ESTIMATION OF FISHERIES RESOURCE SIZE USING POPULATION GENETICS

By

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2002 Churchill Fellow

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Jenny at Bodega Bay Marine Laboratories, California, USA; August 2002
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Report by

Jennifer Ovenden – 2002 Churchill Fellow

Estimation of fisheries resource size using population genetics.

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Introduction

Globally, fishing catch has reached its ecological limit. This includes Australia, where the harvest of numerous species is approaching sustainable limits.

Non-sustainable exploitation is due, in part, to the difficulty in determining the number of fish in the sea 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 can be 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. And stock assessment modelling is expensive, for example 42% of the $8.5 million spend on the northern tiger prawn fishery in Australia over the last five years went directly to data collection and analysis. Currently, there are few independent methods of validating the outputs of stock assessment modelling, apart from the wait-and-see approach.

The objective of the Winston Churchill Fellowship awarded to Jennifer Ovenden in 2002 was to support the development of an independent alternative to stock assessment modelling of for estimating the size of marine populations. This alternative involves making inferences about spawner numbers from genetic information collected from a sample of individuals from the population.

Genetic data can be used in two ways to estimate population size.

1. Measure genetic drift and convert it to effective population size (the number of spawning breeding individuals in the population that effectively contribute offspring to the next generation), or
2. Measure the rate at which fishermen harvest fish whose genotype (genetic fingerprint) has been previously determined.

These two methods were the subject of Jenny’s travels to Universities and marine research institutions in Canada, United States, Norway and United Kingdom.

Both methods are the subjects of current research under Jenny’s leadership in the Molecular Fisheries Laboratory at the Southern Fisheries Centre, Queensland Department of Primary Industries. Effective population size estimates are being made for the tiger prawn (*Penaeus esculentus*) population in Moreton Bay, Queensland. Harvest rate estimates using genotyping (genetagging) are being made for a northern Spanish mackerel (*Scomberomorus commerson*) population.
Executive Summary

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Project Description: Estimation of fisheries resource size using population genetics.

Jenny Ovenden traveled to Fisheries Research Centres and Universities in Canada, US, Norway and Britain to develop genetic monitoring methods for sustainable management of Australian fisheries resources. Jenny consulted international experts about specific aspects of the methodology including the potential to closely integrate fisheries genetics with stock assessment science and fisheries management. Practical solutions were found to mathematical and statistical challenges involved in the implementation of this elegantly simple, but difficult field. As fisheries geneticist for Queensland Department of Primary Industries, Jenny will apply the knowledge gained during the Churchill Fellowship to research projects on tiger prawns (Penaeus esculentus) and Spanish mackerel (Scomberomorus commerson) and other Australian fisheries resources. Contact with the international scientific community about the use of population genetics to estimate the size of fisheries resources has also given Jenny valuable tools for extending the outcomes and implications of the research into the wider scientific, and general fishing community.
## Programme

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Dr David Paetkau is the president of Wildlife Genetics International Pty. Ltd. Their core business is the use of molecular markers on non-invasively obtained tissue samples, such as hairs and scats, to estimate the population size of terrestrial animals such as bears and pine martens. David is a leader in the field of genetic inventories of populations as a result of his postgraduate and post-doctoral studies and his role in the operation of the company (Woods et al., 1999) (Paetkau, in preparation).

At Wildlife Genetics, numerous tissue samples may be received from the same animal from different geographic locations and at different times. Their genotyping process must identify the samples as being identical, and thus from the same animal. A genotyping error with one or more of the samples, could inflate the apparent number of animals ‘counted’ in the population.

Our planned study with Spanish mackerel (Scomberomorus commerson) will take a maximum of two tissue samples per animal; once when a specially-designed fishing lure (Buckworth et al., In Preparation) takes a single tissue sample non-lethally and at depth from a live fish, and again when the fin of that fish is recovered from commercial fishermen after the fish has been harvested. Like the work of Wildlife Genetics, our study needs to ensure the genotyping process is sufficiently accurate to identify the two samples as being from the same individual.

