes for the assemblages, other

than those in South Australia and, to a lesser extent, the Northern Territory, were very small. In addition, the study was based upon patterns of variation at allozyme loci, which exhibit only limited amounts of polymorphism and hence do not always reveal the presence of relatively subtle population structuring (see Shaw *et al.*, 1999). Thus, the evidence in favour of the existence of only a single stock of *P. pelagicus* in Australian waters outside of South Australia is not convincing.

The following lines of circumstantial evidence indicate that it is reasonable to expect the presence of multiple stocks of *Portunus pelagicus* in Australian waters outside South Australia. (1) Since laboratory experiments indicate that the duration of the larval phase of *P. pelagicus* at 25°C is about 21 days (Meagher, 1971; Bryars, 1997), the planktonic phase of the life cycle may provide ample opportunity for dispersal over fine spatial scales but not over long distances. For example, the Leeuwin Current, a southward movement of warm water along the west coast of Australia, is a potential method for the long distance transport of the larvae of *P. pelagicus* in this region. Using the mean speed of this current, *i.e.* 17 cm per sec (Pearce, 1991), as a guide, a larva that spends 21 days in this current will be transported about 308 km from its site of release and not necessarily in a straight line. Similarly, inshore wind-driven currents in the vicinity of Perth, typically flow at rates of 7 - 10 km per day during the summer months (Department of Environmental Protection, 1996), when *P. pelagicus* usually spawns in these waters (Potter, I. *et al.*, 1983), and would be unlikely to disperse a single larvae much more than about 200 km from its natal assemblage. (2) Other widespread marine invertebrates with a planktonic larval phase of less than one month's duration exhibit population genetic subdivision in Australian waters (*e.g.* Salini, 1987; Johnson and Joll, 1993; Williams & Benzie, 1997; Brooker *et al.*, 2000). (3) Although tagging or distributional studies have firmly established that

the juveniles/adults of *P. pelagicus* may move over relatively fine spatial scales (Potter, I. *et al.*, 1983; Potter, M. *et al.*, 1991; Bryars, 1997), there is, to our knowledge, no evidence to suggest that these juveniles/adults migrate over long distances.

Microsatellites are parts of the genome which, unlike genes, do not contain a genetic code that leads to the production of RNA and/or proteins. Indeed, most microsatellite loci have no known function. Specifically, microsatellite loci comprise tandemly arrayed repeats of simple sequences (typically of 1 - 5 bp) and variation at single loci takes the form of different numbers of repeat units (Wright & Bentzen, 1995; O'Connell & Wright, 1997).

Ideally, an analysis of the stock structure of a species requires genetic markers which are (1) inherited in a Mendelian fashion and have co-dominant expression of alleles, (2) selectively neutral, and (3) highly variable. Microsatellite markers appear to combine all of these features (see Wright & Bentzen, 1995; O'Connell & Wright, 1997). In comparison with allozyme loci in particular, microsatellite markers afford two significant advantages in studies of stock structure. Firstly, microsatellite loci generally show levels of underlying polymorphism which are higher, often by an order of magnitude, than those exhibited by allozyme loci (*e.g.* Shaw *et al.*, 1999; Brooker *et al.*, 2000). Secondly, since they are non-genic, microsatellite loci are far less likely to be influenced by selection than is the case with allozyme loci. Accordingly, microsatellites are rapidly replacing allozymes as the marker of choice in population genetic studies and have been successfully applied to address questions about the stock structure of marine invertebrates (*e.g.* Shaw *et al.*, 1999; Brooker *et al.*, 2000) and fishes (*e.g.* O'Connell *et al.*, 1998; Ruzzante *et al.*, 1998).

Need

As with all fisheries, a basic prerequisite for managing the fisheries for the blue swimmer crab is the identification of production units or stocks of a species, since inadequate knowledge of the stock structure may lead to over- or under-exploitation (see Smith *et al.*, 1997). From the background information, it is clear that there is a need for a study aimed at determining the extent to which *Portunus pelagicus* is represented by different stocks in spatially-isolated habitats within and between geographical regions in Australia. Furthermore, it is also evident that such a study should be based on the use of microsatellite markers, since these markers can provide an unambiguous and high resolution method for the detection of different stocks (see Wright & Bentzen, 1995; O'Connell & Wright, 1997; Shaw *et al.*, 1999).

For the following specific reasons, information as to whether there are one or more stocks of blue swimmer crabs, *i.e.* genetically differentiated assemblages, in a given region is crucial for managing the fishery for this portunid.

1. The ability of managers to develop policies to ensure that stocks of the blue swimmer crab are maintained and to be able to respond appropriately to any marked decline in the abundance of an assemblage of this species depends on a knowledge of whether or not different assemblages constitute a single stock or are part of a larger and more widely-distributed stock. In this context, it is highly relevant that the work of Bryars & Adams (1999) showed that, in spite of the apparent vagility of blue swimmer crabs, the assemblages of this species in relatively nearby localities can be genetically different and thus constitute different stocks. It should also be recognised that, as the fishery for blue swimmer crabs increases, the discard mortality associated with repeated captures and releases of undersized crabs could place pressure on those stocks that are being heavily fished.

2. There is a need to identify the different stocks of blue swimmer crabs within a region in order to facilitate adaptive management (and opportunities for research) of separate populations that are exposed to (or experience) different levels of fishing mortality.

3. Any modelling and stock assessment of the blue swimmer crab fishery in a region is dependent on knowing whether the assemblages in that region constitute one or more stocks.

4. Fisheries managers need to know the extent to which the biological characteristics, such as growth rates and fecundity, vary amongst stocks in order to be able to take these variables into account when developing management policies. Furthermore, stock identification is required in order to be able to collect appropriate fishery data.

As well as being of immediate need for stock identification, the proposed project will generate detailed information on the population genetic structure of *Portunus pelagicus* over virtually its entire range in Australia. The resultant information will help to identify the importance of various factors, such as climate, current flow and physical isolation, in determining the stock structure of this species. In turn, this information will be useful should genetic management of this species become a priority, e.g. for conservation of genetic diversity within stocks; for identification of stocks possessing desired performance traits.

Objectives

The primary objective of this study was to determine whether selected assemblages of blue swimmer crabs in nearby and more distant geographic sites in states throughout Australia are genetically differentiated and thus constitute different stocks. More specifically, the three performance indicators of this study were to: (i) determine which of the spatially-isolated assemblages of adult crabs

within and among geographic regions throughout Australia are genetically distinct and should therefore be managed as separate stocks; (ii) determine whether selected assemblages within single ‘habitats’ are genetically homogeneous and thus constitute single stocks; and (iii) identify major genetic breaks, and associated ecological or geographical correlates (*i.e.* north versus south and/or east versus west), in the population structure of *Portunus pelagicus*.

Methods

Sampling sites

In this report, an assemblage refers to the individuals of *Portunus pelagicus* contained within a particular embayment or estuary. However, it should be recognised that, since this species spawns in marine waters, the assemblage within an estuary will be a part of the stock that also encompasses the assemblage outside of the estuary. Samples of juveniles and/or adults of *P. pelagicus* were obtained from 15 assemblages throughout Australia (Fig 1). However, the sizes of the samples obtained from two of these assemblages, namely Darwin in the Northern Territory (N = 6) and Port Stephens in New South Wales (N = 15), were small. The study targeted assemblages of this species that are economically important, separated by a range of spatial scales within a particular geographic region and which collectively encompass the entire distribution of this species in Australia (see Fig. 1).

In some cases, only a single sample was collected from a particular assemblage. However, in order to test for the presence of genetic variation within certain assemblages, samples were collected from both (i) an inshore and offshore site in Moreton Bay in Queensland, (ii) a central and western site in Gulf Saint Vincent in South Australia, and (iii) an eastern and a western site in Spencer Gulf in South Australia. Furthermore, two samples were collected from more or less

the same site within each of the following five water bodies, Hervey Bay in Queensland, and Shark Bay, Port Denison, Cockburn Sound and the Peel-Harvey Estuary in Western Australia. The two samples from Hervey Bay were collected within days of each other and hence can be used to assess the accuracy of our methodology. The pairs of samples from each of the assemblages in Western Australia were also used in this way. However, since these samples were generally collected about 12 months apart, they are not strictly replicates.

Collection and processing of samples

The samples of *Portunus pelagicus* were collected in 1998, 1999 or 2000, usually via baited crab pots. The crabs were frozen within a few hours of their collection and transported to the laboratory in this state, usually on dry ice or in liquid nitrogen. In the laboratory, several grams of muscle tissue were removed from each crab. Each muscle sample was then stored at -80°C. NB. The suitability of several other types of crab tissue and methods of preservation were tested, but frozen muscle tissue consistently yielded the highest quality DNA extracts.

Isolation and characterisation of microsatellite loci

The isolation and characterisation of microsatellite loci from *Portunus pelagicus* was conducted following the protocols of Yap *et al.* (2000). The following four steps were involved in this process.

Firstly, a size-select, genomic library was constructed using an individual of *Portunus pelagicus* from Cockburn Sound in Western Australia. The library comprised 960 recombinant colonies (*i.e.* bacterial colonies that had incorporated DNA from *P. pelagicus*).

Secondly, the library was screened with (CA)₁₂, (AG)₁₂, (AAT)₁₂, (AAG)₈, (AAC)₈, (GCAC)₇, (GATA)₇, (AAAT)₇, and (GACA)₇ oligo probes end-labelled

with [α - 32 P]-dATP, in order to identify the recombinant colonies that had probably incorporated microsatellite sequences from *Portunus pelagicus*. A total of 42 positive colonies were detected.

Thirdly, the nucleotide sequence of plasmids from the positive colonies was determined. The sequencing confirmed that all of the positive colonies contained microsatellite sequences.

Fourthly, the 42 microsatellite loci were assessed for their suitability for an investigation into the stock structure of *P. pelagicus*. Most (34) loci were excluded from consideration because either (i) they comprised a small number of repeat units, (ii) their repeat regions were located close to the cloning site (*i.e.* the sequencing of their flanking regions was insufficient to permit PCR primers to be generated) or (iii) the PCR primers that were generated failed to consistently amplify scorable alleles. Of the remaining eight loci, a further two were excluded because preliminary analyses indicated that they exhibited very high, and potentially 'unmanageable', levels of polymorphism (see Table 1).

In summary, a total of six microsatellite loci, five of which comprised dinucleotide repeat units and one of which comprised a tetranucleotide repeat unit, were isolated from *Portunus pelagicus* and used to investigate the stock structure of this species (see Table 1).

Genetic assays

The genotype of each crab in each sample was determined for each of the six microsatellite loci. The three following steps were involved in this process.

1. DNA extractions

Homogenates from individual crabs were prepared by incubating overnight, at 55°C, about 100 mg of macerated muscle tissue in SDS (sodium dodecyl sulfate) buffer, together with 6 units of proteinase K. Total genomic DNA was isolated from the homogenates using a phenol/chloroform/isoamyl alcohol extraction (25:24:1), interspersed between two chloroform/isoamyl alcohol extractions (24:1). The DNA was then precipitated in ethanol, air dried, resuspended in 1mM Tris-EDTA buffer and stored at -4°C or -10°C.

