

# A New Data Source For Fisheries Resource Assessment:

## Genetic Estimates of the Effective Number of Spawners



**Australian Government**  
**Fisheries Research and  
Development Corporation**

# **A NEW DATA SOURCE FOR FISHERIES RESOURCE ASSESSMENT: GENETIC ESTIMATES OF THE EFFECTIVE NUMBER OF SPAWNERS**

FRDC PROJECT ENTITLED:  
“DEVELOPMENT OF A GENETIC METHOD TO ESTIMATE EFFECTIVE SPAWNER NUMBERS IN THE  
NPF AND QUEENSLAND TIGER PRAWN FISHERIES”

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<b>2001/018</b>	<b>Development of a genetic method to estimate effective spawner numbers in the NPF and Queensland tiger prawn fisheries.</b>
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**OBJECTIVES:**

1. To critically evaluate a variety of mathematical methods of calculating effective population size ( $N_e$ ) by conducting comprehensive computer simulations and by analysis of empirical data collected from the Moreton Bay population of tiger prawns.
2. To lay the groundwork for the application of the technology in the NPF.
3. To produce software for the calculation of  $N_e$ , and to make it widely available.

**OUTCOMES ACHIEVED TO DATE:**

Outcomes from this project were relevant to the Australian fishing industry, and consequently the Australian economy. Specifically, they were

1. *A comprehensive evaluation of a new cost-effective tool for stock assessment methodology.*
2. *The development of a more conservative approach to ecologically sustainable exploitation of fisheries resources.* The project has progressed towards achieving this outcome, but further work is necessary.
3. *Mathematical and statistical collaboration between state and commonwealth fisheries research agencies and the University of Queensland.*

Outcomes from the project were also beneficial to fishery managers, fisheries scientists and operators involved in the Northern Prawn Fishery (NPF) and the Queensland trawl fishery. Specifically, these were

4. *Two separate and independent monitoring methods.* This project has ground-truthed technology that may allow effective spawner numbers to be monitored using a DNA-based approach. Further development of the method is needed, however, to bring the technology on line for the NPF and other trawl fisheries in Queensland waters.
5. *Improvements to the way in which predictions are made about the size of recruitment in the next fishing season.* More development is needed to fully realize the potential of this outcome for fisheries management.
6. *Additional information generated from the genetic analyses.*

Responses to this research from stakeholders were captured in the Proceedings of the Consultative Workshop held in Brisbane in May 2003 (Broderick D, Peel S, Street R, Ovenden JR (2003) 'Genetic methods for the estimation of fisheries spawning stock size: Transcripts of the forum - 9 May 2003.' Queensland Government, Department of Primary Industries, Brisbane, Australia. pp 205.)



## NON TECHNICAL SUMMARY:

**KEYWORDS:** Genetic effective population size, stock recruitment, simulation, *Penaeus esculentus*, Moreton Bay

The development of innovative methods of stock assessment is a priority for State and Commonwealth fisheries agencies. It is driven by the need to facilitate sustainable exploitation of naturally occurring fisheries resources for the current and future economic, social and environmental well being of Australia. This project was initiated in this context and took advantage of considerable recent achievements in genomics that are shaping our comprehension of the DNA of humans and animals.

The basic idea behind this project was that genetic estimates of effective population size, which can be made from empirical measurements of genetic drift, were equivalent to estimates of the successful number of spawners that is an important parameter in process of fisheries stock assessment. The broad objectives of this study were to

1. Critically evaluate a variety of mathematical methods of calculating effective spawner numbers ( $N_e$ ) by
  - a. conducting comprehensive computer simulations, and by
  - b. analysis of empirical data collected from the Moreton Bay population of tiger prawns (*P. esculentus*).
2. Lay the groundwork for the application of the technology in the northern prawn fishery (NPF).
3. Produce software for the calculation of  $N_e$ , and to make it widely available.

The project pulled together a range of mathematical models for estimating current effective population size from diverse sources. Some of them had been recently implemented with the latest statistical methods (eg. Bayesian framework Berthier, Beaumont *et al.* 2002), while others had lower profiles (eg. Pudovkin, Zaykin *et al.* 1996; Rousset and Raymond 1995). Computer code and later software with a user-friendly interface (*NeEstimator*) was produced to implement the methods. This was used as a basis for simulation experiments to evaluate the performance of the methods with an individual-based model of a prawn population.

Following the guidelines suggested by computer simulations, the tiger prawn population in Moreton Bay (south-east Queensland) was sampled for genetic analysis with eight microsatellite loci in three successive spring spawning seasons in 2001, 2002 and 2003. As predicted by the simulations, the estimates had non-infinite upper confidence limits, which is a major achievement for the application of the method to a naturally-occurring, short generation, highly fecund invertebrate species.

The genetic estimate of the number of successful spawners was around 1000 individuals in two consecutive years. This contrasts with about 500,000 prawns participating in spawning. It is not possible to distinguish successful from non-successful spawners so we suggest a high level of protection for the entire spawning

population. We interpret the difference in numbers between successful and non-successful spawners as a large variation in the number of offspring per family that survive – a large number of families have no surviving offspring, while a few have a large number.

We explored various ways in which  $N_e$  can be useful in fisheries management. It can be a surrogate for spawning population size, assuming the ratio between  $N_e$  and spawning population size has been previously calculated for that species. Alternatively, it can be a surrogate for recruitment, again assuming that the ratio between  $N_e$  and recruitment has been previously determined. The number of species that can be analysed in this way, however, is likely to be small because of species-specific life history requirements that need to be satisfied for accuracy. The most universal approach would be to integrate  $N_e$  with spawning stock-recruitment models, so that these models are more accurate when applied to fisheries populations. A pathway to achieve this was established in this project, which we predict will significantly improve fisheries sustainability in the future.

Regardless of the success of integrating  $N_e$  into spawning stock-recruitment models,  $N_e$  could be used as a fisheries monitoring tool. Declines in spawning stock size or increases in natural or harvest mortality would be reflected by a decline in  $N_e$ . This would be good for data-poor fisheries and provides fishery independent information, however, we suggest a species-by-species approach. Some species may be too numerous or experiencing too much migration for the method to work.

During the project two important theoretical studies of the simultaneous estimation of effective population size and migration were published (Vitalis and Couvet 2001b; Wang and Whitlock 2003). These methods, combined with collection of preliminary genetic data from the tiger prawn population in southern Gulf of Carpentaria population and a computer simulation study that evaluated the effect of differing reproductive strategies on genetic estimates, suggest that this technology could make an important contribution to the stock assessment process in the northern prawn fishery (NPF).

Advances in the genomics world are rapid and already a cheaper, more reliable substitute for microsatellite loci in this technology is available. Digital data from single nucleotide polymorphisms (SNPs) are likely to super cede ‘analogue’ microsatellite data, making it cheaper and easier to apply the method to species with large population sizes.

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## Chapter 1 – Introduction

by JR Ovenden and D Peel

### Background and need

Sustainable management aims to safeguard fisheries resources for today and the future by regulating fishing activities when necessary. The keys to success are an understanding of the fishery and its components, the marine environment, fisheries resource and the operators. Despite ongoing research, the marine environment is largely unknown because of the huge variety of species and habitats, and the complex way they interact. The status of the fisheries resource can be estimated using computer models based on data from the fishery and population under study. This process, stock assessment, can provide an estimate of the present size of the resource and the risk of its collapse under current catch rates. It can also evaluate future scenarios under various management options.

This project uses the tiger prawn (*Penaeus esculentus*) population in Moreton Bay (south-east Queensland) as a model system to trial the genetic estimates of spawning stock size (effective population size) as a new data source for stock assessment modelling. Tiger prawns (*P. esculentus*) are key components of the multi-million dollar prawn fishery in northern Australia. Spawning in Moreton Bay occurs annually in October and November, and this three-year study samples the population at successive spawning events to make three temporal estimates (2001 – 2002, 2002 – 2003 and 2001 – 2003) of effective population size across one and two generations.

Stock assessment is essential for sustainable management, but it is expensive. For example, the Research and Environment Committee of NORMAC estimated that 42% of the \$8.5 million spent on the Northern Prawn Fishery (NPF) over five years (1994 - 99) went towards stock assessment. This included the cost of extensive computer modelling, the monitoring of catch and effort data, tagging experiments for mortality and growth rate estimation, standardisation of catch rates, and a host of other biological studies. Genetic effective population size estimates may contribute in a significant way to this process by improving both cost effectiveness and accuracy.

This project arose from previous studies that suggested that the field of population genetics could make a valuable contribution to science of fisheries stock assessment (Lavery and Keenan 1995). Genetic estimates of effective population size ( $N_e$ ) have been made for a marine finfish in the Gulf of Mexico (Turner, Richardson *et al.* 1999) and a freshwater fish in a land-locked freshwater lake in Wisconsin, USA (Miller and Kapuscinski 1997). Genetic estimates of effective population size have the advantage of being independent of fisheries catch and effort data that is used as a surrogate for biomass in the stock assessment process. Catch per unit effort can be biased estimator; for example when both the fishing fleet and target species are aggregated or when increase in fishing power is unrecorded (effort-creep). The genetic estimates are also independent of the fishing sector so effective population size data continues to be relevant when the fishery changes from trawling gear to traps, for example. It is single species data source meaning that it focuses on a particular species in multi-species fisheries. The potential of effective population size estimates to be surrogates for

abundance and recruitment is explored later in the report, along with the additional information it provides about the ecology of recruitment.

## Scope

This study is an atypical application of the principles of population genetics to fisheries science, and it is important to understand the distinction between this study and other uses of population genetics in fisheries. At present population genetics contributes to fisheries science in the following fields

- Conservation biology (particularly for freshwater species, defining conservation units, determining amount of genetic diversity and hence conservation status, suggesting translocation strategies for population enhancement or protection),
- Aquaculture (genetic marker enhanced selective breeding, used along with quantitative genetics), and
- Stock assessment that is a major driver for numerous fisheries genetics projects, which fall into the following types.
  - Population structure (testing for spatial heterogeneity and temporal stability).
  - Genetic tagging (using individual-based genotypes as an alternative for physical tagging in mark recapture and harvest rate estimation), and
  - Assessment of ‘genetic health’ (aimed at preserving genetic diversity in harvested populations that may have a low effective population size).

The estimation of effective population size using genetic methods (this project) falls into the stock assessment category.

The purpose of this chapter is to review the literature relating to the estimation of effective population size based on genetic techniques. The existing methodologies will be assessed and discussed, with an emphasis on possible uses in wild fisheries populations, in particular the tiger prawn fisheries of Moreton Bay and the Gulf of Carpentaria.

## Genetic drift and mutation-drift-migration equilibrium

In naturally occurring populations, random sampling of gametes from generation to generation during reproduction causes changes in population allele (gene) frequencies. This random change in allelic frequency is known as genetic drift. The amount of drift is inversely proportional to population size - allele frequencies will change less over time in large populations compared to small populations. Genetic drift is one of the forces responsible for evolution in naturally occurring populations. The other forces are mutation, natural selection and migration.



Empirical estimates of genetic drift are made assuming it dominates the forces of mutation, selection or migration, or they are under experimental control; for example the choice of an isolated population, selectively neutral genetic loci or short temporal period over which mutation is unlikely to occur (see Table 1.1). Where possible in this study the conformance of the test population (tiger prawns in Moreton Bay) to these assumptions is evaluated to determine whether forces other than genetic drift are responsible for the observed change in allele frequencies.

**Table 1.1: Assumptions of the model of random genetic drift (Hartl and Clark 1989, p. 64).**

(1)	Diploid organism
(2)	Sexual reproduction
(3)	Non-overlapping generations
(4)	Many independent subpopulations, each of constant size N
(5)	Random mating within each subpopulation
(6)	No migration between subpopulations
(7)	No mutation
(8)	No selection

When allele frequencies are not affected by natural selection – for example, at selectively neutral genetic loci – a population will reach a point of equilibrium where the influx of new alleles due to mutation and migration is balanced by the loss due to random genetic drift and migration (Kinnison, Bentzen *et al.* 2002; Turelli, Gillespie *et al.* 1988). Theoretically, a genetically isolated population is expected to arrive at mutation-drift equilibrium after  $4N_e$  generations (Hartl and Clark 1989) where  $N_e$  is the genetic effective population size.

There are many reasons why fisheries species of commercial or conservation value may not be in mutation-migration-drift equilibrium. These include

- Population size may be getting smaller due environmental or anthropogenic effects,
- Population size may be getting larger following recovery from a severe reduction in population size, particularly where generation length is long (for example, several years or more),
- Establishment of new populations or augmentation of existing populations, by one or more translocation events, and
- Population admixture that may have occurred as a consequence of human activities.

The estimation of genetic effective population size from measurements of genetic drift in these types of populations needs to be considered on a case-by-case basis. However, assuming that natural selection, migration and mutation are accounted for,

estimates of genetic effective population size can be made with the temporal method (see below) regardless of whether the population is in equilibrium.

## Hardy-Weinberg equilibrium

Hardy-Weinberg model describes the relationship between the frequencies of genotypes (co-occurrence of two alleles at a single genetic locus) and population allele frequencies.

In a sexually reproducing diploid organism with a large and constant population size that is experiencing random mating and does not have overlapping generations and where effects of mutation, migration or selection are negligible, then for loci that are not on the sex chromosomes that have two alleles

$$(p + q)^2 = p^2 + 2pq + q^2$$

The frequency of the most common allele  $A$  is  $p$  and the frequency of the least common allele  $a$  is  $q$ , such that  $p + q = 1$ . Alleles  $A$  and  $a$  are codominant alleles for a particular trait, that is we can distinguish carriers of the genotypes  $AA$ ,  $Aa$  and  $aa$  based on their appearance or phenotype (Hallerman, Brown *et al.* 2003).

The model is used to check that the observed frequency of a particular genotype is not significantly different to its predicted frequency from known allele frequencies. The model is remarkably robust in naturally occurring populations, and deviations are most likely an indication of methodological errors that can be subsequently checked by the researcher.

## Genetic estimates of effective population size

### *Introduction*

In the science of population genetics the number of individuals that reproduce and pass genetic material onto the following generation is often of interest. This number may differ greatly from the total size of the population (Frankham 1995; Vucetich, Waite *et al.* 1997).

The effective population size ( $N_e$ ) is the number of breeding individuals in an ideal population that would produce the same amount of change in a given genetic measure, due to genetic drift. Common measures include the amount of inbreeding, allelic frequency variation or heterozygosity loss. So the definition of effective population size depends on the measure under consideration. Generally when the population size is not changing over time, the effective population sizes corresponding to the various measures i.e. inbreeding  $N_e$ , temporal  $N_e$  etc. will be similar (Kimura and Crow 1963).

It should be noted that this review is focused on the estimation of short-term (current, extant) effective population size rather than long-term effective population size. Long-term effective population size is estimated from population genetic diversity and the mutation rate of those genetic loci.

There are currently a number of methods available to estimate  $N_e$ , the following sections give a brief overview of the current available approaches, and describes each methods use in selected applications.

### ***Temporal methods***

#### **Theory**

As mentioned, the effects of genetic drift can be seen in the corresponding change in the allele frequencies. The temporal method estimates the effective population size based on observing the temporal (eg over time) variation in the allele frequency ( $F$ ). There are numerous approaches for estimating the effective population size from the observed allele temporal variation. Waples (1989) describes two sampling strategies, sampling with replacement (or sampling after reproduction) and sampling without replacement (or sampling before reproduction). This study focuses on the first strategy of sampling with replacement (sampling plan I).

#### **Moments-based method**

By far the most commonly used approach for estimating the effective population size are the moment-based  $F$ -statistic estimators (Krimbas and Tsakas 1971; Nei and Tajima 1981; Pollack 1983; Waples 1989).

For a single locus with  $A$  alleles, let  $x_1, x_2, \dots, x_A$  and  $y_1, y_2, \dots, y_A$  denote two temporally distinct samples of allele frequency where  $\sum x_i = 1$  and  $\sum y_i = 1$ . Nei (1981) defined the  $F$ -statistic as

$$F = \frac{1}{A} \sum_{i=1}^A \frac{(x_i - y_i)^2}{(x_i + y_i)/2 - x_i y_i} \quad (1)$$

However, Pollack (1983) recommended an alternate form

$$F = \frac{1}{A-1} \sum_{i=1}^A \frac{(x_i - y_i)^2}{(x_i + y_i)/2} \quad (2)$$

to provide better sampling properties.

It follows that an estimate for the effective population size  $N_e$  is given by

$$\hat{N}_e = \frac{t}{2(F - 1/S)} \quad (3)$$

where  $t$  is the number of elapsed generations and  $S$  is the sample size. Basically as expected the estimate for  $N_e$  is inversely proportional to the observed allele temporal variation ( $F$ ) with the  $1/S$  term representing the variation that would occur due to sampling error. It can be seen in (3) that when  $F < 1/S$  then the estimate of  $N_e$  is negative. This corresponds to the observed drift being completely explained by sample variation. In this case a larger sample size may be required or  $N_e$  is taken to be infinity.

These equations are valid for species with non-overlapping generations. Jorde and Ryman (1995) propose a correction factor that can be implemented when generations overlap.

In the case of multiple loci the weighted means of the single locus values are used i.e.  $A_j$  alleles at the  $j^{\text{th}}$  locus

$$F = \sum_{j=1}^L \left[ (A_j - 1)F_j / \sum_{i=1}^L (A_i - 1) \right] \quad (4)$$

The  $\alpha$ -Confidence intervals for  $F$ , and hence  $\hat{N}e$ , are given by

$$\text{CI of } F = \left[ \frac{nF}{\chi_{n,\alpha/2}^2}, \frac{nF}{\chi_{n,1-\alpha/2}^2} \right] \quad (5)$$

where  $n$  is the total number of independent alleles in the data.

The variance of  $\hat{N}e$  is approximately given by

$$V(\hat{N}e) \approx \left[ \frac{8Ne^4}{t^2n} \right] [t/(2Ne) + 1/(2S_0) + 1/(2S_t)]^2 \quad (6)$$

$$= \left[ \frac{8Ne^4}{n} \right] \left[ \frac{1}{4Ne^2} + \frac{1}{N_t S} + \frac{1}{t^2 S^2} \right] \quad (7)$$

From (6) we can see that increasing; the sample size  $S$ , the number of loci (hence the number of alleles  $n$ ) and the number of generations,  $t$ , decreases the variance of our  $Ne$  estimate.

**Sample size** – The estimate of  $Ne$  is calculated from the observed genetic drift over time in the form of the  $F$ -statistic. The  $F$ -statistic contains genetic drift plus sampling error ( $1/S$ ). For large  $Ne$  the genetic drift component of the allele frequency change will generally be quite small, when it is less than the sampling error, all the observed variation in the allele frequency can be explained by the sampling error and so the estimate of  $Ne$  will be negative.

**Number of loci/alleles** – Williamson and Slatkin (1999) showed in their simulation experiment that increasing the number of loci decreases both the variance and bias of the temporal estimate. Although as Kitada et al. (2000) point out, there reaches a point ( $L > 50$ ) where increasing  $L$  further produces only minor gains.

**Number of generations** – The larger the number of generations between the two samples the greater the amount of discernible genetic drift. Williamson and Slatkin (1999) also looked at the effect of generation number in their simulations, finding that increasing the number of generations from 4 to 8 both decreased the variance and bias of the estimate.

Luikart et al. (1999) provide an overview of existing computer software and current statistical approaches, including maximum likelihood, Bayesian and Monte Carlo Markov chain methods.

### **Maximum likelihood**

Williamson and Slatkin (1999) describe a maximum-likelihood based approach to estimate the effective population size from temporal changes in allele frequencies. The estimator is formed under the framework of a Wright-Fisher model (eg discrete, non-overlapping generations). The 'effective population size'  $N$  is taken to be an unknown discrete parameter in the Wright-Fisher model to be estimated via maximum-likelihood. This  $N$  does not have to equal the temporal variance effective population size  $N_e$ , as estimated by the moments-based estimator. However when a population is evolving according to a Wright-Fisher model they are equivalent.

The likelihood function is unusual as the discrete parameter ( $N$ ), we are trying to estimate, is the upper range of a summation. Therefore, we cannot practically differentiate the likelihood function with respect to  $N$ . Evaluating a profile of the likelihood for various values of  $N$  and choosing the  $N$  that corresponds to the largest likelihood can overcome this.

For polymorphic loci computing the likelihood function becomes impractical. In this case a simulation approach such as Monte Carlo integration or Markov Chain Monte Carlo to approximate the likelihood function can be used. Anderson et al. (2000) describe a maximum-likelihood approach, with Monte Carlo integration using importance sampling to reduce the Monte Carlo variance. This allows the analysis of data with more than two alleles per loci without having to bin the data first. The C++ source code to the program, called MCLEEPS is available online from

[www.stat.washington.edu/thompson/Genepi/Mcleeps.shtml](http://www.stat.washington.edu/thompson/Genepi/Mcleeps.shtml).

Williamson et al. (1999) found the maximum-likelihood method to have less bias and greater precision than the moments-based method. Williamson and Slatkin (1999) and Anderson et al. (2000) suggest using the values of  $N_e$  for which the log-likelihood has decreased by two from the maximum to obtain the 95% confidence intervals. The method is computationally intensive, especially when analysing data sets with a large number of highly polymorphic loci.

Wang (2001) points out that the maximum-likelihood based approach described by Anderson et al. (2000) is computationally intensive due to the Monte Carlo approach. Wang (2001) goes on to describe a pseudo-likelihood based approach to estimate a  $N_e$  that is more computationally efficient. Wang and Whitlock (2003) further extend the use of a pseudo-likelihood based approach to estimate both  $N_e$  and the migration rate in the case of a non-isolated population. In this paper we are concerned with an isolated population so the migration factor has not been considered (see Chapter 7). However, in practice this would be a useful feature of the method. The program is called MLNE and available from

<http://www.zoo.cam.ac.uk/ioz/software.htm>.

In their simulation studies Wang (2001) and Wang and Whitlock (2003) found MLNE to provide more precise and accurate estimates than the moments-based temporal method. However, these simulations were limited to relatively small effective population sizes compared to the study given in Chapter 5.

## **Bayesian**

Beaumont (1999) describes a Bayesian approach to estimating population parameters based on coalescence using the Monte Carlo Markov Chain (MCMC) method. The program TM3 is available online from

<http://www.rubic.rdg.ac.uk/~mab/software.html>.

It should be noted that this program currently does not have the facility to handle missing data arising from genotyping failure. Genotyping failures are generally not an issue in the small scale conservation type applications, as the small number of samples allows each sample to be re-genotyped if required, but in large scale samples, as found in our pilot study, this is cost prohibitive and the data may contain missing values. *NeEstimator* includes modified versions of the temporal and linkage disequilibrium methods to estimate  $N_e$  from data containing missing values (Appendix A).

Kitada et al. (2000) outline the use of a Bayesian approach to estimate a posterior distribution of the allele frequencies and using equations (1) to (3) hence a posterior distribution of the effective population size. The purpose for taking this approach is to handle the case of over dispersion in the sample allele frequencies eg. due to spatially divided subpopulations in the sample area. This can cause the effective population size to be underestimated.

## **Examples**

### ***Moments Based Applications***

The examples presented here focus on the estimation of effective population size that are relevant to this study, (1) fisheries populations and (2) large, naturally occurring populations (mosquito).

Jorde and Ryman (1996) studied brown trout (*Salmo trutta*) from four small (300-1500m diameter) lakes in central Sweden. Over 5,800 fish were sampled for allozyme electrophoresis of 14 loci between 1979 and 1993. Pollack's method (1983) was used to estimate  $N_e$ , following correction for the presence of overlapping generations (Jorde and Ryman 1995). Effective population size estimates varied between lakes (97, 52, 480 and 140) and there was circumstantial evidence that there was a reasonable correlation between the estimates and the actual number of fish in the lakes.

Miller and Kapuscinski (1997) examined a natural fish population of Northern Pike (*Esox lucius*). They utilised a historical collection of fish scales, taking samples from 1961, 1977 and 1993. The method of Pollack (1983) was used with seven loci, five of which had two alleles and the remaining two had three alleles. The estimates obtained

were compared to census-based estimates. The results were promising, however the effective population sizes being estimated were relatively small.

Turner et al. (1999) looked at estimating the effective population size of red drum (*Sciaenops ocellatus*) from the northern Gulf of Mexico. The paper examined females only because a maternally inherited genetic locus (mtDNA) was used. Four population samples were taken between 1986 and 1989. One hundred and forty-five mtDNA haplotypes were observed and any with zero allele frequency, for the samples being used, were removed. The effective population size was estimated, using an  $F$ -statistic, as 14308, but the precision of this estimate was very low, and the upper bound for the 95% confidence interval was infinity.

Two examples that involve the estimation of slightly larger  $N_e$  are both insect data sets. The *Drosophila* data set from Begon et al. (1980) (see also Anderson, Williamson et al. 2000; Pollack 1983) had an  $N_e$  estimated in the several hundred. The *Anopheles gambiae* data set from Lehmann et al. (1998) was used to assess the long-term and short-term effective population sizes of two African Mosquito populations. They estimated the effective population sizes to be 6359 and 4258 using the temporal method.

### ***Maximum Likelihood and Bayesian Applications***

Williamson and Slatkin (1999) applied a maximum likelihood based approach to the Northern Pike data from Miller et al. (1997). Since the method as presented was for the specific case of only two alleles per locus, Williamson and Slatkin (1999) grouped the two least common alleles at each locus in the original data set together to form a two-allele data set.

Kitada et al. (2000) also looked at the Northern Pike population, with the same binning modification seen in Williamson and Slatkin (1999). Using their Bayesian approach Kitada et al. (2000) produced similar results but with slightly tighter confidence intervals.

Anderson et al. (2000) applied MCLEEPS to two simulated data sets and a data set relating to a population of *Drosophila* from Begon et al. (1980), which was also analysed in Pollack (1983). Their estimates differed from those of Pollack (1983) although Anderson et al. (2000) gave a number of valid reasons for this.

### ***Point methods***

#### **Linkage/gametic disequilibrium**

##### ***Theory***

Linkage disequilibrium is the difference between the expected co-occurrence of two alleles at two loci, given random mating, independent segregation, and no selection, and their actual instances of co-occurrence (Campton 1987). A population is at linkage equilibrium at a set of loci if the alleles are independently distributed on chromosomes (Hudson 2001). Linkage disequilibrium is often called gametic phase disequilibrium, allelic association or linked loci. Linkage disequilibrium can be

generated by genetic drift, mutation, admixture and selection and its decay is much slower with inbreeding.

When genetic variation at several loci is studied, estimates of effective population size can be made that take advantage of much of the information contained in the multi-locus genotypes determined for individuals sampled for the population. The linkage disequilibrium method uses the deviation from locus-by-locus independence to estimate effective population size. In contrast, temporal methods assume that allelic frequencies at given loci to be independent, and consequently do not access the information that is available in the genotypes among loci.

Linkage disequilibrium methods do not need population samples spaced over at least one temporal interval. Only one population sample is required, provided that sample is truly representative of the population, regardless of generation length. Alternatively, sampling could be done over several generations to ensure that all the year classes are represented. This would have the additional benefit in that changes in  $N_e$  could be documented and evaluated (Bartley, Bagley *et al.* 1992).

Multi-locus data sets that are excluded from temporal methods because they are found to deviate from independence would be suitable for the linkage disequilibrium method. The inclusion of linked loci increases precision, but recombination rates need to be estimated for those loci (Hill 1981). However, there is no *a priori* requirement to use linked loci as long as several loci are used (Waples 1991).

Genetic drift in finite populations causes non-random associations to develop between alleles at different loci. The stochastic fluctuations in allele frequency that occur from generation to generation are responsible for measurable non-random associations between alleles at different loci. Recombination between loci reduces, but does not eliminate these effects. Drift generated linkage disequilibrium ( $D$ ) can be large and either positive or negative, so the squared correlation efficient,  $r^2$  (equation 8, below) is used to show the effect of finite population size.

When the phase of alleles for two polymorphic loci is known, gametic disequilibrium can be estimated directly. Information about the phase tells us which alleles are present together in the one individual, including on the same chromosome. This can be used to directly calculate the gametes produced by each individual, and hence write down all possible genotypes. The frequency of these genotypes can be worked out if the frequency of the alleles is known. Then the frequency of all possible genotypes can be compared to the observed frequency of genotypes. The difference (deviation,  $D$ ) between them is due to the non-random association of alleles within gametes. This is caused when the loci are on the same chromosome, or in special cases on different chromosomes (Hedrick 2000).

Generally the gametic phase is not known, however, as most study animals are not bred in the laboratory, or have no family group data. However, the method can be applied by substituting the frequency of individual alleles with single locus genotypes. For example, instead of using the frequencies of alleles  $a$  and  $b$ , the frequencies of genotypes  $aa$ ,  $ab$  and  $bb$  are used instead. Recently, Kalinowski and Hedrick (2002) reported an expectation-maximization algorithm that can be used to estimate haplotype (gamete) frequencies from multi-locus data in the absence of phase information.



The linkage disequilibrium method measures the deviation of the observed frequency of  $ab$  gametes (or multi-locus heterozygotes) from what is expected from random mating, where  $p$  is the frequency of allele  $a$  at the first locus,  $q$  is the frequency of allele  $b$  at the second locus, so  $D = \text{freq}(ab) - pq$ . The effective population size is then

$$\hat{N}e = \frac{1}{3(\hat{r}^2 - 1/S)} \quad (8)$$

where

$$r = \frac{D}{[p(1-p)q(1-q)]^{1/2}} \quad (9)$$

This quantity,  $r$ , is calculated for the possibly many combinations across loci and the mean taken to provide a final estimate of effective population size, using Equation (8). Since we have no direct measurements of gametes but instead have the offspring's genotypes, the gamete frequencies must be estimated. As suggested by Bartley et al. (1992) we used Burrows composite measure of disequilibrium for  $D$ . In Campton (1987), Burrow's composite measure of disequilibrium is formulated for the case of non-random mating and is given by a simple formula so is more straightforward to estimate than the alternate maximum likelihood approach. Campton (1987) provides excerpts from simulation studies of Weir (1979) which show that even when the mating is random Burrow's  $D^*$  performs the same or better than the maximum likelihood approach.

To estimate  $D^*$  a table of counts is formed for example below for allele  $A_1$  at locus  $A$  and allele  $B_1$  at locus  $B$ . The  $N_{ij}$  values are simply the counts of the corresponding genotypes in the sample.

**Table 1.2: Table of genotype counts.**

		Locus A		
		$A_1A_1$	$A_1A_2$	$A_2A_2$
Locus B	$B_1B_1$	$N_{11}$	$N_{12}$	$N_{13}$
	$B_1B_2$	$N_{21}$	$N_{22}$	$N_{23}$
	$B_2B_2$	$N_{31}$	$N_{32}$	$N_{33}$

Then

$$D^* = \frac{2N_{11} + N_{12} + N_{21} + N_{22}/2}{N} - 2pq \quad (10)$$

This is for the two-allele case. To extend this to polymorphic loci for chosen alleles  $A_1$  and  $B_1$ ,  $A_2$  and  $B_2$  were taken to be the composite of all other alleles. So every combination of loci-pair is taken and for each pair of loci every allele in the first locus is selected and compared to each allele in the other locus. From this we get a large number of values for  $D^*$ . In the case of two alleles, all values of  $D^*$  for each allele pair between two loci will have the same absolute value.

Bartley et al. (1992) go on to say that they use the correlation ( $r$ ) between loci which is obtained by basically standardising the  $D^*$  value

$$r = \frac{D^*}{([p \times (1 - p) + D^*_{\cdot 1}] \times [q \times (1 - q) + D^*_{\cdot 1}])^{1/2}} \quad (11)$$

where  $D^*_{\cdot 1} = N_{\cdot 1}/N - p^2$  in other words the observed frequency of  $A_1A_1$  minus its expected frequency and similarly  $D^*_{\cdot 2} = N_{\cdot 2}/N - q^2$ . We then take the arithmetic mean over all of the  $r^2$  values to get a single  $r^2$  that is used to calculate  $N_e$  using (8).

In an ideal, infinite, random-mating population, both  $r$  and  $D^*$  would be 0. In real finite populations,  $r$  and  $D^*$  will depart from 0 due to genetic drift, migration, selection and physical linkage (Campton 1987; Hill 1981).

Unlike the temporal methods, linkage disequilibrium assumes random mating (Waples 1991). Although Weir et al. (1980) did find that even when the assumption of random mating does not hold (eg monogamous mating) the results were still reasonable. Other assumptions made by the linkage disequilibrium method include neutral alleles, no migration, no subpopulation structure, and a random sampling of the entire population (Hill 1981). Unlike the temporal method only one sample is required, which in some applications can be an advantage.

Approximate confidence intervals for  $r^2$ , and hence  $\hat{N}_e$ , are given by

$$\text{CI of } r^2 = \left[ \frac{Kr^2}{\chi^2_{K, \alpha/2}}, \frac{Kr^2}{\chi^2_{K, 1-\alpha/2}} \right] \quad (12)$$

where  $K$  is the number of pair-wise comparisons of loci ( $K = L(L-1)/2$  for  $L$  loci) Variance estimates of  $\hat{N}_e$  can be calculated by

$$V(\hat{N}_e) \approx \frac{2\hat{N}_e^2}{K} \left[ 1 + \frac{N_e}{.333(S)} \right]^2 \quad (13)$$

where  $S$  is the sample size. Further information is given in Hill et al. (1981), Bartley et al. (1992) and Waples (1991).

Hill et al. (1981) found that even estimates formed from large samples can lack precision. However as Waples (1991) mentions, this problem may be alleviated if data from many loci are available, since for neutral loci each pair-wise comparison provides an independent estimate of the effective population size thus allowing an estimate to be formed by combining the results of many pair-wise comparisons. Bartley et al. (1992) recommended of a sample size greater than 90 and at least six gene loci when using the linkage disequilibrium method. However it would seem that these values would vary depending on the size of the  $N_e$  being estimated and the required precision.

Several authors (Hill 1981; Waples 1991; Waples and Smouse 1990) suggested removing loci with high allele frequency (greater than 0.95) to increase the accuracy of the method, since the expected value of  $r$  may not be zero. Waples (1990) found

that removing these loci increased accuracy but decreased precision. This agreed with the results of Bartley et al. (1992) after removing the same loci from their data. Although, since the true value was unknown it was hard to assess whether removing the alleles was useful.

Hill (1981) showed that the use of tightly linked loci gave the greatest precision. However, a large amount of time is needed for recombination to erode existing disequilibria. This would cause the estimate of the effective population size to reflect the effective population size of the distant past. Schwartz (1998) and Hedrick (1987) discuss another problem of using linkage disequilibrium, as the  $D$  estimates are allele frequency dependent, which may cause problems when using highly polymorphic locus.

### *Examples*

To demonstrate the use of linkage disequilibrium to estimate effective population size Bartley et al. (1992) examined four hatchery populations covering three species; White Sea Bass (*Atractiscion nobilism*), Chinook Salmon (*Oncorhynchus tshawytscha*), and Rainbow Trout (*Oncorhynchus mykiss*). The results (see Table 1.3) included many negative values for  $N_e$  (i.e. infinity), which correspond to all the observed linkage disequilibrium being explained by sampling error. This can be because in the case of large effective population sizes  $r^2$  approaches zero, or because too small a sample size was taken.

**Table 1.3: Results from Bartley et al. (1992) for various hatchery populations.**

Sample	$N_e$	Confidence Interval
Sea Bass 1	$\infty$	[ 1.1 , $\infty$ ]
2	$\infty$	[ 16.9 , $\infty$ ]
3	$\infty$	[ 17.8 , $\infty$ ]
4	$\infty$	[ 2.3 , $\infty$ ]
5	11.6	[ 2.8 , $\infty$ ]
6	$\infty$	[ 6.6 , $\infty$ ]
Chinook Salmon 1	132.5	[ 67.5 , 354.7 ]
Chinook Salmon 2	88.5	[ 44.7 , 265.8 ]
Rainbow Trout	35.8	[ 13.0 , 113.4 ]

### **Heterozygote excess**

#### *Theory*

The basis for the heterozygote excess method is that when the effective population is small, the allele frequencies of males and females will differ, due to binomial sampling error. This will result in a larger number of heterozygotes than would be expected under Hardy-Weinberg equilibrium. Pudovkin et al. (1996) suggested using

a measure of this heterozygote excess as an indicator for effective population size. The expected proportion of heterozygotes ( $H_{Exp}$ ) in a finite population is given by

$$H_{Exp} = \frac{2S}{2S-1} [2p(1-p)], \quad (14)$$

where  $p$  is the frequency of the allele being examined in the population from which the parents were drawn. Letting  $H_{Obs}$  denote the observed total proportion of heterozygotes, then the heterozygote excess ( $D$ ) (Selander 1970) is given by

$$D = \frac{(H_{Obs} - H_{Exp})}{H_{Exp}} \quad (15)$$

This quantity is inversely proportional to effective population size and an estimate for the effective population size is given by

$$\begin{aligned} \hat{N}_e &= \frac{H_{Exp}}{2(H_{Obs} - H_{Exp})} \\ &= \frac{1}{2D} \end{aligned} \quad (16)$$

In the case of multiallelic locus the average of  $D$  is taken over the  $A$  alleles,

$$D = \frac{1}{A} \sum_{i=1}^A D_i \quad (17)$$

where  $D_i$  is the excess of heterozygote containing the  $i^{\text{th}}$  allele.

Approximate confidence intervals can be obtained using the Student  $t$ -distribution, even though  $N_e$  is not normally distributed. Luikart et al. (1999) found that this approximation worked well except in the case of loci with only two alleles, or when the underlying assumption of random mating was broken.

As with the linkage disequilibrium method the heterozygote excess method assumes random breeding and unlike the temporal method only one sample is required.

With regard to the evaluation via a simulation experiment in Pudovkin et al. (1996), Schwartz et al. (1998) point out that they did not include sampling error and that most studies since have had less than the 30 loci used in the simulations.

The method has poor precision (Luikart and Cornuet 1999; Pudovkin, Zaykin *et al.* 1996), with the confidence intervals being too wide unless a large sample of loci and individuals is taken.

### **Examples**

Luikart and Cornuet (1999) demonstrated the heterozygote excess method on simulated and real populations; brown trout (*Salmo trutta*), bull trout (*Salvelinus confluentus*) and harlequin ducks (*Histrionicus histrionicus*). Even though the effective population sizes to be estimated were relatively small, half of the samples

gave infinite estimates (Table 1.4). This would seem to indicate a larger sample size is required or more loci need to be used. To address the problem of precision Luikart and Cornuet (1999) suggested four possible approaches:

- Include 80% confidence intervals as well as the usual 95% to provide more information.
- Use a non-parametric method to estimate confidence intervals.
- Combine estimates from a number of cohorts or generations.
- Combine estimates for  $N_e$  from several sources.

**Table 1.4: Results from Luikart and Cornuet (1999) for various data sets.**

Species	Number of Parents	$N_e$	C.I
<i>Brown Trout</i>	Few	$\infty$	[ 3 , $\infty$ ]
Brown Trout	Few	8.8	[ 4 , $\infty$ ]
Brown Trout	Few	$\infty$	[ 3 , $\infty$ ]
<i>Brown Trout</i>	Few	4.0	[ 3 , 15 ]
Bull Trout	6-15	$\infty$	[ 8 , $\infty$ ]
Bull Trout	6-15	3.2	[ 2 , 7 ]
Bull Trout	3-18	$\infty$	[ 8 , $\infty$ ]
Bull Trout	3-24	6.3	[ 2 , $\infty$ ]
Bull Trout	15-18	6.0	[ 3 , $\infty$ ]
Harlequin Duck	8	$\infty$	[ 4 , $\infty$ ]

The first two approaches do nothing to address the underlying problem of a lack of precision in the estimate being used. The first approach does not change the precision of the estimate, but simply presents the results in a way that would tend to hide the problem. Similarly, with the second approach it would seem that generally the infinite bounds are actually a valid reflection of a precision problem and a non-parametric method to estimate the confidence intervals would produce similar results. The final two approaches seem much more useful and may provide a reliable method to increase precision.

## **Tiger prawns (*Penaeus esculentus*)**

### ***Distribution***

There are over fifty species of penaeid prawn in Australian waters and at least ten of these are of major economic importance (Grey, Dall *et al.* 1983). The dominant commercial genus is *Penaeus* with 11 species that occur in Australian waters, mostly in the tropical north. The majority of these species are also found in the Indo-Pacific

(*P. canaliculatus*, *P. marginatus*, *P. longistylus*, *P. japonicus*, *P. latisulcatus*, *P. merguensis*, *P. indicus*, *P. semisulcatus* and *P. monodon*). There are only two *Penaeus* species (*P. esculentus* and *P. plebejus*) that are endemic to Australia. *P. esculentus* is distributed around the northern coastline from Shark Bay in Western Australia to northern New South Wales on the east coast. *P. plebejus* is restricted to the eastern coast from southern Queensland to northern Victoria (Grey, Dall *et al.* 1983).

### ***Biology and life history***

Prawns have a multi-phase life history with phase specific environmental preferences. Like all penaeids, *P. esculentus* eggs are practically fertilised externally. Spermatophores are held in a cavity (thelycum), which is part of the exoskeleton. Spawned eggs are fertilised by sperm simultaneously released from the thelycum. When a female moults, the contents of the thelycum are shed as well. Females need to mate and be re-impregnated with a fresh spermatophore at each moult (about 30 days). Eggs hatch into planktonic nauplii that give rise to a succession of planktonic stages, protozoa, mysis and post larvae. The subsequent juvenile stage develops in sea-grass beds. They become available to be harvested by fishers as sub-adults. In stock assessment terms, they 'recruit' as sub-adults.

Crococ (1987b) studied the reproductive dynamics of *P. esculentus* in the western Gulf of Carpentaria and found (1) the mean fecundity for females 28 to 51 mm carapace length was 95,750 to 614, 930 eggs, (2) egg production not uniform over his 6 by 6 nm study area near Groote Eylandt, and (3) egg production was spread throughout the year with a peak in late winter and early spring.

Loneragan *et al.* (1994) studied the occurrence of post larvae in open coastline, reef flat and river mouth sea grass beds in north-western Gulf of Carpentaria. They found that 90% of the post larvae occurred in shallow water less than 2.0 m deep, even though many of the beds extended beyond 2.5 m. They suggested that the shallow water sea grass beds were essential settlement and nursery areas for the successful growth and development of juveniles.

The 'advective envelope' was subsequently defined as the near shore spawning area from which nursery ground populations of juveniles are derived (Condie, Loneragan *et al.* 1999; Rothlisberg, Craig *et al.* 1996). Comprehensive studies on penaeids have shown that larval advection is the essential link between offshore spawning populations and inshore nursery grounds. Post larvae have no capacity for horizontal swimming, and rely entirely on currents to advect them to nursery grounds (Rothlisberg, Craig *et al.* 1996). The mechanism that allows directed movement is selective tidal stream transport (Dall, Hill *et al.* 1990; Rothlisberg 1995). Post larvae that experience inshore tidal currents in shallow water (less than 20 metres) switch their behaviour in the water column to a pattern where the larvae stay close to the sediment in the outgoing tide and move to the surface on the incoming tide. This behaviour allows them to ratchet closer to the coastline where they settle in seagrass beds. Post larvae that do not experience inshore tidal flow are lost as recruits to the population. Condie *et al.* (1999) showed that the size of the advective envelope was strongly dependent on (1) the depth at which larvae commence selective tidal transport and (2) annual changes in the timing and magnitude of tides. A

hydrodynamic model of the Gulf showed that winds had little effect on the size of the advection envelopes (Condie, Loneragan *et al.* 1999).

In Moreton Bay, south-east Queensland, the settlement of juveniles of 2 – 3 mm carapace length into seagrass beds occurs between September and July in distinct cohorts. Juvenile growth was proportional to water temperature and varied from 0.03 to 2.1 mm carapace length per week. The weekly instantaneous natural mortality rates ranged from 5.8 to 25%, which apparently is lower than for other species of *Penaeus*, possibly because of protection from fish predation given by the lush seagrass beds (O'Brien 1994).

Courtney and Masel (1997) used histological methods to determine the number of female *P. esculentus* collected throughout the year from Moreton Bay that had mature or ripe ovaries. In both years (1988 and 1989) 57 to 75% of females were in spawning condition between November and December (late spring). The incidence declined rapidly during summer, and was negligible during winter (February to August). The spring peak in egg production was consistent with the records of seasonal abundance of juveniles (O'Brien 1994). For example, eggs hatched in October will produce pelagic larval nauplii that have a three-week duration ending in settlement of post larvae at around 2 mm carapace length. By end of March at an average instantaneous growth rate of 1.2 mm carapace length per week, juveniles will be 27.2 mm carapace length (Courtney and Masel 1997).

Courtney and Masel (1997) also highlighted the life history differences between *P. esculentus* in northern Australia (10 to 17° S) compared to Moreton Bay (27° 15' S) at the southerly extreme of its distribution in eastern Australia. The size at which females first mature in Moreton Bay (about 30 mm carapace length) is larger than the north (25 mm), and the overall incidence of mature females was lower in Moreton Bay (20% of females larger than 35 mm) compared to northern populations (Gulf of Carpentaria, 70 to 80%). The occurrence of mature females is restricted to a few months in late spring and early summer in Moreton Bay, while in the Gulf of Carpentaria mature females were relatively common (at least 20%) in most months of the year and were never completely absent.

### ***Previous genetic analyses***

The taxonomy and fisheries stock structure of Australian penaeids has been studied in the past using genetic methods. Genetic distance estimates based on 37 allozyme loci placed *P. esculentus* in a clade with *P. semisulcatus*, *P. monodon* and *P. merguensis*. Clade members lacked a structure on the cephalothorax (adrostal sulcus) that was possessed by three other *Penaeus* species, *P. latisulcatus*, *P. longistylus* and *P. plebejus* (Mulley and Latter 1980). This study also reported that heterozygosity at allozyme loci was low. Only 0.6 to 3.3% of individuals were heterozygous across six *Metapenaeus* and seven *Penaeus* species for the 37 loci. An average of 14% of loci were polymorphic. Low heterozygosity in crustaceans was also reported by Redfield *et al.* (1980). They assayed nine species representing one stomatopod and four decapod families from the Gulf of Carpentaria. At most, five loci per species out of 12-33 were polymorphic and heterozygosity was low (0.8 – 6%). Genetic diversity in the Penaeidae may be among the lowest recorded for any animals (Dall, Hill *et al.* 1990).

Subsequent studies of four *Metapenaeus* species and five *Penaeus* species (Mulley and Latter 1981a; Mulley and Latter 1981b) collected throughout their range in Australia revealed geographic genetic differentiation to be low, except for *P. latisulcatus* and *M. endeavouri*. For these two species, populations from the Gulf of Carpentaria and Western Australia were significantly different. *P. esculentus* was sampled from southern and northern Queensland, the Gulf of Carpentaria and Exmouth Gulf in Western Australia and genetic variation assayed for four polymorphic loci; *MPI*, *PGI*, *PGM* and *SDH*. Despite the large geographic difference between sampling sites, no gene frequency differences were detected at any of the four loci. This has been interpreted by some as a lack of evidence for genetic stocks of *P. esculentus* in Australia (eg. Kailola, Williams *et al.* 1993). However, the statistical power to reject the null hypothesis of no genetic differentiation between populations was weak due to the lack of polymorphic genetic loci, and their degree of polymorphism (three to five alleles each, Lavery and Keenan 1995). Condie *et al.* (1999) hypothesised there was at least three stocks of *P. esculentus* in the Gulf of Carpentaria based on the geographic distances between the adjective envelopes of spawning stocks and the lack of evidence supporting the ability of adults to migrate across these distances. It awaits genetic testing.

Lavery and Staples (1990) used the allozyme locus *GPI* to distinguish between the post larvae of the two tiger prawn species (*P. esculentus* and *P. semisulcatus*). It was more reliable than existing morphological techniques.

The haploid chromosome number of *P. esculentus* was determined to be 44 (Xiang, Courtney *et al.* 1996). Other species in the genus have 43 and 45 diploid chromosomes.

Several microsatellite loci were developed for analysing the stock structure of *P. esculentus* in Australia (Lavery and Keenan 1995). As part of the same project part of the cytochrome oxidase I region of the mitochondrial genome was sequenced in a small number of individuals. Three haplotypes were found. One haplotype (C) was found only in Western Australia (Shark Bay) and differed by three fixed single nucleotide polymorphisms from two haplotypes (A and B) collected adjacent to the south and north-east coast of Queensland.

### ***Fisheries characteristics***

#### **Moreton Bay (south-east Queensland)**

Moreton Bay is a coastal embayment on the eastern coast of Australia adjacent to the urban centre of Brisbane, the largest city in Queensland of around three million people. The bay ranges from one to 30 km wide (east-west) and is 100 km long (north-south). It is generally shallow with a maximum depth of approximately 35 m. The substrate ranges from sand to mud. On the seaward side the bay is bounded by low sand islands, Stradbroke in the south and Moreton and Bribie in the north. The 1300 km<sup>2</sup> of the Bay are enclosed, except for a northern opening that is 17 km wide and three narrow openings less than two kilometres wide in the eastern sand islands. The Brisbane River flows into the western edge of the bay.

Three of the 12 penaeid species in Moreton Bay comprise the bulk of the commercial catch. They are *P. esculentus*, *P. plebejus* and *Metapenaeus bennettiae*. The



compulsory fishers' logbook programme revealed that 571 tonnes of prawns were removed annually from Moreton Bay between 1989 and 1994. *P. esculentus* comprise approximately 80 tonnes of this catch (about  $2 \times 10^6$  prawns) that are caught between January and October. Management of the fishery includes limitations on the number and size of vessels, length of trawl head rope, net mesh size and days of the week and areas within the Bay permitted for fishing. However, there is no direct control over fishing effort for any penaeid species on the eastern coast of Australia (Courtney and Masel 1997).

Catch rates of spawning *P. esculentus* declined significantly from 1972-4 to 1989-93. For example, at spawning time in Moreton Bay (October to December) eight to 12 kg/boat/day were caught in the 1970's compared to two to eight kg/boat/day in the late 1980's. At the same time, catch rates of recruits also declined, where recruits are defined as prawns available to the fishery in February to April. Rates were about 50 to 75 kg/boat/day in the 1970's and about 15 kg/boat/day in the late 1980's. During this period, fishing methods and levels of fishing effort in the Bay have remained relatively stable and there is no evidence for environmental causes for population decline (Courtney 1996).

The *P. esculentus* population in Moreton Bay appears to be 'closed' and thus does not experience significant immigration or emigration. Spawning, maturation and recruitment occurs internally in the Bay (Courtney and Masel 1997). Habitat containing sea-grass beds that is necessary for post-larval settlement is not found several hundred kilometres north or south of Moreton Bay.

A comparison of the spawning stock dynamics of the three commercial species of prawns in Moreton Bay led Courtney and Masel (1997) to suggest that *P. esculentus* was the most likely to experience recruitment overfishing. The number of post larvae and juveniles at Pelican Banks decreased in southern Moreton Bay over a twenty-year period (1972/3 to 1991/1 Masel and Smallwood 2000) during which fishing pressure increased, and seagrass beds decreased.

### **Gulf of Carpentaria (northern Queensland)**

The Gulf of Carpentaria is significantly larger than Moreton Bay, having a coastline of over 2500km and an area of approximately 500,000 km<sup>2</sup>. It is less than 70 m in depth and is separated from the Coral Sea in the east by Torres Strait, and the Arafura Sea in the west by a sill extending from Cape Wessel (Northern Territory) to Merauke (Papua New Guinea) (Rothlisberg, Craig *et al.* 1996).

The two tiger prawn species (*P. esculentus* and *P. semisulcatus*) and the banana prawn (*P. merguensis*) are the major resources for the Gulf of Carpentaria (Northern Prawn Fishery). Fishers target schools of banana prawns during the day, and tiger prawns at night. The season for banana prawns begins in March to April. Fishers progressively change to tiger prawn fishing as the banana prawn catch rates decline. The banana prawn season is short (several weeks), but the tiger prawn season extends for eight months with a various closures to fisheries to protect spawning populations (Wang and Die 1996).

Fishing effort in the tiger prawn fishery in the Gulf of Carpentaria has increased significantly over the last 30 years. Catches and catch per unit effort in 1986 (3,487

tonnes) had dropped to half of that of 1981, prompting the application of a stock-recruitment model that assumes recruitment overfishing. Subsequent assessments concluded that the 1993 spawning stock of *P. esculentus* was about 60% lower than that in the mid-1970s and recruitment had decreased by 50% (Taylor and Die 1997). On-going management is aimed at sustainable yields while maximizing economic return.

### ***Stock recruitment relationships***

In their influential book Hilborn and Walters (1992) state that the most important problem in the biological assessment of fisheries is relationship between stock size and subsequent recruitment. Recruitment overfishing occurs when future catches are threatened because over harvesting of adult spawning stock has reduced the production by the population of juveniles. Nowadays, fisheries managers and scientists recognize that if you fish hard enough on any stock, you will reduce recruitment. In the past this view was overlooked in some circumstances because of the enormous individual fecundities of most fisheries species, which has the potential to de-couple adult stock size from subsequent recruit numbers.

Stock recruitment analysis consists of studying the empirical relationship between spawning stock size and the subsequent recruitment of the year class produced by that spawning (Hilborn and Walters 1992). The relationship is affected by some underlying biological processes including

- Density-independent mortality, where the causes of natural mortality for young fish (recruits), such as predation, lack of food and currents, are not related to spawner numbers.
- Compensation, where there is a decrease in recruits-per-spawner as spawning stock increases, perhaps due to predation on eggs by adults. However, the reverse can also occur, when for example, egg predators are swamped by high egg production.
- Migration, where the relationship will be affected if samples are taken from a cryptically subdivided fishery where certain subpopulations are being overfished.

Demonstration of a relationship between spawning stock size and subsequent recruitment depends on accurate measurement over the time course of the excessive exploitation of a species. The ideal measurement of the spawning stock is the number of eggs, which is often estimated by multiplying the number of spawning individuals by the average fecundity. There are other methods including (1) the number of females alive at each age times fecundity by age, (2) the number of individuals alive by age times average fecundity by age, (3) total biomass of individuals at or above age of first reproduction and (4) an index of abundance of the population in the year that the eggs are deposited (Hilborn and Walters 1992, p 254). Using catch per unit effort (CPUE) as an index for spawning stock size is imprecise and has reinforced the notion that there stock recruitment relationships do not exist.

Recruitment is defined as the number of individuals still alive at any specified time after the egg stage. The measurement of recruitment is limited to the stage at which it

is most feasible to measure the number alive. In most cases, this occurs when the recruits enter the fishery.

In the early 1980's there was an active debate about the validity of evidence for stock recruitment relationships in penaeid prawns worldwide. Compared to some fisheries species with long-lived adults (eg. orange roughy), prawns were considered to be relatively resistant to overfishing. At the same time significant declines in the catches from two major West Australian *P. esculentus* fisheries were being observed during periods of high fishing effort. Penn and Caputi (1986; 1992) took advantage of (1) the likely presence of a single stock in the Exmouth Gulf due to its geographic isolation, (2) environmental stability experienced by the population in the desert region and (3) availability of detailed catch and effort data to test for a stock recruitment relationship for *P. esculentus*. Even though the data series was relatively short (1970 – 1984) they clearly demonstrated that a stock recruitment relationship existed, except during those years when particularly severe cyclones occurred.

In one of Australia's most important fisheries, the Northern Prawn Fishery (NPF), Wang and Die (1996) used total catch as an index of recruitment and the catch per unit effort during the spawning season as an index of spawning stock in the tiger prawn (*P. esculentus* and *P. semisulcatus*) fishery in the Gulf of Carpentaria. Their stock recruitment analysis confirmed earlier work that both species were being recruitment overfished, *P. esculentus* since 1983 and *P. semisulcatus* from 1983.

These studies show that despite high fecundity, population numbers cannot be maintained from traces numbers of adults. This implies that the reproductive effort of relatively large numbers of adults are needed to overcome the high levels of natural mortality experienced by life stages between egg and adult.

## **Objectives of this study**

The importance of stock – recruitment relationships in fisheries management and the difficulty of measuring stock size and subsequent recruitment was a major stimulus for this study on innovative methods to estimate spawning stock size. Data sources that provide a new window on population processes make it more likely that the goals of sustainability and maximum economic yield for high fecundity species such as prawns will be achieved. This practical application of genetic marker technology and population genetics theory would not have been possible last ten years ago, but recent advances have put it in reach of the project team, on behalf of the fisheries community.