Another similarity between the work of Wildlife Genetics and ours is the importance of minimizing ‘false recaptures’ or ‘shadows’. These are pairs of animals that have identical genotypes by chance. The occurrence of false recaptures depends on the statistical power of genotyping strategy used, but all strategies have their upper limit especially in studies where large numbers of individuals are genotyped. For Wildlife Genetics, false recaptures can lead to the underestimation of the population size. For our work with marine species, it would lead both to the underestimation of population size as well as the
overestimation of the rate at which fishermen harvest individuals from the population. David recommends the use of empirical data, as well as theoretical and simulation methods, to obtain estimates of the ‘false recapture’ rates (Paetkau, in preparation).

We also talked about ‘bio-fraud’ that has occurred in the US recently (eg. Stokstad, 2002). In this case, anti-conservationists apparently leaked information about blind tests to the media (Washington Post). A blind test is where a sample of a known species is included among unknown samples to test the ability of the lab to correctly identify it. The media wrote it up as if biologists had biased the search for rare species (lynx) to further limit human use of wilderness areas. In another case DNA samples were criminally interfered with, presumably by pro-conservationists, to make it appear that the rare animal was actually present among the samples. Both of these cases, and others, have gone to state courts and in some cases, the US congress. They have affected the public and government credibility of DNA-based surveys of animal abundances. David recommends that we should think about situations where we may have to defend our population estimates legally, how we independently validate our results under those circumstances and issues associated with sample security and duplication.

*Marine genetic effective population size estimates – Robin Waples*

Dr Robin Waples is a fisheries conservation geneticist at the National Marine Fisheries Service in Seattle, Washington. He has researched and published extensively on genetic effective population size estimates, from his initial description of the moments-based method (Waples, 1989) to his recent work with salmonid populations (Waples, 2002b) and other endangered species (Waples, 2002a). Robin works with a team of researchers, including Dr Mike Ford.

The field of genetic estimates of effective population size is mathematically challenging and currently under rapid development. Like Robin says
Robin and I discussed the ‘practical’ definition of effective population size. Non-geneticists, such as fisheries scientists and managers, have difficulty relating to the formal definition of effective population size, which 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 (Hedrick, 2000). Instead I have been using a modified, ‘practical’ definition – the number of spawning individuals that effectively contribute offspring in the next generation. Expressed in this way, there is a logical connection between stock assessment science and effective population size estimates as both methods recognize there is a large proportion of breeding individuals, especially in fisheries species, that develop reproductively and participate in spawning, but which may be unsuccessful. Robin agrees that the ‘practical’ definition is appropriate, while not being strictly accurate in theoretical terms.

There are numerous strategies for empirically measuring effective population size; some requiring temporal genetic data from two or more samples taken from the same population at different times, and others requiring data from only a single population sample. For estimates from temporal data, the “given genetic measure” can be allele frequency variance or the amount of inbreeding. Robin made it clear that allele frequency variance estimates are taken from progeny samples, while inbreeding estimates are taken from samples of individuals that are actually spawning. Robin often uses the latter in his work with spawning salmonid populations, while we are using the former for our estimates in tiger prawn populations (Penaeus esculentus, Ovenden, 2000).

**Genetic effective population size estimates in New Zealand snapper fisheries – Lorenz Hauser**

Dr Lorenz Hauser is Assistant Professor at the School of Aquatic and Fishery Sciences at the University of Washington, Seattle. During his postdoctoral work with Gary Carvalho at the University of Hull in the UK, Lorenz collaborated with Peter Smith and Greg
Adcock and others to make effective population size estimates of a harvested population of snapper (*Pagrus auratus*) in New Zealand (Hauser *et al.*, in press). This is similar to the research that is currently underway in my laboratory at the Southern Fisheries Centre, Deception Bay Qld, in that both projects are comparing the magnitude of the effective genetic estimates to estimates of the actual number of spawners in the population.

One of the characteristics of effective population size estimates is that they are most accurate when the size of the estimate is comparatively small, for example $10^2$ to $10^4$. An estimate of this magnitude is not unexpected for marine species as the majority of them have high fecundity, for example snapper produce 1-5 million eggs per female, and a female prawn can produce 0.5 million eggs. Only a small proportion of these offspring need to survive to maintain a stable population size over subsequent generations.

Estimates of effective size in fisheries management will only be useful to fisheries management if they can be accurately measured, but this seems achievable, as the effective number of spawners is likely to be only a small subset of the total number of spawners in the population. Lorenz’s work with New Zealand snapper measured the ratio between effective and actual spawners and came up with five orders of magnitude difference. This result is similar to ratios estimated by Hedgecock (1993; 1992) on marine invertebrates.