2. *PCR amplifications*

Polymerase chain reaction (PCR) was used to amplify the target microsatellite loci from the DNA preparations. The PCR was conducted using reaction mixtures that had a total volume of 15 µL and contained 50 - 100 ng of DNA template, 10 mM Tris-HCl (pH = 8.3) with 50 mM KCl, 1.5 mM of MgCl₂, 0.2 mM of each of the dNTPs, 20 - 80 nmol of each primer, with 25% of the forward primer was end-labelled [γ -³³P]-ATP, and 0.05 U of *Taq* polymerase. The reaction mixtures were subject to (i) an initial denaturation phase of 5 min at 94°C, (ii) 26 amplification cycles, with each cycle consisting of 30 sec of denaturation at 94°C, 30 sec of annealing at T_a °C, 90 sec of extension at 72°C and (iii) a final 7 min extension at 72°C. The annealing temperatures (T_a °C) for each locus were (i) 50°C for the tetranucleotide locus (P19), (ii) 53°C for the P8 and P9 loci, (iii) 58°C for the P2 and P18 loci, and (iv) 63°C for the P4 locus (see Table 1).

3. *Resolution and scoring of alleles*

Amplified alleles were resolved on a 6% denaturing polyacrylamide gel. After electrophoresis, the gel was dried using a gel dryer and an autoradiograph taken by exposing, for 18 - 20 hours, Kodak BioMax MR film to the gel. The exposed film was processed using an automatic film developer. Allele sizes were determined by comparisons with pUC18 DNA sequencing standards. One or more internal standards (*i.e.* samples that had been scored in a previous assay) were run on most gels to ensure internal consistency in the scoring of alleles.

Data analyses

Variation at microsatellite loci is typically in the form of alleles of different sizes (see Background). As expected, the alleles at the tetranucleotide locus varied from each other in increments of 4 bp, while those at the dinucleotide loci typically varied in increments of 2 bp. However, the alleles at the P8 locus varied in increments of 1 bp. This probably reflects the presence of duplications and/or deletions in the region that flanks the repeat units in the alleles at the P8 locus.

The frequency of genotypes and alleles at each locus in each sample were determined and used as the basis for the following analyses.

1. Levels of polymorphism

The level of polymorphism, *i.e.* the information content, of each locus was assessed in terms of both the number of different alleles detected and the expected heterozygosity. Expected heterozygosity (H_E) was calculated as $1 - \sum(f_i)^2$, where f_i is the frequency of the i th allele. Values of H_E range between 0 and 1 and increase as the level of allelic diversity increases.

2. Hardy-Weinberg equilibrium

Simplistically, the proportions of different genotypes at selectively-neutral loci in a sample collected from a randomly-mating group of individuals should match those expected under Hardy-Weinberg equilibrium conditions. The Markov chain method was used to estimate the exact probability that the proportions of different genotypes at each locus in each sample of *Portunus pelagicus* were not significantly different from the proportions expected under Hardy-Weinberg equilibrium conditions (see Raymond & Rousset, 1995). Exact probability tests are not biased by very small samples or low frequencies of alleles or genotypes (Raymond & Rousset, 1995) and hence are suitable for the analyses of variation at highly polymorphic microsatellite loci.

3. Measures of population differentiation

The following three methods were used to analyse the patterns of allele frequency variation among the samples of *Portunus pelagicus*.

(A) Single-locus variation

The statistical significance of differences in the allele frequencies at each locus between pairs of samples was assessed. The associated null hypothesis was that the allele frequency distributions in the two samples were not significantly different. For each locus-sample combination, the Markov chain method was used to estimate the exact probability of a type I error (see above, this section).

(B) Multi-locus variation

The standardised genetic variance, *i.e.* F_{ST} , was used to measure the proportion of the total allele frequency variation that was due to difference among

samples. F_{ST} values greater than zero indicate the presence of significant genetic variation among samples. The F_{ST} values were estimated using the method of Weir & Cockerham (1984) and represent weighted averages of variation across alleles and loci. The F_{ST} analysis was carried at several different levels, *e.g.* among all samples, among the samples from a single geographic region, and between pairs of samples. In order to resolve the patterns of differentiation among samples, the multi-dimensional scaling method was used to ordinate the values of F_{ST} between each pair of samples in two-dimensional space (see below, this section). N.B. The appropriate statistical method for quantifying differentiation at microsatellite loci is a matter of debate and may be study specific (see O'Connell *et al.*, 1996; Gaggiotti *et al.*, 1999; Shaw *et al.*, 1999). Following the criteria of O'Connell *et al.* (1996) and Gaggiotti *et al.* (1999), F_{ST} is the most suitable of the available measures for the analyses of our data set.

(C) Genetic relationships

The overall similarity between pairs of samples was estimated using Nei's (1978) unbiased genetic distance. This particular distance measure was selected because the results of several independent simulations indicate that, when used with data from microsatellite loci, it provides a reliable indication of genetic relationships among recently diverged populations (*e.g.* Goldstein *et al.*, 1995; Paetkau *et al.*, 1997). In order to assess the relationships among the samples, the values of genetic distance for each pair of samples were subjected to multi-dimensional scaling ordination (*e.g.* Hair *et al.*, 1992). This ordination technique has an advantage over cluster analysis, which is traditionally used to summarise the genetic relationships among samples, because it does not force samples into discrete clusters when genetic variation is continuous (Watts, 1991).

4. Other considerations

The extent of allele frequency variation between pairs of samples of *Portunus pelagicus* from the west coast of Australia appeared to increase as the geographic distance between their source assemblages increased (see Figs 1 & 2). Consequently, the Mantel procedure was used to determine if there was a significant positive correlation between the matrix of F_{ST} values (transformed to $F_{ST}/(1-F_{ST})$) for pairs of west coast samples and the matrix of the geographic distance between the corresponding sampling locations (see Rousset, 1997; Schneider *et al.*, 2000).

The exact probability tests were carried using the computer program GENEPOP, version 3.1d (Raymond & Rousset, 1999); the F_{ST} values, and their associated confidence levels, were calculated using the program FSTAT, version 1.2 (Goudet, 1996); the values of Nei's genetic distance were calculated using the program DISPAN (Ota, 1993); the multi-dimensional scaling ordinations were conducted using PRIMER, version 4 (Clark & Warwick, 1994); and the Mantel test was conducted using the program Arlequin, version 2.000 (Schneider *et al.*, 2000). Where multiple tests were conducted as a part of an analysis, a sequential Bonferroni procedure, which controls for group-wide Type I error rates, was used to assess the statistical significance of probability values (see Rice, 1989).

Results/Discussion

Levels of polymorphism

The five dinucleotide loci in *Portunus pelagicus*, i.e. P2, P4, P8, P9 & P18, displayed moderate to high levels of polymorphism (Table 1). The P2, P4 and P18 loci each exhibited 32 or more alleles, averaged 13.4 or more alleles per sample and had an average expected heterozygosity of at least 0.79 (Table 1). The levels of polymorphism at the P8 and P9 loci were more moderate, particularly in the case of the P8 locus, which had a total of 15 alleles, a mean of 8.8 alleles per sample and an average expected heterozygosity of 0.72 (Table 1). As expected, the tetranucleotide locus P19, with a total of five alleles, a mean of 3.1 alleles per sample and a mean expected heterozygosity of 0.51, was conspicuously less polymorphic than any of the dinucleotide loci (Table 1).

The main implications of the above findings for the stock structure analysis are that the dinucleotide loci, in particular, with their moderate to high levels of polymorphism, have a relatively high information content and hence can be used to detect relatively subtle genetic heterogeneity in *Portunus pelagicus*. Indeed, one of the main reasons why this study used microsatellite markers rather than allozymes is because the levels of polymorphism/information content of the former is generally greater than those of the latter (see O'Connell & Wright, 1997). Thus, microsatellite markers have been used to reveal the presence of relatively subtle population genetic differentiation, that was not apparent from an analysis of allozyme markers, in a range of species (e.g. Shaw *et al.*, 1999).

The levels of polymorphism at the tetranucleotide locus in *Portunus pelagicus* were lower than those at the dinucleotide loci. This likely reflects the generally lower rates of mutations at tetranucleotide loci compared to dinucleotide loci in eukaryotes (e.g. Chakroborty *et al.*, 1997). In consequence, it is likely that the patterns of variation at the tetranucleotide locus in *P. pelagicus* will emphasize historical connections among populations, as opposed to contemporary ones, more

strongly than will the patterns of variation at the dinucleotide loci (see below, ‘Variation among geographic regions’).

Reliability of genetic methodology

In order to investigate how accurately the genetic composition of the samples of *Portunus pelagicus* resemble those of the source assemblages, we examined the extent of allele frequency differences between two ‘replicate’ samples collected from the same site from each of five assemblages, namely Hervey Bay in Queensland and the Peel-Harvey Estuary, Cockburn Sound, Port Denison and Shark Bay in Western Australia. In all five cases, the allele frequencies at all six microsatellite loci were similar in ‘replicate’ samples. For example, in a two-dimensional ordination of the values of the genetic distance between all pairs of samples, regardless of whether the distance was measured in terms of F_{ST} or Nei’s unbiased genetic distance, the points for the two ‘replicate’ samples from each of the Peel-Harvey Estuary, Cockburn Sound, Port Denison and Shark Bay were closer to each other than to those of any other sample, or were at least very closely aligned, while those for the two samples from Hervey Bay fell within those of a single relatively homogeneous group of samples from the east coast (Fig. 2). In addition, the F_{ST} value between ‘replicate’ samples was always less than 0.008 and never statistically different from zero (Table 2). Furthermore, the differences in allele frequencies at each of the six loci in any of the five sets of ‘replicate’ samples were never statistically significant (Table 2). Consequently, the ‘replicate’ samples from a single assemblage have been pooled to produce a larger sample for that assemblage and, unless stated otherwise, subsequent analyses have used these combined samples.

Although there were no major, *i.e.* statistically significant, allele frequency differences between ‘replicate’ samples, it is worth noting that there were five sample-locus combinations, *i.e.* the Peel-Harvery Estuary-P8, Cockburn Sound-P2, Port Denison-P9, Shark Bay-P2 and Hervey Bay-P19, for which the differences in allele frequencies in ‘replicates’ approached the level expected for statistical significance, *i.e.* $\alpha \leq 0.05$, but were not significant once the Bonferroni correction was applied (see Table 2). Similarly, the F_{ST} value between ‘replicate’ samples from each of Shark Bay and Hervey Bay approached the level expected for statistical significance (see Table 2).

Since the samples from Hervey Bay were collected at the same time and yet showed at least as much heterogeneity as other ‘replicate’ samples, which were collected up to 12 months apart, it seems unlikely that the minor genetic heterogeneity between ‘replicate’ samples of *Portunus pelagicus* was primarily due to temporal changes in the genetic compositions of the source assemblages. Instead, the minor heterogeneity between ‘replicates’ is more likely to be the result of sampling artefacts and, in particular, of random sampling errors.

Although highly polymorphic loci are associated with a high information content, one of the disadvantages associated with using such loci in population-level studies is that large sample sizes can be required for characterising accurately the distribution of alleles in populations (O’Connell & Wright, 1997). In the current study, we found evidence that sample sizes of ~20 individuals may be inadequate for such, even in the case of the less polymorphic tetranucleotide locus, P19. Thus, for example, during preliminary analyses, when the size of the ‘second replicate’ sample from Port Denison was only 20 or 22 individuals (depending on the locus), the allele frequency differences at each of the P4 and P19 loci between the ‘replicates’ from Port Denison were statistically significant. However, in the final analysis, when the number of individuals assayed in the

second ‘replicate’ had been increased to 40 (P4) or 41 (P19), the allele frequency differences at these loci between the ‘replicates’ from Port Denison were not statistically significant (see Table 2).

