PILOT STUDY OF LARVAL RECRUITMENT AND GENETIC VARIATION OF SOUTHERN ROCK LOBSTER POPULATIONS

88/41

FINAL REPORT

Shellfish Section
Division of Sea Fisheries
DPIF Tasmania

Funded by the
Fishing Research and
Development Corporation

Pilot Study of Larval Recruitment and Genetic Variation of Southern Rock Lobster Populations

Final Report
to the
Fishing Industry Research and Development Council
for project grant 88/41

February, 1991

Shellfish Section,
Division of Sea Fisheries,
Department of Primary Industry,
G.P.O. Box 619F,
Hobart 7001 Tasmania

Fish Research Group,
Department of Zoology,
University of Tasmania,
G.P.0. Box 252C,
Hobart 7001 Tasmania

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Abstract

The occurrence of puerulus and post-puerulus stages of *Jasus novaehollandiae* on artificial collectors was monitored at 5 sites around Tasmania. Three designs of collectors were tested. Crevice collectors usually obtained the highest catches and were the most durable and least costly of the 3 collector designs. Minor settlement of puerulus occurred throughout the study and a peak period of settlement occurred at most sites between November and January. Another peak settlement commenced at east coast sites during June 1990, but was absent during the winter of 1989. Considerable differences in settlement rates were found between sites and between stations within individual sites. The high localised spatial variability in settlement rates suggests that a necessary prerequisite for long term monitoring is to determine the degree to which individual stations are representative of temporal changes in settlement levels over a wider area. At most locations, settlement rates were too low to justify continued sampling, but at 3 stations, a sample size of 10 collectors per station would enable fluctuations in puerulus settlement rates to be detected. Considerable potential exists for obtaining improved settlement rates by manipulating the position of collectors. This has been demonstrated with on-going studies.

Nucleotide sequence polymorphism in the mitochondrial genomes of 132 adult lobsters from Western and southern Australia and from New Zealand was assayed using six restriction endonucleases. Phenetic clustering and gene diversity analyses as well as pairwise comparison of the genetics of specimens from each, or grouped, locales did not detect the presence of genetic subdivision. The mean amount of mtDNA diversity among the 132 mitochondrial genomes was 0.77%. The lack of genetic distinction between the Australian *J. novaehollandiae* and *J. edwardsii* from New Zealand and among Australian populations of *J. novaehollandiae* suggests that larval-mediated gene flow is a potent force which has the potential to genetically unite geographically distant populations.

Preliminary experiments showed that the standard techniques employed to extract and analyze the mtDNA of adult *J. novaehollandiae* could not be applied to puerulus. We describe an alternative technique, in situ gel hybridization, used for the detection of mtDNA restriction fragments from puerulus. Detection of single-stranded mtDNA, or target sequence, involved hybridization with radiolabelled homologous DNA, or probe. These hybrids were then seen as discrete bands after autoradiography. *J. novaehollandiae* target sequence was digested with the restriction enzyme AvaI and electrophoresed through an agarose gel. Following gel dehydration, both denaturation of the target sequence into single strands and subsequent hybridization were carried out in situ. Due to a low degree of sequence homology, puerulus mtDNA restriction fragments

could not be detected using probe synthesized from cloned honeybee mtDNA. Probe synthesized from gel purified adult mtDNA enabled partial genetic characterization of a puerulus larva. This puerulus was identified as having three restriction fragments in common with an adult specimen. Diffusion of small fragments from the gel meant that a small portion of the puerulus mtDNA (~ 10%) could not be characterized. However, by selecting restriction enzymes that produce large fragments from variable regions of the genome, informative genetic markers could be routinely analyzed.

General Introduction

The southern rock lobster (*Jasus novaehollandiae*) occurs throughout southern Australia with highest abundance off Tasmania, Victoria and the southern half of South Australia. The fishery for this species produces an annual catch of approximately 4,500 tonnes and is one of the most valuable fisheries in Australia.

The southern rock lobster fisheries of Australia are managed independently and considerable differences exist in the management policies between states. However it has not been known whether the stocks are discrete and thus whether one fishery can be managed without reference to the others. The phyllosoma stage of *J. novaehollandiae* is planktonic and can be transported considerable distances during a lengthy oceanic phase. Winstanley (1970) and Booth et al. (1990) reported the presence of phyllosoma stages across the Tasman sea between Tasmania and New Zealand. A mechanism whereby Australia contributes to New Zealand recruitment, has often been considered (Winstanley 1970, Phillips and McWilliam 1986, Booth 1987, Booth et al. 1990) and it is even more likely that such a mechanism exists within Australia in which larvae from one State could be a significant source of recruitment to another State. The possibility of single States possessing a number of discrete stocks must also be considered.

Allozyme analysis of adult rock lobster (Smith et al. 1980) indicated that significant recruitment between Australia and New Zealand may be unlikely, but this work was not conclusive. In virtually all allozyme electrophoresis studies on decapods, including lobster, the amount of nuclear gene variation detected between and within species is much lower than for other groups of animals. Thus, it is doubtful that further allozyme studies could assist in stock identification of *J. novaehollandiae*. Mitochondrial DNA (mtDNA) studies on *Panulirus argus* in Florida (McLean et al. 1983) have found significant heterogeneity and possible species subdivision, and MtDNA analysis thus has the potential for stock identification of *J. novaehollandiae*.

The dispersal phase of *J. novaehollandiae* ends with the puerulus larva. This is a transitional settling stage between the planktonic phyllosoma larva and the benthic juvenile. Provided that mtDNA variation in adult *J. novaehollandiae* is geographically partitioned, the mtDNA characteristics of puerulus could be used to identify their parental stock. Such genetic comparisons have the potential to identify the original sources of larval recruitment responsible for maintaining a managed fishery.

Puerulus of some Palinurid species are known to settle on artificial collectors (eg. *Panulirus argus*, Witham et al. 1968; *P. cygnus*, Phillips 1972; *Jasus edwardsii*, Booth

1979 ; *J. novaehollandiae*, Lewis 1977). Prior to the current study, settlement of *J. novaehollandiae* on artificial collectors had not been examined outside South Australia. However, the success of Lewis (1977) in South Australia provided a reason to expect that puerulus could be obtained elsewhere.

Regular monitoring of puerulus collectors can aid in the understanding of larval recruitment patterns and mechanisms. Geographical differences in the temporal pattern of settlement can provide an indication as to whether differing factors are affecting settlement between areas. The presence or absence of settlement in differing locations might provide similar indications. However, the most important requirement of puerulus collectors is to provide an annual index of settlement levels as a means for monitoring the southern rock lobster fisheries. Relationships between settlement indexes and both pre-recruit abundance (Breen and Booth 1979) and the recruited population (Phillips 1986) have been demonstrated in other Palinurid species. Hence, an annual settlement index for *J. novaehollandiae* has the potential to provide an important early warning system of future declines in the fishery.

Funding was provide by the Fishing Industry Research and Development Trust Fund for a one year pilot study to provide preliminary information regarding puerulus settlement and genetic variation. Results from the pilot study are to be used to determine whether a more extensive project would be viable. In particular, this study sought to:

(1) Compare the efficiency of existing *Jasus spp.* puerulus collectors and determine the most suitable collector.
(2) Provide initial estimates of spatial variability and timing of puerulus settlement and determine whether puerulus can be obtained in quantities sufficient for numerical analysis.
(3) Develop mtDNA analysis techniques to genetically identify puerulus larvae.
(4) Obtain preliminary information on the genetic variability of southern rock lobster populations.

CHAPTER 1

Investigation of puerulus settlement of *Jasus novaehollandiae* in Tasmania.

(R.B. Kennedy, D.B. Tarbath, P. Terry and R. Pearn)

1.1 Introduction

The southern rock lobster fisheries of Australia are currently monitored through a combination of commercial catch and effort data and pot sampling studies. These are the basic monitoring tools used by most fisheries throughout the world and they provide the fundamental ingredients required by many stock assessments. Despite the importance of both commercial catch data and pot sampling studies, neither method provides managers with real-time information concerning the status of the resource.

For many Tasmanian fishing regions, the time lag between settlement and subsequent detection by a pot sampling program is approximately 6 years. The time lag is generally greater when using commercial catch data. Hence, a decline in settlement would not be detected by existing monitoring methods for approximately 6 years and a trend could not be established before the decline extended through most year classes of the fishery. The inability to discover trends at an early stage increases the risk of overfishing. Furthermore, late detection of problems is likely to increase the severity of required corrective action (e.g. reductions in effort or allowable catch), as well as causing implementation difficulties due to the financial hardships to industry.

The puerulus larva of *Jasus novaehollandiae* is a transitional settling stage between the planktonic phyllosoma larva and the benthic juvenile. A knowledge of annual variation in puerulus settlement rates would be a considerable aid to managers by indicating potential fluctuations in the resource at an earlier time than is achieved through current monitoring of the fished stocks. This assumes that reproductive output and pre-settlement processes acting upon the planktonic stages are dominant variables influencing recruitment to the fishery, while post-settlement processes are relatively small. Hence, large inter-annual fluctuations in puerulus settlement levels should later be observed as fluctuations in recruitment to the fishery. Phillips (1986) used this assumption with a long time series of puerulus settlement data to advance a predictive model for the fishery catch of *Panulirus cygnus* in Western Australia, while Booth and Bowring (1988) have used settlement information to aid in monitoring the health of the *J. edwardsii* fishery in New Zealand.

In order to provide timely advice to management, it is important to monitor the fishery at the earliest possible stage, so the development of a puerulus monitoring program is

considered to be a high priority for the southern rock lobster fisheries. The aims of this study were to determine a suitable design of collector, to provide preliminary information concerning spatial variation and seasonality of puerulus settlement and to determine whether puerulus could be obtained in sufficient quantities for adequate resolution of inter-annual fluctuations in settlement levels.

1.2 Materials and Methods

The puerulus collectors were of two main types; a 'crevice-type' similar to that described by Booth and Tarring (1986) which provided shelter in the form of crevices (Fig. 1), and a 'pallet-type' which provided shelter via epiphytic growth inside a slatted wooden box (Fig. 2). Two styles of pallet collectors were used; a collector similar to that described by Lewis (1977) and a modified (1/4 size) pallet collector which is easier to service than the standard pallet collector.

Crevice collectors were constructed with either exterior grade (5-ply) or structural grade (3-ply) plywood in order to compare durability versus costs of construction. All plywood was treated as described by Booth and Tarring (1986) to inhibit shipworm infestation. All pallet-type collectors were constructed from *Pinus radiata* timber. Fifty percent of the pallet-type collectors were treated with CCA (copper chromate arsenic) at standard marine grade pressure (24 kpa. c.c.) to inhibit shipworm infestation, while the remaining collectors were untreated as in the original work of Lewis (1977).

All crevice collectors were initially moored 30 cm above the sea floor in depths ranging between 2 to 12 metres. Wherever possible, these collectors were deployed on a sand substrate in order to minimise confounding effects arising from possible migration of puerulus between the collectors and the bottom. After deployment, most moorings were fitted with an extended stand to raise the collectors further from the sea floor and thus reducing the likelihood of sand deposition on collectors. Extended stands were usually fitted to raise the collectors 60 cm above the sea floor, but occasionally longer extensions were used to lift the collectors 90 cm above the sea floor in areas with high sand deposition.

The pallet-type collectors were deployed either near the surface, attached to a long-line (Fig. 3a), or individually at a similar height above the sea floor (Fig. 3b) to that for crevice collectors. Modified pallet collectors were always deployed in pairs, with one collector directly above the other.

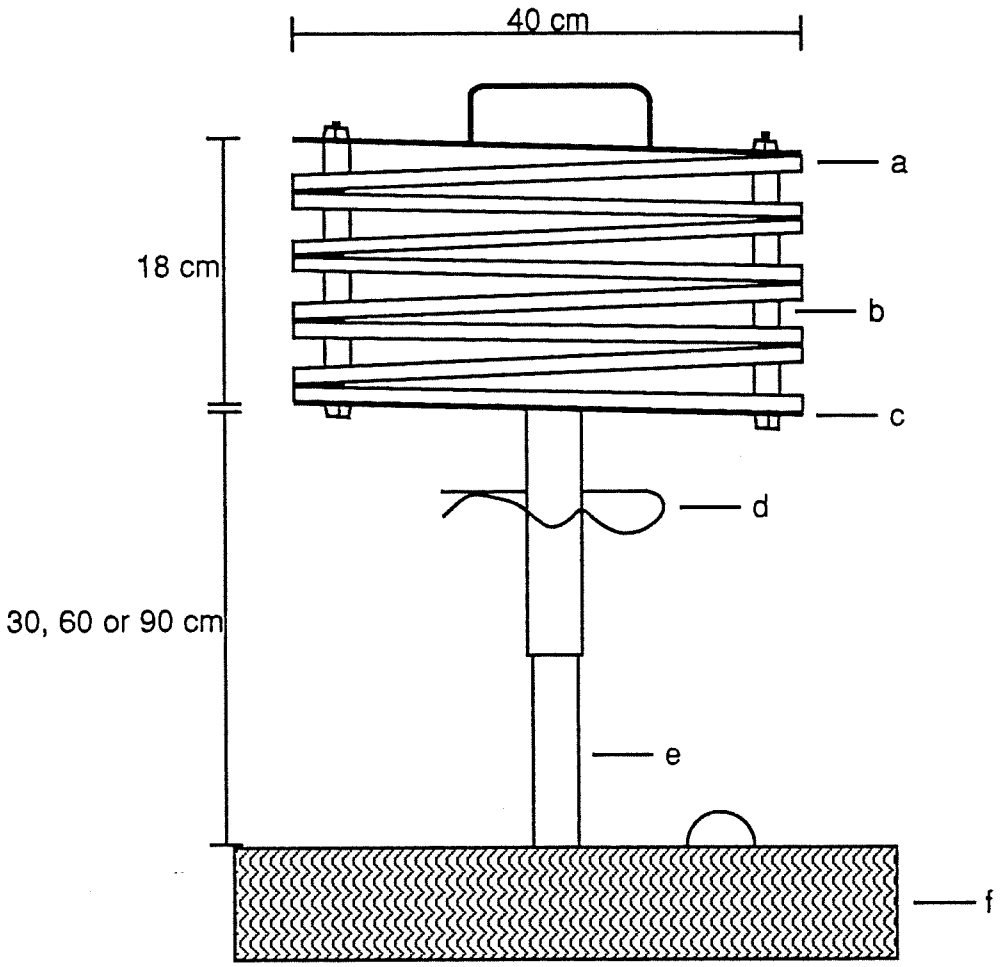

Figure 1: Crevice collector. 40x40 cm plywood sheets (a) form crevices that provide shelter for puerulus. PVC spacers (b) kept the sheets separated. The collector was held together by a galvanised steel frame (c). A clip (d) could be removed so that the collector could be lifted off the stand (e) and 100 kg mooring weight (f) which comprised concrete and iron embedded inside a car tyre.

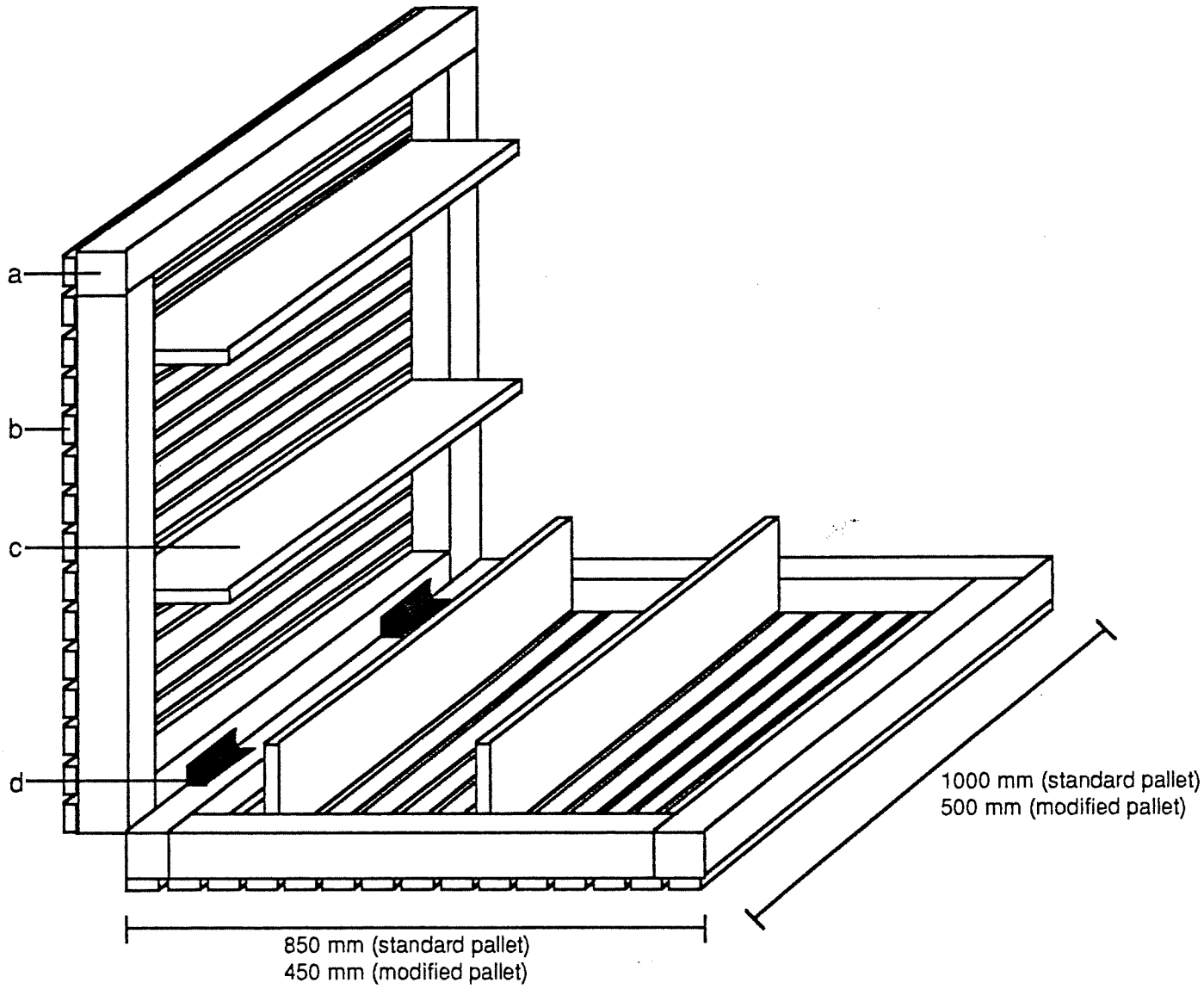

Figure 2: Pallet collector in an open (servicing) position. Pine timber, measuring 45x45 mm in cross section (a) formed the frame of the collector. Pine slats (b), measuring 10x40 mm in cross section were nailed and glued to the frame. A distance of 12-17 mm separated individual slats. Pine baffles (c) provided shelter and reduced water movement within the collector. The collector was hinged (d) so that it could be opened for servicing.

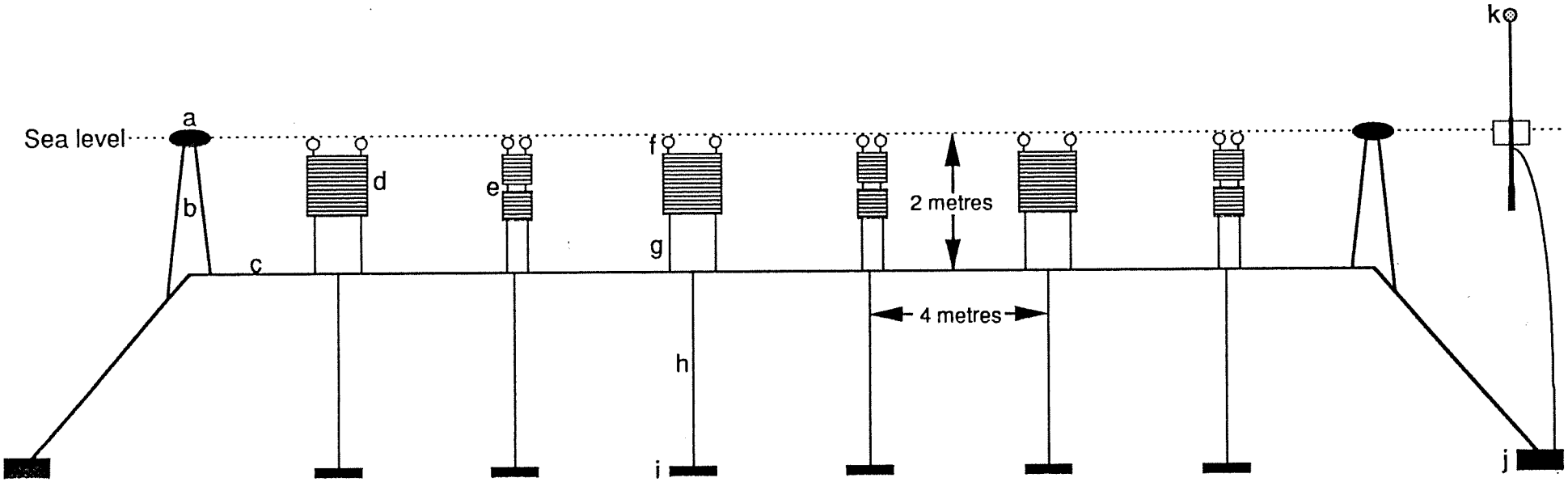

Figure 3a: Long-line mooring system used for pallet-type collectors at Lagoon Bay. A mussel buoy (a) was attached by ropes (b) to support the 20 mm diameter long line (c). Standard pallet (d) and pairs of modified pallet collectors (e) were held above the long-line by 20 cm diameter polystyrene buoys (f) and were attached to the long-line with a length of rope (g) and a stainless steel shark clip for quick removal of the collectors. A drop line (h) beneath each collector, held by a 40 kg iron weight (i) kept the long-line at a constant height above the sea floor. Each end of the long line was anchored with a 200 kg iron weight (j). An amber light (k), flashing 66 times per minute was required to warn shipping of the presence of a navigation hazard.

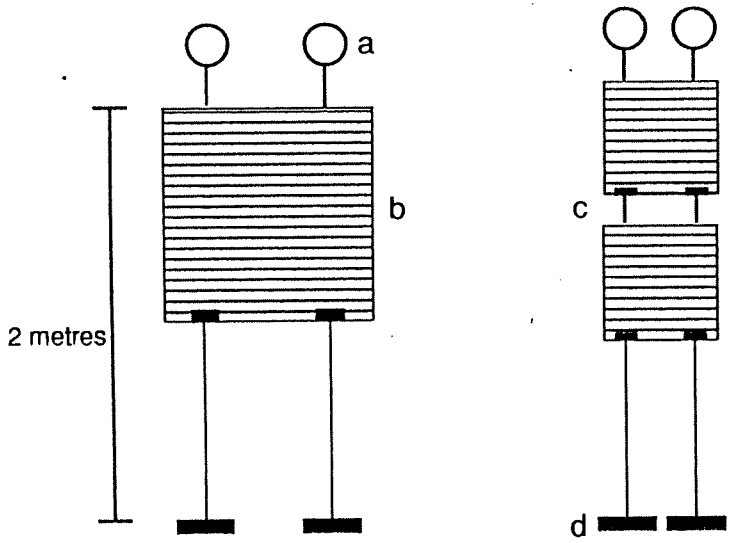

Figure 3b: Individual mooring system used for pallet-type collectors at Bicheno. 20 cm diameter polystyrene buoys (a) support both standard pallet (b) and pairs of modified pallet collectors (c). Both types of collectors were held to the sea floor by two 40 kg iron weights (d).

Five sampling sites were chosen around the eastern half of Tasmania as shown in Figure 4. The presence of many small, juvenile rock lobsters in an area usually suggests that there will be good settlement (Booth and Tarring, 1986). However, such lobsters rarely occur in pots and previous dive surveys in Tasmanian waters have generally not located areas with many small, juvenile lobsters. Station 2 at Bicheno (Fig. 4) was selected on the basis of previous puerulus settlement on scallop spat collectors. The remaining locations were selected to provide an even coverage of the eastern half of Tasmania and to provide a degree of protection for the collectors without reducing the degree of oceanic influence.

Three stations were established at each of the 5 sites, with 3 crevice collectors being deployed per station. Stations were separated by a distance of 300 to 1200 metres, while collectors within each station were spaced approximately 3 metres apart. Catch rates of crevice collectors were compared to those of pallet-type collectors at both Bicheno and Lagoon Bay. Three pallet and 3 pairs of modified pallet collectors were deployed near the sea floor alongside the 3 crevice collectors in station 2 at Bicheno. A near surface long-line containing 3 pallet and 3 pairs of modified pallet collectors was deployed in each station at Lagoon Bay.

Collectors were checked on a monthly basis. Divers placed a nylon bag around each collector before the collector was brought on a boat for inspection. Any lobsters found on collectors were removed, counted and staged according to the criteria of Booth (1979). During the first 3 months of sampling, puerulus were frozen in liquid nitrogen for later genetic analysis (see Chapter 3). After this period, specimens were taken live to the laboratory for further measurements.

Before returning collectors to the sea, fouling organisms were scraped off crevice collectors so that the collectors had a relatively consistent degree of fouling each month. Pallet style collectors were not cleaned as these collectors require considerable fouling in order to provide suitable shelter for puerulus.

1.3 Results

Collectors were installed during May, 1989. A total of 198 specimens (88 puerulus and 110 post puerulus) were caught from June 1989 to June 1990 inclusive. Figure 5 shows the carapace length and weight of captured specimens. Three of the specimens were of a size (carapace length >14 mm, weight > 1 gm) that indicated possible immigration to the

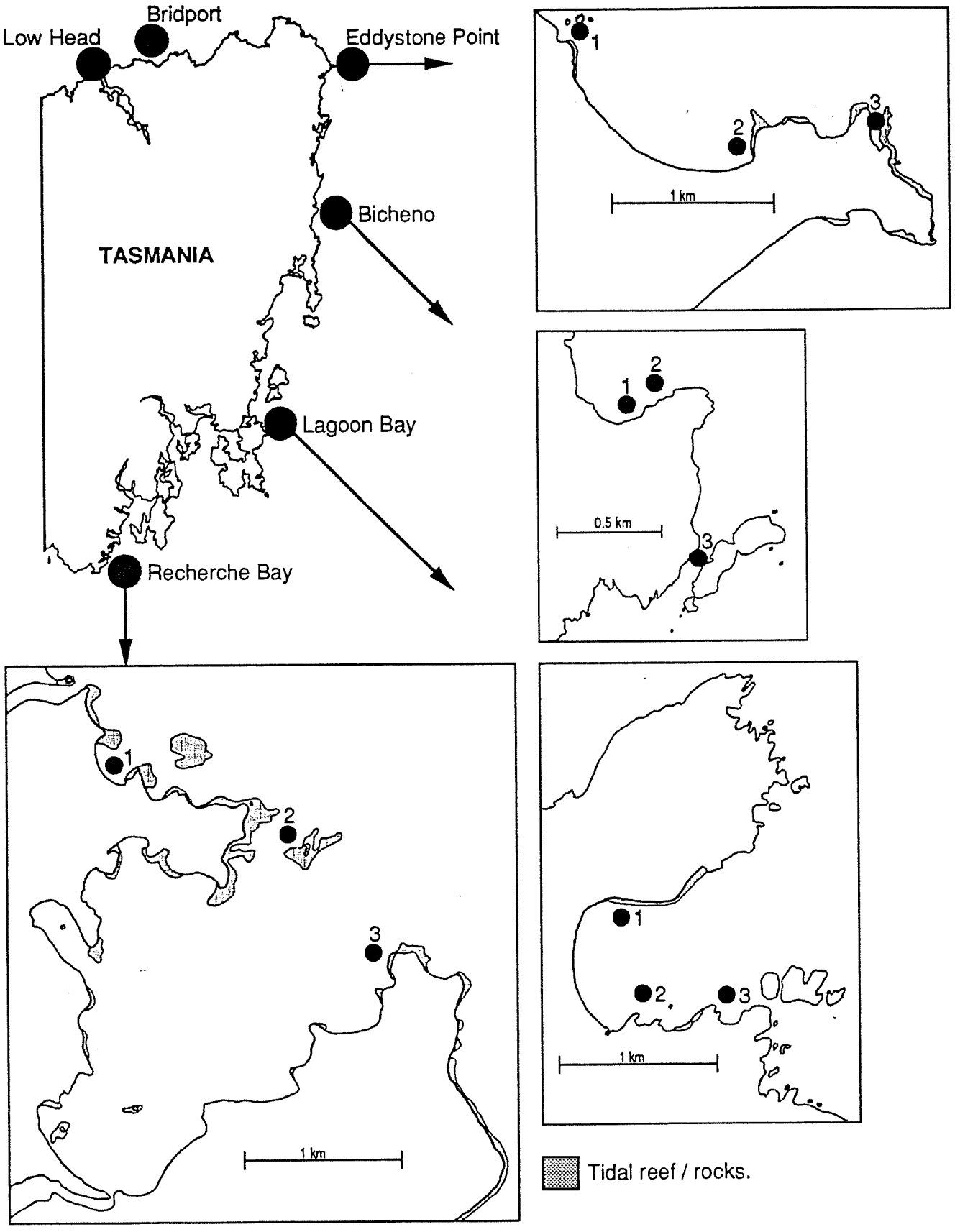

Figure 4: Puerulus Sampling sites. 3 crevice collectors were deployed at each numbered station. In addition, 3 pallet and 3 pairs of modified pallet collectors were deployed at Bicheno, station 2 and at all stations in Lagoon Bay. Collectors were moved from Low Head to Bridport during August 1989. There were 3 stations at each of these sites, but they have not been shown due to lack of settlement.

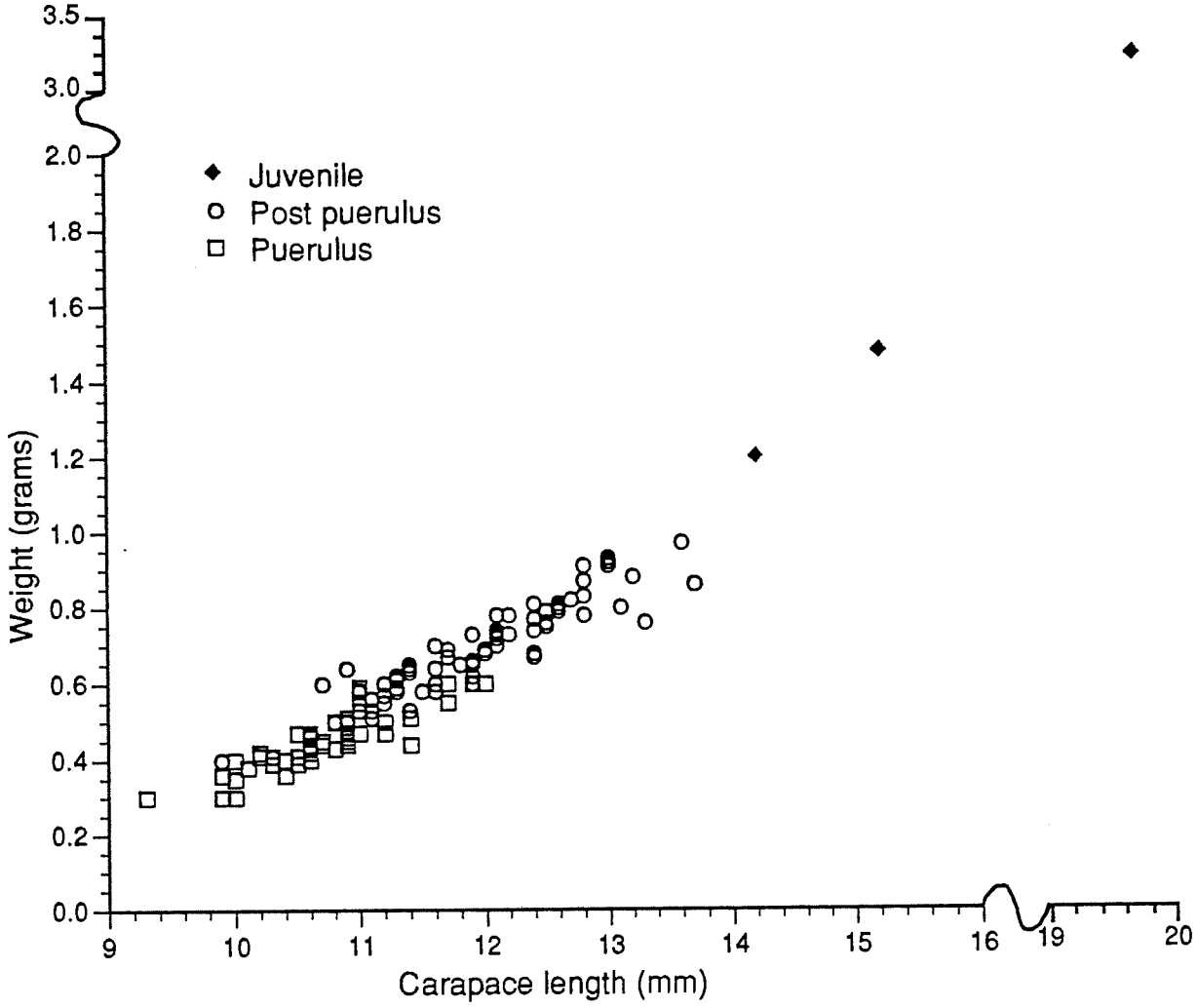

Figure 5: Length and weight of specimens caught on puerulus collectors.

collectors after settlement. Immigrants were only found on crevice collectors and these specimens have been excluded from the results given below.