Lorenz’s work was written up recently in the *Age* newspaper in Melbourne. The headline was “Fish’s gene pool threatened: study”. The journalist reported “…..concluded that commercial fishing practices may leave only a few hundred fish to contribute to the next generation’s gene pool……”

**Genetic tagging for population size estimation in marine populations – Per Palsbøll**

Dr Per Palsbøll is Assistant Professor in the Division of Ecosystem Sciences, at the University of California, Berkeley. He is known for his pioneering work with genetic
mark and recapture of humpback whales (Palsboll, 1997) to estimate the population size in the North Atlantic Ocean.

Per and I discussed the one-way nature of genetic tests for the presence of subdivision in marine populations that are necessary before using genetic mark recapture data for population size estimates. They are one-way because if the $F_{ST}$ is significantly larger than zero, then subdivision is likely; however if it is not larger than zero then it is not possible to conclude that subdivision is absent. Per talked about the usefulness of using genetic methods to determine the proportion of parent-offspring pairs in the population (Palsboll, 1999). If a section of the population is not subdivided from the majority of the population then it will have the same, possibly low, proportion of parent-offspring pairs. However, in the presence of genetic subdivision that section of the population may have a higher proportion of parent-offspring pairs. Per is actively working on this concept through computer modeling and simulation.

Exchanging microsatellites for single nucleotide polymorphisms (SNP) as the genetic marker of choice in gene-tagging studies was another topic that Per and I talked about. With suitable equipment, SNP may potentially be easier and faster to score per individual than microsatellite genotypes. In the human genome SNP appear to occur on average once every 1500 nucleotide pairs. For each genetagging project on a new species, a genomic library would need to be sequenced to locate the ten to 15 SNP’s that would be needed to give enough discrimination among individuals. To facilitate the identification of SNP, the genomic library of the DNA of two animals could be sequenced and SNP should appear as ‘double’ or overlapping peaks in the sequencing chromatograms. Sequence specific PCR primers could target the SNP where the SNP was located at the 3’ end of the primer. Even though scoring SNP may be simpler in a genetagging study, it is likely that the development time for SNP and microsatellite loci may be about equal.
Software for the calculation of effective population size – Eric Anderson

Dr Eric Anderson is a postdoctoral fellow in the Department of Integrative Biology at the University of California, Berkeley. He completed his Ph.D. at the University of Washington under the supervision of Ellen Williamson and Robin Waples.

Eric’s monte-carlo maximum likelihood method for calculating temporal effective population size (MCLEEPS, Anderson et al., 2000) has been incorporated into a beta version our software (Nestimator, Peel, 2002). I explained that the rationale for our development of software was to make a range of techniques readily available to researchers and to simplify data input formats. I formally requested that Eric and his collaborators allow us to use his software in this way, and gave Eric a PC-compatible beta version of our software to trial. Eric suggested that we should consider the inclusion of a pseudo-likelihood method that has recently become available (Wang, 2001).

I discussed with Eric the apparent conundrum in the literature (eg. Heath et al., 2002) concerning populations that have been estimated to have small variance effective population sizes. Population genetic theory predicts that these populations will rapidly experience the fixation or loss of alleles that may reduce their ability to successfully meet environmental perturbations. Eric worked on this problem in during his Ph.D. studies and showed that under non-Fisher-Wright population models the predicted rate of allele loss or fixation could be slower. How much slower is impossible to model as it probably depends on factors that have yet to be discovered.

Affect of allele frequency distribution on estimates of effective population size - Vanessa Rashbrook & Dmitri Churikov

Vanessa Rashbrook is a Staff Research Associate at the Bodega Marine Laboratory that is part of the University of California, Davis. Vanessa works with Dennis Hedgecock and collectively have published numerous papers dealing with the estimation of effective population size in marine populations (Hedgecock et al., 1992, 2000 #3083). Dmitri
Churikov is a postgraduate student in their lab and is working with the estimation of effective population size in wild Chinook salmon populations.

Following the presentation of my seminar describing our research on tiger prawns (*Penaeus esculentus*) in Moreton Bay, Queensland we talked about the affect that allele frequency distribution and allele numbers had on the statistical power of estimates of effective population size. I said that our computer simulations apparently showed an increase in power with increasing number of alleles, but we hadn’t yet investigated the affect of differing frequency distributions. Dmitri said that his work has showed the opposite, and that he had trialled differing pooling strategies to both reduce the allele number and equalize allele frequencies. Dmitri recommended a search of the literature beginning with Tajima (1992) and other papers by this author.