In conclusion, the above results concerning the ‘replicate’ samples indicate that it is highly likely that any statistically significant differences in the allele frequencies between samples of *Portunus pelagicus* collected from different locations, particularly when these differences are associated with a significant value of F_{ST} , will reflect the presence of genetic heterogeneity between the source assemblages. Nevertheless, given that random sampling errors could generate significant genetic differences between samples from homogeneous populations, particularly when the sample size is small, it is important to recognise the need to conduct additional work to clarify those aspects of our results that are based predominantly upon variation at a single locus or upon relatively small samples.

Do assemblages of *Portunus pelagicus* comprise a randomly mating group of individuals?

Ideally, the genotype frequencies at selectively neutral loci, such as microsatellite loci, in samples collected from a randomly-mating group of individuals will conform to those expected under Hardy-Weinberg equilibrium expectations, while samples collected from, for example, an assemblage comprising an admixture of stocks or individuals that inbred will contain an excess of homozygote genotypes relative to Hardy-Weinberg expectations. Thus, ideally, one can use information about the proportions of homozygous and heterozygous genotypes observed in a sample, compared to the proportions of such expected under Hardy-Weinberg equilibrium conditions, to assess the mating system of the source assemblage (see Hartl & Clark, 1989).

The frequency of genotypes at each of the target microsatellite loci in samples of *Portunus pelagicus* collected from assemblages on the east coast of Australia generally approached those expected under Hardy-Weinberg equilibrium expectations (Table 3). In fact, statistically significant departures from Hardy-Weinberg equilibrium expectations were restricted to an excess of homozygotes at the P4 and P9 loci in the sample from Hervey Bay in Queensland (Table 3). However, most of the east coast samples contained an excess of homozygotes at the P8 locus, *i.e.* $H_O < H_E$ and $\alpha \leq 0.05$, but these excesses were not significant once the Bonferroni correction was applied (Table 3).

The above microsatellite evidence is consistent with the view that each of the assemblages of *Portunus pelagicus* sampled on the east coast of Australia, except perhaps that in Hervey Bay, constitutes a single randomly mating group of individuals, *i.e.* a single stock. In the case of the assemblage in Moreton Bay, from which we have samples from an inshore and an offshore site, this view is further supported by the fact that there was no significant heterogeneity in the microsatellite markers between the samples from these two different sites (see below, 'East Coast'). Similarly, the results of tagging studies indicate that individuals of *P. pelagicus* move extensively within this large embayment (Potter *et al.*, 1991). The significant homozygote excesses in the sample from Hervey Bay, and the 'almost significant' excesses at the P8 locus in most of the east coast samples, are probably due to sampling artefacts, although we cannot rule out the possibility that some or all reflect departures from random mating in the source assemblages (see below, this section).

The frequency of genotypes at each of the target microsatellite loci in samples of *Portunus pelagicus* collected from eastern and central sites in Gulf Saint Vincent, eastern and western sites in Spencer Gulf and the west coast region in South Australia generally approached those expected under Hardy-Weinberg

equilibrium expectations (Table 4). However, there was a statistically significant excess of homozygotes at the P4 locus in the sample from the western site in Gulf Saint Vincent and at the P2 locus in the sample from the west coast region (Table 4). Some of the other samples from South Australia also showed evidence of an excess of homozygotes at the P2 and/or the P4 loci, *i.e.* $H_O < H_E$ and $\alpha \leq 0.05$, but these excesses were not significant once the Bonferroni correction was applied (Table 4).

Since the incidence of significant departures from Hardy-Weinberg equilibrium in the samples of *Portunus pelagicus* from South Australia was very low and no sample showed such a departure at more than one locus, it is likely that each of these samples was collected from a randomly mating group of individuals. However, although there were no significant differences in the distribution of the microsatellite markers between samples of *P. pelagicus* from the different sites in Gulf Saint Vincent, there was significant genetic heterogeneity in the microsatellite markers between samples collected from different sites in Spencer Gulf and it is possible that there are multiple stocks of this species in this embayment (see below, 'South Australia'). As was proposed for the east coast samples, the significant and 'almost significant' homozygote excesses that were detected in the samples from South Australia are probably due to sampling artefacts, although, once again, we cannot rule out the possibility that some or all reflect departures from random mating in the source assemblages (see below, this section).

Nine statistically significant departures from Hardy-Weinberg equilibrium expectations were detected from 36 tests (sample X locus combinations) for the samples of *Portunus pelagicus* from the six assemblages in Western Australia (Table 5). The distribution of these nine significant tests among loci was as follows: one at each of the P4, P8 and P18 loci and two at each of the P2, P9, and

P19 loci (Table 5). The distribution of these significant tests among samples was as follows: one in the samples from each of the Peel-Harvey Estuary, Cockburn Sound, Shark Bay and Exmouth Gulf, two in the sample from Geographe Bay and three in the sample from Port Denison (Table 5). Thus, although each locus and each sample showed at least one significant departure from Hardy-Weinberg equilibrium expectations, no single locus or single sample had a particularly high prevalence of such. However, if one also considers the sample-locus combinations that approached the level of statistical significance, then each of the P2 and P9 loci (5/6 samples) and the sample from the Peel-Harvey Estuary (5/6 loci) appear to show a high prevalence (Table 5). As was the case for the samples from outside Western Australia, all significant and ‘almost significant’ departures from Hardy-Weinberg equilibrium expectations were in the form of an excess of homozygous genotypes (Table 5).

Since the above results were based upon pooled samples for the Peel-Harvey Estuary, Cockburn Sound, Port Denison, Shark Bay on the west coast, and for Hervey Bay on the east coast, and since the pooling of genetically heterogeneous samples can generate excesses of homozygotes, we compared the incidence of departures from Hardy-Weinberg equilibrium expectations in the individual ‘replicates’ and the pooled sample for each of the above sets of ‘replicate’ samples. However, these comparisons revealed no clear pattern to the distribution of the homozygote excesses. Thus, for some sample-locus combinations, there was some evidence, *i.e.* $\alpha \leq 0.05$, of an excess of homozygotes in both of the ‘replicate’ samples, whereas, for other combinations, an excess of homozygotes occurred in only one of the two ‘replicates’ (Table 6). Furthermore, in some cases, the homozygote excesses were more extreme (as measured by the extent to which α values were less than 0.05) in a pooled sample,

whereas, in some other cases, they were more extreme in one of the ‘replicates’ than in the pooled sample (Table 6).

The relatively high prevalence of homozygote excesses in the samples of *Portunus pelagicus* from the west coast of Australia is probably due to sampling artefacts, such as random sampling errors and null alleles (see below, this section). However, we cannot exclude the possibility that there is a departure from random mating in one or more of the sampled assemblages within Western Australia, or indeed in other regions (since virtually all samples had a significant or ‘almost significant’ excesses of homozygotes at one locus), especially since Bryars and Adams (1999) detected excesses of homozygotes at allozyme loci in a small proportion of their samples of *P. pelagicus*. Furthermore, it is worth noting that the populations of certain types of benthic marine invertebrates with planktonic larval phases are characterised by excesses of homozygotes. These excesses may be associated with the fine-scale subdivision of breeding groups or differences in the fecundity and/or viability of the presettlement *versus* the post-settlement stages (see Johnson & Black, 1984; Zouros & Foltz, 1984).

One of the major problems associated with using microsatellite markers to address population-level (and other) questions is the fact that, in some organisms, there is a widespread incidence of null alleles at microsatellite loci (*e.g.* see Pemberton *et al.*, 1995; O’Connell & Wright, 1997; Rico *et al.*, 1997). In such cases, it is difficult to use the patterns of variation at the microsatellite loci to assess the mating system of the organism under study. This is because null alleles either do not amplify or only weakly amplify under the specified PCR conditions and hence may not be visible using routine detection methods (O’Connell & Wright, 1997). Thus, since heterozygotes with a null allele will be scored as homozygotes, the presence of null alleles at a locus can generate an apparent excess of homozygous genotypes, relative to the proportion expected under

Hardy-Weinberg equilibrium conditions. Consequently, it can be difficult to distinguish between such apparent excesses and the real excesses of homozygotes that are expected to arise from the sampling of an assemblage comprising an admixture of stocks or individuals that inbreed.

Since ‘replicate’ samples of *Portunus pelagicus* sometimes showed inconsistent results with regard to whether or not they contained an excess of homozygous genotypes, it seems likely that the Hardy-Weinberg results have been influenced by sampling artefacts, such as the presence of null alleles and/or random sampling errors. It is not possible to determine the relative contributions of null alleles *versus* random sampling errors to these results, although it is worth noting the following. (1) The significant or ‘almost significant’ departures from Hardy-Weinberg equilibrium expectations were invariably due to excesses of homozygotes, which is consistent with the presence of null alleles at some loci in some samples. (2) Subtle differences in the PCR assay conditions, such as variation in the quality of the DNA template, can result in the inconsistent amplification of alleles at microsatellite loci in other organisms (*e.g.* O’Connell & Wright, 1997). Such inconsistent amplification of certain ‘weak’ alleles could generate inconsistent results between ‘replicate’ samples. It may therefore be relevant that in the sets of samples from each of the Peel-Harvey Estuary, Cockburn Sound and Hervey Bay, the homozygote excesses were noticeably more common in one ‘replicate’ compared to the other (see Table 6). (NB. Although the same assay conditions were used for all samples, slight variation in the condition of the crab samples when they arrived at the laboratory, and hence of the quality of the DNA template, was unavoidable. In addition, the ‘replicates’ were assayed at different times and hence with different batches of reagents.) (3) The potential for random sampling errors to influence our results, especially those

concerning relatively small samples, was noted above (see ‘Reliability of genetic methodology’).

Genetic variation among assemblages of *Portunus pelagicus* in Australia

Data interpretation

Before discussing our findings regarding the spatial patterns of microsatellite variation in *Portunus pelagicus* in Australia, it is important to highlight three factors that are generally relevant to the interpretation of these findings.

A major strength associated with any genetic approach to stock structure analysis is that genetically differentiated groups of individuals can be unambiguously identified and, provided that selectively neutral markers have been used, such groups will almost certainly correspond to independent breeding units. However, this type of approach is limited because samples collected from different assemblages of a species can appear to be genetically homogeneous for several reasons, including (i) the samples have been collected from a single randomly mating population, *i.e.* a single stock, (ii) the samples have been collected from a series of populations, for which the amount of contemporary migration is sufficient to prevent their genetic divergence, but is negligible in terms of its influence on their abundances, and (iii) the samples have been collected from populations which, although once connected, have become isolated relatively recently and thus the genetic markers emphasise the historic connection rather than the effects of the recent isolation. Consequently, while it is possible that a group of genetically homogeneous assemblages of *Portunus pelagicus* will constitute a single stock, such is not necessarily the case. This interpretational difficulty concerning a finding of ‘no genetic difference’, highlights the

importance of using highly polymorphic markers, such as microsatellites, in stock structure analyses.

Microsatellite loci are non-genic, *i.e.* they do not code for products, and, in fact, most microsatellite regions have no clearly demonstrated function. Thus, although no category of genetic marker is likely to be completely free of the direct effects of selection, so long as a microsatellite locus is not tightly linked to a gene, there is a good chance that the patterns of variation at that locus will predominantly reflect a balance between mutation, random genetic drift and gene flow. This is important because only such selectively neutral genetic markers can be readily used to distinguish between genetic differences that have been promoted or maintained by limited gene flow and those that have arisen via the differential survival or mating success of immigrants. Thus, unless there is strong evidence to suggest otherwise, it is reasonable to assume that differences in the distribution of microsatellite markers between assemblages of *Portunus pelagicus* are the result of restricted gene flow between these assemblages.