The broad objectives of this study were to

1. Critically evaluate a variety of mathematical methods of calculating  $N_e$  by conducting comprehensive computer simulations and by analysis of empirical data collected from the Moreton Bay population of tiger prawns,
2. Lay the groundwork for the application of the technology in the Northern Prawn Fishery (NPF), and
3. Produce software for the calculation of  $N_e$ , and to make it widely available.



## Chapter 2 – Genetic estimation of effective population size in Moreton Bay tiger prawns across one to two generations

by JR Ovenden, R Street, SL Peel and D Peel

### Introduction

Complete enumeration of the total number of individuals in a population is difficult, particularly when there are multiple life stages, each of which can occupy a different habitat in which they are often hard to observe. Generally it is the number of breeding individuals, not the total number that is of prime concern. If it is not possible to directly count breeding individuals, sometimes an indicator of breeding activity can be used, such as nests, egg masses, or breeding colonies. Often, the number of breeding individuals is not always the most appropriate measure, as it does not measure their reproductive success. This may depend on such factors as sex ratio of breeding individuals, offspring per individual, numbers breeding in each generation and type of reproduction. Estimation of genetic effective population size incorporates these factors and allows general predictions irrespective of the particular forces responsible (Hedrick 2000).

The discovery of highly polymorphic microsatellite loci for penaeid prawns led Lavery and Keenan (1995) to suggest that genetic estimates of effective sizes in naturally occurring populations could be obtained. Lavery and Keenan (1995) suggested that genetic effective population size estimates could be used to (1) monitor spawning stock size to provide early warning against decreases that may result in recruitment failure through inadequate numbers, (2) compare to long-term, average effective population size as a measure of virgin compared to current spawning stock size, (3) compare to current census size to test hypotheses about life-time reproductive success. We also believe the estimates can contribute in a significant way to analyses of stock recruitment relationships in fisheries populations (Appendix C).

Hedgecock et al (1992) was the first to measure genetic effective population size in prawn populations. They analysed data from four to eight allozyme loci for a cultured population of Kuruma shrimp (*Penaeus japonicus*) over several generations in a lagoon in Italy. The harmonic mean effective population size was about 10 with 95% confidence limits extending from 0.5 to 41. The actual estimates of the mean numbers of prawns participating in breeding in the lagoon from generation one to seven fell within these confidence limits.

The population of tiger prawns (*P. esculentus*) in Moreton Bay is naturally occurring, and has experienced fishing pressure since the 1970's (Courtney 1996). Like many *r*-selected invertebrate species the population does not have a 'genetic bank' (Gaggiotti and Vetter 1999) of long-lived, reproducing adults that would buffer genetic diversity against declines during significant reductions in population size caused by fishing pressure or environmental catastrophes. Maintenance of genetic diversity of tiger prawns depends on spawning success in each season, as few, if any, prawns survive to spawn in the next season. Theoretically, at neutral marker loci, genetic diversity that is lost prior to (natural or fishing mortality), during (ineffective spawning) or after (poor survival of offspring) spawning would be regained in the longer term by *de-novo* mutation or migration. The amount of genetic change (genetic drift) being

experienced by the population at each generation should be detectable by direct genetic measurements and is proportional to the effective population size. In this chapter, we use temporal change in allele frequencies and linkage (gametic) disequilibrium methods to estimate effective population size. This quantity is distinct from long-term effective population size that is calculated from current population genetic diversity and the estimated mutation rate of microsatellite genetic loci.

Effective population size can also be estimated from non-genetic data where it is interpreted as the number of breeding individuals that produce offspring. This quantity can be estimated using two separate processes (1) direct counting of samples taken from the population - an ecological census, or (2) from life history data on populations. The latter approach involves forming a model based on available demographic or behavioural information and certain assumptions to predict  $N_e$  (Nunney and Elam 1994). Kaeuffer et al. (2004) estimated non-genetic effective population size of two populations of feral cats in France using parameters that included total adult population size, adult sex ratio, generation time, mean adult longevity of each sex and mean fecundity. Their estimates were in good agreement with genetic estimates using the moment-based temporal method (Waples 1989); the effective population size was less than 100. In contrast, a direct counting approach was used in this study to estimate the numbers of prawns that were participating in spawning in Moreton Bay (Chapter 4). Unlike the study by Kaeuffer et al. (2004) our census estimated the numbers that were participating in spawning regardless of their subsequent success at producing recruits.

The range for the magnitude of the relationship between effective population size ( $N_e$ ) and population size ( $N$ ) in natural populations of animals is controversial. When the population is constant in size (i.e. two offspring per pair), the effective population size is

$$N_e = \frac{4N - 2}{V_k + 2}$$

where  $N$  is the census population size and  $V_k$  is the variance in the number of progeny per pair. If there is a Poisson distribution of progeny, where the variance and mean is equal to two, then the ratio of  $N_e$  to  $N$  is unity. When the variance is zero (i.e. all families have exactly two offspring), then  $N_e$  exceeds  $N$  and the ratio is two. However, in some organisms, particularly fisheries species, the variance is much larger than the mean progeny numbers as the recruited young of the year may be offspring of only a few individuals. In marine oysters Hedgecock (1993) presented evidence for a low  $N_e$  to  $N$  ratio. Frankham (1995) summarized the value to be 0.1 across a range of terrestrial vertebrates, and Nunney (1996) has shown theoretically that the ratio should only rarely be outside of the range of 0.25 - 0.75. The value of the ratio to assessment of fisheries populations is that it provides information about the variance of reproductive success that encompasses fishing and natural mortality.

The objective of this section is to estimate the current and long-term genetic effective population size of the *P. esculentus* population in Moreton Bay and to compute the ratio between the current effective and census population size of adults at spawning time. The methods used to estimate the population size of adults at spawning time using a direct counting approach are described in Chapter 4.

## Methods

### *Study site and sampling*

In 2001 and 2002 we sampled Moreton Bay *P. esculentus* that were caught by the fisheries research or commercial trawl vessel in specific 6' x 6' grids. The prawns were subsequently counted for direct estimates of the spawning stock size (Chapter 4). In 2003 all prawns were sampled from the Moreton Bay commercial harvest. All were adults larger than 30 mm carapace length and were sampled in October and November. The reproductive stage of females and carapace length of both sexes was recorded.

Approximately 200mg of muscle tissue, with the carapace removed, was dissected from the abdomen and frozen at  $-80^{\circ}\text{C}$  in 1ml of 20% dimethyl-sulphoxide solution containing 5M NaCl.

### *Laboratory*

Genomic DNA was isolated from 10-25 mg of muscle tissue using a commercial kit (DNeasy™ tissue kit Qiagen P/L, P.O. Box 641 Doncaster, Victoria 3108). DNA was eluted into 100µl of buffer AE (Qiagen P/L) and 5µl was electrophoresed in a 1% agarose gel to check concentration and molecular weight. DNA was diluted 1:40 with buffer AE for use in amplification reactions.

Prawns were genotyped for the eight microsatellite loci used in Ward and Ovenden (Appendix B), except that locus CSGES015 was used instead of CGES190. The primers used for locus 015 were CSGES015.100U20 (GC GGT CAG TGT GGG ACT CGA GAG AAA ATC) and CSGES015.405L20 (GCG GTC AGT AAA GAA TAA AAA CCT TAC CG). One locus was a dinucleotide repeat (Pe1.1), six were trinucleotide repeats (PMCD, CGES120, CGES176, CGES189, CGES015 and CGES268) and one was a tetranucleotide repeat (CGES047).

Microsatellite amplifications were performed in 96-well plates using a Perkin Elmer 9700 thermocycler. Reactions (20µl) contained 2µl of PCR buffer ® (Qiagen P/L) containing Tris-HCl (pH 8.7), KCl and  $(\text{NH}_4)_2\text{SO}_4$ ; 4mM  $\text{MgCl}_2$ ; 0.2µM forward primer (labelled); 0.2µM reverse primer; 0.6 units *Taq* DNA polymerase (Qiagen P/L); 125µM dNTP (Pharmacia Biotech); 1% bovine serum albumin and approximately 50ng genomic DNA template. The DNA template and enzyme were denatured at  $92^{\circ}\text{C}$  for 1 min, followed by 35 cycles consisting of  $92^{\circ}\text{C}$  for 10 sec at the locus-specific annealing temperature (Appendix B) for 20 sec and  $72^{\circ}\text{C}$  for 30 sec. A final extension at  $72^{\circ}\text{C}$  for 5 min was used to ensure complete addition of adenine to the PCR product, essential for consistent allele calling during genotyping. Three loci (176, 189 and 047) were amplified in the same reaction. The amount of each forward and reverse primer in the multiplexed PCR was reduced to 75 to 175 pM.

Microsatellite gel separation and scoring were performed by the AGRF (Australian Genome Research Facility, Melbourne Division, Walter and Eliza Hall Institute, Post Office Royal Melbourne Hospital, Victoria 3050). The PCR amplicons were resolved on gels that were 36 cm long and 0.2 mm thick with 96 lanes. The gels were 4.5% polyacrylamide (19:1 acrylamide-bis) with 6M urea. The running buffer was 1X TBE

diluted from 10x TBE solution containing tris-base (108.0g), boric acid (55.0g), and Na<sub>2</sub>EDTA (9.3g) per litre. Gels were run at 3,000 volts for two hours and 48 minutes at 51°C on a 377 ABI Prism DNA sequencer. For sample loading procedure, 10µl of H<sub>2</sub>O was added to the amplification reaction. From diluted samples, 2.5µl was taken and 0.3µl Genescan-500® TAMRA size standard, 0.5µl blue dextran and 1.7µl formamide was added. This solution was then denatured for 3 min at 95°C and then placed on ice for 5 min. 1µl was loaded onto the gels. The software used for analysis was Genescan® Version 3.1.2 and Genotyper® Version 2.1.

### ***Microsatellite genotype scoring***

The size in base pairs of microsatellite amplicons was calculated to two decimal places. Amplicons were allocated to a 'bin' that represented the mean allele size. The accuracy of the bins was assessed using the least-squares method of Idury and Cardon (1997). When their measure of dispersion for a locus ( $S_w$ ) was greater than 0.30 we used the least-squares method with the 'allelic drift' algorithm to evaluate alternate bin sizes. 'Allelic drift' is the tendency for true allele bins to differ by a value slightly different to the known repeat length. They report that dinucleotide markers with large alleles are more likely suffer allelic drift possibly due to the longer traversal time of large DNA fragments before detection in the electrophoresis procedure.

### ***Data analyses***

#### **Gene diversity**

The gene diversity (heterozygosity) per locus was calculated according to equation 8.1 of Nei (page 177, 1987).

#### **Temporal and spatial tests for genetic subdivision within Moreton Bay**

Tests for spatial and temporal genetic heterogeneity are important to understand the locus-by-locus contribution to allelic variation in the data set. The former tests for evidence of heterogeneity among samples taken from the 6'x 6' logbook grids. Temporal tests explore the allelic variation between samples collected in subsequent years.

The probability of heterogeneity based on allele frequencies at eight loci was evaluated using an analogue of Fisher's exact test (Raymond and Rousset 1995a) implemented in GenePop (Morgan 2000). Similarly, spatial non-differentiation of allele frequencies was tested with Fisher's exact test between prawns sampled each year from spatial grids.

#### **Tests for linkage among marker loci**

Methods used to compute effective population size assume unlinked loci. The presence of linkage disequilibrium between all pairs of loci was tested for using genotype frequencies and a log-likelihood test, where the empirical distribution was obtained by a permutation procedure. The test was implemented in Arlequin (Schneider, Kueffer *et al.* 2000).



### **Test for Hardy Weinberg equilibrium**

The absence of Hardy-Weinberg equilibrium across loci may imply a Wahlund effect (mixing of cryptic subdivided populations, or mixing of cryptic cohorts with among-year differences in allele frequencies). If present, temporal fluctuations in proportions of each subpopulation in the population sample may give rise to significant fluctuation in allele frequencies. If these were assumed to be genetic drift,  $N_e$  estimates would be biased downwards.

Deviation of observed genotype frequencies from Hardy-Weinberg equilibrium may also imply the presence of null alleles. These are alleles that do not amplify possibly as a result of individual specific mutations in the primer-binding site in the sequence flanking the microsatellite locus. When present in a homozygous state, no results are obtained from the individual and this inflates the per locus failure rate. When heterozygous, a null allele is present as a single band and subsequently scored as a homozygote rather than a heterozygote. This is the rationale behind the heterozygote deficit test for the presence of null alleles.

An exact test for Hardy-Weinberg equilibrium was implemented in Genepop-on-the-web (Morgan 2000; Raymond and Rousset 1995b). The Markov chain method was used to estimate the exact p-value using a dememorisation number of 10000, in 100 batches with 1000 iterations per batch. Expected numbers of heterozygotes from this test were compared with direct counts of observed numbers.

### **Tests for genotyping errors**

The program Micro-checker (Van Oosterhout, Hutchinson *et al.* 2004) was used to investigate likely causes for deviation from Hardy-Weinberg equilibrium. The program calculates probabilities for the observed number of homozygotes of various allele size classes. If there was an overall significant excess of homozygotes over all size classes then the presence of null alleles were suggested and the method of Brookfield (1996) was used to adjust allele frequencies to conform to Hardy-Weinberg equilibrium. If there were deficiencies of individuals heterozygous for alleles differing by one repeat unit then homozygous excess due to PCR ‘stutter’ was inferred. Large allele dropout was suggested if excess homozygotes were biased towards the extreme end of the allele size distribution.

### **Tests for selective neutrality**

As relatively low levels of some types of selection can have a large influence on the estimated effective population size (Nunney and Elam 1994), we tested for the possibility that natural selection was acting at marker loci or on loci linked to them. We implemented two statistical tests, the Ewens-Watterson and the Lewontin-Krakauer.

The null hypothesis of selective neutrality, under population mutation-drift equilibrium assuming the infinite alleles model of mutation, was tested using the Ewens-Watterson test. This test is based on Watterson’s (1978) procedure in which the distribution of selectively neutral allele frequencies is summarized by the sum of squared allele frequencies. This is compared to the expected allele frequency distribution for any predetermined sample size and number of observed alleles using

the neutral-allele sampling theory developed by Ewens and Watterson. It is made under the infinite alleles model that states that every time there is a mutation, it creates a new allele that does not already exist in the population (Hartl and Clark 1989). The observed and expected homozygosity was compared using exact tests to simulated neutral data with the same number of alleles in Arelquin v 2.0 (Schneider, Kueffer *et al.* 2000).

The Lewontin-Krakauer test (Jorde and Ryman 1996; Lewontin and Krakauer 1973) is a statistical method for testing the selective neutrality of polymorphic loci through time, although not perhaps through space (Baer 1998; Lewontin and Krakauer 1975; Nei and Maruyama 1975; Robertson 1975). The method involves testing the observed value of interlocus variance ( $s^2$ ) of the estimate of  $F$  (Equation 2, Chapter 1) against the theoretical variance ( $\sigma^2$ ) assuming that (1) the gene frequencies are a random sample and (2)  $F$  is the same for all loci. We computed the test statistic

$$X^2 = \frac{s_F^2}{2\bar{F}^2/df}$$

This statistic is distributed approximately as  $\chi^2/df$  where  $df$  is the degrees of freedom. The test was implemented on a locus-by-locus and allele-by-allele basis.

### **Estimates current effective population size**

This section applies to the estimation of current or extant effective population size. Estimation of long-term effective population size is below.

### ***Handling of Missing Values***

#### *Temporal Method*

Equations are available to estimate temporal  $N_e$  based on a single sample size  $S$ . In this project we are confronted with a more complex situation where the data includes missing values, resulting from variable genotyping success rate for each locus. Therefore the allele frequencies for each locus are estimated from a different sample size and hence contain differing sample errors.

The equation we would normally use to estimate the effective population size  $N_e$  is given by

$$\hat{N}_e = \frac{t}{2[\bar{F} - 1/(2S_0) - 1/(2S_t)]},$$

where  $t$  is the number of generations between samples, and  $1/S_0$  and  $1/S_t$  correspond to the expected sampling error of the statistic  $F$ . In the case of multiple loci the  $F$  is given by the weighted means of the single locus values i.e. For  $L$  loci with  $A_j$  alleles at the  $j^{\text{th}}$  locus

$$\bar{F} = \frac{\sum_{j=1}^L (A_j - 1)F_j}{\sum_{i=1}^L (A_i - 1)}.$$

To provide for unequal sample sizes across loci we incorporated the sample error terms for each locus into the weighted calculation of  $F$ .

$$\bar{F}' = \frac{\sum_{j=1}^L [(A_j - 1)F_j - (A_j - 1)/2S_0 - (A_j - 1)/2S_t]}{\sum_{i=1}^L (A_i - 1)},$$

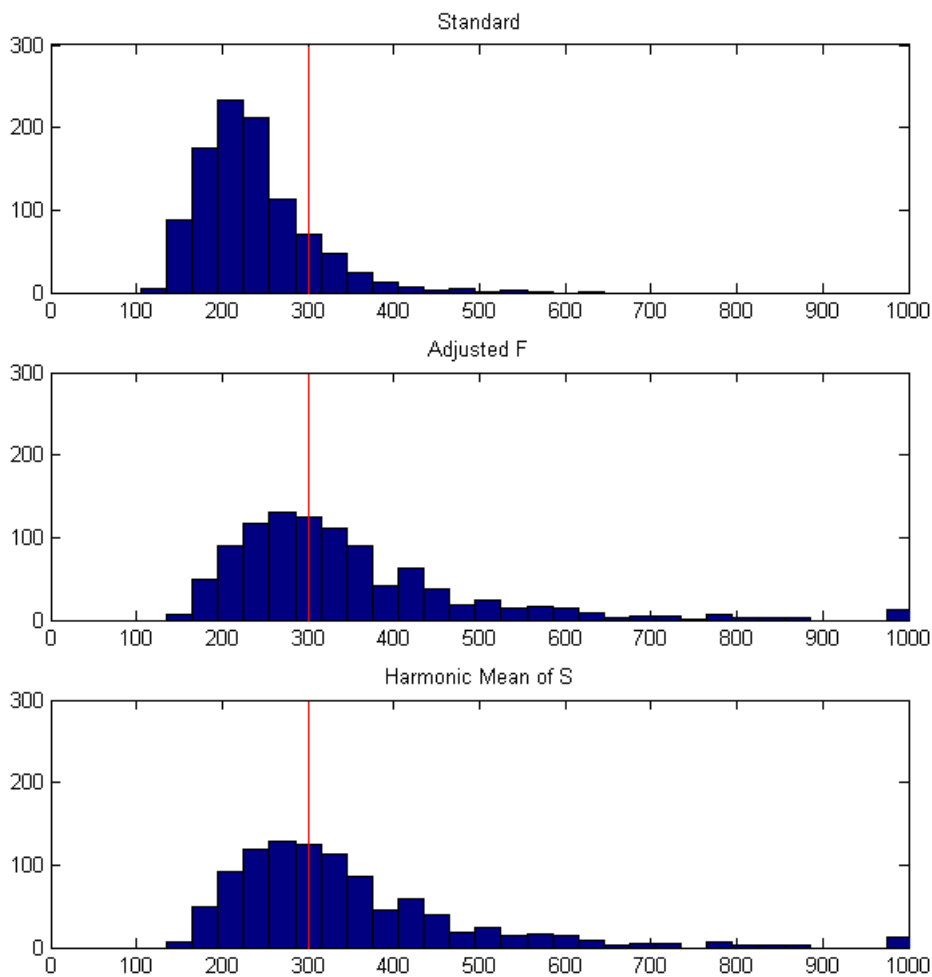
The effective population size is then estimated by

$$\hat{N}_e = \frac{t}{2\bar{F}'}$$

In a similar situation Jehle et al. (2001) used the harmonic mean of the individual loci sample sizes to gain the overall sample size. However, this does not seem to take into account that each locus is providing a weighted contribution to  $F$ , based on the number of alleles at that locus. It would seem more suitable to incorporate the sample sizes weighted by the number alleles as we have proposed. In the case where all loci have a similar number of alleles the two approaches would be almost equivalent.

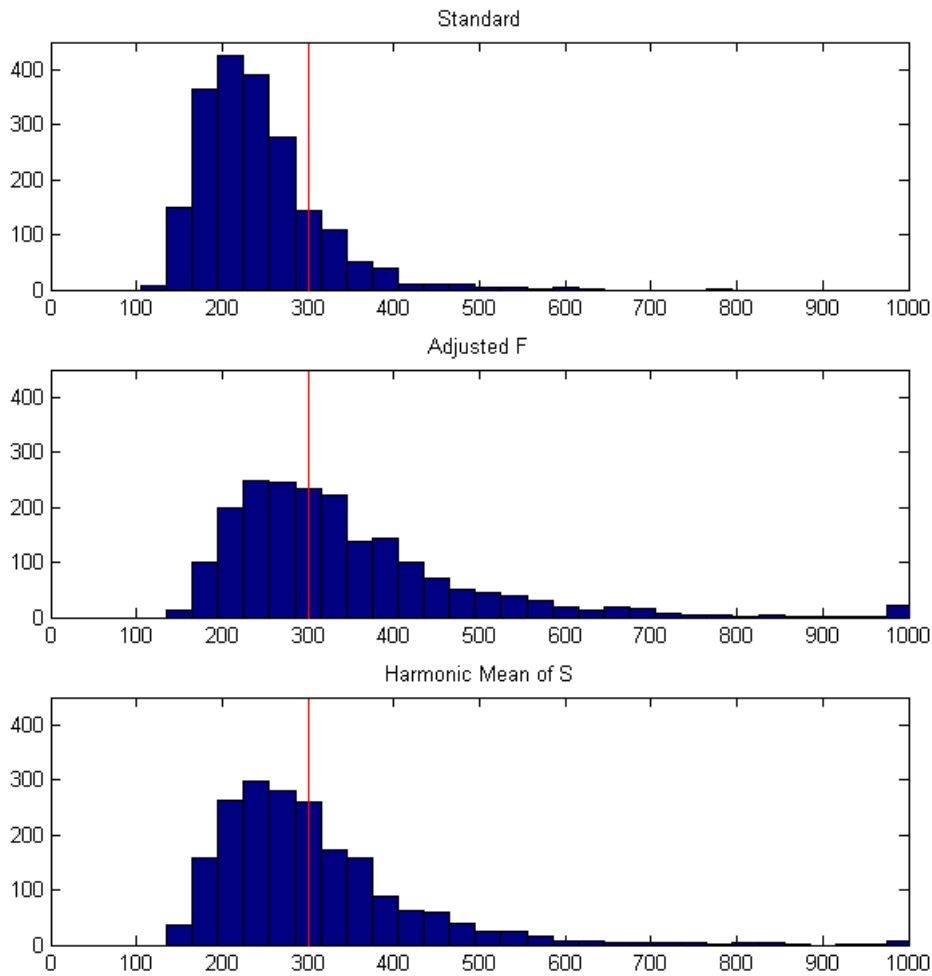
A small simulation experiment was completed to examine the performance of the corrections in the case of missing data. Data was generated using an individual-based model and the individual genotypes of the data assigned to zero (to denote a failure or missing data) with a probability of 0.3 at any given locus. This failure rate of 30% is worse than we have seen in practice but was suitable to fully test the corrections performance.

As can be seen in Figure 2.1 the standard unadjusted calculation, as expected, significantly underestimates the effective population size. This is due to the sampling error being understated since the missing data is presumed present. The excess variation then biases the estimate of genetic drift and hence  $N_e$  is underestimated.



**Figure 2.1: Comparison of correction methods to handle missing data proposed to occur at a rate of 30% (1) standard equation with no adjustment, (2) adjusted  $F$  using our proposed weighted equation and the (3) harmonic mean as used by Jehle et al. The true  $N_e$  is indicated (vertical line), and the frequency (y-axis) of 1000 simulated  $N_e$  estimates (x-axis) are plotted.**

Figure 2.1 shows that the two correction methods are likely to give identical results. But in these simulations, the failure rate was identical across all loci, meaning that our proposed weighting had no effect. In practice, the failure rate can vary greatly across different locus. To provide a more realistic simulation the experiment was repeated with failure rates ranging from 10% to 50% across the individual loci. The results, in Figure 2.2, now show slightly differing results between the two corrections with a slight bias when the weightings are not used (Figure 2.2).



**Figure 2.2: Comparison of corrections to handle missing data proposed to occur at a rate from 1% to 50% across each of eight loci (1) standard equation with no adjustment, (2) adjusted  $F$  using our proposed weighted equation and the (3) harmonic mean as used by Jehle et al. The true  $N_e$  is indicated (vertical line), and the frequency (y-axis) of 1000 simulated  $N_e$  estimates (x-axis) are plotted.**

### *Linkage Disequilibrium*

We also corrected the linkage disequilibrium method for missing data among the prawn microsatellite genotypes. The equation normally used to estimate  $N_e$  using linkage disequilibrium is

$$\hat{N}_e = \frac{1}{3 \times (r^2 - 1/S)} \quad (1)$$

where  $r^2$  is the arithmetic mean of the  $r^2$  value arising from each allele combination. So rewriting (1) we have

$$\hat{N}e = \frac{1}{3 \times \left( \sum_{i=1}^N r_i^2 / N - 1/S \right)} \quad (2)$$

where  $N$  is the number of possible combinations and  $r_i$  corresponds to the  $r^2$  value arising from the  $i^{\text{th}}$  combination. Taking the sample error term into the summation allowed each  $r_i$  to be adjusted for its sampling error,

$$\hat{N}e = \frac{1}{3 \times \sum_{i=1}^N \left( r_i^2 - 1/S_i \right) / N} \quad (3)$$

where  $S_i$  is the number of samples that are not missing in both loci being compared in the particular  $r_i$ .

### ***Temporal Estimates***

Effective population size estimates ( $N_e$ ) were made from allele frequency data from temporally spaced samples over one (2001-2002, 2002-2003) or two (2001-2003) generations. Estimates were made using the following methods implemented in *NeEstimator* version 1.3 (Peel, Ovenden *et al.* 2004)

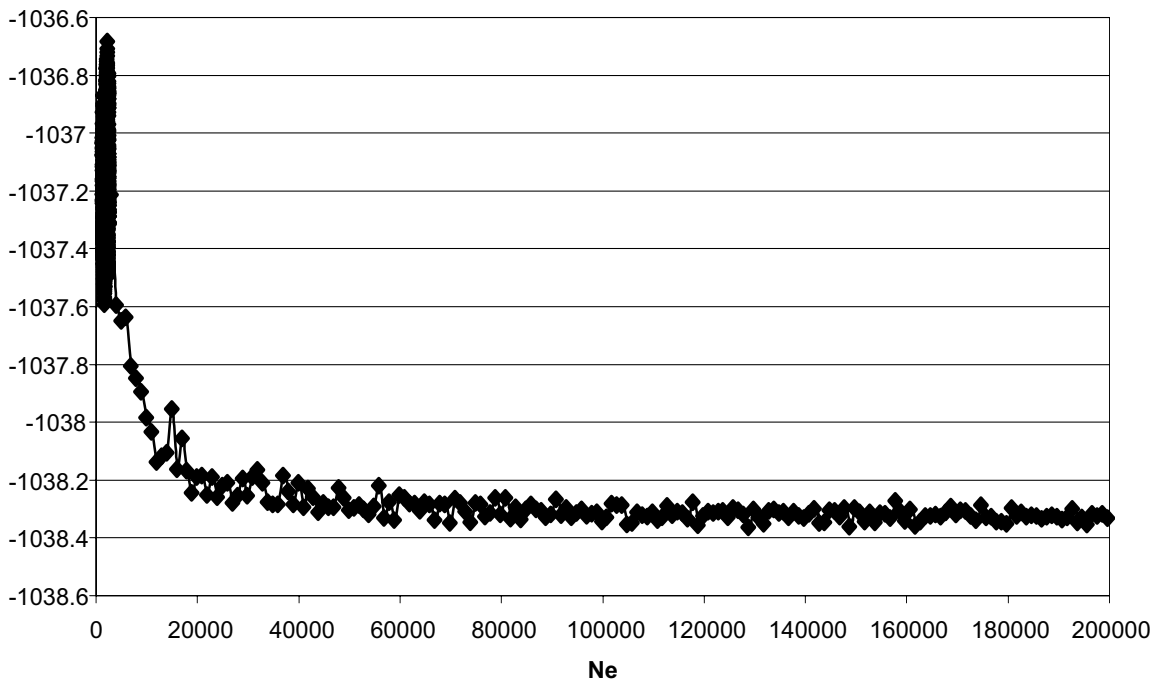
1. Moments-based F-statistics (Krimbas and Tsakas 1971; Nei and Tajima 1981; Pollock 1983; Waples 1989),
2. A temporal method using a maximum likelihood based approach called MCLEEPS (Anderson, Williamson *et al.* 2000), and
3. A temporal method using a pseudo likelihood approach called MLNE (Wang 2001).

We obtained  $N_e$  estimates from MCLEEPS using a process of refinement as suggested in the MCLEEPS documentation. Initially we used a small number of Monte Carlo replicates and a large step size to get a quick first approximation of the log-likelihood curves for each of our data sets. We then chose relevant sections of this curve to investigate at greater accuracy by decreasing the step size and increasing the number of replicates. The areas of interest to us on the log-likelihood curves were

- the maximum log-likelihood value,
- the lower 95% confidence interval,
- the upper 95% confidence interval or the trend of the curve as the  $N_e$  value increased.

We estimated the lower and upper 95% confidence intervals as the values of  $N_e$  where the log-likelihood value was 2.0 less than the maximum log-likelihood value (Williamson and Slatkin 1999). Sometimes it was not possible to estimate the upper 95% confidence interval, as the curve would flatten before decreasing by two (Figure

2.3). In these cases, the upper confidence interval was reported as infinity although the maximum value of  $N_e$  we investigated using MCLEEPS was 200,000.



**Figure 2.3: An example of results from the maximum likelihood method of calculating effective population size (MCLEEPS) with 3000 Monte Carlo replicates.  $N_e$  is the value corresponding to the highest maximum likelihood value. The upper confidence limit of the estimate of  $N_e$  in this case would be infinity, as the plotted values do not decrease by 2 units of likelihood to reach a finite upper 95% confidence limit.**

An example of how the process of successive refinements was implemented follows. From 1000 Monte Carlo replications with a range of 100 to 50100 and a step size of 1000, we surmised that the maximum would be around  $N_e = 1500$ . We performed a second run with the same number of replicates but with a step size of 5 and a range of 100 to 6100. This second run had a maximum at  $N_e = 1385$  and the downward trend of the likelihood estimates suggested that we would be able to get a finite upper confidence interval. A third run with 2000 Monte Carlo replicates around the maximum suggested that the curve was already accurate enough below the maximum but needs more investigation around and above the maximum. Around where we expected the maximum to be we set the step size to be 1, thus estimating  $N_e$  for all integer values between 1000 and 2000. We felt it was important to get this level of accuracy on the maximum, as its log-likelihood value would be used to find the confidence intervals. The maximum log-likelihood of -1060.7 for 3000 Monte Carlo replicates occurred at  $N_e=1025$ . For the lower and upper confidence intervals we would be looking at where the log-likelihood curve was less than or equal to -1062.7.

Below 1000 the results were close to that we had already estimated in previous runs. We reported the lower confidence interval as the first  $N_e$  with

- a log-likelihood less than or equal to -1062.7, and
- all values to the left of it also less than -1062.7.

Above 2000 we ran MCLEEPS with a step size of 100 to make sure that the curve was still falling away from the maximum as for the lower replicates. We reported the upper confidence interval as the first  $N_e$  with

- a log-likelihood less than or equal to -1062.7, and
- all values to the right of it also less than -1062.7.

### ***Linkage (gametic) disequilibrium estimates***

Estimates of effective population size using linkage (gametic) disequilibrium were calculated following Bartley et al. (1992) using Burrow's composite measure of disequilibrium,  $D^*$  (Campton 1987). Simulation studies of Weir (1979) show that even when the mating is random Burrow's  $D^*$  performs the same or better than the maximum likelihood approach. The measure is given by

$$D^* = \frac{2N_{11} + N_{12} + N_{21} + N_{22}/2}{N} - 2pq$$

where the  $N_{ij}$  values are the counts of the corresponding genotypes for allele  $A_1$  at locus  $A$  and allele  $B_1$  at locus  $B$ , for example (Table 1.2). We took  $A_2$  and  $B_2$  to be the composite of all other alleles when  $A_1$  and  $B_1$  are the chosen alleles. A value for  $D^*$  was produced for each pair of alleles across pairs of loci. In the two allele situation all values of  $D^*$  for each allele pair between two locus had the same absolute value.

$D^*$  was standardised using the correlation ( $r$ ) between loci. However the formula given by Bartley et al (1992) (equation below) contained an error

$$r = \frac{D^*}{(p \times (1-p) \times q + (1-q))^{1/2}}$$

and was set to

$$r = \frac{D^*}{(p \times (1-p) \times q \times (1-q))^{1/2}}$$

which agrees with the formula given by Campton (1987)

$$r = \frac{D^*}{([p \times (1-p) + D^*_{\cdot 1}] \times [q \times (1-q) + D^*_{1 \cdot}])^{1/2}}$$

where  $D^*_{\cdot 1} = N_{\cdot 1}/N - p^2$  (the observed frequency of  $A_1A_1$  less its expected frequency,  $p$ ) and similarly  $D^*_{1 \cdot} = N_{1 \cdot}/N - q^2$ .

The arithmetic mean of all of the  $r^2$  values was used to calculate  $N_e$ ,



$$\hat{N}e = \frac{1}{3 \times (r^2 - 1/S)}$$

Confidence intervals were assigned to  $N_e$  using equation (3) and (1) of (Bartley, Bagley *et al.* 1992).

Estimates of effective population size ( $N_e$ ) were made using *NeEstimator* version 1.3 (Peel, Ovenden *et al.* 2004)

### **Estimates of long-term effective population size**

In the absence of selective differences between genotypes and population substructure, when mutation and genetic drift are the principle factors affecting genetic variation, then the equilibrium heterozygosity ( $H_e$ ) in the population under the infinite allele model is

$$H_e = \frac{4N_e\mu}{4N_e\mu + 1}$$

Alternatively,

$$4N_e\mu = \frac{H_e}{1 - H_e}$$

The occurrence of new mutations is generally slow, and population sizes in most species are generally large, the effect of genetic drift is normally small and a long time is required for the conditions of this equation to be satisfied (Hedrick 2000). Consequently, estimates of effective population size based on allelic diversity and heterozygosity are referred to as long-term estimates of effective population sizes.

Mutation rates ( $\mu$ ) for microsatellite loci are estimated to range between  $10^{-5}$  to  $10^{-3}$  per gamete per generation (Bagley, Lindquist *et al.* 1999).

Long-term estimates of effective population size were made under the infinite-alleles model. The infinite alleles model represents an extreme view of microsatellite loci evolution where mutations produce alleles with a random number of tandem repeats. An alternative model is the step-wise mutation model where mutations produce alleles that are a single tandem addition or subtraction to existing alleles.

## **Results**

### ***Characteristics of prawn samples***

#### **Abiotic**

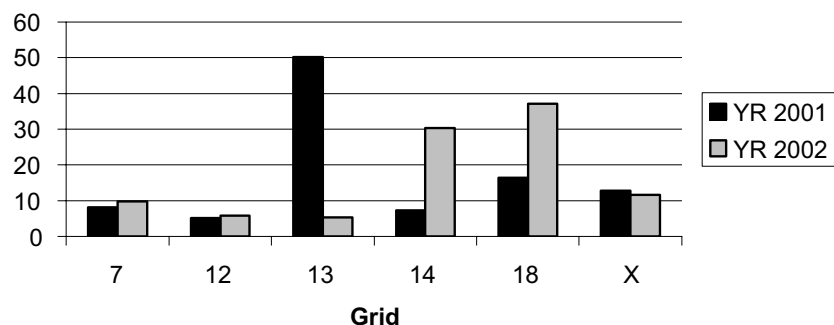
Prawns were sampled from Moreton Bay for genotyping in the spawning season in 2001 (n=655), 2002 (n=685) and 2003 (n=718).

In 2001 and 2002, prawns were captured from pre-determined spatial grids in Moreton Bay for the estimation of number of spawning adults using fisheries survey techniques (Chapter 4). A small number of samples in 2001 and 2002 were taken from

the commercial harvest. In 2003, all samples were obtained from the commercial harvest. The commercial samples were not accompanied by information about spatial grid, depth, temperature or salinity at sampling.

In 2001, survey and commercial prawns were sampled from Moreton Bay on the nights of 30<sup>th</sup> October and 1<sup>st</sup>, 2<sup>nd</sup>, 5<sup>th</sup>, 6<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> November. In 2002, sampling nights were 7<sup>th</sup>, 8<sup>th</sup>, 9<sup>th</sup>, 13<sup>th</sup>, 19<sup>th</sup> and 20<sup>th</sup> November. In 2003, the nights were 28<sup>th</sup>, 29<sup>th</sup> and 30<sup>th</sup> October and 4<sup>th</sup> November.

In terms of the grids sampled, the sampling design for the fisheries census was different in 2001 compared to 2002 (Chapter 4). Consequently, many prawns that were genotyped in 2001 came from grid 13, while in 2002 many prawns came from grids 14 and 18 (Figure 2.4).

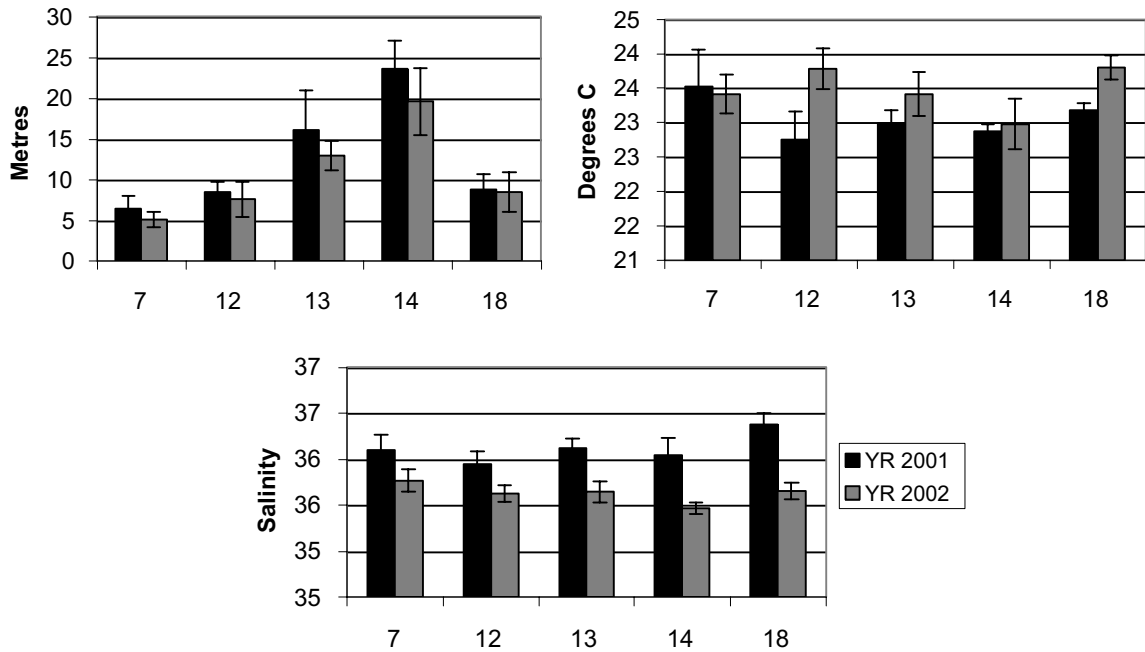


**Figure 2.4: The percentage of prawns (*P. esculentus*) that contributed to the 2001 (n = 655) and 2002 (n = 685) genotyping sample from Moreton Bay grids 7, 12, 13, 14, and 18. Prawns from grid X were sampled from commercial harvest and were not assigned to a grid.**

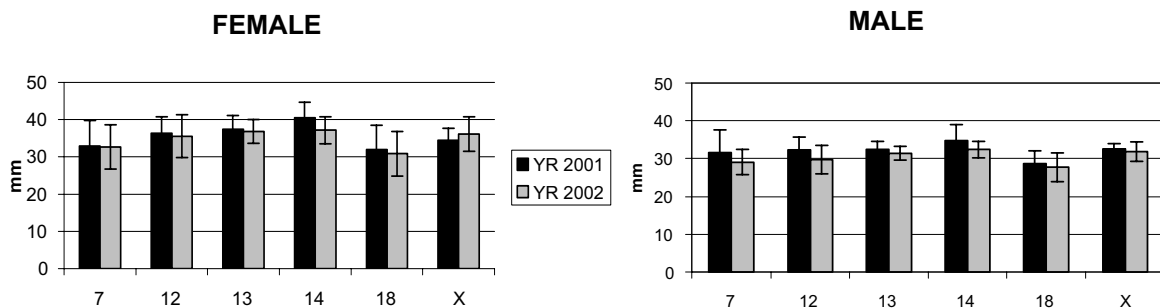
The depth at which the prawns for genotyping were sampled varied according to their sampling location in Moreton Bay. Grid 14 was the deepest (approximately 15 - 25 m) and grids 7, 12 and 18 were the shallowest (5 - 10 m). Water temperature at collection was a consistent 23 - 24°C. Salinity varied between 36 and 37 parts per thousand (Figure 2.5). The apparent significant differences in temperature and salinity between 2001 and 2002 collections may have been related to measuring devices carried by RV Warrego (2001) and the chartered private fishing vessel (2002).

### Biotic

In 2003 female and male prawns were slightly, but not significantly, larger than the 2001 and 2002 samples. The mean ( $\pm$  standard deviation) for females was 38.86 $\pm$ 4.30 mm in 2003. This compares to 36.03 $\pm$ 5.06 for females collected in 2001 and 34.92 $\pm$ 5.33 for females collected in 2002. The mean ( $\pm$  standard deviation) for males was 33.15 $\pm$ 2.93 mm in 2003. This compares to 331.75 $\pm$ 3.85 for males collected in 2001 and 29.81 $\pm$ 3.63 for males collected in 2002. The larger size of 2003 prawns most likely reflects preferential post-catch sorting by commercial fishers.

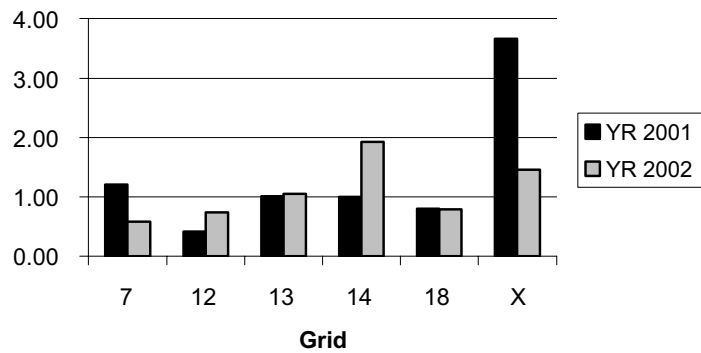


**Figure 2.5: Abiotic characteristics [depth (metres), temperature (degrees C) and salinity (parts per thousand, ppt); means  $\pm$  one standard deviation] for *P. esculentus* collected for genotyping from Moreton Bay in 2001 and 2002 from grids 7, 12, 13, 14, and 18.**



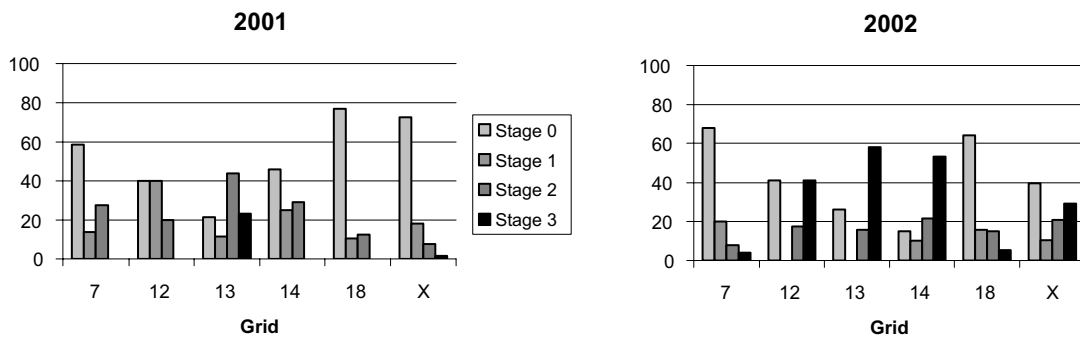
**Figure 2.6: Carapace lengths (mm, means  $\pm$  one standard deviation) for female and male prawns that were included in the genotyping sample for 2001 and 2002, and that were collected from grids 7 – 18 in Moreton Bay. Prawns from grid X were sampled from commercial harvest and were not assigned to a particular grid.**

The ratio of female to male tiger prawns in the genotyping samples taken in 2001 and 2002 was about unity, except for samples taken from grid 14 in 2002 where there was twice the number of females than males. Females were also over-represented in samples taken from the commercial harvest, but this could reflect purposeful selection of the larger females by fishers (Figure 2.7). This was especially true in the commercial samples genotyped in 2003, where the larger females (599) outnumbered males (127).



**Figure 2.7: Ratio of female to male tiger prawns (*P. esculentus*) that were included in the genotyping sample for 2001 and 2002, and that were collected from grids 7 – 18 in Moreton Bay. Prawns from grid X were sampled from commercial harvest and were not assigned to a particular grid.**

There was no theoretical requirement for genotyping samples to be taken from the population at spawning time, provided there is a known temporal interval between them. However, sampling for the fisheries census of spawning prawns was timed to occur just prior to spawning based on previous results (Courtney and Masel 1997). The processes determining reproductive readiness are largely unknown in *P. esculentus*, however in 2002, and to a lesser extent in 2001, a large proportion of females were in an advanced reproductive state (Figure 2.8, Table 2.1).



**Figure 2.8: Percentage of each reproductive stage (0, no development - 3, ready to spawn) of female tiger prawns (*P. esculentus*) that were included in the genotyping sample for 2001 and 2002, and that were collected from grids 7 – 18 in Moreton Bay. Prawns from grid X were sampled from commercial harvest and were not assigned to a particular grid.**

**Table 2.1: Percentage of each reproductive stage (0, no development - 3, ready to spawn) female *P. esculentus* sampled for microsatellite genotyping from Moreton Bay in successive years.**

Reproductive Stage	2001	2002	2003
Stage 0	43	39	27
Stage 1	16	11	15
Stage 2	29	16	29
Stage 3	12	30	29
Unknown	0	3	0
Female Total	357	446	599

### *Microsatellite loci*

#### **Genotyping success rate**

Each prawn was genotyped with eight microsatellite loci, however, for some prawns allelic determination was not achieved at all eight loci. In general, we did not repeat genotyping in these cases. Instead we focused on maximizing sample numbers, rather than obtaining complete eight-locus genotype for all individuals. In this fashion, we genotyped 655 prawns in 2001, 685 in 2002 and 718 in 2003. When genotyping was repeated we found that the likelihood of allelic determination was not improved. Throughout the study we took advantage of every opportunity to optimize locus-by-locus PCR conditions, however we did not achieve this fully. This may have been due to a combination of (1) presence of a PCR inhibitor in DNA extracts from prawn tissue, (2) DNA degradation following extraction, (3) genomic complexity in prawn genomic DNA (eg. excessive repeated sequences) that interfered with primer binding during the PCR.

The most robust loci were 015, 120 and 176 where 91-93% of prawns were successfully genotyped across the three yearly samples (Table 2.3). Locus 268 genotyped 88% of prawns, and loci 047, 189 and Pe1.1 genotyped 81-83%. Locus PMCD genotyped 74% of prawns. Genotyping success rates varied among years, for example, PMCD was 63% successful in 2001, 78% in 2002 and 83% in 2003 (Table 2.3). Attempts to optimize PCR conditions were partly but not fully responsible for fluctuating temporal success rates.

Investigating locus-by-locus failure rates and subsequent conformance of genotype proportions to Hardy-Weinberg equilibrium tested our assumption that genotyping failure was independent of true genotype. Non-conformance could occur if, for example, a heterozygote sample was more likely to fail due to low peak heights. The locus PMCD had the lowest mean success rate (75% over 2001, 2002 and 2003) and subsequent tests (see below) showed that it conformed to Hardy-Weinberg equilibrium. The mean success rate of the three loci that possibly deviated from Hardy-Weinberg equilibrium was 82-83% compared to the success rate of the remaining loci (89-91%). In the case of locus 047 the variation in genotyping success from year to year was not related to conformance to Hardy-Weinberg equilibrium. It

did not conform in all three years (2001, 2002 and 2003), despite wide yearly fluctuations in success rate (2001, 89%; 2002, 68% and 2003, 91%, Table 2.2).

The number of prawns that were genotyped at all eight loci was 236 in 2001, 254 in 2002 and 305 in 2003. The following estimates of effective population sizes were made using the entire data set using *NeEstimator* version 1.3 that implements adjustments for missing data.

**Table 2.2: Percentage of Moreton Bay *P. esculentus* successfully genotyped for eight microsatellite loci in successive years.**

Locus	2001	2002	2003
015	91.15	87.88	92.34
047	89.47	68.76	90.81
120	90.84	89.93	92.62
176	93.44	82.48	94.43
189	87.48	80.15	78.69
268	89.62	84.23	92.76
Pe1.1	79.39	84.23	81.34
PMCD	62.75	78.10	83.15

**Table 2.3: p-values for Hardy-Weinberg equilibrium among *P. esculentus* microsatellite loci in three samples taken from Moreton Bay in successive years (p-values < 0.05 are highlighted).**

Locus Name	2001	2002	2003
015	0.2636	0.0673	0.5611
047	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>
120	0.7116	0.8008	0.5642
176	0.1968	0.3021	0.7427
189	< <b>0.05</b>	< <b>0.05</b>	< <b>0.05</b>
268	0.7244	0.0950	0.1471
Pe1.1	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>
PMCD	0.3315	0.2945	0.0124

### Hardy Weinberg equilibrium

Six of the eight loci conformed to the genotypic proportions of the Hardy-Weinberg equilibrium. Two loci (047 and Pe1.1) deviated from equilibrium ( $p < 0.001$ ) in 2001, 2002 and 2003 (Table 2.3).

The loci 047 and Pe1.1 that deviated from Hardy-Weinberg equilibrium had a deficit of heterozygotes. The deviation of locus 189 was not pronounced (Table 2.4).

**Table 2.4: The observed (O) number of heterozygotes was less than the expected (E) number for the three microsatellite loci (Loci 047, 189 and Pe1.1).**

Locus Name	2001	2001	2002	2002	2003	2003
	E	O	E	O	E	O
047	472	378	372	284	532	414
189	451	436	430	401	476	445
Pe1.1	398	347	437	399	457	412

### Scoring accuracy

Scoring of alleles was accurate according to the guidelines determined by Idury and Cardon (1997). The mean  $S_w$  value for the six tri-nucleotide repeat loci was 0.13 indicating that the average variability around each allele bin was  $\pm 0.38$  bp. The tetra-nucleotide locus (047) varied by  $\pm 0.88$  bp (Table 2.2). The allele size range for each locus is presented in Table 2.2.

**Table 2.5: Normalised standard deviation ( $S_w$ ) for eight microsatellite loci according to the least-squares minimization procedure of Idury and Cardon (1997) on samples of *P. esculentus* from Moreton Bay collected in 2001, 2002 and 2003. Deviations are presented for three binning strategies for locus Pe1.1, where bin size is 1.95, 2 or 2.055 base pairs. The allele size range was determined for loci by comparison to DNA fragments of known size.**

Locus	$S_w$	Repeat type	Allele Size Range (base pairs)
015	0.20	Tri-	316.94 – 390.68
047	0.22	Tetra-	182.91 – 252.46
120	0.06	Tri-	120.58 – 152.97
176	0.11	Tri-	216.81 – 259.05
189	0.10	Tri-	126.12 – 145.72
268	0.15	Tri-	239.94 – 293.99
Pe1.1 bin size 1.95 bp	0.39	Di-	335.21 – 420.07
Pe1.1 bin size 2 bp	0.34		
Pe1.1 bin size 2.055 bp	0.33		
PMCD	0.14	Tri-	188.73 – 226.41

The ‘allelic-drift’ algorithm of Idury and Cardon (1997) was implemented for the di-nucleotide locus Pe1.1 in an attempt to increase the binning accuracy. For a bin size of two base pairs, and without the ‘allelic-drift’ correction, the  $S_w$  was 0.34 suggesting

an average variability around each bin of  $\pm 0.68$  bp. The accuracy of binning increased to  $\pm 0.65$  bp when the bin size was set to 2.055 as determined by the 'allelic-drift' correction method. This bin size indicates that adjacent alleles have an average spacing of 2.055, rather than two base pairs. Idury and Cardon (1997) report that dinucleotide markers with large alleles (eg. over 290 base pairs) are more likely suffer allelic drift possibly due to the longer traversal time of large DNA fragments before detection in the electrophoresis procedure. The allele sizes for Pe1.1 varied between 335 and 420 base pairs (Table 2.5). Bin size of 2.055 was used for subsequent analysis of Pe1.1 data.

**Table 2.6: Observed allele frequencies for locus 047 and allele frequencies that have been adjusted for the presumed presence of a null allele (Brookfield 1996) for samples of *P. esculentus* taken from Moreton Bay in 2001, 2002 and 2003. A single null allele has been proposed.**

Allele	Year 2001 (n=568)		Year 2002 (n=471)		Year 2003 (n=652)	
	Observed	Adjusted	Observed	Adjusted	Observed	Adjusted
183	0.011	0.010	0.030	0.027	0.016	0.015
191	0.030	0.027	0.017	0.015	0.030	0.027
195	0.058	0.053	0.065	0.058	0.077	0.069
199	0.001	0.001	0.002	0.002	0.002	0.001
203	0.034	0.031	0.024	0.022	0.032	0.029
207	0.058	0.053	0.048	0.043	0.052	0.047
211	0.068	0.062	0.078	0.069	0.085	0.077
215	0.252	0.229	0.244	0.219	0.226	0.204
219	0.100	0.091	0.084	0.075	0.104	0.093
223	0.326	0.297	0.358	0.320	0.321	0.289
227	0.019	0.017	0.019	0.017	0.019	0.017
231	0.006	0.005	0.007	0.007	0.010	0.009
235	0.004	0.004	0.002	0.002	0.002	0.001
239	0.002	0.002	0.000	0.000	0.000	0.000
243	0.003	0.003	0.001	0.001	0.001	0.001
247	0.000	0.000	0.001	0.001	0.000	0.000
251	0.028	0.026	0.020	0.018	0.024	0.021
Null	0.000	0.089	0.000	0.104	0.000	0.100

Characteristics of the three loci (047, 189 and Pe1.1) that did not conform to Hardy-Weinberg equilibrium (Table 2.4) were examined in detail with Micro-Checker (Brookfield 1996). For locus 047 for all years (2001, 2002 and 2003) there was a



significant excess of homozygotes at all allele size classes strongly suggesting the presence of a null allele. For this locus, allele frequencies were adjusted for the null allele to contrast with uncorrected data (Table 2.6).

For locus Pe1.1 Micro-Checker showed that there was no large allele drop out despite allele sizes ranging from 335 to 420 base pairs (Table 2.2). Homozygote excess at some allele size classes was insufficient to suggest the presence null alleles. There was a shortage of heterozygote genotypes that differed by one repeat unit (two base pairs). This shortage and the homozygote excess at some size classes were presumably responsible for the lack of conformance to Hardy-Weinberg equilibrium at this locus. For locus 189 there was no lack of heterozygotes that differed by one repeat unit, no large allele dropout, no evidence for the presence of null alleles and some evidence of homozygote excess across size classes. Data from all other loci was shown to be unbiased.