**Harvesting effects on the ratio between effective and census spawning population size - Russ Vetter**

Dr Russ Vetter is the Leader of the Genetics and Physiology Program of the Southwest Fisheries Science Centre. The National Marine Fisheries Service operates the Centre, which is part of the National Oceanic and Atmospheric Administration of the US Department of Commerce. Russ supervises research on the genetic population structure of north-east Pacific species such as rockfish (Sebastes spp. 1999; Rocha-Oliveras *et al.*, 1999), anchovy and sardines (Gaggiotti and Vetter, 1999). The laboratory is also developing single nucleotide polymorphism (SNP) approaches to identify larval fish that have been collected along the Californian coast as indicators of spawning stock size and location since the 1950’s (Moser *et al.*, 2001).

Russ and I talked of the effects of fisheries harvesting on the ratio between effective and census spawning population size. There are at least three ways in which harvesting can affect the ratio.

1. **Non-random harvesting with respect to genotype.** This may occur when the genetic markers are linked to phenotypic traits that were selected for during
harvesting. For example, families of prawns that had a small body size would be under-represented in trawl-net harvests in a series of temporal samples. This would decrease the allele frequency variance of samples taken by trawling compared to a random sample and lead to an over-estimation of effective population size.

2. **Removal of “banked” genetic diversity.** In a long-lived species that experiences patchy recruitment in successive years, the adults in the population are a “bank” of genetic diversity (storage effect Gaggiotti and Vetter, 1999). Many commercial Californian fish and invertebrate species are long-lived (eg. abalone, white seabass and black groper) and experience patchy recruitment related to El Nino oscillations. Recruitment failure year after year does not necessarily decrease population genetic diversity, as the long-lived adults continue to spawn for many years.

Harvesting modifies the adult “bank” of genetic diversity by reducing the mean length of individuals (and consequently their fecundity) and density of the population. Compared to a population unaffected by harvesting, the harvested population will experience more genetic drift because spawner numbers are less and they are being more frequently replaced in the population. Thus in long-lived species subject to patchy recruitment, harvesting may increase genetic drift which reduces the effective population size of a population.

In short-lived species such as tiger prawns, harvesting is unlikely to reduce effective population size unless

   a. The proportion harvested exceeds the effective proportion (for example, where the harvesting takes 99% of the population, and the effective population size is 5%).

   b. As above, but with subsequent recruitment failure associated with environment fluctuations.
Under both these conditions, the genetic effective population size estimate will relate to the proportion of surviving spawners that contribute recruits to the next generation.

3. **Census spawning population size may under-represent the overall spawning population size due to the harvesting of spawning individuals during the spawning period.** When calculating the ratio between the number of effective spawners compared to the total number of spawners, what is the appropriate measure of total number of spawners? The alternatives are

   a. The number of individuals in spawning condition immediately prior to spawning as determined by a random census. This is the strategy being adopted by our current research project on Moreton Bay tiger prawns.

   b. The number of individuals taken by fishermen from the population during the spawning period. This may not be possible in Moreton Bay due to the low catches of tiger prawns by the fishermen, but may be possible in other tiger prawn populations, such as in the Gulf of Carpentaria.

   c. The number of individuals taken by the fishermen from the population during the entire fishing period, perhaps adjusted for natural mortality (ie. the probability that they would have survived until spawning time) and density dependent affects on catch rates. This could be done for the tiger prawn catch in Moreton Bay, and appears to be the strategy used for red drum in the Gulf of Mexico (Turner *et al.*, 1999) and snapper in New Zealand (Hauser *et al.*, in press).

Robin Waples warned that if estimates of Ne/Na ratios are going to be made across several generations, then both the mean Ne and Na estimates need to be harmonic, not arithmetic.
Application of effective size methodology to a fish species with genetic population structure - Tom Turner

Tom Turner is Associate Professor of Biology at the University of New Mexico, Albuquerque. He teaches undergraduate and graduate students, supervises a research laboratory and is the head of the Museum of Southwestern Biology. Tom has published estimates of effective population size in a harvested marine species (red drum Turner et al., 1999). More recently, he collaborated with statistician colleagues to show that the bias associated with making estimates with highly polymorphic loci outweighs the increase in statistical power and precision (Turner et al., 2001).