Finally, it is important to consider a general problem concerning studies of stock structure, particular for those involving widespread species in marine environments. This problem relates to the fact that, when sampling is limited to a small number of discrete points, it can be very difficult to distinguish between a species that comprises a series of discrete sub-units and one which gradually accumulates differences along some geographical gradient(s). Thus, while the microsatellite evidence clearly indicates that, for example, *Portunus pelagicus* in Australia comprises at least three genetically distinct groups of assemblages, corresponding to those on the east, south and west coasts (see below, 'Variation among geographic regions'), more intensive fine-scale spatial sampling is required to resolve whether *P. pelagicus* exhibits major genetic discontinuities or gradually accumulates genetic differences as geographic isolation increases.

Variation among geographic regions

There was a large amount of genetic heterogeneity among the samples of *Portunus pelagicus* from throughout Australia. Accordingly, the value of F_{ST} for all of these samples combined was relatively large, *i.e.* 0.98, and significantly different from zero. Furthermore, multi-dimensional scaling ordination plots of the values of the genetic distance between all pairs of samples, whether measured in terms of either F_{ST} or Nei's genetic distance, indicates that there are at least three major groups samples of *P. pelagicus* (Fig. 2). These groups correspond to:

- (1) samples from the east coast of Australia ranging from Mackay in central Queensland to Port Stephens in central NSW;
- (2) samples from the Gulf Saint Vincent, Spencer Gulf and the west coast region in South Australia;
- (3) samples from the west coast of Australia ranging from Exmouth Gulf in the north to Geographe Bay in the south. However, this 'west coast group' showed a very high level of genetic heterogeneity and could be considered to be separated into a northern group and a southern group (see below, 'West Coast').

The sample from Darwin differed from all other samples (Fig. 2; Tables 7 & 8). However, since the size of this sample is very small ($N = 3 - 6$, depending on the locus), the significance of this result is unclear.

The samples from South Australia were very distinctive, while those from the north-west coast, *i.e.* Exmouth Gulf and Shark Bay, were in some ways intermediate between those from the lower west coast and those from the east coast (Fig. 2). However, all of the samples from Western Australia could be clearly distinguished from all of those from the east coast by the fact that a 142 bp allele at the tetranucleotide locus occurred in a relatively high frequency in the

former (minimum frequency = 0.46), but was extremely rare in the latter (maximum frequency = 0.08).

In general, the amount of genetic heterogeneity between samples from different groupings was large. Thus, the allele frequencies at all or most loci in any two samples from a different group were significantly different (Table 7). The only minor exception to this was that the allele frequencies at between one and four loci only were significantly different for comparisons between either of the two samples from the north-west coast, *i.e.* Shark Bay and Exmouth Gulf, *versus* any of those from the east coast (Table 7). Regardless, the F_{ST} values between any two samples representing a different group were, without exception, significantly different from zero, *i.e.* there was always significant heterogeneity in allele frequencies (Table 8).

The above results indicate that there are at least three major genetic groups of assemblages of *Portunus pelagicus* in Australia, namely those comprising assemblages in South Australia and on the east and west coasts. The west coast assemblages may be further differentiated into a northern and southern group (see below, 'West Coast'). In any case, the location and clarity of the boundaries between the different groups has not been resolved (see above, Data interpretation).

A previous study, based on allozyme markers, identified the presence of a distinct group of assemblages of *P. pelagicus* in South Australia, but found no evidence of any further genetic sub-division of *P. pelagicus* in Australia (Bryars and Adams, 1999). The higher resolution offered by the present study is due to a combination of more intensive sampling on the east and west coasts and the relatively high information content of microsatellite markers (*e.g.* O'Connell and Wright, 1997).

The results of this study add to an increasing body of evidence that challenges the traditional notion that widespread marine species with planktonic larval phases are invariably genetically homogeneous over large geographic areas (see also Benzie, 1998). However, it is important to recognise that the geographic range of *Portunus pelagicus* is centered in tropical waters, while our study has focussed on the temperate margins of this range. Since many of the economically important assemblages of *P. pelagicus* in Australia occur in temperate waters, this focus is appropriate for analysing the stock structure of this species. However, from an academic perspective, a good understanding of the overall population genetic structure of *P. pelagicus* in Australia is dependent on the availability of more information on the genetic composition of the assemblages in northern Australia. In any case, it is worth noting that some other warm water species of marine invertebrates, with a planktonic larval phase of similar duration to that of *P. pelagicus*, show genetic sub-division in tropical waters in Australia, usually at least between the north-west and north-east coasts (e.g. Johnson & Joll, 1993; Williams & Benzie, 1997; Brooker *et al.*, 2000).

The characteristics of the population genetic structure of *Portunus pelagicus* in Australia, as suggested by the microsatellite evidence, raises some interesting questions, and in particular: (1) Why are there three distinct genetic groups that are each associated with a particular geographic area? and (2) Why are the assemblages at Exmouth Gulf, and to a lesser extent Shark Bay on the north-west coast, intermediate in some ways between those on the lower west coast and those on the east coast of Australia? Attempts to answer these questions need to take into account the fact that the populations of a species within a single geographic region may share with each other certain genetic attributes which, in turn, make them characteristically different to populations in a different region because either (i) the populations from a single region have a more recent

common ancestor than those from a different location, or (ii) there are more severe and/or persistent barriers to gene flow between geographic regions than within geographic regions or (iii) a combination of the above. Without information from a phylogenetically powerful marker, such as the mitochondrial genome, it is not possible to provide critical insights into the evolutionary history of *P. pelagicus* and hence into the determinants of the broad-scale population genetic structure of this species in Australia. However, we highlight the following observations.

(1) The samples from the east coast of Australia were relatively homogeneous (see below, 'East Coast') and, when compared to those from the north-west coast, *i.e.* Exmouth Gulf and Shark Bay, showed significant allele frequency differences at a relatively small proportion of loci. These findings suggest that, in eastern and northern Australia, major barriers to gene flow in *Portunus pelagicus* occur only over large geographic distances and, in fact, geographic distance may be the main limiting factor.

(2) There was marked genetic heterogeneity among the samples from the west coast and a significant correlation between the genetic distance between pairs of samples and the geographic distance between their source assemblages (see below, 'West Coast'). In combination with the above point, this indicates that, on the west coast, gene flow in *Portunus pelagicus* is restricted by geographical distance acting in combination with some other factor(s) or by some factor(s) that is correlated with geographical distance.

(3) All of the samples from the west coast could be distinguished from all of those from the east coast by a relatively high incidence of the 142 bp allele at the tetranucleotide locus. Since the distribution of the tetranucleotide markers is more likely to emphasise historical events than that of the dinucleotide markers (see above, 'Levels of polymorphism'), this finding is consistent with the view

that, in the past, there were stronger genetic connections among the assemblages on the west coast than there were between the west and east coast assemblages. Specifically, the west coast assemblages may have had a more recent common ancestry or there may have been relatively extensive gene flow along this coastline at some time in the past.

(4) There were significant allele frequency differences at all or most loci when comparisons were made between the samples from the north-west coast and those on the lower west coast to the south of Port Denison (see below, 'West Coast'). This indicates that, in the present environment, the amount of gene flow between assemblages in tropical and temperate regions on the west coast is either very limited or non-existent.

(5) In South Australia, *Portunus pelagicus* thrives in only three embayments, *i.e.* Gulf Saint Vincent, Spencer Gulf and the west coast region, where water temperatures are sufficiently high in summer to enable this species to grow and reproduce (Bryars, 1997). It has been suggested that the assemblages of *P. pelagicus* in these embayments are the remnants of a once widespread southern population that became fragmented as sea-levels rose and temperatures declined about 6,000 years ago (Bryars & Adams, 1999). This implies that the genetic differences among the assemblages in South Australia have evolved during the past 6,000 years. If this is the case, the fact that the South Australian assemblages, although different, are more similar to each other than to those from elsewhere, indicates that the putative ancestral population has been isolated from populations on the east and west coasts for longer than 6,000 years and possibly for about 10,000 years (Bryars & Adams, 1999).

Management Implications

The microsatellite evidences indicates that there are at least three major genetic groups of *Portunus pelagicus* that occupy different geographic regions in Australia. We have argued that the similarities that unite certain assemblages into a single group, or conversely the differences that distinguish each of the groups, strongly reflect aspects of the evolutionary history of *P. pelagicus* in Australia. A phylogenetically powerful genetic marker, such as the mitochondrial genome, can be used to test the validity of these arguments. In any case, the amount of movement of individuals of *P. pelagicus* among the surveyed regions of the south, east and west coasts of Australia in any one generation is almost certainly negligible, although there may be weak, indirect genetic connections between the assemblages on the north-west and east coasts. It is not possible to use the microsatellite data to determine whether or not the genetic differences between the groups of assemblages are adaptive. However, since the assemblages of this species in different geographical locations have different evolutionary histories and/or are effectively evolving in isolation of each other, sometimes in very different environments, it is possible that there are genetically-based differences in the biological characteristics of crabs from different regions. This could mean that stock management methods that are developed using the characteristics of a stock of *P. pelagicus* in one region might not be appropriate for the management of stock(s) in another region.

Variation within geographic regions

1. East Coast

The allele frequencies at the six microsatellite loci in the samples of *Portunus pelagicus* from the east coast of Australia, *i.e.* from Mackay, Hervey Bay, inshore and offshore Moreton Bay, Wallis Lake and Port Stephens, were

relatively homogeneous. For example, the F_{ST} value for all samples combined was only 0.001 and not significantly different from zero. In addition, the allele frequencies at all loci, except P4, between all pairs of samples were not significantly different (see Table 9). Furthermore, the differences found at the P4 locus were effectively generated by an unusual allele frequency distribution at this locus in the small sample from Port Stephens (see Table 9). In any case, except for that between the sample from offshore Moreton Bay and the small sample from Port Stephens, the F_{ST} values between pairs of samples from the east coast were not significantly different from zero (Table 9).

The above microsatellite evidence indicates that the assemblages of *Portunus pelagicus* on the east coast of Australia, ranging from Mackay (21°09'S) in central Queensland to Port Stephens (32°44'S) in central New South Wales, are effectively homogeneous. The most likely explanation of this finding is that the amount of gene flow in this species along this section of the coast is, or has been in the recent past, sufficient to prevent or retard genetic differentiation of the assemblages in this area. This explanation is consistent with the following observations. (1) Such gene flow could occur through the dispersal of either the planktonic larval phase and/or the juveniles and adults of *P. pelagicus* (Meagher, 1971; Potter, M. *et al.*, 1991; Bryars, 1997; Bryars & Adams, 1999). (2) The Eastern Australian Current, which comprises a predominantly southward flow of warm water along the east coast of Australia, is relatively strong and persistent in the coastal regions from which we obtained our samples (see Briggs, 1978; Murray-Jones & Ayre, 1997) and thus provides an obvious method for dispersing the larvae of *P. pelagicus*. In fact, a variety of marine invertebrates, so long as they have a planktonic phase, are relatively genetically homogeneous over large sections of the east coast (Murray-Jones & Ayre, 1997).