Comparison of collector designs

Crevice collectors caught puerulus more frequently than surface deployed pallet-type collectors, with a shorter time lag between deployment and first settlement (Fig. 6a). There was a 4 fold difference in the total catch per collector for crevice collectors compared to both of these pallet-type collectors. However, no differences were observed between the catch rate of crevice collectors and bottom deployed pallet-type collectors (Fig. 6b). The cumulative catches for these collectors were similar, with considerable cross-over between the lines. Despite a smaller surface area, pairs of modified pallet collectors caught a similar number of puerulus to standard pallet collectors.

After nearly 14 months in the water, all 12 untreated pallet-type collectors were severely infested with shipworm. Of these, 6 were totally destroyed, with the remaining 6 collectors sustaining considerable damaged with broken slats, etc. The 12 treated, pallet-type collectors were in good condition except for the mooring attachment and 3 of these collectors had been rendered unserviceable due to damage in this area. There were no observable differences in the catch rates between treated and untreated pallet-type collectors.

The small, modified pallet collectors proved to be more durable than standard pallet collectors as only 1 in 12 of the modified collectors were destroyed, whereas destruction occurred for 8 of the 12 standard pallet collectors. All crevice collectors were in good condition at the end of the project regardless of the type of plywood which was used in construction. A total of 45 crevice collectors were deployed around Tasmania, of which only 2 were lost in heavy sea conditions.

Seasonality and spatial variability of settlement

Figure 7 shows the daily catch rates of crevice collectors for individual stations at each site. It is evident that minor settlement occurred throughout the year with a summer peak in settlement occurring at both Bicheno (Nov./Dec.) and Recherche Bay (Jan.). Catch rates were too low and varied to deduce seasonality of settlement from crevice collector data at Eddystone Point and Lagoon Bay. However, when the data is pooled from all stations and collector-types, a summer peak in settlement (Dec.) was also apparent at Lagoon Bay (Fig. 8).

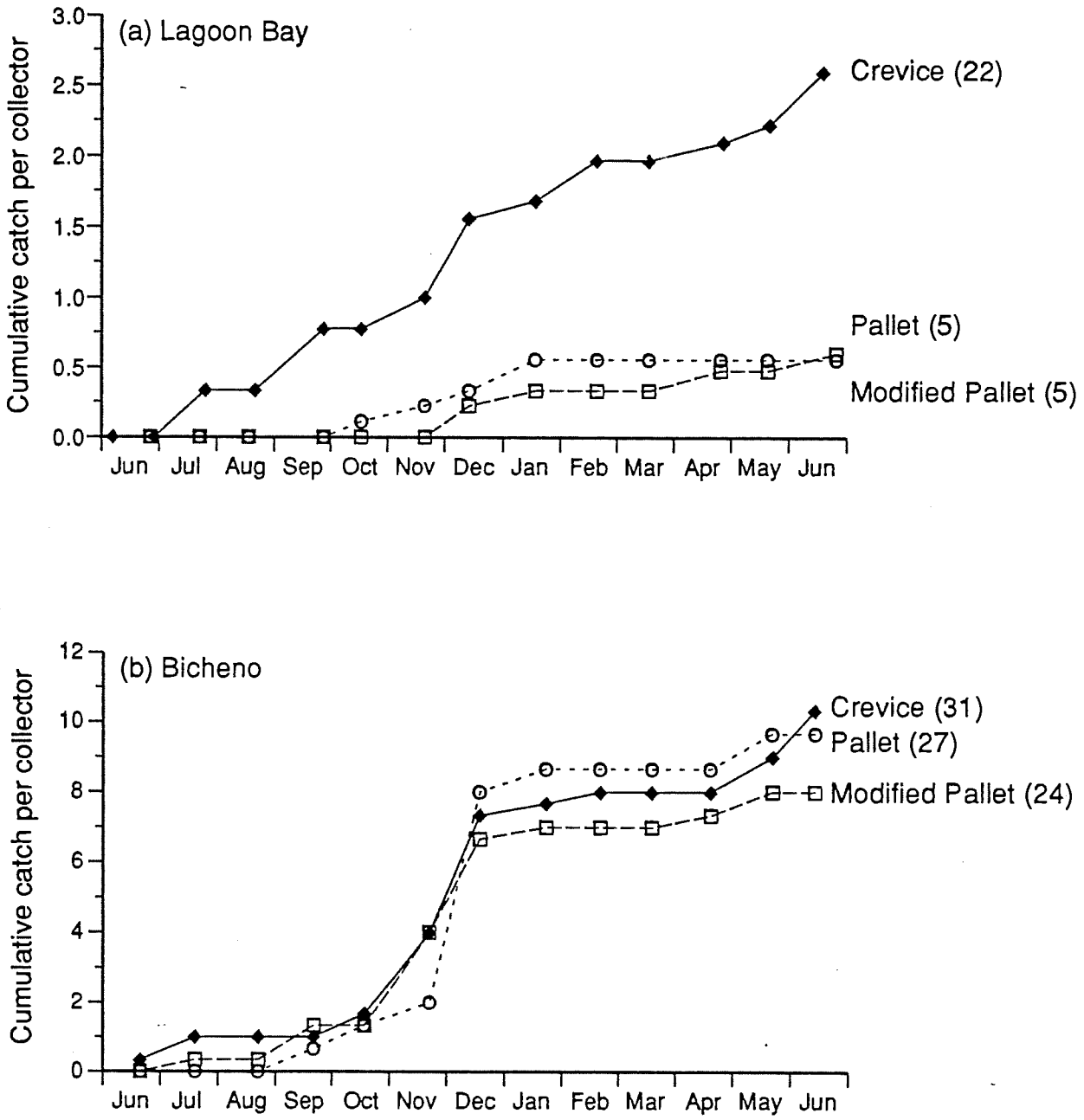

Figure 6: Cumulative catch of puerulus and post puerulus per collector type. Crevice collectors were positioned on sea floor and pallet-type collectors were positioned near the surface at Lagoon Bay (a), whereas crevice and pallet-type collectors were both positioned on the sea floor at Bicheno (b). Numbers in the legend of each graph show the total catch for each collector type.

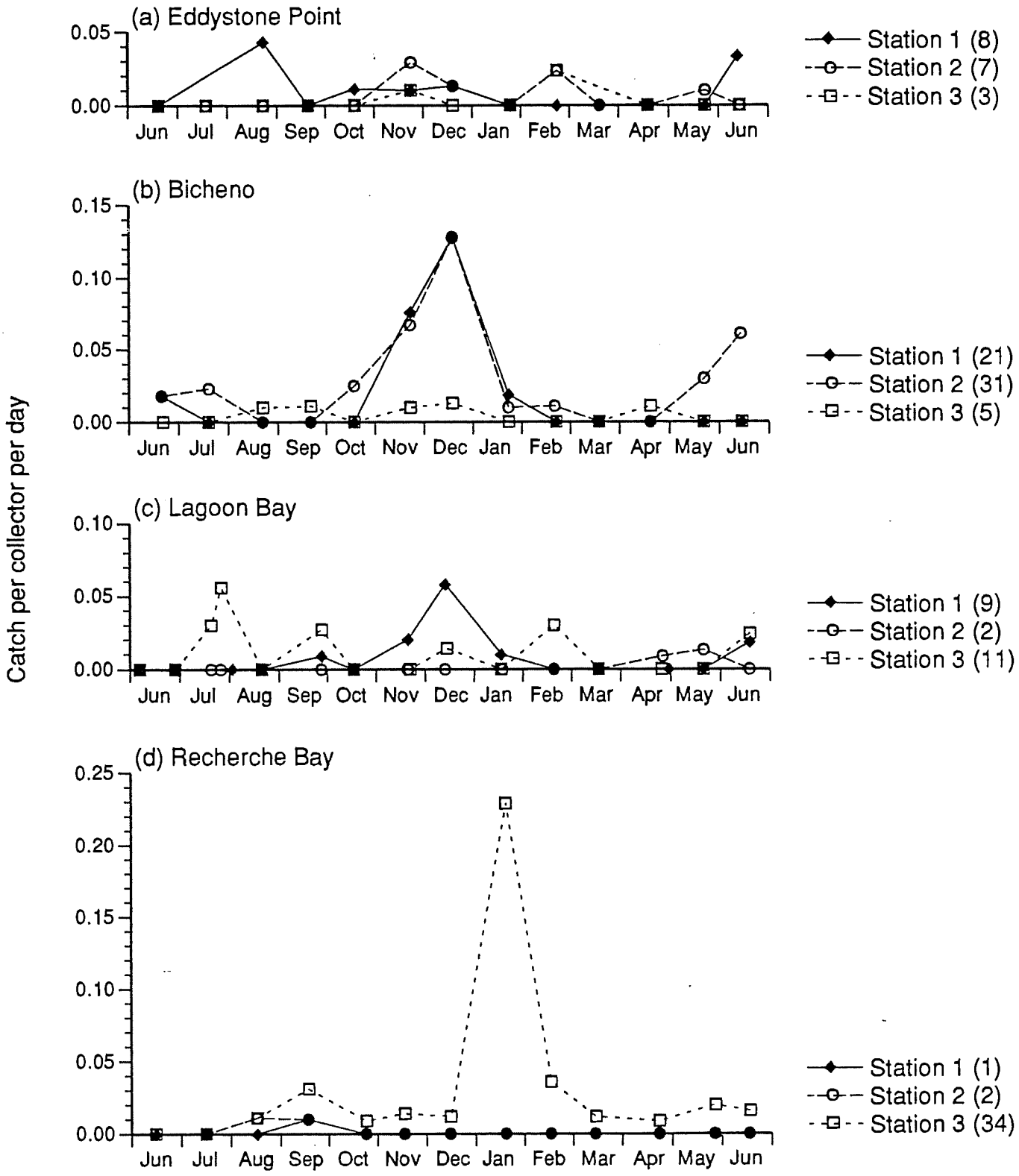

Figure 7: Daily catch rates of puerulus and post puerulus by crevice collectors. Numbers in the legend of each graph show the total catch by crevice collectors at each station.

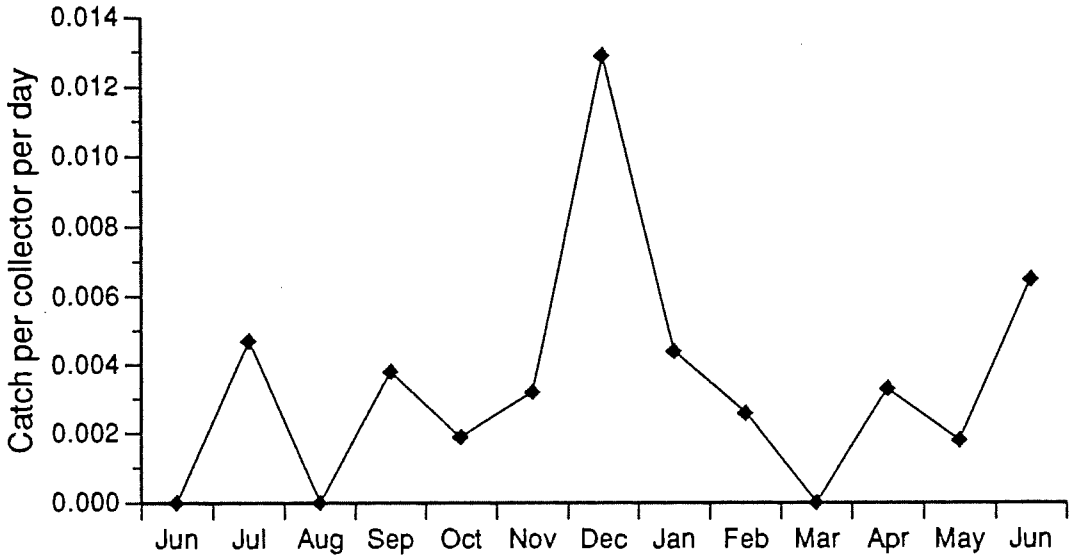

Figure 8: Daily catch rates of puerulus and post puerulus at Lagoon Bay. Points were calculated from the pooled data from all stations and collector-types. A total of 32 specimens were obtained.

During the first 3 months of sampling, Low Head was the only site at which puerulus were not obtained. A high degree of siltation occurred at Low Head and the fauna on collectors differed considerably from that of the other sites. Hence, the Low Head collectors were moved to a new location (Bridport) which showed greater similarity with the other sites. Despite the relocation of collectors, no puerulus have been captured on the north coast.

Bicheno yielded the most specimens. The overall catch by crevice collectors at Bicheno more than doubled the catch from other east coast sites. The highest catch rates for an individual station occurred at Station 3 in Recherche Bay, with 8 specimens on each of two collectors for January 1990. The third collector at this location caught 3 specimens during January. However, this collector was half buried in sand due to servicing problems, hence the result for this collector was not used when calculating the January catch rates shown in Figure 7. The next highest catch rates were at Bicheno (stations 1 and 2) which achieved an average of 3.3 specimens per collector during December, 1989.

Considerable differences existed between the catch rates of stations at individual sites. At all sites where puerulus were obtained, there was at least 1 station with noticeably lower catch rates than for the other stations within the site. A Mann-Whitney U test was used to test for differences in puerulus catches between stations at Bicheno. The combined data from the two peak months of settlement (Nov. and Dec.) were used for these comparisons. Significant differences ($P<0.05$) in puerulus catches were found between station 3 and the other two stations. The difference in catch rate between stations was more apparent at Recherche Bay, where station 3 had the highest average catch rate of all stations containing crevice collectors and station 1 had the lowest catch rate.

An analysis was conducted to determine the required number of collectors to provide an 80% probability of detecting a difference in settlement levels between stations (or years) at the 95% significance level for the peak month of settlement (Sokal and Rohlf, 1969, p.247). Analyses was restricted to Bicheno (stations 1 and 2) and Recherche Bay (station 3), since settlement rates at other stations were not sufficient to justify continued sampling. Prior to analysis, counts were square root transformed. A pooled variance estimate was used for the two Bicheno stations as they had an identical catch rate. Analysis showed than 10 collectors would be required at each of these stations to distinguish a 30% change in settlement levels. This is an absolute difference of 1 puerulus per collector at current settlement rates. Determination of the required number of collectors for station 3 at Recherche Bay is more subjective. All three collectors at this station should be used to provide a variance estimate, but as mentioned above, one

collector was half buried in sand and thus was likely to be catching less efficiently than a exposed collectors. If no corrections are made for this collector, an artificially high variance estimate is obtained and analysis shows that 7 collectors would be required to detect a 30% change in settlement levels. This change is equivalent to an absolute difference of 2 puerulus per collector. By assuming that the half buried collector had half the efficiency of the other collectors, an absolute difference of 1 puerulus per collector (12.5% change in settlement levels) can be detected with only 5 collectors.

1.4 Discussion

The crevice collector performed as well, if not better than pallet-type collectors in terms of catch rate and durability. Furthermore, the cost of a crevice collector ($33., excluding mooring and labour) was less than half of the other collectors ($69. and $73. for standard pallet and pairs of modified pallet collectors respectively). CCA treatment of the pallet-type collectors to prevent shipworm infestation adds an additional $12. to the above prices, but is considered worthwhile as no adverse affects on catch rates were observed and the life-span of the collectors was substantially increased.

Results from the present study and puerulus sampling in South Australia (Kennedy et al., submitted) support the opinion that pallet-type collectors require considerable epiphytic growth before they catch efficiently. At station 2 in Bicheno, crevice collectors caught puerulus 1 month after deployment compared to 2 months for pallet-type collectors. One and 2 months elapsed at Lagoon Bay and South Australia respectively before crevice collectors caught puerulus. Catches were not obtained on pallet-type collectors till 5 and more than 7 months at the same respective locations. The limited life-span of pallet-type collectors would result in major difficulties for a monitoring project when considering the time that might be required to condition replacement collectors. In contrast, the present results and Booth and Tarring (1986) suggest that crevice collectors catch without heavy epiphytic growth, thus reducing the time required to condition replacement collectors as well as reducing the difficulties in standardising for differences in epiphytic growth between sites and times.

The occasional presence of large specimens on crevice collectors indicates that some migration occurs between these collectors and the surrounding substrate. Hence effectiveness of these collectors may be affected by the nature of the nearby substrate. While this may confound the definition of a standard collector, the apparent immigration rate was low (1.5%). Crevice collectors were usually positioned on sand substrates, hence differing substrates are unlikely to have contributed to spatial variability of the

results. It appears that the practice of deploying crevice collectors on sand substrates has reduced immigration to the collectors as the proportion of large specimens found during this study was considerably lower than the 23% which was reported by Booth and Tarring (1986).

The timing of settlement in this study was unexpected since past work in South Australia (Lewis, 1977) found that puerulus settlement occurred primarily between July and August, with only minor settlement during summer. A lack of collector conditioning may have contributed to low catch rates during the winter of 1989. However, at Bicheno, crevice collectors caught specimens during the first sampling (June 1989) and the decline in catches after June indicates that the lack of a winter peak in settlement was not an artifact of collector conditioning. Figures 7 and 8 suggests that a winter peak may have commenced at the 3 east coast sites during June 1990 and continued sampling at Bicheno confirmed a peak in settlement during the following August (Kennedy, 1990). Kennedy et al. (submitted) suggested that settlement levels observed in South Australia during the winter of 1989 was the result of a poor settlement year and thus it is possible that the lack of a winter settlement on Tasmania's east coast during 1989 was also the result of unusual conditions. To date, summer peaks in settlement have been more consistent than winter peaks, a summer peak being evident from 11/89 to 1/90 in the present study and again commencing during 11/90 in on-going studies (FIRDC, grant 90/7).

Throughout Australia, eggs of *J. novaehollandiae* hatch between September and November. Hence, phyllosomes would have been at sea for a minimum of 8-12 months prior to the winter settlement reported by Kennedy (1990). It is likely that a longer period of time (12-16 months) elapsed prior to the summer settlement.

Expected gross regional variations in the numbers of settling puerulus are apparent in the data. No settlement was observed on the north coast. Diver surveys along the central north coast of Tasmania have found few juvenile lobsters and it is believed this result may arise from a circulation pattern which results in low net flow of oceanic water along the central north coast of Tasmania (Fandry 1983). Differences in overall settlement levels between the remaining sites were considerable, but usually less than the variation that existed between stations within individual sites. These results suggest that settlement levels were at least partially dependent on positioning of collectors within a site. A general trend existed within most sites such that the more sheltered stations exhibited the lowest settlement rates. Due to the within site variation, it is difficult to interpret differences in settlement levels between sites. Meaningful comparisons between areas in the future will require either an understanding of the factors that influence settlement on a

localised scale, or extensive sampling of sites so that the full range of localised variation in settlement within each site is known. Furthermore, the high localised variability in settlement rates raises the question of how representative individual stations would be of temporal changes in settlement levels over a wider area. This question needs to be addressed before finalising the sampling procedure of a long term monitoring program.

Unless the year of data collection coincided with an unusually poor period of puerulus settlement, it is doubtful that the number of specimens obtained in the present study would be sufficient to adequately resolve the relative strength of settlement on an inter-annual basis. To achieve this objective, it is likely that an increased number of collectors will be required, or that locations need to be identified in which there is higher settlement of puerulus. Power analysis suggested that a sample size of 10 collectors at the 3 best stations (Bicheno, stations 1 and 2; Recherche Bay, station 3) would be sufficient to provide an 80% probability of detecting an average difference of 1 puerulus per collector at the 95% significance level. Given current settlement rates, this is equivalent to detecting a 30% and 12.5% difference in settlement levels at Bicheno and Recherche Bay respectively. In other locations settlement rates were too low to justify further sampling. However, high localised differences in settlement levels suggest that there is considerable potential for obtaining improved settlement rates by manipulating the position of collectors. The requirements of the present study to provide initial estimates of spatial variability and timing of settlement precluded such manipulation, but on-going studies (FIRDC, grant 90/7) have already demonstrated that manipulation of collector positions can provide large and significant improvements in settlement levels.

CHAPTER 2

Investigation of genetic subdivision among populations of rock lobsters (*Jasus novaehollandiae* and *J. edwardsii*) with an extensive Australasian distribution.

(J.R. Ovenden, D.J. Brasher and R.W.G. White)

2.1 Introduction

Animals and the genes that they carry are not evenly distributed in space. They occupy distinct habitats which are defined by unique combinations of physical, chemical and biological features. The clustering of animals and their genes into large and small populations with different densities and rates of intergroup exchange has profound implications for the evolution of the species. A small amount of effective migration between populations and small effective population sizes enhances the operation of random genetic drift which may cause each population to develop a unique genetic identity.

The detection and measurement of the amount of genetic subdivision is vital to the comprehension of evolution within a species and to commercial resource assessment. To achieve this, Wright's F statistics are often applied to allozyme frequencies to measure the amount of genetic variability within a species that is due to the subdivision of the species into isolated populations. In some species, this approach is not possible because the amount of allozyme frequency variation throughout the range of the species is low. For example, Smith et al. (1980) found an average of only 1.22 alleles per locus for 141 red rock lobsters (*Jasus edwardsii* and *J. novaehollandiae*) amongst 32 protein loci. The proportion of these loci that were polymorphic (0.95 criterion) was only 3%. The overall observed heterozygosity for this species was 1.7%, 1.2% and 1.3% for each of three populations.

In species with low levels of allozyme heterozygosity, polymorphism in the mitochondrial genome provides an alternative genetic system which can be used to measure population subdivision. Nucleotide sequence variation in the genome, detected by the presence or absence of restriction endonuclease cleavage sites, can be analysed with analogs of Wright's F statistics (Takahata and Palumbi, 1985; Rand and Harrison, 1989; Lynch and Crease, 1990). An added advantage of the use of mitochondrial DNA (mtDNA) for the study of population subdivision is that if subdivision is present, the phylogeny of populations can be estimated as the genome is inherited intact from generation to generation. Population subdivision may be present within a species due to the existence in the past of barriers to gene flow. These barriers may or may not be

extant. If they are not, exchange of genes among populations will ensure that the species moves towards an equilibrium without subdivision. Alternatively, the species may be moving away from panmixia due to the recent imposition of barriers to gene flow (Ovenden, 1990). The general occurrence of mitochondrial haplotype subdivision in commercial marine species which have a teleplanic larval stage has yet to be determined.

Many marine species exchange genes between populations at the egg or larval stages. An example of a species in which this occurs is *J. novaehollandiae*. Adults of this species are found on the continental shelf around southern Australia, including Tasmania. Mass migrations are not known to occur in this species as they do in some other *Jasus* and *Panulirus* species. Each female produces 10^4 to 10^6 eggs each year, depending on size. The eggs are brooded by the female for five to six months prior to hatching. They hatch as naupliosoma larvae which quickly moult and develop through a number of phyllosoma stages. These larval stages are capable of vertical movement but are otherwise assumed to be carried passively by wind-induced currents, displacements by upwellings and eddy systems. Eight to 16 months later the last free-living stage, the puerulus, is reached (see Chapter 1). During this phase, the puerulus actively swims toward shallow, rocky reefs where settlement and recruitment occurs into the adult habitat (Phillips and McWilliam, 1986).

After a long planktonic larval existence there appears little chance that *Jasus* recruitment will occur exclusively into natal populations. If this is so, the amount of gene flow between adult populations will be large, preventing the development of genetic subdivision. However, genetic subdivision may develop in marine species under certain combinations of actual compared to potential recruitment. Edwards and Skibinski (1987) have demonstrated that a marine species with planktonic larvae and non-mobile adults (*Mytilus edilus*) consists of a series of genetically unique populations. Reeb and Avise (1990) found distinct mitochondrial haplotypes of the oyster, *Crassotrea virginica*, on either side of a point bisecting the continuous distribution of adults along the south-eastern American coastline.

Genetic subdivision may be present among adult *Jasus* populations if recruitment occurs from larvae which are proximal to adult habitats and if reproductive barriers exist between adult populations. Larvae that successfully recruit may consist only of the small number which are not swept away from adult habitat by prevailing currents (Phillips and McWilliam, 1986). Larvae which have been reported far away from adult habitats, in the Tasman Sea for example (Booth et al., 1990), may be permanently lost to the population (Smith et al., 1980), although the maximum duration of larval existence and the nature of

settlement cues are unknown. *Jasus* larvae may be able able to control their dispersal to a limited extent, in the same way that *Panulirus cygnus* larvae control their distribution on the Western Australian coastline by the judicious choice of water masses by vertical movements (Phillips and McWilliam, 1986). Physical barriers to interbreeding among the benthic adult populations of *J. novaehollandiae* are likely to be common.

Significant adaptation to local habitats may be another factor which may control genetic subdivision in *Jasus*. George (1969) notes that in the marine environment the potential for adaptation via natural selection is just as great as in terrestrial environments as physico-chemical and biological features vary widely between marine habitats. Adaptation to specific habitats could develop and be maintained by selective mortality among post-settlement juveniles. Habitat-specific settlement and recruitment is a cornerstone of theories describing the process of speciation in this genus (Pollock, 1990). On the micro-scale, it could lead to genetic subdivision between *Jasus* populations. Habitat-specific recruitment may be responsible for the lack of *P. cygnus* populations in the north of Western Australia despite the presence of late-stage larvae in surrounding waters (Phillips and McWilliam, 1986). Adaptation to local environmental conditions by breeding adults is found in *P. ornatus*. Adults to the north of Cape York migrate prior to spawning. Adults to the south do not migrate. Eastward migration in the Torres Strait is essential for northerly populations to ensure that larvae are swept back to suitable habitat by prevailing westward currents. Larvae released in Torres Strait would be carried westward along coastline unsuitable for lobsters. Larvae released in situ by the southerly populations are not carried away from suitable habitat (Bell et al., 1987).

The aim of this study is to use mtDNA nucleotide sequence polymorphism to test for the presence of macro and micro-scale genetic subdivision in a marine species in which potential gene flow between populations is mediated almost entirely by the planktonic larval stage. A pair of sibling lobster species, *J. edwardsii* and *J. novaehollandiae* has been chosen. These species occur on either side of the Tasman Sea, in New Zealand and Australia. Booth et al. (1990) suggested that the two species be synonomized as allozyme, morphological and larval distribution studies suggest that gene flow is occurring between them.

2.2 Materials and Methods

Six to 16 *J. edwardsii* or *J. novaehollandiae* individuals were sampled on a single occasion from thirteen localities between April 1989 and June 1990 (Table 1). Nine localities were approximately equidistant along the southern Australian coastline from South Australia to Victoria. They were distributed across western Bass Strait, around the

Table 1: Collection Location, sample size and haplotype designations for *Jasus novaehollandiae* and *J. edwardsii* populations studied.

Location		Sample Size	Haplotypes
Western Australia			
Esperance	34°00'S 122°00'E	15	AAAAAA (3), AAABAB (2), AAACAB (3), AAAEAA (1), AAAFAB (1), AAAMAB (1), AACAAA (1), AADAAA (1), ADABAH (1), CBANAN (1)
South Australia			
Bucks Bay	38°53'S 140°23'E	8	AAAAAA (1), AAAAAJ (1), AAALAB (1), AAGKAA (1), AAJAAA (1), BBAJJA (1), HAAAAA (1), IAABAB (1)
Port Lincoln	34°46'S 135°52'E	10	AAAAAA (2), AAABAA (1), AADAAA (1), AADCAG (1), AAEAAA (1), AAGAAA (1), ABAFAB (1), CAABAB (1), IAABAB (1)
Victoria			
Port Fairy	38°23'S 142°18'E	10	AAAAAM (1), AAACAB (1), AAAEAA (1), AAAIAB (1), AADAAA (1), AAGAAA (1), AAHCAB (1), ABAFAB (2), ABABAD (1)
Bass Strait			
King Island	39°52'S 143°49'E	10	AAAAAA (1), AAABAB (1), AAABAL (1), AAACAB (1), AAADAB (1), AAAMAB (1) AADAAA (1), AAFAAA (1), AAGAAA (1), CAACAB (1)
Flinders Island	40°00'S 148°16'E	10	AAAAAA (1), AAABAB (1), AAACAB (2) AAAEAA (1), AACAAA (1), AADDAB (1) AAEAAA (1), ADAAAA (1), BAFAAA (1)
Tasmania			
Temma	41°14'S 144°35'E	11	AAAAAA (2), AAAADA (1), AAACAB (1), AAAEAA (1), AADAAA (1), BAAAAA (1), BAABAB (1), CAADAB (1), DAAHBA (1), FCABEB (1)
Flying Cloud Point	43°31'S 145°55'E	16	AAAAAA (4), AAAACB (1), AAABAB (3), AAABAF (1), AAAEAG (1), AAAFAB (1), AAAIAB (1), AADAAE (1), AAGGAA (1), ADABAD (1), EBAFAC (1)
Sullivans Point	43°33'S 146°55'E	13	AAAAAA (2), AAACAB (1), AAAIAB (2), AABAAA (1), AACAAA (1), AAEAAA (1), ABAFAB (3), CAABAB (1), GCABAB (1)
Bicheno	41°57'S 148°19'E	6	AAABAA (1), AAACAB (1), AADAAA (1), ADABAD (1), ADABAH (1), JEABAB (1)
New South Wales			
Batemans Bay	35°45'S 150°20'E	4	AAAAAA (1), AAKCAB (1), AFABAB (1), IAABAB (1)
New Zealand			
Gisborne	38°37'S 178°00'E	10	AAAAAA (3), AAACAB (3), AADAAA (2), AAFAAA (1), AAFGAA (1)
Moeraki	45°31'S 176°54'E	9	AAAAAA (3), AAAAAE (1), AAABAB (1), AAACAB (1), AAGAAK (1), CAACAB (1), EAABAB (1)
	Total	132	

coast of southern and eastern Tasmania to eastern Bass Strait. Lobsters were collected from one Western Australian and one New South Wales locality. New Zealand populations were sampled once from the east coast of the North Island and once from the east coast of the south island (Fig. 9). Most individuals were caught in commercial lobster pots at two to 16 fathoms, however, some individuals were captured by divers and some were collected at 38 (Temma) and 65 (Flying Cloud Point) fathoms.

Animals were air-freighted alive to a laboratory where they were killed by osmotic shock in a freshwater ice slurry. Antennal glands were immediately removed and stored in liquid nitrogen until required for mtDNA extraction. MtDNA was recovered, cleaved with restriction endonucleases and visualized by agarose electrophoresis as described by Brasher et al. (submitted). The presence or absence of restriction sites recognized by AflII (CTTAAG), AvaI (CPyCGPuG), BanI (GGPyPuCC), BstYI (PuGATCPy), EcoRV (GATATC) and HindIII (AAGCTT) was determined within the mtDNA from each lobster. To facilitate the scoring of sites from restriction fragment profiles, a restriction map for the genome of a single individual was constructed for these six enzymes as well as for PvuII, NcoI and BglII using double digestion techniques.

The null hypothesis of the absence of genetic subdivision within *J. novaehollandiae* and *J. edwardsii* was tested in two ways. In the first test, nucleotide sequence diversity was calculated between each pair of mitochondrial genomes using the maximum likelihood method of Nei and Tajima (1983). Standard errors of the mean amount of mtDNA sequence divergence among and within populations was then calculated by the method of Nei and Jin (1989) using the UPGMA method to estimate genome phylogeny. If the mean amount of mtDNA sequence divergence between a pair of populations was significantly larger than zero, as judged by the relative magnitude of the standard error, then genetic subdivision may be present. Secondly, the presence of genetic subdivision was tested by nucleotide (G_{ST}; Takahata and Palumbi, 1985; N_{ST}; Lynch and Crease, 1990) and haplotype diversity (G'_{ST}; Rand and Harrison, 1989) analyses. A significantly large value of G_{ST}, N_{ST} or G'_{ST} would indicate that the sampled populations represented naturally occurring, reproductively isolated lobster populations. The significance of G_{ST} was estimated by bootstrapping. Jacknifed estimates of G'_{ST}, in which one sample population was omitted each time, helped to evaluate the significance of G'_{ST}.