Wright (1969) proposed the elegant concept of effective population size under a set of idealized conditions that included panmixia (equal probability of mating among all pairs of males and females), equal sex ratios (1:1), non-overlapping generations, Poisson variance in reproductive success and temporally stable population size. The population of tiger prawns in Moreton Bay was specifically selected as a ‘model’ for our effective size research as it conforms to many of these assumptions.

The goal of our effective population size research is to contribute to resource sustainability in the Gulf of Carpentaria by improving the assessment of the numbers of spawning tiger prawns. The fisheries harvest of this species is worth about $120 million annually to the Australian economy. However, the successful application of the method in the Gulf will depend on the sensitivity of the method to violations of Wright’s (Wright, 1969) assumptions, particularly panmixia and non-overlapping generations.

The Gulf of Carpentaria population of tiger prawns is dispersed among three spatial regions that are defined as ‘stocks’ for the purposes of current stock assessment modeling (Condie et al., 1999). Consequently, the population may contravene Wright’s (Wright, 1969) assumption of panmixia. Unlike tiger prawns in Moreton Bay, the Gulf population appears to have two annual spawning peaks (Ovenden, 2000), suggesting that generations may overlap, which is also contrary to assumptions of the methodology.
Tom Turner’s recent work in estimating temporal effective population size of red drum (*Scianenops ocellatus*) in the Gulf of Mexico investigates the sensitivity of the methodology to lack of panmixia and where generations are not discrete. Violations of other assumptions of the model, such as non-Poisson variance in reproductive success and temporally unstable population sizes, are not dealt with here (but see Table 3 in Turner *et al.*, in press Sept 02)).

Red drum have a life history that is similar to tiger prawns. The fish occurs in coastal and near-shore waters of the northern Gulf of Mexico and the western Atlantic Ocean. Successful recruitment to adult breeding populations depends on bays and estuaries that serve as nursery grounds for larvae and juveniles. Tiger prawns rely on recruitment to inshore sea-grass beds for survival to adults (Rothlisberg *et al.*, 1996). Sexually mature red drum form large, migrating schools offshore, but adults return to inlets adjacent to bays and estuaries for spawning. As for prawns, fertilization is external and individual fecundity is high (3 x 10\(^7\) eggs per 9-14 kg female red drum).

Census population size (N) of red drum in the Gulf of Mexico was estimated by Tom Turner and colleagues to be about 3.4 x 10\(^6\). Census population size of tiger prawns at spawning time in Moreton Bay has been estimated as 200-300,000 and further 2-3 x 10\(^6\) (80 tonnes) are taken from the embayment by fishers throughout the year. In comparison, the Gulf of Carpentaria tiger prawn population may be one order of magnitude larger; 2,000 tonnes of both tiger prawn species (*P. esculentus* and *P. semiculcatus*) are harvested annually. The proportion of the target species (*P. esculentus*) in this harvest is currently unknown.

The genetic structure of red drum populations in the northern Gulf of Mexico conforms to a linear stepping-stone model where the probability of gene exchange between subpopulations in adjacent bays and estuaries decreases as a function of genetic distance. Population structure in tiger prawns in Gulf of Carpentaria is unknown, but likely to be similar to red drum. The presence of undetected genetic structure, or genetic structure where samples from sub-populations are pooled is likely to decrease effective population
size as a portion of the measured allele frequency variance will be due to population structure rather than genetic drift.

Red drum have a large degree of generation overlap. Longevity is known to be 60 years or more, but individuals over 30 years old are relatively rare. Tiger prawns in the Gulf are much more short-lived; most likely 10-12 months, with two annual peaks of spawning. Compared to discrete generations, the presence of overlapping generations is likely to decrease effective population size (Jorde and Ryman, 1995).

Tom used Nunney’s equation 15 (Nunney, 1999) to estimate N/Ne for his red drum genetic data that was pooled across Gulf of Mexico sub-populations (N is the census population size and Ne is the inbreeding effective size). Using empirical F- or R-statistics that describe the degree of inbreeding within a deme (or subpopulation, F\text{IS}) and non-random mating among demes (F\text{ST}), he found that the N/Ne ratio was 0.91 (R) to 0.96 (F). This compares to an observed ratio of 0.001 from temporal estimates of Ne and empirical census data. Tom concluded that hierarchical population structure alone was insufficient to explain the low observed ratio where Ne was three orders of magnitude below N (census population size).