The values of the standardised variance in allele frequencies, *i.e.* F_{ST} , among samples can be used to provide a measure of the amount of gene flow in *Portunus pelagicus*. In particular, the number of genetically effectively immigrants (N_{em}) into each sampling site per generation can be estimated, using the equation $N_{em} = (1 - F_{ST})/4F_{ST}$ (see Murray-Jones & Ayre, 1997). However, N_{em} is related to F_{ST} in this way only if certain assumptions are realised (see Murray-Jones & Ayre, 1997). The microsatellite data for *P. pelagicus* may well conform to the majority of these assumptions. However, the distribution of the microsatellite markers will reflect the patterns of gene flow over multiple generations (Johnson & Black, 1990). Consequently, in certain situations, N_{em} will grossly overestimate of the amount of immigration per generation. We therefore stress that the estimates of N_{em} should be used only as a rough approximation of the average levels of gene flow within a region.

By substituting the F_{ST} value for the samples from the east coast (0.001) into the above equation, we obtained an estimate of $N_{em} = 249.75$ for these samples. Since this value refers to the genetically effective number of immigrants, *i.e.* to those immigrants that actually contribute genes to the breeding pool, the average number of migrants at each site could be much larger (Johnson *et al.*, 1993). On the other hand, this estimate provides little information about site specific patterns of migration and could be inflated because long distance connections may be maintained primarily by multiple rather than single generations (see below, this section). Regardless, the estimated number of immigrants is likely to represent only a small proportion of the total number of individuals at certain sites (see Johnson *et al.*, 1993).

Simplistically, three different patterns of gene flow could maintain homogeneity in *Portunus pelagicus* along the ~1500 km section of coastline surveyed on the east coast (see Richardson *et al.*, 1986). (1) There is a complete

mixing of individuals in this area and thus *P. pelagicus* is represented by a single stock in this region. This situation would imply that the individuals of this species typically move throughout and mate at random in this area. (2) A 'significant' number of individuals in each generation disperse between even the most distant sites. In this regard, it is important to note that, in theory, only a very small number of migrants per generation is sufficient to maintain genetic homogeneity among populations. (3) Within any one generation, 'significant' amounts of mixing occur only over relatively small spatial scales. However, distant connections are maintained predominantly via the cumulative effects of a series of overlapping local interchanges in multiple generations *i.e.* via a 'stepping stone' effect.

The overall results of this study indicate that individuals of *Portunus pelagicus* may rarely disperse over distances of several hundred kilometres or more (see below, 'South Australia & West Coast'). This therefore implies that this species comprises a series of overlapping assemblages in the region surveyed on the east coast (option 3 above). If individuals rarely disperse over distances of the above order, it follows that individuals will be completely mixed, *i.e.* mate at random, only over a much shorter distance. Nevertheless, it is unlikely that this species would be 'genetically homogeneous' in the entire survey region unless there was significant overlap between adjacent 'groups' of randomly mating individuals. These randomly mating 'groups' ('stocks') may be real in the sense that they have definable boundaries, such as would be the case if they occur within a particular embayment or within a discrete series of embayments. However, if individuals mate at random within and between nearby embayments that constitute a part of more or less continuous series of such embayments, there may be no clear boundaries between these groups. This is because an individual in a particular water body will be in the centre of its own 'group' and, although another

individual in a nearby water body may be included in that group, this latter individual will be the centre of its own 'group' and so on (see Richardson *et al.*, 1986).

The only suggestion of significant genetic heterogeneity in *Portunus pelagicus* on the east coast was the unusual allele frequency distribution at the P4 locus in the sample from Port Stephens. Since this sample was of a very small size, it is most likely that this anomaly is the result of a random sampling error. However, it is worth noting that Port Stephens was the most southern sampling point on the east coast and it is possible that dispersal in *Portunus pelagicus* is inhibited by the relatively cold water temperatures that it encounters in the more southern extremes of its range in Australia (see below, 'South Australia & West Coast'). Furthermore, in eastern Australia, the distribution of *P. pelagicus* extends south to at least about Eden (~37°S) and hence into cold temperate waters (see Briggs, 1978). Thus, even if the unusual result concerning Port Stephens is simply due to a sampling error, *P. pelagicus* may well show genetic subdivision further south of Port Stephens on the east coast of Australia.

Management Implications

On the east coast of Australia, *Portunus pelagicus* probably comprises a series of overlapping assemblages, or possibly a semi-continuous stock, ranging from at least as far north as Mackay in central Queensland to at least as far south as Port Stephens in central New South Wales. It is likely that, within any one generation, 'significant' amounts of mixing will occur only over distances of less than three hundred kilometres. Connections over longer distances are probably maintained largely via the cumulative effects of interchange through a 'stepping stone' effect between assemblages over multiple generations. These findings

have two critical implications for the management of the fisheries for *P. pelagicus* in the region surveyed on the east coast. (1) Broad-scale interruptions to the distribution of this species, *i.e.* those that exceed the usual dispersal distance of individuals, will significantly disrupt the genetic continuity between the assemblages that are separated by the interruptions. (2) Although the amount of migration into an embayment in a single generation may not have a significant impact on the abundance of *P. pelagicus* in that water body, it could, in the longer term, significantly enhance the recovery of an assemblage that has been depleted by overfishing.

2. South Australia

In contrast to the situation for the east coast, there was considerable heterogeneity in allele frequencies at the target microsatellite loci among the samples of *Portunus pelagicus* from South Australia, *i.e.* from central and west Gulf Saint Vincent, east and west Spencer Gulf and the west coast region. For example, the F_{ST} value for all these samples combined (0.046) was significantly different from zero and an order of magnitude higher than that for the east coast samples (0.001). Much of the heterogeneity was contributed by the relatively distinctive sample from the west coast region. Thus, the allele frequencies at four or five of the loci (but never the tetranucleotide, P19) in the sample from the west coast region differed significantly from all of those from the two gulfs (Table 10). Furthermore, F_{ST} values for comparisons between the sample from the west coast region and any of the gulf samples always differed significantly from zero (Table 10).

There was also significant genetic heterogeneity between the samples of *Portunus pelagicus* from the neighbouring Gulf Saint Vincent and Spencer Gulf and even between the samples from east and west Spencer Gulf. The sample from

east Spencer Gulf, in particular, was relatively distinct. Thus, the allele frequencies at two or three loci in this sample were significantly different compared to those in the sample from either central or west Gulf Saint Vincent (Table 10). In addition, the allele frequencies at the P18 locus in this east Spencer Gulf sample were significantly different to those in the sample from west Spencer Gulf (Table 10). Furthermore, the F_{ST} value between the east Spencer Gulf sample and either of the samples from Gulf Saint Vincent or the sample from west Spencer Gulf was significantly different from zero (Table 10).

The sample from west Spencer Gulf also showed significant genetic differences in comparison with the samples from Gulf Saint Vincent. In particular, the allele frequencies at the P18 locus or at the P2, P4 and P8 loci in this sample were significantly different to those in, respectively, the one from central Gulf Saint Vincent and the one from west Gulf Saint Vincent (Table 10). Similarly, the F_{ST} value between the sample from west Spencer Gulf and either of the samples from Gulf Saint Vincent was significantly different from zero (Table 10).

Since the significant allele frequency differences between the sample from west Spencer Gulf and those from central and west Gulf Saint Vincent involved different loci (see above, this section), this could imply that there is genetic heterogeneity between the two samples from Gulf Saint Vincent. Thus, it is worth noting that the differences in the allele frequencies at the P2 and P18 loci between the samples from west and central Gulf Saint Vincent approached the level expected for statistical significance (Table 10). However, the allele frequencies at no loci in these two samples were significantly different and the F_{ST} value between these samples was not significantly different from zero, *i.e.* the differences between the two samples from Gulf Saint Vincent were minor (Table 10).

Although the *Portunus pelagicus* from South Australia constitute the most genetically distinct group of this species in Australia, the microsatellite markers of the present study and the allozyme markers used in the study of Bryars & Adams (1999) both indicate that the assemblages of this species in Gulf Saint Vincent, Spencer Gulf and the west coast region are also each genetically distinct. However, both of these studies found that the genetic differences between the assemblage in the west coast region and those of Spencer Gulf and Gulf Saint Vincent were much greater than those between the assemblages in these two neighbouring gulfs. This could imply that the assemblage of this species in the west coast region is completely isolated from those in the two gulfs. Indeed, its isolation could date back to as long as ~6,000 years ago, *i.e.* to a time when rising sea levels and falling temperatures may have cut off the western arm of an ancestral southern population that later became further sub-divided between Spencer Gulf and Gulf Saint Vincent (Bryars & Adams, 1999).

The marked genetic heterogeneity in *Portunus pelagicus* that was found over approximately 500 km of coastline in South Australia contrasts sharply with the general lack of heterogeneity present among the assemblages of this species on the east coast between Mackay and Port Stephens. This contrast implies that, there is, or has recently been, significantly more gene flow in *P. pelagicus* along a 1,500 km section of the east coast of Australia than over a 500 km stretch of the coastline in South Australia. Indeed, the estimate of the average number of genetically effective immigrants (*i.e.* N_{em}) into each sampling site in South Australia was only 5.18, while that for the sites on the east coast was 249.75.

Gene flow between *Portunus pelagicus* on the west coast region of South Australia and either Spencer Gulf or Gulf Saint Vincent is probably restricted through the presence of barrier(s) to the dispersal of this species in this region. The less likely alternative explanation is that gene flow is retarded through the

presence of significant barriers to the reproductive success of immigrants. In particular, dispersal between these two locations is probably limited by one or more of the following factors.

(1) Density fronts that form at the entrances to both Gulf Saint Vincent and Spencer Gulf during the summer months, when *Portunus pelagicus* typically spawns in these waters, could block the passage of the larvae of this species into or out of either gulf (see Bryars, 1997).

(2) Upwellings in the west coast region may tend to disperse any larvae of *Portunus pelagicus* that immigrate to this region away from inshore settlement habitats (see Bryars, 1997).

(3) There is no evidence to suggest that adults or juveniles of *Portunus pelagicus* migrate over long distances and the duration of the larval phase of this species is consistent with the view that the larvae may usually disperse only over relatively short distances (see Background). If this is the case, significant amounts of genetic exchange over relatively long distances could still be possible, but only via a 'stepping stone' effect (see above, 'East Coast'). However, *P. pelagicus* may have a very limited capacity to bridge any discontinuity in its distribution that exceeds the usual dispersal distance of single individuals. Hence, gene flow along the ~400 km of coastline that separates the west coast region from the nearest gulf, *i.e.* Spencer Gulf, could be restricted because single individuals are usually dispersed only over smaller distances and 'stepping stone' dispersal is inhibited by the effective absence of habitats suitable for juvenile and adult crabs in this section of coastline (see Bryars, 1997).

(4) The temperatures of the surface waters of the Southern Ocean surrounding Gulf Saint Vincent, Spencer Gulf and the west coast region in South Australia are at about the lower limit required for the normal development of the larvae of *Portunus pelagicus* (~18°C) (see Bryars, 1997). The juveniles and

adults of this species may also be relatively inactive in such low temperatures (see Bryars, 1997). For these reasons, individuals of *P. pelagicus* may rarely survive in the 'cold' oceanic waters outside the above three embayments and, if so, would be particularly unlikely to traverse the relatively long stretch of 'cold' water between the west coast region and the two gulfs.