For both tests of the presence of genetic subdivision, a sample of lobsters from a particular collection location was not necessarily assumed to represent a single population. Samples from collection locations were grouped together according to the

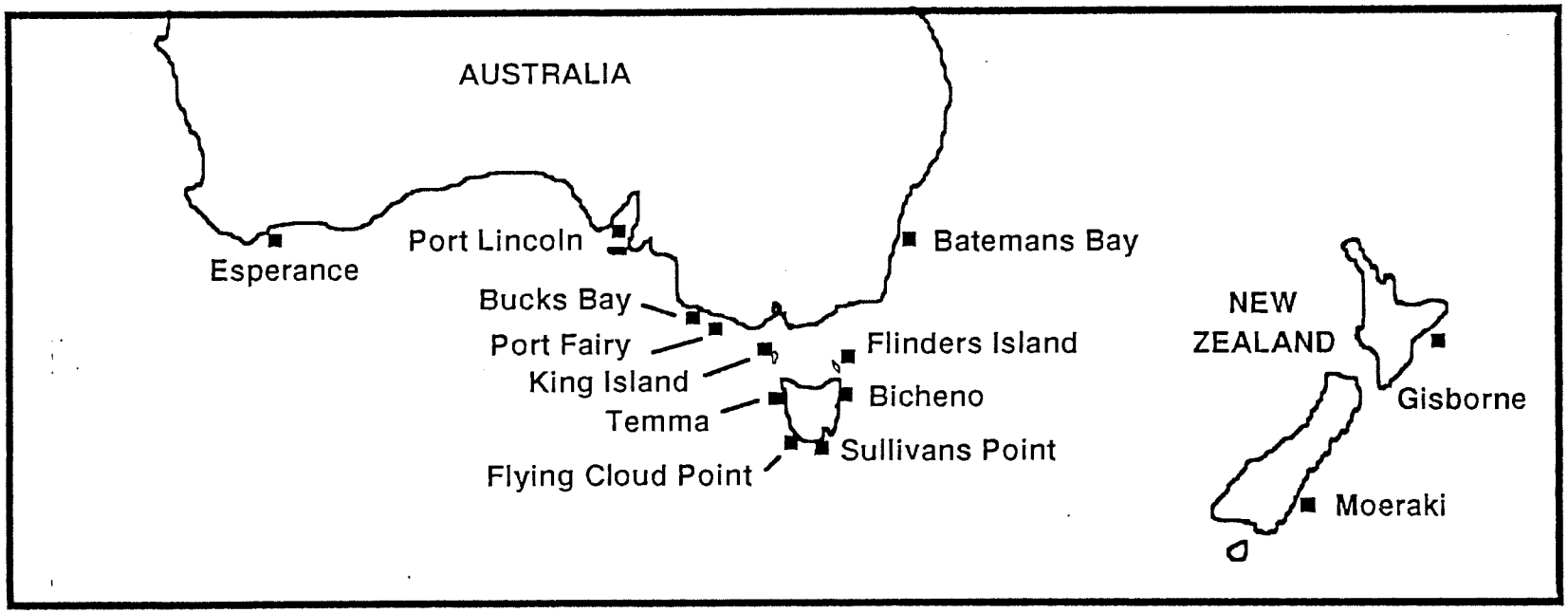

Figure 9: The locations from which *J. novaehollandiae* and *J. edwardsii* were collected during this study.

presence of possible isolating mechanisms between hypothesized interbreeding populations. Two classes of location groupings were made (Table 2). The first assumed populations may be reproductively isolated from each other due to their presence in different current flows. Under this scenario, gene flow between adult populations may be curtailed due to the lack of exchange of larvae between water masses. The second type of locality grouping assumed populations may be reproductively isolated due to their occurrence in physico-chemically different habitats. Pollock (1990) has suggested that lobster larvae have the ability to detect minute changes in the water composition and to metamorphose into the puerulus stage adjacent to parental habitat.

The phylogeny for an alphabetic compilation of mtDNA haplotypes was estimated using the UPGMA (Sneath and Sokal, 1973). The validity of the topology of the UPGMA tree was assessed by the relative magnitude of standard errors on appropriate branch points (Nei et al., 1985).

2.3 Results

A total of 67 restriction sites (11, AflII; 7, AvaI; 12, BanI; 16, BstYI; 9, EcoRV and 12, HindIII) were identified among the 132 lobster mitochondrial genomes (Appendix 1). The relative locations of 36 restriction sites in the genome of a representative lobster is shown in figure 10. The size of the lobster genome was estimated to be 15966±350 nucleotide pairs from all successful endonuclease digests using only one representative morph from each gel. Five to 14 restriction site morphs were detected with each restriction enzyme (10, AflII; 6, AvaI; 11, BanI; 14, BstYI; 5, EcoRV and 14, HindIII; Appendix 1). The pairwise difference among morphs was the simple gain or loss of one or more restriction sites. For example, AflII morph (B) possessed five AflII sites. AflII morph A possessed these five sites and one other, AflII site #6.

There were 55 different haplotypes. Thirty-six of the 55 haplotypes were represented by only one specimen. Six of the 55 haplotypes were found in two or three lobsters. Haplotypes AAAEAA and AAAIAB were represented by four individuals each and ABAFAB by six individuals. Haplotypes AAABAB and AADAAA were represented by eight individuals each. The most frequently identified haplotypes were AAACAB (14) and AAAAAA (22) (Table 1).

The haplotypes were clustered by a UPGMA (Fig. 11) into two major groups of 25 each. The standard errors on the branches leading to these groups were overlapping so this grouping of haplotypes is not definitive. One haplotype group possessed predominantly A morphs for the restriction sites of BstYI (18/25) and HindIII (18/25). Members of this

Table 2: The two classes of groupings for samples of *Jasus novaehollandiae* and *J. edwardsii* from various collection locations for the detection of reproductive isolation between hypothesized populations.

Class I - Collection Locations are grouped according to larval settlement on the first landfall after dispersal in a particular water mass.

- Grouping 1
  - Northern Eastern Australian Current
    - Batemans Bay, New South Wales; Gisborne, New Zealand
  - Southern Eastern Australian Current
    - Flinders Island, Bass Strait; Sullivans Point and Bicheno, Tasmania; Moeraki, New Zealand
  - Great Australian Bight
    - Port Lincoln and Bucks Bay, South Australia; Port Fairy, Victoria; King Island, Bass Strait; Temma and Flying Cloud Point, Tasmania
  - Western Australia
    - Esperance
- Grouping 2
  - New Zealand inshore eddies
    - Gisborne and Moeraki, New Zealand
  - Bass Strait inshore eddies
    - Bucks Bay, South Australia; Port Fairy, Victoria; King Island and Flinders Island, Bass Strait; Temma and Bicheno, Tasmania
  - Southern Tasmanian inshore eddies
    - Flying Cloud Point and Sullivans Point, Tasmania
  - South Australian inshore eddies
    - Port Lincoln
  - Western Australian inshore eddies
    - Esperance

Class II - Larvae settle in habitat having the same environmental parameters as that of their parental population

- Grouping 1
  - Australian carbonate sands, low freshwater runoff
    - Esperance, Western Australia; Bucks Bay and Port Lincoln, South Australia; Port Fairy, Victoria; King Island, Bass Strait; Temma and Flying Cloud Point, Tasmania
  - Australian quartzose sands, high freshwater runoff
    - Flinders Island, Bass Strait; Batemans Bay, New South Wales; Sullivans Point and Bicheno, Tasmania
  - New Zealand, high freshwater runoff
    - Gisborne and Moeraki
- Grouping 2 - Biogeographical
  - Western
    - Esperance, Western Australia
  - Central
    - Bucks Bay and Port Lincoln, South Australia; Port Fairy, Victoria; King and Flinders Island, Bass Strait; Temma, Flying Cloud Point, Sullivans Point, and Bicheno, Tasmania; Batemans Bay, New South Wales
  - Eastern
    - Gisborne and Moeraki, New Zealand
- Grouping 3
  - All collection locations separate.

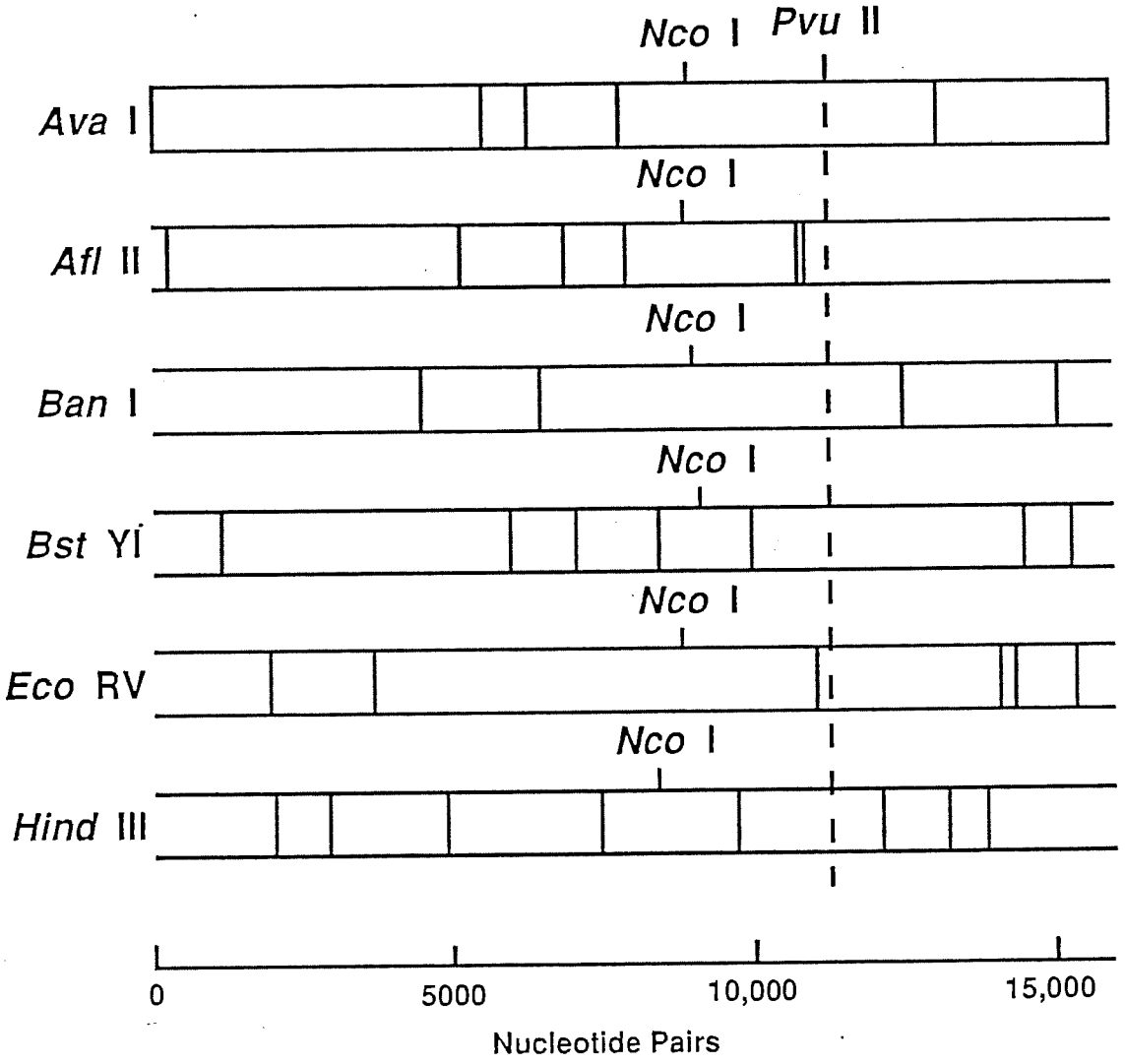

Figure 10: Restriction endonuclease cleavage map for *J. novaehollandiae* (haplotype AAAAAA). The single *Pvu* II restriction site, which was used in mapping experiments, is fixed. To illustrate the error associated with mapping restriction sites, the position of the single *Nco* I site is shown according to the results of double digestion mapping experiments with each of the six restriction enzymes.

collectors after settlement. Immigrants were only found on crevice collectors and these specimens have been excluded from the results given below.

Comparison of collector designs

Crevice collectors caught puerulus more frequently than surface deployed pallet-type collectors, with a shorter time lag between deployment and first settlement (Fig. 6a). There was a 4 fold difference in the total catch per collector for crevice collectors compared to both of these pallet-type collectors. However, no differences were observed between the catch rate of crevice collectors and bottom deployed pallet-type collectors (Fig. 6b). The cumulative catches for these collectors were similar, with considerable cross-over between the lines. Despite a smaller surface area, pairs of modified pallet collectors caught a similar number of puerulus to standard pallet collectors.

After nearly 14 months in the water, all 12 untreated pallet-type collectors were severely infested with shipworm. Of these, 6 were totally destroyed, with the remaining 6 collectors sustaining considerable damaged with broken slats, etc. The 12 treated, pallet-type collectors were in good condition except for the mooring attachment and 3 of these collectors had been rendered unserviceable due to damage in this area. There were no observable differences in the catch rates between treated and untreated pallet-type collectors.

The small, modified pallet collectors proved to be more durable than standard pallet collectors as only 1 in 12 of the modified collectors were destroyed, whereas destruction occurred for 8 of the 12 standard pallet collectors. All crevice collectors were in good condition at the end of the project regardless of the type of plywood which was used in construction. A total of 45 crevice collectors were deployed around Tasmania, of which only 2 were lost in heavy sea conditions.

Seasonality and spatial variability of settlement

Figure 7 shows the daily catch rates of crevice collectors for individual stations at each site. It is evident that minor settlement occurred throughout the year with a summer peak in settlement occurring at both Bicheno (Nov./Dec.) and Recherche Bay (Jan.). Catch rates were too low and varied to deduce seasonality of settlement from crevice collector data at Eddystone Point and Lagoon Bay. However, when the data is pooled from all stations and collector-types, a summer peak in settlement (Dec.) was also apparent at Lagoon Bay (Fig. 8).

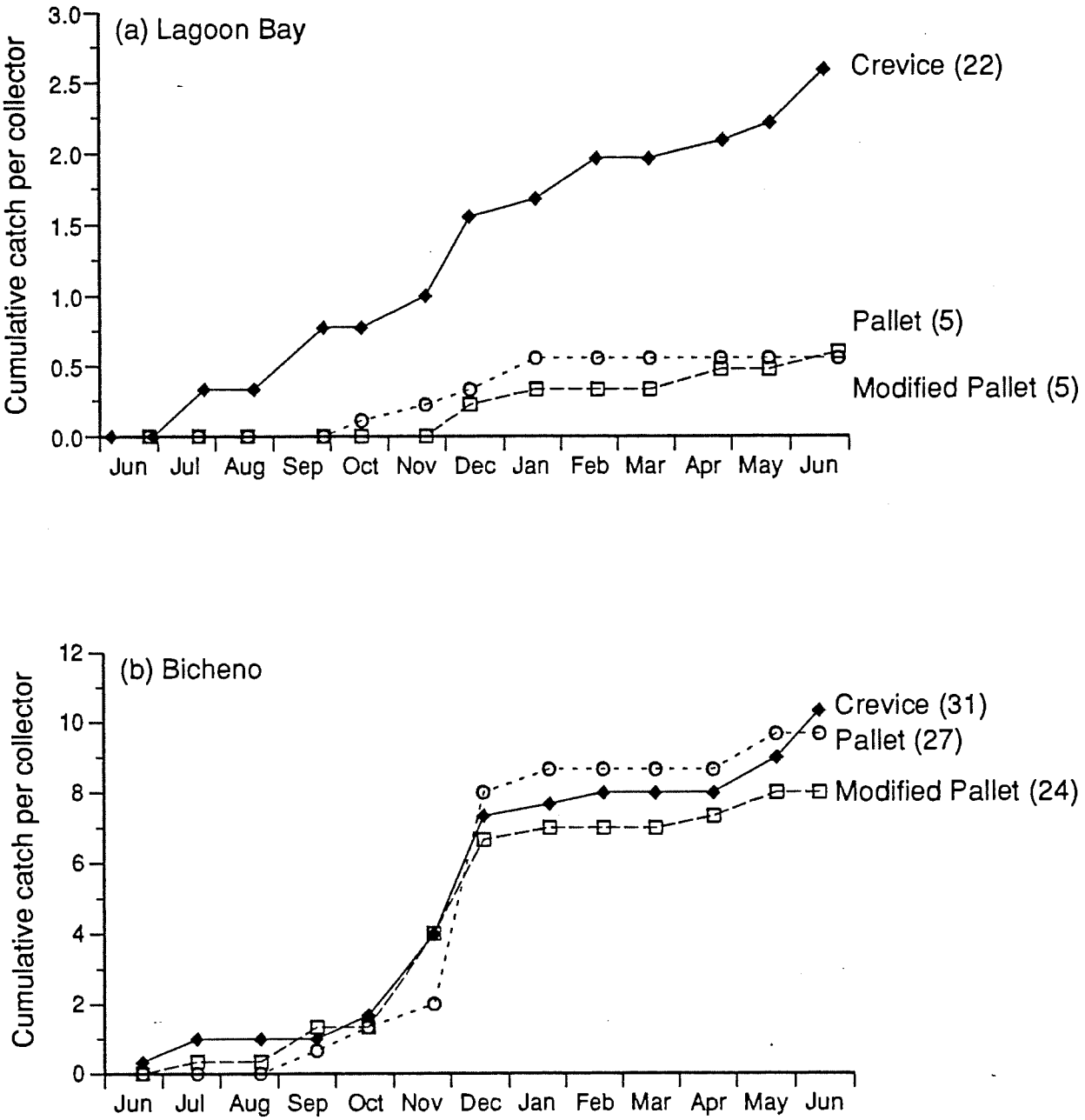

Figure 6: Cumulative catch of puerulus and post puerulus per collector type. Crevice collectors were positioned on sea floor and pallet-type collectors were positioned near the surface at Lagoon Bay (a), whereas crevice and pallet-type collectors were both positioned on the sea floor at Bicheno (b). Numbers in the legend of each graph show the total catch for each collector type.

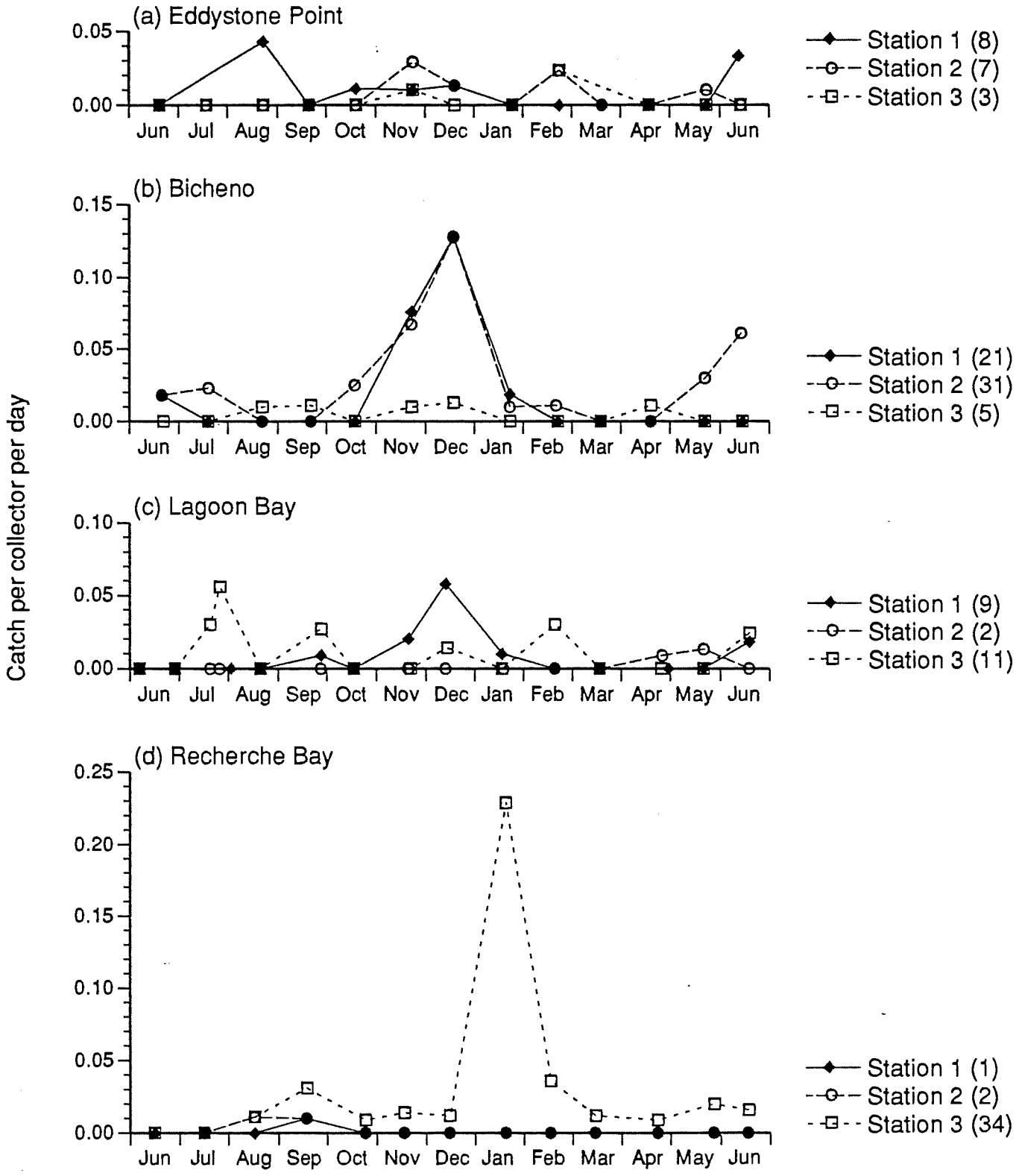

Figure 7: Daily catch rates of puerulus and post puerulus by crevice collectors. Numbers in the legend of each graph show the total catch by crevice collectors at each station.

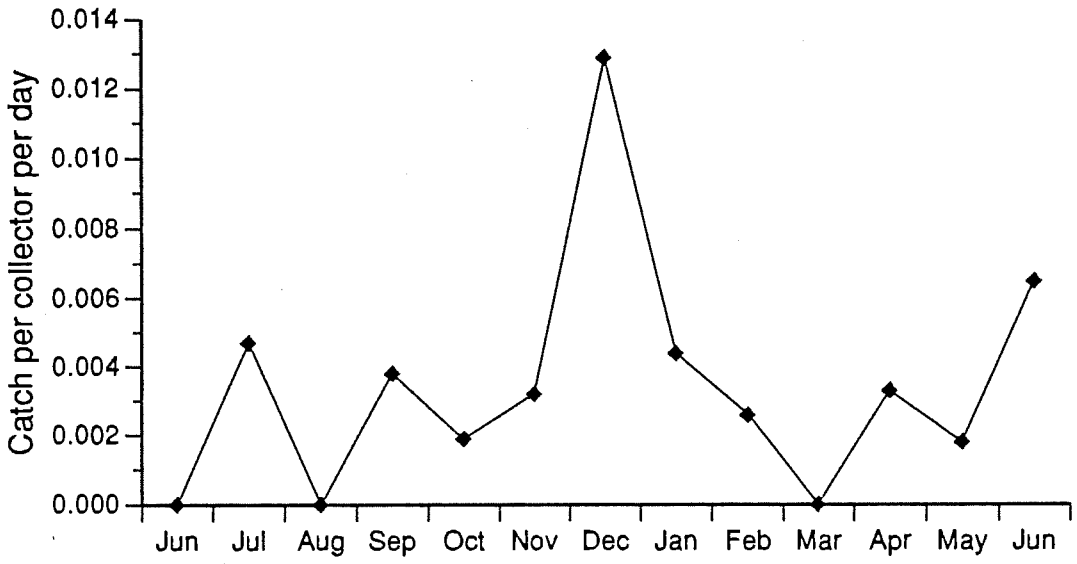

Figure 8: Daily catch rates of puerulus and post puerulus at Lagoon Bay. Points were calculated from the pooled data from all stations and collector-types. A total of 32 specimens were obtained.

During the first 3 months of sampling, Low Head was the only site at which puerulus were not obtained. A high degree of siltation occurred at Low Head and the fauna on collectors differed considerably from that of the other sites. Hence, the Low Head collectors were moved to a new location (Bridport) which showed greater similarity with the other sites. Despite the relocation of collectors, no puerulus have been captured on the north coast.

Bicheno yielded the most specimens. The overall catch by crevice collectors at Bicheno more than doubled the catch from other east coast sites. The highest catch rates for an individual station occurred at Station 3 in Recherche Bay, with 8 specimens on each of two collectors for January 1990. The third collector at this location caught 3 specimens during January. However, this collector was half buried in sand due to servicing problems, hence the result for this collector was not used when calculating the January catch rates shown in Figure 7. The next highest catch rates were at Bicheno (stations 1 and 2) which achieved an average of 3.3 specimens per collector during December, 1989.

Considerable differences existed between the catch rates of stations at individual sites. At all sites where puerulus were obtained, there was at least 1 station with noticeably lower catch rates than for the other stations within the site. A Mann-Whitney U test was used to test for differences in puerulus catches between stations at Bicheno. The combined data from the two peak months of settlement (Nov. and Dec.) were used for these comparisons. Significant differences ($P<0.05$) in puerulus catches were found between station 3 and the other two stations. The difference in catch rate between stations was more apparent at Recherche Bay, where station 3 had the highest average catch rate of all stations containing crevice collectors and station 1 had the lowest catch rate.

An analysis was conducted to determine the required number of collectors to provide an 80% probability of detecting a difference in settlement levels between stations (or years) at the 95% significance level for the peak month of settlement (Sokal and Rohlf, 1969, p.247). Analyses was restricted to Bicheno (stations 1 and 2) and Recherche Bay (station 3), since settlement rates at other stations were not sufficient to justify continued sampling. Prior to analysis, counts were square root transformed. A pooled variance estimate was used for the two Bicheno stations as they had an identical catch rate. Analysis showed than 10 collectors would be required at each of these stations to distinguish a 30% change in settlement levels. This is an absolute difference of 1 puerulus per collector at current settlement rates. Determination of the required number of collectors for station 3 at Recherche Bay is more subjective. All three collectors at this station should be used to provide a variance estimate, but as mentioned above, one

collector was half buried in sand and thus was likely to be catching less efficiently than a exposed collectors. If no corrections are made for this collector, an artificially high variance estimate is obtained and analysis shows that 7 collectors would be required to detect a 30% change in settlement levels. This change is equivalent to an absolute difference of 2 puerulus per collector. By assuming that the half buried collector had half the efficiency of the other collectors, an absolute difference of 1 puerulus per collector (12.5% change in settlement levels) can be detected with only 5 collectors.

1.4 Discussion

The crevice collector performed as well, if not better than pallet-type collectors in terms of catch rate and durability. Furthermore, the cost of a crevice collector ($33., excluding mooring and labour) was less than half of the other collectors ($69. and $73. for standard pallet and pairs of modified pallet collectors respectively). CCA treatment of the pallet-type collectors to prevent shipworm infestation adds an additional $12. to the above prices, but is considered worthwhile as no adverse affects on catch rates were observed and the life-span of the collectors was substantially increased.

Results from the present study and puerulus sampling in South Australia (Kennedy et al., submitted) support the opinion that pallet-type collectors require considerable epiphytic growth before they catch efficiently. At station 2 in Bicheno, crevice collectors caught puerulus 1 month after deployment compared to 2 months for pallet-type collectors. One and 2 months elapsed at Lagoon Bay and South Australia respectively before crevice collectors caught puerulus. Catches were not obtained on pallet-type collectors till 5 and more than 7 months at the same respective locations. The limited life-span of pallet-type collectors would result in major difficulties for a monitoring project when considering the time that might be required to condition replacement collectors. In contrast, the present results and Booth and Tarring (1986) suggest that crevice collectors catch without heavy epiphytic growth, thus reducing the time required to condition replacement collectors as well as reducing the difficulties in standardising for differences in epiphytic growth between sites and times.

The occasional presence of large specimens on crevice collectors indicates that some migration occurs between these collectors and the surrounding substrate. Hence effectiveness of these collectors may be affected by the nature of the nearby substrate. While this may confound the definition of a standard collector, the apparent immigration rate was low (1.5%). Crevice collectors were usually positioned on sand substrates, hence differing substrates are unlikely to have contributed to spatial variability of the

results. It appears that the practice of deploying crevice collectors on sand substrates has reduced immigration to the collectors as the proportion of large specimens found during this study was considerably lower than the 23% which was reported by Booth and Tarring (1986).

The timing of settlement in this study was unexpected since past work in South Australia (Lewis, 1977) found that puerulus settlement occurred primarily between July and August, with only minor settlement during summer. A lack of collector conditioning may have contributed to low catch rates during the winter of 1989. However, at Bicheno, crevice collectors caught specimens during the first sampling (June 1989) and the decline in catches after June indicates that the lack of a winter peak in settlement was not an artifact of collector conditioning. Figures 7 and 8 suggests that a winter peak may have commenced at the 3 east coast sites during June 1990 and continued sampling at Bicheno confirmed a peak in settlement during the following August (Kennedy, 1990). Kennedy et al. (submitted) suggested that settlement levels observed in South Australia during the winter of 1989 was the result of a poor settlement year and thus it is possible that the lack of a winter settlement on Tasmania's east coast during 1989 was also the result of unusual conditions. To date, summer peaks in settlement have been more consistent than winter peaks, a summer peak being evident from 11/89 to 1/90 in the present study and again commencing during 11/90 in on-going studies (FIRDC, grant 90/7).

Throughout Australia, eggs of *J. novaehollandiae* hatch between September and November. Hence, phyllosomes would have been at sea for a minimum of 8-12 months prior to the winter settlement reported by Kennedy (1990). It is likely that a longer period of time (12-16 months) elapsed prior to the summer settlement.

Expected gross regional variations in the numbers of settling puerulus are apparent in the data. No settlement was observed on the north coast. Diver surveys along the central north coast of Tasmania have found few juvenile lobsters and it is believed this result may arise from a circulation pattern which results in low net flow of oceanic water along the central north coast of Tasmania (Fandry 1983). Differences in overall settlement levels between the remaining sites were considerable, but usually less than the variation that existed between stations within individual sites. These results suggest that settlement levels were at least partially dependent on positioning of collectors within a site. A general trend existed within most sites such that the more sheltered stations exhibited the lowest settlement rates. Due to the within site variation, it is difficult to interpret differences in settlement levels between sites. Meaningful comparisons between areas in the future will require either an understanding of the factors that influence settlement on a

localised scale, or extensive sampling of sites so that the full range of localised variation in settlement within each site is known. Furthermore, the high localised variability in settlement rates raises the question of how representative individual stations would be of temporal changes in settlement levels over a wider area. This question needs to be addressed before finalising the sampling procedure of a long term monitoring program.

Unless the year of data collection coincided with an unusually poor period of puerulus settlement, it is doubtful that the number of specimens obtained in the present study would be sufficient to adequately resolve the relative strength of settlement on an inter-annual basis. To achieve this objective, it is likely that an increased number of collectors will be required, or that locations need to be identified in which there is higher settlement of puerulus. Power analysis suggested that a sample size of 10 collectors at the 3 best stations (Bicheno, stations 1 and 2; Recherche Bay, station 3) would be sufficient to provide an 80% probability of detecting an average difference of 1 puerulus per collector at the 95% significance level. Given current settlement rates, this is equivalent to detecting a 30% and 12.5% difference in settlement levels at Bicheno and Recherche Bay respectively. In other locations settlement rates were too low to justify further sampling. However, high localised differences in settlement levels suggest that there is considerable potential for obtaining improved settlement rates by manipulating the position of collectors. The requirements of the present study to provide initial estimates of spatial variability and timing of settlement precluded such manipulation, but on-going studies (FIRDC, grant 90/7) have already demonstrated that manipulation of collector positions can provide large and significant improvements in settlement levels.

CHAPTER 2

Investigation of genetic subdivision among populations of rock lobsters (*Jasus novaehollandiae* and *J. edwardsii*) with an extensive Australasian distribution.

(J.R. Ovenden, D.J. Brasher and R.W.G. White)

2.1 Introduction

Animals and the genes that they carry are not evenly distributed in space. They occupy distinct habitats which are defined by unique combinations of physical, chemical and biological features. The clustering of animals and their genes into large and small populations with different densities and rates of intergroup exchange has profound implications for the evolution of the species. A small amount of effective migration between populations and small effective population sizes enhances the operation of random genetic drift which may cause each population to develop a unique genetic identity.

The detection and measurement of the amount of genetic subdivision is vital to the comprehension of evolution within a species and to commercial resource assessment. To achieve this, Wright's F statistics are often applied to allozyme frequencies to measure the amount of genetic variability within a species that is due to the subdivision of the species into isolated populations. In some species, this approach is not possible because the amount of allozyme frequency variation throughout the range of the species is low. For example, Smith et al. (1980) found an average of only 1.22 alleles per locus for 141 red rock lobsters (*Jasus edwardsii* and *J. novaehollandiae*) amongst 32 protein loci. The proportion of these loci that were polymorphic (0.95 criterion) was only 3%. The overall observed heterozygosity for this species was 1.7%, 1.2% and 1.3% for each of three populations.

In species with low levels of allozyme heterozygosity, polymorphism in the mitochondrial genome provides an alternative genetic system which can be used to measure population subdivision. Nucleotide sequence variation in the genome, detected by the presence or absence of restriction endonuclease cleavage sites, can be analysed with analogs of Wright's F statistics (Takahata and Palumbi, 1985; Rand and Harrison, 1989; Lynch and Crease, 1990). An added advantage of the use of mitochondrial DNA (mtDNA) for the study of population subdivision is that if subdivision is present, the phylogeny of populations can be estimated as the genome is inherited intact from generation to generation. Population subdivision may be present within a species due to the existence in the past of barriers to gene flow. These barriers may or may not be

extant. If they are not, exchange of genes among populations will ensure that the species moves towards an equilibrium without subdivision. Alternatively, the species may be moving away from panmixia due to the recent imposition of barriers to gene flow (Ovenden, 1990). The general occurrence of mitochondrial haplotype subdivision in commercial marine species which have a teleplanic larval stage has yet to be determined.

Many marine species exchange genes between populations at the egg or larval stages. An example of a species in which this occurs is *J. novaehollandiae*. Adults of this species are found on the continental shelf around southern Australia, including Tasmania. Mass migrations are not known to occur in this species as they do in some other *Jasus* and *Panulirus* species. Each female produces 10^4 to 10^6 eggs each year, depending on size. The eggs are brooded by the female for five to six months prior to hatching. They hatch as naupliosoma larvae which quickly moult and develop through a number of phyllosoma stages. These larval stages are capable of vertical movement but are otherwise assumed to be carried passively by wind-induced currents, displacements by upwellings and eddy systems. Eight to 16 months later the last free-living stage, the puerulus, is reached (see Chapter 1). During this phase, the puerulus actively swims toward shallow, rocky reefs where settlement and recruitment occurs into the adult habitat (Phillips and McWilliam, 1986).