Tom further argues that very large variances in reproductive success (far in excess of Poisson expectation) are unlikely to fully explain the small size of the Ne/N ratio. Allele frequency shifts caused by immigration of new alleles into the study area are also unlikely to contribute as their effect is likely to be small. He concludes that variance in productivity (recruitment success) may explain the low Ne/N ratio. Tom’s demonstration that deme-wide temporal estimates of Ne can be corrected for the presence of population structure is critically important to the expansion of our work into the Gulf of Carpentaria where we expect that populations will be sub-structured as it shows the affect of population structure on Ne/N ratios can be independently assessed and accounted for. The variance in productivity hypothesis may become relevant to our work with prawns where the extent and condition of sea-grass is likely to be a critical factor in recruitment,
Tom used the method developed by Jorde and Ryman (Jorde and Ryman, 1995) to correct Ne estimates for overlapping generations. The critical fishery data variables that Tom used were average life span, standardized variance of life span and generation time. These were calculated from age-specific survivorship where survivorship was estimated from a tag-recapture study of pre-reproductive fish and from the age-structure data of sexually mature fish. Tom assumed an equal probability of surviving from one year class to the next, and equal probability of survival of males and females. Birth rate was estimated by calculating the mean (wet) weight of gonads, as an indicator of relative gamete contribution at each age class. This gives us an idea of the type of fishery data that is likely to be needed to correct temporal Ne estimates in the Gulf of Carpentaria population of tiger prawns.

Extension of fisheries genetics research - Anne Kapuscinski

Anne Kapuscinski is a Professor in the Department of Fisheries, Wildlife and Conservation Biology at the University of Minnesota, St Paul campus. Anne is also an Aquaculture and Biotechnology Extension Specialist. In this capacity she is the Director of the Institute for Social, Economic and Ecological Sustainability (ISEES).

Extension of fisheries genetics research into the community is important for the uptake of the outputs of the research and for the coupling of research objectives with community needs. Analogies are useful to researchers in order to explain concepts to audiences who vary in educational and life experiences.

Anne and I discussed the difficulty of developing an analogy for the concept of effective population size. Anne suggested using the “wind-chill factor”. For example, winter temperatures are reported by the weather bureau, but the actual experience of that temperature is affected by the strength of the wind. A strong wind can effectively drive the temperature lower. Likewise, the number of spawning individuals in a fishery can be counted using ecological census techniques. But the number of successful spawning individuals (that contribute to the next generation) is less than the ecological census number. It is driven down by the slim chance that one of millions of offspring per adult
survives the cruel “wind” of food and other resource unavailability in a patchy and unreliable marine environment.

Recent work by Anne and a postgraduate student (William Ardren, Ardren and Kapuscinski, in press) is relevant to the affects of harvesting on effective population size (Ne) and the Ne/N (N is census population size) ratio. Following discussions with Russ Vetter (above), I wrote

In species, such as the Moreton Bay population of tiger prawns that do not have over-lapping generations, harvesting is unlikely to reduce Ne unless

a. The proportion harvested exceeds the effective proportion (ie. If harvesting takes 99% of the population, and the effective population size is 5%).

b. As above, but with subsequent recruitment failure associated with environment fluctuations.

Anne and her student studied a population of steelhead trout (Oncorhynchus mykiss) in Washington State in the northwest of the US. Using temporal estimates of Ne and census estimates of spawning individuals (N) they found that the mean Ne/N ratio over 17 years was 0.73, except that when N was low when the ratio increased. This implied that Ne did not decrease as N decreased. They concluded that a reduction in variance associated with reproductive success was responsible for this compensatory affect. A smaller number of spawning individuals may have caused a relative increase in their spawning success because of increased access to some limiting spawning resource; in this case most likely gravel beds in the stream where the fish deposit their fertilized eggs (redds).