Although the genetic composition of the assemblages of *Portunus pelagicus* in Gulf Saint Vincent and Spencer Gulf are more similar to each other than either is to that of the assemblage in the west coast region, this species nevertheless shows genetic sub-division between these two gulfs. Certainly, both the present microsatellite study and the allozyme study of Bryars & Adams (1999), which used independent samples, found that the allele frequencies at certain loci in samples of this species from Gulf Saint Vincent were significantly different to those in samples from Spencer Gulf. Furthermore, in the present study, the values of F_{ST} between the samples from these two gulfs were always significantly different from zero, although such was not the case in the allozyme study. This difference between the two studies is likely due to the higher information content/greater sensitivity of the microsatellite markers.

The presence of genetic heterogeneity in *Portunus pelagicus* between Gulf Saint Vincent and Spencer Gulf might seem surprising in the sense that it indicates that gene flow is restricted between the assemblages in two water bodies that are in very close geographical proximity. However, as implied in points 1 & 4 above, there are certain aspects of hydrological conditions in this region of South Australia that could hinder the dispersal of this species, not only to or from the more distant west coast region, but also between the neighbouring Gulf Saint Vincent and Spencer Gulf. Thus, the genetic and hydrological evidence suggest a complementary picture - there is restricted gene flow between the *P. pelagicus*

found in Gulf Saint Vincent and Spencer Gulf because of the presence of hydrological barriers to the dispersal of this species.

From the available evidence, it is not possible to determine whether the assemblages of *Portunus pelagicus* in Gulf Saint Vincent and Spencer Gulf are completely isolated from each other in the contemporary environment. Given the close geographical proximity of the two gulfs, it seems reasonable to expect either that (i) a very small number of individuals of *P. pelagicus* migrate between the two gulfs every generation or so, or (ii) the temporary loss of a dispersal barrier could allow for occasional pulses of migration. Such types of weak genetic connections can retard the evolution of substantial differentiation and hence could explain why the differences in the genetic compositions of the assemblages in Gulf Saint Vincent and Spencer Gulf are relatively minor in comparison with their distinctiveness from the west coast assemblage. However, it is also possible that the relative similarity of assemblages in the two gulfs more strongly reflects patterns of historical connections among these assemblages rather than any patterns of contemporary gene flow (Bryars & Adams, 1999).

Local fishers have speculated about the presence of an inshore and an offshore stock of *Portunus pelagicus* within Spencer Gulf in South Australia (see Bryars and Adams, 1999). Although the allozyme study of Bryars and Adams (1999) found no evidence of genetic heterogeneity within the assemblages of this species in Spencer Gulf, the present microsatellite study found a significant allele frequency difference at one locus, and a significant value of F_{ST} , between samples from a western and eastern site in this water body. Neither of these studies found significant genetic differences between samples collected from different sites within Gulf Saint Vincent.

Models, that incorporate information about the hydrological conditions and distribution of the larvae of *Portunus pelagicus* within Gulf Saint Vincent,

indicate that, in an average year, the zoeae of this species are dispersed throughout the upper reaches of this system (Bryars, 1997). Similar detailed study of the situation in Spencer Gulf has not been undertaken, although the available evidence indicates that the zoeae of *P. pelagicus* are likely to be similarly dispersed within Spencer Gulf (Bryars, 1997). In any case, most of the juveniles and adults of this species in Gulf Saint Vincent and Spencer Gulf are found in the upper regions of these systems (Bryars, 1997), and all of our samples were obtained from these regions. The juveniles and adults undergo a distinct pattern of seasonal movements within these two water bodies, retreating from shallow inshore waters, where they spend the warmer months (September to April), to deeper offshore waters during the colder months of May to August (Bryars, 1997). Thus, there is seemingly a high potential for the larvae, juveniles and adults of *P. pelagicus* to mix within both Gulf Saint Vincent and Spencer Gulf.

The microsatellite evidence, together with the above information, indicates that it is unlikely that there are genetically isolated sub-groups, *i.e.* multiple stocks, of *Portunus pelagicus* within Gulf Saint Vincent. The situation for *P. pelagicus* in Spencer Gulf is less clear. The heterogeneity in the distribution of the microsatellite markers between samples collected from different sites coincides with anecdotal claims about the presence of multiple stocks within this embayment, although the microsatellite differences were detected between two sites within the upper reaches of this system and not between inshore and offshore environments, as suggested by these claims. On the other hand, most of the available ‘non-genetic’ evidence indicates that there is likely to be a significant amount of mixing among individuals of *P. pelagicus* within Spencer Gulf, although this evidence is not as detailed as that for Gulf Saint Vincent. Additional fine-scale, genetic sampling of *P. pelagicus* within Spencer Gulf, combined with

more detailed information about the movements of individuals of this species within this embayment, is required to resolve this uncertainty.

Management Implications

Different stocks of *Portunus pelagicus* occur in each of Gulf Saint Vincent, Spencer Gulf and the west coast region in South Australia. The fisheries for this portunid in each of these three locations should therefore be managed separately. There is probably only a single stock of *P. pelagicus* within Gulf Saint Vincent, but it is possible that there are multiple stocks within Spencer Gulf. The stock of this species in the west coast region is geographically and genetically isolated in a 'marginal' environment and may have a relatively limited capacity to recover from disturbance.

3. West Coast

There was considerable genetic heterogeneity among the samples of *Portunus pelagicus* from the west coast of Australia, *i.e.* from Exmouth Gulf (22°05'S), Shark Bay (24°45'S to 26°36'S), Port Denison (29°15'S), Cockburn Sound (32°11'S), the Peel-Harvey Estuary (32°26'S) and Geographe Bay (33°37'S) (see Fig. 1). The F_{ST} value for all these samples combined was 0.042 and hence was comparable to that for the samples from South Australia (0.046), but much greater than that for the east coast samples (0.001).

The main results concerning the patterns of microsatellite variation in the samples of *Portunus pelagicus* from the west coast were as follows.

Firstly, the genetic distance between pairs of samples from the west coast was positively correlated with the geographic distance between their source assemblages ($r = 0.892$, $p \leq 0.05$).

Secondly, there was considerable heterogeneity between samples from the north-west coast, *i.e.* Shark Bay and Exmouth Gulf, *versus* those from the lower west coast south of Port Denison, *i.e.* Cockburn Sound, the Peel-Harvey Estuary and Geographe Bay (see Figs 1 & 2). Thus, the allele frequencies at all six loci in either the sample from Exmouth Gulf or Shark Bay were significantly different from those in any of the samples from south of Port Denison (Table 11). Accordingly, the values of F_{ST} between either the sample from Shark Bay or the one from Exmouth Gulf and any of the samples from south of Port Denison, always differed significantly from zero (Table 11).

Thirdly, there was heterogeneity between the two samples from the north-west coast, although the extent of this heterogeneity was relatively limited. In particular, the allele frequencies at the P8 locus in the sample from Exmouth Gulf were significantly different to those in the sample from Shark Bay (Table 11). In addition, the value of F_{ST} between these two samples was significantly different from zero (Table 11).

Fourthly, the sample from Port Denison was in some ways intermediate between the samples from the north-west coast and those from the south-west coast. However, it was more closely aligned with the ‘southern’ samples, in particular those from Geographe Bay and the Peel-Harvey Estuary, than with the ‘northern’ samples (see Figs 1 & 2). Hence, when the sample from Port Denison was compared to either the sample from Shark Bay or that from Exmouth Gulf, the F_{ST} value was significantly different from zero and significant allele frequencies differences were apparent at four of the six loci (Table 11). On the other hand, when the sample from Port Denison was compared to either the sample from Geographe Bay or the sample from the Peel-Harvey Estuary, the F_{ST} value was not significantly different from zero, but the differences in the allele frequencies at one locus were statistically significant and those at another two to

four loci approached the level expected for statistical significance, *i.e.* $\alpha \leq 0.05$ (Table 11).

Fifthly, the allele frequencies at all six loci in the samples from the two most southerly locations, *i.e.* Geographe Bay and the Peel-Harvey Estuary, were effectively homogeneous (Table 11). However, the allele frequencies at the P4 and P18 loci in either of these samples *versus* those in a sample from near-by Cockburn Sound were significantly different (Table 11). Similarly, the value of F_{ST} between the sample from either Geographe Bay or the Peel-Harvey Estuary and that from Cockburn Sound was significantly different from zero (Table 11). Furthermore, the extent of the genetic differences between the sample from Cockburn Sound *versus* that from Port Denison was greater than those between the sample from either the Peel-Harvey Estuary or Geographe Bay *versus* that from Port Denison (see Table 11). This is despite the fact that Cockburn Sound is located only ~50 km and ~130 km north of, respectively, the Peel-Harvey Estuary and Geographe Bay, while Port Denison is located ~450 km north of Geographe Bay (see Fig. 1).

The genetic heterogeneity among the assemblages of *Portunus pelagicus* on the west coast provides a stark contrast with the homogeneity found among those from the east coast. This contrast can be clearly illustrated by comparing the estimates of the number of genetically effective migrants N_{em} into each sampling site per generation for each region: N_{em} equals only 5.3 for the west coast but 249.75 for the east coast. The contrast is important considering that (i) sampling was conducted over similar latitudes in both regions, and (ii) there is a southward movement of warm water along both the western and eastern seabords of Australia. This movement on the west coast, termed the Leeuwin Current, flows faster within the sampled latitudes than its east coast counterpart, *i.e.* the Eastern Australian Current (see Hamon *et al.*, 1975; Boland & Church, 1981;

Pearce 1991). Furthermore, although the Leeuwin Current tends to move off the continental shelf and flow along the shelf break south of about 27°S (Hutchins, 1991), it may nevertheless still spread onto the shelf at higher latitudes (Cresswell *et al.*, 1989). Regardless, the currents that do flow on this shelf south of 27°S could disperse the larvae of *P. pelagicus* as effectively as the Leeuwin Current (see Cresswell *et al.*, 1989).

The following observations provide two plausible explanations for the marked genetic heterogeneity of *P. pelagicus* in the region surveyed on the west coast.

(1) Permanent estuaries and sheltered coastal waters are rare and tend to be widely spaced within the northern sector of the surveyed section of the west coast, specifically from about ~200 km north of Port Denison to Exmouth Gulf (see Kirkman & Walker, 1989). Since the juveniles and adults of *Portunus pelagicus* are largely restricted to these types of environments, the assemblages of this species will presumably be similarly distributed in this region. Furthermore, although Exmouth Gulf and Shark Bay are very large, the other juvenile/adult habitats that occur in this region are small in area. These features may severely restrict the opportunity for 'stepping stone' dispersal in the region of the west coast between Port Denison and Exmouth Gulf. Thus, if the distance between 'findable' habitats typically exceeds the usual dispersal distance of individuals, gene flow would be expected to be restricted.

(2) The Leeuwin Current flows predominantly during autumn and winter. Consequently, it will typically transport the larvae of tropical species into temperate latitudes when temperatures are falling and hence when conditions are least suitable for the survival and/or settlement of these larvae (Hutchins, 1991). In contrast, the Eastern Australian Current flows mainly during the summer months and the reverse situation will apply (Hutchins, 1991). The seasonal

difference in the flow of these two current is thought to explain why recruitment in tropical fish species virtually ceases at 32°S on the west coast, but extends to as far south as 37°S on the east coast (Hutchins, 1991). A parallel situation may be occurring with respect to dispersal in *Portunus pelagicus*, *i.e.* dispersal from tropical to temperate waters on the west coast in particular may be restricted by the fact that the larvae will usually be delivered during times of falling water temperatures. Since the above-mentioned shelf currents also flow southwards during the coldest months of the year (Cresswell *et al.*, 1989), this suggestion is not dependent upon the Leeuwin Current being the main transport medium of the larvae of *P. pelagicus* on the west coast.