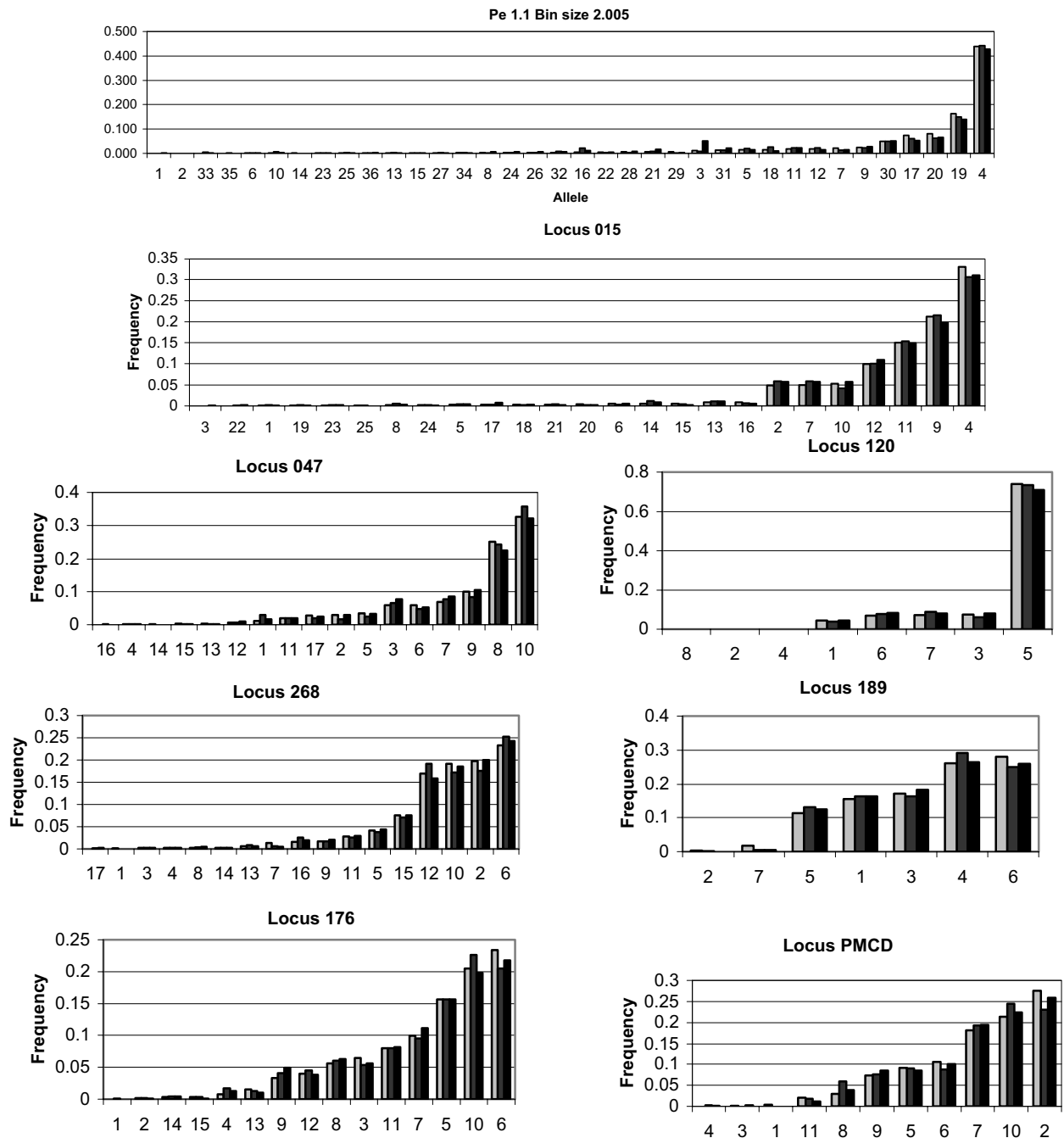
### Allelic variation

The number of alleles per locus per individual ranged from seven (locus 189) to 37 (locus Pe1.1) for all years combined. Allele number fluctuated across years. For example, in the 2001 sample locus 015 was represented by 23 alleles and by 24 in years 2002 and 2003 (Table 2.7).

**Table 2.7: Number of alleles per microsatellite locus for *P. esculentus* in three samples taken from Moreton Bay in successive years.**

Locus Name	2001	2002	2003	All years
015	23	24	24	25
047	16	16	15	17
120	7	5	6	8
176	14	15	14	15
189	7	7	6	7
268	16	16	16	17
Pe1.1	33	34	35	37
PMCD	10	9	10	11

The allele frequencies are presented in Figure 2.9.



**Figure 2.9: Allelic frequencies of *P. esculentus* microsatellite loci (in ascending frequency) for population samples collected from Moreton Bay in 2001 (pale grey bar), 2002 (grey bar) and 2003 (black bars).**

The per locus heterozygosities were high - locus 176 was the most heterozygous (0.85), and locus 120 was the least (0.43 – 0.47, Table 2.8). Mean heterozygosity was generally stable across the samples taken in consecutive years (0.7625, 0.7657 and 0.7742).

**Table 2.8: Per locus heterozygosity for microsatellite loci for *P. esculentus* in three samples taken from Moreton Bay in successive years.**

Locus Name	2001	2002	2003
015	0.8058	0.8174	0.8205
047	0.8055	0.7902	0.8160
120	0.4351	0.4423	0.4767
176	0.8521	0.8562	0.8585
189	0.7874	0.7834	0.7869
268	0.8327	0.8303	0.8323
Pe1.1	0.7630	0.7819	0.7838
PMCD	0.8187	0.8241	0.8189
Mean	0.7625	0.7657	0.7742

**Linkage disequilibrium**

In the 2001, 2002 and 2003 population samples from Moreton Bay, no linkage was detected between the eight loci ( $p > 0.05$ ).

**Selective neutrality**

The hypothesis of selective neutrality under the infinite allele model was rejected by the Ewens-Watterson test for three loci (176, 189 and PMCD) in the 2001, 2002 and 2003 samples and an additional locus (047) in 2003 (Table 2.9). For these tests, the observed  $F$  (sum of squared allele frequencies) was 40 to 70% less than the  $F$  expected under the infinite allele model for the given sample size and allele number. This implied that the observed allele frequency distribution was too even and some low frequency alleles may be missing from the sample. The observed to expected  $F$  ratio for locus 268 also implied a frequency distribution that was too even, although it was not significant. The absence of low frequency alleles under the infinite allele model was reflected by the difference between the observed and expected number of alleles for these loci (Table 2.9). The most extreme case was locus 189 where less than half of the expected number of alleles was observed (2001, expected 18 alleles, observed 7; 2002, expected 17, observed 7; 2003, expected 18, observed 6).

Two loci (015 and 120) conformed to neutral expectations in the three yearly samples.

The observed  $F$  for one locus (Pe1.1) was greater than the expected  $F$  suggesting that the most frequent allele was too common. The observed number of alleles for this locus was 33 to 35 for each yearly sample and the number expected under the infinite alleles model was 15 to 17.

**Table 2.9: Ewens-Watterson test for selective neutrality under the infinite alleles model (IAM) for eight microsatellite loci assayed in *P. esculentus* sampled from Moreton Bay in successive years, where expected F is homozygosity expected under the infinite alleles model, and observed F is homozygosity calculated by the sum of squares of allele frequencies.**

	Locus							
	015	047	120	176	189	268	Pe1.1	PMCD
<b>Year 2001</b>								
2N	1194	1172	1190	1224	1146	1174	1042	822
Observed number of alleles	23	16	7	14	7	16	33	10
Expected number of alleles under IAM	20	19	5	26	18	23	15	20
Expected F	0.197	0.276	0.508	0.310	0.509	0.275	0.134	0.384
Observed F	0.194	0.194	0.565	0.148	0.213	0.167	0.237	0.182
Watterson's <i>F</i> p-value	0.592	0.074	0.469	0.016	0.004	0.105	0.961	0.014
	NS	NS	NS	S	S	NS	NS	S
Comments	Neutral	Too even	Neutral	Too even	Too even	Too even	Common too high	Too even
<b>Year 2002</b>								
2N	1204	942	1232	1130	1098	1154	1178	1070
Observed number of alleles	24	16	5	15	7	16	34	9
Expected number of alleles under IAM	21	17	5	27	17	23	16	21
Expected F	0.190	0.266	0.616	0.289	0.506	0.274	0.132	0.430
Observed F	0.190	0.201	0.557	0.143	0.217	0.170	0.236	0.175
Watterson's <i>F</i> p-value	0.566	0.183	0.461	0.039	0.005	0.117	0.967	0.004
	NS	NS	NS	S	S	NS	NS	S
Comments	Neutral	Too even	Neutral	Too even	Too even	Too even	Common too high	Too even
<b>Year 2003</b>								
2N	1326	1304	1330	1356	1130	1332	1166	1194
Observed number of alleles	24	15	6	14	6	16	35	10
Expected number of alleles under IAM	22	21	6	28	18	23	17	21

Expected $F$	0.193	0.293	0.564	0.312	0.556	0.281	0.129	0.403
Observed $F$	0.180	0.184	0.524	0.141	0.213	0.168	0.217	0.180
Watterson's $F$ p-value	0.530 NS	0.046 S	0.502 NS	0.010 S	0.011 S	0.136 NS	0.768 NS	0.046 S
Comments	Neutral	Too even	Neutral	Too even	Too even	Too even	Common too high	Too even

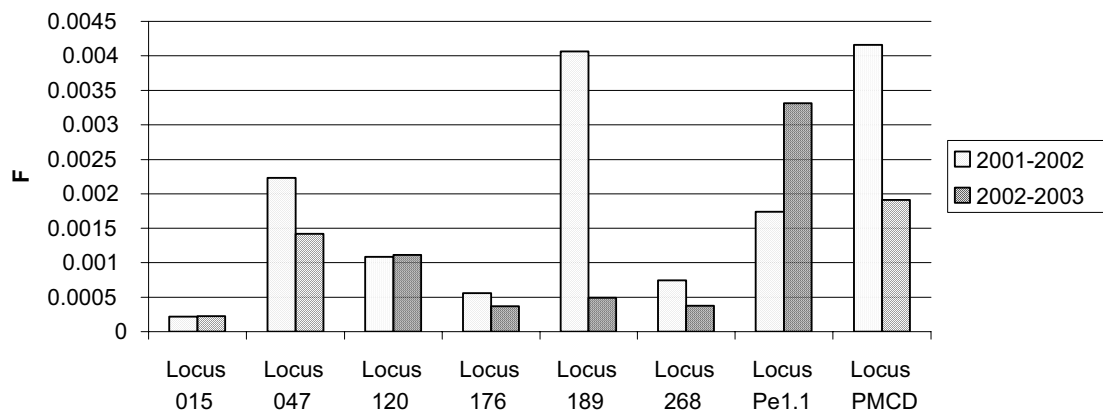
**Table 2.10: Amount of allele frequency change ( $F$ ; equation 1, Chapter 1) adjusted for sample size ( $S$ ) and tests for heterogeneity of  $F$  among loci for samples of *P. esculentus* taken from Moreton Bay in successive years.  $X^2$  is the Lewontin-Krakauer test statistic that has a  $\chi^2$  distribution with  $(k-1)/k$  d.f. for the locus-by-locus tests and d.f. = 1 for the allele-by-allele tests where  $k$  = number of alleles.**

	Locus								
	015	047	120	176	189	268	Pe1.1	PMCD	$X^2$
Locus-by-locus									
2001-2002									
No alleles	24	17	7	15	7	17	34	11	
$F$	0.001049	0.003182	0.001910	0.001411	0.004958	0.001646	0.002657	0.005214	2.592348 <sup>NS</sup>
$F - 1/S$	0.000215	0.002236	0.001085	0.000562	0.004066	0.000742	0.001744	0.004157	
2002-2003									
No alleles	25	16	6	15	7	16	34	11	
$F$	0.001013	0.002306	0.001891	0.001173	0.001388	0.001176	0.004174	0.002796	2.286349 <sup>NS</sup>
$F - 1/S$	0.000223	0.001416	0.001110	0.000369	0.000490	0.000372	0.003313	0.001912	
Allele-by-allele									
2001-2002									
$F$ per allele	0.001005	0.002994	0.001637	0.001317	0.004249	0.001581	0.002579	0.004740	0.973048 <sup>NS</sup>
2002-2003									
$F$ per allele	0.000973	0.002162	0.001576	0.001095	0.001190	0.001103	0.004051	0.002796	4.854463 *

The Lewontin-Krakauer test for heterogeneity in the amount of allele frequency change per locus across the two intervals (2001-2002 and 2002-2003) was non-significant when the test was implemented on locus-specific  $F$ -values (Table 2.10). This implied that there was no allele or locus specific effects such as natural selection, genotyping errors or non-equilibrium on the estimates of genetic drift across these

periods. A significant result was obtained for the period 2002-2003 when the test was implemented on allele-specific F-values (Table 2.10). Trials were conducted by omitting data from this test one locus at a time. This identified locus Pe1.1 as contributing the bulk of the heterogeneity to the F-values. Further work is needed to clarify the apparently different levels of statistical power for the two alternative methods (locus or allele-specific) of implementing the Lewontin-Krakauer test; both computer simulations and theoretical studies would be appropriate.

The locus-specific F-statistics for 2001 to 2002 and 2002 to 2003 are shown in Figure 2.10.



**Figure 2.10: Magnitude of F (per locus variance in allele frequency change, equation 2, Chapter 1) for eight microsatellite loci for the *P. esculentus* population in Moreton Bay across two temporal intervals of one generation in length (2001 to 2002 and 2002 to 2003). In this figure, genetic drift was not adjusted by  $1/S$  where  $S$  was the per locus sample size.**

### Spatial and temporal genetic heterogeneity

#### *Spatial*

There was no significant difference in allelic frequencies between population samples collected from grids 7, 12, 13, 14 and 18 in Moreton Bay in 2001 and 2002 (Fisher's exact test,  $p > 0.05$ ).

#### *Temporal*

There was a significant difference in allelic frequencies between population samples collected from Moreton Bay in 2001, 2002 and 2003 at loci 189, Pe1.1 and PMCD (Table 2.11). The  $p$ -value of a fourth locus (047,  $p = 0.067$ ) also revealed variation in allele frequencies between years.

**Table 2.11: Locus-by-locus probability of differentiation (Fisher's exact test) and standard error of probability (S.E.) between allelic frequencies at eight microsatellite loci from samples of *P. esculentus* collected from Moreton Bay in three successive years (2001, 2002 and 2003).**

Locus	p-value	S.E.
015	0.952	0.008
047	0.067	0.012
120	0.321	0.031
176	0.754	0.023
189	0.010	0.004
268	0.854	0.019
Pe1.1	<0.001	<0.001
PMCD	0.036	0.011

### *Current effective population size estimates*

#### **Temporal estimates**

##### *Over one generation*

Using the moments-based method, the temporal estimate of effective population size for *P. esculentus* in Moreton Bay in 2001 (temporal interval 2001 – 2002) was 797 (Table 2.12). The corresponding estimate using the maximum likelihood method (MCLEEPS) was 1165. The pseudo-maximum likelihood estimate (MLNE) was 1013. The upper 95% confidence limits of these estimates were 4182 (moments-based), and 2950 (MCLEEPS) and 2887 (MLNE). The lower confidence limits varied between 366 and 700.

Simulation experiments predicted that these estimates would be likely to have non-infinite upper confidence limits. For example, if the true effective population size was less than 100, then sampling 600 individuals in two consecutive years should yield estimates that were non-infinite 96 to 100% of the times (Figure 5.6 and Figure 5.26). Simulation experiments also predicted that maximum likelihood estimates would exceed moments-based estimates. According to the simulations all methods slightly overestimate the true effective population size, so the estimate for Moreton Bay may be slightly less than 797 (Figure 5.7 and Figure 5.27).

The temporal estimates of effective population size for 2002 (temporal interval 2002 – 2003) were similar to the previous period (2001). The moments-based estimate was 866, and the maximum likelihood methods were 1304 (MCLEEPS) and 1087 (MLNE, Table 2.12). Their confidence intervals spanned from 392-710 to 3072-4960, which showed they were not significantly different from the 2001 estimates.

**Table 2.12: One generation estimates of effective population size ( $N_e$ , with upper and lower 95% confidence limits) for *P. esculentus* in Moreton Bay made using temporal methods. Three temporally based methods (moments, and two maximum likelihood methods) were used. Estimates were made by setting a maximum  $N_e$  of 25000 for the MLNE method and a maximum  $N_e$  of 50000 for MCLEEPS method. Confidence intervals for the maximum likelihood methods are approximate and were calculated using a likelihood value of negative 2.**

Temporal Interval	Applies to the effective population size of	Method	$N_e$	Lower confidence limit	Upper confidence limit
2001 to 2002	2001	Moments based	797.9	366.1	4182.0
		MCLEEPS ~	1165.0	700.0	2950.0
		MLNE	1013.8	580.5	2887.7
2002 to 2003	2002	Moments based	866.7	392.8	4960.5
		MCLEEPS ~	1304.0	710.0	3130.0
		MLNE	1087.3	624.4	3072.4

### *Over two generations*

Temporal genetic effective population size was also estimated from this data over two generations, from 2001 to 2003. The moments-based estimate was 2798 and the maximum likelihood estimates were 2877 (MCLEEPS) and 2543 (MLNE, Table 2.13).

**Table 2.13: Two generation estimates of effective population size ( $N_e$ , with upper and lower 95% confidence limits) for *P. esculentus* in Moreton Bay made using temporal methods. Three temporally based methods (moments, and two maximum likelihood methods) were used. Estimates were made by setting a maximum  $N_e$  of 25000 for the MLNE method and a maximum  $N_e$  of 50000 for MCLEEPS method. Confidence intervals for the maximum likelihood methods are approximate and were calculated using a likelihood value of negative 2.**

Temporal Interval	Applies to the effective population size of	Method	$N_e$	Lower confidence limit	Upper confidence limit
2001 to 2003	2001 - 2002	Moments based	2798.1	1020.3	Infinity
		MCLEEPS ~	2877.0	1430.0	8800.0
		MLNE	2543.3	1235.4	19191.7

The simulation experiments (Chapter 5) predicted that the statistical power of two generation estimates would be greater than one generation estimates. The logic behind this is that for the same sample size, the amount of drift over two generations would be more than over one generation that would be more likely to have non-infinite upper confidence limits. However, although MCLEEPS and MLNE estimates have finite



upper confidence limits, the moments-based estimate that we obtained here has an infinite upper confidence limit (Table 2.13). This suggests that overall the statistical power of two generation estimates is not as high as one generation estimates.

However, the upper confidence intervals of the MCLEEPS and MLNE estimates for the two generation estimates encompassed those of the one-generation estimates. For example, for the MCLEEPS and MLNE methods the upper confidence limits of one generation estimates (2,900, 3,100 and 2,800 and 3,000) is within the equivalent estimates for two generation estimates (8,800 and 19,000, Table 2.13). This shows that there may be no significant difference between the one and two generation estimates.

In theory, the  $N_e$  over a large temporal interval is the harmonic mean of the  $N_e$ 's of the intervening temporal intervals (Hartl and Clark 1989). Estimates of effective population sizes made over a longer temporal interval (for example, 10 years) may conform to the predictions of higher statistical power, and it may important in the future to test this.

There is an alternate method of calculating the average  $N_e$  over two generations. This is the equivalent of averaging the  $N_e$  from 2001-2002 and 2002-2003, and is calculated from the average F-statistic per locus from the first and second period. For this data the 'averaged'  $N_e$  is 825.9 with 95% confidence intervals of 462 - 2083. Note that the confidence intervals are even tighter than the one generation estimates. This is due to the inclusion of three sets of temporal samples in the calculations. The software *NeEstimator* that was produced as part of this project does not implement this method of calculating average  $N_e$ .

### Linkage disequilibrium estimates

Genetic estimates of effective population size made using the linkage disequilibrium method have the advantage of not needing samples over a temporal interval, so have a great deal of inherent appeal. The genetic data from the population sampled in 2001, 2002 and 2003 yielded estimates of effective population size in the preceding years (2000, 2001 and 2002).

**Table 2.14: Estimates of effective population size ( $N_e$ ) with upper and lower 95% confidence limits for *P. esculentus* in Moreton Bay made using the linkage disequilibrium method. Estimates were made using population samples taken in 2001, 2002 and 2003 and apply to the effective population size of the preceding year.**

Applies to the effective population size of	Population sample	$N_e$	Lower confidence limit	Upper confidence limit
2000	2001	926.4	747.5	1199.1
2001	2002	3583.4	1953.4	17263.0
2002	2003	10939.3	3532.1	infinity

The linkage disequilibrium estimate for 2000 was 926.4 (95% CI 747.5 – 1199.1,

Table 2.14). There was no temporal  $N_e$  for 2000 to compare to this. The estimate for the next year (2001) was significantly larger than for 2000; 3583.4 (95% CI 1953.4 – 17,263.0). It is also larger, but not significantly, than the temporal estimate for 2001; for example the MCLEEPS estimate was 1165.0 (95% CI 700.0 – 2950.0). Simulation experiments predicted that linkage disequilibrium method would overestimate effective population size compared to the maximum-likelihood temporal method (Figure 5.27) and a preliminary correction was proposed (Chapter 5).

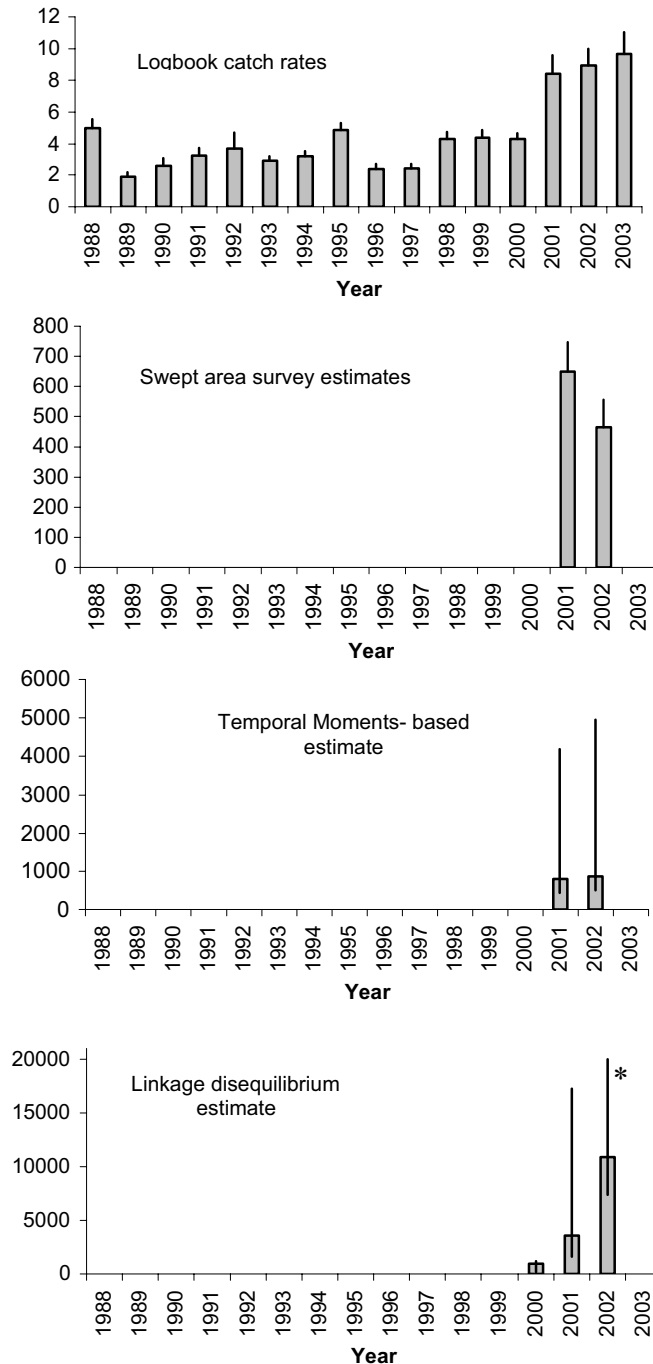
Overestimation may also be the case for the linkage disequilibrium estimates for 2002, which are both larger than the linkage disequilibrium estimates for the preceding year and larger than the temporal estimates for the same year. The infinite upper 95% confidence limit on the 2002 estimate (10,939.3, 95% CI 3532.1 – infinity) was predicted by the simulation experiments where the true effective population size was set to 10,000 in the individual-base model (Figure 5.28).

### ***Relationship between genetic estimates of effective population size and spawning population size***

In Chapter 4, two methods of directly counting spawning prawns in Moreton Bay were implemented; swept area census and fishers logbook data. The stability between the temporal effective population size estimates in 2001 and 2002 mirrors the stability in the logbook estimates of kilograms of prawns caught during the spawning season (Figure 2.11). As discussed in Chapter 4, the swept area survey estimates show a decline in numbers from 2001 to 2002, compared to the logbook estimates that show a stable catch rate.

The linkage disequilibrium estimates made in three subsequent years span the period in the logbook data that show a contrast between low catches prior to 2000 compared to higher catches from 2001 onwards. The linkage disequilibrium estimates reflect this upward trend from 2000 to 2002. However, the linkage estimates in 2001 and 2002 do not show the stability of the temporal genetic estimates and the logbook estimates, but neither do the swept area census estimates for 2001 and 2002 (Figure 2.11).

Its important to note that any relationships, or lack of relationships between the a) survey estimates of spawning stock size, b) commercial logbook catch rate data and c) equilibrium estimates of spawning stock size are very preliminary and require several more annual estimates in order to confirm any co-variance.



**Figure 2.11: Comparison of commercial logbook, swept area survey, and moments-based temporal estimates of spawning stock size for tiger prawns *Penaeus esculentus* in Moreton Bay. All estimates apply to the population during the spawning season (October-November). Vertical bars are 95% confidence intervals. See Chapter 4 for methods used to obtain the fisheries estimates. Starred (\*) upper 95% confidence interval is infinite.**

Nunney and Elam (Nunney and Elam 1994) describe the difficulties in interpreting  $N_e/N$  ratios from published studies and suggest the best definition of  $N$  is the number of adults, where adulthood is defined by likelihood of breeding. Our estimates of  $N_e/N$  use the number of adults in the population at spawning time that have a reasonable expectation of participating in breeding (Chapter 4). In accordance with

the suggestion by Wright (1938), we took  $N$  as the harmonic mean of  $N_0$ 2001 and  $N_1$ 2002, such that

$$\frac{1}{N} = \left( \frac{1}{2N_{2001}} + \frac{1}{2N_{2002}} \right)$$

$$\frac{1}{N} = \left( \frac{1}{2,648,000} + \frac{1}{2,464,000} \right)$$

$$N = 540,777$$

The genetic effective population size estimate was taken as the mean of the moments-based estimates for 2001 and 2002 (797 and 866), as the maximum likelihood estimates are known to be over-estimates (Chapter 5). Thus, the  $N_e/N$  ratio for *P. esculentus* in Moreton Bay during 2001 and 2002 was 0.00154.

An alternate approach would be to calculate separate  $N_e/N$  ratios for each temporal period. For the 2001-2002 period the ratio was 0.001230 and for the 2002-2003 period it was 0.001865, which gives an average of 0.00154. The results are the same for the two methods of calculating the ratio.

### ***Long-term estimates of effective population size***

The average of heterozygosity ( $H_e$ ) for the eight microsatellite loci ranged from 0.4515 for locus 120 to 0.8556 for locus 176, and averaged 0.7675 across loci for the three years of sampling (Table 2.6 and Table 2.12).

There was a seven-fold difference between the estimates of  $4N_e\mu$  for various loci (Table 2.15), most likely indicating a difference in the mutation rate per locus.

Assuming the mutation rate was  $10^{-4}$  per locus, the long-term estimates of effective population size for *P. esculentus* in Moreton Bay were 2,000 to 14,000 across loci based on the infinite alleles model. These estimates were sensitive to variations in average mutation rate. For example, tenfold decrease in the average mutation rate from  $10^{-4}$  to  $10^{-5}$  resulted in a tenfold increase in the long term effective population size estimates.

Assuming average mutation rates of  $10^{-4}$  for the eight microsatellite loci, the long-term effective population size of the Moreton Bay population of *P. esculentus* was five to ten times larger than the current (extant) effective population size.

**Table 2.15: Estimates of long-term effective population size under the infinite alleles model (IAM) for eight microsatellite loci assayed in *P. esculentus* sampled from Moreton Bay in 2001, 2002 and 2003. The standard deviation (SD) is for the error associated with estimating heterozygosity (He) in three successive years. The term  $4N_e\mu$  was calculated by assuming that it was equal to  $He(1-He)$ . The mutation rate ( $\mu$ ) was set at  $10^{-4}$  per gamete per generation.**

Locus Name	He	SD	$4N_e\mu$	Long-term Ne
015	0.8146	0.0077	4.4	10982
047	0.8039	0.0130	4.1	10249
120	0.4514	0.0222	0.8	2057
176	0.8556	0.0032	5.9	14813
189	0.7859	0.0022	3.7	9177
268	0.8318	0.0013	4.9	12360
Pe1.1	0.7762	0.0115	3.5	8672
PMCD	0.8206	0.0031	4.6	11433
Mean	0.7675	-	3.3	9968

## Discussion

### *Accuracy and precision of Ne estimates*

The positive aspects of the accuracy and precision of the estimates of genetic effective population size that are presented in this chapter for the tiger prawn population in Moreton Bay are

1. Successive temporal genetic estimates were similar in magnitude,
2. All but one of the genetic estimates had finite upper confidence limits indicating a high degree of statistical power, and
3. Two independent methods of calculating genetic effective population size (temporal and linkage disequilibrium) gave similar results.

We have confirmed the predictions of simulation experiments (Chapter 5). When genetic effective population size is small, as it is in the population of tiger prawns in Moreton Bay, genotyping 600-700 individuals can produce statistically powerful estimates over one generation with eight polymorphic microsatellite loci.

An important issue to be resolved in the future is the overestimation of effective population size by the linkage disequilibrium method. The computer simulations in Chapter 5 show that the latter method overestimates effective population size for all but small populations by about 25%. Applying this correction to the linkage disequilibrium estimates for 2001 in Table 2.13 reduces Ne to about 2700 compared to 3583. This makes it more in line with the temporal estimates. Furthermore, the performance of the linkage disequilibrium method was shown to be less accurate with

allele frequency distributions that were skewed rather than random (Chapter 5). This may also account for the discrepancy between the temporal and linkage disequilibrium estimates, as the Moreton Bay allele frequencies were not random, but skewed (Figure 2.10).

### ***Short-term compared to long-term $N_e$***

A bottleneck or decline followed by recovery in population size reduces genetic diversity in proportion to the magnitude of the bottleneck and the number of elapsed generations (Nei, Maruyama *et al.* 1975). This is reflected by measurements of long-term population size, that stay depressed until the population returns to mutation/migration/drift equilibrium. During this time, the population continues to experience genetic drift, so estimates of current and long-term effective population size can be compared. If a population is at equilibrium, then long term and short term effective population size will be similar.

In a study of red drum (*Sciaenops ocellatus*), a fin-fish from the northern Gulf of Mexico, Turner *et al.* (2002) reported that the current (short-term)  $N_e$  (3516; 95% CI 1785-18148) calculated from variance in microsatellite allele frequencies and long-term  $N_e$  (317 to 7266 depending on per-gene mutation rate) yielded nearly identical estimates to  $N_e$ . They cited this as evidence that neither contemporary or historical fluctuations had occurred in adult population size. Hauser *et al.* (2002) also found that long term and current genetics estimates of population size of the New Zealand snapper (*Pagrus auratus*) in a coastal embayment were similar, although low (long term  $N_e$ , 46; current  $N_e$  176 with 95% confidence limits of 80-720) that they linked to excessive fishing pressure since the 1950's

The long-term effective population size estimates for *P. esculentus* in Moreton Bay may have been influenced by a past decrease in population size, and consequent reduction in genetic diversity, that may have occurred when prawn populations on the east coast of Australia became physically isolated by rising sea levels. Populations could have been isolated in shallow embayments such as Wallis Lakes (NSW), Moreton Bay (Queensland) and Hervey Bay (Queensland) since the last glacial (10-15,000 years ago, Chappell and Shackleton 1986; McManus 1985) as the extent of shallow-water habitat on the coast of eastern Australia was reduced. Genetic equilibrium in Moreton Bay may have returned since then in this species with a short generation time. Alternatively, the long-term effective population size may reflect species-wide levels of genetic diversity that are maintained by small numbers of migrants per generation. This is supported by similarity between levels of heterozygosity in adjacent populations on the Western Australian coast (Exmouth Gulf; 0.74, Shark Bay 0.75). Heterozygosity levels between Gulf of Carpentaria and Moreton Bay differ (0.69 compared to 0.76) suggesting that migration may not occur on this spatial scale (Appendix B).

Unlike the studies on red-drum and New Zealand snapper, the long-term and current effective population size for the tiger prawn population in Moreton Bay differ by an order of magnitude, at least, depending on the rate of mutation assumed for microsatellite loci. This could imply that there has been a decrease in the size of the tiger prawn population in Moreton Bay. The decrease could be linked to anthropogenic effects such as environmental degradation or harvesting, or more long-

term climatic cycles that could affect population size through environmental productivity.

### ***Selective neutrality***

Relatively low levels of some types of selection at marker loci or on loci linked to them can have a large influence on the estimated effective population size (Nunney and Elam 1994). Two statistical tests were implemented to determine if the amount of allele frequency change through time and locus-per-locus were likely to be due to factors other than sampling error or random genetic drift, such as selection or genotyping errors.

The Ewens-Watterson test determines if the genetic diversity (ie. as homozygosity) in a population sample of known size with a certain number of different alleles is consistent with neutrality (ie. no selection) hypothesis under the infinite alleles model. Five (047, 176, 189, 268 and PMCD) of eight microsatellite loci in *P. esculentus* samples from Moreton Bay had allele frequency distributions that were too even, although not significantly so in the case of locus 268. This deviation from neutrality could be attributed to balancing selection acting to promote existing genetic diversity. However, other explanations could be departures from the assumptions of the model, such as (1) failure of the population to be at mutation-drift equilibrium, (2) a mutation model other than the infinite allele model.

We applied the Ewens-Watterson test to microsatellite data from other *P. esculentus* populations (Appendix B) to contrast to the test performed on the Moreton Bay population samples. Loci 176, PMCD, 189 and 268 were tested for neutrality in the larger populations of the Gulfs of Carpentaria (NT, Qld) and Exmouth (WA) and Shark Bay (WA) in population samples of approximately 100 tiger prawns. All loci conformed to neutral expectations at Shark Bay, three loci (PMCD, 189 and 176) conformed at Exmouth Gulf and two (189 and 176) in the Gulf of Carpentaria (results not shown). While it is possible that a locus would experience balancing selection in one population but not other, it would be unlikely. We conclude that the significant deviation from the Ewens-Watterson model for the Moreton Bay samples is not due to departure from selective neutrality but to departure from another assumptions, possibly the mutation model (IAM).

The step-wise mutation model (SMM), rather than the infinite allele model (IAM) may be more accurately describe evolution in microsatellite loci, or they may have characteristics of both models (Luikart and Cornuet 1998). Under the SMM, mutations can occur to existing states, whereas under the IAM mutations always result in new, non-existing states. For a given data set, the IAM predicts a higher equilibrium (expected) homozygosity than the SMM. For microsatellite data from *P. esculentus* in Moreton Bay, expected homozygosities calculated under the SMM would have been more similar to the observed homozygosities, and loci would have been more likely to appear neutral with respect to selection.

Other violations of the Ewens-Watterson model may be tested for in the data; for example, population expansion following a recent bottleneck. A significant freshwater flood event in Moreton Bay in the summer of 1974 may have caused a significant population bottleneck. Survival of juveniles may have been affected by the reduced extent of seagrass beds that was caused by freshwater scouring in Moreton Bay.

Cornuet and Luikart (1996) proposed that following a bottleneck the mutation-drift equilibrium is transiently disrupted and heterozygosity will exceed the heterozygosity calculated assuming SMM or IAM. A locus-by-locus sign test can be performed to test deviation from expectations. This test has not been applied to the Moreton Bay data, because despite large population sample sizes, the number of loci (eight) is below the 10-20 loci recommended by Cornuet and Luikart (1996) for adequate power.

The outcome of the Lewontin-Krakauer test supported the selective neutrality of bulk of microsatellite loci used to measure genetic drift. Only one locus (Pe1.1), in the period 2002-2003 only, had an amount of allele frequency change that could be considered to be divergent from the change in remaining loci, indicating that perhaps it was influenced by factors other than drift. Despite this divergent amount of allele frequency change the estimates of  $N_e$  for the 2002-2003 period were similar to the previous period (2001-2002). Furthermore, the locus Pe1.1 was relatively free of genotyping errors that could have been due to allele binning and large allele dropout.

Hedgecock (1992) suggested that an independent, a posteriori test of whether alleles behave as neutral markers is to compare the number of alleles remaining in the population with the numbers expected to remain if the estimated effective population size was the true effective population size. Our measurements of the effective population size of tiger prawns in Moreton Bay are finite, but large. The expected number of alleles in would be unlikely to be significantly different from the observed number unless effective population size was smaller.

### ***Locus-by-locus departures from Hardy Weinberg equilibrium***

In this study there was evidence that two loci (Pe 1.1 and 047) may not conform to Hardy-Weinberg proportions. However, for locus Pe1.1 there was no significant evidence for the presence of null alleles or genotyping errors. Furthermore, the Ewens-Watterson test and the Lewontin-Krakauer test showed that random drift at this locus, rather than other evolutionary forces such as natural selection, were most likely to be responsible for deviation of allele frequency variance from the remainder of the loci. For locus 047, there were two sources of evidence for the presence of null alleles. Microchecker (Van Oosterhout, Hutchinson *et al.* 2004) analysis showed significant evidence of null alleles based on the consistent overestimation of homozygote frequencies compared to the expected frequencies. The study by Ward *et al.* (Appendix B) showed that genotypic proportions of locus 047 in *P. esculentus* populations around northern Australia conformed to Hardy-Weinberg equilibrium except in the Moreton Bay population, strongly suggesting the presence of a null-allele in Moreton Bay. Frequencies for 047 that were adjusted for the presence of null alleles by Microchecker (Van Oosterhout, Hutchinson *et al.* 2004) compared favourably to the unadjusted allele frequencies.

### ***Ne/N ratio***

Under the basic assumptions of an 'ideal' population, the distribution of the number of progeny (or gametes) per parent ( $k$ ) approaches the Poisson distribution when  $N$  is large. One of the most important characteristics of the Poisson distribution is that the mean number of progeny ( $k$ ) is equal to the variance in the number of progeny (Hedrick 2000). In some species many factors (genetic, environmental or accidental)



produce a non-Poisson distribution of progeny. This is most likely to be the consequence of family-related mortality where whole batches or clutches of eggs survive or perish as a group. If the population is constant in size (ie  $k = 2$ ), then the effective population size is

$$N_e = \frac{4N - 2}{V_k + 2}$$

$V_k$  is the variance in the number of progeny and  $N_e$  is inversely proportional to  $V_k$ . Variance in number of progeny is the same as variation in family size and variance in reproductive success. In some organisms, with type III survivorship curves (high fecundity and high mortalities in early life) such as oysters that produce large numbers of progeny and most of the recruited young in a given year may be from a few individuals, then  $V_k$  is large and consequently  $N_e$  can be very small.

Nunney (1995) compares the theoretical size of the ratio  $N_e/N$  when populations are overlapping or non-overlapping. When they are non-overlapping, reproduction is limited to one season only and  $N_e$  is exposed to the effects of extreme mating systems and short-term environmental instability. Thus, low ( $N_e/N < 0.25$ ) values may occur. This is important because if the ratio is markedly below 0.1, then random drift can be strong although the population is large. Consequently, a population that is highly resistant to random extinction because it has a large population size can lose genetic variation and suffer the effects of low genetic diversity.

Furthermore, Nunney (1996) found that variation in female fecundity was unlikely to reduce the  $N_e/N$  ratio below 0.1 unless there was high family mortality (90% or more). Hedgecock et al (1992) and (Hedgecock 1993) published the lowest example to date of an  $N_e/N$  ratio ( $\approx 10^{-6}$ ) obtained for a natural population of oysters in Washington state, US based on allozyme gene frequencies between samples collected in two consecutive years.

This study has demonstrated that the ratio between  $N_e$  and  $N$  for *P. esculentus* in Moreton Bay in 2001 and 2002 was 0.00154. This is similar to a ratio of 0.001 for a vertebrate fin-fish species (red drum) in the Gulf of Mexico, US (Turner, Wares *et al.* 2002). For tiger prawns the ratio implies that the variance in family size is large, and on average only one in every thousand matings produce recruits that make it to the breeding population in the next generation. Although mortality is experienced throughout the life cycle, theory shows that the ratio is more affected by family mortality. This suggests that the majority of mortality (eg. 90% *sensu* Nunney) experienced by prawns occurs early in the life cycle when family members are spatially and temporally associated. If this is true, protection of spawning animals, and protection of spawning and larval habitat is most likely to lead to increased resource size resulting in significant benefits to fishers. Furthermore, if most of the mortality that contributes to genetic drift is experienced early in the reproductive cycle and empirical estimates of drift can be made on individuals sampled directly after this mortality, then the genetic effective population size estimates have the potential to predict the subsequent size of recruitment, or the subsequent spawning stock size.



## **Chapter 3 – Estimation of genetic effective population size over multiple generations using wax-embedded prawn specimens.**

*by JR Ovenden, R Street and AJ Courtney*

### **Introduction**

The polymerase chain reaction (PCR) takes minute amounts of specific DNA and produces larger quantities making further analysis possible. Conservation and forensic genetics depend upon it entirely and most other fields that involve molecular biology or genetics also depend upon it to a certain extent. Some components of the reaction are readily controlled. These include (1) sequence similarity between small lengths of artificially created DNA sequence (primers) and the target, or template DNA, (2) the enzyme (DNA polymerase) that is tolerant to multiple changes in temperature from 50 to 96°C and is readily available in many forms from commercial suppliers, and (3) other components such as magnesium and dinucleotide concentration. However, the integrity and purity of the starting or template DNA is often out of direct experimental control despite the availability of a large number of commercial and generic methods to extract and purify template DNA.

Higuchi and colleagues (1987) were the first to report the application of PCR on preserved tissue from an extinct animal (quagga) that allowed its genetic relatedness to zebras to be determined. Since then PCR has provided DNA for the analysis of plants and animals often thousands of years old (Austin, Smith *et al.* 1997). Estimation of effective population size by genetic methods requires temporally spaced population samples, and the larger the temporal spacing the smaller the confidence limits of the estimate (Hedrick 2000). Biological research institutions often possess preserved specimens from which DNA can be extracted and amplified using PCR. The allele frequencies of these samples can be compared across generations to make estimates of effective population size with tight confidence limits.

Wax-embedded tissue from Moreton Bay *Penaeus esculentus* have been stored at the Southern Fisheries Centre since 1989. The wax blocks contain preserved ovary and muscle tissue. Preliminary DNA extractions to recover template from the wax-embedded tissue suggested that PCR may be used to amplify microsatellite alleles from these samples. Genetic estimates of the mean effective population size of the population over the period 1989 to 2003 could be made by comparison to extant allele frequencies. It was expected that the statistical power of estimates made over 12 generations will be larger than those of estimates made over one or two generations. Computer models were used to simulate the effect on the statistical power of genetic estimates of effective population size when the size of the temporal interval between population samples of Moreton Bay tiger prawn samples was increased.

The objective of this chapter was to determine the allele frequencies of eight microsatellite loci (Chapter 2) from archived Moreton Bay tiger prawns. A critical step in this procedure was the evaluation of the integrity and purity of template DNA extracted from wax-embedded prawn tissue for subsequent PCR of microsatellite alleles.

## Methods

### *Tissue samples*

*P. esculentus* ovary and muscle tissue was collected from Moreton Bay in 1988-90, during or subsequent to spring spawning (October to January). Thawed tissue was dissected in the laboratory from adults previously frozen on board the research trawler, and fixed in buffered formalin solution. It was desiccated with xylene and embedded in paraffin wax blocks that were later used for histology mounts (Courtney and Masel 1997). The blocks were stored at room temperature in the dark until 2002.

*P. esculentus* tissue was collected during 2002 from adults captured in Moreton Bay and held at 4°C on board the research trawler. About 10-20 mg of tissue was stored in DMSO solution (20% dimethyl sulphoxide in 5M sodium chloride) at room temperature in the field and – 80°C in the laboratory (see Chapter 2 for further details).

### *Template DNA isolation*

#### **DNeasy Tissue Kit**

DNeasy™ tissue kit (Qiagen P/L, P.O. Box 641 Doncaster, Victoria 3108) was used to recover DNA from prawn tissue preserved in paraffin wax or DMSO solution

For wax-embedded tissue (1) a small (<25 mg) section was finely chopped with a sterile scalpel blade and 1200 µl of either xylene or HistoClear™ (National Diagnostics, 305 Patton Drive, Atlanta, Georgia 30336 US) was added to dissolve the wax, (2) vigorous vortexing was followed by centrifugation at full speed (13000 g) for 5 min at room temperature, (3) 1200 µl of absolute ethanol was added to the tissue pellet, gently vortexed and spun as before and (4) pellet was washed again with ethanol, and then air dried at 37°C for 10-15 min. A paraffin wax section containing no tissue was similarly treated in a separate tube to monitor the removal of wax from the tissue.

Genomic DNA from the wax-derived pellet or DMSO-preserved tissue was extracted using the DN-easy protocol for animal tissue, and eluted into 100 µl of storage buffer (AE). Complete lysis of the tissue pellet was ensured by the addition of an extra 180 µl of lysis buffer (ATL) and 20 µl of proteinase K solution.

#### **Chelex**

The Chelex method was also used to prepare prawn genomic DNA as PCR template. Using sterile scalpel and forceps a pinhead amount of DMSO-preserved tissue was soaked for three hours in 1 ml STE (100mM NaCl, 10mM Tris-HCl ph 8.0, 1mM EDTA). The tissue was placed into 500µl of 10% Chelex 100 (Biorad P/L, P.O. Box 210 Regents Park, New South Wales 2143) solution, and 5µl Proteinase K (20mg/ml) added.

For wax-embedded prawn tissue, a finely chopped section (< 25 mg) was placed in Chelex 100 solution and heated to 80°C for 10 min, then spun for five minutes at 13,000 g at room temperature. The wax plug on the surface was pierced and the

Chelex solution containing the tissue was withdrawn to a fresh tube to which proteinase K was added .

For DMSO and wax-preserved tissue, the Chelex solution was incubated for three hours or overnight at 55°C, with gentle mixing from time to time. It was then boiled for eight mins, cooled to room temperature and 55µl TE solution was added. The solution was again gently mixed and centrifuged for five mins at 13,000 g. Supernatant was removed into clean, labelled tubes and stored at -20°C.

### **Levi's method**

Levi's method was the third method evaluated for the production of template DNA for PCR.

Several thin sections of paraffin embedded prawn tissue were incubated with proteinase K (10 mg/ml) in a detergent solution (400 µl of Levi lysis buffer, 10mM Tris-HCL, 50mM KCL, 2.5mM MgCl<sub>2</sub> with 18 µl of 10% Tween 20) overnight at 55°C to hydrolyze proteins that were covalently linked to the DNA, as well as other proteins in the tissue. After denaturing the proteinase K by boiling for 15 mins, the sample was centrifuged (5,000 g for two mins). The aqueous phase was removed from under the paraffin layer and 8 µl of 0.05M EDTA was added for DNA storage.

### **RN-ase digestion**

RNase A (DNase free, Sigma-Aldrich P/L, Sydney Australia) was added to 15 µl of genomic DNA extract to a final concentration of 50 µg/ml in 20 µl. Aliquots were removed during incubation at 37°C after 10, 20 and 30 mins.

### ***PCR Amplification***

Eight microsatellite loci (120, 189, 047, PMCD.001, 176, 268, PE 1.1 and 015) developed specifically for *P. esculentus* were amplified using PCR methods (Chapter 2). Briefly, the initial denaturation step was 92°C for 1 min, followed by subsequent denaturing steps at 92°C for 10 sec. The annealing temperature was either 60 or 64°C for 35 cycles. Extension temperature was 72°C for either 30 or 60 secs and the final extension step was five mins. The primer concentration per primer pair was 5 µM, with 0.8 mM dNTP's and 5 µl of a 1:40 template dilution. Multiplexed PCR's used 0.3 µl per reaction, and single reactions used 0.12 µl. The reaction volume was 20 µl.

### ***Agarose Electrophoresis***

Amplicons from microsatellite PCR amplifications and extracted genomic DNA were visualised on 2% and 1.5% agarose gels, respectively, using Tris-Borate-EDTA buffer. Gels were run at 80 volts for approximately 45 mins. They were stained with ethidium bromide for 10 min, destained in water for 10 mins, and photographed on a UV lightbox with a polaroid camera. Each gel included DNA size standards.

### ***Inhibition Test***

The potential of genomic DNA extracted from wax-embedded prawn tissue to inhibit microsatellite allele amplification was tested by comparing the amplification of a

control sample in either the presence or absence of wax-derived DNA. The control sample was amplified in two sets of four reactions containing (1) normal strength, (2) 1:10, (3) 1:100 and (4) 1:1,000 diluted template, respectively. Wax-derived genomic DNA (5  $\mu$ l) at normal template strength was added to the second set of control template reactions.

### ***ABI electrophoresis and scoring***

For allele scoring using laser detection, microsatellite loci were amplified with fluorescently labelled primers (Chapter 2). Loci were labelled with blue (047, PMCD and PE 1.1), green (189 and 268), or yellow (120 and 176) labels. Gel separation and scoring was performed at the Australian Genome Research Facility (AGRF, Melbourne).

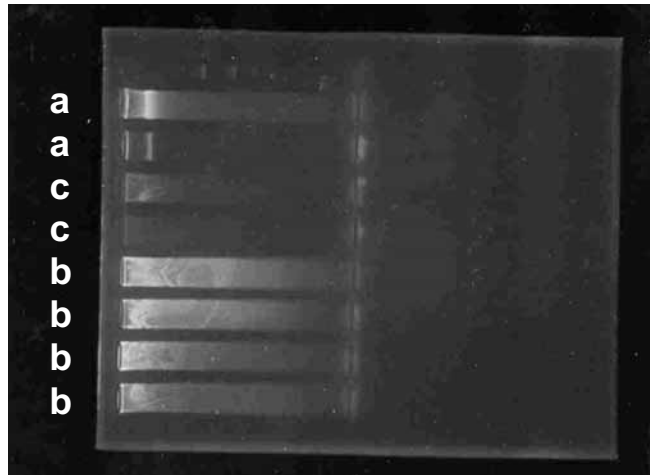
### ***Statistical evaluation***

The expected increase in statistical power on estimates of genetic effective population size made over a temporal interval of 12 generations compared a temporal interval of one generation to make was tested. This was done by comparing estimates of effective population size produced from an individual-based model (Chapter 5) where effective population size was known. Upper and lower 95% confidence limits were calculated for a given effective population size ( $N_e$ ) and sample size ( $S$ ) by substituting  $F$  (equation 11) into equation 16 (Waples 1989). The evaluation was performed assuming that eight microsatellite loci could be resolved for the fresh tissue, but only one locus for the wax preserved tissue.

## **Results**

### ***Genomic DNA extracts from ovary and muscle prawn tissue preserved in wax.***

Genomic DNA extracted from *P. esculentus* muscle preserved in DMSO for a short period (around six months) contained high molecular weight molecules discernable on an agarose gel as a bright band near the origin. Muscle tissue preserved in paraffin wax for a longer period (about 12 years) did not show this high molecular weight band, but electrophoresis showed that nucleic acids were present in a smear from large to small molecules. Paraffin-preserved ovary tissue apparently yielded more nucleic acid than muscle tissue across the same molecule size range (Figure 3.1).



**Figure 3.1: Genomic DNA from *P. esculentus* tissue stored in DMSO (a, muscle) and paraffin wax (b, ovary; c, muscle), extracted using the DN-easy tissue kit™.**

These results were confirmed using the Chelex method of DNA extraction (results not shown). As DN-easy extracted DNA was supposedly more free from contaminants and potential PCR inhibitors, the Chelex method of extracting DNA was discontinued.

***Initial amplification of *P. esculentus* microsatellite loci with DNA from wax-embedded tissue as template.***

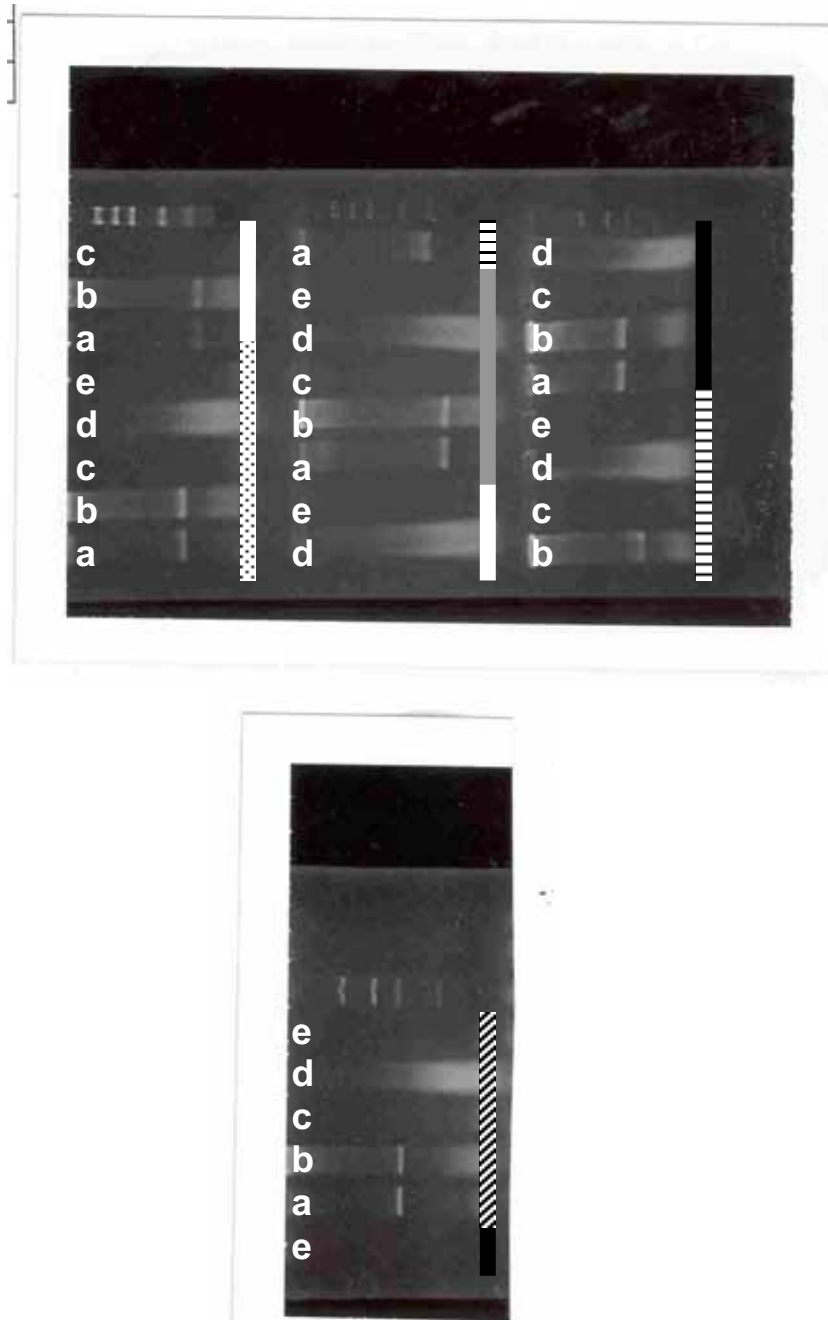
Alleles were amplified for six of the eight *P. esculentus* microsatellite loci, and were visualised on an agarose gel (2%). Genomic DNA from muscle and ovary tissue preserved with DMSO and stored for about six months gave discernable bands in the predicted size range. Similar tissue preserved in paraffin wax and stored for about 12 years did not yield visible bands (Figure 3.2).

We proposed that PCR template from wax-embedded tissue may contain substances that may be inhibiting the amplification reaction as gel electrophoresis of the template (Figure 3.1) had showed that template of sufficient length was present.

***Removal of potential PCR inhibitors***

**RNA**

Comparison of RN-ase A treated and untreated genomic DNA derived from wax-preserved tissue showed that the majority of nucleic acid in the extraction was not DNA, but RNA (Figure 3.3). However, subsequent amplification of RNA-free DNA template derived from wax-preserved prawns was unsuccessful (results not shown).