I would not expect “genetic compensation” to occur with the Moreton Bay population of tiger prawns, however. The ability of post-larvae to gain access to seagrass beds is the most obvious factor limiting recruitment in this species, assuming that the capacity of seagrass beds to host post-larval development is not limiting. A reduction in the census size due to harvesting or other affects is unlikely to increase the proportion of the post-larvae population that are able to gain access to seagrass.
Effective population size estimates in populations with overlapping generations - Per Erik Jorde

Per Erik Jorde is a researcher based at the Norwegian Institute of Marine Research, Flødevigine Marine Research Station in Ardenal on the coast of southern Norway. He has close links to the Division of Zoology at the University of Oslo. Per Erik has published a method for correcting effective population size estimates when generations overlap (Jorde and Ryman, 1995) in addition to empirical studies of brown trout populations (Laikre et al., 1998; Palm et al., in press).

The goal of my effective population size research in Australia is to ultimately apply the methodology to populations of brown (P. esculentus) and grooved (P. semisulcatus) tiger prawns in the Gulf of Carpentaria in northern Australia. The fisheries harvest of these species is worth about $120 million annually to the Australian economy. However, the successful application of the method in the Gulf will depend, in part, on the sensitivity of the method to violations of Wright’s (Wright, 1969) assumptions, particularly panmixia and non-overlapping generations. Sensitivity to lack of panmixia was discussed with Tom Turner (above). My work with Per Erik Jorde addresses sensitivity to overlapping generations that occurs in grooved, and to a lesser extent in brown tiger prawns in the Gulf of Carpentaria.

Crocos studied the spawning patterns of grooved (P. semisulcatus, Crocos, 1987a) and brown (P. esculentus, Crocos, 1987b) tiger prawns in the north-west of the Gulf of Carpentaria adjacent to Groote Eylandt. For grooved tigers, Crocos 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). For brown tiger prawns egg production was more spread throughout the year, with eggs being produced most consistently in late winter and early spring.

Temporal estimates of effective population size are made from genotype data from a random sample of the population separated in time by a known temporal interval. The
temporal interval can be less than one generation, where a generation is the average age of spawning individuals. For example, samples taken from the spawning population of grooved tiger prawns six months apart, in spring (Aug – Oct) and autumn (Feb), would be taken less than one generation apart, as a proportion of the spawning prawns are more than six months old. This sampling strategy contravenes the assumption of non-overlapping generations.

When generations overlap, estimates of standardized allele frequency variance \( (F, \text{ genetic drift}) \) among single cohort samples will be biased upward compared to non-overlapping generations. The upward bias occurs because several (rather than just one) cohorts or year classes are contributing to allele frequency variance between the temporal samples. Estimates of effective population size made from these \( F \)’s will be biased downwards.

In deterministic models developed by Per Erik and myself at Flødevigne, overlapping generations and seasonal fluctuations in spawning peaks between spring and autumn had little effect (≈10%) upon effective population size estimates in grooved tiger prawns (Ovenden et al., in preparation). The addition of fluctuating spawning peaks between spring and autumn reduced this bias further, for reasons that are unclear at present. These results imply that estimates of effective population size in grooved tigers could be made relatively accurately under the discrete population model.

Gulf of Carpentaria brown tiger prawn spawning cycle differs from grooved tigers 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.
**Coalescent estimates of effective population size - Mark Beaumont**

Mark Beaumont is a Research Fellow at the School of Animal at the University of Reading, UK. His specialty is theoretical and statistical population genetics. He was a co-author on a recent paper that reported estimation methods for effective population size from temporal data using a genealogical, or coalescent, approach with Bayesian statistics (Berthier et al., 2002). Mark kindly gave me permission to use the computer program that he wrote for the implementation of this method in our project in Australia. Introductions to the concept of coalescence in population genetics include Nordberg (2001) and Stephens (2001).

We contrasted the pseudo-maximum-likelihood method of Wang (Wang, 2001) with Mark’s coalescent approach. Eric Anderson (Berkeley, see above) had previously recommended Wang’s method as a computationally faster alternative to his multi-allelic maximum likelihood method (Anderson et al., 2000). Wang takes a non-coalescent approach that assumes a Fisher-Wright population model with the common set of assumptions (closed populations, panmixia, equal sex ratios, non-overlapping generations, etc.). It also converts data from multi-allelic loci into bi-allelic system that greatly simplifies the production of a probability matrix. Wang’s system is similar to this; for a multi-allelic locus with three alleles (a, b and c) the bi-allelic equivalent would be a and b+c, b and a+c and c with a+b. Only the significantly large near-diagonal elements of the probability matrix are used in the maximum likelihood computations. In Mark’s opinion, the estimates from both methods are similar in magnitude, but the pseudo method of Wang gives tighter confidence limits than the true confidence limits given by the full maximum likelihood.