Although *Portunus pelagicus* inhabits temperate waters in Australia, it is essentially a tropical species that has adjusted its biology in order to grow and reproduce in temperate waters (Smith, 1982). For example, while this species spawns more or less continuously in tropical waters (*e.g.* Ingles & Braum, 1989), it typically spawns during the late spring and summer in temperate locations (*e.g.* Bryars, 1997; Potter *et al.*, 1983). Furthermore, the larvae of *P. pelagicus* exhibit significantly better survivorship at 25°C than at 20°C in a laboratory environment (Bryars, 1997). On this basis, it is reasonable to expect that the larvae of this species will not usually survive if dispersed into temperate waters during times of falling water temperature.

There are a few scattered reports of the distribution of genetic variation in other species along a comparable section of the coastline of Western Australia (*e.g.* Johnson & Black, 1984; Watts *et al.*, 1990; Watts, 1991; Thompson *et al.*, 1996; Chaplin *et al.*, 1998). The species that have been studied can be separated into four broad categories: (a) two invertebrates with a relatively long-lived planktonic larval phase and sedentary adults that inhabit shallow water reefs (Johnson & Black, 1984a; Watts *et al.*, 1990); (b) one invertebrate with a long-

lived planktonic phase, and mobile juveniles and adults that inhabit benthic environments on the continental shelf (Thompson *et al.*, 1996); (c) one teleost that has a long-lived planktonic phase and utilizes estuaries as a nursery area, but spawns in the ocean (Watts, 1991); and (d) two teleosts that typically complete their entire life-cycle in sheltered coastal waters (Watts, 1991; Chaplin *et al.*, 1998). The genetic compositions of the populations of the species with a long-lived planktonic phase were effectively homogeneous along a large section of the west coast. In contrast, the genetic compositions of the other two species exhibit a major break in the boundary between tropical and temperate waters and further genetic heterogeneity on either side of this break. NB. The boundary between tropical and temperate waters on the west coast is diffuse and extends from about Port Denison to Shark Bay (see Briggs, 1978; Morgan & Wells, 1991). Thus, there is evidence that the population genetic structure of a species in this region is strongly influenced by its powers of dispersal. If these powers are limited, both climatic boundaries and discontinuities in the distribution of suitable habitat may restrict gene flow. However, it is also worth noting that all of the above studies used allozyme markers and hence significant population genetic sub-division in certain of these species may have gone undetected.

The significant correlation between the genetic and geographic distances between pairs of samples of *Portunus pelagicus* from along the west coast indicates that the assemblages of this species in this region become increasingly genetically differentiated as the geographic distance between the assemblages increases. This apparent relationship implies that gene flow in this species in this region becomes increasingly restricted as the extent of geographic isolation between assemblages increases. However, the results for the east coast indicate that significant amounts of gene flow in this species can occur over distances comparable to the entire distance that was surveyed on the west coast. Thus, if

geographic distance plays a role in restricting gene flow along the west coast, it is probably acting in combination with some other factor(s), such as gaps in the distribution of suitable habitats for the juvenile/adult phase. Indeed, although the data overwhelmingly indicate that there are major genetic differences between the crabs from the southern *versus* northern ends of the sampling range (see below, this section), the evidence of an association between genetic distance and geographic distance was largely provided by the three samples from within the stretch of coastline where these habitats were widely-spaced, *i.e.* from Port Denison, Shark Bay and Exmouth Gulf (see Figs 1 & 2).

If the amount of contemporary gene flow between assemblages of *Portunus pelagicus* on the west coast is inversely correlated with the geographic distance between assemblages, this has important implications for the management of the stocks in this region. In particular, it implies that, although, within any one generation, there may be negligible amounts of gene flow between relatively distant assemblages, weak genetic connections could be indirectly maintained via the cumulative effects of the movements of individuals along the west coast over multiple generations (see Richardson *et al.*, 1986). Thus, although these weak connections would have no direct implications for stock abundance, they could nevertheless be important in determining how the stock(s) in particular localities will respond to major environmental changes or perturbations (see Richardson *et al.*, 1986).

The incidence of significant allele frequency differences was higher when comparing samples from north and south of Port Denison on the west coast than when comparing the samples from the north-west coast with those from the east coast. This could imply that, in the present environment, the genetic connections between the assemblages on the north-west and east coast are stronger than those between the assemblages on the north-west coast and those south of Port Denison

on the west coast. If this is the case, some factor(s) in addition to the discontinuities in crab habitats, is likely to be restricting gene flow in this species along the west coast. Certainly, the idea that there is a group of genetically distinct assemblages in temperate waters on the west coast coincides with the above-mentioned view that dispersal in *P. pelagicus* from tropical to temperate regions on the west coast may be restricted by the fact that the larvae will be transported southwards during times of falling water temperatures.

The microsatellite data indicate that the genetic compositions of the assemblages of *Portunus pelagicus* in Exmouth Gulf and Shark Bay, although relatively similar, are significantly different. This finding is important because it indicates that gene flow in this species can be restricted over a moderate geographic distance (~450 km), even in tropical waters. However, this finding may have been influenced by certain unique features of the Shark Bay environment. In particular, it is likely that gene flow in *P. pelagicus* is restricted between Shark Bay and Exmouth Gulf by one or both of the following factors.

(1) The amount of dispersal between these two embayments could be restricted. This is because there are (i) limited sheltered environments, *i.e.* juvenile/adult habitats, along the ~450 km of coastline that separates these two embayments (see Kirkman and Walker, 1989); and (ii) limited rates of water exchange within Shark Bay, and between Shark Bay and the ocean (see Hagan & Logan, 1974; Johnson & Black, 1990).

(2) The success of immigrants to Shark Bay, and of individuals that disperse from this embayment to elsewhere, may be limited. This relates to the possibility that the assemblage of *Portunus pelagicus* in Shark Bay has become genetically adapted to the unusual ecological conditions within this embayment, such as the presence of salinities as high as 65 ppt in its inner reaches (see Black *et al.*, 1990; Johnson & Black, 1990). In which case, these conditions may not be suitable for

immigrants to Shark Bay and individuals from Shark Bay may have reduced fitness in other environments.

The genetic composition of the assemblage of *Portunus pelagicus* in Port Denison appears to be much more similar to those of the assemblages at the two most southerly sampling points, *i.e.* Geographe Bay and the Peel-Harvey Estuary, than to those of the sampled assemblages on the north-west coast, *i.e.* Shark Bay and Exmouth Gulf. This is an interesting finding because Port Denison is located approximately equal distance (~450 km) between Geographe Bay and Shark Bay (see Fig. 1). However, the extent to which this result may have been influenced by the unusual conditions in Shark Bay (see above, this section), as opposed to reflecting general trends in north-south patterns of dispersal along the west coast, is not clear. Nevertheless, even though they are separated by over 300 km of coastline, it is clear that the genetic differences between the assemblage in Port Denison and those in the Peel-Harvey Estuary and Geographe Bay are relatively minor. This may be related to the fact that, between Geographe Bay and Port Denison, there is a more or less continuous fringe of limestone reefs that runs parallel to the coast and breaks the Indian Ocean swell and hence of relatively sheltered inshore environments (see Kirkman & Walker, 1989). These inshore environments provide 'suitable' habitat for the juveniles and adults of *Portunus pelagicus* and hence the opportunity for 'stepping stone' dispersal between distant sites within this area (but see below, this section). However, such environments and thus presumably also the potential for 'stepping stone' dispersal, become increasingly rare on the west coast north of Port Denison (see Kirkman & Walker, 1989).

The microsatellite data indicate that the genetic composition of the assemblages of *Portunus pelagicus* in Geographe Bay and the Peel-Harvey Estuary are effectively homogeneous. This is perhaps not surprising given that

these two water bodies are located only about 130 km from each other and within the above-mentioned section of the west coast where there is continuity in the distribution of relatively sheltered inshore environments. Hence, presumably there is opportunity for both the direct exchange of individuals of *P. pelagicus* between Geographe Bay and the Peel-Harvey Estuary as well as for ‘stepping stone’ dispersal. Indeed, there is an assemblage of blue swimmer crabs in the Leschenault Estuary and an associated marine embayment (Koombana Bay), which are located approximately half-way between Geographe Bay and the Peel-Harvey Estuary (e.g. see Potter & de Lestang, 2000). Since there are no obvious features of the inshore environment in this area that are likely to impede gene flow in *P. pelagicus*, it is likely that there are relatively strong genetic connections among the assemblages of this species in Geographe Bay, the Peel-Harvey Estuary and also in the Leschenault Estuary/Koombana Bay. However, it is not possible to determine if *P. pelagicus* is represented in this area by a single stock, a semi-continuous stock or a series of overlapping stocks (see above, ‘Data Interpretation & East Coast’).

Although relatively sheltered coastal environments are more or less continuously distributed between Geographe Bay and Port Denison, *Portunus pelagicus* nevertheless shows evidence of genetic subdivision within this section of coastline, as follows.

(1) The assemblage of *Portunus pelagicus* in Cockburn Sound was genetically distinct compared to all other sampled assemblages. The distinctiveness of this assemblage is unusual in the sense that Cockburn Sound is located near-by to Geographe Bay (~180 km south) and especially to the Peel-Harvey Estuary (~50 km south) and yet the differences between the assemblage in Cockburn Sound and that in either Geographe Bay or the Peel-Harvey Estuary are greater than those between the assemblages in Geographe Bay and the Peel-

Harvey Estuary (which are separated by ~130 km) and even between the assemblage in either Geographe Bay or the Peel-Harvey Estuary and that in Port Denison (which is located over 300 km to the north). The distinctiveness of the assemblage in Cockburn Sound probably relates to the semi-enclosed nature of this embayment, which may limit dispersal/gene flow into and out of this system. The topography and hydrology of this system is particularly likely to restrict dispersal/gene flow in a southward direction. This is because the southern end of Cockburn Sound is largely enclosed by land and a series of shallow reefs (Department of Environmental Protection, 1996). Furthermore, wind is the major driving force on the waters within and around Cockburn Sound and, during the summer months, when *P. pelagicus* typically spawns on the lower west coast (*e.g.* Potter *et al.*, 1983), strong southerly winds generate a predominantly northward flow of water (Department of Environmental Protection, 1996).

(2) The microsatellite data indicate that the genetic composition of the assemblage of *Portunus pelagicus* in Port Denison is different to that of all of the sampled assemblages further south, although it is relatively similar to those at the two most southerly sampling points, *i.e.* Geographe Bay and the Peel-Harvey Estuary. Since the east coast assemblages were effectively homogeneous over a much longer section of coastline, these differences are unlikely to be purely a function of the moderate geographic distances (300 - 400 km) that separate these assemblages. In fact, the reason for these differences could be related to the fact that, although the coastline between Port Denison and Geographe Bay is relatively sheltered, *i.e.* not directly exposed to oceanic swells, there are no large estuaries or highly protected embayments in the ~250 km stretch of coastline between Port Denison and the Swan River Estuary/Cockburn Sound. Such habitats are particularly important nursery areas for *P. pelagicus* (see Potter *et al.*, 2001). Consequently, the abundance of this species may be reduced in this and other

areas where these types of habitats are limited. If only a very small proportion of the total pool of larvae of *P. pelagicus* in an area disperse over moderate or large distances, then it is likely that the amount of dispersal/gene flow across areas of sub-optimal habitat and low crab abundance will be relatively low.