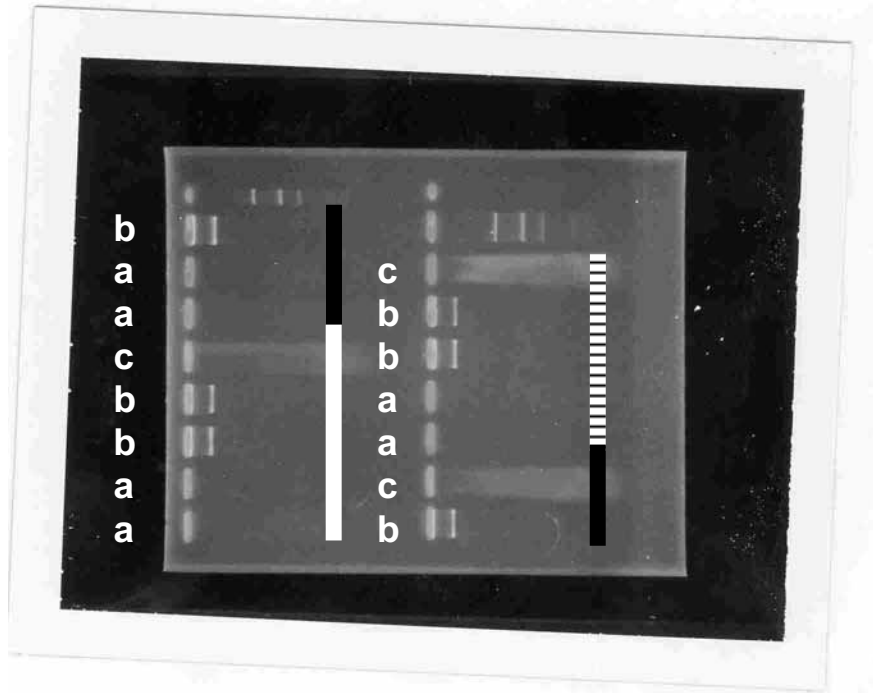


**Figure 3.2:** *P. esculentus* microsatellite alleles from muscle and ovary tissue stored in DMSO (a, b) and from muscle and ovary tissue paraffin wax (c, d) for six loci (176, dotted; 120, white; 189, grey; 047, horizontal lines; Pe1.1, black; 015, diagonal lines and negative control, e).

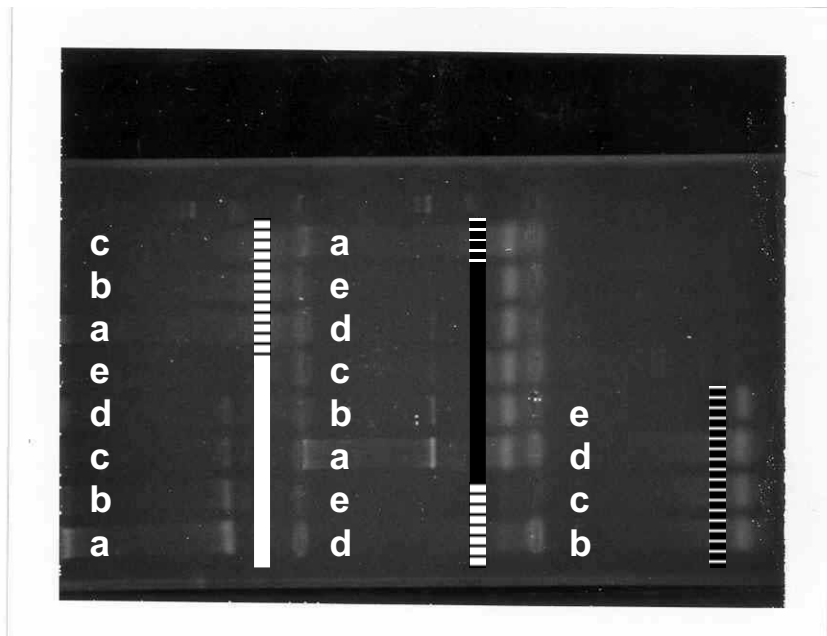
#### Other contaminants associated with Histoclear<sup>TM</sup> or paraffin wax

Further experiments showed that substances other than RNA were inhibiting amplification from template derived from wax-embedded prawn tissue. We amplified two loci (189 and 015) from serial dilutions (1 to  $10^{-3}$ ) of DMSO-derived template and, as expected, the intensity of the microsatellite band for both loci decreased as template became more dilute (Figure 3.4). However, the addition of 5  $\mu$ l of wax-derived template into a set of identical reactions inhibited amplification of both loci (Figure 3.4).

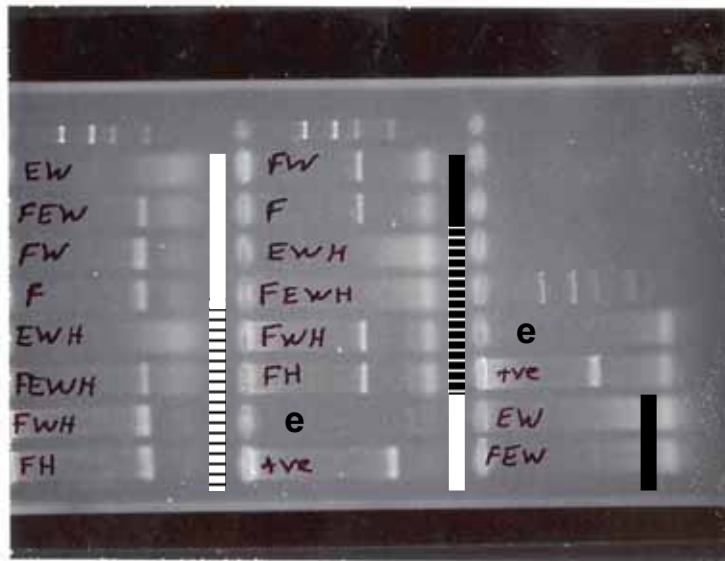




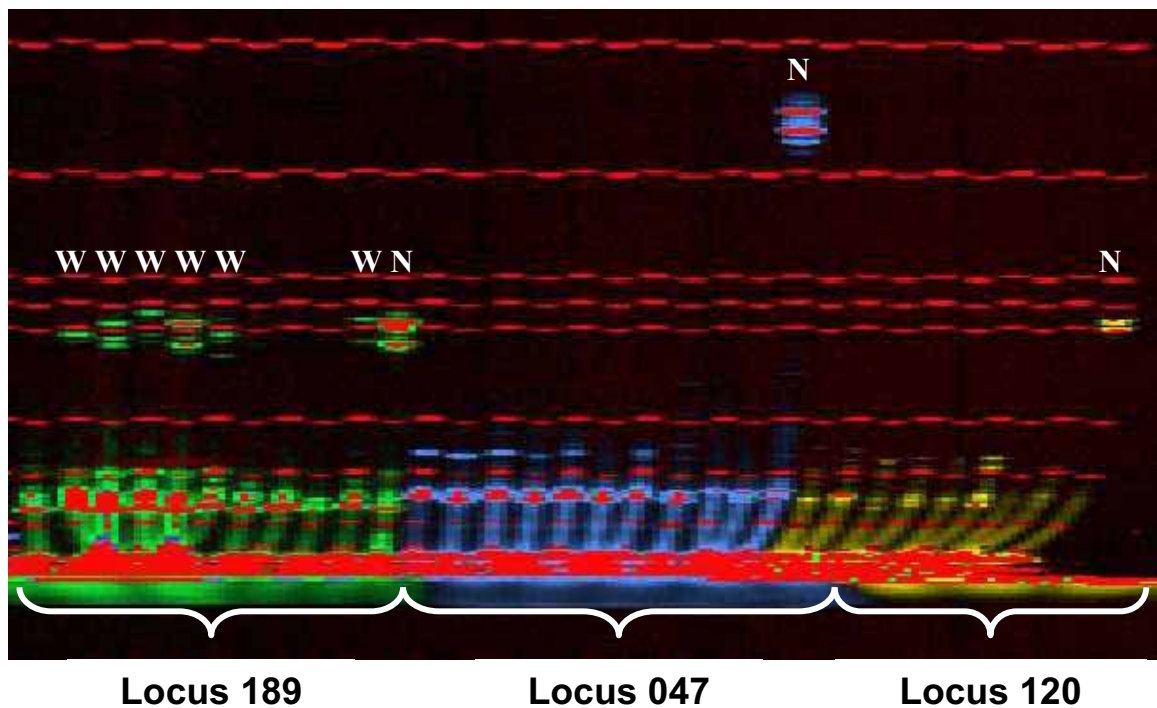
**Figure 3.3: Removal of nucleic acid smear by RN-ase A treatment for 10 mins (white), twenty mins (horizontal lines) and thirty mins (black) in genomic DNA extracted using DN-easy™ tissue kits from wax (a) and DMSO (b) preserved *P. esculentus* tissue. Untreated tissue from wax preserved tissue (c) was included as a control.**



**Figure 3.4: Inhibition of amplification of locus 189 (white) and 015 (black) by the addition of 5 µl of template derived from wax embedded prawn tissue (horizontal lines). The control sample was amplified in four reactions containing normal strength (a), 1:10 (b), 1:100 (c), 1:1,000 (d) and no template (e – negative control). No products were evident when template from wax-embedded tissue was added to the reactions (horizontal lines).**



**Figure 3.5:** Using Histoclear™ (H, horizontal lines) to aid template extraction does not affect amplification of locus 189 (white) and 015 (black). Amplification of both loci occurred from templates prepared from fresh (DMSO-derived, F) tissue, with or without the addition of plain wax (W), or tissue embedded in wax (EW), except for locus 015. The presence of tissue embedded in wax (EW) inhibited the amplification of locus 015 (black). Positive (+ve) and negative (e) controls for each locus are shown.



**Figure 3.6:** Gel image from AGRF of microsatellite alleles for loci 189, 047 and 120 for prawn template from wax-embedded tissue (W) and from normal template (N). Internal size standards are red-labelled.

We proposed that some component of the DNA solution extracted from wax template may be inhibiting amplification. We performed two experiments to test the effect of wax-derived and Histoclear™-derived inhibitors. The more widely used wax removal chemical, xylene, requires strict Workplace Health and Safety standards for its use in the laboratory and was not routinely used in this study. In our first experiment, we extracted template DNA from fresh tissue in the presence of either (1) tissue embedded in wax or (2) wax without tissue using the DN-easy tissue kit. We expected that if a component of the paraffin wax was the inhibitor, then amplification would be inhibited by both the wax with and without the embedded tissue.

We repeated this experimental design by omitting the wax dissolving agent, Histoclear™. If the Histoclear™, or its components, was inhibiting the amplification, then template extracted without using it would amplify regardless of the presence of wax or wax-embedded tissue. Instead of using Histoclear™ to remove wax prior to DNA extraction, we melted the wax in the presence of the DN-easy ATL buffer in the same way as for the Chelex 100 method.

The amplification of locus 189 from fresh template was not inhibited by the presence of tissue embedded in wax, or by wax alone. Amplification of locus 015 from fresh template was inhibited by the presence of tissue embedded in wax, but not by wax alone (Figure 3.6). Even though the presence of template from wax-embedded tissue had previously inhibited the amplification of locus 189 (Figure 3.4), these results indicate that a contaminant associated with the presence of wax-preserved tissue may be responsible.

The use of Histoclear™ in the extraction of template did not affect the amplification of locus 189 or 015 (Figure 3.5); the template derived from fresh tissue with various additives (plain wax, wax-embedded tissue) produced bands in most cases. However, omitting the Histoclear™ did not produce bands from wax template for either locus (189 or 015).

### ***ABI results for wax-embedded template for loci 189, 047 and 120***

When we were unable to obtain PCR products for microsatellite loci with wax-derived template extracted with the DN-easy protocol and were unable to find out why, we abandoned it in favour of the Levi's method of preparing template. Additionally, the DN-easy method was expensive compared to the Levi's method.

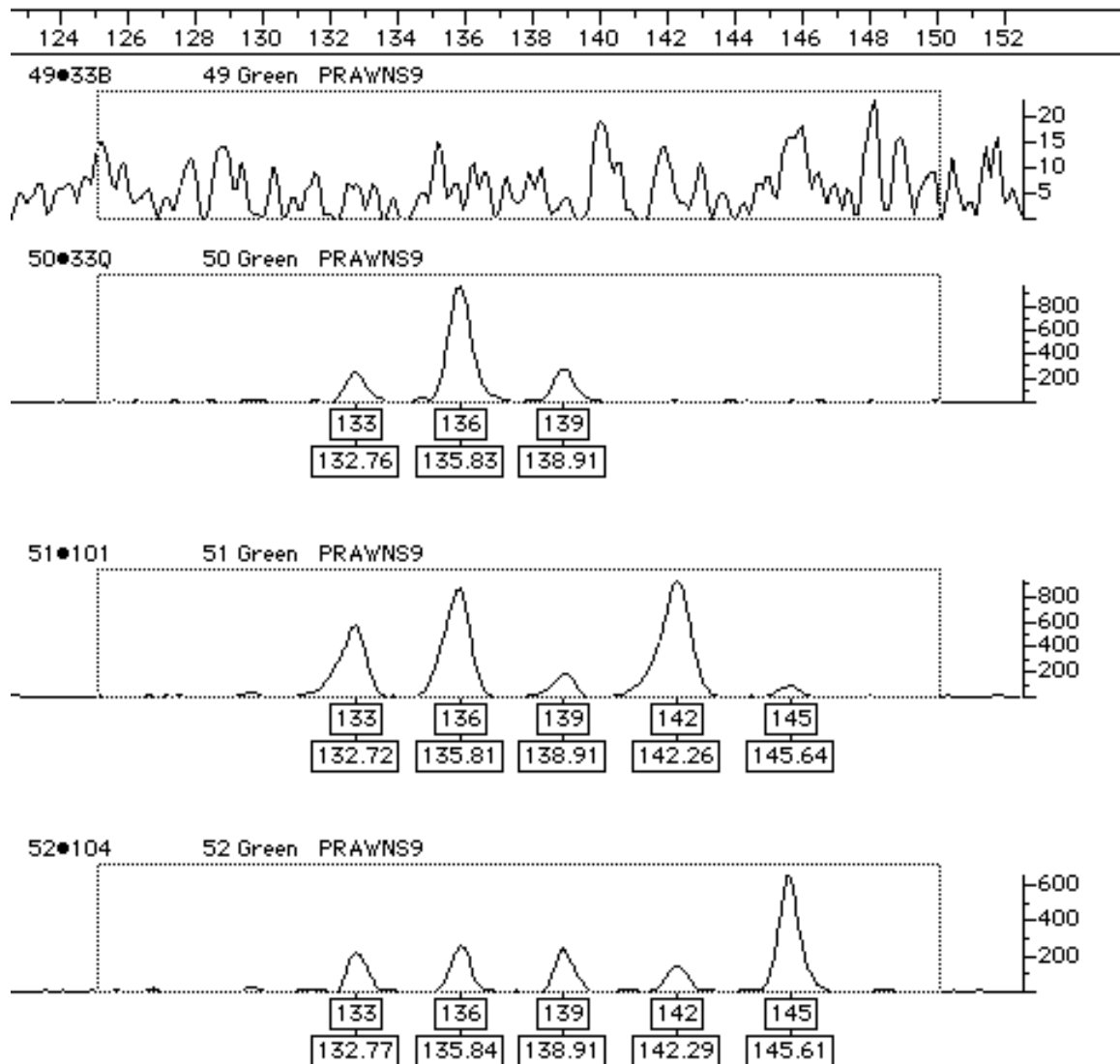
Tissue from eight wax-embedded prawn samples was extracted using the Levi method, and amplified for three loci (120, 047 and 189) along with a positive (normal template) and negative control.

Wax template produced bands on the AGRF gel for most of the amplifications for locus 189 (Figure 3.6). No bands were produced for loci 120 and 047.

Densitometer traces for the 189 gel lanes showed that most amplifications contained three or more bands (Figure 3.7). As microsatellite markers are codominant loci, the maximum number of bands expected per lane is two. The extra bands were most likely to be contamination of the amplification reaction, which was confirmed by subsequent agarose gel electrophoresis of the remaining 10 µl that showed the

negative control also contained bands. Subsequent contamination control procedures in new PCRs were successful, yielding negative lanes with no bands.

Allele determination for microsatellite loci from template derived from wax-embedded tissue was abandoned, as various experiments involving different DNA extraction methods would not produce PCR product consistently for any locus.



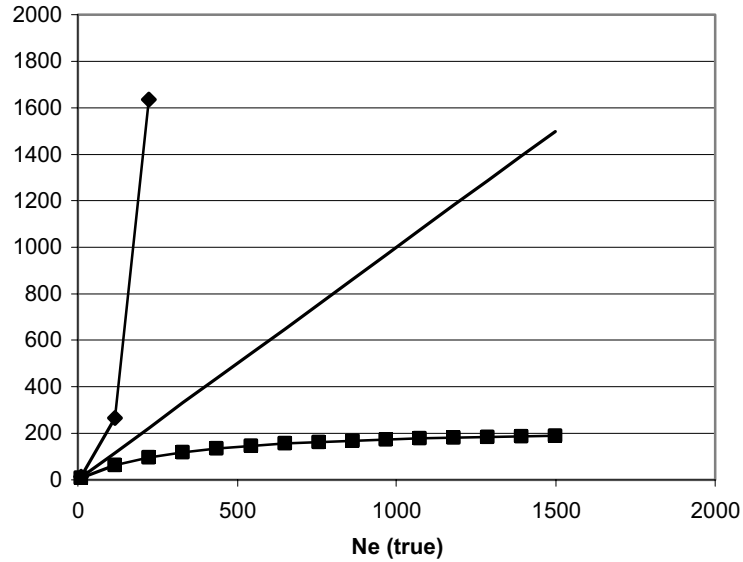
**Figure 3.7: Densitometer traces of the first four lanes for locus 189 from the AGRF gel in Figure 3.6. The intensity (height) of each band is given on the vertical axis and the size in base pairs is indicated based on red-labelled, internal size standards.**

***How does the statistical power of  $N_e$  estimates improve when wax samples are used?***

When effective population size lies between 200 and 1500, the 95% confidence limits of estimates are broader when the temporal interval is one generation compared to 12 generations (Figure 3.8, Figure 3.9 and Figure 3.10). In order to obtain the same

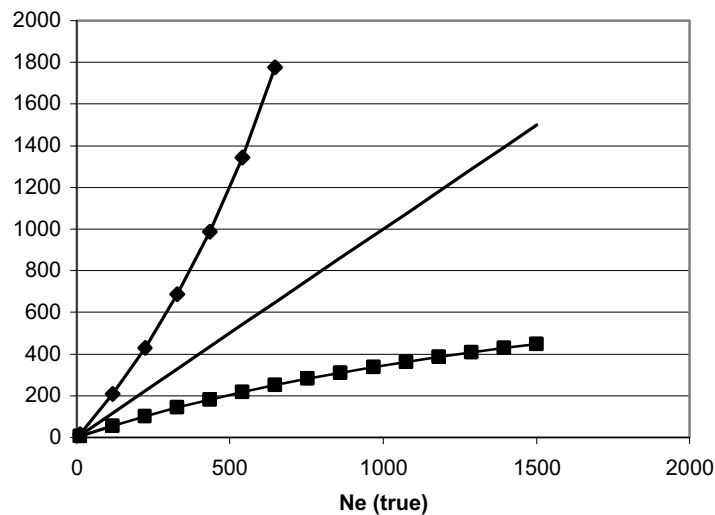
statistical power over one generation as over 12 generations, then approximately 730 samples need to be analysed one generation apart.

**One generation, S=[100,400]**

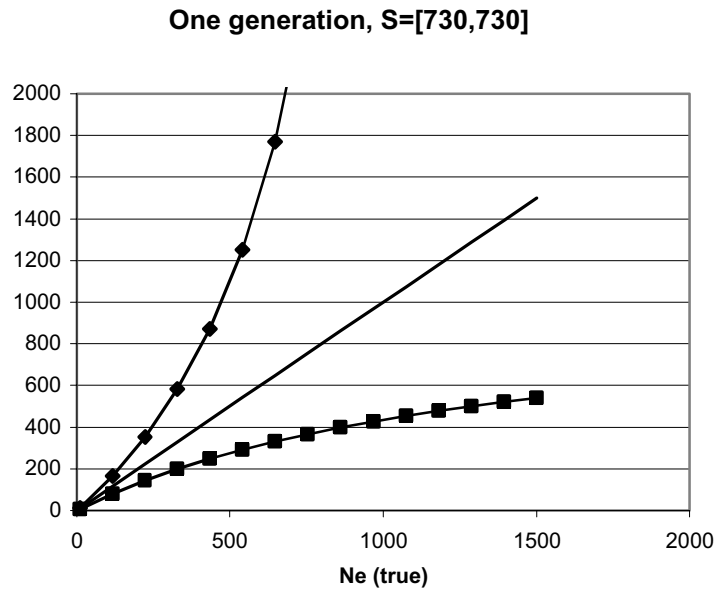


**Figure 3.8: Upper (diamonds) and lower (squares) 95% confidence limits for effective population size calculated using the temporal method by taking 100 samples and 400 samples one generation later. The diagonal indicates estimates obtained for increasing values of true effective population size.**

**12 generations, S=[100,400]**



**Figure 3.9: Upper (diamonds) and lower (squares) 95% confidence limits for effective population size calculated using the temporal method by taking 100 samples and 400 samples twelve generations later. The diagonal indicates estimates obtained for increasing values of true effective population size.**



**Figure 3.10: Upper (diamonds) and lower (squares) 95% confidence limits for effective population size calculated using the temporal method by taking 730 samples and 730 samples one generation later. The diagonal indicates estimates obtained for increasing values of true effective population size.**

## Discussion

We recommend that the use of archival tissue samples for increasing the temporal interval for the estimation of effective population size is considered on a case-by-case basis. We were unable to utilize wax-embedded prawn samples in this study, possibly due to denaturation of the starting DNA with formalin that was used as an initial preservative. However, for our purposes, computer simulations showed that similar increases in statistical power could be achieved by increasing the number of prawns sampled over a shorted temporal interval.

For animals that have a longer generation time it is more critical to access archival tissue because sample collection in a single three to five year project will not encompass a temporal interval that includes a whole generation. Sampling and computational methods are available to adjust estimates of effective population size for overlapping generations (Chapter 6). Case studies for the denaturation of DNA over time in various species are becoming available (eg. Wandeler, Smith *et al.* 2003).

It is also important to have the most stringent PCR contamination controls in the laboratory when working with archival tissue. Abundant PCR products in the laboratory produced from amplifications on extant animals are highly likely to contaminate reactions when archival tissue is used as template. The PCR products are dispersed as aerosols and on surfaces and the archival template is easily out-competed as template by the contaminating PCR product. Practically this often means have distinct separation between the DNA extraction and PCR areas of the laboratory (Austin, Smith *et al.* 1997).

Other genetic marker systems, such as single nucleotide polymorphisms (SNPs), may be more reliably scored in archival samples. Unlike microsatellite loci that depend on

the PCR amplification of a product that is 150 to 300 bases in length, a SNP locus can be assayed in a PCR amplification of 10 to 15 bases. When DNA has broken down during archival storage, it is more likely that this class of loci will yield results. SNP loci have other advantages also, such as more streamlined data collection.





## **Chapter 4 – Swept area trawl survey estimates of tiger prawn spawning stock size**

*by AJ Courtney, HM Podlich and JR Ovenden*

### **Introduction**

This section of the report details results from a trawl survey that was designed to estimate the size of the spawning population ( $N_a$ ) for tiger prawns (*Penaeus esculentus*) in Moreton Bay during the spawning seasons of 2001 and 2002. It also examines long-term trends in the catch rate of tiger prawn spawning stocks using the reported commercial logbook data. The survey and logbook estimates are compared against the effective genetic population estimates ( $N_e$ ) in Chapter 2.

### **Methods**

*P. esculentus* is endemic to Australia's tropical and sub-tropical waters from mid-New South Wales to Shark Bay, Western Australia (Grey, Dall *et al.* 1983) and generally fished in areas associated with, or adjacent to shallow seagrass meadows. The population in Moreton Bay (27°30'S) may be considered as relatively isolated because commercial logbook data indicate that catches immediately north and south of the Bay are negligible and because the majority of the seagrass in the region is located in the Bay. Tagged adults generally do not migrate more than a few kilometres and so the population in Moreton Bay could be considered as a separate unit.

The spawning period for *P. esculentus* in Moreton Bay is relatively short, mainly because the area approaches the southern geographical limit for the species, and is characterised by a single peak in egg production in October-November (Courtney and Masel 1997). Egg production declines markedly thereafter although there is still some production through to March. The spawning period for brown tiger prawns increases with decreasing latitude and in the Gulf of Carpentaria (13°30') it occurs year-round with peaks in July-September (Crococ 1987a).

### **Survey design**

To estimate the number of adult spawning tiger prawns in Moreton Bay surveys were designed and undertaken during the main spawning periods (October-November) in 2001 and 2002. Logbook data for the months of October and November for each year from 1988-99 were examined to determine the reported variation in the temporal and spatial distribution of tiger prawn catches in the Bay. These data showed that 0.779 of the reported catch from the Bay were caught from five 6' x 6' logbook grids (Log book grids 7, 12, 13, 14 and 18). The general approach used in the surveys was to sample the five grids and then extrapolate the population estimate upwards (by 1/0.779) to give the total population size estimate for the Bay.

Funding was obtained for approximately 6 nights of trawl sampling each year. The survey design for the first year (2001) assumed equal variance in catch rates across the five grids and consequently, approximately equal sampling effort was applied to each grid. This equated to allocating approximately 15 one-nautical mile trawl

transects to each grid. Grids with reduced area (i.e. those infringing the mainland or with island masses) received proportionally less sampling effort.

The location of transects was randomly allocated within each grid, but conditional upon avoiding untrawlable ground. Each 6' x 6' grid was divided into 36 one square nautical mile sub-grids and each transect randomly allocated to a one-nautical mile sub-grid. The skipper was asked to locate the centre of the transect on the centroid of the one-nautical mile sub-grid.

The variance of mean estimates obtained for each individual grid sampled in 2001 and their contribution to the variance of the overall stratified mean were used to allocate the sampling effort in 2002. The variance estimates were derived using the method described by Haddon (1997) appropriate for a stratified design that calculates the total variance of the overall mean and the variance of the mean within each strata (=grid). The proportion of the variance due to each grid (in 2001) was then used to apply the level of sampling effort to each grid in the following year (2002). Thus, if the variance of the mean of a particular grid accounted for 50% of the overall mean variance then it received approximately 50% of the sampling effort.

### ***Estimating population size***

The mean catch rate (number per one-nautical mile transect) of the adult tiger prawns in each of the five 6' x 6' grid was derived from the sampling program data.

An overall stratified mean ( $\hat{U}g$ ) was derived as a weighted sum of the five grid means for each year. Weights were assigned according to the relative proportion of each grid area to the total area sampled. The stratified mean was assumed to conform to normality and 95% confidence intervals computed. The assumption of normality was investigated and verified by carrying out simulations (the results of which are presented later).

The total number of adult tiger prawns ( $T$ ) was estimated as the product of the stratified mean catch rate ( $\hat{U}g$ ) and the total area encompassed by the grids, thus:

$$T = \hat{U}g \times \left( \frac{\text{total area of all grids}}{\text{area of one nautical mile transect}} \right)$$

Complete or entire grid area = 6nm x 1,852m = 123.47654 km<sup>2</sup>. Area of one-nautical mile transect was 1,852 m x beam width of 5 m = 9,260 m<sup>2</sup> or 0.00926 km<sup>2</sup>. Because some grids included land masses or islands the actual area covered by seawater was calculated using geographic mapping software (Arcview). The precise area for each grid is provided in Table 4.1.

Because the 5 grids represent only 0.779 of the total number of prawns in the entire Bay, based upon logbook catch data, the total number of adult prawns and their associated confidence intervals were adjusted upwards by a factor of 1.2837 (=1/0.779).

The capture efficiency of the trawl gear for *P. esculentus* is unknown. Joll and Penn (Joll and Penn 1990) have shown that for western king prawns *P. latisulcatus* capture efficiencies vary from 15-31% and 50-53%, for males and females, respectively. In

the absence of specific estimates for *P. esculentus* a capture efficiency of 50% was chosen, which is common practice for such surveys (Sparre and Venema 1992). Thus, to correct for capture efficiency of 50% the total annual estimates were further adjusted upwards by a factor of 2 (100/50).

### ***Defining adults***

The survey was designed to estimate the population size of adult tiger prawns only. The definition of adults used herein was based on the reproductive biology of females. In the Gulf of Carpentaria, Crocos (1987) found that the minimum size at which *P. esculentus* matured was 25 mm CL. The smallest size at which vitellogenesis occurs in *P. esculentus* in Moreton Bay is 30 mm CL (Courtney and Masel 1997) and for this reason adults were defined as those  $\geq 30$  mm CL. It was assumed that all females in this size category ( $\geq 30$  mm CL) developed mature ovaries, were inseminated and spawned during the October-November spawning period. In the absence of any reproductive data for males the definition was applied to both sexes.

### ***Trawl gear***

The trawl gear consisted of a 5m beam trawl fitted with a Florida Flyer net composed of 38 mm mesh. Analysis of the selectivity ogives for trawl nets used in Moreton Bay (see Courtney 1997) suggest that 38 mm mesh would retain approximately 100% of adult tiger prawns (i.e. size classes  $\geq 30$  mm CL). A beam trawl was chosen because the area swept by the beam is less variable than the standard otter-board configuration used by commercial fishers in the Bay.

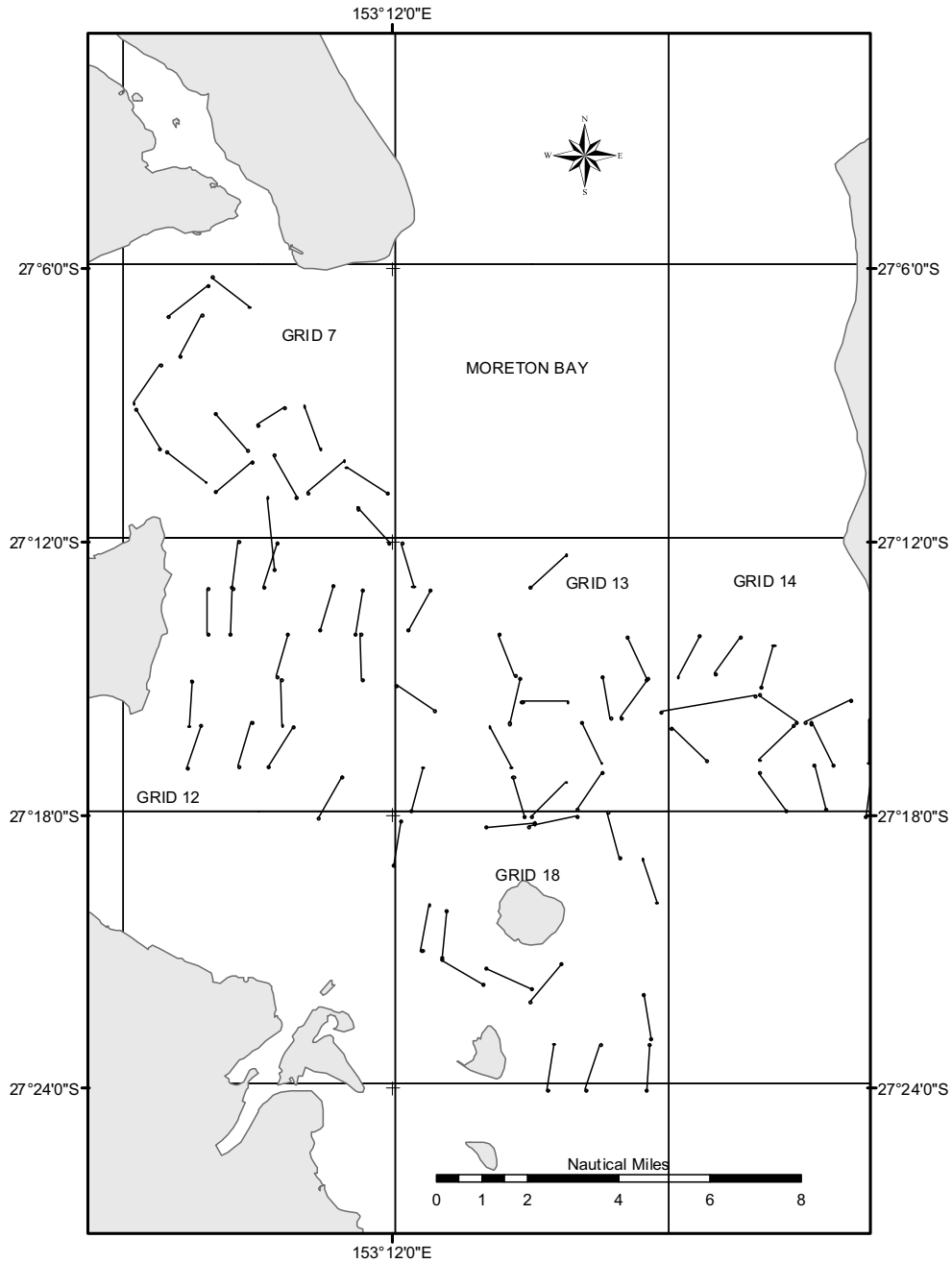
## **Results and Discussion**

### ***Ecological estimate of effective population size***

Estimates of  $N_a$  can be converted to ecological estimates of effective population size using the 'minimal' method of Nunney and Elam (1994). Their method depends on six parameters (1) mean maturation times to adulthood for both males and females, (2) average life span for each sex, (3) generation time, (4) variation in male, and (5) female reproductive success per breeding season and (6) the adult sex ratio. For tiger prawns in Moreton Bay the adult sex ratio is one, and the generation time is also one, but beyond that the remaining parameters are largely unknown. Consequently, ecological estimates of the number of adults participating in spawning were not made using the method of Nunney and Elam (1994).

### ***Swept area survey estimates of the spawning population size***

In 2001, 72 one-nautical mile transects were sampled (Figure 4.1). The mean catch rates varied between 7.7500 adult prawns per one-nautical mile in grid 13 and 2.2143 adult prawns per one-nautical mile in grid 12 (Table 4.1).



**Figure 4.1: Location of 72 one-nautical mile trawl transects in Moreton Bay sampled during the peak tiger prawn spawning period (October-November) 2001.**

**Table 4.1: The mean catch rates of adult tiger prawns in Moreton Bay from the 2001 survey.**

Grid	Mean catch rate of adults ( $\geq 30\text{mm CL}$ )	Number of observations (transects)	SE
7	2.5333	15	0.4008
12	2.2143	14	0.4709
13	7.7500	16	0.6614
14	3.6923	13	0.7372
18	3.7857	14	1.0596
Total		72	

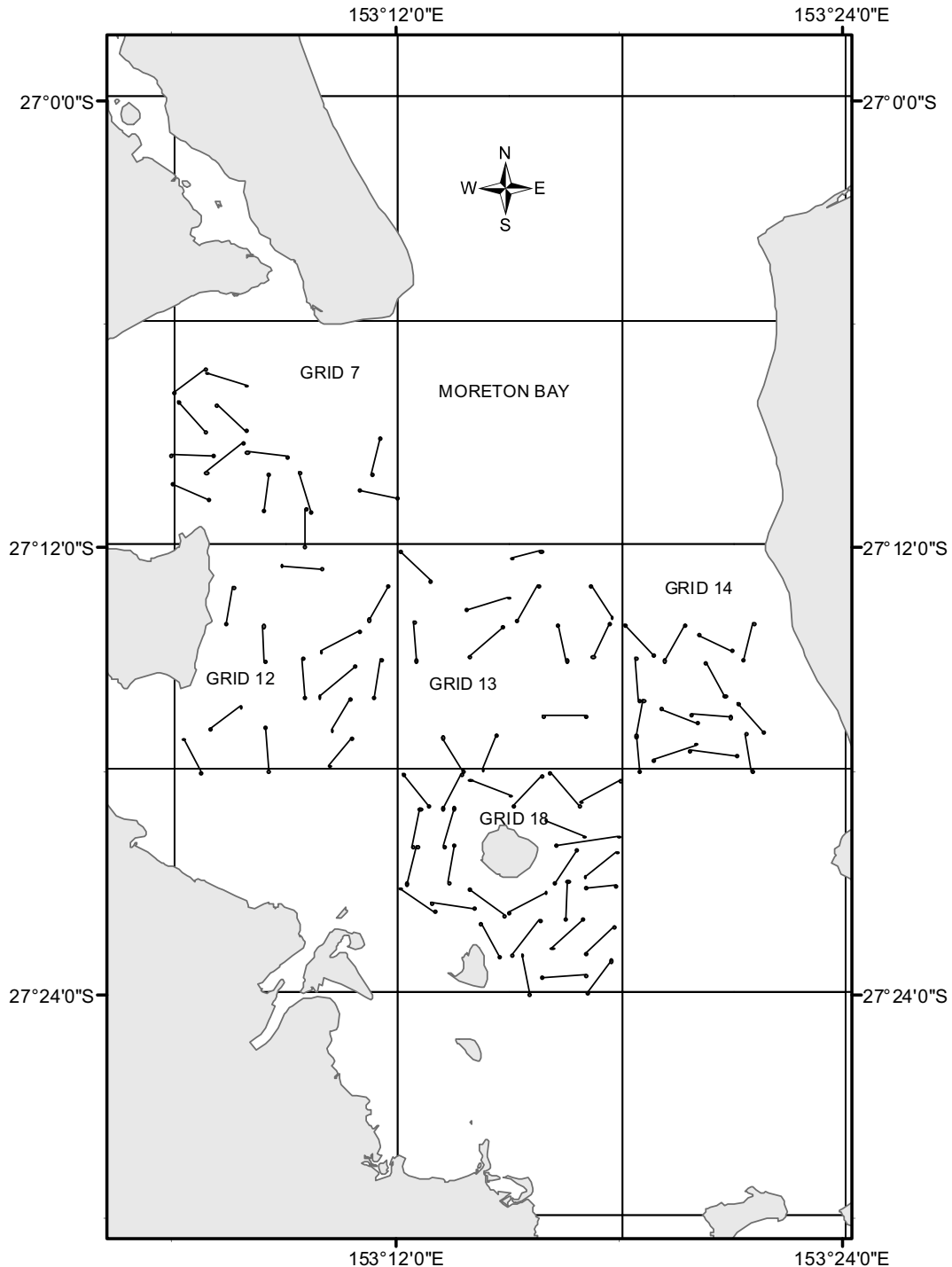
The variance of mean ( $SE^2$ ) in each grid was examined and their relative contribution to the variance of overall stratified mean used to apportion sampling effort across grids in the 2002 survey. In general, this analysis revealed that much of the variance in the 2001 survey occurred in grid 18 (about 40%) and that the remaining variance from the other grids were similar (Table 4.2). Consequently, grid 18 received a proportionally high level of sampling effort in 2002 and the remaining grids each received similar and lower levels of sampling effort.

**Table 4.2: Summary results for grids sampled in 2001. The weighted means are the contributions for each grid to the overall stratified mean. The variance estimates are contributions for each grid to the variance of the overall stratified mean. This approach was used to apportion the sampling effort across grids for the 2002 survey (Haddon 1997).**

Stratum (or grid)	Grid 7	Grid 12	Grid 13	Grid 14	Grid 18	Totals
Mean catch rate of prawns	2.53	2.21	7.75	3.69	3.79	
No. stations sampled	15	14	16	13	14	72 stations sampled
Strata area sq km	122.4	113.1	123.5	103.8	116.5	579.3sq. km total area
SD of mean	2.21	2.21	2.21	2.21	3.96	
Strata weight	0.21	0.20	0.21	0.18	0.20	1
Weighted mean	0.53	0.43	1.65	0.66	0.76	4.04 (stratified mean)
Variance	0.0145	0.0133	0.0139	0.0121	0.0455	0.0992127
						Stratified SE 0.31
						Coefficient of variation 7.79
Number of stations to be sampled in 2002	11	10	10	9	33	

In 2001 the stratified mean catch rate was 4.04 adult prawns per one-nautical mile trawled and the survey coefficient of variation (CV) was 7.793%. In 2002 a total of 79

transects were sampled (Figure 4.2). The mean catch rates varied between 4.3333 adult prawns per one-nautical mile in grid 18 and 2.2143 adult prawns per one-nautical mile in grid 14 (Table 4.3). The stratified mean for 2002 was 2.8946 adult prawns per one-nautical mile and CV was 10.03%.



**Figure 4.2: Location of 79 one-nautical mile trawl transects in Moreton Bay sampled during the peak tiger prawn spawning period (October-November) 2002.**

**Table 4.3: The mean catch rates of adult tiger prawns in Moreton Bay from the 2002 survey.**

Grid	Mean catch rate of adults ( $\geq$ 30mm CL)	Number of observations (transects)	SE
7	2.6923	13	0.6735
12	2.2308	13	0.3947
13	2.9167	12	0.7330
14	2.2143	14	0.5947
18	4.3333	27	0.7434
Total		79	

***Estimation of population size and confidence intervals***

The confidence intervals assume normality of the stratified mean, that the 5 sampled grid areas account for 77.9% of the population of Tiger prawns in Moreton Bay and 50% capture efficiencies. The overall stratified mean estimate,  $\hat{U}_g$ , of the 5 sampled grids in each year is computed as a weighted sum of the individual means from each grid, with weights given by their area as a fraction of the total area sampled (see Table 4.2 for grid areas).

When normality of the mean stratified catch rates  $\hat{U}_g$  are assumed, the confidence intervals can be calculated as  $\hat{U}_g \pm 1.96 * s.e.(\hat{U}_g)$  which equate to:

2001: 4.0426 (3.4278, 4.6574), and

2002: 2.8946 (2.3254, 3.4637).

The confidence interval for the total number of adult prawns in the sampled area was obtained by multiplying these endpoints by (total area of grids sampled/area of trawl) = 579.253586/0.009265 sq km giving and estimated number of adults in sampled area:

2001: (214 310.3, 291 184.5), and

2002: (145 388.4, 216 554.3).

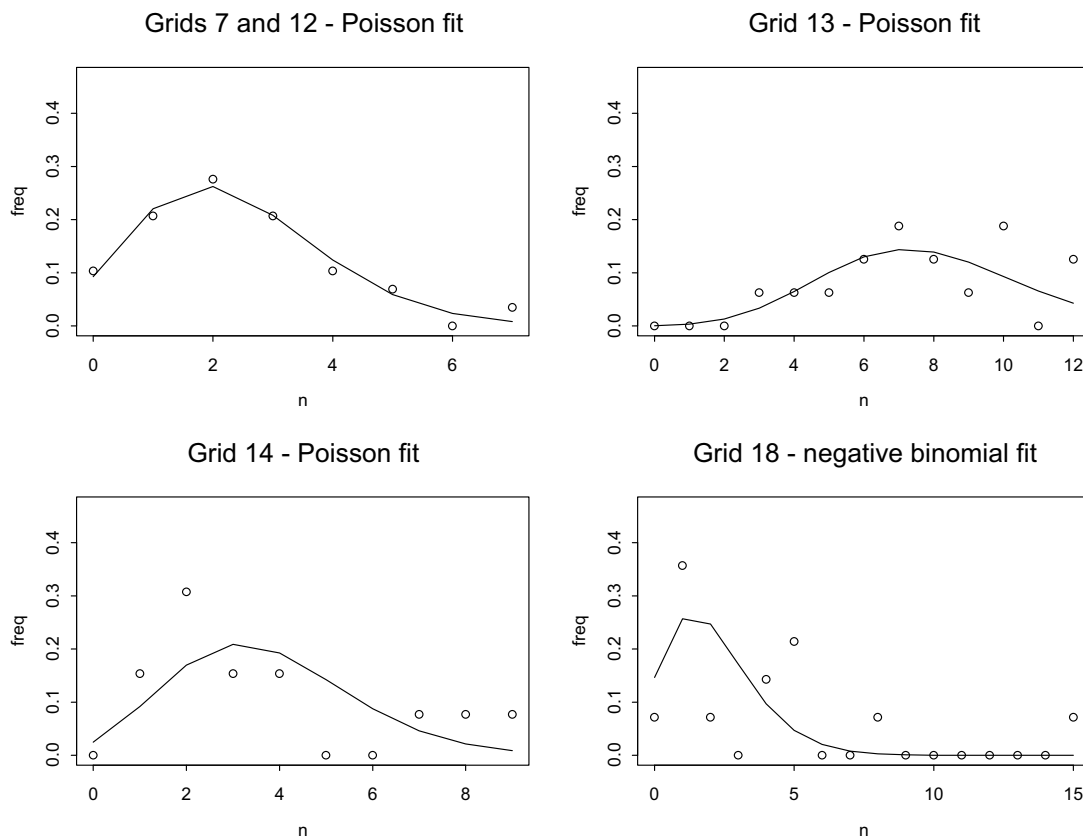
By taking into account a) the sampling grids account for approximately 77.9% of the population of adult prawns in Moreton Bay (ie., extrapolating upwards by 1/0.779), b) the 50% capture efficiency of the net (ie., extrapolating further by a factor of 2), the final estimated number of adult prawns in Moreton Bay were:

2001: **648,898** (550 219.1, 747 585.4), and

2002: **464,627** (373 269.3, 555 980.2).

### ***Checking assumption of normality of stratified mean***

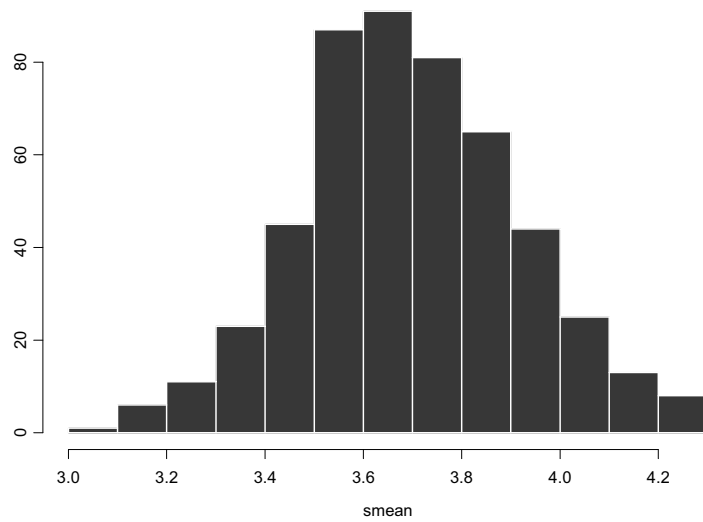
To check this assumption we propose to construct 95% confidence intervals for the stratified mean in 2001 and 2002 by simulation. To simulate re-sampling, models for the distribution of counts in the 5 sampled grids are required. Shown in Figure 4.3 are the empirical relative frequency distributions of catch rates for 2001 from each grid (points) with fitted models (solid lines) superimposed on top. Since the log fitted means from the Poisson models for grids 7 and 12 were within a standard error of one another, a common Poisson model was fitted to the combined data from grids 7 and 12.



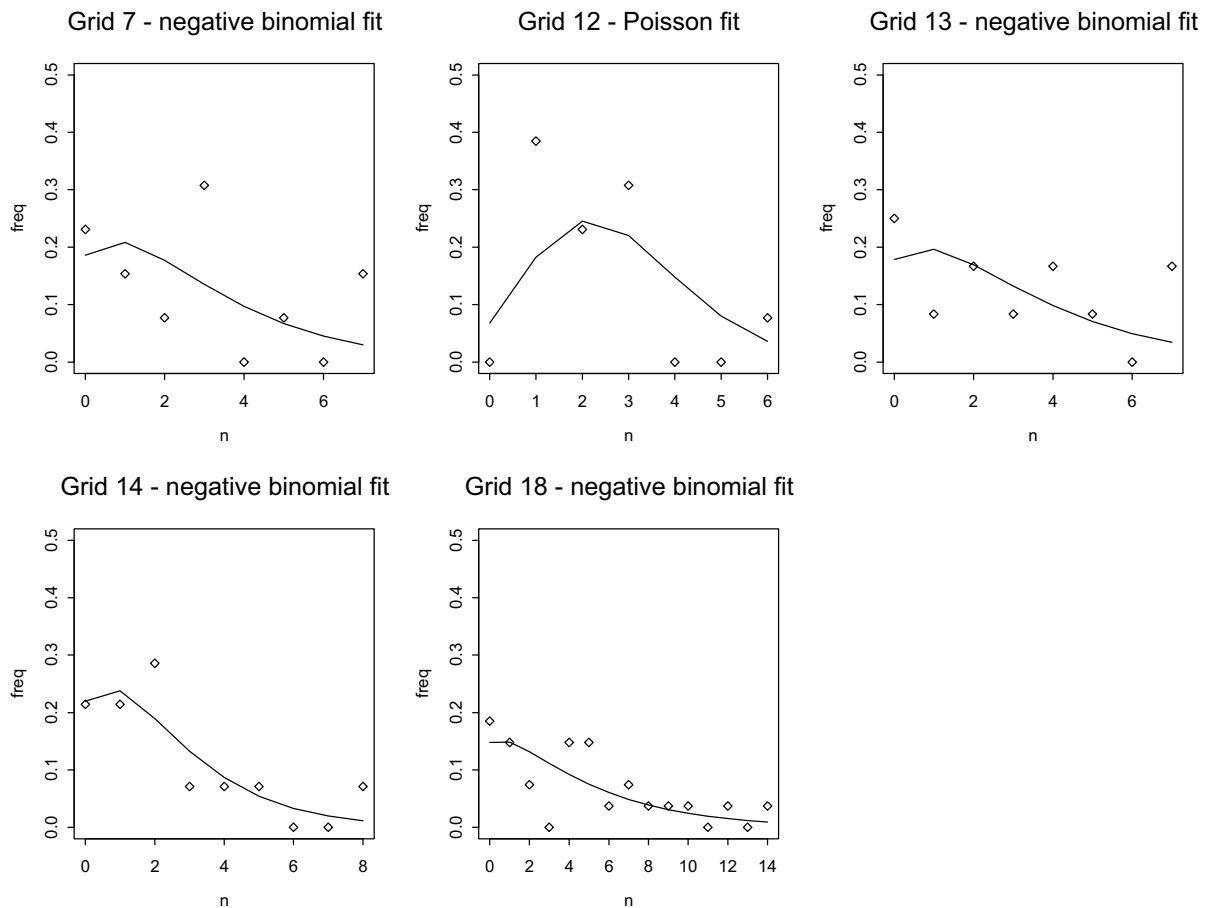
**Figure 4.3: Relative frequency distributions and fitted models (solid lines) of catch rates of adult tiger prawns in the 6'x6' grids in 2001.**

Frequency histograms of 500 simulated stratified means, computed by continually resampling from the fitted models in Figure 4.3, are provided in Figure 4.4. A Kolmogorov-Smirnov goodness of fit test for normality suggests that normality of the stratified mean can be assumed ( $p$ -value=0.5). The simulated confidence interval for the stratified mean is (3.2526, 4.1528), where the lower and upper values represent the 2.5 and 97.5th percentiles of the simulated values, respectively. Recall the corresponding confidence interval assuming normality is (3.4278, 4.6574). Although normality holds, both endpoints of the simulated interval are slightly lower than those of the computed interval. It is believed this result can be attributed to under-estimation of the mean from the negative binomial fit to the grid 18 data in the simulations.





**Figure 4.4: Frequency distribution of 500 simulated stratified means for the 2001 data.**

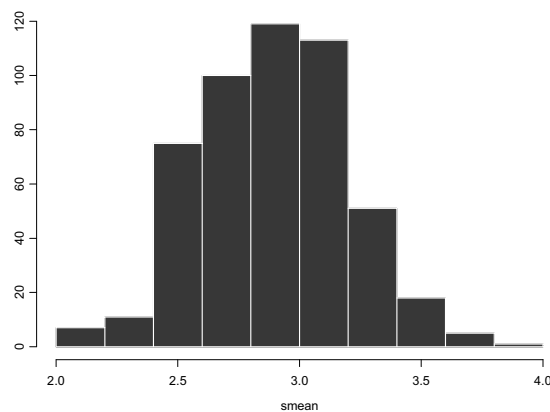


**Figure 4.5: Relative frequency distributions and fitted models (solid lines) of catch rates of adult tiger prawns in the 6'x6' grids in 2002.**

Shown in Figure 4.5 are the empirical relative frequency distributions of catch rates for 2002 from each grid (points) with fitted models (solid lines) superimposed on top. Frequency histograms of 500 simulated stratified means, computed by continually resampling from the fitted models in Figure 4.5, are provided in Figure 4.6. A Kolmogorov-Smirnov goodness of fit test for normality suggests that normality of the stratified mean can be assumed ( $p$ -value=0.5). The simulated confidence interval for the stratified mean is (2.2658, 3.4384), where the lower and upper values represent the 2.5 and 97.5th percentiles, respectively. Recall the corresponding confidence interval assuming normality is (2.3254, 3.4637) which is very similar to the simulated interval. Figure 4.5. Relative frequency distributions and fitted models (solid lines) of catch rates of adult tiger prawns in the 6'x6' grids in 2002.

The simulations presented in this section serve to justify the use of the normal approximation to the distribution of the stratified mean and therefore the use of the normal distribution in constructing 95% confidence intervals in both the 2001 and 2002 data.

While the uncertainty in the mean catch rate of adult tiger prawns was considered in the population size estimates, no uncertainty was considered for a number of other parameters. For example, we assumed that the proportion of adult females contributing to the spawning stock was fixed at 100%. This does not take into account any possible variation in the proportion of adult females with mature ovaries. Furthermore, we assumed a fixed capture efficiency of 50%, whereas Joll and Penn (1990) reported a range in values for both males and females. If the uncertainty in these parameters was incorporated in the spawning stock population size estimates the confidence intervals reported above would increase.



**Figure 4.6: Frequency distribution of 500 simulated stratified means for the 2002 data.**

## Chapter 5 – A simulation study of methods to estimate effective population size

by D Peel and JR Ovenden

### Introduction

The effective population size ( $N_e$ ) is the number of breeding individuals in an ideal population that would produce the same value of a given genetic measure such as the amount of inbreeding, change in allelic frequency variation or loss of heterozygosity. Consequently, the definition of effective population size depends on the measure under consideration. Generally when the population size is not changing over time, the effective population sizes corresponding to the various measures will be similar.

Effective population size can also be estimated without genetic data and can be estimated using (1) a survey approach (see Chapter 4) or (2) using life history data. The latter approach involves forming a model based on available demographic or life history information and certain assumptions to predict  $N_e$  (Nunney and Elam 1994). Non-genetic estimates of  $N_e$  are not considered in this chapter. Similarly, this chapter is not concerned with long-term estimates of  $N_e$ .

In parallel with this simulation study, software to estimate effective population sizes has been developed called *NeEstimator* (Appendix A). A study was also conducted on a genetically isolated tiger prawn (*Penaeus esculentus*) population in Moreton Bay on the east coast of Australia to test the methodology in a fisheries context (Chapter 2).

The methods available to estimate  $N_e$  are described in Chapter 1.

The literature contains a number of simulation studies to investigate the properties of estimators of  $N_e$ . Turner et al. (2001) conducted a simulation experiment to evaluate the performance of the two forms of the moments-based temporal method given by (1) and (2). The simulation examined a true  $N_e$  from 50 to 500 with sample sizes of 50 and 200. They found in all cases that the moments-based methods over-estimated  $N_e$ .

Jorde et al. (1999) also completed a simulation study which included results for codominant markers for reference. They examined a true  $N_e$  from 20 to 500 and sample sizes of 20 and 100. Generally they found the estimates to underestimate  $N_e$ .

Wang (2001) included a small simulation study to compare the performance of the MLNE program and the moments-based temporal method. The effect of sample size, allele frequency and number of generations was investigated. However, only small populations where  $N_e$  was equal to or less than 100 were considered.

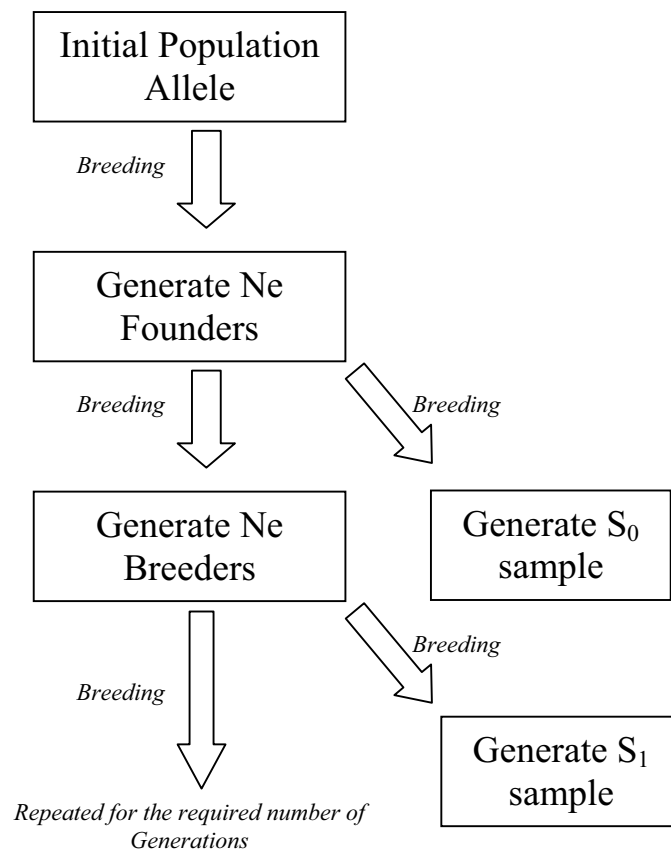
Waples (1989) looked at the performance of the moments-based temporal method for  $N_e$  of 100 and 500 with sample sizes of 50. Luikart and Cornuet (1999) examined the heterozygote excess method in the case of very small  $N_e$ .