After a long planktonic larval existence there appears little chance that *Jasus* recruitment will occur exclusively into natal populations. If this is so, the amount of gene flow between adult populations will be large, preventing the development of genetic subdivision. However, genetic subdivision may develop in marine species under certain combinations of actual compared to potential recruitment. Edwards and Skibinski (1987) have demonstrated that a marine species with planktonic larvae and non-mobile adults (*Mytilus edilus*) consists of a series of genetically unique populations. Reeb and Avise (1990) found distinct mitochondrial haplotypes of the oyster, *Crassotrea virginica*, on either side of a point bisecting the continuous distribution of adults along the south-eastern American coastline.

Genetic subdivision may be present among adult *Jasus* populations if recruitment occurs from larvae which are proximal to adult habitats and if reproductive barriers exist between adult populations. Larvae that successfully recruit may consist only of the small number which are not swept away from adult habitat by prevailing currents (Phillips and McWilliam, 1986). Larvae which have been reported far away from adult habitats, in the Tasman Sea for example (Booth et al., 1990), may be permanently lost to the population (Smith et al., 1980), although the maximum duration of larval existence and the nature of

settlement cues are unknown. *Jasus* larvae may be able able to control their dispersal to a limited extent, in the same way that *Panulirus cygnus* larvae control their distribution on the Western Australian coastline by the judicious choice of water masses by vertical movements (Phillips and McWilliam, 1986). Physical barriers to interbreeding among the benthic adult populations of *J. novaehollandiae* are likely to be common.

Significant adaptation to local habitats may be another factor which may control genetic subdivision in *Jasus*. George (1969) notes that in the marine environment the potential for adaptation via natural selection is just as great as in terrestrial environments as physico-chemical and biological features vary widely between marine habitats. Adaptation to specific habitats could develop and be maintained by selective mortality among post-settlement juveniles. Habitat-specific settlement and recruitment is a cornerstone of theories describing the process of speciation in this genus (Pollock, 1990). On the micro-scale, it could lead to genetic subdivision between *Jasus* populations. Habitat-specific recruitment may be responsible for the lack of *P. cygnus* populations in the north of Western Australia despite the presence of late-stage larvae in surrounding waters (Phillips and McWilliam, 1986). Adaptation to local environmental conditions by breeding adults is found in *P. ornatus*. Adults to the north of Cape York migrate prior to spawning. Adults to the south do not migrate. Eastward migration in the Torres Strait is essential for northerly populations to ensure that larvae are swept back to suitable habitat by prevailing westward currents. Larvae released in Torres Strait would be carried westward along coastline unsuitable for lobsters. Larvae released in situ by the southerly populations are not carried away from suitable habitat (Bell et al., 1987).

The aim of this study is to use mtDNA nucleotide sequence polymorphism to test for the presence of macro and micro-scale genetic subdivision in a marine species in which potential gene flow between populations is mediated almost entirely by the planktonic larval stage. A pair of sibling lobster species, *J. edwardsii* and *J. novaehollandiae* has been chosen. These species occur on either side of the Tasman Sea, in New Zealand and Australia. Booth et al. (1990) suggested that the two species be synonomized as allozyme, morphological and larval distribution studies suggest that gene flow is occurring between them.

2.2 Materials and Methods

Six to 16 *J. edwardsii* or *J. novaehollandiae* individuals were sampled on a single occasion from thirteen localities between April 1989 and June 1990 (Table 1). Nine localities were approximately equidistant along the southern Australian coastline from South Australia to Victoria. They were distributed across western Bass Strait, around the

Table 1: Collection Location, sample size and haplotype designations for *Jasus novaehollandiae* and *J. edwardsii* populations studied.

Location		Sample Size	Haplotypes
Western Australia			
Esperance	34°00'S 122°00'E	15	AAAAAA (3), AAABAB (2), AAACAB (3), AAAEAA (1), AAAFAB (1), AAAMAB (1), AACAAA (1), AADAAA (1), ADABAH (1), CBANAN (1)
South Australia			
Bucks Bay	38°53'S 140°23'E	8	AAAAAA (1), AAAAAJ (1), AAALAB (1), AAGKAA (1), AAJAAA (1), BBAJJA (1), HAAAAA (1), IAABAB (1)
Port Lincoln	34°46'S 135°52'E	10	AAAAAA (2), AAABAA (1), AADAAA (1), AADCAG (1), AAEAAA (1), AAGAAA (1), ABAFAB (1), CAABAB (1), IAABAB (1)
Victoria			
Port Fairy	38°23'S 142°18'E	10	AAAAAM (1), AAACAB (1), AAAEAA (1), AAAIAB (1), AADAAA (1), AAGAAA (1), AAHCAB (1), ABAFAB (2), ABABAD (1)
Bass Strait			
King Island	39°52'S 143°49'E	10	AAAAAA (1), AAABAB (1), AAABAL (1), AAACAB (1), AAADAB (1), AAAMAB (1) AADAAA (1), AAFAAA (1), AAGAAA (1), CAACAB (1)
Flinders Island	40°00'S 148°16'E	10	AAAAAA (1), AAABAB (1), AAACAB (2) AAAEAA (1), AACAAA (1), AADDAB (1) AAEAAA (1), ADAAAA (1), BAFAAA (1)
Tasmania			
Temma	41°14'S 144°35'E	11	AAAAAA (2), AAAADA (1), AAACAB (1), AAAEAA (1), AADAAA (1), BAAAAA (1), BAABAB (1), CAADAB (1), DAAHBA (1), FCABEB (1)
Flying Cloud Point	43°31'S 145°55'E	16	AAAAAA (4), AAAACB (1), AAABAB (3), AAABAF (1), AAAEAG (1), AAAFAB (1), AAAIAB (1), AADAAE (1), AAGGAA (1), ADABAD (1), EBAFAC (1)
Sullivans Point	43°33'S 146°55'E	13	AAAAAA (2), AAACAB (1), AAAIAB (2), AABAAA (1), AACAAA (1), AAEAAA (1), ABAFAB (3), CAABAB (1), GCABAB (1)
Bicheno	41°57'S 148°19'E	6	AAABAA (1), AAACAB (1), AADAAA (1), ADABAD (1), ADABAH (1), JEABAB (1)
New South Wales			
Batemans Bay	35°45'S 150°20'E	4	AAAAAA (1), AAKCAB (1), AFABAB (1), IAABAB (1)
New Zealand			
Gisborne	38°37'S 178°00'E	10	AAAAAA (3), AAACAB (3), AADAAA (2), AAFAAA (1), AAFGAA (1)
Moeraki	45°31'S 176°54'E	9	AAAAAA (3), AAAAAE (1), AAABAB (1), AAACAB (1), AAGAAK (1), CAACAB (1), EAABAB (1)
	Total	132	

coast of southern and eastern Tasmania to eastern Bass Strait. Lobsters were collected from one Western Australian and one New South Wales locality. New Zealand populations were sampled once from the east coast of the North Island and once from the east coast of the south island (Fig. 9). Most individuals were caught in commercial lobster pots at two to 16 fathoms, however, some individuals were captured by divers and some were collected at 38 (Temma) and 65 (Flying Cloud Point) fathoms.

Animals were air-freighted alive to a laboratory where they were killed by osmotic shock in a freshwater ice slurry. Antennal glands were immediately removed and stored in liquid nitrogen until required for mtDNA extraction. MtDNA was recovered, cleaved with restriction endonucleases and visualized by agarose electrophoresis as described by Brasher et al. (submitted). The presence or absence of restriction sites recognized by AflII (CTTAAG), AvaI (CPyCGPuG), BanI (GGPyPuCC), BstYI (PuGATCPy), EcoRV (GATATC) and HindIII (AAGCTT) was determined within the mtDNA from each lobster. To facilitate the scoring of sites from restriction fragment profiles, a restriction map for the genome of a single individual was constructed for these six enzymes as well as for PvuII, NcoI and BglII using double digestion techniques.

The null hypothesis of the absence of genetic subdivision within *J. novaehollandiae* and *J. edwardsii* was tested in two ways. In the first test, nucleotide sequence diversity was calculated between each pair of mitochondrial genomes using the maximum likelihood method of Nei and Tajima (1983). Standard errors of the mean amount of mtDNA sequence divergence among and within populations was then calculated by the method of Nei and Jin (1989) using the UPGMA method to estimate genome phylogeny. If the mean amount of mtDNA sequence divergence between a pair of populations was significantly larger than zero, as judged by the relative magnitude of the standard error, then genetic subdivision may be present. Secondly, the presence of genetic subdivision was tested by nucleotide (G_{ST}; Takahata and Palumbi, 1985; N_{ST}; Lynch and Crease, 1990) and haplotype diversity (G'_{ST}; Rand and Harrison, 1989) analyses. A significantly large value of G_{ST}, N_{ST} or G'_{ST}, would indicate that the sampled populations represented naturally occurring, reproductively isolated lobster populations. The significance of G_{ST} was estimated by bootstrapping. Jacknifed estimates of G'_{ST}, in which one sample population was omitted each time, helped to evaluate the significance of G'_{ST}.

For both tests of the presence of genetic subdivision, a sample of lobsters from a particular collection location was not necessarily assumed to represent a single population. Samples from collection locations were grouped together according to the

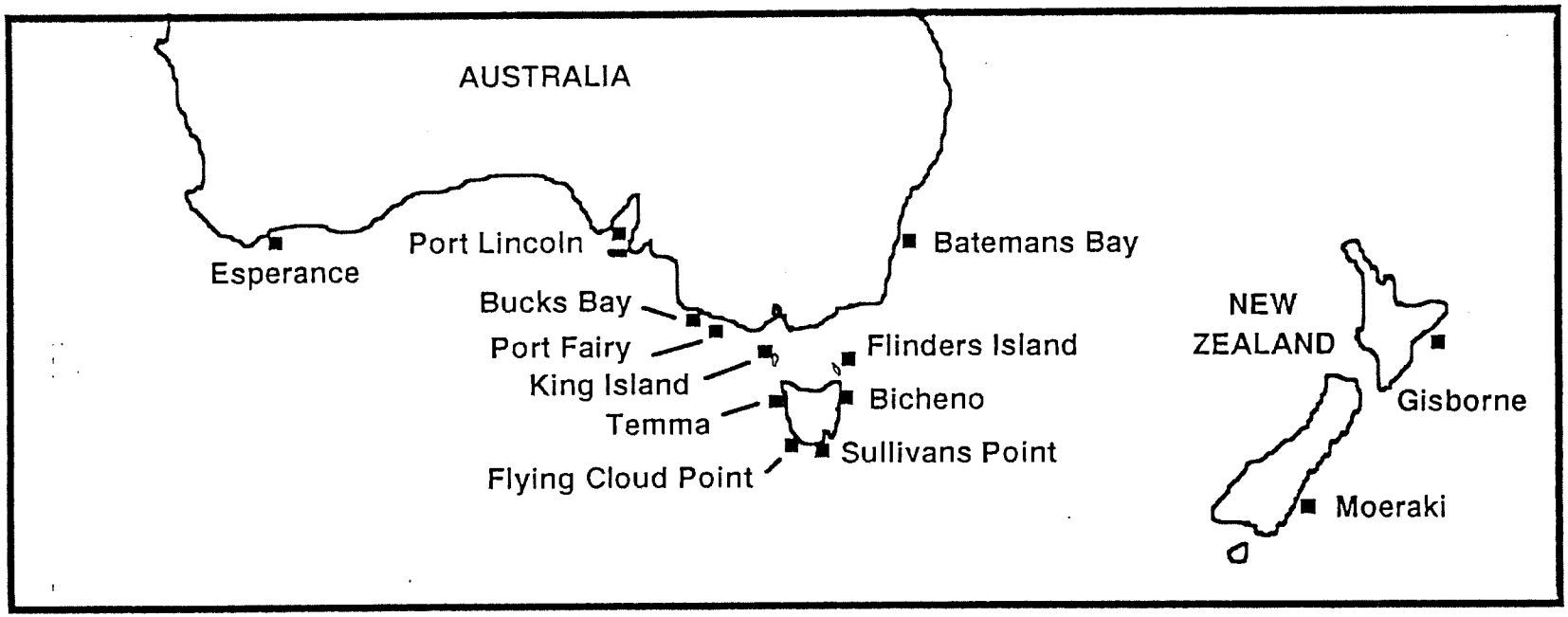

Figure 9: The locations from which *J. novaehollandiae* and *J. edwardsii* were collected during this study.

presence of possible isolating mechanisms between hypothesized interbreeding populations. Two classes of location groupings were made (Table 2). The first assumed populations may be reproductively isolated from each other due to their presence in different current flows. Under this scenario, gene flow between adult populations may be curtailed due to the lack of exchange of larvae between water masses. The second type of locality grouping assumed populations may be reproductively isolated due to their occurrence in physico-chemically different habitats. Pollock (1990) has suggested that lobster larvae have the ability to detect minute changes in the water composition and to metamorphose into the puerulus stage adjacent to parental habitat.

The phylogeny for an alphabetic compilation of mtDNA haplotypes was estimated using the UPGMA (Sneath and Sokal, 1973). The validity of the topology of the UPGMA tree was assessed by the relative magnitude of standard errors on appropriate branch points (Nei et al., 1985).

2.3 Results

A total of 67 restriction sites (11, AflII; 7, AvaI; 12, BanI; 16, BstYI; 9, EcoRV and 12, HindIII) were identified among the 132 lobster mitochondrial genomes (Appendix 1). The relative locations of 36 restriction sites in the genome of a representative lobster is shown in figure 10. The size of the lobster genome was estimated to be 15966±350 nucleotide pairs from all successful endonuclease digests using only one representative morph from each gel. Five to 14 restriction site morphs were detected with each restriction enzyme (10, AflII; 6, AvaI; 11, BanI; 14, BstYI; 5, EcoRV and 14, HindIII; Appendix 1). The pairwise difference among morphs was the simple gain or loss of one or more restriction sites. For example, AflII morph (B) possessed five AflII sites. AflII morph A possessed these five sites and one other, AflII site #6.

There were 55 different haplotypes. Thirty-six of the 55 haplotypes were represented by only one specimen. Six of the 55 haplotypes were found in two or three lobsters. Haplotypes AAAEAA and AAAIAB were represented by four individuals each and ABAFAB by six individuals. Haplotypes AAABAB and AADAAA were represented by eight individuals each. The most frequently identified haplotypes were AAACAB (14) and AAAAAA (22) (Table 1).

The haplotypes were clustered by a UPGMA (Fig. 11) into two major groups of 25 each. The standard errors on the branches leading to these groups were overlapping so this grouping of haplotypes is not definitive. One haplotype group possessed predominantly A morphs for the restriction sites of BstYI (18/25) and HindIII (18/25). Members of this

Table 2: The two classes of groupings for samples of *Jasus novaehollandiae* and *J. edwardsii* from various collection locations for the detection of reproductive isolation between hypothesized populations.

Class I - Collection Locations are grouped according to larval settlement on the first landfall after dispersal in a particular water mass.

- Grouping 1
  - Northern Eastern Australian Current
    - Batemans Bay, New South Wales; Gisborne, New Zealand
  - Southern Eastern Australian Current
    - Flinders Island, Bass Strait; Sullivans Point and Bicheno, Tasmania; Moeraki, New Zealand
  - Great Australian Bight
    - Port Lincoln and Bucks Bay, South Australia; Port Fairy, Victoria; King Island, Bass Strait; Temma and Flying Cloud Point, Tasmania
  - Western Australia
    - Esperance
- Grouping 2
  - New Zealand inshore eddies
    - Gisborne and Moeraki, New Zealand
  - Bass Strait inshore eddies
    - Bucks Bay, South Australia; Port Fairy, Victoria; King Island and Flinders Island, Bass Strait; Temma and Bicheno, Tasmania
  - Southern Tasmanian inshore eddies
    - Flying Cloud Point and Sullivans Point, Tasmania
  - South Australian inshore eddies
    - Port Lincoln
  - Western Australian inshore eddies
    - Esperance

Class II - Larvae settle in habitat having the same environmental parameters as that of their parental population

- Grouping 1
  - Australian carbonate sands, low freshwater runoff
    - Esperance, Western Australia; Bucks Bay and Port Lincoln, South Australia; Port Fairy, Victoria; King Island, Bass Strait; Temma and Flying Cloud Point, Tasmania
  - Australian quartzose sands, high freshwater runoff
    - Flinders Island, Bass Strait; Batemans Bay, New South Wales; Sullivans Point and Bicheno, Tasmania
  - New Zealand, high freshwater runoff
    - Gisborne and Moeraki
- Grouping 2 - Biogeographical
  - Western
    - Esperance, Western Australia
  - Central
    - Bucks Bay and Port Lincoln, South Australia; Port Fairy, Victoria; King and Flinders Island, Bass Strait; Temma, Flying Cloud Point, Sullivans Point, and Bicheno, Tasmania; Batemans Bay, New South Wales
  - Eastern
    - Gisborne and Moeraki, New Zealand
- Grouping 3
  - All collection locations separate.

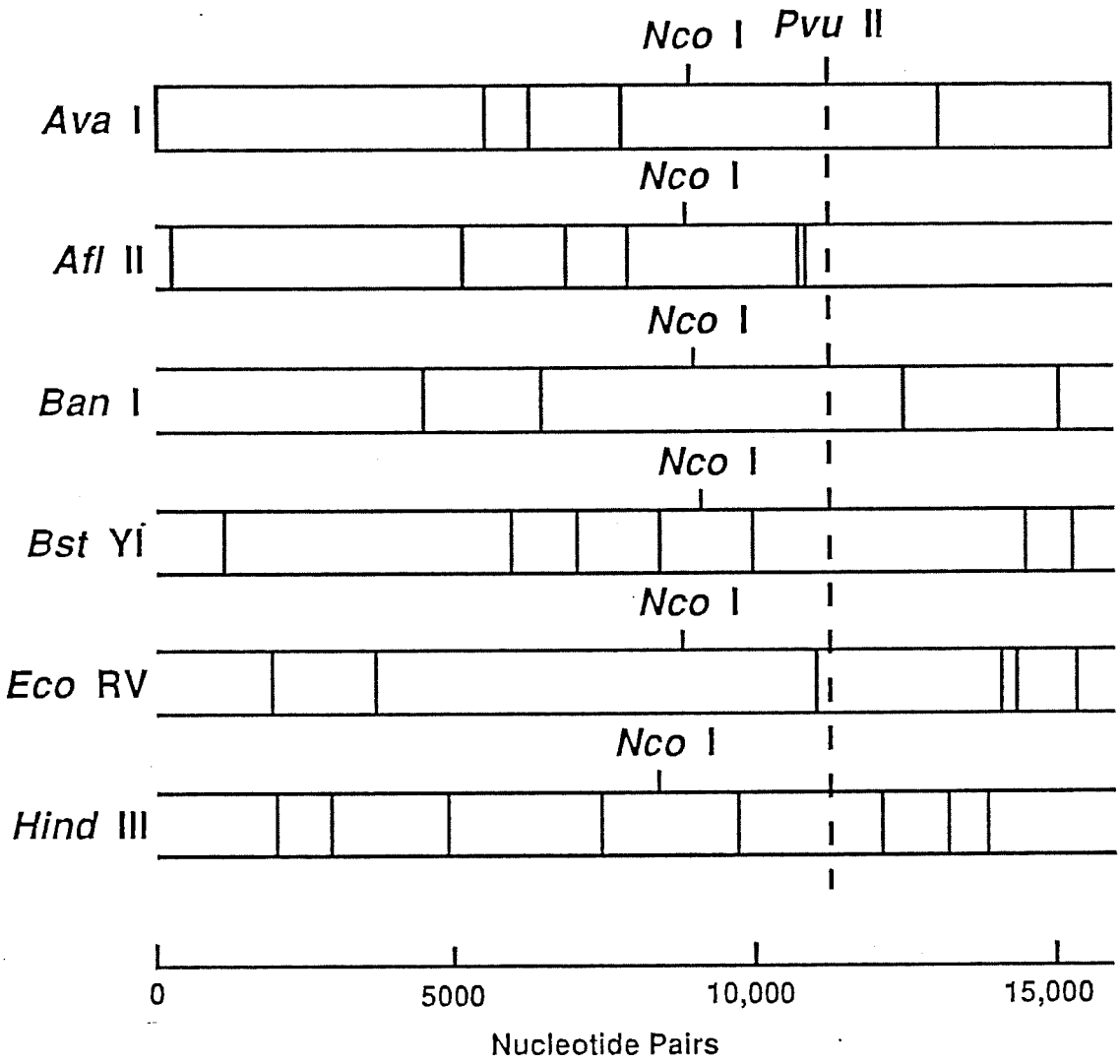

Figure 10: Restriction endonuclease cleavage map for *J. novaehollandiae* (haplotype AAAAAA). The single *Pvu* II restriction site, which was used in mapping experiments, is fixed. To illustrate the error associated with mapping restriction sites, the position of the single *Nco* I site is shown according to the results of double digestion mapping experiments with each of the six restriction enzymes.

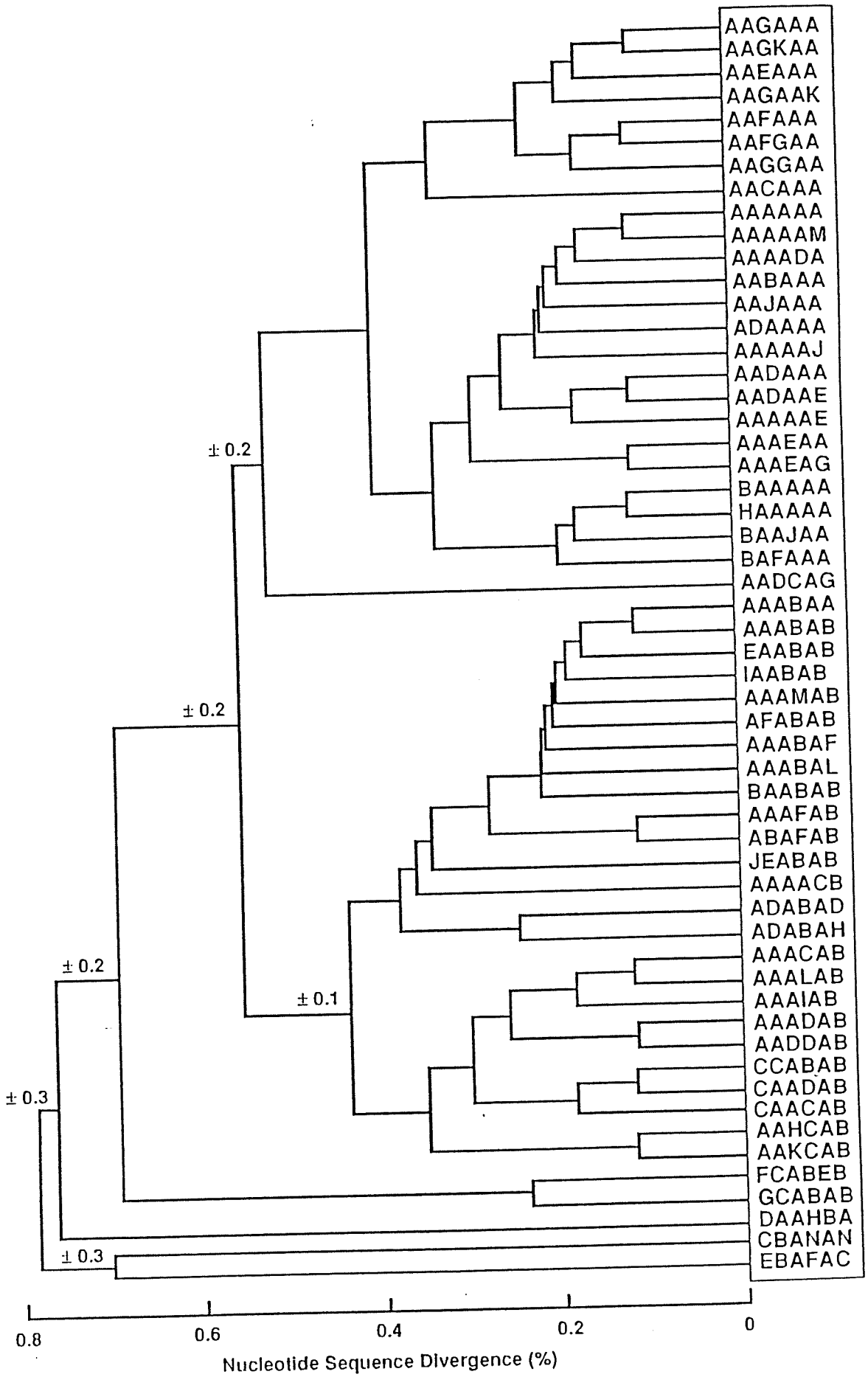

Figure 11: A UPGMA describing the relationship between 55 *J. novaehollandiae* and *J. edwardsii* haplotypes based on pairwise mtDNA nucleotide sequence divergence. The size of the standard error for selected branch points is indicated.

haplotype group were found at each collection location (Esperance, 6/15; Bucks Bay, 6/8; Port Lincoln, 6/10; Port Fairy, 4/10; King Island, 4/10; Flinders Island, 6/10; Temma, 6/11; Flying Cloud Point, 7/16; Sullivans Point, 5/13; Bicheno, 1/6; Batemans Bay, 1/4; Gisborne, 7/10; Moeraki, 4/9). The haplotypes belonging to the other group possessed mostly B morphs for the BstYI (12/25) and HindIII (20/25) restriction sites. Five haplotypes belonged to neither group. Two of these (FCABEB, DAAHBA) belonged to lobsters collected from north-western Tasmania (Temma, Table 1). Another two (EBAFAC, Flying Cloud Point; GCABAB, Sullivans Point) were from lobsters from southern Tasmania. The remaining haplotype (CBANAN) was identified once amongst the 15 lobsters collected from Esperance, Western Australia.

The mean number of base substitutions per nucleotide (mtDNA diversity) for the 132 lobster mitochondrial genomes was 0.7781±0.1946% (mean±standard error). A pair of lobsters, one from Flying Cloud Point, Tasmania (EBAFAC) and the other from Temma (DAAHBA) had the most different mitochondrial genomes (2.4111±0.8173%). The locality with the most diverse set of mitochondrial genomes was Port Fairy, Victoria (n=10, diversity=0.9565±0.2486%, Table 3). The ten mitochondrial genomes sampled from fish at Gisborne, New Zealand were the most similar (0.4382±0.2421%).

The amount of nucleotide diversity between the mtDNA of lobsters from pairs of collection locations, corrected for intra-populational diversity, was less than or equal to zero for 51 of the 78 possible comparisons. The magnitude of the corrected inter-populational mtDNA diversities for the remaining pairs of collection locations ranged from 0.0016% to 0.1179%, with most of the values lying between 0.01% and 0.06% (Table 4). The size of the standard errors for some of these measurements were larger than the magnitude of the measurement itself. For example, the amount of inter-populational mtDNA nucleotide diversity between Flying Cloud Point, Tasmania and Temma, Tasmania was 0.0016%. The standard error of this diversity was 0.0209%. Standard errors exceeded the inter-populational diversity measurements in 16 of the pairwise comparisons. For these comparisons, there is no evidence of historical or contemporary restrictions in gene flow between populations. However, for 11 measurements of pairwise mtDNA diversity between collection locations the standard error of the measurement was smaller than measurement itself (Table 4). In four of these comparisons, the inter-populational diversity may be significantly larger than zero (0.0421±0.0264%, Temma, Tasmania v Bicheno, Tasmania; 0.0632±0.0386%, Sullivans Point, Tasmania v Gisborne, New Zealand; 0.1011±0.0343%, Bucks Bay, South Australia v Bicheno, Tasmania; 0.1179±0.0480%, Gisborne, New Zealand v Bicheno, Tasmania) suggesting that gene flow may be restricted between these locations.

Table 3: Intra-populational mean mtDNA nucleotide sequence diversity and standard errors (%) for thirteen *Jasus novaehollandiae* and *J. edwardsii* populations.

Collection Location	Diversity	Standard Error
King Island, Bass Strait	0.6872	0.2497
Flying Cloud Point, Tasmania	0.7799	0.2363
Temma, Tasmania	0.8938	0.2486
Sullivans Point, Tasmania	0.9193	0.2605
Flinders Island, Bass Strait	0.7699	0.2462
Port Lincoln, South Australia	0.7334	0.2381
Gisborne, New Zealand	0.4382	0.2421
Moeraki, New Zealand	0.7114	0.2616
Bucks Bay, South Australia	0.8516	0.2235
Bicheno, Tasmania	0.8630	0.2865
Port Fairy, Victoria	0.9565	0.2486
Batemans Bay, New South Wales	0.8763	0.3309
Esperance, Western Australia	0.7681	0.2499

Table 4: Inter-populational mean mitochondrial DNA nucleotide sequence diversity and standard errors (%) between samples collected from pairs of collection locations. The diversities between the remaining pairs of locations were less than or equal to zero.

Collection Locations	Diversity	Standard Error
King Island, Bass Strait v Gisborne, New Zealand	0.0177	0.0277
King Island, Bass Strait v Bucks Bay, South Australia	0.0214	0.0229
Flying Cloud Point v Temma, Tasmania	0.0083	0.0158
Flying Cloud Point, Tasmania v Flinders Island, Bass Strait	0.0016	0.0209
Flying Cloud Point, Tasmania v Gisborne, New Zealand	0.0552	0.0432*
Flying Cloud Point, Tasmania v Bucks Bay, South Australia	0.0315	0.0219*
Temma v Sullivans Point, Tasmania	0.0163	0.0185
Temma, Tasmania v Gisborne, New Zealand	0.0125	0.0215
Temma v Bicheno, Tasmania	0.0421	0.0264*
Temma, Tasmania v Port Fairy, Victoria	0.0017	0.0180
Sullivans Point, Tasmania v Gisborne, New Zealand	0.0632	0.0386*
Sullivans Point, Tasmania v Bucks Bay, South Australia	0.0466	0.0333*
Sullivans Point v Bicheno, Tasmania	0.0213	0.0298
Flinders Island, Bass Strait v Bicheno, Tasmania	0.0336	0.0302*
Port Lincoln, South Australia v Gisborne, New Zealand	0.0158	0.0325
Port Lincoln, South Australia v Bicheno, Tasmania	0.0122	0.0239
Gisborne v Moeraki, New Zealand	0.0021	0.0213
Gisborne, New Zealand v Bicheno, Tasmania	0.1179	0.0480*
Gisborne, New Zealand v Port Fairy, Victoria	0.0161	0.0254
Gisborne, New Zealand v Batemans Bay, New South Wales	0.0565	0.0644
Gisborne, New Zealand v Esperance, Western Australia	0.0386	0.0356*
Moeraki, New Zealand v Bucks Bay, South Australia	0.0068	0.0203
Moeraki, New Zealand v Bicheno, Tasmania	0.0379	0.0289*
Bucks Bay, South Australia v Bicheno, Tasmania	0.1011	0.0343*
Bucks Bay, South Australia v Port Fairy, Victoria	0.0228	0.0214*
Bucks Bay, South Australia v Batemans Bay, New South Wales	0.0298	0.0423
Bucks Bay, South Australia v Esperance, Western Australia	0.0270	0.0287

* Comparisons in which the magnitude of the standard error is less than that of the diversity.

A large distance separated lobster specimens collected from Western Australia (2057±407km in a straight line) and from New Zealand (2605±456km in a straight line) from those collected in south-eastern Australian waters. This large geographical distance was not reflected by the amount of mtDNA sequence divergence. The net amount of sequence diversity between Western Australian lobster genomes and those from south-eastern Australia was zero. That between genomes from New Zealand and south-eastern Australia was also close to zero (0.0129%).

None of the gene diversity analyses on groups of collection locations (Table 2), based either on coastal current patterns or environmental parameters of the adult habitats, yielded a significant result. For example, when lobster collection locations were grouped according to their occurrence in either the northern Eastern Australian Current, the southern Eastern Australian Current, the Great Australian Bight or in southern Western Australian waters (Table 2) the calculated nucleotide (N_{ST} = -0.012; G_{ST} = 0.168, range 0.138-0.232) and haplotype diversities (G'_{ST} = 0.009, range 0.003-0.011, Table 5) indicated a lack of genetic subdivision.

2.4 Discussion

In this analysis of mtDNA nucleotide sequence polymorphism in Australian and New Zealand populations of *Jasus*, genetic subdivision was not detected. This is clearly shown by the lack of geographical partitioning of haplotypes in each of two major clades and by the lack of significant measurements of gene diversity. The significantly large inter-populational mtDNA sequence divergences involving lobsters collected from Bicheno, on the eastern coast of Tasmania (3/4 comparisons), and Gisborne, on the eastern coast of the North Island of New Zealand (2/4 comparisons), indicate that these locales are the most likely representatives of isolated populations. We believe that the lobsters at these locales are not genetically isolated as the efficiacy of the analysis may have been affected by the small number of animals sampled from Bicheno (6) and the low intra-populational mtDNA sequence divergence for the 10 animals sampled from Gisborne, New Zealand. The probable absence of genetic subdivision among *Jasus* populations suggests that larval exchange includes the population at Gisborne. The relatively small amount of mtDNA sequence diversity in the Gisborne sample was probably a result of sampling error.

The absence of genetic breaks within these widespread *Jasus* species is in contrast to the well-defined boundaries reported by Saunders et al. (1986) for horseshoe crabs (*Limulus polyphemus*) and by Reeb and Avise (1990) for the American oyster (*Crassotrea*

Table 5: Nucleotide (N_{ST}, G_{ST}) and haplotype subdivision (G'_{ST}) between regional populations of *Jasus novaehollandiae* and *J. edwardsii*. Population groupings are described in Table 2.