Management Implications

It is realistic to separate the stocks of *Portunus pelagicus* in tropical and temperate waters on the west coast into two distinct groups. The amount of gene flow between these two groups of stocks is likely to be negligible, even over multiple generations. Furthermore, certain characteristics of the stocks in temperate waters are very different to those on the north-west coast (*e.g.* in temperate waters this species typically spawns only during the warmer months of the year) and it is possible that the temperate stocks have become acclimated and/or genetically adapted to a 'cool' water environment. The stock, *i.e.* Port Denison, that occurred in the transition from tropical to temperate influences on the west coast was intermediate in certain respects between the tropical and temperate stocks. In theory, this indicates that the tropical and temperate groups may not be completely isolated from each other, *i.e.* there is a leaky boundary. In practice, in the contemporary environment, the assemblages on the north-west coast are probably more closely aligned with those in northern Australia and those on the east coast, than they are to those in temperate waters on the west coast. Thus, the 'northern' and 'southern' groups of stocks in Western Australia should be managed separately and the management methods that are applicable to one group might not necessarily be applicable to the other.

On the west coast, gene flow in *Portunus pelagicus* is apparently also restricted by large discontinuities in the distribution of suitable habitats for the juveniles and adults of this species. Consequently, the spatially-isolated

assemblages of this species are each likely to constitute separate stocks and should be managed as such. However, weak genetic connections between isolated assemblages may be maintained by rare long distance dispersal and could be important in determining how isolated stocks will respond to major environmental change and perturbations in the long-term. Since the amount of gene flow in *P. pelagicus* appears to be inversely proportional to geographic distance over all but relatively fine spatial scales, further fragmentation of this species should be avoided if possible.

Even in the region from Cockburn Sound to Geographe Bay on the southwest coast, where there are only small gaps in the distribution of habitats for the juveniles and adults of this species, gene flow is apparently restricted by the semi-enclosed nature of Cockburn Sound and possibly also the northward flow of the prevailing currents at the time of spawning. Whatever the reason, the assemblage of *P. pelagicus* in Cockburn Sound is a separate stock from those in nearby water bodies further south and it is therefore desirable to manage the fishery for this species in Cockburn Sound separately from those in these other nearby water bodies.

Overview

The foregoing findings regarding the stock structure of *Portunus pelagicus* within certain geographic regions can be combined to produce hypotheses regarding the patterns of contemporary gene flow in this species in Australia. In this regard, it is particularly relevant that the genetic structure of *P. pelagicus* is far more heterogeneous along the coastline of Western Australia than in eastern Australia. This difference is believed to be related largely to the fact that embayments and estuaries, which contain the habitats occupied by the juveniles and adults of *P. pelagicus*, are far more widely distributed on the central

west coast than on the central east coast of Australia. In other words, the distances between adjacent embayments and/or estuaries are often so large on the central west coast that they restrict the exchange of larvae and/or juvenile/adult crabs between those water bodies, whereas the shorter distances between adjacent water bodies on the east coast facilitate intermixing between assemblages.

The results of this study cannot be used to determine the dispersal distances of *Portunus pelagicus*. However, the following information suggests that individuals of this species usually disperse over distances of less than 300 kilometres, although 'rare' individuals may move over longer distances. Firstly, outside the east coast, any of the sampled assemblages that were separated from each other by 300 or more kilometres were invariably genetically differentiated. Secondly, given that the duration of the planktonic phase of *P. pelagicus* is about three weeks, the speed of the currents in coastal regions on the east and west coasts are such that they are unlikely to transport a larva more than about 300 kilometres from its natal site (*e.g.* see Background).

Portunus pelagicus is essentially a tropical species. At higher latitudes, the temperatures during the coolest times of the year are not optimal for the survival and/or settlement of the larvae of this species. Consequently, gene flow from tropical to temperate waters may be inhibited, at least during these times. Furthermore, even during the summer months, the relatively cold temperatures of offshore waters in South Australia, and possibly also of those in the southern extremes of its range on the east and west coasts of Australia, may restrict gene flow within temperate latitudes.

Finally, the assemblages of *Portunus pelagicus* contained within certain semi-enclosed embayments, such as Gulf Saint Vincent, Spencer Gulf and Cockburn Sound, were each genetically distinctive, even in comparison with the assemblages in nearby water bodies. The rates of water exchange between these

embayments and outside marine waters are restricted in some ways and this may limit the ability of individuals of *P. pelagicus* to immigrate from or into these water bodies.

In conclusion, gene flow in *Portunus pelagicus* appears to be limited by one or more of the following factors (i) a modest planktonic larval phase, (ii) habitat availability, (iii) relatively cold waters at high latitudes and (iv) local features of coastal environments. In any case, the 'popular' view that *P. pelagicus* has a high potential for dispersal simply because it has planktonic larval stages and mobile adults and juveniles is misleading.

Benefits

The main benefits of this study reside in the fact that the results and conclusions provide managers of the fisheries for *Portunus pelagicus* in Australia with detailed information about the stock structure of this species and the associated management implications. The most important of these implications are specified below.

The assemblages of *Portunus pelagicus* in different embayments along the west coast of Australia and in South Australia often constitute genetically-differentiated stocks. The amount of migration between these different stocks is probably negligible. Hence, the dynamics of the assemblages in these regions will often be determined 'solely' by internal patterns of recruitment and mortality. Furthermore, in the event that a particular stock is depleted by overfishing, it is unlikely to be replenished from outside sources. Hence, the critical scale for the management of the fisheries for *P. pelagicus* in South Australia and Western Australia is at the level of the individual assemblage.

In eastern Australia, at least between Mackay and Port Stephens, *Portunus pelagicus* comprises either a series of overlapping assemblages or a single semi-continuous stock. Hence, in this region, there may be sufficient immigration into a water body to aid in the recovery of an assemblage that has been depleted by overfishing. However, significant amounts of replenishment may not occur within a single generation of crabs. Furthermore, individuals of *P. pelagicus* usually disperse over relatively short distances. The genetic connections that are maintained between geographically distant assemblages of this species on the east coast presumably reflect the cumulative effects of a series of overlapping local interchanges in multiple generations. Any broad-scale fragmentation of this species would cause disruptions to these local interchanges and could serve to isolate certain assemblages. The most critical issue for the long-term sustainable

management of this species in this region is, therefore, the maintenance of the continuity in its geographical distribution.

This study identified four genetically distinctive groups of stocks of *Portunus pelagicus* that occur in definable geographic regions, *i.e.* South Australia, the east coast, north-west coast and south-west coast. On this basis, it is realistic to expect that the stocks within a particular region are more likely to share certain fundamental biological similarities than those from a different region. This information can be used by managers to develop efficient strategies for collecting the biological data necessary to develop stock assessment methods.

In more general terms, this study has helped to identify the factors that are likely to play an important role in restricting the dispersal of *Portunus pelagicus*. These factors include a modest planktonic life, discontinuities in the distribution of habitats for the juveniles and adults, the semi-enclosed nature of certain embayments and the relatively cold water in the southern regions of the distribution of this species in Australia. This information can be used by managers to predict the locations of the boundaries between the different stocks in locations not included in the survey area.

Finally, while the results of a previous allozyme study were consistent with the view that there was a single stock of *Portunus pelagicus* in Australian waters outside South Australia, the microsatellite markers used in this study identified the presence of numerous genetically-differentiated stocks of this species in these waters. This finding should be taken into account when interpreting the results of previous allozyme-based studies of the stock structure of species and when considering the type of approach that is appropriate for future studies of this nature.

Further Development

More information about the distribution of microsatellite markers in *Portunus pelagicus* in tropical waters in Australia would lead to a more sophisticated understanding of the stock structure of this species in Australia. This is partly because this information would resolve more clearly how many major genetic groups and genetically differentiated stocks of this species are present in the coastal waters of Australia. Furthermore, it would provide the opportunity to investigate more thoroughly the aspects of the environment that present barriers to dispersal in *P. pelagicus*, the spatial scales over which these barriers operate and how these factors may or may not vary among geographic regions. Thus, ultimately, it would become easier to make specific predictions about the locations of the stock boundaries of this species and how this species may respond to major environmental perturbation.

Conclusions

This project has used microsatellite markers to determine the stock structure of *Portunus pelagicus* in Australia. The primary objective of this project was to determine whether selected assemblages of blue swimmer crabs in nearby and more distant geographic sites in states throughout Australia are genetically differentiated and thus constitute different stocks. More specifically, the study proposed to focus on determining the extent of any such differences and providing managers with an interpretation of the biological implications of those differences. The project has achieved these objectives, as is illustrated by the following conclusions.

Variation Among geographic regions

- * The microsatellite markers demonstrated that there are a series of genetically-differentiated stocks of *Portunus pelagicus* in Australian waters.
- * There are at least three highly genetically-distinct groups of *P. pelagicus* in these waters.
- * Each of these groups occurs in a different geographical region.
- * Specifically, one group occurs on the east coast, another group occurs in South Australia and a third group is found on the west coast.
- * In addition, the 'west coast group' exhibits considerable genetic heterogeneity and comprises distinctive 'northern' and 'southern' groups of stocks.
- * Within any one generation, the amount of genetic exchange in *P. pelagicus* between these geographic regions is likely to be negligible.
- * However, the cumulative effects of 'stepping stone' dispersal in multiple generations in waters in northern Australia may maintain weak genetic connections between the *P. pelagicus* on the north-west coast and those on the east coast.
- * Historic connections among the assemblages on the west coast may be relatively strong but, in the contemporary environment, the amount of gene

flow between assemblages on the north-west coast and those in temperate waters on the west coast is probably less than that between the assemblages on the north-west coast and those on the east coast.

- * It is possible that the biological characteristics of the crabs in the different distinctive groups of stocks are fundamentally different.
- * It is likely that individuals of *P. pelagicus* usually disperse over relatively short distances, *i.e.* less than 300 km.

Variation within geographic regions

- * On the east coast of Australia, between Mackay and Port Stephens, *Portunus pelagicus* comprises either a series of overlapping assemblages or a single semi-continuous stock.
- * Genetic connections between the more distant assemblages in this region are probably maintained via the cumulative effects of a series of overlapping local interchanges in multiple generations.
- * These long distance connections are made possible by the fact that there is a more or less continuous series of estuaries and relatively sheltered embayments in this region, *i.e.* of suitable habitats for the adults and juveniles of *P. pelagicus*.
- * The assemblages of *P. pelagicus* in South Australia and between Exmouth Gulf and Geographe Bay on the west coast often constitute different stocks.
- * Habitat availability, cold water in the more southern regions of its range and the hydrological characteristics of certain embayments appear to play an important role in keeping assemblages of *P. pelagicus* isolated.
- * The critical level for the management of the fisheries for *P. pelagicus* in South Australia and on the west coast is at the level of the individual assemblage.
- * The most critical issue for the long-term sustainable management of this species on the east coast is the maintenance of continuity in its geographical distribution.

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Appendix 1: Intellectual Property

N/A

Appendix 2: Staff

This project was conducted by the following researchers, at the Centre for Fish and Fisheries Research, Murdoch University.

Principal Investigator: Dr. Jennifer Chaplin

Co-investigator: Prof. Ian C Potter

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