The objective of this chapter was to evaluate the performance of the various methods for estimating  $N_e$ , particularly in large populations, but also across generations and in relation to the number and frequencies of alleles available for sampling.

## Method

To examine the performance of each method we simulated 1000 data sets for each circumstance. The effective population size was then estimated from each of these data sets using selected methods. The profile of these resulting estimates gives an insight into the behaviour of each method. This allows the comparison of the methods performance as well as a study of the behaviour of the method when various experiment parameters are changed eg sample size.

Of particular interest was the power of the methods, and in terms of our simulation experiment, power was defined as the proportion of simulations replications that provided a finite result. Infinite estimates of  $N_e$  were obtained when the sampling error ( $1/S$ ) was larger than the allele frequency variance ( $F$ , equation 3 Chapter 1).



**Figure 5.1: Flowchart showing the process to produce simulated populations.**

To generate the simulated data sets we used an individual-based model and attempted to replicate the natural life history of prawns in Moreton Bay. The model represents each individual prawn by its genotype. At each generation  $N_e$  breeding individuals are selected at random from the entire population and mated together randomly to produce offspring. Only the individuals sampled and the  $N_e$  individuals that breed in the following generation need to be generated as non-breeding individuals are not of concern in the model. This greatly simplifies the simulation as the total population size does not have to be specified, only  $(S + N_e)$  individuals need be modelled at each generation, and biological parameters such as mortality do not have to be known. The overall process is depicted in Figure 5.1.

The simulation study required a large computational effort and was accomplished using the distributed computer software Condor (<http://www.cs.wisc.edu/condor/>).

Several factors affected the power or performance of these methods. From the literature, we know that the larger the true  $N_e$  the worse the performance. The literature also points out that the number of samples, loci, alleles and generations between samples are all positively correlated with the power to estimate effective population size. So in the following sections we examine the effect of changing these parameters. When a parameter was considered all other parameters were fixed at the values used in the Moreton Bay study; sample sizes of 700; the number of loci equal to eight, and one generation between the two temporal samples (Chapter 2). Simulations were done with both the allelic frequencies randomly generated and also set to the estimated population frequencies obtained from the pilot study (Figure 2.9).

To generate random allele frequencies, a random number was sampled from the uniform distribution for each allele and then the collection of numbers standardised by the sum for all alleles to ensure that the frequencies summed to one. The allele frequency distribution that is generally produced using this method is a relatively flat distribution with the average frequency close to  $1/A$ , where  $A$  is the number of alleles. Although this approach can produce allele frequency values of close to zero the probability of producing an allele frequency close to one is small.

Initial simulations found the heterozygote excess method gave very low power for even moderately sized  $N_e$ , so it was not considered further in this paper. The TM3 and MCLEEPS methods were quite computationally intensive and so could not be included in the overall simulation. However, initial tests seem to indicate a similar behaviour to MLNE in that they provide an improvement over the moments-based temporal method.

Due to the relative computational simplicity of the moments-based temporal method and the linkage disequilibrium method, a large-scale simulation was conducted for these methods. This involved 1000 simulations for each scenario, where each scenario corresponds to a certain true  $N_e$  value and a set of experimental parameters. The two methods were examined for a range of true  $N_e$  (400-19,000) in steps of 1000 for each parameter we are interested in.

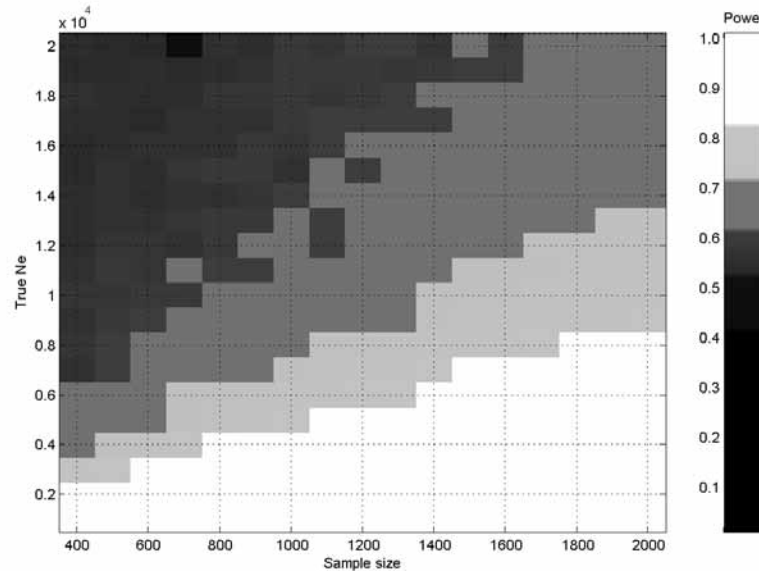
As the computation time for MLNE is quite reasonable a further simulation was conducted to compare MLNE with the moments-based temporal and linkage disequilibrium methods for a small number of test cases, rather than a full range of true  $N_e$ . One hundred simulated samples were taken for the cases of small  $N_e$  (1000), medium  $N_e$  (10 000) and in some cases large  $N_e$  (18 000). This provides us with an approximate guide to the precision and accuracy of the MLNE method in these cases.

## Results

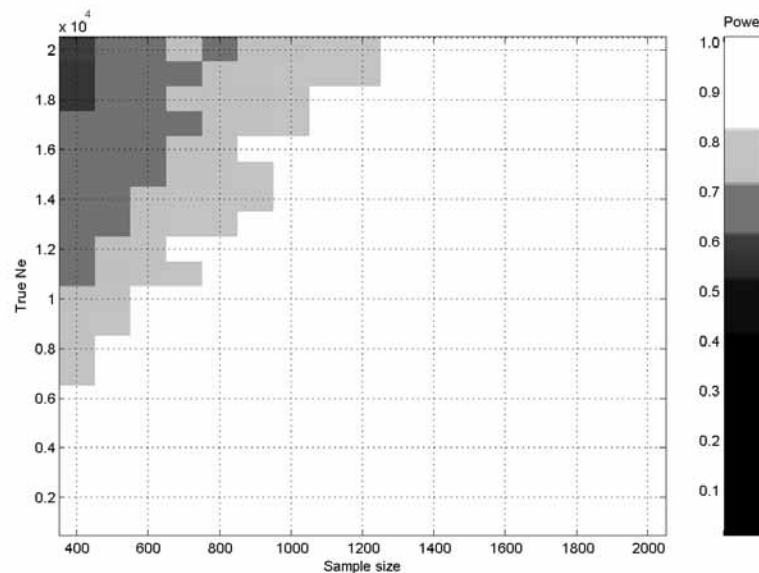
Due to the large amount of data produced by these simulations it was felt that plots would be more informative than tables of results. To summarise the data we use greyscale grid plots for the simulations where we varied  $N_e$  as well as another parameter and line plots for when we used a fixed  $N_e$ . In the grid plots the various intensities of grey correspond to a range of power or error depending on the plot. The ranges were chosen to best show the pattern in the data. It should be noted in the

power plots that the boundary between the grey and white regions corresponds to a power of 0.8, which is what we have taken to be acceptable.

### *Sample Size*



**Figure 5.2: Plot of the power obtained by the moments-based method for various sample sizes, over a range of true  $N_e$ .**



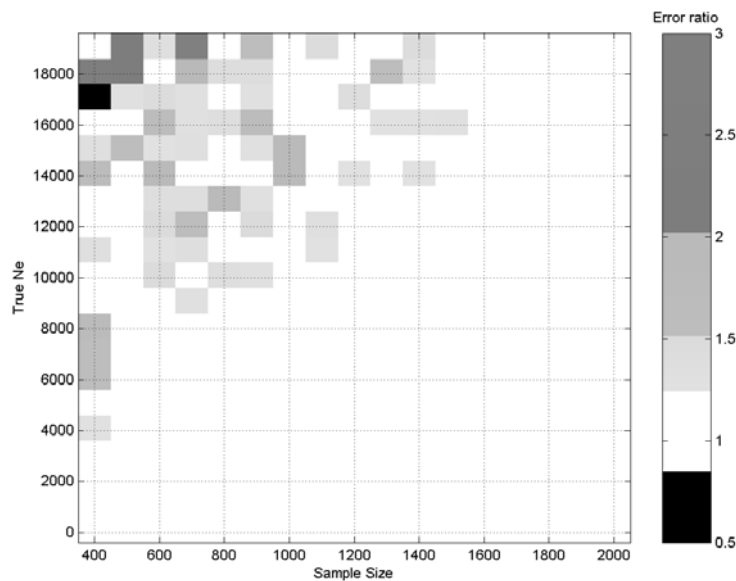
**Figure 5.3: Plot of the power obtained by the Linkage Disequilibrium method for various sample sizes, over a range of true  $N_e$ .**

As seen in the literature, increasing the sample size increased the power. This is because the sampling error, which can mask the genetic quantity being measured, is reduced. Figure 5.2 shows a plot of the power of the moments-based temporal method for a range of true  $N_e$  values and different samples sizes. Similarly, Figure 5.3 shows the power plot of the power of linkage disequilibrium method. The other experimental parameters were taken to be the same as the Moreton Bay study and the allele

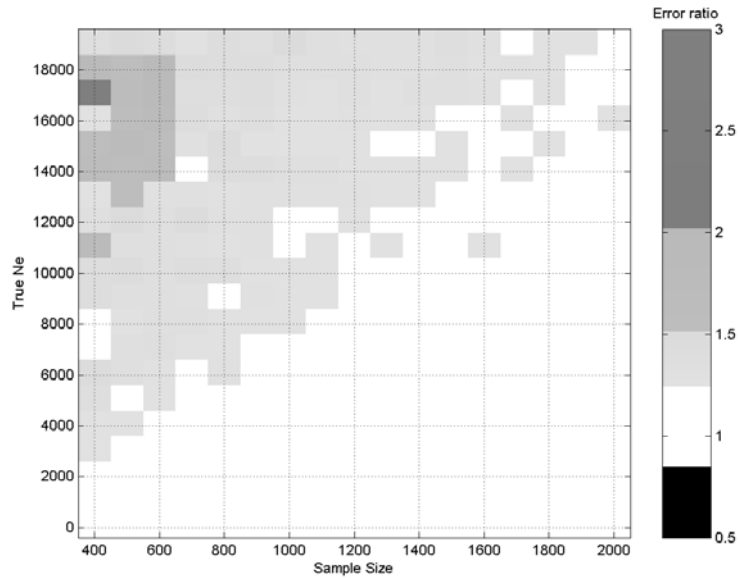
frequencies were generated randomly. It can be seen from these figures that for a given sample size that the linkage disequilibrium methods gives greater power than the moments-based temporal method.

It should be noted that when looked at in a cost per sample basis the temporal methods in these comparisons used twice as many samples as the point estimate linkage disequilibrium since two samples are taken. So without other considerations twice as many samples would have to be taken for the linkage disequilibrium method for the same cost.

Also of concern is the accuracy of the methods. Since the distribution of simulated estimates was skewed and often contained a small number of extremely high values, the mean of the simulated estimates does not adequately reflect the behaviour of the estimate. It was felt a better indication of performance was the median of the simulated estimates. For comparison, the ratio of the median of the simulated estimates to the true  $N_e$  value is given in Figure 5.4 and Figure 5.5. Obviously, a value of one is ideal and the larger the difference from one the worse the bias of the median. Examining Figure 5.4 and Figure 5.5 we can see that the moments-based method provides a slightly less biased estimate than the linkage disequilibrium. So although the linkage disequilibrium method provides more precise estimates (ie greater power) than the moments-based method it would seem it lacks some accuracy (ie. greater error/bias).



**Figure 5.4:** Plot of the ratio of the median of the simulated estimates to the true  $N_e$  using the moments-based method for various sample sizes, over a range of true  $N_e$ .



**Figure 5.5: Plot of the ratio of the median of the simulated estimates to the true  $N_e$ , using the linkage disequilibrium method for various sample sizes, over a range of true  $N_e$ .**

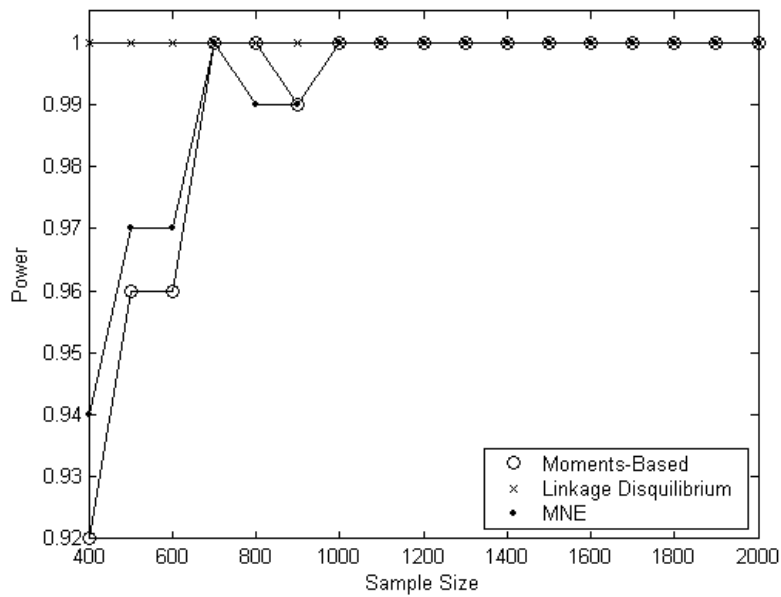
As discussed in “Method” of this chapter, a smaller scale simulation was also done to investigate the performance of the MLNE method (see Chapter 1). A comparison to the results given by the moments-based temporal and linkage disequilibrium methods on the same samples for the cases of small (1000), medium (10 000) and large (18 000) true  $N_e$  is given in Figure 5.6 to Figure 5.11. Figure 5.6, Figure 5.8 and Figure 5.10 are plots of the power of each method over a range of sample sizes for each sized true  $N_e$ . Similarly, the median estimates over a range of samples sizes for each case are given in Figure 5.7, Figure 5.9 and Figure 5.11.

The random fluctuation or jaggedness in the plots, especially in the temporal methods, is largely due to bootstrap error, since we used only 100 replicated samples for this part of the study. Even with this variation it is still possible to see the some strong traits in the each of the methods' performance.

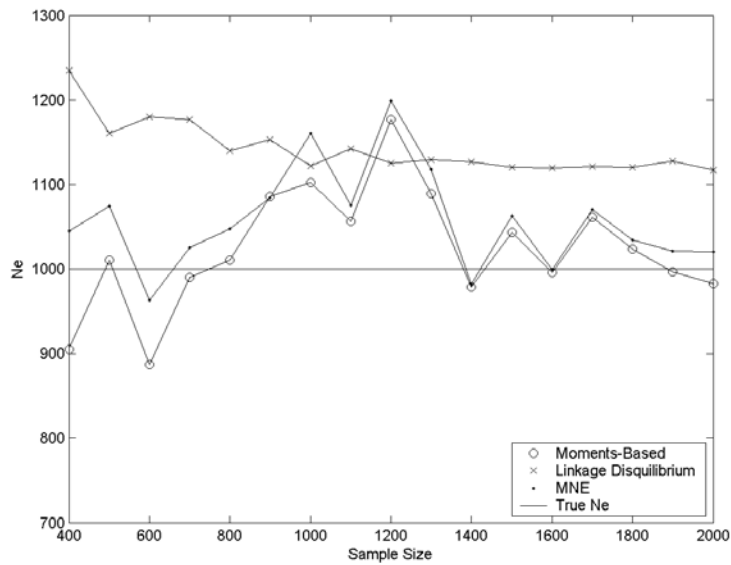
In terms of power MLNE did give a small improvement over the moments-based temporal but still not as good as the linkage disequilibrium method. It should be noted that an input parameter of MLNE is a maximum  $N_e$  (we used a value 30 000 for small and medium  $N_e$  and 40 000 for the large  $N_e$  case), where estimates greater than this value considered to be infinite. To estimate power we assumed that estimates that are close to the maximum  $N_e$  are considered as infinite estimates.

Looking at the accuracy of each method, as we saw in Figure 5.4 and, the linkage disequilibrium method gives slightly less accurate estimates than the moments-based method. We can see more clearly from Figure 5.7, Figure 5.9 and Figure 5.11 that the Linkage disequilibrium method consistently overestimates the  $N_e$ . The MLNE method behaves similarly to the moments-based temporal method but with slightly greater accuracy, especially in the case of large true  $N_e$ .

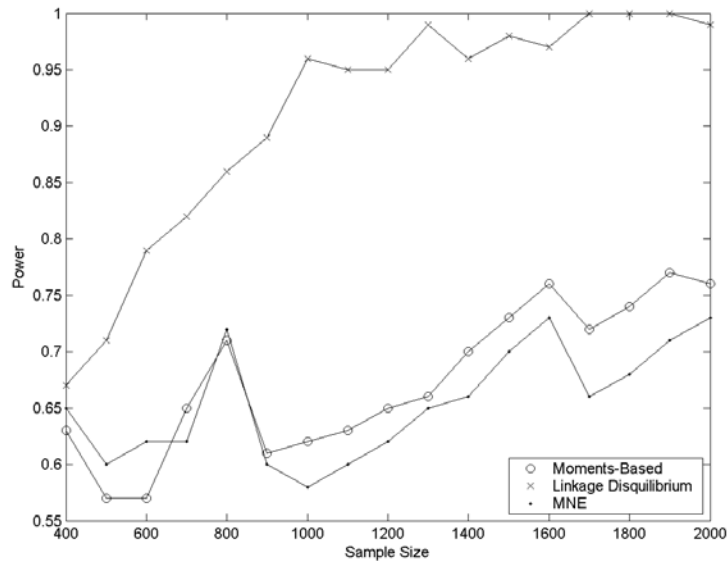




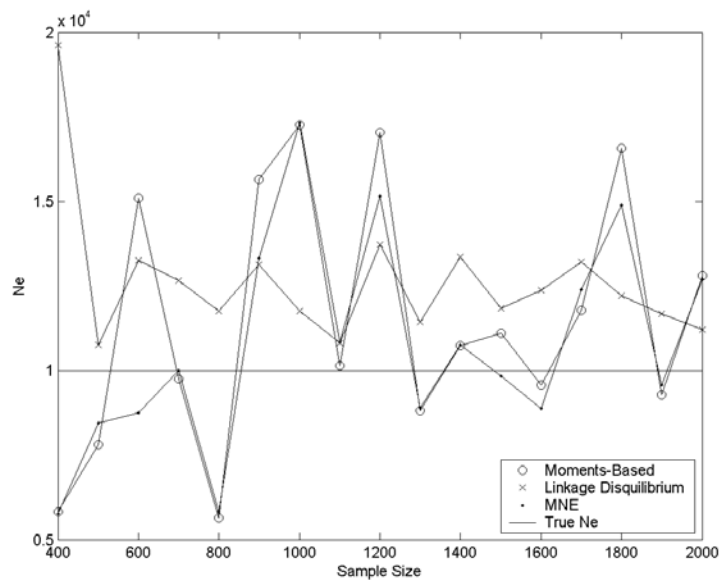
**Figure 5.6: Plots of the power of selected methods for a range of sample sizes with a small true  $N_e$  (1000).**



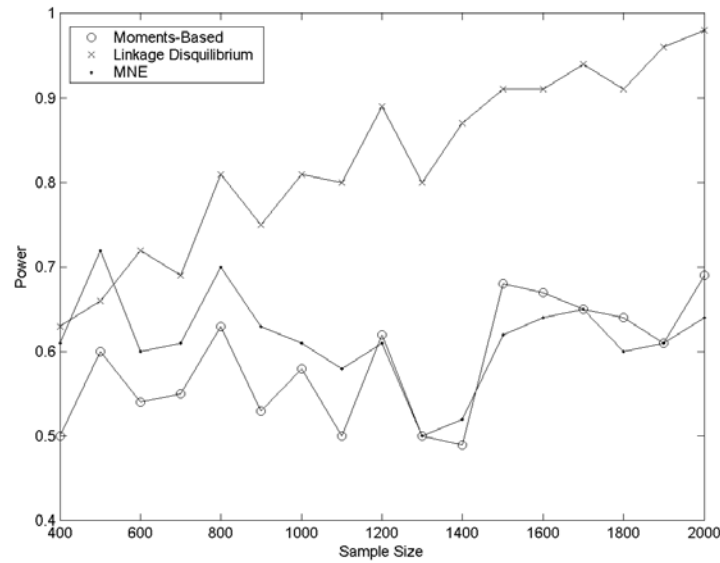
**Figure 5.7: Plots of the median estimate of selected methods for a range of sample sizes with a small true  $N_e$  (1000).**



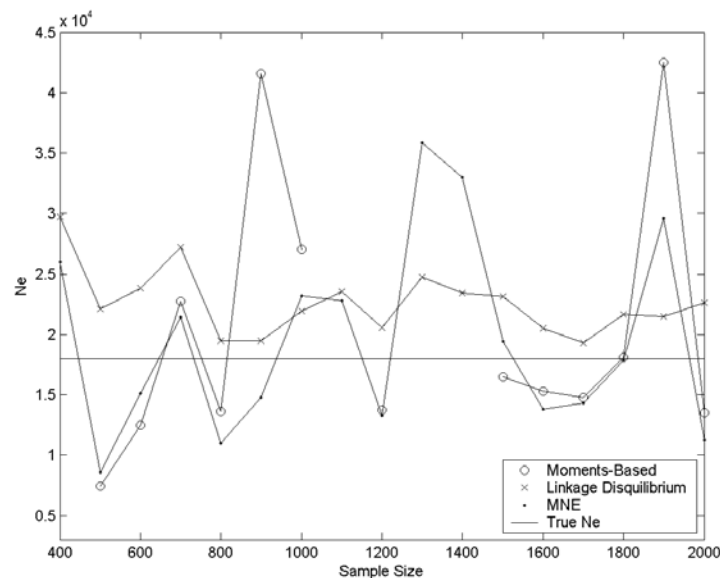
**Figure 5.8: Plots of the power of selected methods for a range of sample sizes with a medium true Ne (10 000).**



**Figure 5.9: Plots of the median estimate of selected methods for a range of sample sizes with a medium true Ne (10 000).**



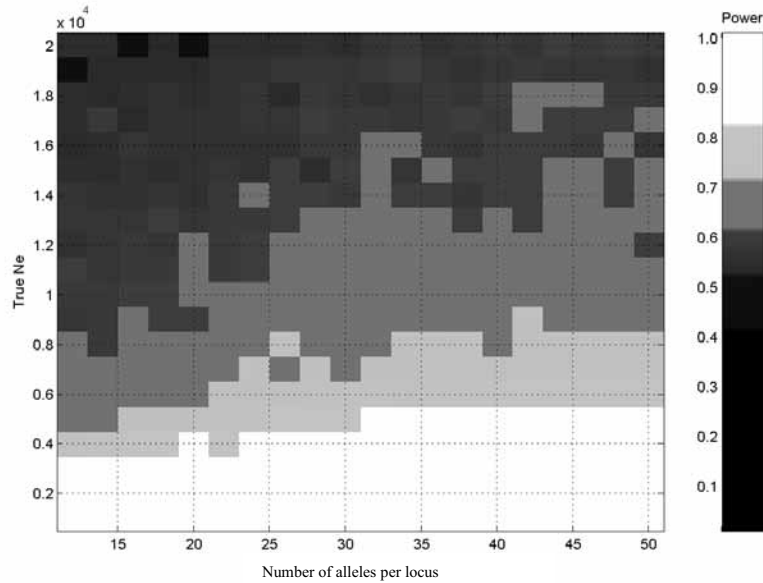
**Figure 5.10: Plots of the power of selected methods for a range of sample sizes with a large true  $N_e$  (18 000).**



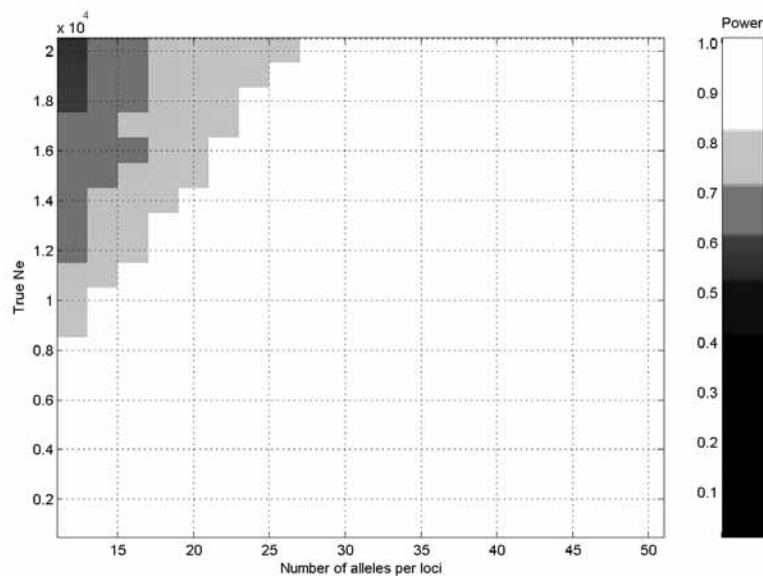
**Figure 5.11: Plots of the median estimate of selected methods for a range of sample sizes with a large true  $N_e$  (18 000). Infinite values for the moments-based temporal method are not shown and seen as gaps in the line.**

### *Number of alleles*

Another parameter that affects the performance of the methods is the number alleles per loci. Figure 5.12 and Figure 5.13 show the power of the methods with respect to the true  $N_e$  and the number of alleles at each of the 8 loci for the linkage disequilibrium method and the moments-based temporal method. As before all other parameters were taken to be the same as the Moreton Bay pilot study. Again the linkage disequilibrium method had greater power than the moments-based method and also seems to provide a greater benefit in terms of power from an increased number of alleles per loci.

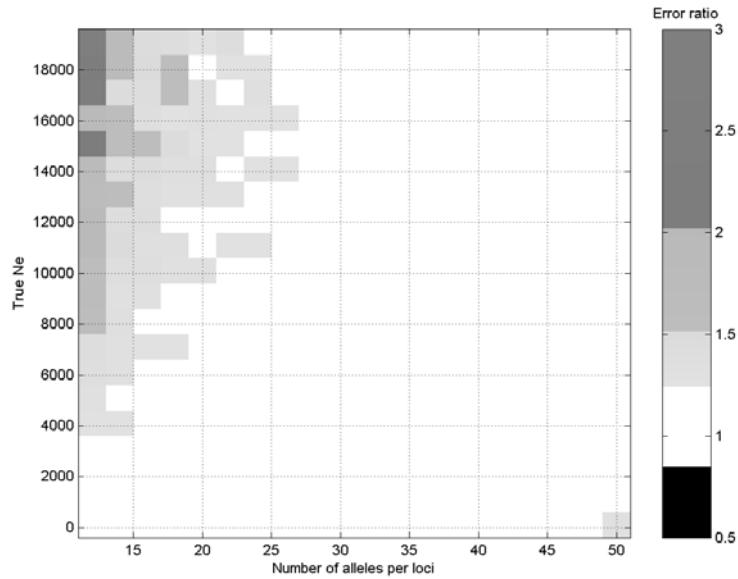


**Figure 5.12: Plot of the power obtained by the moments-based method for various number of alleles per loci, over a range of true Ne. The solid line denotes the boundary of 80% power.**

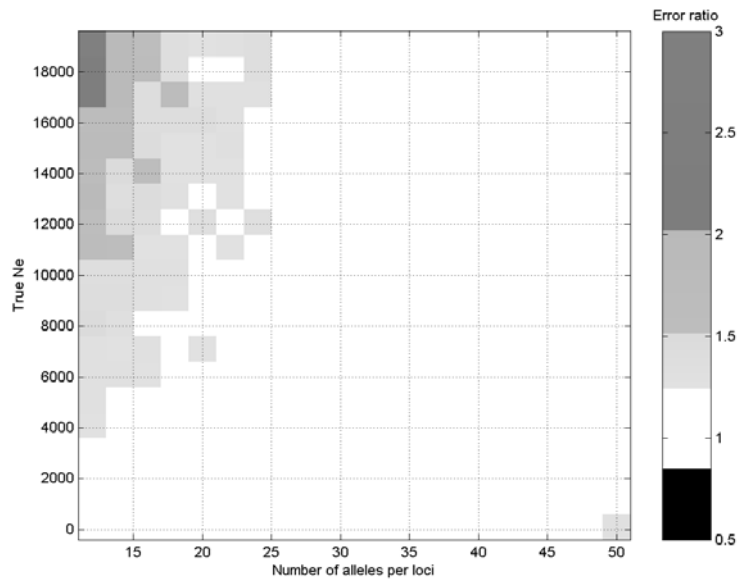


**Figure 5.13: Plot of the power obtained by the linkage disequilibrium method for various number of alleles per loci, over a range of true Ne.**

Looking at the accuracy of the methods, Figure 5.14 and Figure 5.15 show the ratio of the median of the simulated estimates to the true Ne value for the moments-based and linkage disequilibrium method respectively. Interestingly, as the number of alleles per locus increases the accuracy of the linkage disequilibrium and moments-based temporal method is comparable.



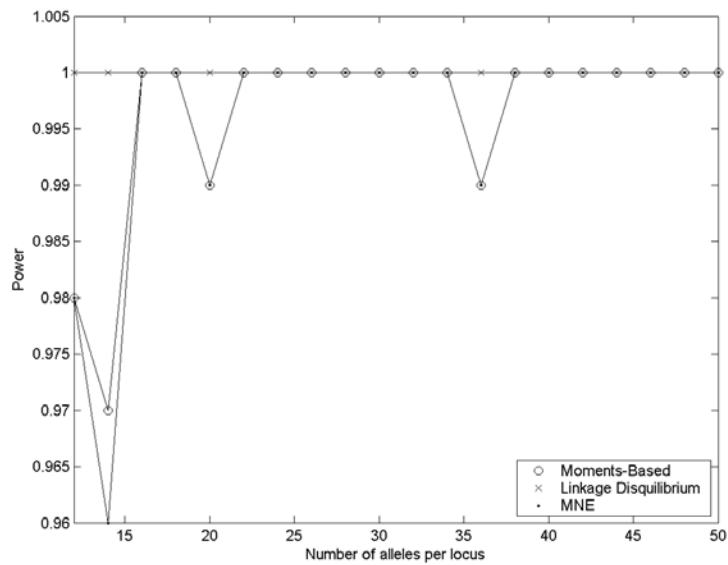
**Figure 5.14: Plot of the ratio of the median of the simulated estimates to the true  $N_e$ , using the moments-based method for various number of alleles per loci, over a range of true  $N_e$ .**



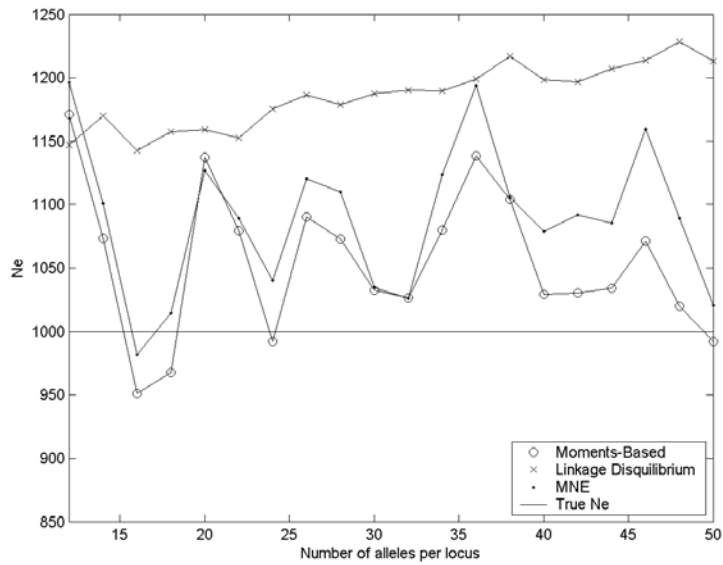
**Figure 5.15: Plot of the ratio of the median of the simulated estimates to the true  $N_e$ , using the linkage disequilibrium method for various number of alleles per loci, over a range of true  $N_e$ .**

The cases of small, medium true  $N_e$  were examined for the MLNE method for a range of number of alleles per locus, and the results given in Figure 5.16 to Figure 5.19. As expected the MLNE method behaved in a similar manner to the moments-based temporal method but provided a small improvement in accuracy and precision. As seen previously, it would seem that for small true  $N_e$  with a reasonable number of alleles, that the temporal methods outperform the linkage disequilibrium method in terms of accuracy. This is not seen in Figure 5.14 and Figure 5.15 as the colour classification is not fine enough to show the small difference in performance.

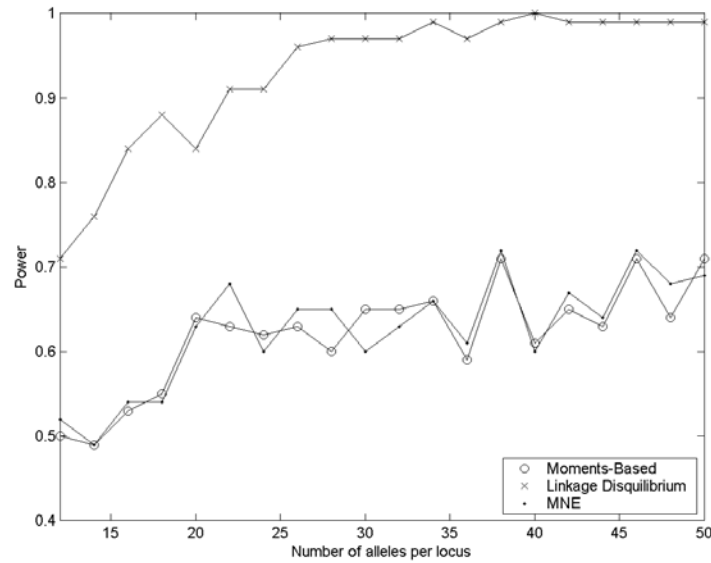
However, when the number of alleles is very small the temporal methods give biased large estimates of  $N_e$ .



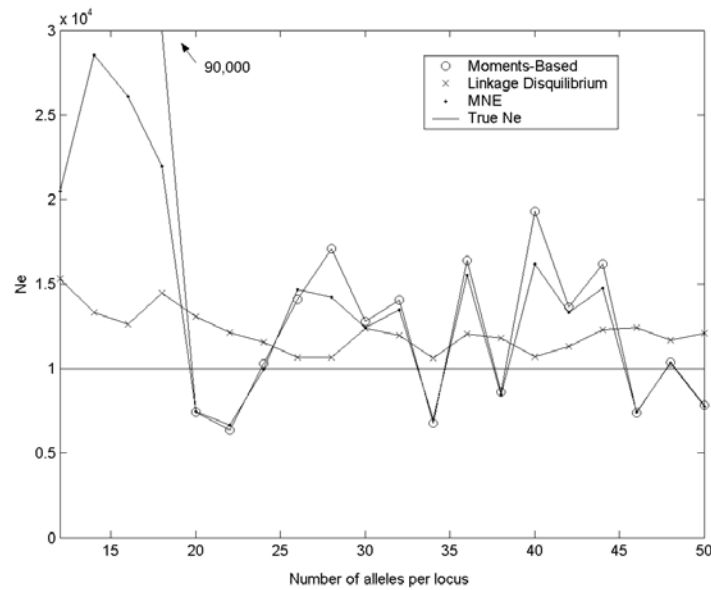
**Figure 5.16: Plots of the power of selected methods for a range of number of alleles per locus with a small true  $N_e$  (1000).**



**Figure 5.17: Plots of the median estimate of selected methods for a range of number of alleles per locus with a small true  $N_e$  (1000).**



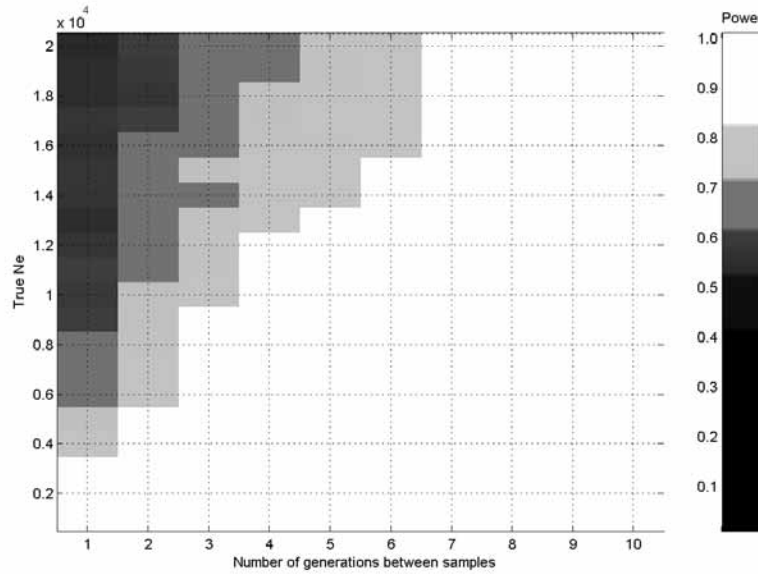
**Figure 5.18: Plots of the power of selected methods for a range of number of alleles per locus with a medium true  $N_e$  (10 000).**



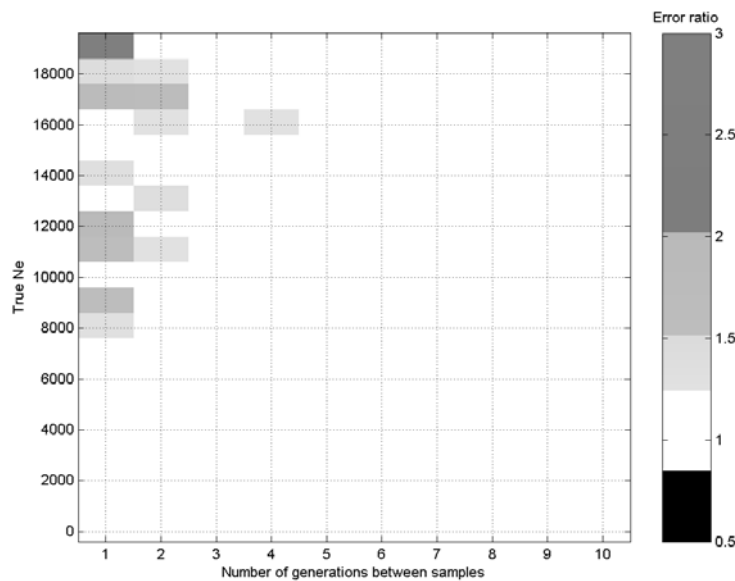
**Figure 5.19: Plots of the median estimate of selected methods for a range of number of alleles per locus with a medium  $N_e$  (10 000).**

***Number of Generations***

The number of generations between the temporal samples is only of concern in the case of the temporal method. The greater the number of generations elapsed between the measurements of allele frequencies, the greater the observed amounts of drift, giving rise to more statistically powerful estimates of  $N_e$ . However, it is important to remember that estimates of  $N_e$  across several generations are the harmonic mean of intervening intergenerational estimates. Figure 5.20 gives a plot of the power of the moments-based method for a range of temporal distances. Similarly, Figure 5.21 provides the ratio of the median of the simulated estimates to the true  $N_e$  value.



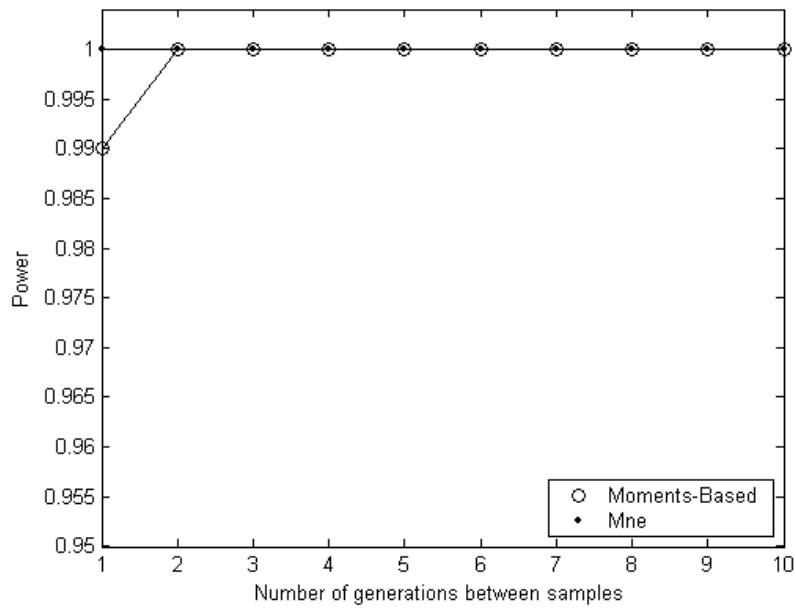
**Figure 5.20: Plot of the power of the estimates of  $N_e$  obtained by the moments-based method for various Number of generations, over a range of true  $N_e$ .**



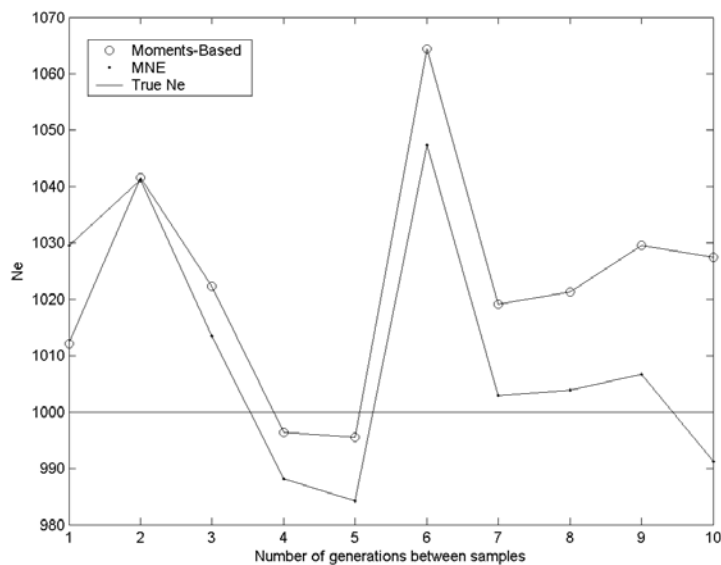
**Figure 5.21: Plot of the ratio of the median of the simulated estimates to the true  $N_e$ , using the moments-based method for various number of generations between samples, over a range of true  $N_e$ .**

Comparing the moments-based method with MLNE, we again looked at the cases of small and medium  $N_e$ . Figure 5.22 and Figure 5.24 show the difference in power between the two methods. As expected for small  $N_e$  both methods provide almost perfect power. For medium sized  $N_e$  the power of both methods is quite low but as seen in Figure 5.20 the power increases steadily for both methods as the number of generations between samples increases. The moments-based method appears to have greater precision than MLNE, although it may not be to the extent shown as the definition of an infinite estimate with respect to the maximum  $N_e$ , in the MLNE method, greatly affects the calculated observed power.



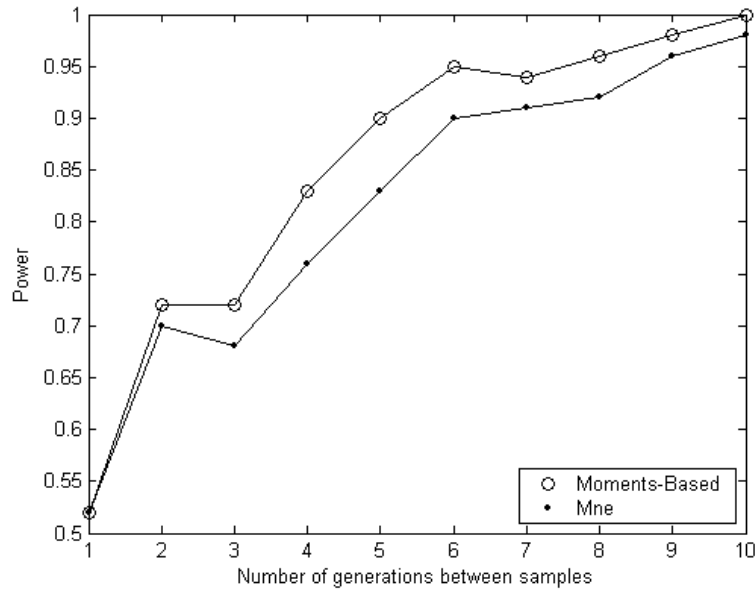


**Figure 5.22: Plots of the power of the moments-based and MLNE temporal method with a range of number of generations between samples with a small true  $N_e$  (1000).**

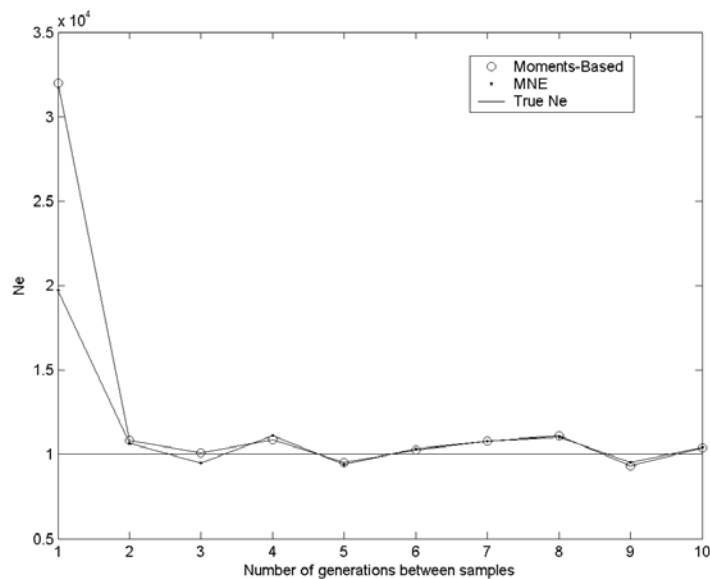


**Figure 5.23: Plots of the median estimate of the moments-based and MLNE temporal method with a range of number of generations between samples with a small true  $N_e$  (1000).**

Figure 5.23 and Figure 5.25 show the ratio of the median estimate to the true  $N_e$ . In these simulations MLNE came out as being slightly more accurate. Of note is in Figure 5.25, that for a single generation both methods in this simulation considerably overestimated  $N_e$ .



**Figure 5.24: Plots of the power of the moments-based and MLNE temporal method with a range of number of generations between samples with a medium  $N_e$  (10 000).**

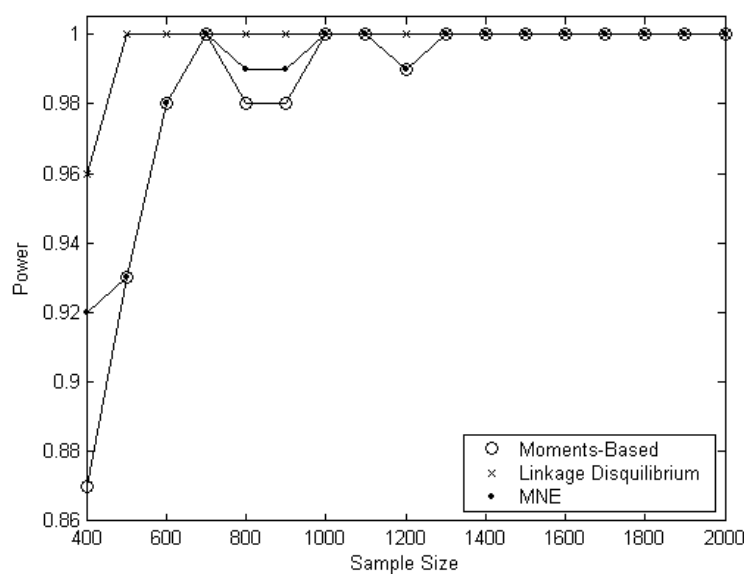


**Figure 5.25: Plots of the median estimate of the moments-based and MLNE temporal method with a range of number of generations between samples with a medium  $N_e$  (10 000).**

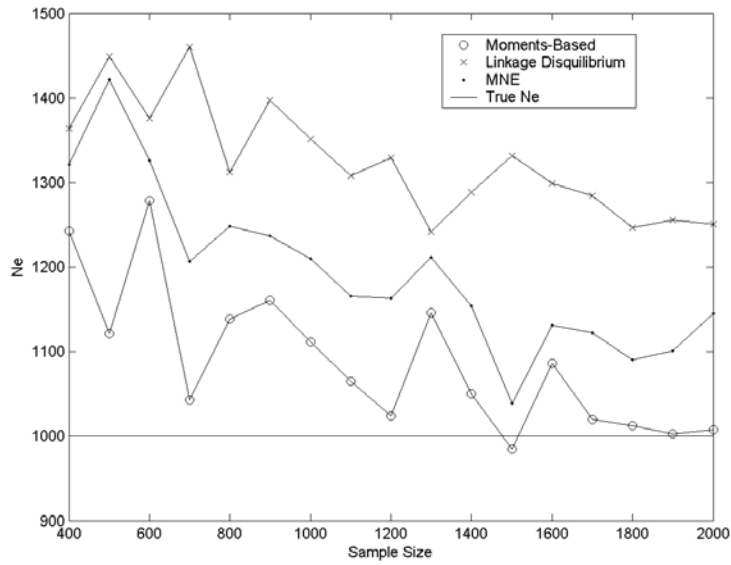
***Allele Frequency Distribution***

The simulations presented so far have all simulated the populations with random allele frequencies. For large sample sizes, this generally leads to a rather uniform allele frequency distribution. However, in practice the allele frequencies of actual real world samples may be far from uniform. To examine the effect of allelic distribution we repeated the sample size simulation using the actual empirical allele frequency distribution from Moreton Bay (Figure 2.9).

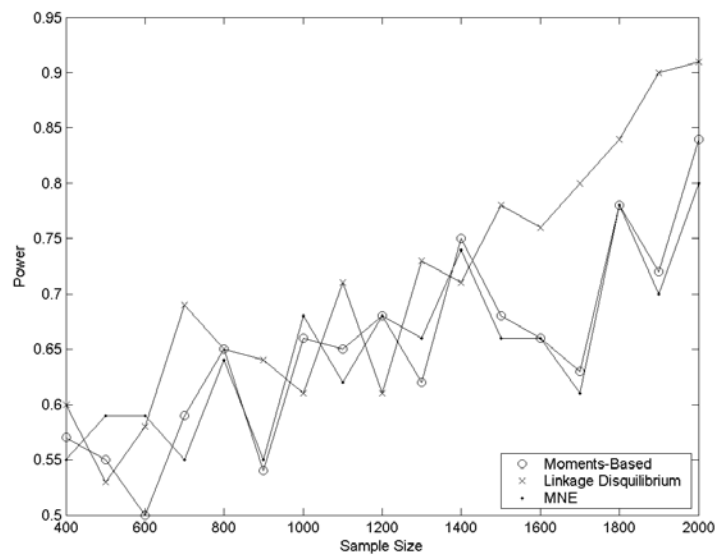
The power and accuracy plots for the three methods are given in Figure 5.26 to Figure 5.28. Comparing these results to the corresponding results seen in “Results, Sample Size” in this chapter, we can see that the allelic frequency does effect the performance of the methods. In particular using the pilot study allelic frequencies we see an increased positive bias in all of the methods. This maybe due, in part, to the presence of low frequency alleles in the parent population. Interestingly, in Figure 5.27 as the sample sizes increase the observed bias decreases. Also for a moderate  $N_e$  (10,000) the linkage disequilibrium method is less statistically powerful when allele frequencies are skewed (Figure 5.28) compared to its strong comparative performance when allele frequencies were randomly determined (Figure 5.8). The sensitivity of the temporal methods to allele frequency and the subsequent bias is discussed in Wang (2001) and Turner et al. (2001).



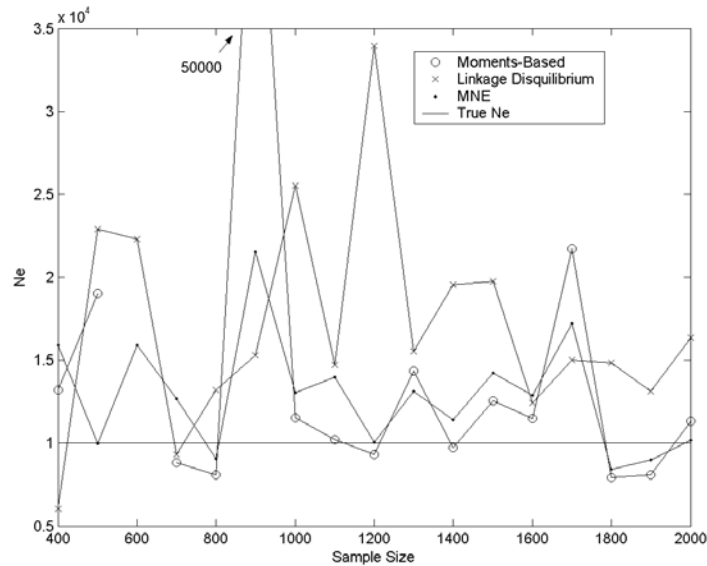
**Figure 5.26: Plots of the power of selected methods for a range of sample sizes with a small true  $N_e$  (1000) and the empirical allele frequencies from the pilot study used.**



**Figure 5.27: Plots of the median estimate of selected methods for a range of sample sizes with a small true  $N_e$  (1000) and the empirical allele frequencies from the pilot study used.**



**Figure 5.28: Plots of the power of selected methods for a range of sample sizes with a medium true  $N_e$  (10 000) and the empirical allele frequencies from the pilot study used.**



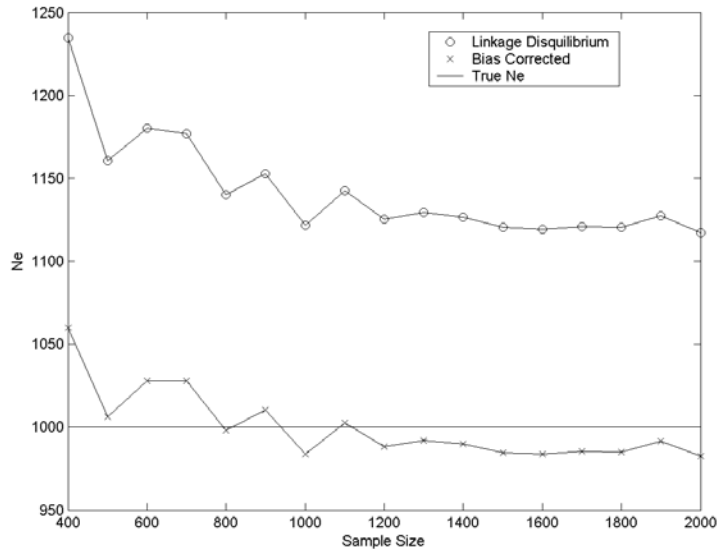
**Figure 5.29: Plots of the median estimate of selected methods for a range of sample sizes with a medium true  $N_e$  (10 000) and the empirical allele frequencies from the pilot study used.**

### *Linkage Disequilibrium Correction*

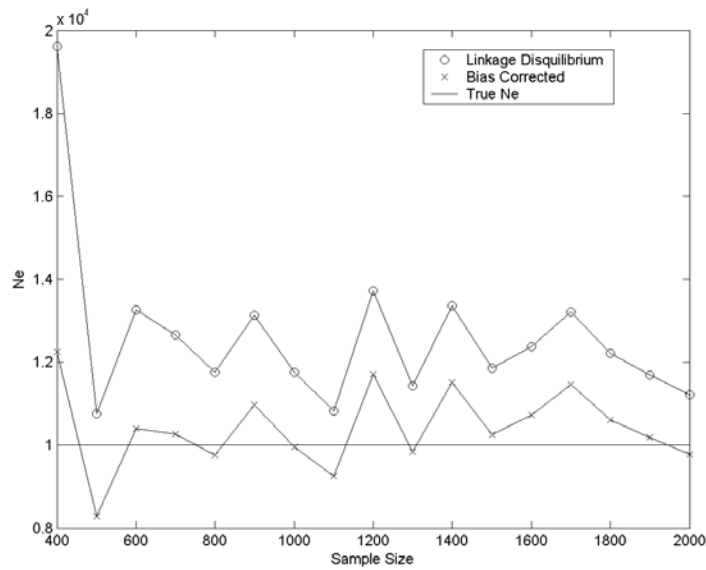
It is obvious that for all but small  $N_e$  the linkage disequilibrium method consistently overestimates  $N_e$ . To examine this bias further we examined a simple corrected form of the linkage disequilibrium formula to estimate  $N_e$ . Although, any bias correction we form will be based on limited simulations only and hence will not provide a suitable correction for the general use of the method. We used a simple correction of the form

$$\hat{N}_e = \frac{1}{K(\hat{r}^2 - 1/(S+B))}, \quad (15)$$

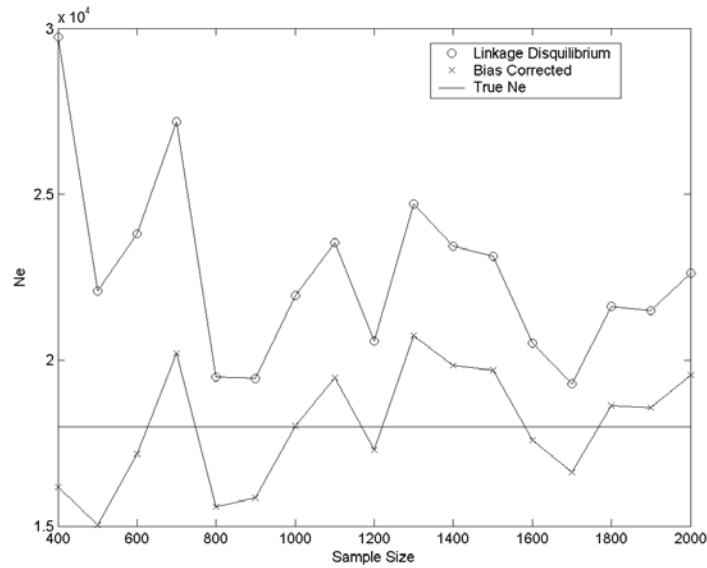
The original formulation seen in (7) corresponds to values of  $K=3$  and  $B=0$ . Doing a simple optimisation on the simulated results seen in “Sample Size” of this chapter, gives values of approximately  $K=3.4$   $B=1$ . The effect of applying this correction is given in plots of the median estimate in Figure 5.30 to Figure 5.32 for the linkage disequilibrium.