	Nucleotide			Haplotype	
Grouping Type	N_{ST}	G_{ST}	Range[a]	G'_{ST}	Range[b]
Current Flow					
1	-0.012	0.168	0.138 – 0.232	0.009	0.003 – 0.011
2	-0.004	0.188	0.164 – 0.246	0.016	0.012 – 0.016
Environmental					
1	0.009	0.190	0.127 – 0.222	0.009	0.001 – 0.005
2	0.001	0.193	0.135 – 0.250	0.004	-0.001 – 0.007
3	-0.007	0.238	0.212 – 0.279	0.005	0.004 – 0.005

a Range of 1000 bootstrapped estimates of G_{ST}
b Range of jacknifed estimates of G'_{ST}

virginica). Both of these species have a continuous distribution along the eastern coast of the United States, including the coast of the Gulf of Florida, and a planktonic larval stage, although the larvae of the horseshoe crab is thought to have a low vagility. The location of the genetic boundary between northern and southern assemblages of horseshoe crab and oyster populations, the mid-Atlantic coast of Florida, is the same for both species. The boundary coincides with a long-recognized transitional zone between warm-temperate and tropical faunal assemblages (Reeb and Avise , 1990). The presence of this transition zone, and the patterns of past and present surface currents appears to have produced a unique barrier to gene flow within marine species having planktonic larvae.

The lack of genetic subdivision in *J. novaehollandiae* and *J. edwardsii* reported here does not prove that genetic subdivision is absent in these species. Although the technique of mtDNA analysis is one of the most sensitive available for stock assessment (Ovenden, 1990), the detection of a small amount of subdivision may be below the level of resolution of the method. It remains to be seen whether the magnitude of either contemporary or historical gene flow which would be associated with a small amount of genetic subdivision are relevant to the requirements of resource management. A refinement of mtDNA analysis, polymerase mediated DNA amplification (Erlich, 1989) followed by nucleotide sequence determination, may increase the resolution of the technique in future analyses.

One of the approaches used here to test the null hypothesis of the absence of genetic subdivision is novel. A statistically significantly large mtDNA sequence divergence between a pair of populations would have been accepted as evidence that gene flow between them was restricted. As a large number of pairwise comparison between populations were made, and the level of statistical significance used was 5%, one in twenty tests would have been expected to yield a significant result. The test uses the sampling variance of net mtDNA sequence divergence between pairs of populations (Nei and Jin, 1989) to evaluate the null hypothesis. However, in an analysis of population subdivision using mtDNA polymorphism there are three levels of variance; that which accounts for error in the sampling of nucleotides from the genome, individuals from the population and populations from the range of the species (Lynch and Crease, 1990). The variance used here is the sampling variance associated with the assay of a subset of nucleotides between pairs of genomes, one genome from each of two populations. The variance calculated by the algorithm of Nei and Tajima (1983) appears to be associated with the choice of a particular pair of individual genomes, one from each of two populations. Until an algorithm is available which calculates a single variance for the

error associated with nucleotide and genome sampling combined, the gene diversity approach of Lynch and Crease (1990) is recommended.

The lack of a detectable amount of genetic subdivision among populations of *J. novaehollandiae* and *J. edwardsii* suggests that habitat-specific settlement of larvae, as proposed by Pollock (1990) to account for the allopatric adult and sympatric larval distributions of *J. tristani* and *J. lalandii*, may not occur in these species. The southern Australian and New Zealand coastline is likely to present as much diversity of settlement cues as experienced by *J. tristani* and *J. lalandii* larvae along the South African coastline and the area surrounding the Vema seamount and the island of Tristan da Cunha. In the absence of geographical isolation or isolation by distance among the adult *Jasus* populations studied here, further speciation may be unlikely to occur within these taxa. Speciation has produced mtDNA differences between other *Jasus* species. Brasher et al. (submitted) has shown that *J. lalandii*, *J. tristani* and *J. verreauxi* are separated from *J. novaehollandiae* and *J. edwardsii* by 4.4%, 7.3% and 15.0% mtDNA sequence divergence respectively.

The lack of genetic distinction between *J. novaehollandiae* and *J. edwardsii* suggests that larvae may travel thousands of kilometres prior to settlement. The minimum number of larvae which would need to be exchanged each generation between Australian and New Zealand populations to prevent subdivision is approximately one. The high mortality which would be associated with extended larval life and travel across large distances may dilute the trans-Tasman survivors at the recruitment site to such small numbers. Alternatively, large numbers of larvae of Australian origin may be carried by the prevailing currents for recruitment into New Zealand populations. Australian lobster populations may be a major source of larvae if it is found that the majority of larvae from New Zealand populations are swept offshore and need to complete a global or Pacific circumnavigation in southern ocean currents before encountering adult habitat. Other factors, such as differential survival of larvae of Australian and New Zealand origin, may favour Australian populations as a source of recruitment. Analyses of the species composition of lobster larvae collected throughout the southern oceans is necessary to investigate these alternatives. The presence of *J. novaehollandiae* and *J. edwardsii* larvae in the southern Indian Ocean to the west of Australia would raise the possibility of New Zealand lobsters contributing to Australian populations.

CHAPTER 3

Development of mitochondrial DNA (mtDNA) analysis techniques to genetically identify puerulus larvae.

(D. J. Brasher, J. R. Ovenden & R. W. G. White)

3.1 Introduction

The genetic characterization of *Jasus novaehollandiae* puerulus larvae has the potential to reveal much about the population dynamics of this species. If mtDNA variation in adult *J. novaehollandiae* is geographically patterned, the mtDNA haplotype of puerulus would reflect the geographical origin of their parents. The information derived from these genetic comparisons could help clarify aspects of larval ecology, such as the likely paths of larval transport in oceanic current systems, the principal source of larval recruitment to benthic populations and the importance of post-settlement selection on the genetic composition of benthic populations.

Preliminary experiments showed that the standard techniques employed to extract and analyse the mtDNA of adult *J. novaehollandiae* could not be applied to puerulus larvae. Briefly, these techniques include differential centrifugation for isolation of mitochondria, organic extraction for mtDNA purification, restriction enzyme digestion, radioactive end-labelling of restriction fragments and gel electrophoresis. Because of the poor quality of puerulus tissue, mtDNA extracts were of low yield and heavily contaminated with nuclear DNA. A more sophisticated technique, such as in situ gel hybridization, was required for the detection of larval mtDNA.

The conventional Southern blotting technique involves transferring restriction fragments from an agarose gel to a nitrocellulose filter, or nylon membrane, prior to hybridization with a specific radioactive probe. The ability to perform hybridizations within dried agarose gels eliminates the need for a transfer step. Following gel dehydration, both DNA denaturation and hybridization are carried out in situ, providing a method which is faster and more sensitive than hybridization of probes to DNA on a solid-support filter or membrane.

This report contains a description of the in situ gel hybridization technique used for the detection of mtDNA restriction fragments from puerulus larvae. Two probe systems, purified *J. novaehollandiae* mtDNA and cloned honeybee mtDNA, were used. Future developments of the techniques and their potential applications are discussed.

3.2 Materials and Methods

Experimental Design

Total DNA and mtDNA extracts from puerulus were enzymatically digested prior to application to the hybridization gel. The total DNA extraction procedure is simpler and less time consuming than that for mtDNA. If restriction fragments could be detected from a crude extract, this form of DNA preparation would be the method of choice for large-scale screening of puerulus.

The ability to remove an existing probe from the dried gel and then rehybridize with a new probe enabled us to test the efficacy of two different probe systems. In the first part of the experiment, cloned mtDNA sequence from honeybee (*Apis mellifera ligustica*) was used as probe template. Cloning has the advantage of providing highly purified sequences in large quantities. Since no cloned crustacean mtDNA was available, a clone of a species belonging to the same phylum, Arthropoda, was chosen. Studies generally show a high degree of mtDNA sequence similarity between closely related taxa.

The second approach was to use gel purified adult mtDNA extracted from antennal glands. Probes synthesized from this template would essentially be 100% homologous to the target sequence. However, large quantities of mtDNA are generally difficult to recover using this method, and contamination by extraneous DNA can result in poor resolution of bands following autoradiography.

Also present on the gel was a radiolabelled digest of adult *J. novaehollandiae* mtDNA. This represents the most frequent restriction profile for the restriction enzyme AvaI (see Chapter 2), and served as a control to confirm suspected mtDNA:mtDNA hybrids. Three lanes, each containing a different quantity of AvaI digested adult mtDNA (unlabelled), were used to assess the sensitivity of the technique. The suitability of hybridization conditions was determined with linearized pUC18 plasmid. Molecular weight markers, labelled HindIII digested lambda DNA, were used to calibrate the size of DNA fragments.

Sample Collection

All *J. novaehollandiae* puerulus larvae used in hybridization experiments were collected from the east coast of Tasmania using either crevice-type or pallet-type collectors (Figs 1 & 2). The live puerulus were immediately frozen whole in liquid nitrogen.

Isolation of Target Sequence from Puerulus Larvae

Mitochondrial DNA isolation - The methods described by Chapman and Powers (1984) were employed to isolate mitochondria and extract mtDNA from *J. novaehollandiae* puerulus larvae. Because the soft portions of a puerulus weigh as little as 0.3 g, re-extraction of phenol with a single 500 µl aliquot of distilled water at both deproteinization steps was performed to maximize mtDNA recovery. Both aqueous phases were pooled prior to ethanol precipitation.

Total DNA isolation -Both mitochondrial and nuclear DNA was extracted from puerulus using a modified version of the method described by Maniatis et al. (1982). The carapace and hard portions of the tail and legs were removed from the puerulus, and the remaining soft tissue minced with a scalpel. This tissue was transferred to a ceramic mortar, overlaid with liquid nitrogen, and ground to a fine powder. The liquid nitrogen was allowed to evaporate before transferring the powder to 10 volumes of lysis solution (0.5 M EDTA pH 8.0, 0.5% Sarcosyl, 100 $mg.ml^{-1}$ proteinase K). This suspension was gently agitated in an incubator at 65°C for 30 min. The resulting DNA solution was extracted three times with an equal volume of equilibrated phenol, and once with an equal volume of 24:1 chloroform: isoamyl. Proteins, polysaccharides and cell metabolites that may have inhibited subsequent enzymatic digestion of the DNA were removed from the solution with a Qiagen anion-exchange resin tip (Phoenix Scientific Industries, Victoria). DNA eluted in 600 µl of buffer was precipitated with 0.8 volumes of isopropanol and pelleted by centrifugation. The pellet was washed with 70% ethanol, vacuum dried, resuspended in 100 µl of distilled water, and stored at -20°C until required for restriction enzyme analysis.

Preparation of Dried Agarose Gel

DNA containing target sequences was enzymatically digested and electrophoresed (electrophoresis buffer, TPE) at 24 V for 20 hr through a 1.4% agarose gel (dimensions, 15 X 15 X 0.3 cm). The gel was transferred to a thick plastic sheet, covered with two sheets of blotting paper (Whatman 3MM), and placed in a vacuum drier where it was dehydrated at room temperature for 30 min, and then at 50°C for 30 min. Extraneous areas of gel were removed before autoradiography. The gel was stored in a plastic bag at 4°C until required for DNA hybridization. The prepared gel, which had retained a small amount of water, was thin, flexible and surprisingly resilient to handling and transfer steps.

Probe Synthesis

Jasus novaehollandiae mtDNA - Mitochondrial DNA isolated from approximately 6 g of antennal gland, using the method of Chapman and Powers (1984), was partially purified

via agarose gel electrophoresis. So that stained mtDNA could be visualized as single, sharp bands under UV radiation, the circular genomes were first linearized with the restriction enzyme PvuII. This singularly cleaved mtDNA was electrophoresed through a 1% agarose minigel at 70V for 1 hr. The gel was placed in its electrophoresis buffer (TPE) and the mtDNA stained by the addition of 20 µl ethidium bromide (10 $mg.ml^{-1}$). Under a longwave UV lamp and using a scalpel, blocks of agarose containing the mtDNA bands were excised from the gel. Nuclear contamination was minimized by removing the smallest amount of agarose with the mtDNA band. Excised bands were pooled and the mtDNA recovered using the Geneclean DNA purification kit (Bio 101, Inc., California).

Apis mellifera ligustica mtDNA - The pUC18 plasmid, containing the cytochrome c oxidase subunit I (CO-I), (CO-II), and tRNA genes of *Apis*, was donated by R. H. Crozier, Y. C. Crozier (School of Biological Science) and A. G. Mackinlay (School of Biochemistry), University of New South Wales. Competent *E. coli*, XL1-Blue (Stratagene, California), were transformed by pUC18 using standard techniques (Ausubel et al., 1989). The protocol used for subsequent large-scale preparation of plasmid DNA (Ausubel et al., 1989) produced 30 µg of pUC18. This DNA was resuspended in 300 µl of water prior to storage at -20°C. The oligo-labelling method used to produce radioactive probes is most efficient when linear DNA is provided as template for the reaction. So that high specificity *Apis* probe would be generated during the subsequent labelling step, approximately 100 ng of plasmid was linearized with HindIII.

Radiolabelling - Both *J. novaehollandiae* mtDNA and plasmid were radiolabelled with alpha-^{32}P-dCTP using a kit for the oligo-labelling of DNA restriction fragments (Biotechnology Research Enterprises, South Australia). The DNA solution was boiled for 4 min in a microcentrifuge tube, denaturing the double-stranded DNA to produce single-stranded templates. Random oligonucleotides (prepared by DNAase digestion of calf thymus DNA) were added to the chilled solution, which annealled to the templates at complimentary base sequences. DNA polymerase I, 50 mCi alpha-^{32}P-dCTP and the three remaining unlabelled dNTPs were added to the tube and the solution incubated at 40°C for 40 min. The 3'-5' polymerase activity of DNA polymerase I adds the introduced nucleotide residues to the 3'-OH termini of the oligonucleotides, which act as primers for DNA synthesis. A Sephadex G-50 column (5 cm high X 1 cm diameter), equilibrated in TE buffer (10 mM Tris pH 7.4, 1 mM EDTA, pH 8.0), was used to separate labelled mtDNA from unincorporated radionucleotides. The solution was made to 120 µl with distilled water and 20 µl bromophenol blue, and applied to the top of the column. Labelled DNA is excluded from the gel and exits the column before the radionucleotides,

while bromophenol blue filters through the gel at a similar rate to the radionucleotides, thus serving as an indicator. The sample was allowed to run into the gel before topping up the column with TE. The sample was eluted into 20 fractions, each containing approximately 500 µl. Collection of eluent stopped when bromophenol blue appeared in the fractions. The radioactivity of the fractions was determined with a hand-held minimonitor. The first large peak of radioactivity represented labelled mtDNA, the second, radionucleotides. Fractions constituting the first peak were pooled.

DNA Hybridization

The hybridization method employed was developed by Roger Drinkwater, Stephen Moore, William Barendse, Robbie Hediger and Jay Hetzel of the Molecular Genetics Group, CSIRO, Division of Tropical Animal Production, Rockhampton, Queensland. DNA within the dried gel was denatured into single strands with gentle agitation in a tray containing 100 ml of denaturing solution (0.4 M NaOH, 1.0 M NaCl) for 30 min at room temperature. The gel was given two 15 min washes in 100 ml neutalizing solution (0.5 M Tris pH 7.4, 1.0 M NaCl), then a 10 min wash in 100 ml 5XSSC (20XSSC: 3 M NaCl, 0.3 M Na_3citrate.$2H_2O$ pH 7.0), each wash being performed at room temperature. The gel was then transferred to a tray containing 100 ml pre-heated hybridization buffer (5XSSC, 1.0% SDS, 0.5% skim milk, 20mM Tris pH 7.4) and ^{32}P-labelled DNA probe which had been denatured into single strands by boiling for 5 min. It was crucial that that the ^{32}P-probe be ready to add to the hybridization buffer when the gel has finished its washing sequence. The double-stranded DNA in the gel does not move far apart when denatured, and reanneals quickly under the hybridization conditions. Hybridization was performed, with gentle agitation, for 18hrs in an incubator. Following hybridization, the gel was washed in three 100ml volumes of 2XSSC/0.1% SDS for 10 min each to eliminate unhybridized probe and background signals caused by nonspecific strand re-association. The gel was vacuum dried for 30 min at room temperature and transferred onto a thick plastic sheet for autoradiography. Probe was removed from the gel by repeating the denaturation, neutralization and washing (2XSSC/0.1% SDS) steps above.

3.3 Results and Discussion

Figure 12 is an autoradiograph of the dried agarose gel prior to the addition of probe. It shows the AvaI restriction profile of mtDNA extracted from adult antennal gland and a lambda DNA weight marker, both of which were radiolabelled prior to electrophoresis. The approximate sizes of the four AvaI restriction fragments, in descending order, are 5500, 5300, 2900 and 1500 base pairs (bp).

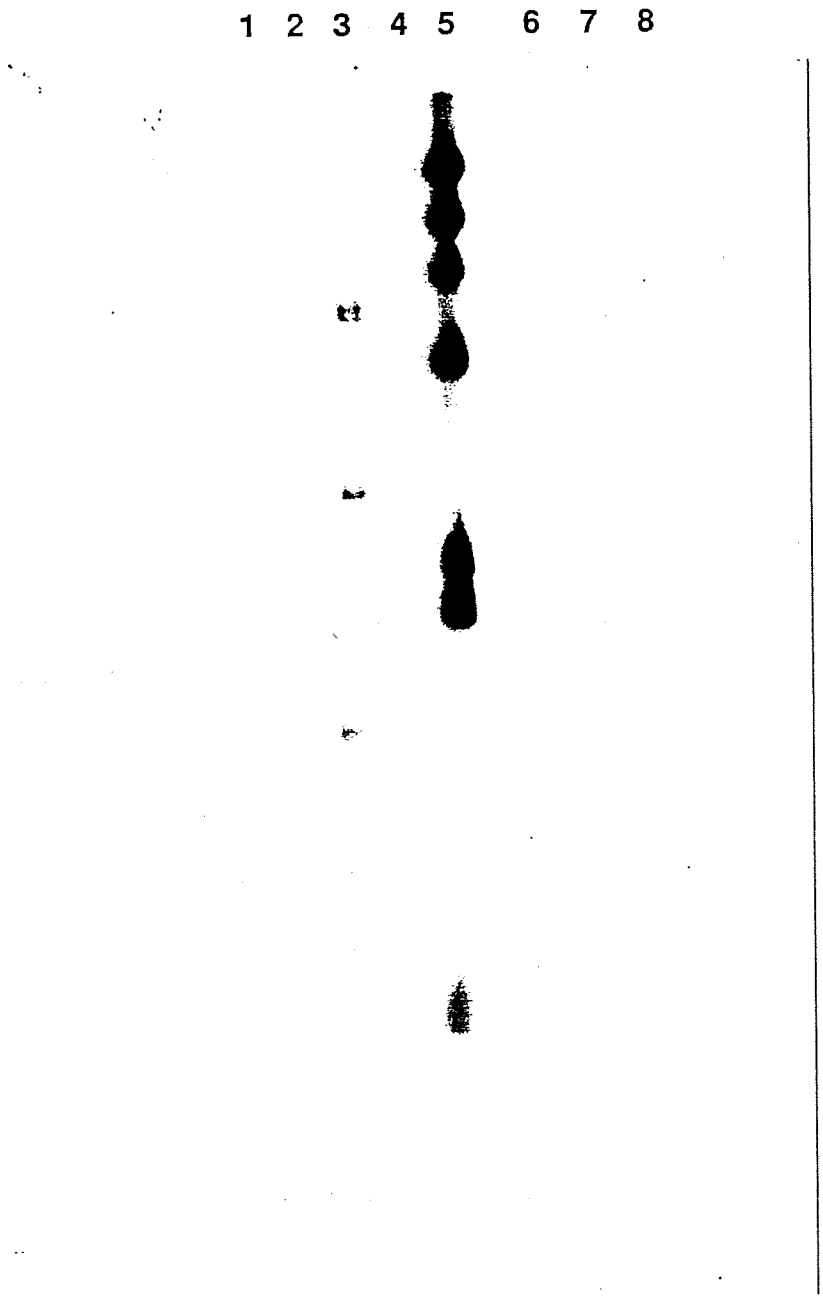

Figure 12: Autoradiograph (4 hr exposure) of dried agarose gel prior to addition of probe. All *J. novaehollandiae* samples were digested with AvaI. The contents of each lane are as follows: 1) 10% of total DNA extract from puerulus; 2) 20% of mtDNA extract from puerulus; 3) 4% of mtDNA extract from adult antennal gland, radiolabelled with alpha-^{32}P dCTP; 4) 4% of mtDNA extract from adult antennal gland; 5) 2.5 ng of lambda DNA weight marker, digested with HindIII and radiolabelled; 6) 100 ng of pUC18 plasmid with *A. mellifera ligustica* insert, linearized with HindIII; 7) 20% of mtDNA extract from adult antennal gland; 8) 70% of mtDNA extract from adult antennal gland.

No observable hybridization occurred between the *Apis* probe and *Jasus* mtDNA, as shown by autoradiography (Fig. 13). However, a signal from the *Apis* target sequence indicates correct hybridization conditions. One of the disadvantages of in situ hybridization is the inability of the gel matrix to retain target fragments smaller than ~ 500 bp during the denaturation, hybridization and washing steps. This is demonstrated by the labelled AvaI digest of adult mtDNA. Although five fragments are generated by this enzyme for this particular individual, only four can be detected after autoradiography; the smallest fragment (size ~ 500bp) has diffused from the gel.

In light of this, it is possible that the *Jasus* target sequence was present on this lost fragment. Diffusion, however, is probably not responsible for the negative result, since the *Apis* insert is larger than the lost mtDNA and, consequently, should have hybridized to one of the larger restriction fragments. The absence of a signal is most likely due to a low degree of homology between the probe and mtDNA target. Gel treatment was nonstringent, i.e. the low temperatures used for hybridization (45°C) and washing (25°C) would have allowed hybridization between related, rather than only complementary, sequences. On the basis of melting temperature (Tm) values for DNA:DNA hybrids, we estimate that there is less than 65% homology between *Jasus* and *Apis* genes. *Jasus* and *Apis* belong to two major groups within the Arthropoda, the Crustacea and Insecta respectively. Crozier et al. (1989) raised taxonomic questions about the Insecta group after finding a low degree of sequence homology (~ 71%) between the CO-I and CO-II genes of *Apis* and *Drosophila*. They concluded that existing views were valid and that the low homology was attributable to a faster rate of mtDNA evolution in honeybees. By demonstrating an even lower homology between *Jasus* and *Apis*, our results give support to the traditional phylogenetic view, i.e. the grouping of insects to the exclusion of other arthropod taxa.

The recovery of adult mtDNA from agarose using the Geneclean kit was poor. Using ethidium bromide quantitation, we estimated that ~ 60 ng (80%) of electrophoresed mtDNA was lost through this procedure. Consequently, only ~ 15 ng of *Jasus* mtDNA was available for the labelling reaction, resulting in a probe of low specific activity. Nevertheless, hybridization of the Jasus probe to mtDNA restriction fragments from both adult and puerulus mtDNA extracts was observed (Fig. 14). Although the restriction profile of the puerulus mtDNA is incomplete (approximately 2000 bp of the 16000 bp genome cannot be detected), it appears that the puerulus has the same AvaI genotype as the adult control. As expected, there was no hybridization between the *Jasus* probe and *Apis* target sequence. A relatively low signal for the fourth band in the restriction profile

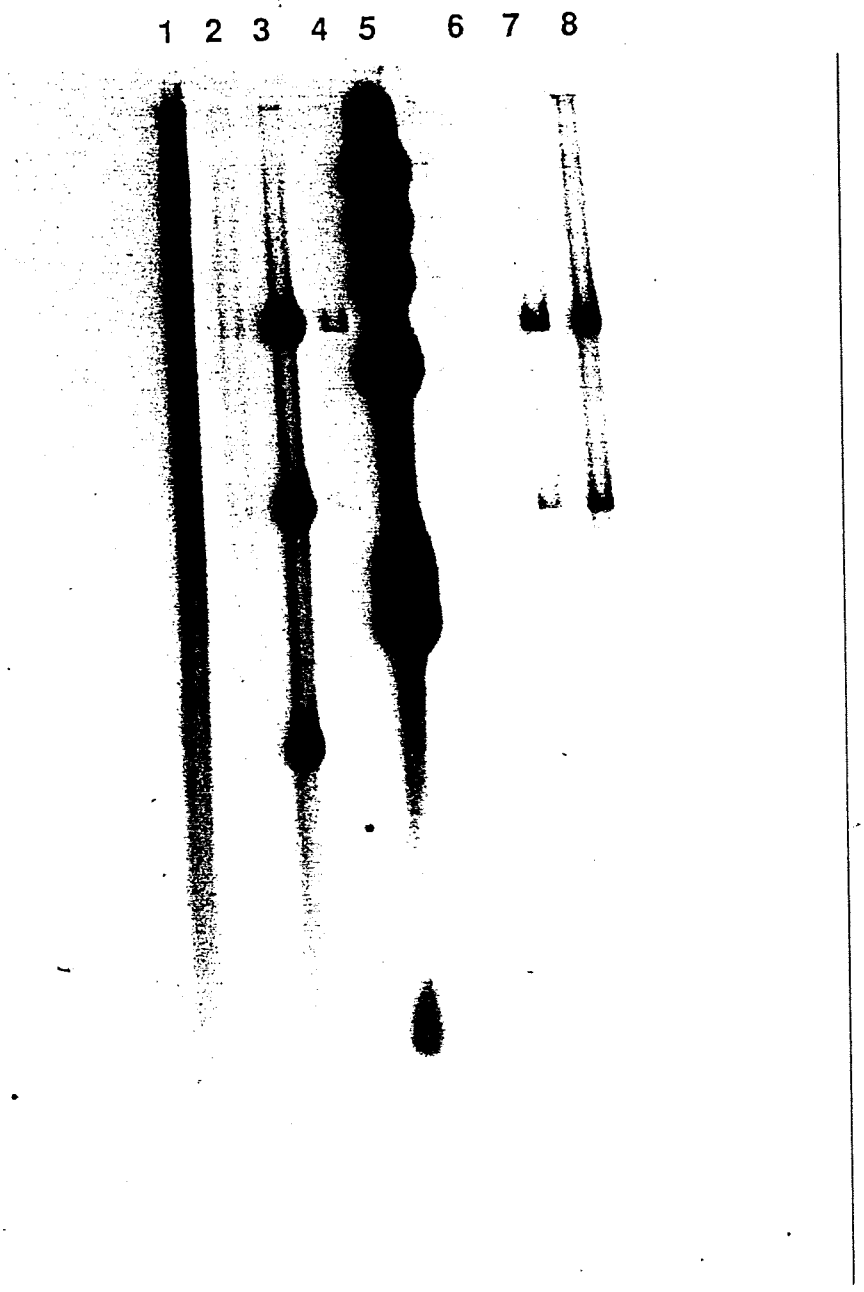

Figure 14: Autoradiograph (95 hr exposure) of dried agarose gel after addition of ~15 ng of *J. novaehollandiae* mtDNA probe, followed by high-stringency washing. For content of lanes see figure 12.

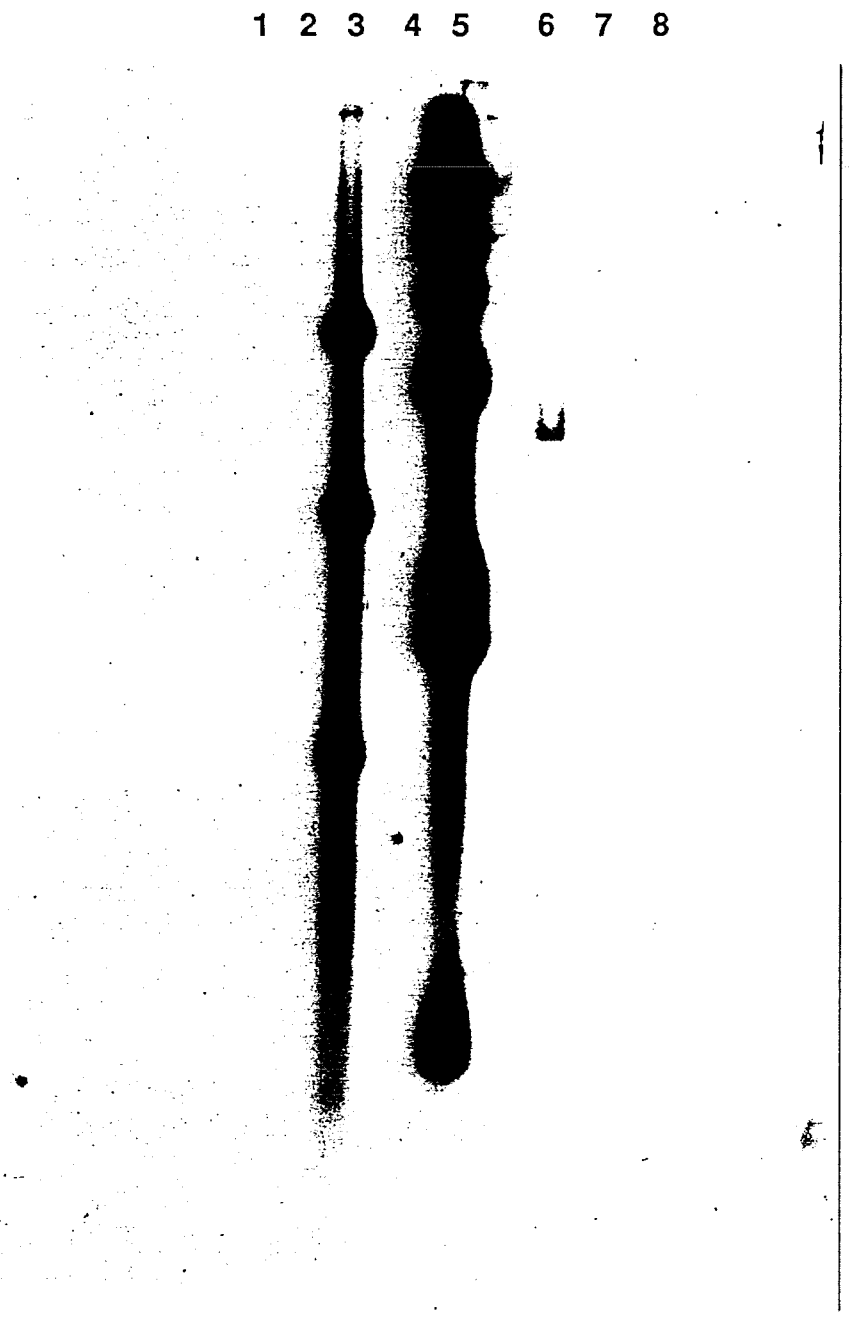

Figure 13: Autoradiograph (34 hr exposure) of dried agarose gel after addition of 100 ng of *A. mellifera ligustica* mtDNA probe, followed by nonstringent washing. For content of lanes see figure 12.

of the adult digests indicates that fragments up to ~ 1500 bp in size are diffusing from the gel.

Following nonstringent gel treatment (described above), fragments identified from the puerulus mtDNA extract appeared as weak bands and were obscured by background radiation. A subsequent high-stringency wash (60°C for 15 min) failed to eliminate this background (Fig. 14), suggesting that contaminating nuclear DNA was excised and labelled with the gel purified *Jasus* mtDNA. This nuclear DNA probe then hybridized with homologous contamination present in the mtDNA extract. Alternatively, background may be the result of target mtDNA which has been sheared into a range of sizes by the vigorous manipulation necessary during the extraction procedure.

No mtDNA restriction fragments are discernible within the lane containing the puerulus total DNA extract; the dark smear observed, which obliterates any mtDNA band that may be present, is most likely due to nonspecific hybridization between the probe and nuclear DNA. The presence of a dark band at ~ 16000 bp suggests that the mtDNA was not cleaved by AvaI. If this was the case, it would seem that the high proportion of nuclear DNA in the extract has resulted in competitive inhibition of the enzyme, a problem that may be circumvented by adding more enzyme to the digestion mixture.

Concluding Remarks

The technique of in situ gel hybridization was successfully employed to enzymatically characterize a large section of the of the mtDNA of a puerulus larva. This puerulus was identified as having three AvaI restriction fragments homologous to those of the adult control, despite the low activity of the *Jasus* probe and its likely contamination with labelled nuclear DNA. Further developments to intensify these bands and eliminate background would include the use of highly purified probe at higher concentrations in the hybridization buffer. Total purification of mtDNA preparations is achieved in other laboratories with cesium chloride equilibrium centrifugation. Although small mtDNA fragments are lost from the gel, by selecting restriction enzymes that produce large fragments from variable regions of the genome, informative genetic markers could be routinely analyzed. Apart from its potential application to population research, in situ gel hybridization could also be employed for species identification of egg masses, animal remains and early life-cycle stages that are morphologically conserved across taxa.

Acknowledgements

We thank John Booth (Ministry of Agriculture and Fisheries, New Zealand), Bruce Phillips (CSIRO Marine Laboratories, Western Australia) and Bruce Wallner (South Australian Department of Fisheries) for useful discussions and for providing providing either specifications or prototypes for the puerulus collectors used in this study. We also thank John Booth, Bruce Wallner, Robert Grove-Jones (South Australian Department of Fisheries), Rhys Brown and Eric Barker (Fisheries Department of Western Australia), David Molloy (Marine Science Laboratories, Queenscliff, Victoria), Steve Montgomery (Fisheries Research Institute, Cronulla, N.S.W.), Steve Bishop (King Island, Tasmania), Lee Cox (Flinders Island, Tasmania), George Greaves (Esperance, Western Australia), Mick Murphy (Temma, Tasmania) and Charles Wessing (Hobart, Tasmania) for obtaining adult lobsters for use in genetic analysis.