Mark has developed a population-based model to estimate population growth or decline in genetically monitored populations (Beaumont, in press). This method may be an alternative to repeated pair-wise statistical testing to successive estimates of effective population size in prawn populations through time. As well as avoiding bonferroni
adjustments to the level of significance in repeated statistical tests (Ryman and Jorde, 2001); (Rice, 1989), it circumvents the need for the setting of alpha levels.

Mark is also the senior author on Bayesian approaches to population genetics that may pave the way towards the integration of a range of temporal and point estimation methods to a single, robust estimate of effective population size (Beaumont et al., in press). Both of these advances in theoretical population genetics may be important for the future of fisheries population monitoring in Australia.
Conclusions and dissemination plan

During her Winston Churchill Fellowship in 2002, Jenny Ovenden met with leading researchers in the Canada, US, Norway and Britain who are using effective population size estimates for genetically monitoring increases or declines in animal populations. As a result of collaboration and consultation, Jenny believes methodology has an excellent potential for contributing to the sustainable management of harvested fisheries species.

So far only a small amount of this potential has been realised because of the mathematical complexity of the elegantly simple population genetic theory on which it is based. Consequently, computational and technical approaches are common in the literature while treatments of empirical data that could lead to management outcomes are rare. The robustness of the population genetics framework surrounding the methodology, and the high calibre of theoreticians who are currently working toward mathematical and statistical solutions suggests that the path will soon be opened for the more widespread practical application of the methodology.

Jenny sought answers during her trip to specific aspects of the methodology that were perceived to be impeding progress in the short term. It is likely that statistically powerful estimates of effective population size may be able to be made for tiger prawns in Moreton Bay based on new data for two marine fish species (New Zealand snapper and red drum) which has shown that the effective numbers of spawners is three to five orders of magnitude smaller than actual numbers of spawning individuals. Furthermore, Jenny worked on the effects of lack of panmixia (random breeding), harvesting and discrete generations on effective population size estimates that may allow the methodology to be applied to the larger, and commercially important population of tiger prawns in the Gulf of Carpentaria.

Jenny learnt new ways to communicate the concept of effective population size in a fisheries context to scientists, policy makers and members of the interested public. The use of a simplified definition of effective population size was validated by discussions with experts and she developed a new analogy for use in explaining the concept to a non-
scientific audience – the “wind-chill factor”. These communication tools will be used during discussions of the outcomes of Jenny’s Churchill Fellowship with fisheries and scientist colleagues, policy makers, community fisheries organizations and research students following Jenny’s return to Queensland.
Recommendations

Fisheries scientists who are working with genetic monitoring techniques such as effective population size estimation and genotyping for harvest rate estimation should

- Assess the risk of ‘bio-fraud’ and take action, if necessary, to prevent or manage the contamination, or other interference, of genetic samples by special-interest groups.

- Monitor the development of single nucleotide polymorphisms (SNP) as genetic markers to evaluate their usefulness as replacement for microsatellite loci currently in use by both genetic monitoring methods.

To maximize the research outcomes of the current research project on tiger prawns in Moreton Bay, the research team should

- Make successive estimates of the ratio between effective and census spawning population size over several consecutive years to investigate possible correlation with environmental fluctuations that may be important to survival to one or more life history stages.

- Experiment with alternate life history stages of the tiger prawn as genetic samples to assess the importance of the timing of effective population size estimates in management.

- Continue to incorporate new statistical methods for effective population size estimates into user friendly software to ensure that this Australian research team is, and is cited as being on the fore-front in this field.

To lay the groundwork for the estimates of effective population size to be made for the numerically larger, and economically important population of tiger prawns in the Gulf of Carpentaria, the research team should
• Take genetic samples from the five ‘stocks’ of tiger prawns in the Gulf to assess their degree of population structure.

• Obtain ‘ball-park’ estimates of effective population size of tiger prawns in the Gulf by applying the ratio between effective and census spawning population size for Moreton Bay tiger prawns to fisheries estimates of the spawning census population size for tiger prawns in the Gulf.
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