**Figure 5.30: Plots of the median estimate for the linkage disequilibrium method with and without bias correction for a range of sample sizes with a small true Ne (1000).**

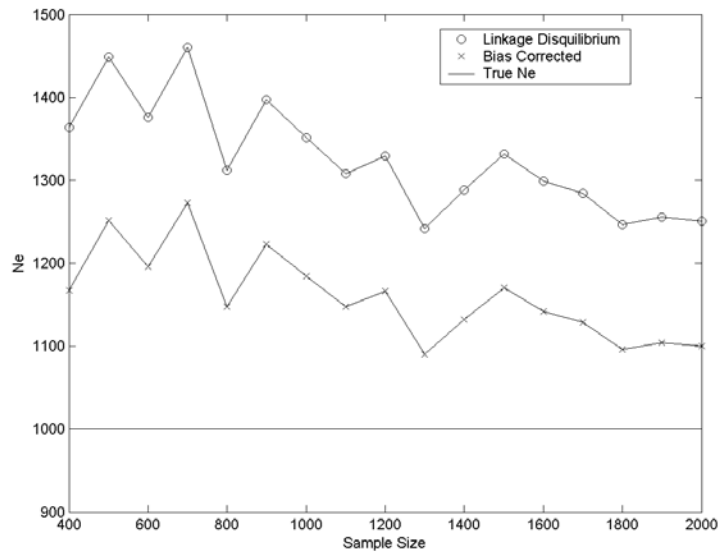


**Figure 5.31: Plots of the median estimate for the linkage disequilibrium method with and without bias correction for a range of sample sizes with a medium true Ne (10 000).**



**Figure 5.32: Plots of the median estimate for the linkage disequilibrium method with and without bias correction for a range of sample sizes with a large true  $N_e$  (18 000).**

It can be seen from Figure 5.30 to Figure 5.32 that some form of bias correction could work very well. However, as Turner et al. (2001) found with regard to the bias in the moments-based temporal method, the bias term may be a function of a number of parameters; the true  $N_e$ , sample size and allele frequency. As we have seen in “Allele Frequency Distribution” of this chapter, the allele frequency seems to definitely be a factor in the bias. The correction used in this section is based on random allele frequencies and we can see when we apply our correction in Figure 5.33 in the case of using the pilot study allele frequencies that the bias correction is not sufficient. A further simulation study to generate a bias correction term based on some sort of allele frequency parameter may provide a more general bias correction. Further investigation would be required to find what characteristic of the allele frequency effects the bias for example denoting the allele frequency as  $A_1, \dots, A_n$ ; we could investigate a measure of the dominance of a single allele  $\max(\mathbf{A})$ , a measure of very rare alleles  $\min(\mathbf{A})$ , or the variation of allele frequency  $\text{var}(\mathbf{A})$ .



**Figure 5.33: Plots of the median estimate of selected methods for the linkage disequilibrium method with and without bias correction for a range of sample sizes with a small true  $N_e$  (1000) and the empirical allele frequencies from the pilot study used.**

## Discussion

This simulation experiment has provided an insight into the performance of the various methods to estimate large effective population sizes, most notably the effect of the various experimental parameters on the precision and accuracy of the resulting estimates. By mimicking the real world situation in the Moreton Bay population these simulations provide a guide as to the resources that we would require to gain a specified expected power and accuracy from our estimates for our specific project. By examining the effect of each parameter we can evaluate which parameter provides the best improvement for the cost.

For these large effective population size simulations the linkage disequilibrium method gave much better performance than the temporal moments-based method in terms of power. Although it should be noted in other simulations we have found this is reversed when the sample size or number of alleles is very small. Although we found the linkage disequilibrium method to be powerful it was also biased; overestimating  $N_e$  in all of our simulations. One possible solution that was examined was to use a bias corrected estimate. It was seen that the bias was dependent on allele frequency, in particular rare alleles. Further simulations are required to develop a bias correction related to some allele frequency parameter.

The use of the more complex temporal method MLNE did provide some benefit over the standard moments-based method but the linkage disequilibrium method still gave the best performance for large  $N_e$ . As noted in the literature, the best approach may be to apply all of the available methods and use the results collectively to determine the effective population size.

The effect of the various experiment parameters on the power of the methods was found to be substantial. Increasing the sample size or the number of alleles per loci



increased the power for both methods, with the linkage disequilibrium method improving the most. Increasing the sample size will often be the easiest to implement and generally will simply be a cost issue. Interestingly, increasing the sample size or number of alleles per locus improved the linkage disequilibrium method to a greater extent than it did for the moments-based temporal method.

The bulk of the simulations were performed with random allele frequency distributions, but when realistic allele frequency distributions are used all the  $N_e$  estimation methods, including MLNE, the estimates are upwardly biased. The temporal method appears to be the less biased. In terms of the large confidence intervals around the point estimates, and provided the upper confidence limit is non-infinite, then the uncertainty is not a major issue.

Increasing the number of generations between the temporal samples as expected increased power and accuracy. However, as was mentioned previously the quantity being estimated has changed. When the temporal samples are greater than one generation apart the  $N_e$  being estimated is the harmonic mean of all of the intermediate  $N_e$ 's.

This paper has examined the improvements gained by increasing the experiment resources (eg number of samples, number of alleles per loci) in isolation. The most feasible way to use these methods in the case of large  $N_e$  would be to improve all of the resources in conjunction. This would greatly improve the power and accuracy and allow the precise estimation of quite large  $N_e$ .

From these simulations we have built up a much stronger understanding of the methods' behaviour for large effective population sizes. The number of replicated samples in some of the simulations was small (100) due to computational issues and in some cases the variation in the simulated estimates did not allow an accurate picture of the estimates behaviour. This may be addressed in the future with further simulations.



## Chapter 6 – Estimation of effective population size when migration is occurring

by JR Ovenden

### Introduction

Estimates of effective population size ( $N_e$ ) are based on the measurement of genetic drift within a fisheries resource, namely a single-species population. It is well known that migration into and out of the population and overlapping generations can bias this measurement. The objective of this chapter is to review two correction methods that are available in the literature to account for the bias due to migration. Correction factors for the life history traits of *Penaeus*, in particular the presence of overlapping generations in the populations of the Gulf of Carpentaria, are evaluated in Appendix D.

There are a range of methods for the estimation of  $N_e$  from genetic data. They include the linkage disequilibrium (Hill 1981) and heterozygote excess methods (Pudovkin, Zaykin *et al.* 1996) and the several methods based on temporal genetic data (see Chapter 1 for a review). Temporal genetic methods assume that if other population genetic processes, such as mutation, migration and selection are negligible, then observed changes in allele frequencies over the time-frame of the study can be assumed to be due to genetic drift. Genetic drift is then inversely proportional to  $N_e$ .  $N_e$  can be estimated without genetic data from demographic parameters (reviewed by Wang and Caballero 1999). Direct survey counts of the adult population at breeding time yield measures that are related to  $N_e$ , but are subject to considerable sampling error (Chapter 4).

Generally it is reasonable to ignore the effects of mutation, as it will be negligible over the time-frame of most projects. Similarly, it may be reasonable to ignore the effects of selection as the genetic markers normally chosen for these studies (microsatellite loci) are regarded as neutral with respect to selection (Li, Korol *et al.* 2002). In addition, the operation of selection, whether direct or indirect, can be tested for *post-hoc* and affected loci can be excluded from the study. However, for many populations the effects of migration are not negligible. In this project, the population of tiger prawns in Moreton Bay was specifically chosen as a test population because it was unlikely to be significantly influenced by migration due to its spatial isolation from adjacent populations to the north or south. The population genetics of the more commercially valuable populations of tiger prawns (*Penaeus esculentus* and *P. semisulcatus*) in the Gulf of Carpentaria and other invertebrate and vertebrate fisheries resource populations are not spatially isolated and will be affected by migration.

As an example of the spatial scale over which migration may occur in the tropical north, Derbyshire *et al.* (1990) tagged 19,156 *P. esculentus* in western and eastern Torres Strait in January and February 1987. Tagged prawns released in the west moved an average of 25 nautical miles (nm, males) to 27 nm (females) with the greatest movement being 69 nm by a single female. The average time at liberty was 73 days, with the greatest time being 297 days. In the east, prawns moved much less; only 4 nm on average over a mean time at liberty of 28 days. The greatest distance traveled was 58 nm. The longest time at liberty was 243 days. Somers and Kirkwood

(1984) had previously shown movements of a similar magnitude from inshore to offshore in western Gulf of Carpentaria for both tiger prawn species. Areas outside commercial fishing grounds were not sampled in these studies, so some long distance movements may be undetected.

Wang and Whitlock (2003) describe how migration affects estimates of  $N_e$  in theory. In the short term, migration can change the allele frequencies of a population quickly by mimicking a large amount of genetic drift that causes the underestimation of  $N_e$ . Large amounts of migration and drift over a longer time-frame would cause allele frequency change in the population to approach that of all donor or recipient populations. Thus, the  $N_e$  of the population would become the  $N_e$  of the entire metapopulation, and consequently be an overestimate of the per-population  $N_e$ . If the population was at mutation/drift/migration equilibrium then temporal estimates of current  $N_e$  may be similar to long-term  $N_e$ . Valid measurements of current  $N_e$  can be made in the absence of mutation/drift/migration equilibrium and indeed the ratio between long-term and current  $N_e$  may be indicative of whether the population is at equilibrium.

By far the most widely used method of estimating migration using genetics is to assume that the population is at equilibrium, and conforms to the island model of Wright (Hedrick 2000; Wright 1969).  $F$ -statistics are used to calculate  $m$ , the local immigration rate using the equation

$$F_{ST} = \frac{1}{(4N_e \cdot m + 1)}$$

The majority of modern studies do not present estimates of  $m$  due to the unrealistic assumptions that need to be made about population structure. Furthermore,  $N_e$  must be known to convert migration into actual numbers of successfully recruiting individuals exchanged between populations. A useful application of the  $F$ -statistics method however, is to test predictions that the population being studied is, or is not, experiencing migration.

An alternate approach to estimating migration is to use assignment testing (Berry, Tocher *et al.* 2004; Castric and Bernatchez 2004; Davies, Villablanca *et al.* 1999). This method uses multi-locus genotypes to identify possible migrants, or close descendants of migrants that have been sampled. They appear as genetic outliers, and those individuals can be removed from the analysis as required. For example, Kinnison *et al.* (2002) compared the estimated extent of gene flow between subpopulations of chinook salmon in New Zealand using assignment testing. Their estimate of immigration rates was an order of magnitude (30-50%) above conventional  $F_{ST}$  - based methods ( $\approx 2.3\%$ ). Assignment tests may have limited practical power in cases where population structure is recent and hence gene frequency differences among populations are subtle. Assignment testing also potentially suffers from the inability to distinguish the exchange of non-breeding individuals from migration. The latter results in gene flow when the immigrant participates in breeding in its adopted population (Endler 1977).

Two methods (Vitalis and Couvet 2001b; Wang and Whitlock 2003) have appeared in the literature during the project that allow the simultaneous estimation of  $N_e$  and  $m$  under robust and realistic assumptions. Both have the potential to correct single

population estimates of  $N_e$  for migration. They will be described briefly in the next section, and their strengths and weaknesses will subsequently be highlighted.

### **One- and two-locus identity probabilities**

Vitalis and Couvet (2001) proposed a method-of-moments method to jointly infer local effective population size and immigration rate based on estimates of functions of one- and two-locus identity probabilities. The method requires only a single population sample and obtains confidence limits by bootstrapping. The assumption of an infinite-island model was evaluated with simulations involving true  $N_e$  up to 100. When the method was applied to a simulated finite island population, some bias was introduced but the results were still reasonable.

Software (ESTIM 1.0 Vitalis and Couvet 2001a) is available to implement this method on up to 100 populations represented by less than 1000 samples and assayed with up to 100 loci. We are currently unaware of examples of application of this method, however it appears to provide a pathway to separate the variance due to drift from the variance due to migration in a data set from a single population. The method could be evaluated by comparing the per-population amount of migration in a pair of populations where gene-flow had been measured and quantified using Wright's  $F$ -statistics or assignment testing. Alternatively, the method could be applied to an isolated population not experiencing gene-flow where the estimated amount of migration would be expected to be zero.

### **Maximum-likelihood and moment estimation of $N_e$ and $m$**

Wang and Whitlock (2003) extend previous methods (Anderson, Williamson *et al.* 2000; Wang 2001; Williamson and Slatkin 1999) for the temporal estimation of  $N_e$  in a single population, in order to make estimates of  $N_e$  and  $m$  for a metapopulation situation. Their aim was two-fold; 'to assess and correct for the population genetic effects of immigration on estimates of the effective population size and to estimate the rate of migration itself.' (p. 442, Wang and Whitlock 2003). They checked the performance of their method under several situations; a focal small population receiving immigrants from an infinitely large population, two small populations connected by migration and a small number of populations (demes) in island or stepping-stone migration models.

Software for the infinite source model MLNE (maximum likelihood estimate of  $N_e$ ) is available for free download (Wang and Whitlock 2003). The performance of MLNE to estimate  $N_e$  was evaluated against a variety of temporal and point-estimation methods and the results are presented in Chapter 5. This study did not collect empirical spatial and temporal data from two populations that would have been needed to evaluate performance of MLNE in estimating migration ( $m$ ), but it has been comprehensively tested by simulations and applied to an empirical data set in Wang and Whitlock (2003).

### **Discussion**

Wang and Whitlock (2003) state that for reasonably good estimates of migration rate and effective population size their MLNE method requires a large amount of genetic marker information. They recommend at least a few hundred individuals per

population and the use of 10 to 20 loci that will produce 20-140 alleles in total. For other temporal methods of estimating effective population size the statistical power of the method increases with the generation or sampling interval. For the MLNE method the situation is slightly different. The power does increase as the interval increases, but it reaches a plateau at intermediate values. From then on the power begins to decrease. Wang and Whitlock (2003) explain that as the interval is large, then  $N_e$  is being estimated for the whole species rather than for the local population.

Vitalis and Couvet (2001b) recommend using eight to 12 polymorphic loci for their identity probability method. The estimation of linkage relationships among loci is essential in this method, with more tightly linked markers giving larger values for identity disequilibrium. For large population sizes, a large value for the identity disequilibrium would produce more accurate estimates of  $N_e$ , but to achieve this it would be necessary to select genetic markers with known, and tight, linkage relationships. For non-model species, such as fisheries species, this information may not be available. However, genetic loci are being used for selective breeding in aquaculture species such as the giant tiger prawn (*Penaeus monodon*) and may be a source of the required information on linkage among genetic markers.

Wang and Whitlock (2003) state that they do not make any assumptions about genetic equilibrium, but their simulations were run assuming an equilibrium level of initial genetic differentiation among populations. Populations receiving large amounts of migration are unlikely to be in migration/drift equilibrium as the inflow of new alleles from migration would outbalance the numbers lost through drift or created by mutation. The method of Vitalis and Couvet (2001b) assumes genetic equilibrium.

Temporal and spatial sampling of tiger prawn populations from the Gulf of Carpentaria at different scales would yield empirical data that could be analysed using both methods (Vitalis and Couvet 2001b; Wang and Whitlock 2003). This would have the advantage of cross-verifying the estimates of  $N_e$  and  $m$  from both methods. Varying spatial scales would investigate the genetic connectedness of populations on a fine scale (eg. around Mornington Island in the southern Gulf) or broader scale (eg. eastern compared to western Gulf of Carpentaria). Varying temporal scales would allow the increase of statistical power for the estimation of effective population size. Accompanying computer simulations to investigate the effects of departure from equilibrium would be valuable.

The method of Wang and Whitlock (2003), in combination with the method of Vitalis and Couvet (2001b), potentially increases the power of genetic stock identification in fisheries species by taking advantage of the combination of spatial and temporal data. More significantly, however, their methods present roadmaps for the estimation of effective population size in metapopulations. This capability, combined with (1) the demonstration by simulations (Chapter 5) that powerful estimation of large effective population sizes is within the limits of practical resources currently available and (2) ground-truthing of the accuracy and precision of  $N_e$  from naturally occurring fisheries population may allow the application of genetic methods for the estimation of  $N_e$  to assessment stock assessment of fisheries species, including tiger prawns in the Gulf of Carpentaria.

## **Chapter 7 – Overall discussion and conclusion, benefits, further development and planned outcomes**

*by JR Ovenden*

### **Overall discussion and conclusion**

This project trials a new, independent and complementary data source for computer modelling of naturally occurring populations. Genetic information from individuals from a population is used to estimate the genetic effective population size, a concept closely related to the number of successful spawning individuals in the population. In a fisheries context, for example, Crocos (1987a; 1987b) proposed the term "effective spawning stock" as the proportion of spawning fish that contribute to stock renewal. In population genetics, effective population size is formally the number of breeding individuals in an idealized population that would be experiencing the same amount of genetic drift in a given genetic measure (Hedrick 2000). This equates to the number of spawning individuals that effectively contribute offspring in the next generation. Effective size is inversely proportional to the amount of random genetic drift in the population, which is the chance fluctuation in gene frequencies across generations. Measurements of genetic drift form the basis for estimating effective population size.

Using eight polymorphic microsatellite genetic markers and three annual population samples taken at spawning time, we measured the number of Moreton Bay tiger prawns that successfully contributed recruits to the next generation. In 2001, successful breeders were estimated to number about 800. In 2002 they numbered about 900. At the same time fisheries trawl survey methods showed that the number of prawns participating in spawning was about 1000 times greater. The large discrepancy between animals in spawning condition and those that successfully spawn is expected for high fecundity species where survival is dependent on environmental conditions. For example, the average fecundity of female tiger prawns is 350,000 eggs per female. If there were 900 effective females this would result in  $3.15 \times 10^6$  eggs released at the beginning of the year. This would be more than enough to account for the 80 tonnes of tiger prawns harvested annually from Moreton Bay – equivalent to  $1.6 \times 10^6$  individuals (assuming average weight of 50 grams) – as well as the  $0.5 \times 10^6$  animals (Chapter 4) that were still alive at the end of the year.

Making use of this potentially valuable data source in fisheries management depends, in part, on how it can be integrated into existing stock assessment models. The pathway is not clear at the moment, however, significant steps forward have been made. We believe the key is understanding the factors that affect the magnitude of  $N_e$  in natural populations. These factors are the number of animals that reach reproductive condition and the mortality that affects offspring of these spawners. In a fisheries context, mortality is a consequence of both natural features of the environment and harvest mortality. Natural mortality can affect single individuals or groups of individuals essentially at random throughout the population. If mortality affects groups of individuals that are genetically related, then the mortality is called family-related mortality. The occurrence of this type of mortality depends on the degree of clustering that occurs in space and time of the offspring of a single female. Harvest mortality occurs when animals are removed from the population by fishers and is generally non-random. Harvest mortality can also affect groups of genetically

related individuals. Some families may be genetically, and thus phenotypically, more able to avoid being caught – for example by possessing superior swimming speed or small body size.

In this study, and as discussed by Hoyle (Appendix C), effective population size could be seen as a function of spawning stock size. This function is simply the ratio between the estimated the size of the spawning stock (about  $0.5 \times 10^6$  animals, Chapter 4) and the estimated effective population size (about 900, Chapter 2). At each generation the size of the spawning stock could be estimated by knowing the effective spawning stock size and the function between them. This approach assumes that mortality on offspring is constant - if it was variable, then the function would no longer apply. An example could be a species where recruitment was strongly dependent on density, but the number of spawning individuals was unregulated.

An alternative view, again outlined in Appendix C, is that the number of recruits or offspring in a population is a function of effective population size. The function is related to the degree of post-spawning natural and harvest mortality and was not empirically determined in this study. Technically, the function could be measured. It would be the ratio between the number of recruits alive at a particular stage, and the effective population size. If it was known, the effective population size could be used to predict the number of recruits. This approach assumes that the same number of adults each year were able to spawn, as the relationship would not hold if this number was variable. An example may be a species such as salmon where the number of adults that spawn is determined by the finite number of gravel beds to deposit eggs.

Both these cases, where  $N_e$  is related to either spawning stock size or recruitment, represent the extremes. While examples may exist, the number of species that would conform to these extremes would be small. However, there is no doubt that effective population size contains new information about the relationship between spawning stock size and recruitment. If this is the case, then we believe that it can be most profitably used in fisheries stock assessment in the context of the stock-recruitment relationship. The stock-recruitment relationship describes the relationship through time between the spawning stock size (often expressed on the x-axis) and subsequent recruit numbers (y-axis). There has been doubt about whether stock-recruitment relationships exist for fished species given their high fecundity. However, meta-analyses have increasingly shown that the concept is a useful one for the majority of harvested species, even marine invertebrates such as prawns that have high fecundities and rapid growth rates.

The two relationships described above,

$$N_e = f(\text{spawning biomass})$$

and

$$\text{Recruitment} = g(N_e)$$

can be inserted into equations that describe the theoretical stock-recruitment relationship as described by Hoyle (Appendix C). We contend that if  $N_e$  was known, then the accuracy with which the relationship can be used in fisheries modeling may increase. However, as Hoyle points out, adding parameters into relationships is not



without cost – they are also subject to error in estimation. We agree that computer simulations may be the most cost-effective way to evaluate the usefulness of this new approach.

Until a pathway is clear for the utilization of  $N_e$  in fisheries modeling, it is not appropriate at this stage to try to evaluate the cost-effectiveness of this new data in fisheries management. Compared to the cost of collecting fisheries independent data with field personnel and equipment and subsequent data analysis, genetic estimates of spawning stock size are relatively inexpensive. We do not advocate that the latter replace the former, merely that the overall goal of achieving sustainability of fisheries resources justifies the collection and analysis of a variety of data sources albeit in the most cost effective manner. The utilization of this new data source requires collaboration between population modelers who are not risk adverse and population geneticists willing to work in a new frame of reference.

In addition to potentially strengthening the stock recruitment relationship,  $N_e$  could be a potential monitoring tool. If the same methods of measuring  $N_e$  were applied to the same population over time, then a decline in  $N_e$  would most likely indicate a fall in population numbers. However, Ardren and Kapuscinski (2003) reported that  $N_e$  stayed constant during falls in the breeding population size of steelhead trout (*Oncorhynchus mykiss*) in North America that they attributed to more survivorship per family. This highlights the species-specific differences in the dynamics between  $N_e$  and spawning population size and that in this salmonid species  $N_e$  is more likely to predict recruitment rather than spawning population size. As a monitoring tool it would be desirable to increase both accuracy and precision by following the resource requirement guidelines suggested by computer simulations in Chapter 5. The advantages of  $N_e$  as a monitoring tool is that (1) it doesn't rely on data from the fishery, (2) it is species specific, (3) it doesn't rely on a time-series of data to produce an estimate so its good for data-poor fisheries, and (4) its relatively cheap.

This study predicts that similar methods could be used to examine the spawning stock dynamics of larger, and economically more important populations of tiger prawns, such as those in the Gulf of Carpentaria. Computer simulation experiments have shown that statistically powerful estimates could be made in populations larger than the Moreton Bay population. Further simulation studies have shown that the bias introduced into the estimates because generations of tiger prawns in the Gulf overlap would be minimal. Two recent theoretical papers explain how estimates of effective population size could be made when populations are experiencing migration. With appropriate validation, this method may be applicable to tiger prawn populations may be in the Gulf of Carpentaria. New genetic markers, such as single nucleotide polymorphisms (SNPs) may be methodically easier and cheaper to implement on large populations than the microsatellite loci used in this study.

## **Benefits**

This research was knowingly funded as “blue-sky” research. This means that not only was the project rated as having a relatively low chance of success, but also that the genetic methodology for fisheries stock assessment was innovative and likely to be of international significance. This was confirmed by Ovenden (2002) during a study tour of Canada, the United States, and Europe in 2002.

As it turned out the project was successful. It did measure effective population size with a high level of statistical power in a fisheries resource and it showed that the method was likely to be free of year-to-year process error. These results are new to science, and benefits to the fishing industry have not yet begun to accrue.

The potential beneficiaries of this project are the fishery managers, fisheries scientists and operators of Australian fishing industry, and consequently the Australian economy. Those involved in the Northern Prawn Fishery (NPF) and the Queensland trawl fishery may be more directly affected through the provision of more robust stock assessment advice.

## **Further development**

In light of the initial investment made by the FRDC to trial the technology, and the successful completion of the project, it is strongly recommend that the technology be further developed.

An important first step is to disseminate the new knowledge to stakeholders. The scientific audience will be addressed as part of the current project with the production of scientific publications. The final report will also be made widely available. There are numerous ways to present this information to stakeholders outside this audience. Members of the research team can personally meet with and speak to individuals or groups. A good example of this would be presentations to management advisory committees that oversee various fisheries and that have a representative membership. Following on from the successful consultative workshop held during this project, a further workshop would be an excellent forum for presentation and feedback of ideas from a general audience.

After a period of dissemination, and if the consensus of scientific and management advisory bodies was favorable, then we would recommend several follow-on projects for the further development of the technology. These would include

- a. Trialing of the method as a monitoring tool by collecting a time-series of genetic and log-book estimates on the spawning population of *P. esculentus* in Moreton Bay. The substitution of digital data from single nucleotide polymorphisms (SNPs) for microsatellite data could be tested at the same time.
- b. Transferring the technology to the tiger prawn (*P. esculentus*) resource in the Gulf of Carpentaria.
- c. Evaluation of the benefit of the technology to stock assessment science by computer simulations of stock assessment models incorporating the stock recruitment relationship and management cycles. This would be essential before a cost-benefit evaluation of the technology could be undertaken.
- d. A desk-top study of the transferability of the technology to non-prawn species such as finfish, shellfish and other crustaceans.

There is no intellectual property generated by this project, and the results will be placed in the public domain by publication in scientific journals. Consequently, there is little or no opportunity to commercially exploit the results of this research.

## Planned outcomes

FRDC provides guidelines to distinguish between outcomes and outputs. Outcomes are the results, impacts or consequences of actions by the FRDC and its research and development partners on the fishing industry and Australia's economic, environmental and social resources. Outputs are the goods and services (mainly knowledge, processes and technology) that the FRDC and partners produce for external organizations and individuals. This section summaries outputs and outcomes for this project and discusses the role of outputs in the outcomes.

The outputs of this project were:

1. *Web site* – The web site (<http://www.dpi.qld.gov.au/fishweb/11629.html>) contains a description of this project, and contact details for the collaborative research team. The web site has been used to
  - a. Distribute and support software for the estimation of genetic effective population size (*NeEstimator*).
  - b. Facilitate outcomes from the Consultative Workshop. It was used to obtain and maintain a list of registrants and interested researchers. The web site was also used to distribute the proceedings of the workshop via download of .pdf documents.
2. *Genetic analysis software* – Software called *NeEstimator* accompanied by help files has been developed during this project. It is distributed via the web site along with FAQ and version information. It is freely available to the scientific community in Australia and worldwide. By November 2004 there were 188 registered users of *NeEstimator*.
3. *Consultative workshop* - A consultative workshop was held in Brisbane in the second year of the project in May 2003. It was attended by fisheries scientists, managers and commercial operators. The topics addressed included the problems that could be solved by the implementation of this methodology, the long and short term benefits and the development and implementation of the technology for fisheries management.
4. *Proceedings of the Consultative Workshop* – These were written up and made available to participants, FRDC and the remainder of the fishing industry in hard copy, on CD and on the web. Citation for the proceedings is “ Broderick D, Peel S, Street R, Ovenden JR (2003) Genetic methods for the estimation of fisheries spawning stock size: Transcripts of the forum - 9 May 2003. Queensland Government, Department of Primary Industries, Brisbane, Australia. pp 205.”

5. *New scientific knowledge*– Three scientific publications based on the results of the project are being prepared for submission to genetics, fisheries and statistical science journals.
6. *Final report* – The FRDC final report will be made available in print and on CD.
7. *Alliance of researchers* – As a result of this project, an alliance has been established between the Queensland Department of Primary Industries and Fisheries, the University of Queensland and CSIRO Marine Laboratories, Cleveland. The alliance captures a unique combination of genetics, statistical, computational and mathematical methodologies. The alliance will focus its efforts on contributing to sustainable production from State and Federally managed tiger prawn fisheries for the economic benefit of Australians.

Outcomes from this project were relevant to the Australian fishing industry, and consequently the Australian economy. Specifically, they were

1. *A comprehensive evaluation of a new cost-effective tool for stock assessment methodology.* All of the outputs of the project have been important in achieving this outcome. A natural consequence of this outcome is the provision of assistance to fisheries managers to achieve sustainable development of resources. This may be especially important for stocks that do not have a long-time-series of data needed for conventional stock assessment. The project and its outputs have progressed towards this follow-on outcome, but it has not been achieved as yet.
2. *The development of a more conservative approach to ecologically sustainable exploitation of fisheries resources.* The outputs of this project, particularly the new scientific knowledge, the consultative workshop and the final report, have emphasized that the number of breeding prawns that effectively contribute to stock renewal is much smaller than expected. It is recommended that spawning stocks are identified and quantified and, where necessary, the fishing mortality upon them is reduced. Spatial management rather than controlling effort and catch could achieve this. Again, the project has progressed towards achieving this outcome, but further work is necessary.
3. *Mathematical and statistical collaboration between state and commonwealth fisheries research agencies and the University of Queensland.* The research team that carried out this project represented these institutions. Regular meetings were held during the project to encourage communication of ideas and research collaboration that culminated in the production of the project outputs. It is anticipated that the project team will progress this line of research towards the application of the new methodology to other heavily exploited and commercially valuable fisheries such as finfish, molluscs and other crustacean species.

Outcomes from the project were also beneficial to fishery managers, fisheries scientists and operators involved in the Northern Prawn Fishery (NPF) and the Queensland trawl fishery. Specifically, these were

4. *Two separate and independent monitoring methods.* This project has ground-truthed technology that may allow effective spawner numbers to be monitored using a DNA-based approach. This is far-removed from the conventional stock assessment approach that relies on computer models and estimates of demographic parameters. The web site has provided information about the project to a wide audience, as well as disseminating the software developed in the project for converting genetic estimates into estimates of effective spawning stock size. The new knowledge generated by this project will be published in scientific journals, making it widely available. Finally, non-scientific parties learned about the method from the consultative workshop, workshop proceedings and the final report. Further development of the method is needed, however, to bring the technology on line for the NPF and other trawl fisheries in Queensland waters.
5. *Improvements to the way in which predictions are made about the size of recruitment in the next fishing season.* Accurate, advance knowledge on recruitment is essential for short-term management of the resource, as well as for harvesting and marketing strategies. Monitoring effective spawning stock size with the tools developed here will improve the quality of advice from stock assessment modelers. Contributions to this outcome were made by all of the project's outputs; however, more development is needed to fully realize the potential of these outputs for fisheries management.
6. *Additional information generated from the genetic analyses.* A good example of this outcome is new knowledge about the population structure of *P. esculentus* in the tropical north of Australia. Population structure is relevant when fisheries populations straddle state or other administrative borders and as are results are potentially subject to different management plans. The project outputs that contributed to this outcome were the generation of new knowledge and the final report where the separate study by Ward et al. is reported in Appendix B.



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## **Appendix A – *NeEstimator* software**

*by D Peel, JR Ovenden and SL Peel*

### **Description**

*NeEstimator* v 1.3 is a tool for estimating effective spawning numbers ( $N_e$ ) in a genetically closed population using multi-locus diploid genotypes from a sample of the population. Six methods are available to calculate  $N_e$ ; two point estimation methods and four temporal methods. Three of the temporal methods are third-party programs that can be used from within *NeEstimator*. *NeEstimator* converts the input data to the correct format for each method. Thus the user need only provide the input data in one of the accepted formats (GenePop, Arlequin, simple columns or counts) for all six methods. The methods are as follows.

### **Internal Methods**

These are

1. A point estimation method using linkage (gametic) disequilibrium (Hill, 1981).
2. A point estimation method using heterozygote excess (Pudovkin, Zaykin and Hedgecock, 1996).
3. A temporal method using moments based F-statistics (Krimbas and Tsakas, 1971; Nei and Tajima, 1981; Pollock, 1983; Waples, 1989).

### **Third Party (External) Methods**

These are

1. A temporal method using a Bayesian based approach called TM3 (<http://www.rubic.rdg.ac.uk/~mab/software.html>).
2. A temporal method using a maximum likelihood based approach called MCLEEPS (<http://www.stat.washington.edu/thompson/Genepi/Mcleeps.shtml>).
3. A temporal method using a pseudo likelihood approach called MLNE (<http://www.zoo.cam.ac.uk/ioz/people/wang.htm>).

### **Specifications**

*NeEstimator* can be run on one of these Microsoft Windows operating system versions

1. Microsoft Windows 95, 98, or ME
2. Microsoft Windows 2000, NT (version 4.0 with service pack 3 or later) with access to an Administrator account

It is assumed that if you are able to run one of these operating systems, you have enough computing power to run the *NeEstimator* software.

You will require approximately 4MB free hard disk space (not including user data space requirements or operating system requirements) and a mouse or compatible pointing device. Certain options require Microsoft Excel 97, 2000 or 2002.

## **Contacts and Updates**

Please use the web site <http://www.dpi.qld.gov.au/fishweb/11629.html> to find the latest contact information and updates of the program.

## Appendix B – Population genetic structure of the brown tiger prawn, *Penaeus esculentus*, in tropical northern Australia

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### Abstract

Eight polymorphic microsatellite loci were analysed in six samples of the Australian endemic brown tiger prawn, *Penaeus esculentus*. Tests of Hardy-Weinberg equilibrium were generally in accord with expectations, with only one locus, in two samples, showing significant deviations. Three samples, collected in different years, were taken from Exmouth Gulf. There was no significant heterogeneity between them, and it was concluded that they were from a single panmictic population. A sample from Shark Bay, also on the west coast of Australia, showed barely detectable differentiation from Exmouth Gulf ( $F_{ST} = 0$  to 0.0014). A north-east sample from the Gulf of Carpentaria showed low ( $F_{ST} = 0.008$ ) but significant differentiation from Moreton Bay, on the east coast. However, Exmouth Gulf/Shark Bay samples were well differentiated from the Gulf of Carpentaria/Moreton Bay ( $F_{ST} = 0.047$  to 0.063). The data do not fit a simple isolation-by-distance model. It is postulated that the west-east differentiation largely reflects the isolation of east and west coast populations that occurred at the last glacial maximum, by a landbridge between north-eastern Australia and New Guinea.

### Introduction

Two *Penaeus* species (*P. esculentus* and *P. plebejus*) are endemic to Australia. *P. esculentus* is a tropical species distributed around the northern coastline from Shark Bay in Western Australia to northern New South Wales on the east coast, while *P. plebejus* is a more subtropical species restricted to the eastern coast from southern Queensland to northern Victoria (Grey *et al.* 1983). The remaining Australian commercial *Penaeus* species (*P. canaliculatus*, *P. marginatus*, *P. longistylus*, *P. japonicus*, *P. latisulcatus*, *P. merguensis*, *P. indicus*, *P. semisulcatus* and *P. monodon*) are not Australian endemics, being found in other regions of the Indo-Pacific.

As part of a genetic population structure survey of Australian penaeids, Mulley and Latter (1980) found that heterozygosity at allozyme loci was low. Few individuals

(0.6 to 3.3%) were heterozygous across six *Metapenaeus* and seven *Penaeus* species examined for 37 loci; an average of 14% of loci were polymorphic per species. Low heterozygosity in crustaceans was also reported by Redfield *et al.* (1980). They assayed nine species representing one stomatopod and four decapod families from the Gulf of Carpentaria. At most, five loci per species out of 12 to 33 examined per species were polymorphic and heterozygosity was low (0.8 – 6%). In fact, penaeid prawns have lower allozyme variability than most other taxonomic groups (Benzie 2000).

Spatial genetic variation was assayed by Mulley and Latter (1981a) for four polymorphic allozyme loci (*MPI*, *PGI*, *PGM* and *SDH*). Populations were sampled from southern and northern Queensland, the Gulf of Carpentaria and Exmouth Gulf in Western Australia. Despite the large geographic difference between sampling sites, no gene frequency differences were detected at any of the four loci. This has been interpreted by some as a lack of evidence for genetic stocks of *P. esculentus* in Australia (e.g. Kailola *et al.* 1993). However, the statistical power to reject the null hypothesis of no genetic differentiation between populations was low. Only *MPI* was strongly polymorphic, and that only had two common alleles and one very rare allele.

The supposed lack of stock structure for *P. esculentus* in Australia's tropical north, based on allozyme analysis, is not consistent with data from other genetic and biological sources. For example, as part of their original allozyme study, Mulley and Latter (1981a, 1981b) found that two penaeid species (*P. latisulcatus* and *M. endeavouri*) showed geographic genetic differentiation across the same range as *P. esculentus*. In addition, a preliminary study of the cytochrome oxidase I region of the mitochondrial genome of *P. esculentus* by Lavery and Keenan (1995) revealed three haplotypes. Haplotype C was found only in Western Australia (Shark Bay) and differed by three fixed single nucleotide polymorphisms from haplotypes A and B collected adjacent to the south and north-east coast of Queensland.

Life history characteristics of *P. esculentus* populations also suggest the presence of separate stocks. Courtney and Masel (1997) highlighted life history differences between *P. esculentus* in northern Australia (10 to 17° S) compared to Moreton Bay (27° 15' S) at the southerly extreme of its distribution in eastern Australia. The size at which females first mature in Moreton Bay (about 30 mm carapace length) is larger than the north (25 mm), and the overall incidence of mature females was lower in Moreton Bay (20% of females larger than 35 mm) compared to northern populations (Gulf of Carpentaria, 70 to 80%). The occurrence of mature females is restricted to a few months in late spring and early summer in Moreton Bay, while in the Gulf of Carpentaria mature females were relatively common (at least 20%) in most months of the year and were never completely absent (Crococ 1987). Longshore migration, which would promote genetic homogeneity between populations, is not a general feature of *P. esculentus*. In Moreton Bay *P. esculentus* do not appear to undergo significant immigration or emigration as spawning, maturation and recruitment occurs internally in the Bay (Courtney and Masel 1997). Sea-grass beds, necessary for post-larval settlement (Loneragan *et al.* 1998), are absent several hundred kilometres north or south of Moreton Bay. Additionally, adult *P. esculentus* in the Gulf of Carpentaria do not move great distances. Derbyshire *et al.* (1990) tagged 19,156 *P. esculentus* in western and eastern Torres Strait in January and February 1987. Tagged prawns moved an average of 4 to 27 nm with the greatest movement being 69 nm by a single

female. The average time at liberty was one to two months, with the greatest time being about 250 days.

Fisheries management consortiums need stock information on tiger prawns to assist with plans for sustainable resource use. Fishing effort in the tiger prawn fishery in the Gulf of Carpentaria has increased significantly over the last 30 years. Catches and catch per unit effort in 1986 (3,487 tonnes) had dropped to half of that of 1981, prompting the application of a stock-recruitment model that assumes recruitment overfishing. Subsequent assessments concluded that the 1993 spawning stock of *P. esculentus* was about 60% lower than that in the mid-1970's and recruitment had decreased by 50% (Wang and Die 1996; Taylor and Die 1997). A comparison of the spawning stock dynamics of the three commercial species of prawns in Moreton Bay led Courtney and Masel (1997) to suggest that *P. esculentus* was the most likely to experience recruitment overfishing. The number of postlarvae and juveniles in Moreton Bay decreased significantly between two assessments 20 years apart (1972/3 to 1991/1, Masel and Smallwood 2000), while fishing pressure increased and seagrass cover decreased.

Delineation of stock structure in penaeids is needed to lay the groundwork for estimation of spawning stock size. The discovery of highly polymorphic microsatellite loci for penaeid prawns led Lavery and Keenan (1995) to suggest that genetic estimates of effective population size may be likely in the near future. As well as contributing in a significant way to analyses of stock recruitment relationships, they suggested that genetic effective population size estimates could be used to (1) monitor spawning stock size, (2) compare to long-term, average population size as a measure of virgin compared to current spawning stock size and (3) compare to current census size to test hypotheses about life-time reproductive success. An assumption of the genetic model is no migration, so it is important to determine the degree of genetic subdivision and hence gene flow among populations.

Six microsatellite loci, selected from 23 originally tested, were found to be polymorphic and reliable in *P. esculentus* (Meadows *et al.* 2003). These loci, together with two pre-existing loci (Preston 1997), form the panel of eight loci used in the current study. These loci have the potential to be statistically more powerful than allozyme loci, since all eight are highly polymorphic with multiple alleles. The objective of this study was therefore to revisit the null hypothesis of lack of genetic stock structure for *P. esculentus* throughout its range in tropical northern Australia.

## Materials and Methods

Mature prawns ( $n = 546$ ) were collected from commercial prawn trawlers, with each sample coming from a single trawl. Ex99, Ex00 and Ex02 were collected in Exmouth Gulf in the Novembers of 1999, 2000 and 2002 respectively. ShB came from Shark Bay in November 2002, GoC came from the southern Gulf of Carpentaria, adjacent to Mornington Island, in October 2001 and MorB came from Moreton Bay in November 2001. These locations are identified in .

Eight microsatellite loci were examined. Two (PMCD and Pe1.1) were described in Preston (1997) and six (CGES047, CGES120, CGES176, CGES189, CGES190 and CGES268) in Meadows *et al.* (2003). One locus was a dinucleotide repeat (Pe1.1), six were trinucleotide repeats (PMCD, CGES120, CGES176, CGES189, CGES190 and

CGES268) and one was a tetranucleotide repeats (CGES047). Total genomic DNA for microsatellite analysis was extracted from 40 mg of prawn pleopod tissue, shattered after freezing in liquid nitrogen (Ex and ShB) or from muscle tissue without shattering (GoC and MorB) using a modified DNeasy™ 96 Tissue Kit (QIAGEN®). Methods are described in Meadows *et al.* (2003). Results were collected using an ABI 377 Prism DNA autosequencer and analysed using GeneScan® 3.1 and Genotyper® 2.5 software.

Most statistical analysis used the Arlequin v2.0 package (Schneider *et al.* 2000). Observed ( $H_{obs}$ ) and Hardy-Weinberg expected ( $H_{exp}$ ) heterozygosity were determined, and goodness-of-fit to HW equilibrium estimated from 100,000 Markov chain lengths. Comparisons of allele frequencies across all loci gave pairwise population estimates of  $F_{ST}$  and associated P values, based on 100172 permutations.  $F_{ST}$  analyses over all populations considered together were based on the AMOVA (analysis of molecular variance) procedures of Arlequin, with 16000 permutations. A Mantel test correlating  $F_{ST}$  with geographic distance was carried out with 10000 permutations.

GenePop v.3.3 (Raymond and Roussett 2000), with 500 batches and 5000 iterations per batch, was used to examine genotypic disequilibrium and single locus comparisons of allele frequencies in population pairs.

Null allele frequencies were derived from the program NULLTEST (Bill Amos, Department of Zoology, Cambridge, CB2 3EJ).

## Results

All eight loci were highly polymorphic in each population (Table B.1 and Appendix 1). Some prawns from the Shark Bay sample proved difficult to type – this was attributed to transport delays and sample degradation prior to arrival in the laboratory. Only one pairwise locus comparison of genotypes across all populations showed any evidence of genotypic disequilibrium. This was the locus pair CGES047 and CGES120 ( $P = 0.013$ ); however, this apparent association becomes non-significant given the 28 possible pairwise combinations examined.

Observed heterozygosity ranged from 0.247 for CGES190 in the Gulf of Carpentaria to 0.936 for Pe1.1 in Exmouth Gulf 2000. Numbers of alleles per locus ranged from 5 for CGES190 in Shark Bay to 26 for Pe1.1 in the Gulf of Carpentaria. Mean numbers of alleles per locus per population and mean locus heterozygosities were similar across populations. Of the 48 tests for fits to Hardy-Weinberg equilibrium (Table B.1), ten showed significant deviations at the 5% level. Nine of these showed excess homozygosity. Adjusting significance levels using a standard Bonferroni correction for 48 tests reduced the number of significant deviations to two: CGES047 in the Gulf of Carpentaria and Moreton Bay, both showing excess homozygosity. Possible explanations for such deviations include population mixing, selection, or null allele presence. If the cause was a null or non-amplifying allele, likely frequencies can be estimated – at 0.143 and 0.149 respectively. Genotype distributions for this locus in the four other populations accorded with HWE, with the (non-significant) deviations producing negligible estimates of null frequencies ranging from 0 to 0.020.

All possible pairwise population comparisons were carried out over all loci to examine the extent of population differentiation (Table B.2).  $F_{ST}$  values among all three Exmouth Gulf samples were zero. The three Exmouth Gulf to Shark Bay comparisons were non-significant, with an average  $F_{ST}$  value less than 0.001. In contrast, all the Exmouth Gulf/ Shark Bay comparisons with Gulf of Carpentaria and Moreton Bay were highly significant (all  $P < 0.0001$ ), with  $F_{ST}$  values ranging from 0.047 to 0.063, and averaging 0.055. The Gulf of Carpentaria and Moreton Bay pair showed a small ( $F_{ST} = 0.008$ ) but significant difference ( $P = 0.0004$ ).

Examination of individual loci indicated the possibility of some small allele frequency differences between Shark Bay and the three Exmouth Gulf samples at CGES047 ( $P$  values of 0.002, 0.349 and 0.036). All loci contributed to the Exmouth Gulf / Shark Bay to Gulf of Carpentaria / Moreton Bay differentiation. Particularly striking was PMCD\*174, present at frequencies of about 0.19 in both the Gulf of Carpentaria / Moreton Bay but totally absent in all the Exmouth Gulf and Shark Bay samples. Only locus CGES176 contributed in a significant way ( $P < 0.0001$ ) to the Gulf of Carpentaria to Moreton Bay differentiation. At this locus, GoC had higher frequencies of alleles \*229 and \*232 and lower frequencies of \*244 and \*247 than MorB.

Populations were split into two groups, a western group (comprising the Exmouth Gulf and Shark Bay populations) and a north-eastern group (Gulf of Carpentaria and Moreton Bay). An AMOVA analysis of variance estimated the overall  $F_{ST}$  as 0.055 ( $P < .00001$ ). Unsurprisingly, given the earlier analyses, this was almost entirely due to differentiation between these groups ( $F_{ST} = 0.054$ ,  $P < 0.00001$ ). Differentiation among group members was very small ( $F_{ST} = 0.001$ ,  $P = 0.064$ ).

A Mantel test examining a pairwise population matrix of  $F_{ST}$  and geographic distance gave a just significant correlation coefficient of 0.855 ( $P = 0.047$ ).

## Discussion

No instances of Bonferroni adjusted deviations from di-locus genotypic equilibrium were observed, and thus the eight loci can be taken as independent gene markers. This is not unexpected as the haploid chromosome number of prawns is high, that of *P. esculentus* being 44 (Xiang *et al.* 1996), and so the *a priori* probabilities of any two of eight loci being closely co-located on the same chromosome are low.

Generally satisfactory fits to Hardy-Weinberg expected genotype distributions were observed at each locus. There were two exceptions, both at the CGES047 locus, and both were due to excess homozygosity (heterozygote deficiency). The two populations showing these deviations were the Gulf of Carpentaria and Moreton Bay. The deviations appear to be locus-specific since all other loci accorded with Hardy-Weinberg equilibrium, and might well represent the presence of a null or non-amplifying allele. If true, then estimated null allele frequencies in both populations were similar at about 0.145 and the proposed null allele would be a shared, derived character. Population mixing may not be a tenable alternative explanation for the disequilibrium at locus CGES047 in the north-east populations, as allele frequencies for these populations were very similar while other populations had genotype frequencies consistent with panmixia. Across all loci, observed and expected average heterozygosities were similar, at 0.694 and 0.736 respectively. Benzie (2000) reported the average observed microsatellite heterozygosity of four species of prawns ( $P$ .

*esculentus* was not one of those assessed) to be 0.666, substantially less than the average expected heterozygosity of 0.927. The generally good fits to equilibrium in *P. esculentus* probably reflect our concerted initial efforts at developing reliable microsatellite primer sets. Similarly good fits to Hardy-Weinberg equilibrium were observed by Brooker *et al.* (2000) for three microsatellites in Australian populations of *P. monodon*.

The three population samples from Exmouth Gulf, collected from different prawn trawlers on different fishing transects in different years (1999, 2000 and 2002), showed no significant differentiation for any of the eight loci and  $F_{ST}$  estimated across the three samples was zero. There are therefore no grounds to reject the hypothesis that Exmouth Gulf comprises a single panmictic population. Furthermore, the effective population size must be large enough that allele frequencies were not significantly different between the three different cohorts each of about 93 individuals.

Shark Bay, about 300km south of Exmouth Gulf on Australia's west coast, showed very little differentiation from Exmouth Gulf. The average  $F_{ST}$  between Shark Bay and the three Gulf populations was less than 0.001. There was a suggestion that one of the eight loci (CGES047) showed weak differentiation between Shark Bay and the Exmouth Gulf samples, but this would need to be corroborated with further samples. At the moment, we tentatively suggest some weak restriction on gene flow between these areas. Given the relatively poor vagility of this species, similarity between west coast populations may be more indicative of shared ancestry from a putative contiguous population at times of low sea level than extant exchange of migrants.

There was substantial and highly significant differentiation between these west coast samples and the Gulf of Carpentaria / Moreton Bay samples from Australia's northeast and eastern coast. Over all six samples, the  $F_{ST}$  was 0.055, almost entirely due to this west to northeast differentiation. All loci contributed to this separation. Especially striking was allele PMCD\*174, the smallest PMCD allele, present at frequencies of about 0.19 in the northeast but completely absent from the west coast samples. Also striking was the likely occurrence of null alleles for locus CGES047 in the northeast and their absence from the west. Clearly there is a major restriction to *P. esculentus* gene flow across the top-end of Australia, and further sampling in this area is warranted. Exmouth Gulf and the Gulf of Carpentaria are separated by about 2500 km.

The Gulf of Carpentaria and Moreton Bay, areas separated by about 2000 km, show significant but very limited differentiation. The  $F_{ST}$  between these two samples was 0.008, low, but statistically greater than zero ( $P = 0.0004$ ). Allele frequencies at most loci were quite homogeneous between these two samples, homogeneity even reflected in the similar estimated frequency of null alleles at locus CGES047. Locus CGES176 contributed most of the differentiation.

The null hypothesis of lack of genetic stock structure for *P. esculentus* throughout its range in tropical northern Australia is unambiguously rejected. This contrasts with the earlier study that found genetic homogeneity across a similar geographic range (Mulley and Latter 1981a). That study was based on four polymorphic allozyme loci, only one of which was strongly polymorphic (but only had two common alleles). Clearly, the extra power afforded by the increased variability of the microsatellite loci



has better resolved the population structure of this species. More in accord with the present findings were observations by Lavery and Keenan (1995) of a diagnostic mitochondrial DNA haplotype difference between Australian west coast (Shark Bay) and east coast (Cairns, Moreton Bay) samples, although only ten prawns were sampled in each locality.

These findings of significant differentiation among populations of *P. esculentus* are not particularly surprising, as the dispersal powers of prawns are likely to be less than many other invertebrates and fish. Prawns have internal fertilisation so there are no planktonic gametes that might disperse. Egg release to settlement of small juveniles is about 21 to 23 days (*P. Crocos*, pers. com.); during this period the larvae and early post-larvae are planktonic and can be dispersed. Settlement in shallow water on sea grass beds is regarded as essential (Condie *et al.* 1999), and as animals mature they move into progressively deeper water of 10 to 200m. Tagging studies have revealed little adult migration (Hynd 1974; Derbyshire *et al.* 1990; Courtney and Masel 1997).

A simple isolation by distance model would seem to be insufficient to describe the heterogeneous population structure of this prawn species. The geographic distance between Exmouth Gulf and the Gulf of Carpentaria is not a lot greater than that between the Gulf of Carpentaria and Moreton Bay, yet the degree of genetic differentiation as assessed by  $F_{ST}$  is about 0.052 for the former comparison and only 0.008 for the latter. Rather, it seems that there is a genetic break somewhere between the Gulf of Carpentaria and Exmouth Gulf, with relative genetic uniformity either side of the postulated break. When  $F_{ST}$  is plotted against geographic distance, the influence of this discontinuity can be seen (Figure B.2). Without the discontinuity, differentiation is small, with  $F_{ST}$  values appearing to rise by only about 0.01 for every 2000km separation. A Mantel test showed a high correlation coefficient (0.855) between  $F_{ST}$  and geographic distance, but this correlation is only just significant ( $P = 0.047$ ). The marginal significance likely reflects the discontinuity evident in Fig 2.

How did this break arise? Sea change levels in the Pleistocene were sufficient on several occasions to open and close the confluence of the Indian and Pacific Oceans in the Arafura Sea/Torres Strait region (Chappell and Shakleton 1986; Chivas *et al.* 2001). Most recently, in the period 70,000 to 10,000BP, sea level was often 50m below current levels, leading to a substantial land bridge between Australia and New Guinea in the Gulf of Carpentaria region. At the lowest sea levels, the landbridge included not only the Carpentaria Basin but also the Arafura Sea. Within the central depression of the Carpentaria Basin there then existed a freshwater or brackish lake, which did not become marine until sea level rise 11,000BP led to sea water inundation over the Arafura Sill at -53m (Jones and Torgerson 1988). Torres Strait is shallower, -12m, and only opened to the Gulf of Carpentaria 7,000BP (Chappell 1983). Clearly then, there was a substantial period of time when east coast populations of *P. esculentus* were entirely isolated from those on the west coast; remember that this is a tropical Australian endemic species and dispersal through more northerly or southerly routes would not be possible. This isolation probably accounts for the genetic break between east and west coast populations. The genetic similarity of the Gulf of Carpentaria to Moreton Bay suggests that the Gulf population was primarily founded from immigrants from the east rather than the west, against the direction of the initial marine incursion. Further, genetic diversity in the Gulf of Carpentaria is no lower than

that in Moreton Bay, suggesting that any population bottleneck effects that might have occurred at the time of this immigration are no longer detectable.

Several other species have been investigated for their genetic population structure in this region. Mitochondrial DNA data for the coastline-restricted catadromous barramundi (*Lates calcarifer*) suggests a similar migration history (Chenoweth *et al.* 1998) to that postulated for *P. esculentus*, with Gulf of Carpentaria samples being more similar to Coral Sea samples than to western Arafura Sea samples. However, more extensive allozyme data indicated a rather more complex pattern of migration and colonisation (Keenan 1994). Allozyme and mitochondrial DNA analyses of a second teleost, the saddle-tail sea perch (*Lutjanus malabaricus*), suggested a recolonization of the more northern waters from a west coast stock, with the Torres Strait being the break point (Elliott 1996). Torres Strait was also identified as a major genetic disjunct for mitochondrial DNA clades in the mud crab, *Scylla serrata* (Gopurenko and Hughes 2002). Benzie *et al.* (1993) and Brooker *et al.* (2000) proposed two scenarios to account for similarities among collections of the tiger prawn *P. monodon* from north-east Australia (from the Arafura Sea to southern Queensland) yet differences from a Western Australia population (north of Exmouth Gulf). These differences included reduced variability in the Western Australia population (not observed in our *P. esculentus* data). They suggest either a recent bottleneck in Western Australia or colonization by a small founding population from the east coast when sea links were re-established about 7,000BP. While the proposed evolutionary histories of these species differ in detail, all agree that the ancient landbridge between Australia and New Guinea has been pivotal in the genetic structuring of fish and invertebrate stocks in this region.