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Appendix 1A: Site presence and absence information for 10 AflII morphs identified amongst 132 *Jasus novaehollandiae* and *J. edwardsii*.

	Site Number										
Morph	1	2	3	4	5	6	7	8	9	10	11
A	1	1	1	1	1	1	0	0	0	0	0
B	1	1	1	1	1	0	0	0	0	0	0
C	0	1	1	1	1	1	0	0	0	0	0
D	0	1	1	1	1	1	1	0	0	0	0
E	1	1	1	1	1	1	0	1	0	0	0
F	1	1	1	1	0	1	1	0	0	0	0
G	1	1	1	1	0	1	0	0	0	0	0
H	1	1	1	1	1	0	0	0	1	0	0
I	1	1	1	1	1	1	0	0	0	1	0
J	1	1	1	1	1	1	0	0	0	0	1

Appendix 1B: Site presence and absence information for 6 AvaI morphs identified amongst 132 *Jasus novaehollandiae* and *J. edwardsii.*

	Site Number						
Morph	1	2	3	4	5	6	7
A	1	1	1	1	1	0	0
B	1	1	0	1	1	0	0
C	0	1	1	1	1	0	0
D	1	0	1	1	1	0	0
E	1	1	1	1	1	1	0
F	1	1	1	1	1	0	1

Appendix 1C: Site presence and absence information for 11 BanI morphs identified amongst 132 *Jasus novaehollandiae* and *J. edwardsii.*

	Site Number											
Morph	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	0	0	0	0	0	0	0
B	1	1	1	1	1	1	0	0	0	0	0	0
C	1	1	1	1	1	0	1	1	1	1	0	0
D	1	1	1	1	1	0	0	0	0	0	1	0
E	1	1	1	1	1	0	1	1	0	0	0	0
F	1	1	1	1	1	0	0	1	0	0	0	0
G	1	1	1	1	1	0	1	1	0	0	0	0
H	1	1	1	1	1	0	0	0	0	1	0	0
I	1	1	1	1	1	0	0	0	0	0	0	1
J	1	1	1	0	1	0	0	0	0	0	0	0
K	1	1	1	1	0	0	0	0	0	1	0	0

Appendix 1D: Site presence and absence information for 14 BstYI morphs identified amongst 132 *Jasus novaehollandiae* and *J. edwardsii.*

	Site Number															
Morph	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
B	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
C	1	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0
D	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0
E	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0
F	1	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0
G	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
H	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0
I	1	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0
J	1	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0
K	1	1	1	1	1	1	1	0	0	0	0	0	1	0	0	0
L	1	0	1	1	1	1	1	0	0	0	0	0	0	1	0	0
M	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	0
N	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1

Appendix 1E: Site presence and absence information for 5 EcoRV morphs identified amongst 132 *Jasus novaehollandiae* and *J. edwardsii.*

	Site Number								
Morph	1	2	3	4	5	6	7	8	9
A	1	1	1	1	1	1	0	0	0
B	1	1	1	1	0	1	0	0	0
C	1	1	1	1	1	1	1	0	0
D	1	1	1	1	1	1	0	1	0
E	1	1	1	1	1	1	0	0	1

Appendix 1F: Site presence and absence information for 14 HindIII morphs identified amongst 132 *Jasus novaehollandiae* and *J. edwardsii.*

	Site Number											
Morph	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	0	0	0
B	1	1	1	0	1	1	1	1	1	0	0	0
C	1	1	1	0	1	1	1	1	1	1	0	0
D	1	1	1	0	1	0	1	1	1	0	0	0
E	1	1	0	1	1	1	1	1	1	0	0	0
F	1	1	0	0	1	1	1	1	1	0	0	0
G	0	1	1	1	1	1	1	1	1	0	0	0
H	0	1	1	0	1	1	1	1	1	0	0	0
I	1	1	1	1	1	1	1	1	1	0	1	0
J	1	1	1	1	1	1	1	0	1	0	0	0
K	1	1	1	1	1	1	1	1	1	0	0	0
L	1	0	1	0	1	1	1	1	1	0	0	0
M	1	0	1	1	1	1	1	1	1	1	0	0
N	1	1	1	0	1	1	1	1	1	0	0	1

Appendix 2: Achievements in dissemination of information.

Manuscripts

Brasher, D.J., Ovenden, J.R. and White, R.W.G. Mitochondrial DNA variation and phylogenetic relationships of *Jasus* spp. (Decapoda: Palinuridae). submitted for publication in J. Zool.

Kennedy, R.B., Wallner, B. and Phillips, B.F. Preliminary investigations of puerulus settlement of the southern rock lobster *Jasus novaehollandiae* in southern Australia. Submitted for publication in the proceedings of the International Workshop on Lobster Ecology and Fisheries, June 1990, Havana, Cuba.

Ovenden, J.R., Brasher, D.J. and White, R.W.G. Lack of genetic subdivision among populations of rock lobsters (*Jasus novaehollandiae* and *J. edwardsii*) with an extensive Australasian distribution. In preparation.

Popular articles for industry

Kennedy, R.B. (1989) Puerulus collectors go to sea. Fishing Today **2(3):** 28.

Kennedy, R.B. (1990) Juvenile crays: where do they settle and when? Fishing Today **3(6):** 22-23.

White, R.W.G. (1989) It's all in the genes. Fishing Today **2(5):** 19.

Presentations

Kennedy, R.B. and Ovenden, J.R. (May, 1989) A new project to study larval recruitment and genetic variation of the southern rock lobster. Public rock lobster seminar day, Wrest Point Casino, Hobart.

Kennedy, R.B. (January, 1990) Progress in puerulus settlement studies - spatial variation in puerulus settlement off eastern Tasmania. Southern Rock Lobster Research Group, DSF Research Laboratories, Taroona.

Kennedy, R.B. (June, 1990) Progress in tagging and larval recruitment studies. General meeting of the Tasmanian Rock Lobster Fishermen's Association. Federal Country Club Casino, Launceston.

Kennedy, R.B., Wallner, B. and Phillips, B.F. (June, 1990) Preliminary investigations of puerulus settlement of the southern rock lobster *Jasus novaehollandiae* in southern Australia. Poster display at the "International Workshop on Lobster Ecology and Fisheries". Havana, Cuba.

Ovenden, J.R. and Brasher, D.J. (October, 1989) Progress in studies of genetic variation in the southern rock lobster. Typed summary provided to the AGM of Tasmanian Rock Lobster Fishermen's Association, Federal Country Club Casino, Launceston.

Ovenden, J.R. and Brasher, D.J. (January, 1990) Results of genetic analysis. Southern Rock Lobster Research Group, DSF Research Laboratories, Taroona.

Ovenden, J.R., Brasher, D.J. and White, R.W.G. (June, 1990) Genetic variation in the southern rock lobster and the phylogenetic relationships of 5 *Jasus* species. Poster display at the conference of "Population genetics and its application to fisheries management and aquaculture". University of N.S.W., Sydney.

Ovenden, J.R. Results from genetic analysis of the southern rock lobster. Public seminar, date and venue to be announced.

Other dissemination of information

Numerous informal discussions have been held with individual fishermen and members of the executive of the Tasmanian Rock Lobster Fishermen's Association by both the Division of Sea Fisheries and the Fish Research Group, University of Tasmania.

Appendix 3: List of slides submitted with report.

1: Recently settled puerulus of *Jasus novaehollandiae.* Note the dark brown tips of the antenna and the absence of visible digestive glands
2: Puerulus stage of *Jasus novaehollandiae.* Note the clearly visible, white digestive glands and pale banding along the length of the antenna. This specimen is at a later developmental stage than the specimen in slide 1.
3: Two puerulus stages of *Jasus novaehollandiae.* The specimen without digestive glands is at the earlier developmental stage.
4: Juvenile of *Jasus novaehollandiae.*
5: Crevice collector in-situ. This collector has a short (300mm) stand.
6: Crevice collector in-situ. This collector has a long (900mm) stand as it is located in an area with high sand transport.
7: A pair of modified pallet collectors deployed near the surface on a long-line.
8: A pair of modified pallet collectors deployed near the sea floor.
9: Collectors were placed inside a nylon bag before being brought to the surface for inspection. In this slide, a crevice collector is being hauled into the boat.
10: The crevice collectors complete with bag were placed in a fish bin prior to removing the collector from the bag.
11: A crevice collector being removed from the bag.
12: Inspection of a crevice collector.
13: A standard pallet collector being removed from its bag prior to inspection.
14: Inspection of a standard pallet collector.
15: Large *Jasus novaehollandiae* specimens from Esperance, Western Australia.
16: Dissection of adult *Jasus novaehollandiae.* The antennal gland, from which mtDNA is extracted, is being removed.
17: High-speed centrifuge used to isolate mitochondria from other cell components.
18: Addition of radioactive label to mtDNA restriction fragments.
19: Assembly of electrophoresis apparatus for gel separation of mtDNA restriction fragments.
20: Autoradiograph of dehydrated gel. MtDNA restriction fragments are visualized as dark bands.

Preliminary investigations of puerulus settlement of the rock lobster *Jasus novaehollandiae* in southern Australia

R. B. Kennedy, B. Wallner and B. F. Phillips

Abstract

The occurrence of puerulus and post-puerulus stages of *Jasus novaehollandiae* on artificial collectors was monitored in three southern Australian states, namely, Tasmania, South Australia and Western Australia. Four designs of collectors were tested, with highest settlement rates most frequently occurring on collectors deployed near the sea floor. Considerable differences in settlement rates were found both within and between areas, and localised positioning of collectors appeared to influence the settlement results. Settlement rates at most sites were too low and varied to deduce seasonality of settlement. However, a summer peak in settlement occurred at two sites off Tasmania.

Estudios preliminares sobre la fijación de estados de puerula de la langosta *Jasus novaehollandiae* en el sur de Australia

Resumen

La ocurrencia de estados pos-larvales (puerula y post-puerula) de *Jasus novaehollandiae* en colectores artificiales fue estudiada en los estados del sur de Australia: Tasmania, South Australia y Western Australia. Se provaron cuatro diferentes tipos de colectores obteniéndose las mayores tasas de fijación en aquellos situados cerca del fondo. Se observaron diferencias considerables en las tasas de fijación dentro de la misma area y entre diferentes areas, indicando que la posición localisada de los colectores parece tener algun efecto sobre la fijación. No obstante haber un incremento en la fijación en verano en dos sitios de Tasmania, las tasas de fijación en la mayoría de los sitios fue demasiada baja y variable como para concluir estacionalidad en la fijación de los estados post-larvales de esta especie.

Introduction

The southern rock lobster (*Jasus novaehollandiae*) occurs throughout southern Australia with highest abundance off Tasmania, Victoria and the southern half of South Australia. A fishery for this species produces an annual catch of approximately 4,500 tonnes and is one of the most valuable fisheries in Australia.

The puerulus larva of *J. novaehollandiae* is a transitional settling stage between the planktonic phyllosoma larva and the benthic juvenile. A knowledge of annual variation in puerulus settlement rates may be a considerable aid to managers by indicating potential fluctuations in the resource at an earlier time than is achieved through current monitoring of the fished stocks. This assumes that reproductive output and pre-settlement processes acting upon the planktonic stages are dominant variables influencing recruitment to the fishery, while post-settlement processes are relatively small. Hence, large inter-annual fluctuations in puerulus settlement levels should later be observed as fluctuations in recruitment to the fishery. Phillips (1986) used this assumption with a long time series of puerulus settlement data to advance a predictive model for the fishery catch of *Panulirus*

cygnus in Western Australia. Puerulus settlement information may also aid in the understanding of recruitment patterns and mechanisms.

Independent investigations into puerulus settlement of *J. novaehollandiae* were conducted in Western Australia, South Australia and Tasmania. The aims of these investigations were to determine a suitable design of collector and to provide preliminary information concerning spatial variation and seasonality of puerulus settlement.

Methods

Collectors were deployed at 9 sites around southern Australia (Figure 1). Site 1 was monitored from June 1987 to October 1989. Monitoring at the other sites commenced during June 1989.

Four designs of collectors were used. These comprised: a 'crevice collector' similar to that described by Booth and Tarring (1986); an 'artificial seaweed collector' as described by Phillips (1972); a 'pallet collector' used by Lewis (1977), and a 'modified pallet collector' which is a scaled down (1/4 size) version of the pallet collector. Approximately 50 percent of the pallet-style collectors were prepared with a protective marine treatment to reduce shipworm infestation while the remaining collectors were untreated as in the original work of Lewis.

Table 1 provides details of collector deployment. Collectors were checked on a monthly basis although sea conditions and other factors occasionally disrupted this routine. Crevice collectors were surrounded by a nylon bag before being brought to the surface by divers. A bag was also used for pallet style collectors at sites 7 and 8. However, use of a bag is generally considered unnecessary for pallet and seaweed collectors so such techniques were not employed at other sites. Any lobsters found on collectors were removed, counted and staged according to the criteria of Booth (1979). Crevice collectors were scraped to remove epiphytic growth before being returned to the sea.

Results

A total of 220 specimens (98 puerulus and 122 post-puerulus) were caught by April 1990. Five of the specimens were of a size (total carapace length >15 mm) that indicated immigration to the collectors after settlement. Immigrants were only found on crevice collectors and these specimens have been excluded from the results given below.

Comparison of collector designs

After 12 months in the water, all crevice and seaweed collectors were in good condition. The majority of treated pallet-style collectors were also in good condition although some had been damaged at the mooring attachment. The untreated pallet-style collectors had been severely infested by shipworm and the majority were not serviceable after 12 months. No differences in catch rates occurred between treated and untreated collectors.

Despite a smaller surface area, pairs of modified pallet collectors caught a similar number of puerulus to standard pallet collectors (Figure 2, Sites 7 & 8).

No clear differences were observed between the catch rates of crevice collectors and bottom-deployed pallet-style collectors (Figure 2, site 7). However, some differences were apparent between the catch rates of crevice collectors and surface deployed pallet-style collectors (Figure 2, sites 3 & 8).

Crevice collectors caught puerulus more frequently than surface deployed pallet-style collectors and with a shorter time lag between deployment and first settlement. Crevice collectors were the most effective collectors at site 8 and for most of the sampling at site 3. Pallet collectors had the highest catch in the last two sampling periods at site 3. However, the number of pallet collectors decreased during this period (from 6 to 3) and confidence in the results is therefore reduced. Furthermore, a greater number of days elapsed between the last two samplings at site 3, and when the results for this site are standardised to catch per collector per day, the crevice collectors appear to have the highest catch rates.

Spatial variability and seasonality of settlement

No specimens were obtained from sites 1, 4 and 5. Collectors were removed from site 4 during December 1989 due to the lack of settlement. Only one specimen was obtained from site 2. However, this site was affected by polluted freshwater from 15 September 89 to 10 November 89.

Figure 3 shows the catch rates of crevice collectors for individual stations at each site. Settlement rates were too low and varied to deduce seasonality of settlement at sites 3, 6 and 8. However, a summer peak was clearly evident at both sites 7 and 9.

Considerable differences existed between the catch rates of stations at individual sites. A Mann-Whitney test was used to test for differences in puerulus catches between stations at Site 7. The combined data from the two peak months of settlement (Nov. and Dec.) were used for these comparisons. Significant differences ($P<0.05$) in puerulus catches were found between station C and the other two stations. The difference in catch rate between stations is even more apparent at Site 9 where station A had the highest average catch rate of all stations containing crevice collectors and station C had the equal lowest catch rate.

Discussion

The crevice collector performed as well, if not better, than the other 3 designs of collector in terms of both catch rate and durability. Similar results were obtained by Booth (1979) when comparing crevice and seaweed collectors. Furthermore, results from the present study and Booth (1986) suggest that crevice collectors catch without heavy epiphytic growth, thus reducing the difficulties of standardising for differences in growth between sites and times. The unit cost of the crevice collector ($35 US) is also about half that of the other types of collectors.

The occasional presence of large specimens on the crevice collectors indicates that some migration occurs between these collectors and the surrounding substrate. Hence collector effectiveness may be affected by the nature of the surrounding substrate. While this may confound the definition of a standard collector, the apparent immigration rate was low (<2.5%). Furthermore, crevice collectors were usually positioned on sand substrates at sites 5-9 and hence differing substrates are unlikely to have contributed to spatial variability of the results.

Expected gross regional variations in numbers of settling puerulus are apparent in the data. No settlement was observed at site 1 which is located at the western edge of the geographic range for this species and supports low adult densities. Similarly, no settlement was observed at site 5 despite its central geographic location within the species range. Diver surveys along the central north coast of Tasmania have found few juvenile lobsters and it is believed this result may arise from a circulation pattern which results in low net flow of oceanic water through this region (Fandry 1983).

Results indicate that settlement levels were at least partially dependent on positioning of collectors within a site. Hence, it is difficult to interpret differences in settlement levels between sites. Meaningful comparisons between areas in the future will require either an understanding of the factors that influence settlement on a localised scale, or extensive sampling of sites so that the full range of localised variation in settlement within each site is known.

Settlement rates at site 3 were considerably lower than in a previous survey at the same location (Lewis 1977), indicating that the present results for this area may be due to a poor settlement year. It is not known whether a similar phenomena has occurred at the remaining sites. Differing factors may be influencing settlement between the areas since Lewis (1977) found a consistent winter peak in settlement at site 3, whereas the peak settlement occurred during summer at sites 7 and 9 in the present study. Conversely, it is possible that the timing of settlement in the present study was a result of unusual conditions.

Unless the year of data collection coincided with an unusually poor period of puerulus settlement, it is doubtful that the number of specimens obtained in the present study would be sufficient to adequately resolve the relative strength of settlement on an inter-annual basis. To achieve this objective, it is likely that an increased number of collectors will be required or that locations need to be identified in which there is higher settlement of puerulus. High localised differences in settlement levels suggest that there is considerable potential for obtaining improved settlement rates by manipulating the position of collectors.

Acknowledgements

We thank D. Tarbath, M. Clarke, P. Terry and R. Pearn for help with construction, maintenance and checking of the collectors. The Tasmanian section of this study was conducted by the Department of Sea Fisheries, Tasmania with funding from the Fishing Industry Research and Development Trust Fund. The work in South Australia and Western Australia was conducted by the South Australian Department of Fisheries and CSIRO respectively.

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Site	Station	Depth (m)	Number of Each Collector Type			
			Crevice	Pallet	Modified Pallet	Seaweed
1	A	4			3 *	
2	A	5		6 *		
3	A	2	11	6 *		6 *
	B	2	6			
4	A	5		6 *		
5	A	3	3			
	B	4	3			
	C	5	3			
6	A	3	3			
	B	3	3			
	C	2	3			
7	A	12	3	3	3	
	B	3	3			
	C	2	3			
8	A	4	3	3 *	3 *	
	B	3	3	3 *	3 *	
	C	5	3	3 *	3 *	
9	A	3	3			
	B	3	3			
	C	3	3			

Table 1. Deployment of Puerulus Collectors.
Stations within each site were separated by 300 to 1200 metres. Collectors marked with an asterix were positioned on the surface. The remaining collectors were positioned on the sea floor. Collectors within each station were separated by 3 to 6 m except for floating collectors at sites 2-4 which were separated by 25 m.

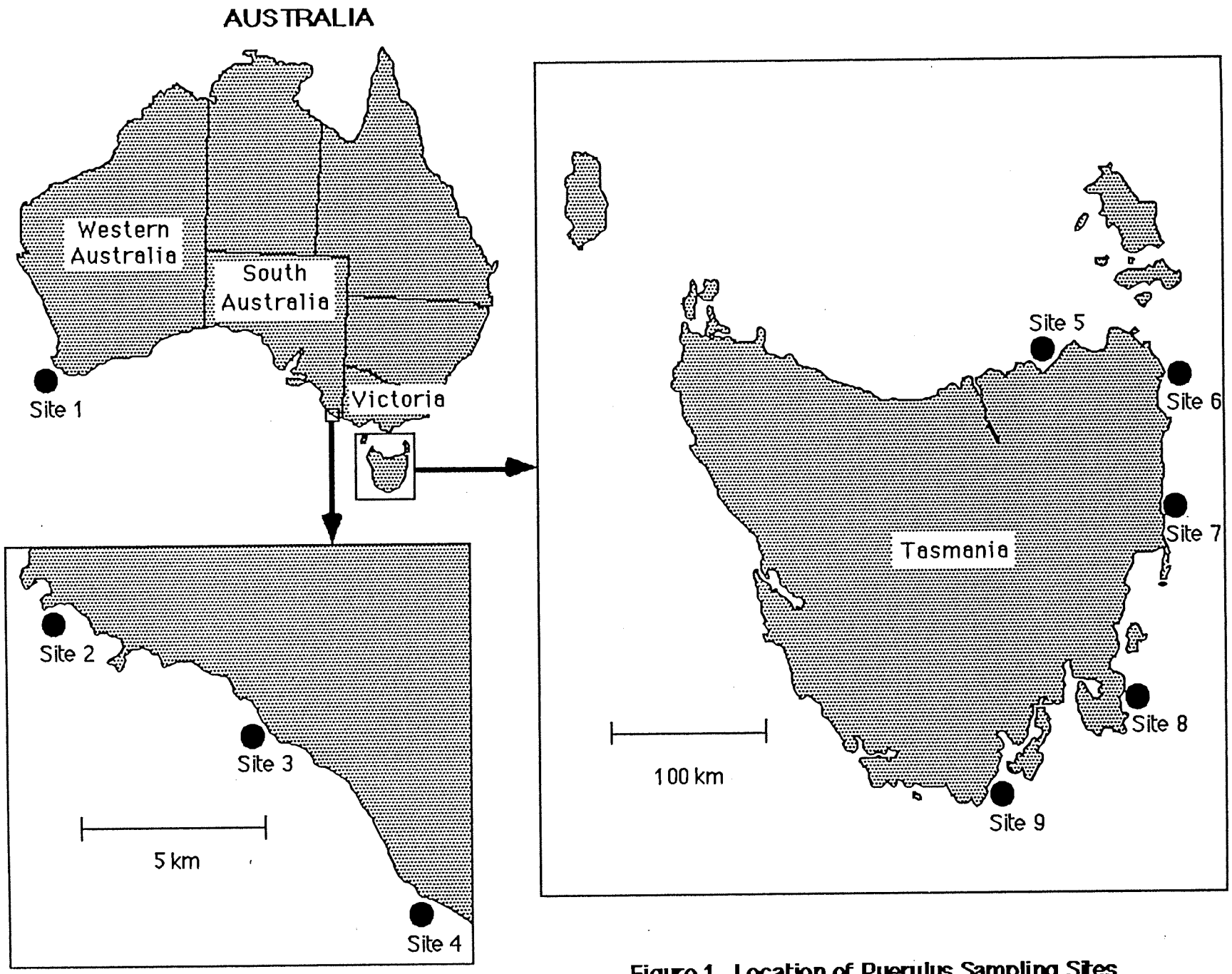

Figure 1. Location of Puerulus Sampling Sites

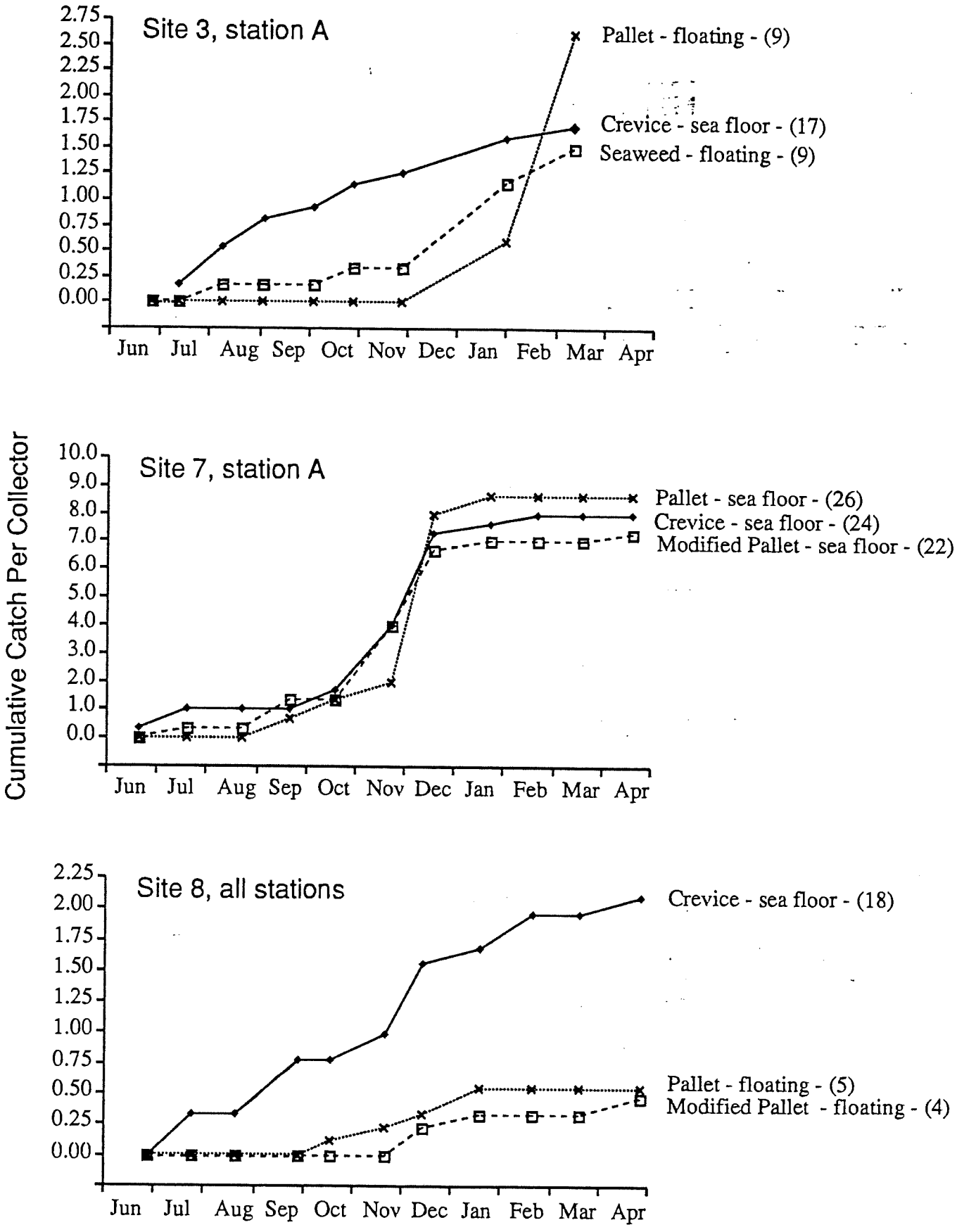

Figure 2. Cumulative Catch of Puerulus Per Collector
Numbers in the legend of each graph show the total catch for each collector type.

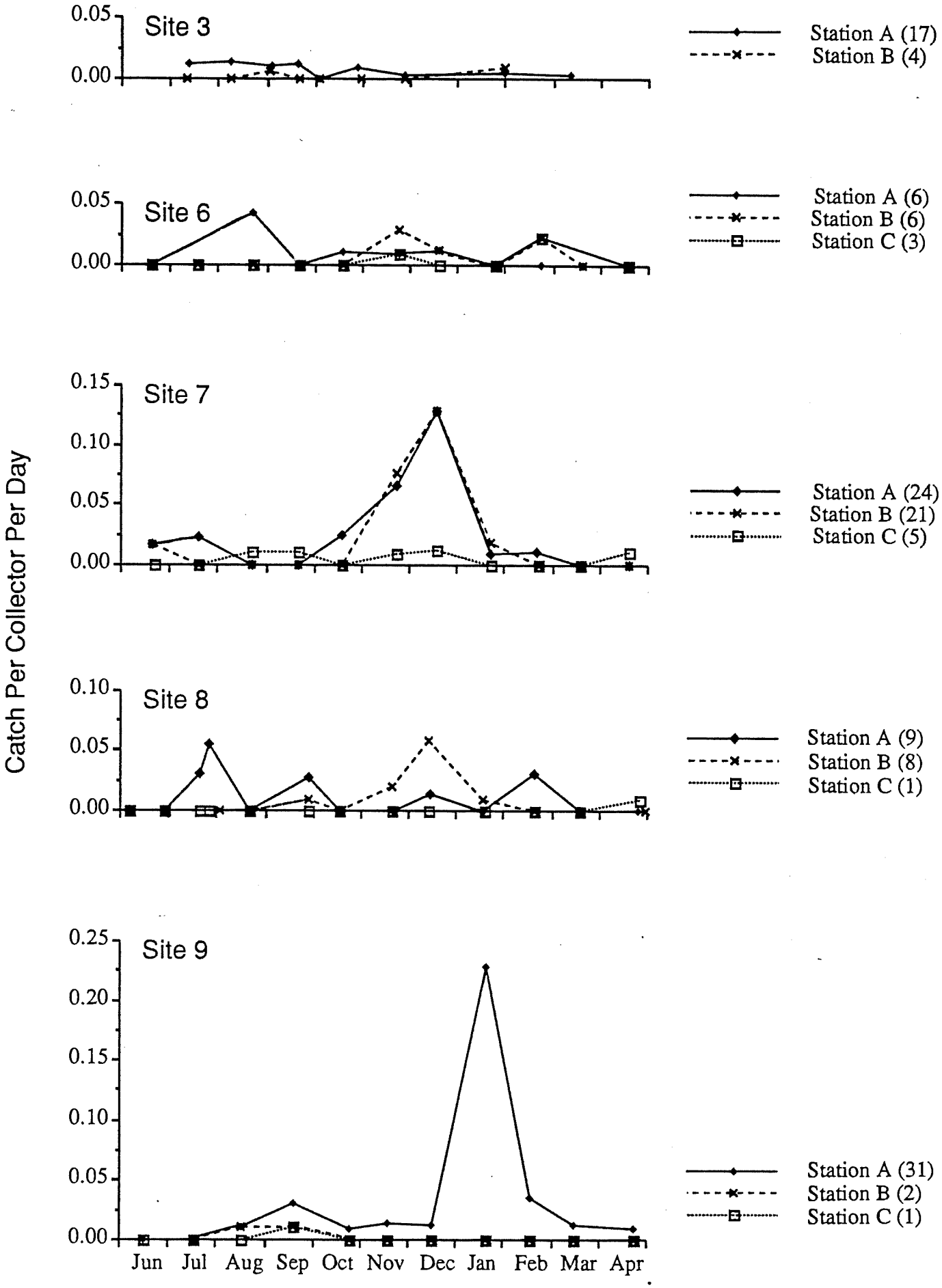

Figure 3. Daily Catch Rates of Puerulus by Crevice Collectors.
Numbers in the legend of each graph show the total catch at each station.

Mitochondrial DNA variation and phylogenetic relationships of Jasus spp. (Decapoda: Palinuridae)

(Short title: mtDNA phylogeny of Jasus spp.)

D. J. Brasher, J. R. Ovenden & R. W. G. White

Fish Research Group, Department of Zoology, University of Tasmania, G.P.O. Box 252C, Hobart, Tasmania. Australia 7001

Abstract

The seven species of rock lobster in the genus Jasus have a fragmented circumpolar distribution, inhabiting continental or island waters of the Southern Ocean. Gene flow between nominal species is possible as the planktonic larval stages of Jasus are widely dispersed in major oceanic gyres. Restriction endonuclease analysis of mitochondrial DNA (mtDNA) from five species (J. verreauxi, J. novaehollandiae, J. edwardsii, J. lalandii and J. tristani) was used to assess taxa previously defined only by morphological criteria. Intraspecific mtDNA nucleotide sequence diversity was generally high (0.33-0.99%). An absence of episodic population bottlenecks and extinctions, attributable to a teleplanic (far wandering) and prolonged pelagic stage, may be a significant factor contributing to this variation. New Zealand (J. edwardsii) and Australian (J. novaehollandiae) populations appear to be conspecific and should be referred to as J .edwardsii; however, a significant difference in the magnitude of mean sequence diversities between these populations may indicate restrictions to gene flow across the Tasman Sea. The genome of J. verreauxi is highly distinct from the genomes of the other species (nucleotide sequence diversity: 14.92-16.67%), supporting the existence of "verreauxi" and "lalandii" groups within Jasus. High sequence diversities separating J. edwardsii, J. lalandii and J. tristani (4.41-7.36%) indicates long term reproductive isolation. Hypotheses for the evolution of "lalandii" group Jasus, which suggest a relatively recent divergence of J. lalandii and J. tristani, are not supported by phylogenetic reconstruction. Instead, it gives systematic validity to the grouping of J. lalandii with

J. edwardsii as proposed by the existing taxonomy.

Contents

Introduction

The seven species recognized in the genus Jasus have a fragmented circumpolar distribution, inhabiting continental or island waters of the Southern Ocean (George and Main, 1967). Holthuis and Sivertsen (1967) recognized two groups within this genus, probably deserving of subgeneric status. The "lalandii" group comprises six species characterized by squamiform sculpturation on the abdomen. The "verreauxi" group, formed by the remaining species, J. verreauxi, lacks this character. Jasus verreauxi is primarily found in New South Wales and adjacent coasts, Australia, and the north-east coast of the North Island, New Zealand (Kensler, 1967).

For over 150 years there has been much uncertainty about the status of the

various forms belonging to the "lalandii" group. Holthuis and Sivertsen (1967) were the first to study material of all forms and identified a separate species for each of six major geographical areas. They divided the "lalandii" group into two subgroups, each of which contains three species: the "lalandii" subgroup with J. lalandii (South Africa), J. edwardsii (New Zealand) and J. novaehollandiae (S. and S.E. Australia); and the "frontalis" subgroup with J. frontalis (Juan Fernandez), J. paulensis (St. Paul and New Amsterdam Islands) and J. tristani (Tristan da Cunha, Gough Island and Vema Seamount). The two subgroups are differentiated primarily on the basis of carapace spine dimensions and sculpturation of the abdomen.

George and Kensler (1970) supported the division of the "lalandii" group into two subgroups after reviewing the existing morphological classification with additional characters. Within the "lalandii" subgroup, these workers claimed that only a single character, i.e. broad, pale bands on the antennal flagellum of J. novaehollandiae, could reliably separate this species from J. edwardsii. Booth et al. (1990), however, found it impossible to distinguish New Zealand and Australian samples on the basis of morphology and recommended that crayfish populations from both countries be referred to as a single species, which by priority is J. edwardsii. Smith et al. (1980) had previously concluded from an electrophoretic analysis of 33 enzyme gene loci that these species were probably conspecific; the small genetic distance separating New Zealand and Tasmanian samples was due to differences only in allele frequencies at the Ldh locus.

The two existing hypotheses describing the evolution of the "lalandii" group and their colonization of oceans of the southern hemisphere suggest a relatively recent divergence of J. tristani and J. lalandii, thus implying that the "lalandii" and

"frontalis" subgroups are phylogenetically misleading.

In the first hypothesis, by George (1969), J. tristani and J. lalandii are envisaged as using the Mid-Atlantic Ridge to move northwards to their current distributions from high latitude areas in the South Atlantic Ocean. Although not explicitly stated by George, the implication is that J. lalandii and J. tristani shared a common ancestor before or during the northward shift. These population shifts were forced by temperate waters moving to lower latitudes during climatic cooling. Under the same influence, J. edwardsii and J. novaehollandiae moved northwards from a marine province south of New Zealand. Speciation is believed to have occurred when selection for a shortening of the larval life (the planktonic phyllosoma stage) led to a cessation of gene flow between isolated populations.

The alternative hypothesis is presented by Pollock (1990). He suggests that the potential for long range dispersal conferred by a prolonged larval stage enabled circumpolar colonization. He accepts that J. novaehollandiae and J. edwardsii arose from an ancestral species which evolved in waters to the south of New Zealand, but goes on to suggest that larvae from this ancestor also colonized islands and seamounts in the South Atlantic (the ancestral J. tristani) via the Antarctic circumpolar current. Some time later, these Atlantic populations distributed larvae to the south-western coast of southern Africa, establishing the ancestral J. lalandii.

Phyllosoma are believed to develop through most of their stages well offshore and are transported over large distances, encountering a number of water masses before recruiting back to benthic stocks (Phillips and McWilliam, 1986). Phyllosoma of J. novaehollandiae and J. edwardsii appear to share a gyre system in the South

Pacific (Pollock, 1990) and those of J. tristani and J. lalandii share the South Atlantic gyre (Pollock, 1989; 1990). Therefore, to explain speciation and maintenance of reproductive isolation between populations of "lalandii" group Jasus within the South Pacific and South Atlantic ocean basins, Pollock's hypothesis requires that metamorphosis into the settling puerulus stage must occur only at "home" environments. Selection for this recognitive faculty in phyllosoma is thought to have come about very gradually as a result of genotype-specific post-settlement mortality (Pollock, 1990).

Allozyme studies suggest that decapods possess low levels of nuclear genetic variation (Hedgecock et al., 1976; Smith et al., 1980). Since the rate of evolution of mtDNA observed in higher animals is frequently faster than the rate for single copy nuclear DNA (scnDNA) (Brown et al., 1979; Vawter and Brown, 1986; Moritz et al., 1987), mtDNA may be an appropriate molecule for systematic evaluation of low-level taxa within Jasus. High-resolution analysis of the evolutionary process provided by mtDNA has already been useful in systematic studies of bird species (Kessler and Avise, 1984; Ovenden et al., 1987), taxa also shown to be allozymically and morphologically similar.

Successful hybridization between J. novaehollandiae and each of J. edwardsii and J. lalandii (Kittaka, 1987) suggests that there are no isolating mechanisms preventing gene exchange between "lalandii" group Jasus. Given that phyllosoma larvae are widely dispersed, larval mixing between J. tristani and J. lalandii in the South Atlantic and J. novaehollandiae and J. edwardsii in the South Pacific is likely. Larvae may even move between these ocean basins. It is possible, therefore, that the small number of morphological features used to divide these Jasus into four taxa are

attributable to ecological rather than reproductive factors and that gene flow between nominal "lalandii" species is occurring. To test this possibility, we use data from restriction endonuclease analysis of mtDNA from five Jasus species to define species boundaries. Phylogenetic reconstruction also enables us to assess the conflict between the existing taxonomy and the hypotheses describing evolution of the "lalandii" group in oceans of the southern hemisphere.

Materials and methods

Forty-nine Jasus specimens were collected from five localities in the Southern Ocean (Fig. 1). Antennal glands, weighing 0.5-2.0 g, were removed and stored in liquid nitrogen. Mitochondrial DNA was extracted from this tissue using the method of Chapman and Powers (1984). Purified mtDNA was resuspended in 100 ml water per gram of starting tisue and stored at -20°C until required for restriction enzyme analysis. Three microlitres of mtDNA solution were added to a 20 µl reaction mixture containing digestion buffer (as recommended by vendor) and 5-20 units of restriction endonuclease. Due to an unidentified property of the Jasus mtDNA extract, enzymatic cleavage was absent or attenuated unless bovine serum albumin (BSA) or spermidine, or both, were present at final concentrations of 100-200 $mg.ml^{-1}$ and 1 mM respectively. Complete digestion of the mtDNA with the six endonucleases used (Table I) required 1.5-3.0 h incubation. The mtDNA restriction fragments were end-labelled with either α-^{32}P-dCTP, α-^{35}S-dCTP or α-^{35}S-dATP using the polymerase and exonuclease activity of the Klenow fragment DNA

polymerase I (Biotechnology Research Enterprises, South Australia) as described by Ovenden et al. (1989). The labelled fragments were separated by electrophoresis through 1.4-1.8% agarose gels. Gels were heat dried prior to fragment visualization by autoradiography. The molecular weight standard used to estimate fragment sizes was lambda-phage DNA (New England Biolabs, California) digested with Hind III.

Figure 1 here

Restriction site rather than fragment homology data were used to assess intra- and interspecific variation, since the latter method is unreliable if there is length variation, or if the overall sequence diversity exceeds 10-15% (Upholt, 1977; Kessler and Avise, 1985). The relative gain or loss of restriction sites between samples was determined by the additive loss or gain of appropriately sized fragments. For each individual, every unique restriction morph was denoted a character from a set used to identify the following species: A to G for J. novaehollandiae and J. edwardsii, Z to V for J. lalandii, 1 to 4 for J. tristani, and 9 to 7 for J. verreauxi. The first character of each set represents the most frequent morph. However, no attempt was made to assign consecutive characters to the most similar morphs. Jasus novaehollandiae and J. edwardsii morph designations were assigned to J. tristani and J. lalandii in the case of shared restriction profiles. Mitochondrial genomes were described by a six character summary representing the haplotype of each individual. Site presence or absence was binary coded for computer analysis for each genome analyzed.

Restriction site data were converted to estimates of nucloetide sequence diversity

(∂) between pairs of genomes using the maximum likelihood method of Nei and Tajima (1983). Taxa were clustered according to sequence diversity using the unweighted pair group method (UPGMA - Sneath and Sokal, 1973). Standard errors were assigned to branching points using the method of Nei et al. (1985). Mean intra- and interspecific diversities with group variances were calculated using the method described by Nei and Jin (1989). Genetic polymorphism within species was expressed in terms of the number of unique haplotypes identified by site differences using the nucleon diversity ($\hat{h}$) equation of Nei and Tajima (1981): $\hat{h} = n(1-\Sigma x_i^2)/(n-1)$, where x_i is the frequency of the ith mtDNA haplotype in a sample of size n. The FITCH method in J. Felsenstein's PHYLIP program package (version 3.2, Department of Genetics, University of Washington, Seattle, Washington) was used to construct a mtDNA phylogeny without the assumption of constant rates of evolution between lineages. Data were analyzed cladistically with MIX (PHYLIP) by considering the presence and absence of restriction sites as binary characters. As suggested by Felsenstein, the MIX program was run ten times with global optimization and a different input order of operational taxonomic units each time. FITCH could only be run three times due to time constraints imposed by the large data set. The PHYLIP majority-rule consensus tree and compatability programs, CONSENSE and CLIQUE respectively, were used to test the strength of the phylogenetic hypothesis obtained with MIX. Characters regulating the topologies of alternative cladistic trees were identified with MacCLADE (version 2.1, written by Wayne Maddison and David Maddison, Harvard University).

Results

Digestion of all Jasus mtDNA genomes with six restriction endonucleases produced cleavage at a total of 107 sites (Appendix I), with an average of 39 sites per genome (Table I). Based on restriction profile fragment totals, we estimate the mtDNA of Jasus species to be approximately 16.0 kilobases (kb) in size. The only example of mtDNA size variation among individuals was observed in a J. lalandii specimen (haplotype XZZBWA, genome size ~17.0 kb) which possessed a 1.0 kb insert, detected by all six enzymes as a restriction fragment 1.0 kb larger than its homolog. This mutation event was not included in any analyses, which were based solely on the presence and absence of restriction sites.

Table I here

Restriction endonuclease analysis demonstrated a high level of mtDNA polymorphism within each species. Thirty-six haplotypes were observed among the 49 individuals (Table I). Jasus lalandii had the largest genotypic diversity possible with each individual having a unique mitochondrial genome (Table II). Generally, the genotypic diversities found for Jasus fall at the higher end of a continuum of mtDNA variabilities observed in vertebrate and other invertebrate species (see Table II in Avise et al., 1989). Mean intraspecific sequence diversities approaching 1% for single populations of J. novaehollandiae, J. lalandii and J. tristani (Table II) are relatively high when compared to values from species that exhibit no geographic localization, i.e. are effectively single populations (see Fig. 3 in Avise, 1989).

However, the smaller intraspecific diversities for J. edwardsii (0.44%) and J. verreauxi (0.33%) are within the range of values previously found by Avise (1989). The maximum intraspecific sequence diversity was 2.19% (or nine restriction sites) between haplotypes XZWBWZ and ZZVZVX of J. lalandii.

Table II here

No net diversity was found between J. novaehollandiae and J. edwardsii (Table II). Three haplotypes (AAAAAA, AAAADA and ABCAAA) were shared by these nominal species. The two haplotypes unique to J. edwardsii (AAAAFA and AAGAFA) differed from the most similar J. novaehollandiae haplotype (AAAAAA) by only one and two restriction site(s) respectively (Y1 and B6, Appendix I). The maximum sequence diversity between these species was 1.95% (AAGAFA versus CBBFAE), which is comparable to that found within each Jasus species: J. novaehollandiae, 1.95%; J. edwardsii, 0.98%; J. tristani, 1.55%; J. lalandii, 2.19%; and J. verreauxi, 0.74%. As thus expected, J. novaehollandiae and J. edwardsii haplotypes clustered at a low level of genetic distance in subsequent phenetic and cladistic analyses (Figs 2 & 3), constituting a major monophyletic group. These species will be regarded as conspecific populations of J. edwardsii in all further discussion.

There is a significant difference ($t=2.09$ $P<0.001$) in the mean intraspecific sequence diversity values of the Tasmanian and New Zealand populations of J. edwardsii (Table II), which may be a function of population size.

The analyses support the recommended specific status of J. edwardsii, J.

lalandii and J. tristani. Twenty-four of the 107 sites were shared between all three "lalandii" species; J. tristani and J. lalandii shared an extra three sites, J. tristani and J. edwardsii shared an extra two sites, and J. lalandii and J. edwardsii shared an extra six sites (Appendix I). Despite this high degree of homology, the large number of sites unique to each of these species (J. edwardsii, 14; J. lalandii, 18; J. tristani, 16) resulted in high interspecific sequence diversities (Table II), indicating long-term reproductive isolation.

The genome of J. verreauxi was found to be highly distinct from the genomes of the other species. In no cases were single-enzyme profiles shared between J. verreauxi and the other species, nor could the differences between these profiles be attributed to a single site gain or loss. Jasus verreauxi shared 14 sites with the other three species, one extra site with J. lalandii and J. edwardsii and another extra site with J. lalandii and J. edwardsii (Appendix I). This relatively small amount of site homology combined with the 24 sites unique to J. verreauxi resulted in the largest interspecific sequence diversities found (Table II). These values, and the clear taxonomic status of J. verreauxi (Holthius and Siversten, 1967), justifies the use of this species as an outgroup in the following phylogenetic analyses.

Ninety-seven site characters (Appendix I), which were either autapomorphic (20) or synapomorphic (77) with respect to haplotypes, were used in a cladistic analysis. The ten characters ommitted were present in all haplotypes, i.e. plesiomorphic (F2, F3, F4, A5, B3, Y3, H1, H7, H8, H9). Using the Wagner method, at least 100 parsimonious trees with treelengths of 122 steps were found. A consensus tree (Fig. 2) showed that each of these trees had the same topology above

the species level, with J. edwardsii and J. lalandii more similar to each other than to J. tristani (topology A). Because of the variable nature of the Jasus mtDNA, and the large nucleotide diversities between each of the species (the latter saturates the genome with nucleotide substitutions [Brown et al., 1979]), this tree exhibits considerable convergence, viz. 43 homoplasious changes involving 19 characters.

Figure 2 here

The shortest trees for the two alternative species topologies were also investigated. When J. lalandii and J. tristani were grouped to the exclusion of J. edwardsii (topology B), the shortest tree had a length of 123 steps. Grouping J. edwardsii and J. tristani to the exclusion of J. lalandii (topology C) gave a minimum length tree of 126 steps. By identifying characters that were present in or absent from at least two, but not all species, i.e. species' synapomorphies, the differences between the three topologies were found. Alternative treelengths were primarily determined by five characters; three of these (A1, Y4 and Y5) united J. edwardsii and J. lalandii, two (B9 and Y12) united J. tristani and J. lalandii, and none united J. tristani and J. edwardsii. Although several of these characters regulated the difference in treelength between topologies A and C and B and C, when any one of the three characters supporting topology A was omitted from the data, subsequent cladistic analysis showed the most parsimonious tree to have topology B.

The compatability program CLIQUE excluded B9 and Y12 from the 97 character data set to find four cliques, each with topology A. Both UPGMA (Fig. 3) and FITCH phenograms possessed topology A (FITCH sum of squares = 5.12).

The inclusion of standard errors on the UPGMA branching points collapses the nodes between the three "lalandii" species, thus forming an unresolved trichotomy.

Figure 3 here

No evidence for geographic partitioning of J. edwardsii, was provided by the phylogenetic analyses. Standard errors on the UPGMA intraspecific branching points (not shown) collapses the nodes between all haplotypes. Although two distinct J. edwardsii clades supported at the 100% level are shown by the consensus tree (Fig. 2), haplotypes found in Tasmania and New Zealand occur in both these clades.

Discussion

The existence of " verreauxi" and "lalandii" groups within Jasus is supported by the mtDNA sequence diversity data. The fossil of a warm-water Jasus, J. flemingi, has close affinities to J. verreauxi (George and Main, 1967) and is thought to have inhabited the New Zealand coastline during the early Miocene (George, 1969). Jasus verreauxi is also a warm-water species inhabiting a sub-tropic marine zone whereas species within "lalandii" occupy temperate waters (George, 1969). Pollock (1990) explains, in general terms, the origins of each group, suggesting J. flemingi to be the ancestral form for both lineages: A cool-water Jasus (the ancestor of the "lalandii" group) evolved near the Pacific coast of the Antarctic continent with climatic cooling during the Palaeocene and Eocene epochs, 38-65 million years ago. Warm-water

forms remained in a more northerly position near Australia and New Zealand, eventually giving rise to the highly distinct J. verreauxi.

Since the expected mtDNA sequence diversity within a random-mating population or species is directly related to effective population size (under a neutral model with given mutation rate) (Engels, 1981; Nei, 1987), the difference in diversity between New Zealand and Tasmanian J. edwardsii populations may indicate restrictions to gene flow across the Tasman Sea. If larvae are exchanged freely between these locations, population diversities should be similar irrespective of the numbers of breeding females at each location. Even a small number of migrants exchanged between populations per generation is sufficient to prevent genetic differentiation (Hartl, 1981).

Additional evidence to support a hypothesis of isolated J. edwardsii populations has been provided by allozyme and morphological studies. On the basis of different allele frequencies at the Ldh locus between individuals from New Zealand and Tasmania, Smith et al. (1980) concluded that gene flow across the Tasman Sea was unlikely. George and Kessler (1970) were unable to distinguish between animals from New Zealand and Tasmania using sculpture index (the percentage of the tergum covered by sculpture), although they could distinguish between animals from New Zealand and the mainland coast of southern Australia. Given the long pelagic life of phyllosoma (Phillips, 1977; Phillips and McWilliam, 1986), the widespread occurrence of phyllosoma in the central and southern Tasman Sea (Booth et al., 1990) and the absence of any hydrological barriers to larval exchange across the Tasman Sea (Heath, 1985), genetic differentiation between New Zealand and Australian populations is difficult to explain. If the difference found is a result of

incipient speciation between these populations, this may be occurring via selection for individuals which metamorphose and settle at home environments. Phillips and McWilliam (1986) suggest that stimuli for this metamorphosis are changes in temperature or salinity, or both, either by mixing of the water containing the larvae with another water body or by movement of the larvae into another water body.

Evolutionary explanations for the high mtDNA restriction site polymorphism observed in Jasus are unclear. Avise et al. (1989) suggests that such variation may relate to either one or a combination of the following: enormous effective population sizes; extensive historical population subdivision, which has the effect of buffering mtDNA lineages against extinction; and enhanced mutation rates. An absence of episodic population bottlenecks and extinctions, attributable to a teleplanic (far wandering) and prolonged pelagic stage, may be a significant factor contributing to the high variation shown here.

The validity of conclusions made from the phylogenetic analyses relies largely on an assumption of constant rates of mtDNA evolution across each lineage. Ohta (1974, 1976) predicted that, under a model of nearly neutral or slightly deleterious mutations, reductions in population size that may occur through bottleneck or founder events are crucial in giving rise to heterogeneous rates of molecular evolution. This prediction is strongly supported by a study of Hawaiian Drosophila (DeSalle and Templeton, 1988) in which the mtDNA rate of change in a group of flies that have undergone repeated bottlenecks due to inter-island colonization was found to be three times faster than in a group of flies that have had contact during speciation on the same island complex. If the high polymorphism found in Jasus is

indicative of historical population stability, an assumption of constant mtDNA evolution across each lineage is probably valid and, therefore, the topology recommended by the phylogenetic reconstructions should be a good representation of interspecific relationships.

Jasus tristani at Vema Seamount and J. lalandii on the south-west coast of Africa are separated by less geographic distance than New Zealand and Australian J. edwardsii. Larvae from both J. tristani and J. lalandii are likely to share the same or similar pathways in the South Atlantic anticyclonic gyre system (Pollock, 1989), yet each of these species forms a monophyletic unit and the degree of mtDNA sequence divergence indicates long-term reproductive isolation. Assuming that J. lalandii and J. tristani arose from the same stock, as suggested by Pollock (1990) and implied by George (1969), it would appear that the specific larval metamorphois response has been operating in synchrony with other isolating mechanisms to accelerate speciation. Pollock (1990) proposes that genetic differentiation of an ancestral Jasus living in the South Atlantic into J. tristani and J. lalandii has been accelerated by the temporary formation of palaeocirculation cells which acted as barriers to gene flow (larval exchange) between populations. Such cells would have arisen if a period of sea level regression occurred in concert with shoaling of the Walvis Ridge, resulting in a South Atlantic anticyclonic gyre to the west of the ridge and a partially separated cell on its eastern flank within the confines of the Cape Basin (see Fig. 8 in Pollock, 1990). If this hypothesis is correct, it must be assumed that Vema Seamount, which lies within the Cape Basin, was colonized by J. tristani originating west of the Walvis Ridge after speciation of J. lalandii was completed. This may have occurred during a period of sea level advance when the effectiveness of the ridge to act as a

barrier to larval movement was reduced.

Our phylogenetic reconstruction does not support existing hypotheses for the spread of "lalandii" group Jasus around the southern hemisphere. If J. lalandii was recently derived from a J. tristani ancestor (Pollock, 1990) or both species moved to their current positions using the Mid-Atlantic Ridge (George, 1969), the mtDNA of J. edwardsii should be highly divergent from the mtDNAs of J. tristani and J. lalandii. Although the relationships of these three species were essentially unresolved, the grouping of J. edwardsii and J. lalandii by the cladistic analysis and the small genetic distance separating these species despite vast geographical separation (the same amount of genetic distance separates J. tristani and J. lalandii whose larvae are sympatric) gives systematic validity to the "frontalis" and "lalandii" subgroupings of Holthuis and Sivertsen (1967). Future hypotheses delineating "lalandii" group evolution need to accommodate the interspecific relationships identified here.

Apart from morphological similarities, members within each of the subgroups have the same distributional patterns: "lalandii" members are continental and those belonging to "frontalis" occupy oceanic islands. Newman (1979) found the same correlation between two genera of pan-austral balanomorph barnacles. Species in one genus (Notomegabalanus) failed to colonize oceanic islands and, as inhabitants of high latitudes, suffered severe extinction during the Pleistocene. Members of the other genus (Austromegabalanus) are characterized by their ability to colonize oceanic islands by long-range epiplanktonic dispersal via the West Wind Drift. Similarly, the recognition of continental ("lalandii") and insular ("frontalis") Jasus as natural groupings may assist in understanding biogeographical origins and factors

influencing historical population shifts for this genus. Mitochondrial DNA analysis of the two remaining insular species, J. frontalis and J. paulensis, is required before the systematic importance of these subgroups can be fully evaluated.

It is possible that introgressive hybridization has confounded phylogenetic analyses. Mitochondrial DNA is able to penetrate the boundaries between species because, unlike nuclear genes, it is not closely linked to genes responsible for maintaining reproductive isolation (Barton and Jones, 1983). Consequently, the distribution of mtDNA clones may lack concordance with taxonomic boundaries based on morphological and reproductive criteria (Barton and Jones, 1983; Tegelström, 1987). Wide dispersal of phyllosoma may have led to hybridization between disjunct populations in the past, yielding a mtDNA phylogeny which obscures the evolutionary history of Jasus. Even if this had occurred, it is difficult to reconcile the existing evolutionary scenarios with the equivalent genetic distances separating the three "lalandii" species. Historical and contemporary sympatry purported for J. tristani and J. lalandii would have facilitated periods of secondary hybridization between these species, rather than between J. edwardsii and South Atlantic Jasus, thus yielding a phylogeny in conflict with the one obtained.

The initial rate of 2% per million years per lineage for primate mtDNA divergence (Brown et al., 1979) appears to hold for some invertebrate species (Vawter and Brown, 1986). Applying this provisional rate to Jasus, the "verreauxi" and "lalandii" groups separated about 8 million years ago and species within "lalandii" about 2-4 million years before the present. These divergence times greatly understate those inferred from fossil and biogeographical evidence. Lack of

concordance may be explained by either episode(s) of secondary hybridization between populations, or a slower rate of mtDNA evolution in Jasus. There is increasing evidence that mtDNA evolutionary rates can vary, even between closely related lineages (Moritz et al., 1987). Therefore, applicability of the primate mtDNA evolutionary rate cannot be assumed, and a slower rate in Jasus could be a major factor in explaining this discrepancy. Since biogeographical, fossil and molecular information describing the evolutionary history of Jasus is scarce, calibrations to estimate the absolute rate of mtDNA evolution of species in this genus, and subsequently their divergence times, cannot be made at present.

We thank the Fishing Industry Research and Development Council (grant 88/41) for financially supporting this study. We are also grateful to Bob Kennedy, Mick Murphy and colleagues, John Booth, Dave Pollock and Roy Melville-Smith for the provision of samples; Jenny Jarrett, Adam Smolenski and Wayne Kelly for their assistance in the laboratory; and Russell Bywater for computer programming. Bob Kennedy, Bruce Phillips and Dave Pollock made constructive reviews of earlier drafts of the manuscript.

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TABLE I

Frequencies and number of restriction sites for the 36 mtDNA haplotypes in Jasus. Morph designations, from left to right, were assigned from the following 5.33 and 6.0 class restriction endonucleases: Ava I (recognition sequence: CPyCGPuG), Hind III (AAGCTT), Bst YI (PuGATCPy), Afl II (CTTAAG), Ban I (GGPyPuCC) and Eco RV (GATATC).

Haplotype	Number of restriction sites scored	Individuals with haplotype	Haplotype	Number of restriction sites scored	Individuals with haplotype
J. novaehollandiae/ J. edwardsii			J. lalandii		
AAAAAA	38	5	ZZZBYA	41	1
ABCAAA	36	4	WZZBZA	41	1
AAAADA	39	3	VZXBXA	40	1
AAAAFA	39	1	XZWBWZ	37	1
AAGAFA	38	1	ZZVZVX	40	1
AAHDAB	37	1	YZZBZA	41	1
AAEAAA	39	1	ZZZBUA	40	1
ABDCAA	35	1	XZZBWA	40	1
ABBBAA	37	1	ZZZBXA	38	1
AAABAA	37	1	ZZYBWA	38	1
CBBFAE	38	1	ZZZBTY	40	1
AAAAAD	39	1			
			J. tristani		
J. verreauxi					
			113211	43	2
999999	37	4	124212	42	1
979999	38	1	111A11	41	1
999899	36	1	114211	44	1
979799	37	1	112A11	42	1
799999	38	1	111A33	41	1
979979	39	1	111241	41	1

TABLE II

Jasus intraspecific genotypic diversities calculated from haplotype frequencies (Nei aand Tajima, 1980), and mean intra- and interspecific nucleotide divergence estimates ± standard deviation (Nei and Jin, 1989).

Species	Genotypic Diversity	Mean Intraspecific ∂ (%)	Mean Interspecific ∂ (%) - above diagonal Standard deviation - below diagonal				
			(1)	(2)	(3)	(4)	(5)
(1) J. novaehollandiae	0.882	0.894 ± 0.235	-	0.013	4.439	7.362	14.918
(2) J. edwardsii	0.733	0.438 ± 0.227	0.015	-	4.406	7.279	15.071
(3) J. lalandii	1.000	0.990 ± 0.243	1.163	1.197	-	6.758	16.462
(4) J. tristani	0.821	0.715 ± 0.245	1.648	1.679	1.639	-	16.672
(5) J. verreauxi	0.833	0.333 ± 0.172	3.130	3.161	3.119	3.145	-

Restriction sites generated using the six enzymes listed in TABLE I. A=Ava I. H=Hind III. Y=Bst YI. F=Afl II. B=Ban I. E=Eco RI. Numbers represent frequency of restriction sites in each sample.

Enzyme site	J.tristani	J. verreauxi	J. lalandii	J. edwardsii	J. novachollandiae
A1	0	0	1	1	0.9
A2	0	0	0	1	1
A3	0	0	0	1	1
A4	1	0	1	1	1
A5	1	1	1	1	1
A6	0	0	0.9	0	0
A7	0	0	0.2	0	0
A8	0	0	0.1	0	0
A9	0	0	0.1	0	0
A10	1	0	0	0	0
A11	0	1	0	0	0
A12	0	0.1	0	0	0
H1	1	1	1	1	1
H2	0	0	0	1	1
H3	1	0	1	1	1
H4	0	0	1	0.7	0.6
H5	1	1	0	1	1
H6	1	0	1	1	1
H7	1	1	1	1	1
H8	1	1	1	1	1
H9	1	1	1	1	1
H10	0	0	1	0	0
H11	0	0	1	0	0
H12	0	0	1	0	0
H13	1	0	0	0	0
H14	0.9	0	0	0	0
H15	0	1	0	0	0
H16	0	1	0	0	0
H17	0	1	0	0	0
H18	0	0.3	0	0	0
Y1	0	0	0	0.9	1
Y2	1	1	1	0.7	0.8
Y3	1	1	1	1	1
Y4	0	0	1	1	1
Y5	0	0	1	1	1
Y6	0	0	0	1	1
Y7	0	0	0	1	1
Y8	0	0	0	0	0.3
Y9	0	0	0	0	0.1
Y10	0	0	0	0	0.1
Y11	0	0	1	0	0
Y12	1	0	0.9	0	0
Y13	1	0	0.9	0	0
Y14	0	0	0.1	0	0
Y15	0	0	0.1	0	0
Y16	1	0	0	0	0
Y17	1	0	0	0	0
Y18	0.4	0	0	0	0
Y19	0.5	0	0	0	0

Enzyme site	J.tristani	J. verreauxi	J. lalandii	J. edwardsii	J. novachollandiae
Y20	0	1	0	0	0
Y21	0	1	0	0	0
Y22	0	1	0	0	0
Y23	0	1	0	0	0
Y24	0	1	0	0	0
Y25	0	1	0	0	0
Y26	0	1	0	0	0
Y27	0	1	0	0	0
Y28	0	1	0	0	0
Y29	0	1	0	0	0
F1	1	0	1	1	0.8
F2	1	1	1	1	1
F3	1	1	1	1	1
F4	1	1	1	1	1
F5	1	0.9	1	1	0.9
F6	1	0	0	1	0.8
F7	0	0	0	0	0.2
F8	0	0	0.1	0	0
F9	0.6	0	0	0	0
F10	0	0.9	0	0	0
F11	0	1	0	0	0
F12	0	1	0	0	0
B1	0	1	0.7	1	1
B2	0.9	0	1	1	1
B3	1	1	1	1	1
B4	1	0	1	1	1
B5	1	0	1	1	1
B6	0	0	0	0.2	0
B7	0	0	0	0.2	0.1
B8	0	0	0.3	0	0
B9	1	0	1	0	0
B10	0	0	0.9	0	0
B11	0	0	0.1	0	0
B12	0	0	0.1	0	0
B13	0	0	0.1	0	0
B14	0	0	0.1	0	0
B15	1	0	0	0	0
B16	1	0	0	0	0
B17	0.8	0	0	0	0
B18	0.9	0	0	0	0
B19	1	0	0	0	0
B20	0	1	0	0	0
B21	0	1	0	0	0
B22	0	1	0	0	0
B23	0	1	0	0	0
B24	0	0.1	0	0	0
E1	1	1	0.9	1	1
E2	0	0	0.9	1	1
E3	1	0	1	1	1

Enzyme site	J.tristani	J. verreauxi	J. lalandii	J. edwardsii	J. novaehollandiae
E4	1	0	1	1	1
E5	1	1	1	1	0.9
E6	1	0	0.9	1	1
E7	0	0	0	0	0.1
E8	0	0	0	0	0.1
E9	0	0	0.1	0	0
E10	1	0	0	0	0
E11	1	0	0	0	0
E12	0.1	0	0	0	0

Figure Legends

Figure 1

Origin of Jasus samples investigated. Exact locations of collection sites and sample sizes are in parentheses: A - J. novaehollandiae, Temma Harbour (41°14'S 144°35'E, n=11). B - J. edwardsii, Gisborne (38°37'S 178°00'E, n=10). C - J. verreauxi, Matakaoa Point (37°33'S 178°26'E, n=9). D - J. lalandii, Hout Bay (34°07'S 18°19'E, n=11). E - J. tristani, Vema Seamont (31°38'S 8°19'E, n=8). Contour for Walvis Ridge approximately 2000m below sea level.

Figure 2

Rooted consensus tree produced from cladistic analysis of mtDNA restriction site data. Character state changes for species synapomorphies (see text) are placed along the branches; however, branch lengths are not proportional to the number of changes between taxonomic units. Shaded and unshaded bars represent homoplasious and nonhomoplasious changes respectively. Values at each node represent the the number of times the group defined by that node occurred in 100 parsimonious 122 step trees. Haplotypes shared by J. novaehollandiae (n) and J. edwardsii (e) are denoted n, e.

Figure 3

Tree topology inferred from the mtDNA data produced by clustering of sequence divergence estimates with UPGMA. Solid bars representing standard errors at the major nodes were calculated using the algorithm of Nei et al. (1985). Denotations as described in Figure 2.