The suggestion by Condie *et al.* (1999) that there are at least three stocks of *P. esculentus* in the Gulf of Carpentaria, based on the geographic distances between the adjective envelopes of spawning stocks and limited adult vagility, remains to be genetically assessed. The present results (homogeneity within Exmouth Gulf, very limited differentiation between Exmouth Gulf and Shark Bay, and between Gulf of Carpentaria and Moreton Bay) suggest that any genetic differentiation between these fishery stocks is likely to be small. Nevertheless, such studies deploying hypervariable microsatellites will help to resolve this issue and, in addition, might well enable estimates of biologically effective population size to be made. We therefore anticipate the continuing deployment of genetic markers to assist the sustainable management of the tiger prawn fishery.

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**Table B.1: Genetic diversity parameters for *Penaeus esculentus* from six samples.**

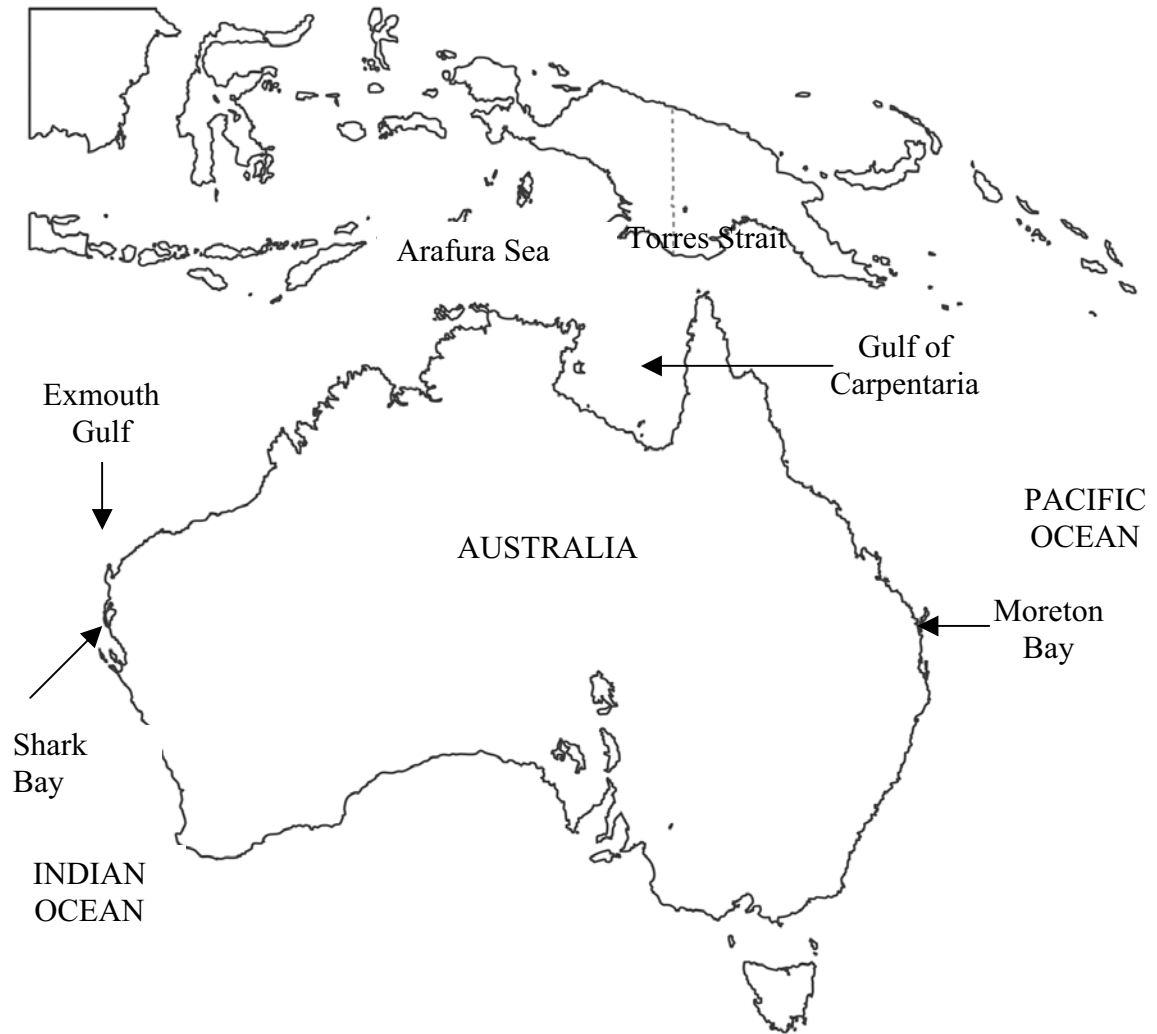
N is number of individuals. Figures in parenthesis are total numbers of alleles per locus.  $H_{obs}$  and  $H_{exp}$  are the observed and Hardy-Weinberg expected heterozygosities respectively. Significant deviations from Hardy-Weinberg equilibrium are indicated by asterisks: \*,  $0.05 > P > 0.01$ ; \*\*,  $0.01 > P > 0.001$ ; \*\*\*,  $P < 0.001$

Locus	Parameter	ExG99	ExG00	ExG02	ShB	GoC	MorB
PMCD	N	93	96	94	70	93	94
	Alleles (12)	9	9	9	9	8	9
	$H_{obs}$	0.806	0.740	0.862	0.771	0.849	0.862
	$H_{exp}$	0.833	0.831	0.831	0.827	0.822	0.826
CGES268	N	91	96	94	74	92	93
	Alleles (19)	15	15	16	18	10	12
	$H_{obs}$	0.648	0.719	0.734	0.770	0.848	0.892
	$H_{exp}$	0.729	0.713	0.714	0.796*	0.844	0.859
CGES190	N	89	95	91	49	85	86
	Alleles (8)	7	6	7	5	6	7
	$H_{obs}$	0.506	0.495	0.451	0.408	0.247	0.326
	$H_{exp}$	0.550	0.489	0.538*	0.386	0.311*	0.333
CGES047	N	91	96	94	74	94	94
	Alleles (20)	18	17	16	15	15	14
	$H_{obs}$	0.846	0.865	0.904	0.865	0.638	0.585
	$H_{exp}$	0.905	0.883	0.894	0.906	0.852***	0.800***
CGES176	N	94	96	93	63	92	94
	Alleles (18)	13	14	12	12	11	13
	$H_{obs}$	0.809	0.833	0.807	0.619	0.772	0.830
	$H_{exp}$	0.841	0.849	0.829	0.855**	0.816	0.865

CGES120	N	91	92	94	41	90	92
	Alleles (12)	8	10	7	6	7	7
	H <sub>obs</sub>	0.396	0.500	0.575	0.439	0.300	0.489
	H <sub>exp</sub>	0.472	0.531	0.577	0.554*	0.336	0.542
CGES189	N	91	95	94	74	94	94
	Alleles (11)	9	8	7	8	7	8
	H <sub>obs</sub>	0.681	0.800	0.713	0.689	0.787	0.713
	H <sub>exp</sub>	0.709	0.767*	0.728	0.760	0.763	0.794
Pe1.1	N	92	94	94	63	93	71
	Alleles (35)	23	22	23	21	26	15
	H <sub>obs</sub>	0.859	0.936	0.840	0.873	0.763	0.648
	H <sub>exp</sub>	0.920	0.907	0.925*	0.933	0.789	0.778*
Average	N	91.5	95	93.5	63.5	91.625	89.75
	Alleles	12.750	12.625	12.125	11.750	11.250	10.625
	H <sub>obs</sub>	0.694	0.736	0.736	0.679	0.651	0.668
	H <sub>exp</sub>	0.745	0.746	0.755	0.752	0.692	0.725

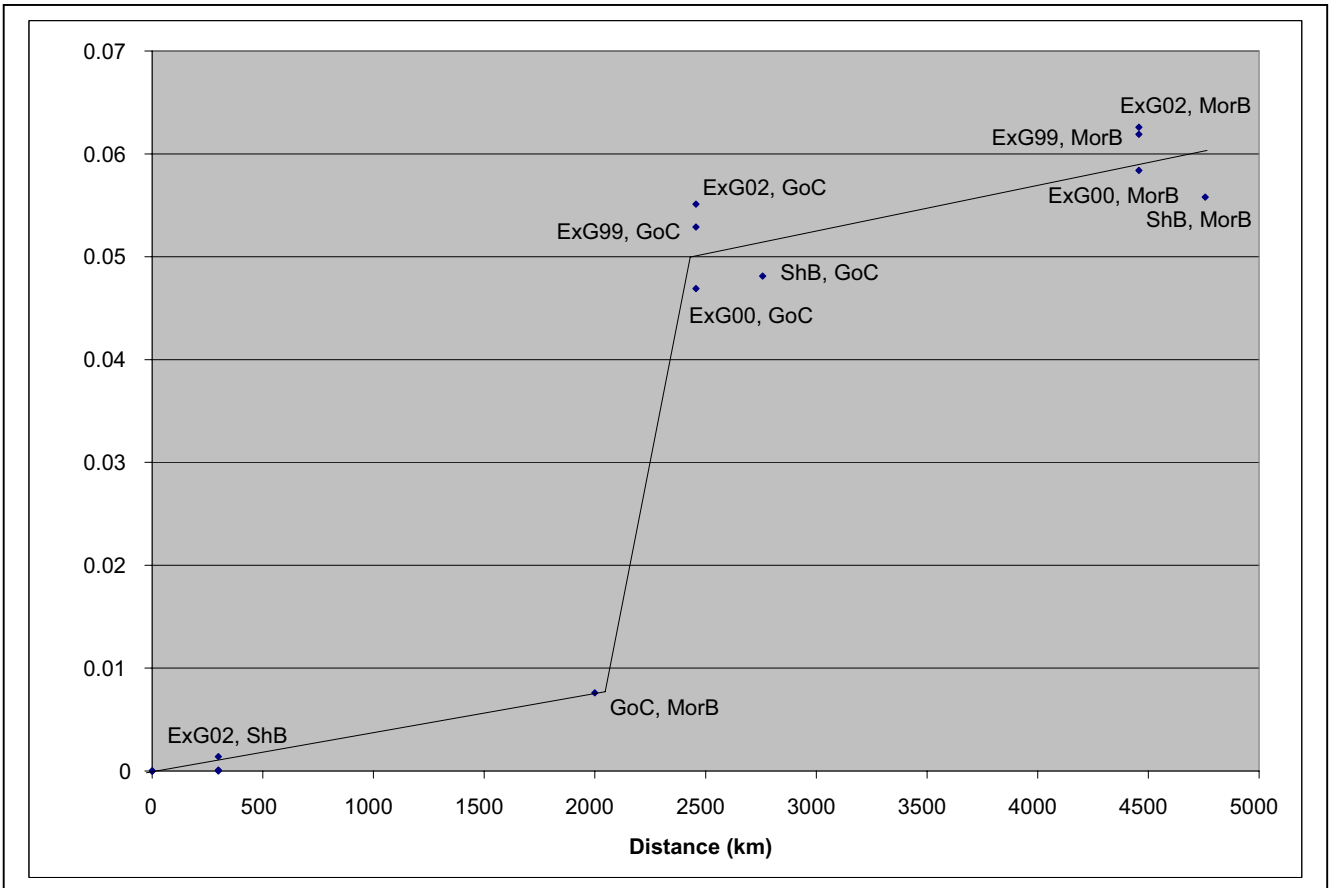
**Table B.2: *Penaeus esculentus* - pairwise population  $F_{ST}$  values above diagonal,  $P$  values below. Negative  $F_{ST}$  values rounded up to zero.**

	ExG99	ExG00	ExG02	ShB	GoC	MorB
ExG99		0	0	0	0.0529	0.0619
ExG00	ns		0	<0.0001	0.0469	0.0584
ExG02	ns	ns		0.0014	0.0551	0.0626
ShB	ns	ns	ns		0.0481	0.0558
GoC	<0.0001	<0.0001	<0.0001	<0.0001		0.0076
MorB	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	



**Figure B.1: Map of Australia showing the four sampling localities for *Penaeus esculentus*.**





**Figure B.2: Pairwise  $F_{ST}$  values plotted against geographic distances between contributing population pairs of *Penaeus esculentus*. The 0:0 point includes the three comparisons within Exmouth Gulf, and the 0:300 point is two of the three comparisons between Exmouth Gulf and Shark Bay.**

**Appendix 1: Allele frequencies and numbers of genes examined per locus in each sample of *Penaeus esculentus*.** Alleles are described by their size in base pairs. These sizes include 9 bp tags added to each primer: true bp sizes are therefore 18 bp less than presented.

Locus	Allele*	ExG99	ExG00	ExG02	ShB	GoC	MorB
PMCD	174	0	0	0	0	0.183	0.197
	177	0.005	0	0	0	0	0
	186	0.038	0.036	0.027	0.014	0	0.005
	189	0.108	0.083	0.117	0.071	0.065	0.117
	192	0.086	0.109	0.08	0.1	0.097	0.08
	195	0.167	0.141	0.154	0.129	0.253	0.25
	198	0.231	0.229	0.16	0.229	0.054	0.027
	201	0.247	0.266	0.298	0.279	0.091	0.059
	204	0.086	0.104	0.112	0.121	0.253	0.234
	207	0.032	0.026	0.043	0.05	0.005	0.032
	210	0	0	0.011	0.007	0	0
	213	0	0.005	0	0	0	0
	total		186	192	188	140	186
CGES268	240	0	0	0.005	0.007	0	0
	243	0.005	0.016	0.011	0.014	0	0
	246	0	0	0	0.007	0	0
	249	0.082	0.12	0.117	0.162	0.152	0.183
	252	0	0	0	0	0	0.016
	255	0	0.005	0	0.007	0	0
	258	0.005	0.01	0.005	0.014	0.098	0.059
	261	0.038	0.042	0.027	0.041	0.212	0.204
	264	0.022	0.021	0.027	0.007	0	0.011
	267	0.038	0.047	0.043	0.047	0	0
	270	0.044	0.01	0.027	0.074	0.005	0.032
	273	0.06	0.063	0.069	0.034	0.125	0.194
	276	0.11	0.073	0.064	0.061	0.043	0.048

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	279	0.495	0.516	0.516	0.405	0.234	0.145
	282	0.011	0.005	0.011	0.034	0.005	0.016
	285	0.033	0.026	0.027	0.047	0	0
	288	0.016	0.026	0.027	0.02	0.114	0.065
	291	0.033	0.021	0.021	0.014	0.011	0.027
	294	0.005	0	0.005	0.007	0	0
	total	182	192	188	148	184	186
CGES 190	261	0.022	0	0.016	0	0.012	0.041
	264	0	0.005	0	0	0	0
	267	0.022	0.026	0.044	0.031	0.006	0.006
	270	0.006	0	0.038	0.01	0	0
	273	0.64	0.684	0.659	0.776	0.829	0.814
	276	0.129	0.068	0.077	0.041	0.047	0.023
	279	0.022	0.016	0.022	0.031	0.006	0
	282	0.157	0.2	0.143	0.112	0.1	0.116
	total	178	190	182	98	170	172
CGES 047	160	0	0	0.005	0	0	0
	180	0.005	0	0	0	0	0
	184	0.005	0.01	0.011	0.027	0.011	0.005
	188	0.016	0.021	0.064	0.034	0	0
	192	0.099	0.057	0.074	0.162	0.021	0.027
	196	0.027	0.026	0.021	0.027	0.064	0.064
	200	0.038	0.021	0.032	0.02	0.005	0
	204	0.159	0.182	0.202	0.122	0.021	0.053
	208	0.154	0.198	0.16	0.149	0.096	0.085
	212	0.077	0.089	0.064	0.061	0.09	0.043
	216	0.082	0.063	0.074	0.088	0.218	0.271
	220	0.082	0.068	0.053	0.115	0.144	0.053
	224	0.132	0.156	0.133	0.061	0.255	0.335
	228	0.049	0.057	0.069	0.068	0.027	0.021
	232	0.044	0.016	0.027	0.034	0.011	0.005

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	236	0.005	0.01	0.005	0	0	0.005
	240	0.011	0	0.005	0.027	0.005	0.005
	244	0.005	0.01	0	0	0.005	0
	248	0	0	0	0.007	0	0
	252	0.005	0.01	0	0	0.027	0.027
	260	0	0.005	0	0	0	0
	total	182	192	188	148	188	188
CGES 176	220	0	0	0	0	0.005	0.016
	223	0.059	0.068	0.048	0.079	0.06	0.053
	226	0.08	0.052	0.032	0.071	0.038	0.005
	229	0.112	0.125	0.102	0.04	0.261	0.16
	232	0.319	0.302	0.328	0.286	0.283	0.213
	235	0.128	0.115	0.108	0.175	0.13	0.059
	238	0.112	0.104	0.151	0.079	0.065	0.053
	241	0.069	0.109	0.118	0.119	0.033	0.053
	244	0.069	0.068	0.07	0.048	0.114	0.202
	247	0.032	0.026	0.027	0.056	0.005	0.117
	250	0.005	0.01	0.005	0.024	0.005	0.043
	253	0	0	0.005	0.016	0	0.021
	256	0	0.005	0	0.008	0	0.005
	259	0.005	0.005	0	0	0	0
	262	0.005	0.005	0	0	0	0
	265	0	0	0.005	0	0	0
	268	0	0.005	0	0	0	0
	271	0.005	0	0	0	0	0
	total	188	192	186	126	184	188
CGES 120	118	0	0.005	0	0	0	0
	121	0.016	0.005	0	0	0.022	0.043
	124	0	0	0	0	0.006	0
	127	0.005	0	0.011	0.012	0	0.016
	130	0.181	0.179	0.239	0.159	0.067	0.098

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	133	0.016	0.033	0.037	0.073	0	0
	136	0.011	0.005	0.016	0.037	0.006	0.005
	139	0.703	0.663	0.601	0.659	0.817	0.658
	144	0.049	0.076	0.069	0.061	0.039	0.114
	147	0.016	0.016	0.027	0	0.044	0.065
	150	0	0.005	0	0	0	0
	156	0	0.011	0	0	0	0
	total	182	184	188	82	180	184
CGES 189	123	0.005	0	0	0	0	0.005
	126	0.016	0.026	0.027	0.02	0.096	0.16
	129	0.005	0.021	0.005	0.014	0.005	0
	132	0.297	0.263	0.335	0.284	0.101	0.17
	135	0.418	0.321	0.34	0.338	0.229	0.229
	138	0.055	0.111	0.09	0.088	0.207	0.133
	141	0.181	0.232	0.197	0.223	0.356	0.293
	144	0.016	0.021	0	0.027	0	0.005
	147	0	0	0.005	0.007	0.005	0.005
	150	0	0.005	0	0	0	0
	156	0.005	0	0	0	0	0
	total	182	190	188	148	188	188
Pe1.1	339	0.016	0	0	0	0	0
	341	0.005	0	0.005	0	0	0
	351	0	0.005	0.005	0.008	0	0
	353	0.125	0.223	0.154	0.143	0	0.007
	355	0.054	0.096	0.069	0.063	0.425	0.415
	357	0.076	0.053	0.048	0.048	0.027	0.028
	359	0.103	0.085	0.08	0.095	0.005	0.007
	361	0.043	0.053	0.08	0.063	0.005	0.021
	363	0.027	0.048	0.08	0.024	0.011	0
	365	0.027	0.021	0.032	0.048	0.102	0.12
	367	0.179	0.112	0.117	0.119	0.005	0

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369	0.054	0.043	0.08	0.071	0.022	0.007
371	0.011	0.043	0.069	0.048	0.022	0.035
373	0.011	0.021	0.005	0.008	0	0
375	0.005	0.005	0.011	0.008	0.005	0
377	0.038	0.037	0	0.04	0	0
379	0.043	0.043	0.043	0.071	0.005	0
381	0.054	0.043	0.032	0.04	0.048	0.077
383	0.049	0.027	0.027	0.032	0.005	0.014
385	0.049	0.016	0.016	0.032	0.145	0.19
387	0.005	0.005	0.027	0.024	0.032	0.056
389	0	0.011	0.005	0	0.027	0
391	0	0	0	0	0.005	0.007
393	0.011	0	0	0	0.005	0
395	0.005	0	0	0.008	0	0
397	0	0	0.005	0.008	0.011	0.007
399	0.005	0	0	0	0.005	0
403	0	0.005	0	0	0	0
405	0	0	0.005	0	0.011	0
407	0	0	0	0	0.005	0.007
409	0	0.005	0	0	0.032	0
411	0	0	0	0	0.016	0
413	0	0	0.005	0	0	0
415	0	0	0	0	0.011	0
417	0	0	0	0	0.005	0
total	184	188	188	126	186	142

## **Appendix C – The prawns who say ‘Ne’: how and when to use Ne in stock assessment**

*by Simon Hoyle*

This talk was presented to a project forum “Genetic methods for the estimation of fisheries spawning stock size” held in Brisbane on 9th May 2003.

### **Abstract**

Fish stock assessment is at the cutting edge of methods for quantitative risk assessment in general, but new methods for data collection have lagged behind. Stock assessment is greatly limited by data availability. Effective population size may provide a new data source with which to increase the precision and accuracy of stock assessment, and improve fishery management decisions. Advantages include fishery independence; focus on individual species, and a unique ecological perspective.

Effective population size may be included in stock assessment models by deconstructing the stock recruitment relationship into a two-stage ‘genetic stock recruitment relationship’: the stock to  $N_e$  relationship, and the  $N_e$  to recruit relationship. Equations are proposed and placed in a state-space modelling context. This context permits estimation of the requisite parameters and hypothesis testing of alternative models, and uses the power of the additional information to reduce uncertainty in management outputs. Results from a simple simulation show increased precision of parameter estimates in some scenarios but not others. Further model development and simulation are recommended.

Factors that affect the usefulness of  $N_e$  estimates are complex and context-dependent, and include observation error and process error in  $N_e$ , the length and contrast of the  $N_e$  and fishery time series, and the quantity and quality of other fishery data.

Fisheries more likely to benefit from  $N_e$  estimates will have short generation time, non-overlapping generations, small stock size, low fecundity, and/or a high  $N_e$  to  $N$  ratio. Fisheries with a longer generation time and overlapping generations, but reliable ageing, may also benefit given further development of theory and practice.

Management of the northern prawn fishery is likely to benefit in the long term from a time series of fishery-independent, single species, substock-based  $N_e$  estimates. The sooner sample collection and storage begins, the sooner the data will become useful. Samples should be stored long-term to obtain the benefits of future technology advances, which will reduce observation error. This fishery may contain a series of sub-stocks. Lack of awareness of existing stock substructure will bias  $N_e$  estimates, which will reduce their utility (for some purposes) and increase observation error. For this reason, stock discrimination analyses are recommended. A method is suggested which may permit stock discrimination even where migration has equalised allele frequencies.

The following steps are suggested to further develop the use of  $N_e$  in stock assessment: 1) develop SNP technology, since it reduces annual costs and observation error in  $N_e$ ; 2) further develop the integrated stock assessment model, and simulate

alternative fishery and life history scenarios; 3) begin sampling in the northern prawn fishery; and 4) begin cataloguing existing sampling in suitable candidate fisheries, and identify fisheries where additional data collection may provide greatest benefit.

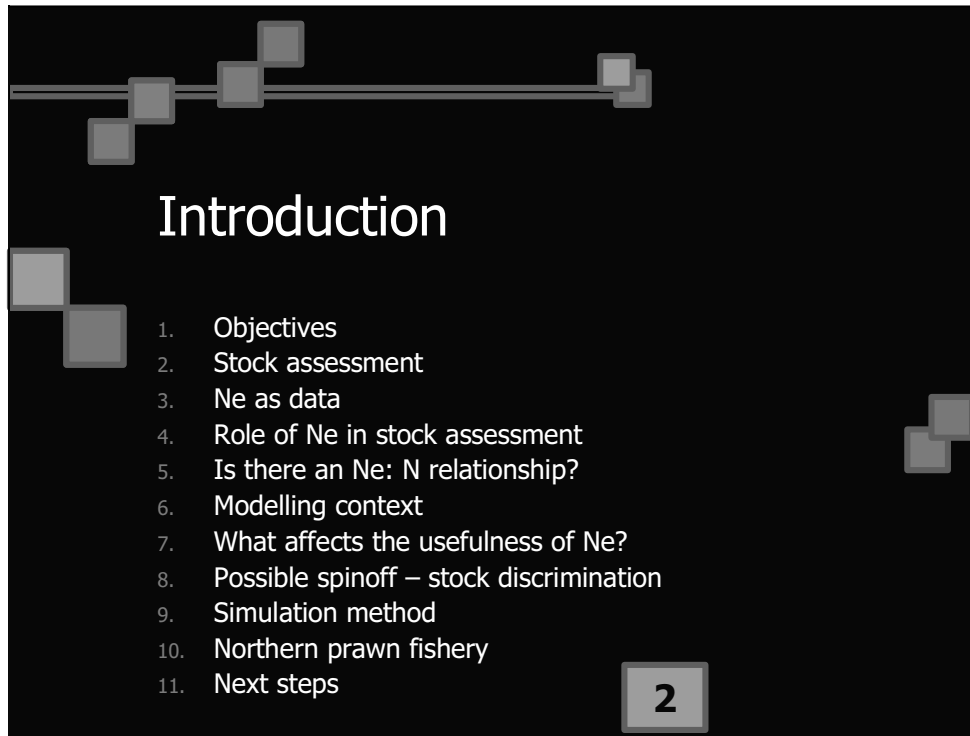


## Presentation

The slide features a header with logos for AFMA (Australian Fisheries Management Authority), Fisheries Research & Development Corporation, The University of Queensland Australia, CSIRO Marine Research, and Queensland Government Department of Primary Industries. The main title is "The prawns who say 'Ne'" followed by the subtitle "When and how to use Ne in stock assessment?". Below this is a navigation bar with three squares. The presenter's name, "Simon Hoyle", and affiliation, "Southern Fisheries Centre, Agency for Food and Fibre Sciences, DPI", are listed. A small logo for the Southern Fisheries Centre, Deception Bay, is in the bottom left, and a large number "1" is in the bottom right.

### Slide 1: The prawns who say 'Ne'.

Today I am going to try and answer the question of when and how to use effective population size in stock assessment.



**Slide 2: Introduction.**

There is a long list of things to go through in this talk and the structure of the talk is outlined here.

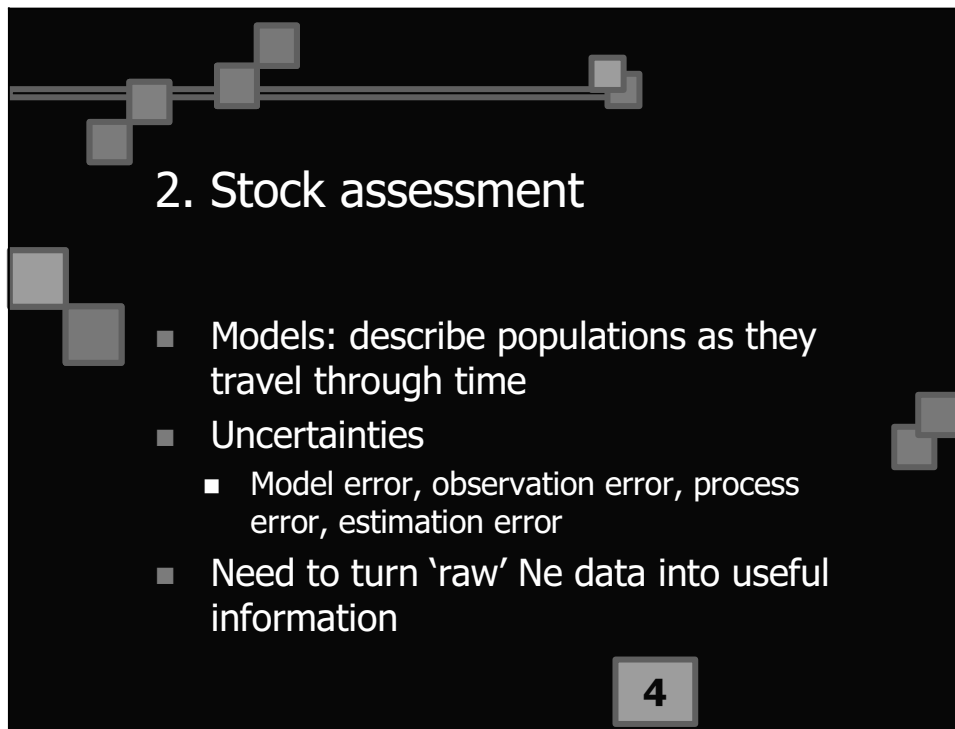
# 1. Objectives

- Overall goal is more accurate stock assessment models
- Project objective: determine conditions where  $N_e$  can be used to improve stock assessments

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**Slide 3: Objectives.**

The overall goal of this project is to get more accurate stock assessment models and better management outcomes. In this presentation I will discuss the various conditions where effective population size can be used to improve stock assessment models.



## 2. Stock assessment

- Models: describe populations as they travel through time
- Uncertainties
  - Model error, observation error, process error, estimation error
- Need to turn 'raw'  $N_e$  data into useful information

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**Slide 4: Stock assessment.**

Stock assessment models describe populations as they travel through time. The model we use to describe that process contains various kinds of uncertainty. Model error is where you are unsure as to the exact nature of the processes that are going on in the population and are therefore unsure as how to best model the population. Observation error, or sampling error, is a well-known source of error and involves the things, like age structure or catch per unit effort, that we assume we can observe. Process error is the variation in the process from year to year, for example natural mortality may be different each year or there may be a different ratio between stock size and effective population size each year. Estimation error is a combination of the three sources of error above. It is the uncertainty in estimating the parameters within your model because of the combined effect of those sources of error. What you are trying to do as part of your model is determine whether the raw effective population size data will give you useful information.



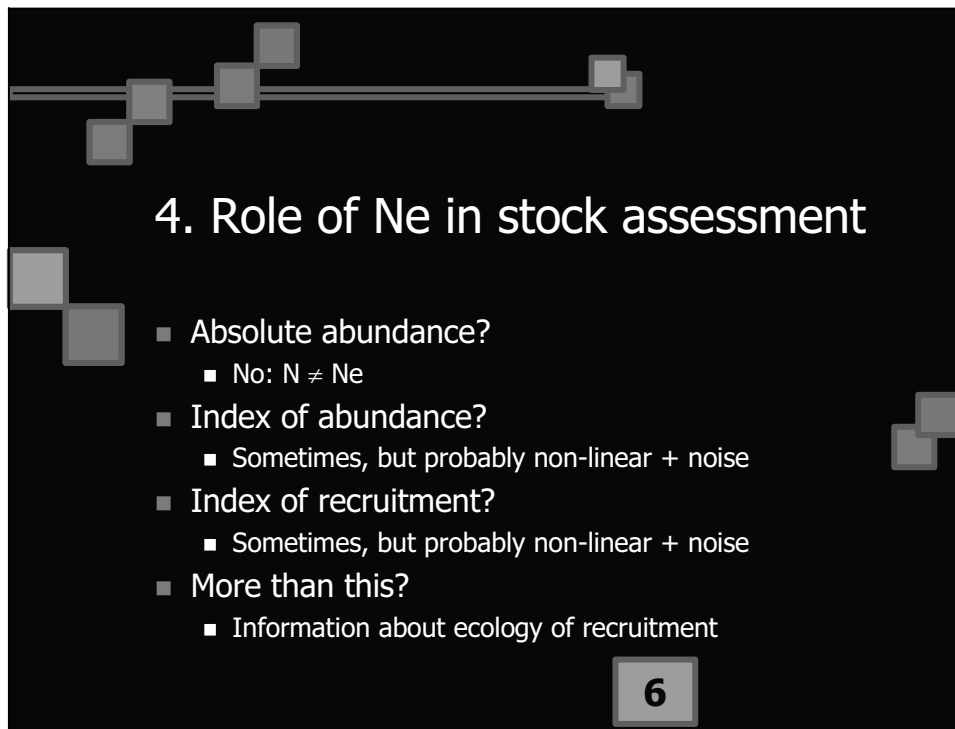
### 3. Ne as data

- New & independent data source
  - Goes beyond the 'usual suspects'
  - Unique perspective
- Fishery independent
  - No effort creep problems
  - Independent of fishing sector
- Single species
  - Even in multi-species fishery
- Cheap

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**Slide 5: Ne as data.**

Ne as raw data has a lot of good features. It is a new and independent data source going beyond the usual kinds of data like catch and CPUE data that are typically available for stock assessment. Ne data gives a unique perspective on the fishery that has not been previously available. It is fishery independent data enabling it to avoid some of the problems with fishery dependent data like effort creep using CPUE data; Cathy Dichmont talked in detail earlier about some of these problems. It is also independent of the fishing sector so that you can still use your effective population size data even if the fishery completely transforms itself from using say trawling gear to traps. It is also single species data meaning that it is possible to focus on a particular species even in multi-species fisheries. In the long term the technology may become relatively cheap to use as a monitoring tool on a yearly basis.



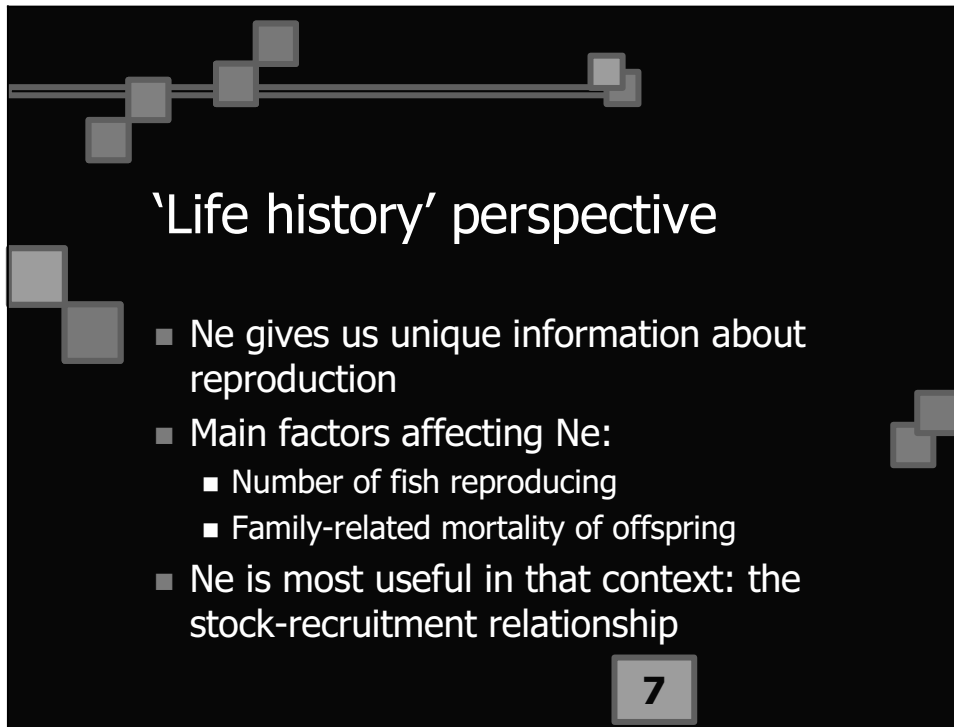
## 4. Role of $N_e$ in stock assessment

- Absolute abundance?
  - No:  $N \neq N_e$
- Index of abundance?
  - Sometimes, but probably non-linear + noise
- Index of recruitment?
  - Sometimes, but probably non-linear + noise
- More than this?
  - Information about ecology of recruitment

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**Slide 6: Role of  $N_e$  in stock assessment.**

How can stock assessment use effective population size? As we have already discussed it is not the same as absolute abundance. Sometimes, effective population size may have some of the features of an index of abundance but as I will discuss later the relationship between  $N_e$  and  $N$  is probably non-linear and it is a fairly noisy relationship. There may be some relationship between  $N_e$  and recruitment but it is probably also non-linear and noisy. Beyond these two relationships,  $N_e$  provides some additional information about the ecology of recruitment.



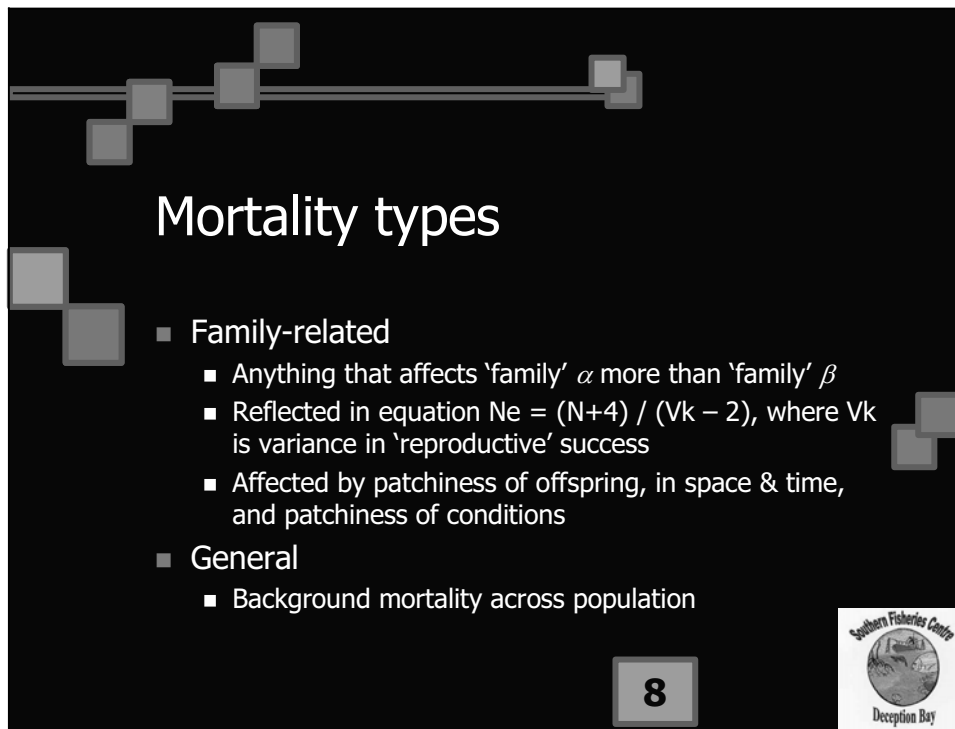
'Life history' perspective

- $N_e$  gives us unique information about reproduction
- Main factors affecting  $N_e$ :
  - Number of fish reproducing
  - Family-related mortality of offspring
- $N_e$  is most useful in that context: the stock-recruitment relationship

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Slide 7: 'Life history' perspective.

A good way to look at effective population size is that it gives unique information about reproduction, because the main factors effecting  $N_e$  are the number of individuals reproducing and the family related mortality of the offspring.  $N_e$  is most useful in a fisheries context when it is looked at as a component of the stock recruitment relationship.



## Mortality types

- Family-related
  - Anything that affects 'family'  $\alpha$  more than 'family'  $\beta$
  - Reflected in equation  $N_e = (N+4) / (Vk - 2)$ , where  $Vk$  is variance in 'reproductive' success
  - Affected by patchiness of offspring, in space & time, and patchiness of conditions
- General
  - Background mortality across population

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Southern Fisheries Centre  
Deception Bay

**Slide 8: Mortality types.**

When eggs are first spawned they are in close proximity to their siblings, resulting in strong spatial patchiness of relatedness. Over time this patchiness will reduce, but on some scales it may linger for a long time. Anything that affects one group of related individuals (eggs / larvae / juveniles) more than another group of related individuals is going to reduce the effective population size. It is difficult to conceive how the offspring of different individuals could have the same survivorship, given variable environmental conditions and the patchiness in space and time of recruitment in a natural population. So this is the first type of mortality – family-related mortality. In addition there is the general background mortality across the entire population. Sources of mortality from spawning right through to eventual recruitment back into the spawning population can be classified into general and family-related mortality.



## Standard stock-recruitment relationship

- A well-established perspective on fish reproduction
  - Recruits as function of stock / spawning biomass
- Standard Beverton-Holt model (q.v. Ricker)

$$R = \frac{S}{\alpha + \beta S} \cdot e^{\varepsilon}$$

Stock (S)	Recruitment (R)
1	2
2	4
3	6
4	8
5	10
6	15
7	20
8	18
9	15
10	14
11	13
12	12
13	11
14	18
15	22
16	25
17	28
18	26
19	24
20	22
21	20
22	25
23	28
24	26

**Slide 9: Standard stock-recruitment relationship.**

One of the standard models used for looking at stock recruitment relationships in fisheries is the Beverton-Holt model (q.v. Ricker) where recruitment is a function of stock and spawning biomass. The model has a fairly flexible shape that describes various possible relationships between stock and recruitment. The error parameter describes the degree of variability in the relationship. A typical relationship between stock and recruitment is shown in the chart. In this example there is a good relationship between stock and recruitment at low stock sizes, but large amounts of noise quickly flatten out this relationship for larger stock sizes.

## Proposed model – genetic SRR

- Divide SRR process into spawning variation + family-related mortality (f) and general (g) mortality
- $N_e = f(\text{Spawning biomass})$
- Recruitment =  $g(N_e)$

$$R = \frac{S}{\alpha + \beta S} \cdot e^{\varepsilon}$$

$$N_e = \frac{S}{a + bS} \cdot e^{\varepsilon_1}$$

$$R = \frac{N_e}{c + dN_e} \cdot e^{\varepsilon_2}$$

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**Slide 10: Proposed model – genetic SRR.**

The genetic stock recruitment relationship (SRR) divides the stock recruitment process into two components. The first component is the spawning variation and family related mortality, denoted here as the function  $f$ , where  $N_e = f(\text{spawning biomass})$ . The second component is the general mortality function  $g$ , where recruitment =  $g(N_e)$ . Both of these model components have the same form as the standard stock recruitment relationship where  $N_e$  is a function of stock size and recruitment is a function of  $N_e$ . The level of spawning and family related mortality will determine the effective population size, and the general background mortality will produce the recruitment that results from that effective population size.

**Substitution**

- Beverton-Holt

$$R = \frac{S}{\alpha + \beta S} \cdot e^{\epsilon}$$

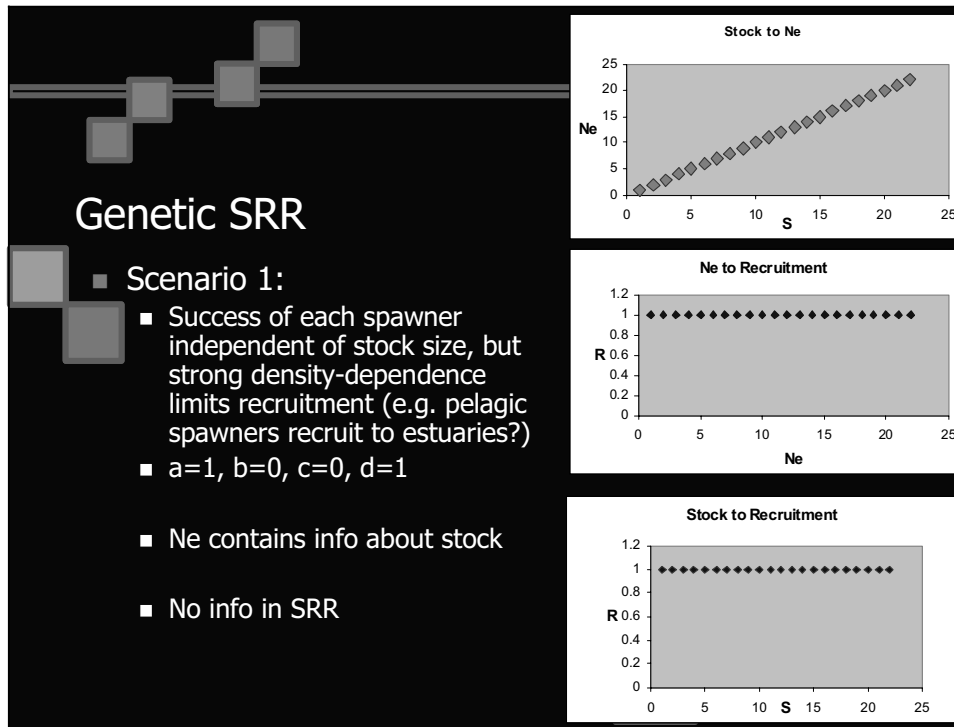
$$R = \frac{S}{c(a + bS) + dSe^{\epsilon_1}} \cdot e^{\epsilon_1 + \epsilon_2}$$

- Still the same form as the original model

**11**

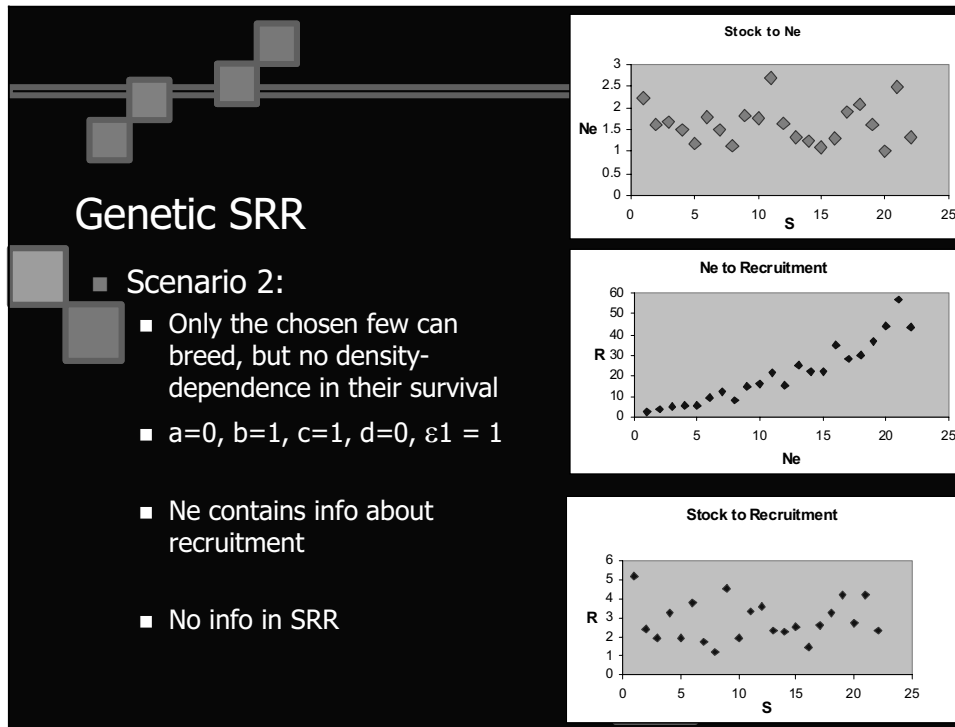
**Slide 11: Substitution.**

Here I have just substituted one formula into the other, showing that it still retains the same form as the standard stock recruitment relationship. The standard Beverton-Holt stock recruitment model can now be considered as a special case of the genetic stock recruitment model.



Slide 12: Genetic SRR – Scenario 1.

I'll now go through a couple of scenarios, exploring what effect different values of the parameters have on the stock recruitment relationship. The values of these parameters represent extreme cases. In this first example  $N_e$  is directly proportional to stock size, effectively making it an index of abundance, and there is no relationship between recruitment and  $N_e$ . Under this scenario the success of each spawner is independent of stock size so that the greater the spawning biomass the bigger the effective population size. Very strong density dependent factors limit recruitment so that no matter how many individuals are spawning recruitment is constant. This scenario may be applicable to pelagic spawners where once the larvae are fully mixed they recruit to estuaries, where their survivorship becomes density dependent. In this case  $N_e$  contains information about stock size but bears no information about recruitment or the stock recruitment relationship.



**Slide 13: Genetic SRR – Scenario 2.**

This scenario describes another extreme example where only the chosen few are able to breed, perhaps due to some sort of territoriality. However, once they have bred there is no density dependence acting on their survivorship. In this case there is no relationship between stock and effective population size, because only a few animals in any given situation are able to breed. Because there is no density dependence influencing survivorship, more recruits result in higher effective population sizes. Under this scenario  $N_e$  contains information about recruitment but not about stock size or the stock recruitment relationship.

5. Is there Ne Ne relationship?

- Is  $R \propto S$ ? Recent meta-analyses suggest SRR's fairly common
- If so,  $N_e = f(S)$  and  $R = g(N_e)$  probably even stronger relationships?
- But not necessarily:  $S$  can affect  $R$  along ecological pathways – say if adults prey on species  $B$ , which compete with juveniles

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**Slide 14: Is there Ne Ne relationship?**

Recent meta analyses suggest that there is a relationship between stock and recruitment for most fisheries. If that were the case then we would logically imagine that there should be even stronger relationships between  $N_e$  and stock and between recruitment and  $N_e$ , if the relationships are as I have described. However this may not always be the case, as there are mechanisms where stock can affect recruitment indirectly without going through the normal path of  $N_e$ . For example, if an adult stock preys on another species that competes with its juveniles then a higher stock size will result in higher recruitment, but this stock recruitment relationship is not mediated through  $N_e$ .

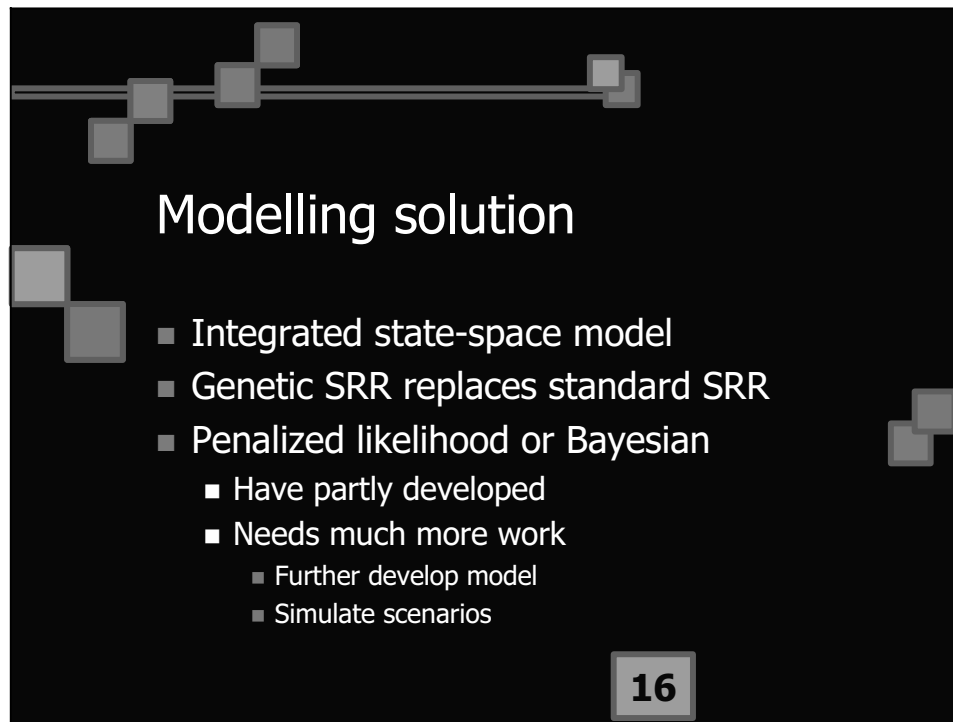
## 6. Modelling context

- Separate confirmation?
- Integrated model 'ultimately united'
  - Statistical hypothesis tests
  - Estimate & reduce uncertainty
- What's needed to use  $N_e$ 
  - Must estimate Genetic SRR parameters  $a, b, c, d, \varepsilon_1, \varepsilon_2$
  - To do this, need other information

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**Slide 15: Modelling context.**

The genetic stock recruitment relationship in a modelling context can be used as a confirmation of other stock recruitment models based on traditional information. However a more powerful approach is to unify all of the models into one. Among the benefits are that this permits statistical testing of various hypotheses, to see if different models are suitable for the data. Uncertainty is also substantially reduced, and can be estimated better when all of the data are combined into a single model. However, there are more parameters ( $a$ ,  $b$ ,  $c$ ,  $d$ ,  $\varepsilon_1$  &  $\varepsilon_2$ ) in the genetic stock recruitment relationship than in the standard SRR. There is a basic statistical principle that estimating more parameters requires more information.  $N_e$  estimates do provide new information, but additional information from the fishery such as longer time series of fishery data may also be needed. We need to simulate to examine this issue further.



## Modelling solution

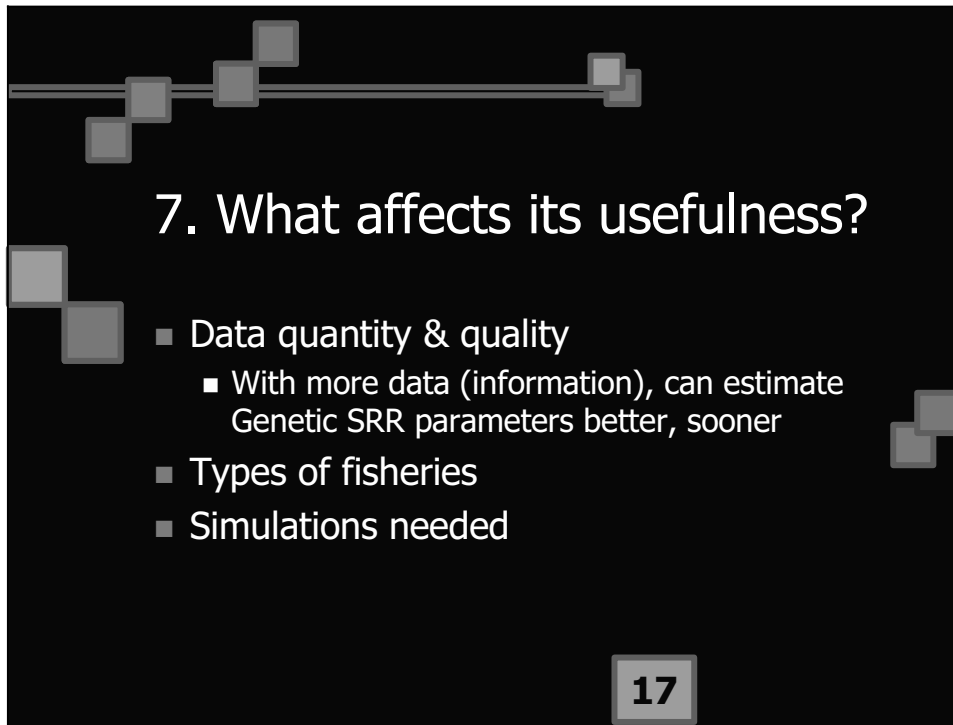
- Integrated state-space model
- Genetic SRR replaces standard SRR
- Penalized likelihood or Bayesian
  - Have partly developed
  - Needs much more work
    - Further develop model
    - Simulate scenarios

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**Slide 16: Modelling solution.**

A solution is to use an integrated state space model, in which the genetic stock recruitment relationship replaces the standard stock recruitment relationship. We have partly developed this model in a Bayesian framework. We believe that further development of this model through simulations is highly desirable, but was unfortunately outside the scope of the current project.



A presentation slide with a black background and white text. The title is '7. What affects its usefulness?'. Below the title is a bulleted list with three main items: 'Data quantity & quality', 'Types of fisheries', and 'Simulations needed'. The first item has a sub-bullet: 'With more data (information), can estimate Genetic SRR parameters better, sooner'. The slide number '17' is in a small box at the bottom right. There are several grey squares of varying sizes scattered around the text, some connected by thin lines, resembling a network or data flow diagram.

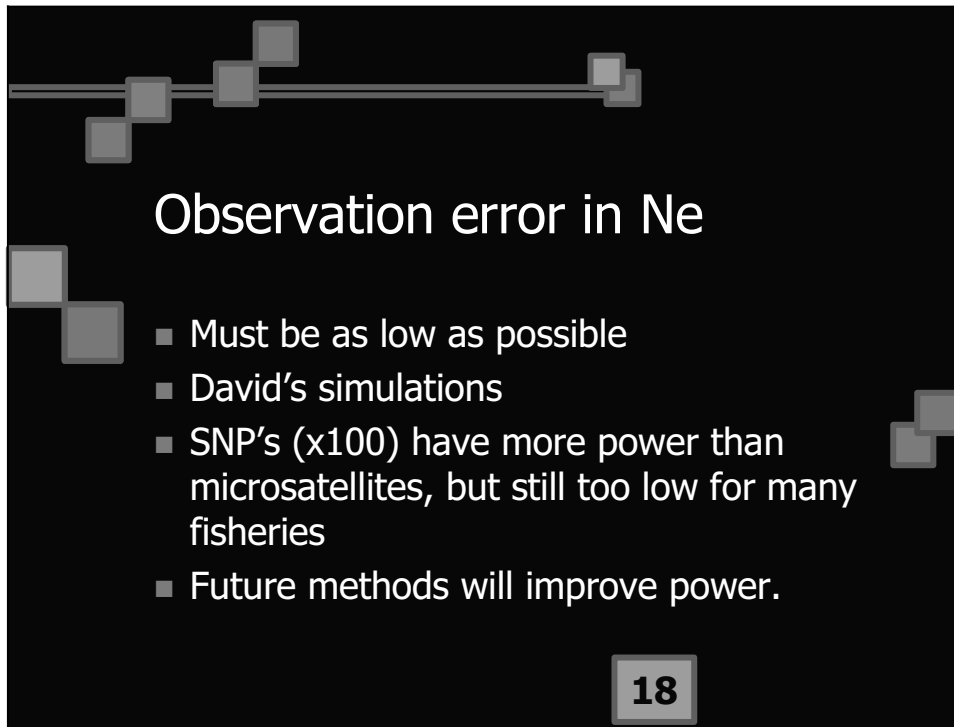
## 7. What affects its usefulness?

- Data quantity & quality
  - With more data (information), can estimate Genetic SRR parameters better, sooner
- Types of fisheries
- Simulations needed

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**Slide 17: What affects its usefulness?**

There are several things that are likely to affect the usefulness of  $N_e$  as part of an integrated stock assessment model. In the next few slides I'll go through some of those things. In general, data quality and the amount of data affect the amount of information about the genetic stock recruitment relationship, which obviously affects the speed and precision with which genetic parameters can be estimated. The genetic stock recruitment relationship may be more useful in certain kinds of fisheries than in others. We really need to use simulation to explore in more detail the behaviour of the genetic stock recruitment relationship parameters, and the way features of the fishery and data affect the usefulness of  $N_e$ .



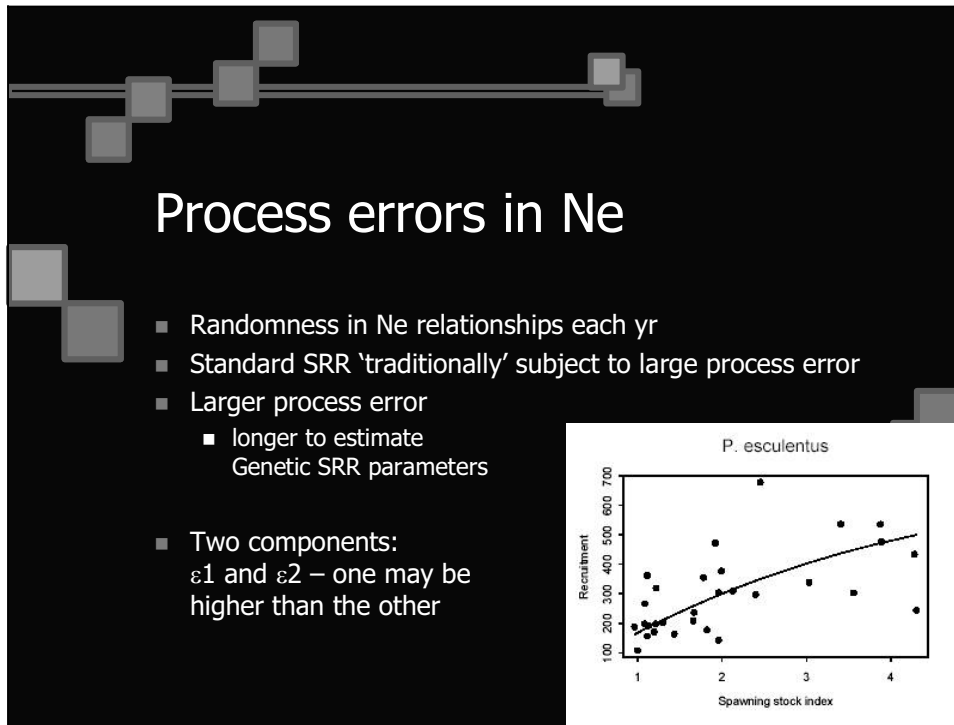
## Observation error in $N_e$

- Must be as low as possible
- David's simulations
- SNP's (x100) have more power than microsatellites, but still too low for many fisheries
- Future methods will improve power.

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**Slide 18: Observation error in  $N_e$ .**

Observation error in  $N_e$  is a very big issue, and David Peel's simulations (Chapter 5) have shown ways to reduce and quantify this source of error. It appears as though while single nucleotide polymorphisms (SNPs) have more power than microsatellites, even they may not have sufficient power for many fisheries that have large effective population sizes. Future developments will improve the power of these methods and reduce costs so that more samples can be analysed. It is difficult to speculate on how long it will take until observational error is reduced to acceptable levels but it is already moving rapidly in the right direction.



**Slide 19: Process errors in  $N_e$ .**

Process error in the estimate of effective population size is the randomness in the true  $N_e$  relationship each year. Standard stock recruitment relationships are traditionally subject to large process error. The larger the process error the longer it will take to estimate the genetic stock recruitment parameters and the longer it will take before estimates of  $N_e$  will be useful to the fishery. The example shown here is data from a tiger prawn fishery, including both estimation and process error. Reducing the fishery biomass to very low levels as in this example would allow us to get good estimates of the parameters; but we would be less certain of those relationships in a fishery maintained at higher levels of biomass. There are two components to process error in the genetic stock recruitment relationship model:  $\varepsilon_1$  &  $\varepsilon_2$ . One may be higher than the other and their relative magnitudes will be unknown until you have a time series of effective population size estimates. Comparing species with similar life histories may provide priors for novel fisheries but this is a long way off and will require  $N_e$  estimates from a large number of fisheries.

## Process errors in $N_e$ (cont)

- Unknown: species and stock-dependent
- May preclude use as index
- But  $N_e$  may still be very useful:
  - Uncover causes of process error:
    - Life history?
    - Environment?

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**Slide 20: Process errors in  $N_e$  (cont).**

Process errors are generally unknown and are species and stock dependent. If the process errors are too large then it may not be possible to use  $N_e$  as an index of abundance or as an index of recruitment. However, this does not mean that  $N_e$  is not useful because information about the life history on the species may be uncovered during the process of investigating the causes of process error.

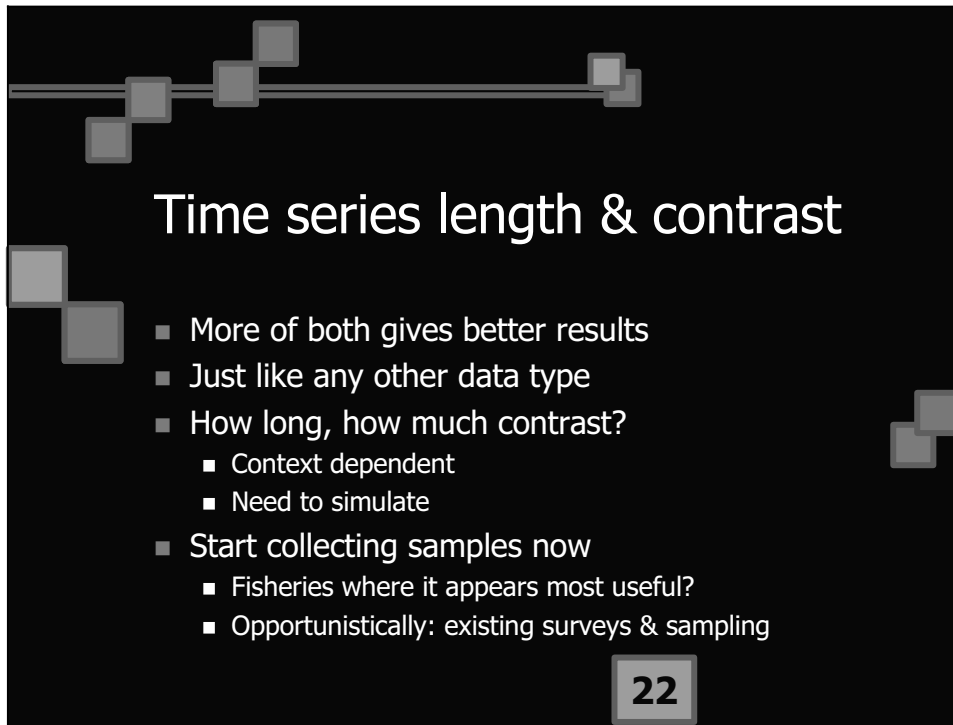
## Existence of comparable data

- If abundance or recruitment indices exist
  - Faster to estimate Genetic SRR parameters
  - Ne not redundant
  - Validate, reject, or adjust other index
  - E.g. show that hyperstability affects CPUE
  - Reduce model error
- If such indices don't exist
  - Slower to estimate Genetic SRR parameters

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**Slide 21: Existence of comparable data.**

The existence of comparable data improves the usefulness of  $N_e$ . The more that is known about a stock the more quickly the relationship with  $N_e$  can be deduced. If you already have alternative abundance or recruitment indices then  $N_e$  estimates may help to validate the information you have or reject some of the assumptions you are making about the fishery. Knowledge of  $N_e$  can therefore reduce your model error and help to ensure that you are using the right sort of model. If alternative indices do not exist then the genetic parameters will be much slower to estimate. Once the genetic parameters are estimated the model can then be effectively used for stock assessment and management.



## Time series length & contrast

- More of both gives better results
- Just like any other data type
- How long, how much contrast?
  - Context dependent
  - Need to simulate
- Start collecting samples now
  - Fisheries where it appears most useful?
  - Opportunistically: existing surveys & sampling

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**Slide 22: Time series length and contrast.**

As with any data type, results are much better with longer time series and more contrasting data. By contrasting data I mean that the power to uncover a signal in the data is limited unless you can compare data from stocks in both good and bad situations. Poorly managed or highly variable fisheries can provide this sort of contrast. How much time and how much contrast you need is very context dependent, given the other parameters in the model, and can only be answered through simulation of each fishery. Because long time series are so valuable it would be a good idea to start collecting tissue samples now, particularly in fisheries where we think  $N_e$  may be most useful. Opportunistic sampling during existing surveys and at the market place is perhaps the most efficient way to do this.

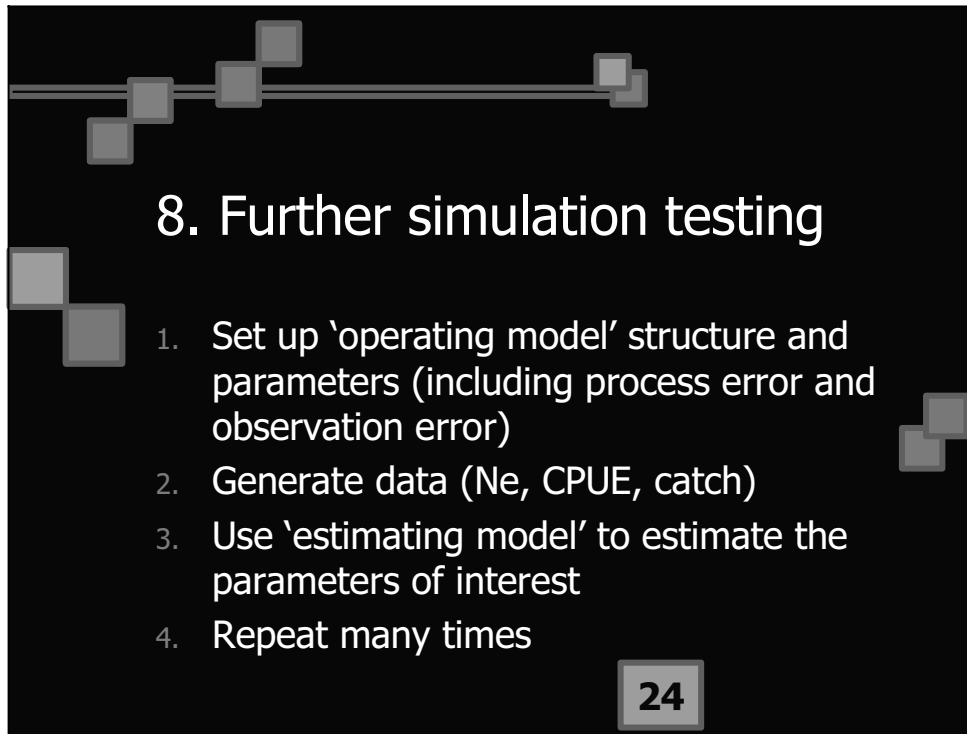
## Types of fisheries

- Non-overlapping, short generation time
- Long generation but reliable ageing
  - Theory & practice need development
- Lower process error
  - High  $N_e/N$  ratio?
- Lower observation error
  - Smaller  $N_e$ , more markers available

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**Slide 23: Types of fisheries.**

The types of fisheries where  $N_e$  may be most useful include those with non-overlapping and short generation times.  $N_e$  is easiest to estimate in these fisheries and results can be forthcoming in reasonable timescales. Fisheries with overlapping and long generation time are more problematic and the theory is still being developed. Accurate ageing of individuals will be important in these sorts of fisheries.  $N_e$  is likely to be more useful in fisheries with smaller process error because the relationship between  $N_e$  and stock size will be easier to elucidate. Likewise,  $N_e$  will be more useful in fisheries that have high  $N_e/N$  ratios and in fisheries with lower stock sizes, since this will reduce effective population size. Fisheries with low observation error, such as those with smaller effective population sizes and those with more molecular markers giving more precision, are likely to be good candidates for the genetic stock recruitment models.



## 8. Further simulation testing

1. Set up 'operating model' structure and parameters (including process error and observation error)
2. Generate data ( $N_e$ , CPUE, catch)
3. Use 'estimating model' to estimate the parameters of interest
4. Repeat many times

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**Slide 24: Further simulation testing.**

This is the proposed simulation method that we will use to validate our genetic stock recruitment models. Unfortunately there is no simulation data as yet to present.



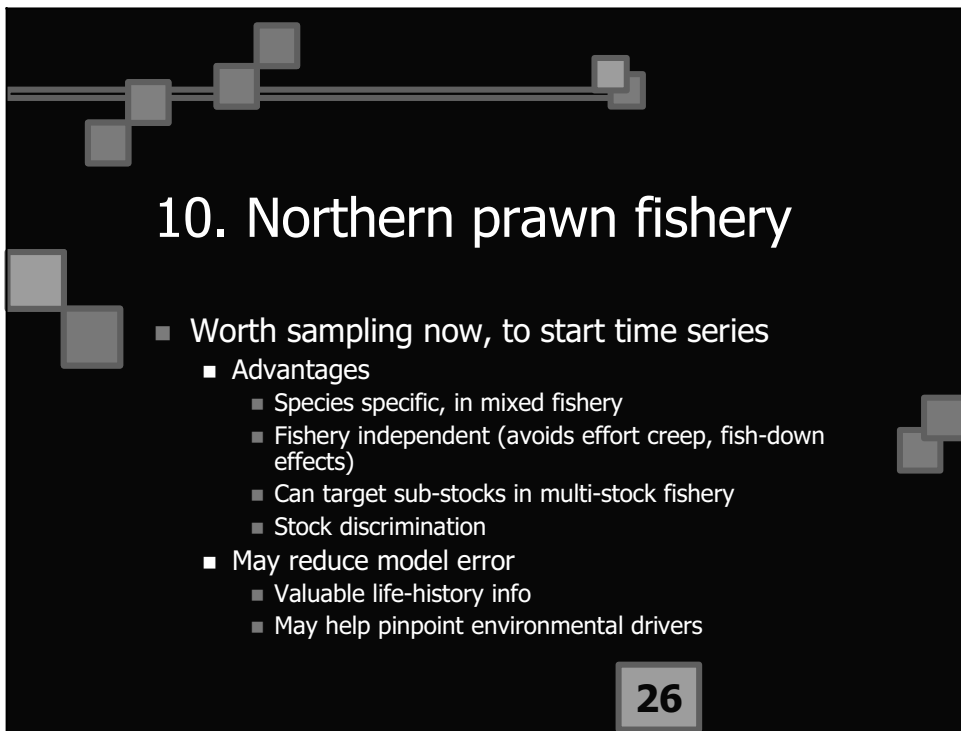
## 9. Possible spin-offs: stock discrimination

- Separate but cryptic sub-stocks may exist
- Managing separately gives better outcomes
- Sampling for  $N_e$  gives power to detect small allele frequency differences
- If  $> 1$  migrant per generation, allele frequencies will not differ: stocks may be separate on management but not on evolutionary timescales
- May use  $N_e$  estimates to infer stock subdivision

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**Slide 25: Possible spin-offs: stock discrimination.**

There are possible spin-offs beyond estimating  $N_e$  from collecting genetic data. Cryptic stocks may exist in the fishery that adversely influences the efficacy of management. Management outcomes are much better if separate stocks are managed separately, and it is therefore advantageous that cryptic stocks be recognised. Sample sizes needed to estimate  $N_e$  are very large and therefore typically have the power to detect very small allele frequency differences between potential cryptic stocks. However differences in allele frequencies between stocks will not be observed if migration is greater than one migrant per generation. In these cases stocks may be separate on management time scales but not on evolutionary timescales. While beyond the scope of this presentation, we have been considering ways to recognise this kind of stock subdivision using changes in the effective population size estimates among the cryptic stocks.



## 10. Northern prawn fishery

- Worth sampling now, to start time series
  - Advantages
    - Species specific, in mixed fishery
    - Fishery independent (avoids effort creep, fish-down effects)
    - Can target sub-stocks in multi-stock fishery
    - Stock discrimination
  - May reduce model error
    - Valuable life-history info
    - May help pinpoint environmental drivers

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**Slide 26: Northern prawn fishery.**

One of the aims of this project was extension into the northern prawn fishery. Sample collection began in 2001. An advantage of extending the effective population size work into the northern prawn fishery is that it provides species specific information in a mixed species fishery. It is a fishery independent data source and could be very useful at identifying cryptic stocks. Additional life history information could result from estimating  $N_e$  and this would then in turn help to reduce model error. Given the expected size of  $N_e$  in the northern prawn fishery we could do a cost benefit analysis and simulation to determine the number of samples and loci required to estimate  $N_e$ . This information could be used to make judgements about what technologies to use or whether it is even worthwhile to pursue estimating the effective population size in the northern prawn fishery. These simulations are needed before proceeding to analyse northern prawn samples.



## 11. Next steps

- Develop SNP's
  - Appear better than microsatellites
- Develop integrated model, simulate scenarios
- Sample in NPF
- Opportunistic sampling in candidate fisheries to build time series
  - Catalogue 'library' samples
- Develop stock discrimination

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Slide 27: Next steps.

Finally, future directions of this project include:

- i. The development of the SNP technology, as it appears to have more potential than microsatellites. Higher levels of automation and their straightforward scoring mean that SNPs markers have substantially less observation error than microsatellites.
- ii. Further develop integrated fisheries models and use simulation of different fisheries scenarios to investigate under what conditions  $N_e$  may be useful.
- iii. Conduct cost benefit simulations in the northern prawn fishery to establish whether it is useful to pursue estimating  $N_e$ .
- iv. Collect tissue samples opportunistically in candidate fisheries to establish important time series data.
- v. Develop stock discrimination methodology.



## **Appendix D – Estimation of effective population size in Gulf tiger prawns from temporal changes in allele frequencies.**

*by Per Erik Jorde<sup>†</sup>, Jenny Ovenden\* and Tony Courtney\**

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\*Southern Fisheries Centre, Department of Primary Industries and Fisheries, Brisbane, Australia.

### **Summary**

We used computer simulations to characterize random genetic drift in model populations and evaluated the possibility of using empirical genetic data to estimate the genetic effective sizes of tiger prawn populations. The simulations show that neither overlapping generations nor seasonal fluctuations in demographic characteristics of tiger prawns significantly affects the pattern of genetic drift in these species, and methods developed for species with discrete, non-overlapping generations are adequate for tiger prawns too. We discuss various sampling strategies for collecting genetic data and how they affect estimates of effective size.

### **Introduction**

#### *Fisheries imperative*

Fisheries scientists believe that global fishing catch has reached its ecological limit (Jackson and others, 2001). This is partly due to inadequate scientific estimates of the number of fish that are available to be harvested. Fisheries managers cannot set effective controls on fishing effort without this essential information.

Computer modelling of population size is commonly used by fisheries stock assessment scientists to guide fisheries managers. But models must be based on accurate biological measurements of the population, which are time-consuming and often difficult to obtain. Modellers use the rate of harvest of the population (catch per unit effort or CPUE) to measure abundance; but it can be seriously biased if, for example, catches are taken from spawning schools that are a feature of the population regardless of the numbers of fish remaining (Hilborn and Walters, 1992).

An independent alternative to stock assessment modelling of marine populations is being developed at the Molecular Fisheries Laboratory at the Southern Fisheries Centre, Queensland (Department of Primary Industries, Deception Bay, Queensland). This alternative involves making inferences about stock size from genetic information collected from samples of individuals from the population. Such genetic information is used to estimate the genetically effective population size, a concept closely related to the number of successful spawning individuals in the population. Effective size is inversely proportional to the amount of random genetic drift in the population (*i.e.*, chance fluctuation in allele frequencies across generations: Wright 1969, Waples 1989a), and measurements of genetic drift form the basis for the so-called temporal method of estimating effective population size. In the last decade or so, the temporal

method has become increasingly popular, and several studies employing various molecular genetic techniques have applied this method to different organisms (cf. Krimbas & Tsakas 1971; Begon *et al.* 1980; Waples 1990; Hedgecock *et al.* 1992; Jorde & Ryman 1996; Miller & Kapuscinski 1997; Saavedra 1997; Scribner *et al.* 1997; Laikre *et al.* 1998; Funk *et al.* 1999; Sitnikov *et al.* 1999; Turner *et al.* 1999, 2002; Queney *et al.* 2000; Jehle *et al.* 2001; Heath *et al.* 2002; Palm *et al.* 2002). Estimates of effective size are currently being made for the brown tiger prawn (*Penaeus esculentus*) population in Moreton Bay, Queensland.

The population of tiger prawns in Moreton Bay was selected as a 'model' for our research into developing effective population size as a stock assessment tool, because it conforms to many of the simplifying assumptions that are typically made when estimating effective size. Such assumptions include random mating (panmixis), even sex ratio, discrete (non-overlapping) generations, and random (Poisson distributed) number of offspring (*e.g.* Wright 1969). However, our goal is to apply the methodology to populations of brown (*P. esculentus*) and grooved (*P. semisulcatus*) tiger prawns in the Gulf of Carpentaria in northern Australia, which do not conform so well to the simplifying assumptions. The fisheries harvest of these species is worth about \$120 million annually to the Australian economy. The successful application of the method in the Gulf will depend on how sensitive the method is to violations of the underlying assumptions, such as panmixia and non-overlapping generations. Sensitivity to lack of panmixia is being dealt with elsewhere; this paper addresses the effects of overlapping generations that occurs in grooved, and to a lesser extent in brown tiger prawns in the Gulf of Carpentaria.

### ***Tiger prawn spawning cycles***

Crococ studied the spawning patterns of grooved (*P. semisulcatus*) and brown (*P. esculentus*) tiger prawns in the north-west of the Gulf of Carpentaria adjacent to Groote Eylandt (Crococ, 1987a; b). His estimates of population fecundity index (equal to egg production) used the product of the proportion of spawning females in the population ( $p$ ) and the abundance of females ( $a$ ). The logic here is that if female numbers are low at a certain time of year then the population's total egg production will also be low, even if the ovaries are ripe and spawning is occurring. Alternatively, egg production may be low if only a fraction of females participate in breeding.

For grooved tigers, Crococ reported two peaks of population fecundity occurring each year; egg production was high from August to late October with a peak in September (spring), followed by a drop and then a slight increase in February (autumn). During these peak times only a proportion of the females came into spawning condition (90% of the total population in August-October and 20% in February). Abundance of prawns varies throughout the year. For brown tiger prawns egg production is more spread throughout the year, with eggs being produced most consistently in late winter and early spring. Adult female tiger prawns take about 28 days to complete a moult/spawning cycle, so in each of these spawning peaks ('seasons') individual prawns probably spawn twice. Individual length is generally used as a measure of age for prawns but the size of the individuals is not a particularly important factor when quantifying egg production. Instead, the main factors affecting seasonal variation in egg production are abundance and the proportion mature or ripe.

Mortality rates for prawns are high; about 90% of the population die within 12 months so the vast majority would probably only experience a single spawning season. Hence, the autumn spawners should be dominated by individuals born in spring whereas most spring spawners are born in autumn. Old spawners that may have spawned in the previous season are present in the population, but probably account for less than 10% of egg production (ref needed – ask Tony). Not all egg production contributes to stock renewal (recruitment into the next generation). Crocos (1987a, 1987b) put forward the term "effective spawning stock" which is that proportion of spawning which contributes to stock renewal.

### *Aim of study*

The aim of the present study is to evaluate how the so-called temporal method can be applied to estimate effective sizes of tiger prawn populations. We do this evaluation on the basis of computer simulations directed towards exploring the genetic consequences of limited population size in such populations, given their demographic characteristics as outlined above. Further, the computer simulations address how to most effectively sample individuals from the population for genetic analysis and how sampling affects the estimates of effective population size.

## **Method**

### *Population models*

We design a simplified model of tiger prawn demography that includes the characteristic spawning peaks in spring and autumn and accounts for their relative intensities. Briefly, we assume that spawning occurs twice a year, in the spring and in the autumn season. Two age classes (cohorts) participate in breeding each season: a cohort of roughly six-months old (hereafter age class 1) which were born the preceding spawning season ( $N_1$  individuals) and a smaller cohort of older individuals (age class 2) which were born one year ago ( $N_2$  individuals). This latter, older cohort consists of individuals that may have spawned the previous season and, thus, some of these are parents of individuals in the younger cohort. This overlap in generations is the reason that we need to model the transmission of genes in prawn populations before we can use observations on temporal genetic change to estimate effective size. Nevertheless, the proportion of 12 months old in the population is relatively small because of a high mortality (survival rate from 6 to 12 months:  $l_x = 0.1$ ). The demography of the reproduction event is generally less well known and for this reason we make two alternative models. In the first scenario (hereafter Model I: the seasonally fluctuating spawner proportion model) a fraction  $bp$  of individual from each age class enters spawning condition and participate in breeding. This fraction differs between the two seasons and we assume  $bp = 0.9$  in the spring and 0.2 in autumn, respectively. We count only the offspring that survive to the next season, because those who die don't contribute to the population in any way, so that the smaller number of spawners in the autumn is counterbalanced by a higher proportion of surviving offspring and, we assume, the population size stays constant. As indicated above, we do not know if this assumption is correct and therefore also explore a second, alternative model (Model II: the seasonally fluctuating birth rates model), where population size fluctuates seasonally. In this second model all adults (*i.e.*, all members of age class 1 and 2) participate in breeding each season, so that  $bp = 1$  is constant. Instead, we let the birth

rate ( $bx$ ) differ among seasons, being highest in autumn and lowest in spring so that, as a result, the population size fluctuates seasonally with a peak in spring.

Mathematically, the demographic models can be phrased as follows. Number of young in autumn,  $N_1$  = number of spawners in spring,  $(N_1+N_2)*bp$ , times the average number of (surviving) offspring per spawner ( $B$ ). In Model I  $bp = 0.9$  and  $B = 1.01$  so that the average birth rate,  $bx = B*bp = 0.909$  per adult in spring. Thus, each adult in spring delivers on average 0.909 offspring that enters the population as age class 1 in the next (autumn) season. For Model II  $bp = 1$  and we set  $B = 0.1$ , yielding  $bx = 0.1$  for the spring season, resulting in a low number of young ones (age class 1) in the autumn. Similarly for the spring population: in Model I  $B = 4.545$  in the autumn, to account for the lower proportion of spawners ( $bp$ ) in that season, so that  $bx = bp*B = 0.2*4.545 = 0.909$  is the same as in spring and the population remains constant. In Model II we set  $B = 5$  so that  $bx = 5.0$ , which is much larger than in spring and the population size fluctuates accordingly. In either model and season the number of older individuals (age class 2),  $N_2$  = number of young in the preceding season times the survival rate,  $lx = 0.1$ . The models are depicted graphically in Figure D.1 and Figure D.2.

On the basis of the above -highly simplified- demographic models we ask the following question. If we sample genes from such populations in two different spawning seasons, say, in the spring and autumn of the same year, how will the observed genetic change over that time relate to the effective size of the population? Clearly, an answer to that and related questions depends on both the real changes that occur in the population and also on how the samples were taken, *i.e.*, what we sample. In principle, it is possible to evaluate these processes theoretically [see Waples (1989a) for a general treatment of the discrete generation situation and Jorde & Ryman (1995) for an extension to populations with overlapping generations], but so far no complete theory exists that allow us to include all properties of even our simplified models. We therefore used computer simulations to gain insight into the allele frequency dynamics of the modeled prawn populations as well as on sampling issues.

### ***Computer simulations***

Computer simulations were carried out by repeated random drawings of genes (gametes) according to the demographic models above. Every second iteration were treated as 'spring' (odd iteration numbers) or 'autumn' (even numbers) and demographic parameters ( $bp$  and  $B$ ) were set according to season. We used an arbitrary (not intended to be realistic) size for the simulated populations. For Model I we constructed an initial population consisting of  $N_1 = 450$  individuals in age class 1 and  $N_2 = 45$  individuals in age class 2, for a total of 495 individuals. For Model II the spring population consists of  $N_1 = 472$  young ones but only  $N_2 = 5$  old individuals, whereas in autumn the numbers in the two age classes change to  $N_1 = 48$  and  $N_2 = 47$ , respectively. Hence, there is a pronounced seasonal shift in age structure accompanying the fluctuations in population size in this model. For either model we initiated the population at the start of each simulation with two alleles at equal proportions (frequencies) of  $p = q = 0.5$  in both age classes, and we monitor the change in allele frequency during the simulation. Each breeding season we picked out at random a proportion  $bp$ , according to the demographic model, of the individuals from each age class to constitute the breeders for the next generation. The offspring



generation was produced by randomly drawing (with replacement) an average number  $2B$  gametes per breeding individual, yielding a number of individuals ( $N_1$ ) that enter the population as age class 1 in the next season. Survival from age 1 to 2 was simulated by random drawing (without replacement) a proportion ( $lx = 0.1$ ) of the individuals of age class 1, yielding  $N_2$  survivors that constitute age class 2. All individuals in this latter age class were removed after breeding so that no individuals live more than one year. The same survival rate was used in the spring and the autumn seasons: all differential survival in the models between seasons refers to larvae, post-larvae and juveniles (before they enter age class 1) and were implemented by using different spawner proportions (Model I) or birth rates (Model II), as described above.

Sampling of genes for genetic analysis was done by randomly drawing (without replacement) about 100 individuals (actually, 200 genes) from the total population with the two age classes represented in their population proportions. For Model I this corresponds to 20% of each age class, yielding total sample sizes of  $n = 99$  individuals: 90 from age class 1 and 9 from age class 2. For Model II, we sampled 21% of the spring population ( $n=100$  individuals for the two age classes combined; 99% of them from age class 1) but included all (95) individuals from the autumn population (approx. 50% from each age class). These sample sizes should be fairly realistic: about one hundred individuals is a reasonable compromise between precision of estimation and cost of analysis. (Obviously, while the sample *sizes* are realistic the sample *proportions* are hardly so since the size of the actual populations are likely to be much larger than the simulated ones. However, the temporal method is insensitive to the actual population sizes and this latter quantity only enters in the sampling-correction step, as described in the legend to Table D.2. All sampled individuals were returned to the population after their genes were counted, before reproduction. Thus, sampling followed plan 2 of Nei & Tajima (1981) and Waples (1989a). We ran each simulations for 50 iterations ('seasons') before the first sample (at  $t = 51, i.e.$ , in spring), so as to allow the system to become independent of its initial conditions. Sampling was repeated in the next two seasons (at  $t = 52$  and  $53$ ), after which a new simulation was initiated. A total of 20 000 replicate simulations were performed.

Observed temporal genetic change was calculated from the sample allele frequencies of each simulation, using Pollack's (1983) estimator  $F_k$ :

$$F_k = \frac{1}{(a-1)} \sum \frac{(x-y)^2}{(x+y)/2} \quad (1)$$

where  $x$  and  $y$  are the observed (sample) frequency of one allele in the first ( $x$ ) and second ( $y$ ) sample, respectively, and the summation is over all  $a$  alleles at the locus (here,  $a = 2$ ).  $F_k$  was averaged over the 20 000 replicate computer runs, corrected for sampling bias, and used to estimate the effective population sizes as described in the legend to Table D.2. We addressed different sampling strategies for estimating effective size, viz. sampling of a single age class (1) in consecutive seasons (spring to autumn) or over a year (spring to spring), and sampling at random from both age classes without using any age information. The first approach was recommended by Jorde & Ryman (1995), whereas the latter is more accessible for tiger prawns, as it does not require ageing individual prawns. The effective sizes were estimated as the

inverse of twice the amount of drift (cf. Table D.2), ignoring any effect of overlapping generations.

The effective sizes thus estimated were compared to the true effective size of the modeled population. At present, there is no simple formula to calculate true effective size for this kind of models and we used computer simulations to assess effective size. Running 100 000 replicate computer run of the simulations above, this time skipping the sampling steps, we measured the variance in allele frequencies ( $\text{var}_p$ ) among computer runs and calculated effective sizes for the spring-autumn and spring-spring intervals from the increase in this variance over the interval:

$$N_s(t) = \frac{pq - \text{var}_p(t)}{2[\text{var}_p(t+1) - \text{var}_p(t)]} \quad (2)$$

where  $p$  and  $q$  are the average allele frequencies among replicate runs (practically identical to the initial allele frequency of 0.5). This equation is slightly modified from equation 14 of Felsenstein (1971), and defines the inverse of the effective size as the ratio of the increase in drift variance in the population from time  $t$  to  $t + 1$  (the quantity in the denominator above) to the maximum possible increase (*i.e.*, the amount left until fixation: the expression in the nominator above). An analogous expression for the inbreeding effective size is given by Choy and Weir (1978, p.596), and the latter authors also make explicit reference to the increase per year and equate that with the so-called 'annual' effective size. This annual effective size is also discussed by Hill (1979), although he express it in terms of demographic parameters (variance in reproductive success, etc), rather than in genetic terms. Our modification is to replace year with season and relate this to the 'seasonal' effective size ( $N_s$ ), or the size of an ideal population that have the same amount of drift as the actual population over one season. Because one season is less than a generation, the amount of drift is less and, hence,  $N_s$  is larger than the more generally used effective size *per generation* ( $N_e$ ).

It may be noted in passing that the above equation (2) could in principle be applied directly to estimate effective size in natural populations by substituting  $\text{var}_p$  for the variance among loci. However, the statistical properties of estimates based on this equation would be very poor because  $\text{var}_p$  cannot generally be estimated from natural populations with any precision.

### ***Sensitivity analysis***

In order to check the sensitivity of the conclusions from the computer simulations - that estimation of  $N_e$  can be done in prawns on the basis of discrete-generation theory - we performed additional computer simulations. These were done using our model I (with constant population size and fluctuating spawner proportions) but varying the birth rates, as follows. In the original model (Model Ia), we used a birth rate of  $b=0.9$ . This we changed to 0.8 (Model Ib) and then to 0.6 (Model Ic). In doing so, we obviously reduced the number of young individuals (age class 1: the six months old) and must compensate by allowing a larger fraction of individuals to survive to age 2 (12 months): otherwise the population will go extinct. Thus, changing birth rate has the important side-effect of changing the age composition, by increasing the proportion of older individuals (Table D.1). As before, we sampled a total of 100

individuals (both age classes included in the samples, in the proportion above). True and estimated effective sizes were obtained over 100 000 and 20 000 replicate computer runs, respectively.

## Results

The result of the computer simulations are depicted in Table D.2. With regards to the temporal pattern of genetic change and true effective sizes the most obvious characteristics of the models are the seasonal fluctuations in effective size and the asynchrony between the two models. In model I, with seasonally fluctuating proportions of spawners, the effective population size follows roughly the number of spawners each season with a pronounced peak in spring and low size in autumn. In the second model, which fluctuate seasonally in numbers and age structure, the pattern is reversed and effective size is greatest in autumn when the number of spawners is least. This apparent paradox arises in model II because spring breeding leaves a relatively small number of offspring, as compared to autumn breeding, and resulting in more genetic drift and smaller effective size during spring. In model I, on the other hand, the numbers of offspring are the same for both seasons and differences in drift and effective size among seasons are a consequence of the bottleneck in the number of spawners participating in autumn breeding. Both models have similar effective sizes when monitored over a full year (two seasons), averaging out the seasonal differences.

### *Sampling and estimation of effective size*

Generally, samples drawn from a single age class (here, age class 1) show slightly larger temporal shifts ( $F_k'$ ) than do samples drawn from the total population (*i.e.*, age class 1 and 2 combined). This effect is especially pronounced in model II during the autumn to spring transition, when the temporal allele frequency shift is six times higher for age class 1 than for the total population. This age-class effect is less pronounced in model I and for samples drawn one year (two seasons) apart. A larger temporal shift for single age classes is expected in species with overlapping generations because each age class is produced by a limited set of parents and there is incomplete mixing of genes among age classes (Jorde & Ryman 1995).

The consequences of the seasonally fluctuating demographic parameters is - not unexpected - that the amount of drift and the estimated effective population sizes fluctuate from seasons to season, as do the true effective sizes. For samples from the total population the estimates closely matches the fluctuations in the true effective size, never deviating more than 10% from the true value in either model (cf. Table D.2). The reasonably good fit of the estimated effective sizes to the true value is despite that we have made no attempt to 'correct' the estimates for bias. A certain amount of bias is expected because we are applying an estimator that assumes discrete (non-overlapping) generations to a more complex situation. Nevertheless, the computer simulations show that such bias is small for tiger prawn populations as long as samples are drawn at random from the total population. Sampling a single age class, on the other hand, may result in a biased estimate (cf. Table D.2).

Measuring drift and effective size over one full year we include two breeding seasons and there is thus more drift than in each season. This results in an (true) effective size per year that is smaller than the effective sizes for each season separately. However,

one year includes more than one generation, and when comparing effective sizes among populations and species we should ideally consider the effective size per generation ( $N_e$ ) rather than the annual size. Hill (1979) has shown that, for populations with overlapping generations, the effective size per generation is simply the annual effective size,  $N_a$ , divided by the average generation interval ( $G$ ):  $N_e = N_a/G$ . The average generation interval is defined as the average age of parents, or  $G = \sum p_i \cdot i$  (e.g. Felsenstein 1971), where  $p_i$  is the proportion of offspring whose parents are of age  $i$ . Here, age is measured in breeding seasons and there is just two age classes. For model I, the proportions are  $450/495 = 0.909$  and  $45/495 = 0.091$ , for age classes 1 and 2, respectively, yielding a generation length of  $G = 0.909 \cdot 1 + 0.091 \cdot 2 = 1.091$  seasons, or 6.55 months. Thus the average effective size per generation is  $N_e = 91 \cdot 12 / 6.55 = 167$ , and represents the harmonic mean over time. For model II, the corresponding proportions differ among seasons. For offspring produced during the spring season,  $472/477 = 0.99$  have parents of age 1, whereas in autumn this proportion is only  $48/95 = 0.5$ . More offspring are born during autumn, however, and the weighted proportion of offspring born during a year that have parents of age 1,  $p_1 = (0.99 \cdot 48 + 0.5 \cdot 472) / (48 + 472) = 0.55$ . Similar,  $p_2 = 0.45$  and the generation length for this model is  $G = 0.55 \cdot 1 + 0.45 \cdot 2 = 1.45$  seasons, or 8.7 months, which is substantially longer than for model I. The effective size per generation,  $N_e$ , in model II is therefore  $82 \cdot 12 / 8.7 = 113$ .

As noted before (Methods) changing the birth rates in order to assess the sensitivity of the outcomes has the important side effect of changing the age composition of the population: the proportion of old individuals increases as the birth rate is lowered. There is little point in simulating *higher* birth rates because the proportion of old individuals is already very low in the original model. With a higher proportion of older individuals both estimates of  $N_s$  as well as true effective size change relative to the original model (Table D.3). The true effective size per season is seen to fluctuate less than before (compare spring-autumn with autumn-spring), and flattens out completely in model Ic, even though the spawner proportion fluctuates as before (alternating between 0.9 to 0.2). This is probably a direct consequence of the persistence of older individuals in the population, which should dampen temporal allele frequency fluctuations. Also, the effective size over a full year increases somewhat, probably as a consequence of increased generation lengths.

The change in true effective size is not very well reflected in estimates of effective size, however. When basing such estimates on the younger age-class alone, gross underestimation of effective size may result if the effect of overlapping generations is not taken into account. Estimates based on samples from the total population (both age classes included) fare somewhat better and are not wildly off. However, such estimates are seen to differ by a factor of two or more if a large fraction (here: 40%) of individuals survive to enter a second season of reproduction (model Ic).

## Discussion

We have found that overlapping generations and seasonal fluctuations in spawning peaks between spring and autumn has little effect upon effective population size estimates if such estimates are based on random samples from the total population (all age groups included). The presence of a certain amount of overlap among generations

in tiger prawns cause estimates of effective population size by the temporal method to be underestimated by less than 10%. No doubt this low figure stems from the rather restricted overlap in generations in tiger prawns, as only a small fraction of individuals live beyond one spawning season. The results imply that estimates of effective population size can be made fairly accurately under the discrete population model and that the temporal method can be applied in a straight-forward manner to tiger prawns. The computer simulations further show that the least biased results are expected if we take samples from all (both) age classes combined, rather than from just one single age class. Care should then be taken to ensure a representative proportion of both age classes in the samples to avoid erroneous estimates. For instance, as a 'worst-case' scenario, if both samples are from the same cohort, which is possible even when the samples are drawn 6 months apart, there will be no event of genetic drift between samplings. In such situations the estimated effective size has no biological meaning (since there is no genetic drift), and will most likely be infinite. On the other hand, samples for genetic analysis need not be of spawning individuals if non-spawning samples can be shown to be equally representative.

The above discussion is in apparent contrast to the findings of Jorde & Ryman (1995), who recommended that samples should be stratified into age classes and estimation of temporal change and effective size be made from comparisons of consecutive year classes. This approach is useful in several situations that do not seem to apply to tiger prawns. Such situations include broad overlap in generations and when representative sampling from the population is impractical or impossible, say, when different age classes live in different habitats possibly in different areas. Also, the generally larger temporal allele frequency shift for single age classes are easier to estimate, for statistical reasons, than are the smaller shifts characterizing samples from the total population. This phenomenon can be utilized in practice to get more accurate estimates of genetic drift and effective size. This requires age information on all sampled individuals as well as an evaluation of the relationship between such shift and the effective size (see Jorde & Ryman 1995). For tiger prawns, the benefit of such an approach appears limited, however, because the temporal allele frequency shifts ( $F_k$ ) among samples from single age classes are only marginally larger than for samples from both age classes combined, except for model II (cf. Table D.2). The difference among models in this respect, and our present lack of demographic data to conclude which model is the most realistic one for tiger prawns, dictate that care should be exercised when interpreting genetic data from single age classes. This care should be extended to estimates of seasonal effective sizes ( $N_s$ ) even if samples are from the total population, as different demographic aspects of the species population biology may dominate such estimates (cf. the difference in seasonal effective sizes between the two model populations). To put it another way, temporal genetic data from tiger prawns clearly have the potential to uncover very interesting demographic properties of these organisms in addition to provide estimates of effective population size.

*Precision and sensitivity:* The above computer simulations and discussion were directed towards addressing potential problems and bias associated with the temporal method when applied to tiger prawn populations. As such, no consideration has been made to statistical precision. However, the finding that discrete-generation methodology and theory apply at least approximately implies that published theoretical and numerical evaluations of the temporal method and its statistical properties

(Waples, 1989a, 1989b; Richards & Leberg 1996; Luikart *et al.* 1998; Jorde *et al.*, 1999) are useful for tiger prawns too. These studies have shown that the expected precision in the estimated effective population size is a function of the true (but unknown) effective size, sample sizes, sample interval(s), and number of genes screened. Briefly, for large populations increasing sample sizes is often more effective than increasing the number of loci. However, a reasonable large number of polymorphic loci must nevertheless be screened in order to obtain useful estimates of effective size because each allele is just one incarnation of a stochastic process. No exact numbers can be given at present since we have little or no idea how large the effective size of prawn populations are: estimates from other organisms range from about half the actual number of individuals down to only a millionth (reviewed by Frankham 1995).

Sensitivity analysis shows that our finding that discrete-generation theory applies approximately to tiger prawns is valid to the extent that only a small fraction, say, 25% or less, of individuals live to participate in a second season of reproduction. Beyond that, estimates of effective size becomes progressively more biased. These findings are consistent with those of Jorde & Ryman (1995). Further, because it may be difficult to develop adequate corrections for overlapping populations for tiger prawns as we do not have detailed demographic data, and demographic parameters appear to fluctuate between seasons, estimates of effective size using the temporal method (and probably other methods as well) should be based on random samples of individuals from the entire population, and include both age classes.

*Application to other species and populations:* This study models the spawning characteristics of grooved, not brown tiger prawns. Gulf of Carpentaria brown tiger prawn spawning cycle differs from the grooved by having a less pronounced autumn spawning peak. Consequently, it seems unlikely that spawning in this species occurs on a six-month cycle or that it has overlapping generations. Accurate estimates of effective population size could be made with temporal samples taken at 12-month intervals from the spring spawners in the same way as the methodology is being currently applied to the brown tiger prawn population in Moreton Bay.

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**Table D.1: Range of birth rates used in sensitivity analysis for Model I and the corresponding values of survival and proportion of older individuals.**

	Model Ia (original)	Model Ib	Model Ic
Birth rates (b):	0.90909	0.8	0.6
Survival (lx):	0.1	0.25	0.6667
Proportion age2:	9.1%	20%	40%

**Table D.2: Result of computer simulation of genetic drift and estimates of effective population sizes in two tiger prawn models. Effective size is estimated from temporal shifts in age class 1 alone (1) and for samples from the total population (All), ignoring any effect of overlapping generations, using the estimator  $N_s = 1/(2F_k')$ . Both sampling and measurements of true effective size were done just before reproduction in the indicated seasons.**

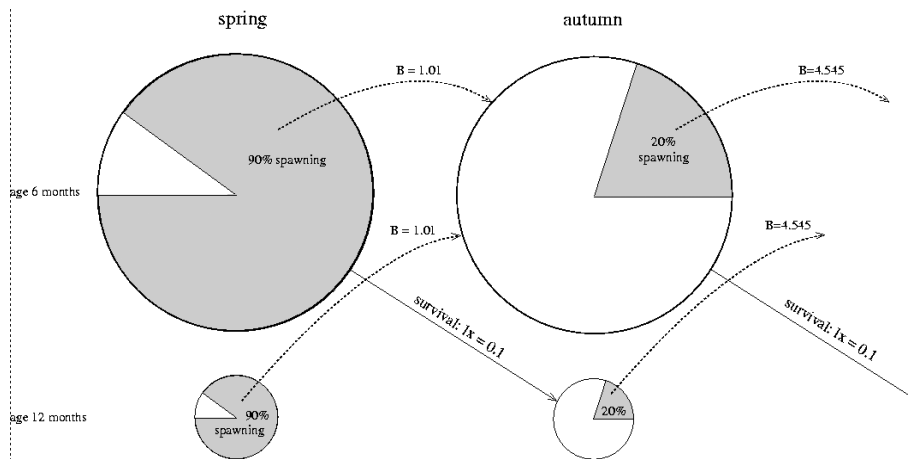
		Estimated genetic drift and effective size (interval)					
		Spring-autumn (t=51-52)		Autumn-spring (t=52-53)		Spring-spring (t=51-53)	
	Sample sizes	$F_k'$	$N_s$	$F_k'$	$N_s$	$F_k'$	$N_s$
Model I:							
1	90 + 90	0.0012148	412	0.0050744	99	0.0062982	79
All	99 + 99	0.0010139	493	0.0041851	119	0.0054161	92
True effective size			454		113		91
Number of spawners			495		99		
Model II:							
1	99 + 48	0.0103997	48	0.0060791	82	0.0059497	84
All	100 + 95	0.0049606	101	0.0010151	493	0.0059252	84
True effective size			97		508		82
Number of spawners			477		95		

The  $F_k'$  values in the table are the average of the 20 000 replicate  $F_k$  -values calculated from equation (1), and corrected for sampling according to scheme 2 of Nei and Tajima (1981) and Waples (1989a):  $F_k' = F_k - 1/2n_x - 1/2n_y + 1/2N_x + 1/2N_y$ , where  $n_x$  is the sample size (number of diploid individuals) in season 'x' (spring or autumn) and  $N$  is the number of individuals in the population from which the sample were drawn, *i.e.* the number in age class 1 or the total population at the time when the sample was drawn. (Note that in actual application of the temporal method to tiger prawn populations,  $N$  will be so large that its inverse can be safely ignored when estimating effective size.)

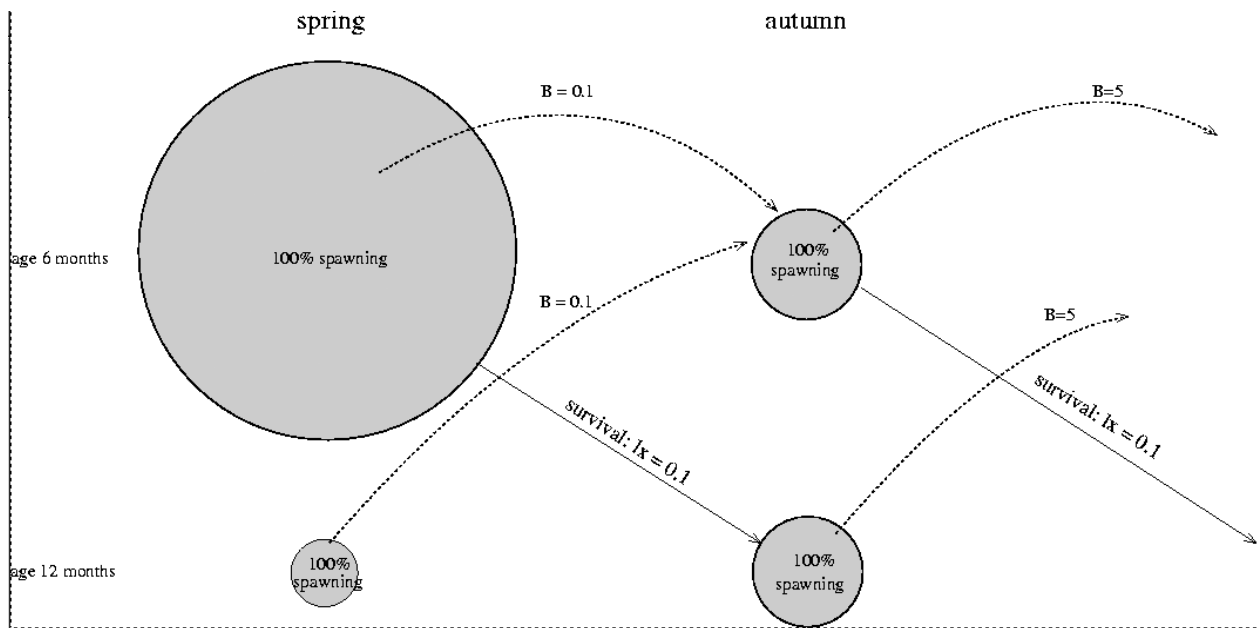
**Table D.3: The estimated amount of genetic drift (Fk') and effective size (Ns) over one or two six-monthly intervals.**

	Spring-autumn		Autumn-Spring		Spring-spring	
Age (No. samples) (t=51-52)			(t=52-53)		(t=51-53)	
	Fk'	Ns	Fk'	Ns	Fk'	Ns
<i>Model Ia (b=0.9)<sup>1</sup></i>						
1 ( 90+ 90)	0.0012148	412	0.0050744	99	0.0062982	79
All ( 99+ 99)	0.0010139	493	0.0041851	119	0.0054161	92
True effective size <sup>2</sup>		454		113		91
<i>Model Ib (b=0.8)</i>						
1 ( 80+ 80)	0.0018271	274	0.0055822	90	0.0066688	75
All (100+100)	0.000939	532	0.0035537	141	0.0047013	106
True effective size		386		142		104
<i>Model Ic (b=0.6)</i>						
1 ( 60+ 60)	0.0027505	182	0.0066857	75	0.0070343	71
All (100+100)	0.0009726	514	0.0024082	208	0.0032956	152
True effective size		239		261		125

<sup>1</sup> Model Ia is Model I from Table D.2.<sup>2</sup> As before, the true effective size relates to drift (increase of allele frequency variance among 100,000 replicate runs) over the corresponding time period – one season (six months) or one full year.



**Figure D.1: Schematic representation of tiger prawn model I. The proportion of spawner (indicated with grey) differs between seasons, from 0.9 in spring to 0.2 in autumn, as do the average number of offspring per spawner ( $B$ ). The population size remains constant.**



**Figure D.2: Tiger prawn model II. All individuals participate in spawning but the birth rate differs between seasons, yielding a larger spawning population in spring.**

## **Appendix E – Intellectual property**

No intellectual property was generated during this project.



## **Appendix F – Beneficiaries statements**

### **Review by Steve Montgomery**

My comments on this project are qualified by the fact that I am not an expert in the field of genetics. As such, I will wait to see the results published in external, peer reviewed journals as a guide to the validity of the methodology used in the project.

Notwithstanding the above, I found the project extremely interesting. The technology developed in this project is helping to break new ground in understanding spawner-recruit relationships. Concepts such as “source-sink” dynamics recognise that not all spawning contributes to what is known as the fishable population. Much of the recruitment is lost to the defined fishable population through the interaction of environmental factors such as currents, water temperature and the availability of space and food. The results of this project contribute greatly to this concept. These are some of the first to demonstrate the difference between the number of animals spawning and the number whose offspring actually contribute to the fishable population.

I would like to encourage the authors to investigate the use of gene technology for the purposes of estimating rates of mortality and growth, and levels of exploitation in particularly short-lived invertebrate species. Stock assessments of particularly short-lived invertebrates are lacking in methods to estimate rates of mortality and growth. Gene technology offers great opportunities in this regard.

Dr Steve Montgomery  
Senior Research Scientist  
NSW Department of Primary Industries

### **Review by Simon Hoyle**

This review is from the perspective of a stock assessment scientist, not an expert in the genetic techniques being used. It is not an independent review since I had some involvement in the research while working at the Southern Fisheries Centre: I was investigating how to take the techniques developed in this project and apply them to stock assessment – see Appendix D. I am working with Dr's Ovenden and Peel on a manuscript that goes into more detail.

I think this work is very valuable and has a lot of potential for helping stock assessment in the long term. Stock-assessment is generally data-poor and data-hungry, and this new data type has some very useful attributes – fishery independence, single species focus and, potentially, cost. Given the expense of some methods currently used to generate data (e.g. northern prawn trawl surveys), and the potential financial and sustainability benefits for Australian and international fisheries from better information, the potential of this method makes it worth an ongoing investment.

I also think it needs more development work before it can be used in stock assessment. Effective population size is a very different thing from spawning stock size, as is demonstrated by this project, with the very low  $N_e/N$  ratio calculated.

Further development should involve two parallel processes: simulation, and more Ne estimates. Each of these processes should be relatively inexpensive. Simulation is needed to examine just how useful Ne estimates might be, what situations and fisheries they will be useful in, how many samples will be needed, which assumptions must be validated, and what the cost-benefit ratio is likely to be.

Biological data is needed from a time series of Ne estimates for a fishery where recruitment and stock size are quite variable, and existing assessments are accurate and precise. This would let real-life relationships between Ne, spawning stock, recruitment, and environmental conditions be worked out. A fishery where there are good estimates of recruitment (e.g. a fishery for a short-lived prawn where catch is a good measure of recruitment) would also be a good laboratory for working out how Ne fits into the stock assessment picture. However, tighter confidence intervals on Ne estimates than those in the current study are needed to give the investigation the necessary power. More power in each year's estimates will produce answers with a much shorter time series of Ne estimates. In other words, more individual prawns should be sampled, and at more loci. Great care should also be taken to sample representatively across the whole spatial population, but with appropriate use of technology it might be possible to do this via commercial sampling. Samples can be collected and stored for several years before processing them, thus reducing costs.

Migration and population subdivision remain major concerns both for this study and for future work. A longer time series of Ne estimates for this population would be very useful for investigating and solving these problems, as would an intensive simulation study. The trawl-based estimates of spawning stock size have quite wide and very optimistic confidence intervals, and I'm not sure it is worth continuing with them given the cost.

Mr Simon Hoyle  
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