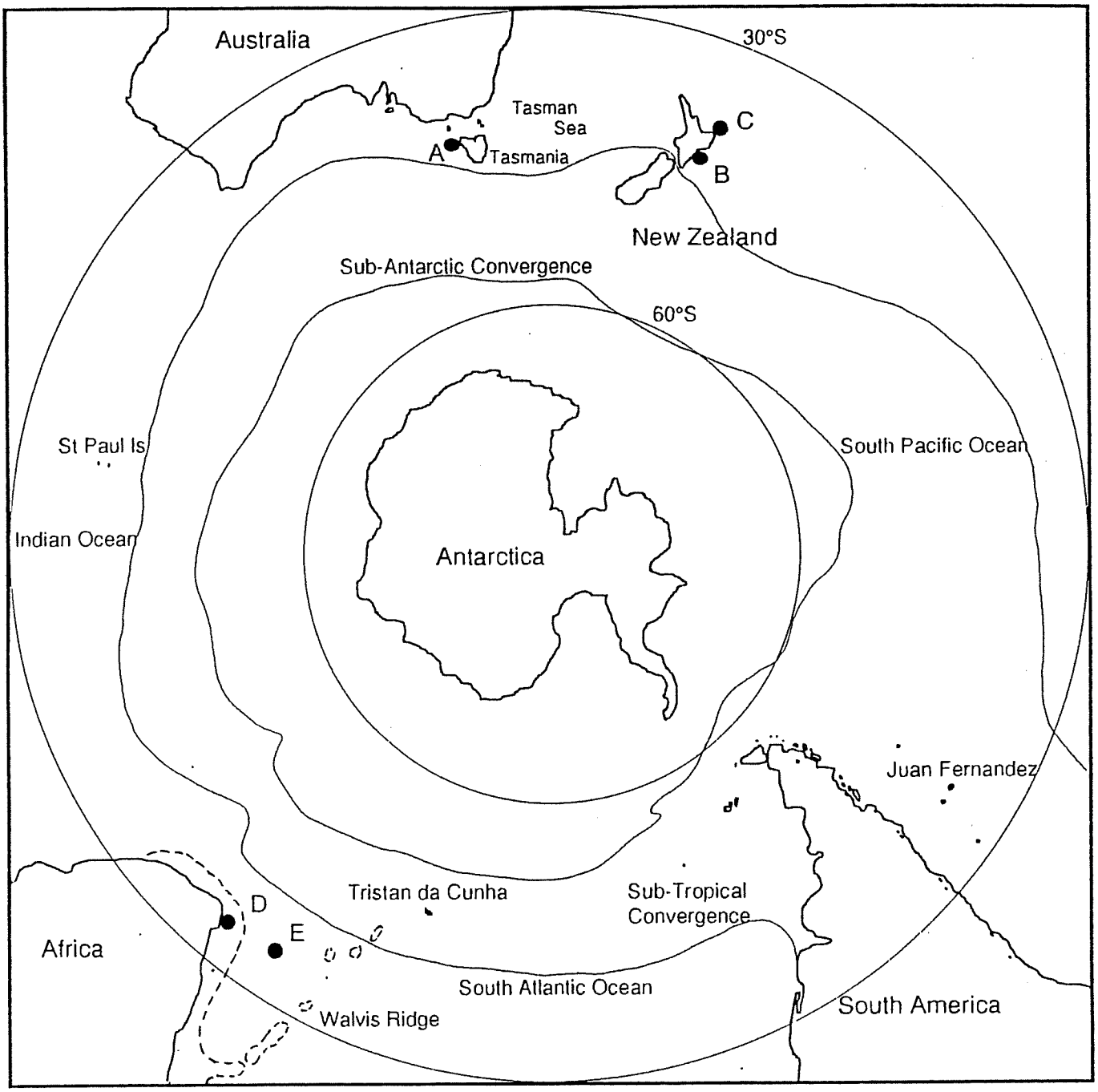

Australia
30°S
Tasman Sea
A
Tasmania
C
B
New Zealand
Sub-Antarctic Convergence
60°S
St Paul Is
South Pacific Ocean
Indian Ocean
Antarctica
Juan Fernandez
Tristan da Cunha
Sub-Tropical Convergence
D
E
Africa
South Atlantic Ocean
Walvis Ridge
South America

Figure 7

Figure 2

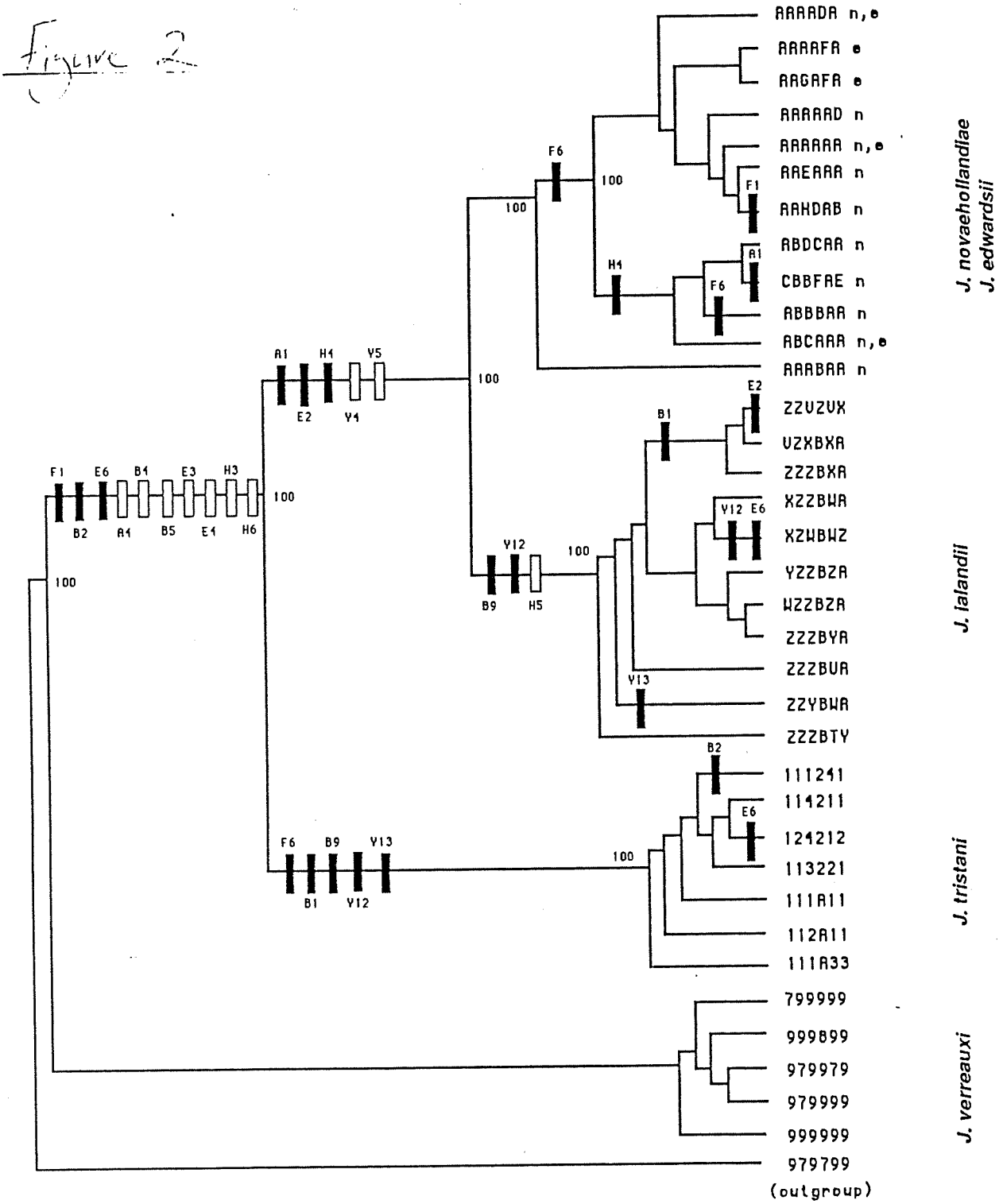
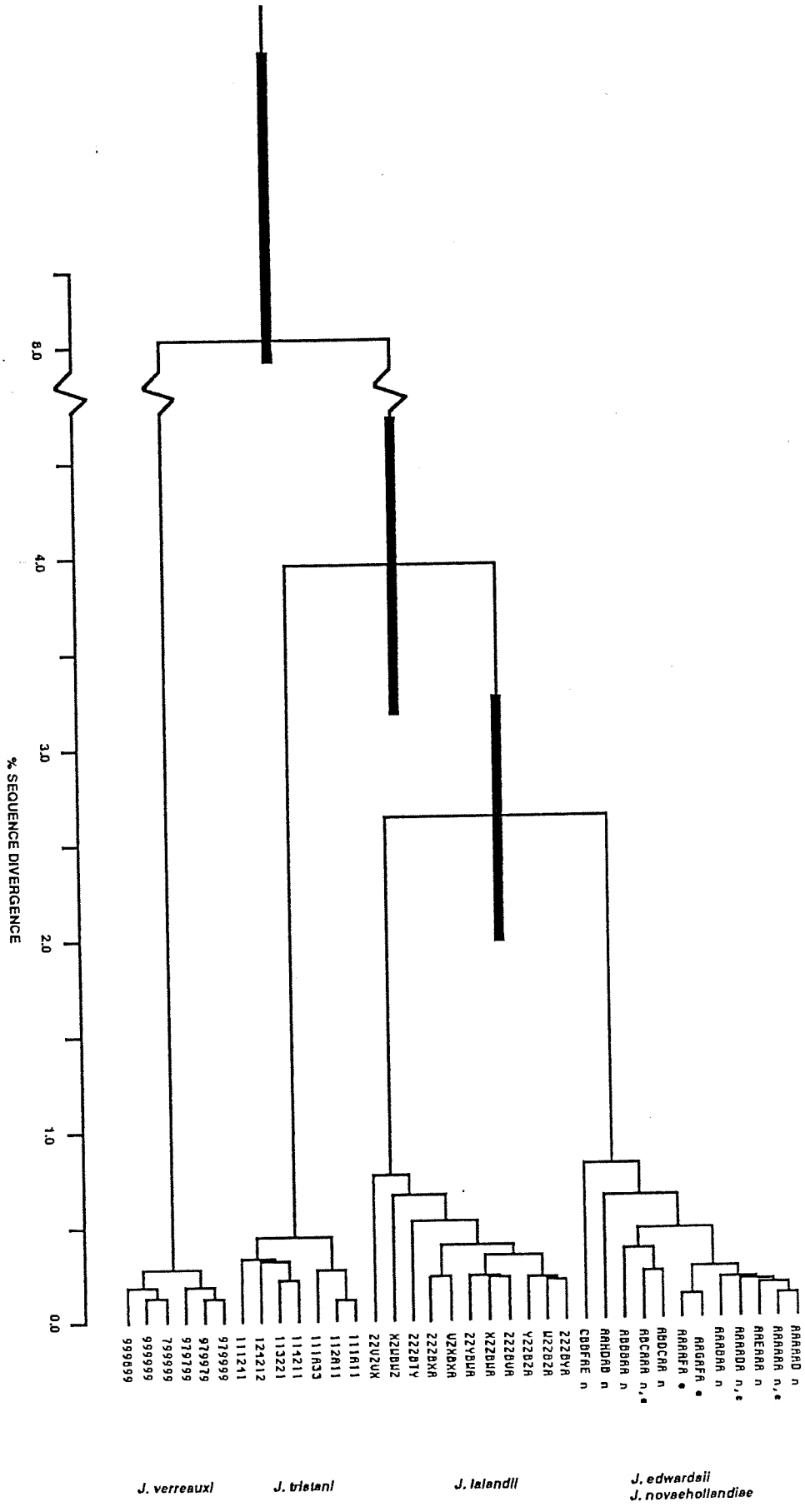

AAAADA n,e
AAAAFA e
AAGAFA e
AAAAAD n
AAAAAA n,e
AAEAAA n
AAHDAB n
ABDCAA n
CBBFAE n
ABBBAA n
ABCAAA n,e
AAABAA n
ZZUZUX
UZXBXA
ZZZBXA
XZZBWA
XZWBWZ
YZZBZA
WZZBZA
ZZZBYA
ZZZBUA
ZZYBWA
ZZZBTY
111241
114211
124212
113221
111A11
112A11
111A33
799999
999899
979979
979999
999999
979799
(outgroup)
J. novaehollandiae
J. edwardsii
J. lalandii
J. tristani
J. verreauxi
100
F6
H1
A1
F6
F1
A1
E2
Y5
Y1
F1
E6
B1
E3
H3
B2
A1
B5
E1
H6
Y12
B9
H5
E2
B1
Y12
E6
Y13
B2
E6
F6
B9
Y13
B1
Y12

Figure 3


8.0
4.0
3.0
2.0
1.0
0.0
% SEQUENCE DIVERGENCE
J. verreauxi
J. tristani
J. lalandii
J. edwardsii
J. novaehollandiae

FISHING INDUSTRY RESEARCH TRUST ACCOUNT
APPLICATION FOR GRANT 1988/1989

1. Title of Proposal

Pilot Study of Larval Recruitment and Genetic Variation of Southern Rock Lobster Populations.

2. Name of Applicants

- Department of Sea Fisheries, Tasmania (D.S.F.) - Co-ordinating the project.
- University of Tasmania

3. Division

- Fisheries Section (D.S.F.)
- Zoology Department (University of Tasmania)

4. Proposal

This proposal seeks funding for a one year pilot study to develop techniques and obtain baseline data which will allow a more extensive project to proceed. In particular, this proposal will:

- Compare the efficiency of existing *Jasus spp.* puerulus collectors and determine the most suitable collector.
- Provide initial estimates of spatial variablity in puerulus settlement of *Jasus novaehollandiae* to determine whether puerulus can be obtained in sufficient quantities for numerical comparisons of settlement levels.
- Develop mitochondrial DNA (mtDNA) analysis techniques to genetically identify puerulus.
- Obtain preliminary information on the genetic variability of southern rock lobster populations to determine whether mtDNA analysis will provide a suitable method of stock discrimination.

Very little information concerning larval recruitment is available for the southern rock lobster fisheries. The phyllosoma of *J. novaehollandiae* have a long oceanic phase and it is possible for one population within state boundaries to be providing significant recruitment to another state. The southern rock lobster fisheries are managed independently but it is not known whether the stocks are discrete and thus whether one fishery should be managed without consideration of the others.

Due to the long oceanic phase and associated high mortality, it is not possible to estimate recruitment levels or consider recruitment patterns and mechanisms without some form of larval sampling programme. This information is required for effective management.

The pilot study will be concentrating its sampling effort in Tasmania although some sampling will be conducted in Western Australia and the South Australian Department of Fisheries intends to resume its puerulus sampling programme. *J. novaehollandiae* is distributed throughout southern Australia so sampling should be conducted simultaneously in all states. If the pilot study is successful, funding for a three year overall project will be sought (see end of application) and this project will involve sampling and co-ordination between all southern states (Tas., Vic., S.A. & W.A). In brief, the overall project will seek to determine and compare the spatial and temporal variations in puerulus settlement throughout southern Australia. It will also aim to determine whether the "stocks" are genetically discrete and whether the source of recruitment is constant over time.

5. Persons Responsible for Programme

- Mr. K.R. Evans B.Sc.(Hons.), Acting Assistant Director (Fisheries), Department of Sea Fisheries, G.P.O. Box 619F, Hobart, 7001.
- Dr. R.W.G. White, Zoology Department, University of Tasmania, G.P.O. Box 252C Hobart, 7001.

6. Qualifications of Staff to be Employed on Programme

- Mr. R.B. Kennedy B.Sc.(Hons.), Research Officer (Rock Lobster), Department of Sea Fisheries.
- Dr. J.R. Ovenden, Research Fellow, Zoology Department, University of Tasmania.
- Dr. B.F. Phillips, Senior Research Scientist , C.S.I.R.O.
- Two Technical Officers - to be appointed.
- Graduate assistant - to be appointed.

7. Objectives of pilot Study

(1) Compare the efficiency of existing *Jasus spp.* puerulus collectors and determine the most suitable collector.

(2) Provide initial estimates for spatial variability and timing of puerulus settlement and determine whether puerulus can be obtained in quantities sufficient for numerical analysis.

(3) Develop mtDNA analysis techniques to genetically identify puerulus larvae.

(4) Obtain preliminary information on the genetic variability of southern rock lobster populations.

Objectives of Overall Project

The following are subject to successful completion of the first four objectives; funding is not requested for these at this stage, but will be requested in 1989.

(1) Determine the spatial and temporal variations in the levels of puerulus settlement for the southern rock lobster fisheries.

(2) Determine whether differential stock abundance between areas can be related to differential settlement levels.

(3) Determine whether southern rock lobster fisheries are genetically discrete by examining differentiation of mtDNA on a spatial scale.

(4) Determine whether the source of recruitment is constant over time (between settlement periods) by examining differentiation of mtDNA of puerulus on a temporal scale.

(5) Further examine stock discreteness with morphological comparisons of puerulus and analysis of puerulus settlement patterns.

8. Justification, Including Practical Application

This application only seeks funding for a 12 month pilot study. However, if sampling and development of genetic techniques are successful, it is proposed that the study be continued in order to achieve the objectives of the overall project. Since the pilot study is the initial step towards achieving the overall objectives, the first section of the justification relates to the overall project. The second section of the justification explains our reasons for examining mitochondrial DNA rather than using allozyme electrophoresis in the genetic section of this project.

a) Justification of the Overall Project

The combined value of the southern rock lobster (*Jasus novaehollandiae*) fisheries is over $50 million, but almost nothing is known about larval recruitment in these fisheries. The only work conducted so far has been puerulus sampling in South

Australia during the mid 1970's (Lewis 1977). No work of this nature has been conducted for *J. novaehollandiae* in Tasmania, Victoria, or Western Australia. Monitoring of puerulus settlement for *Panulirus cygnus* in Western Australia has been ongoing since 1967. The monitoring programme has yielded valuable information concerning larval recruitment and has enabled successful prediction of future catches. This has been extremely valuable for the management of the fishery. However, differences between the ocean circulation patterns and differences between the species (particularly concerning settlement behaviour) prevent the information gained in Western Australian from being used for the southern rock lobster fisheries.

Southern rock lobster phyllosoma spend 8 to 15 months in the plankton and can disperse widely during this period. For example, stage I phyllosoma have been found in the Tasman Sea over 800 miles east of Tasmania (Winstanley, 1970). An open-loop system, whereby Australia contributes to New Zealand recruitment, has often been considered (Winstanley 1970, Phillips and McWilliam 1986, Booth 1987) and it is even more likely that such a system exists within Australia in which larvae from one state could be a significant source of recruitment to another state. Allozyme analysis (Smith et al. 1980) indicates that significant recruitment between Australia and New Zealand may be unlikely, but this work was not conclusive. The possibility of a single state possessing a number of discrete stocks must also be considered. If such a situation exists, zoned management policies should be developed in order to optimize the sustainable yield.

The project will seek to determine whether discrete stocks occur amongst the southern rock lobster fisheries. A fundamental concept in fisheries biology is that of a unit stock and without some knowledge of stock delineation management policies are likely to be ineffective. The southern rock lobster fisheries of Australia are all managed independantly and there are considerable differences in the management policies between the states. However it is not known whether the stocks are discrete and thus whether one fishery can be managed without reference to the others.

The project also aims to determine important information concerning larval recruitment, ie. what is the variation in puerulus settlement between areas, how does this relate to stock abundance, when does the major settlement occur and how does settlement vary over time. Knowledge of the patterns and levels of larval recruitment is essential to understand the structure of the fisheries. Larval settlement patterns can have major management implications, for example:

- In Tasmania, there is a distinct trend of increasing size and decreasing lobster density from south to north. It has been hypothesised that this may be due to habitat suitability or availability of larvae for settlement. The latter explanation involves the possible existence of a north south larval drift in which larvae from the northern regions settle in the south and larvae from the southern regions are lost to the system. This could place the stocks in jeopardy because recruitment would be from the north where the stocks are least abundant and have the shortest period of sexual maturity before reaching legal size. It would also mean that the southern stocks are overprotected and that a zoned management plan should be adopted. While the proposed project may not determine the existence of a north south larval drift, it would determine whether the concern is justified (whether settlement is predominantly in the south).

Numerous other scenarios could be presented to demonstrate the implications of different settlement patterns on management of the southern rock lobster fisheries. However, it should be recognised that with so little known about larval recruitment, any information will be of benefit to the industry.

For the project to be successful, the co-operation of all southern states (Tasmania, Victoria, South Australia, and Western Australia) is required. Sampling will be performed concurrently in all states with the same sampling equipment. The

southern states have agreed that this project is necessary and they are willing to work together to achieve the goals.

b) Justifcation of Genetic Analysis

Mitochondrial DNA is a small molecule which occurs in all organisms in the cell cytoplasm. In many circumstances, it provides the best system for the genetic identification and investigation of animal stocks. The unique features of the molecule which allow it to be used as an accurate genetic marker are its rapid rate of mutation, its maternal mode of inheritance and the ease with which its nucleotide sequence can be studied.

We predict that allozyme electrophoresis will not be useful in this study of southern Australian *Jasus novaehollandiae* stocks because of the lack of allozyme variation within lobster species. For example, Smith and McKoy (1980) found allele frequency differences only at the lactate dehydrogenase locus in a study of the variation at 33 scoreable genetic loci of *J. novaehollandiae* and *J. edwardsii*. The complete lack of definitive genetic differences between the species will preclude the use of allozyme electrophoresis for this and any other stock identification problem within *J. novaehollandiae*.

In virtually all allozyme electrophoresis studies on decapods, including lobster and crayfish, the amount of nuclear gene variation detected between and within species is much lower than for other groups of animals. For example, the amount of genetic heterozygosity within *J. novaehollandiae* and *J. edwardsii* was 1.2 to 2.8% (Smith and McKoy, 1980) and for *Panulirus ornatus*, the ornate tropical rock lobster, heterozygosity was 3.3% (Salini et al, 1985). Similarly, only 0.003 units of genetic distance was measured between *J. novaehollandiae* and *J. edwardsii* in the study by Smith and McKoy (1980). Powell (1976) calculated that mean heterozygosity for invertebrate and vertebrate species was about 9.3% while Avise and Aquadro (1982) concluded that the mean amount of genetic distance between vertebrate species varied from a low of about 0.1 units for birds to a high of about 0.8 units for amphibians. Because allozyme electrophoresis does not provide information on the genetic differentiation of lobster stocks, the use of mtDNA to analyse stocks of southern rock lobsters is essential.

There are two studies which show that mtDNA does vary between lobster populations. Komm et al (1982) reported variation in the mtDNA of individual Florida spiny lobsters, *P. argus*, and McLean et al (1983) found significant heterogeneity and possible species subdivision in a subsequent study. MtDNA analysis of lobster populations is technically demanding, but this part of the project will be carried out at the Fish Genetics Laboratory at the University of Tasmania which has a proven record of successful mtDNA analyses of numerous types of animal populations (eg. Galaxiids).

9. Location(s) of Operation

The project has three primary bases of operation:

a) D.S.F. Research Laboratories, Taroona. The project will be coordinated from this location. In addition, The D.S.F laboratories will be the base for Tasmanian puerulus sampling and will be the collection point for interstate samples where initial identification and morphological comparisons will be made.

b) Fish Genetics Laboratory, Zoology Department, University of Tasmania, Hobart. All genetic analysis will be conducted at this location.

c) CSIRO Marine Laboratories, Perth. This will be the base for Western Australian puerulus sampling.

Samples of puerulus and adults will also be obtained from South Australia (via the South Australian Department of Sea Fisheries) and from New Zealand (via the Ministry of Agriculture and Fisheries, Fisheries Research Centre).

10. Proposal in Detail

a) Plan of Operation

(i) Methods of procedure

Puerulus Sampling - Pilot Study

During the pilot study, the majority of sampling and all experimentation will be conducted in Tasmania. Some sampling will also be conducted in Western Australia and South Australia will recommence its puerulus sampling programme. Samples of adults and puerulus from both locations will be sent live or in liquid nitrogen to Tasmania for mtDNA analysis.

Comparisons of puerulus collectors

Three existing types of *Jasus* puerulus collectors will be compared. These are:

- The New Zealand collector
- The South Australian "pallet" collector
- A modified South Australian collector

The New Zealand collector contains a series of 8 wooden crevices which provide shelter for puerulus. It rests on a detachable weighted base plate to allow divers to retrieve the collector for sampling. The South Australian collector is essentially a slatted, wooden crate which can be opened to allow for removal of puerulus. This collector is suspended from a surface buoy and is attached to a mooring. In areas where recreational activity is high, sub-surface buoys will be used to avoid interference problems and divers will be needed to place and retrieve the collectors. The modified South Australian collector is a scaled down version (measuring 50cm x 50cm x 12cm) of the original which allows for easier handling.

Nine collectors of each type will be deployed on the north east Tasmanian coast (near St.Helens). Scallop spat collectors have indicated that this is an area in which puerulus settlement occurs. Three sites will be chosen in the St. Helens area, each site being separated by approximately 2 kilometres. At each site, 3 collectors of each type will be deployed (a total of 9 collectors per site) and the individual collectors will be separated by a distance of between 10 and 20 metres.

Sampling will be conducted monthly and comparisons will be made to determine the most efficient and reliable collector. The decision as to the most suitable collector for future sampling will also involve a consideration of costs, ease of use and durability of the different collectors.

Spatial Variablity of Puerulus Settlement

Prior to commencing the overall project, it is important to determine the relative levels of spatial variablity both within and between areas. This will determine the scale of sampling required to obtain representative samples from an area, which in turn affects the viability of the overall project.

Three general areas will be sampled monthly in Tasmania. These are:

- North east coast (St. Helens)
- South east coast (Bruny Island)
- North coast (Rocky Cape)

Three sites will be selected in each area and the sites will be separated by a distance of approximately two kilometres. At each site, 3 modified South Australian puerulus collectors will be deployed and the collectors will be separated by a distance of 10 to 20 metres. The modified South Australian collector is being used because of its ease of use and relatively low construction cost.

Comparisons will be made of settlement levels both between sites (within areas) and within sites to determine localized variations in settlement levels. Large scale spatial variations in settlement levels will also be examined by comparing settlement levels between areas and between the three states (Tas., S.A. and W.A.).

Puerulus Sampling - Overall Project

If the pilot study is successful, future sampling will also be conducted in Victoria and all states will use the same type of puerulus collector (as determined by the pilot study).

It is expected that the number of collectors used per area will be lower than for the pilot study and that a greater number of areas per state will be sampled. However, this is dependent on the results of the pilot study.

Genetic Analysis

The specific aims of this part of the project are :-

i) to develop appropriate techniques for the extraction and restriction enzyme analysis of lobster mtDNA.

ii) to apply these techniques to adult lobster collected throughout southern Australia, New Zealand and possibly from South Africa, in order to measure the amount and type of mtDNA variation within and between localities.

iii) to identify and characterize partially or fully reproductively isolated lobster stocks from the amount and type of mtDNA variation within and between adult populations

iv) to use restriction enzyme analysis of the mtDNA of puerulus larvae to determine the adult lobster stock from which they are most likely to be derived.

The quickest and easiest way of isolating suitable mtDNA from adult lobsters will be determined from published protocols. The selected isolation technique will be used to extract mtDNA from adult lobsters from southern Australia. Restriction enzyme analysis will be used to determine the type and numbers of mutations which are present in the mtDNA of these individuals. This information will be analysed with existing computer software to determine if the populations sampled are part of discrete or continuous stocks. If discrete stocks are identified, their mtDNA will be characterized as fully as possible with restriction enzymes.

Having defined potential parental stocks, restriction enzyme analysis will be used to characterize the puerulus larvae. Because the larvae are very small, the amount of mtDNA extracted from individuals will be low. Initially, the same technique will be used to extract and analyse mtDNA from the larvae as we will have used for the adults. If this is unsuccessful an alternate method, called 'Southern Blotting', will be used. For this method all of the nucleic acids from the larvae will be extracted and subjected to restriction enzyme analysis. The results of the analysis which relate to mtDNA are separated from the background nuclear DNA using purified mtDNA from adult lobsters. This technique is technically challenging but has produced unambiguous results in similar studies in other laboratories.

The results of restriction enzyme analysis of puerulus larvae will be compared to those obtained for adult lobsters. Because mutations within the mitochondrial genome are independent of the developmental stage of the individual, adults and their puerulus offspring from the same stock will share the same type of mtDNA. Thus the mtDNA characteristics of the puerulus larvae will be used to identify their parental stock.

(ii) Facilities available

The D.S.F. Research Laboratories at Taroona will provide office and laboratory space, computing and administrative support, workshop facilities, boats for field work and some vehicle usage. The University of Tasmania is equipped for mtDNA analysis and will also provide laboratory and office space. CSIRO (Marmion Laboratories) will provide office space, boats, vehicle usage and workshop facilities.

b) Supporting Data

The project involves the co-operation of most institutions currently working on *J. novaehollandiae*. Successful monitoring of *J. novaehollandiae* puerulus settlement has been achieved in South Australia (Lewis 1977) and projects on *J. edwardsii* (which appears to be synonymous with *J. novaehollandiae*) are continuing in New Zealand (eg. Booth 1979, Booth and Tarring, 1986). CSIRO and the Department of Sea Fisheries are currently conducting tests on a modified South Australian "pallet" collector and D.S.F. is also conducting trials with the New Zealand puerulus collector and has obtained some preliminary information concerning puerulus settlement from occasional settlement on scallop spat collectors.

Mr. Kennedy is in charge of D.S.F. rock lobster research and is currently running rock lobster catch sampling and stock assessment programmes. Dr. Phillips co-ordinatesCSIRO rock lobster research and has extensive experience and expertise in this field particulary with respect to puerulus settlement of *Panulirus cygnus*.

Drs. Ovenden and White, of the Zoology Department, University of Tasmania, are currently conducting two related projects. These are:

- 'Evolutionary, population and molecular genetics of fishes of the family Galaxiidae' funded by the Australian Research Grants Scheme. This grant provides salary for Dr. Ovenden and a small amount of equipment and consumable funding. This three-year project is a continuation of a previous two-year project entitled 'Biochemical and karyological studies of the phylogenetic relationships of the Australasian Galaxiid fishes'.
- 'The assessment of restriction enzyme analysis of mitochondrial DNA for the identification of stocks of commerically important marine species and for the detection of genetic markers for use in salmonid husbandry' funded by the Fishing Industry Research Account in 1987 with possible extension to 1990. The funds received under this grant are fully committed to the analysis of teleosts.

11. Proposed Commencement Date and Anticipated Completion Date

July 1988 to June 1989.

12. Funds Requested

(a) Total Salaries and Wages	$66,976
(b) Total Operating Expenses	$16,000
(c) Total Travel Expenses	$11,224
(d) Total Capital Items	$11,100
GROSS TOTAL COST	$105,300

13. Funds to be Provided by the Applicant(s)

a) Funds to be Provided by D.S.F.

- Salaries of R.B. Kennedy, 40% time	$11,000
- Overtime / Travel Allowance	$ 5,112
- Boat (full time use)	$20,000
- Vehicle usage	$ 3,000
- 1 x Diving Equipment	$ 2,000
	$41,112

D.S.F. will also provide laboratory and office accomodation, workshop and storage facilities, and the administrative, secretarial and clerical support required for the running and co-ordination of this project. Full access to the department's in house computing network will be provided.

b) Funds to be Provided by University of Tasmania

The University will provide full laboratory and office accommodation for the genetics section of this project together with access to administrative and secretarial services. The Fish Genetics laboratory is fully equiped for the analysis of mtDNA; the minor equipment requested is to provide extra facilities needed specifically for this project. The University has central and microcomputing facilities.

14. Co-operating Agencies and their Functions

The South Australian Department of Fisheries and the Ministry of Agriculture and Fisheries, Fisheries Research Centre (New Zealand) will be co-operating by providing samples of adults and puerulus for mtDNA analysis.

General liaison will also be conducted with the Department of Conservation (Victoria) and the New South Wales Department of Agriculture as the project has relevance to both states.

If the project continues beyond the preliminary year, co-operation of the Department of Conservation (Victoria) will be required. At this stage, all states would provide puerulus for analysis and puerulus settlement data would be compared to determine variations in settlement between states.

15. Is Similar Work being Undertaken in Australia ?

No work on puerulus settlement or larval recruitment of any *Jasus* species is being conducted in Australia. Similarly, no work on genetics or stock discrimination of *Jasus* is being conducted in Australia.

Drs. Ovenden and White are currently investigating mtDNA in marine teleosts to define stock structures of the fisheries.

Monitoring of *P. cygnus* puerulus settlement is continuing in Western Australia.

16. Plans for Reporting or Publishing Results

Reporting to:
- D.S.F. Research Reviews
- Western Fisheries Research Committee
- Conferences and Seminars as appropriate

Papers to be submitted for publication in
- FINTAS
- Australian Fisheries
- Appropriate Australian and International Scientific Journals

17. References

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Detailed Statement of Funds Requested

	1988/89
a) Salaries and Wages	
Technical Officer - Class III	$18,432
a	
Technical Officer - Class II	$17,545
a	
Coxswain and Diving Allowances	$ 1,634
a	
9% on costs (payroll Tax, Workers Comp.)	$ 3,385
a	
Graduate/Senior Graduate Research Assistant	$23,835
b	
9% on costs	$ 2,145
b	
Total Salaries and Wages	$66,976
b) Operating Expenses	
Materials for puerulus collectors and replacement collectors	$ 8,000
a	
Boat and Field Running Costs	$ 2,000
a	
Sample storage and transport	$ 2,000
b	
Consumables	$ 4,000
b	
Total Operating Expenses	$16,000
c) Travel Expenses	
Travel Allowance (field work)	$10,224
a	
Large Vehicle Hire	$ 1,000
a	
Total Travel Expenses	$11,224
d) Capital	
2 x Diving and Field Equipment	$ 4,000
a	
Microcentrifuge	$ 2,500
b	
Liquid Nitrogen Dewar	$ 1,600
b	
Minor equipment (pipettors, homogenizers etc.)	$ 3,000
b	
Total Capital Expenses	$ 11,100
Gross Total Cost	$105,300

Note: The annotations (a and b) to the right of the cost of each item refers to:
- a Items required for puerulus sampling.
- b Items required for genetic analysis.

Funding for capital items and materials for puerulus collectors will be required in July 1988. In addition, all staff will be appointed at this time.

Indication of Funding Likely to be Sought if the Pilot Study is Successfull

	1989/90	1990/91	1991/92
Salaries and Wages	$80,100	$80,100	$80,100
Operating Expenses	$16,000	$13,000	$11,000
Travel	$30,000	$30,000	$30,000
Capital	$ 7,500	-	-
Total	$133,600	$123,100	$121,100