

LONG-TERM GENETIC CHANGES IN EXPLOITED POPULATIONS: EFFECTS OF FISHING ON NEW ZEALAND SNAPPER

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Summary

The exploitation of wild populations has been one of the most damaging anthropogenic effects on biodiversity, with many species becoming extinct as a direct result. While such species extinctions have become an issue of global concern, relatively little consideration has been given to the effects of exploitation on genetic diversity *within* species. Fish are of particular importance because they are the only major food source harvested from wild populations. Here, we have the unique opportunity to investigate the effects of almost 50 years of exploitation of New Zealand snapper (*Pagrus auratus*) by using novel molecular techniques to analyze the extensive scale collection of the National Institute of Water and Atmospheric Research (NIWA) in New Zealand. DNA will be extracted from fresh specimens and scales using standard laboratory procedures. Microsatellites and sections of the mitochondrial DNA will be PCR amplified and used for the estimation of genetic variability. The amount of variation will be examined for populations from different times and localities, and will be related to levels of exploitation and environmental events. The analysis will be enhanced by examining different types of molecular genetic variation differing in their mode of inheritance, which will provide complimentary and independent data sets.

1. Background

1.1. Biodiversity conservation and fisheries management

The loss of biodiversity has become an issue of worldwide importance, in particular where biological resources form the basis of ongoing or potential exploitation and significant commercial returns. Fish are of particular interest as they are the only major food source harvested from wild populations (Ryman *et al.* 1995) and because current fishery yields have reached or exceeded sustainable limits (Graves 1996). Management efforts have focused on the estimation of sustainable yields and the maintenance of sufficient stocks (Hilborn & Walters 1995), giving little consideration to how fishing may affect genetic diversity and selection pressures in the exploited stocks. Ever increasing levels of exploitation have generated an urgent need for a better understanding of genetic effects caused by harvesting in order to improve long-term management strategies and thus ensure the conservation of biological resources.

The foundation for such understanding comes from models in evolutionary biology and population genetics, which suggest that exploitation may affect fish species in several different ways (Nelson & Soule 1987): (i) reproductively isolated populations may become extinct due to local overfishing, (ii) population sizes may be reduced to levels where genetic variability cannot be maintained in the long-term and (iii) fishing may selectively remove fish with certain characteristics and thus cause irreversible genetic changes in exploited populations. Although these effects are well known from theoretical considerations (Ryman *et al.* 1995), convincing empirical evidence for their occurrence in wild populations remains scant. Molecular genetic techniques allow the identification of fish stocks and the estimation of genetic variability. Such techniques, however, have only been extensively applied to fishery problems after most fisheries had been established, and molecular studies on temporal genetic changes in exploited populations are almost entirely missing. Limited data comparing a sample collected in the past with the present day situation gave some indications of loss of genetic variability (Smith *et al.* 1991, Nielsen *et al.* 1997) or changes in the genetic structure (Purcell *et al.* 1996) in exploited fish populations, but as there were no continuous temporal data series, the interpretation of results is difficult and factors other than fishing may have caused the observed effects. Indeed, recent data suggest that in one of the above studies initial data may have been misleading (Smith *et al.*, in press).

Time series of genetic diversity, on the other hand, would allow a more thorough investigation of genetic effects of fishing, ideally starting before the onset of heavy exploitation and covering several decades of exploitation. Fortunately, recent developments in molecular genetics now make it possible to use archived scale collections for population analysis, allowing temporal studies covering decades of exploitation histories. Here we have the unique opportunity to use the extensive scale collection of New Zealand snapper (*Pagrus auratus*) of Dr. Peter Smith of the National Institute of Water and Atmospheric Research (NIWA) in New Zealand, covering almost 50 years, to assess the effects of fishing on population structure and genetic variability. The species is ideal for the investigation of temporal genetic changes in fish populations, because the available scale collection links with the wealth of background information on biology and fishery and specific features of the environment. Furthermore, New Zealand snapper is confined to coastal habitats, representing a discrete unit free of immigration from unknown offshore populations, and is thus

particularly amenable to genetic investigations. The species is extremely long-lived and the use of 30 year old fish will allow the investigation of year classes spawned in the 1920's. Data will be of value not only for the management of the snapper fishery in New Zealand, but also provide information relevant to other fisheries and to the long-term exploitation of biological resources in general.

1.2. The New Zealand snapper fishery as a model system

The history of the snapper fishery in the north of New Zealand (Fig 1) is typical for many other fisheries in the world (Annala *et al.*, 2000): yields of the commercial fishery in Hauraki Gulf, which developed in the mid 1800s, increased slowly up to the 1970s, when the introduction of pair trawls raised catches to 12,000 tons per year (Fig. 2). By the mid-1980s annual catches had declined to 6,000 tons, and stocks showed signs of overfishing.

According to age structured maximum likelihood models, the spawning stock biomass had by then decreased from an estimated 280,000 t to 37,000 t, though population abundance never fell below 37 million individuals (Annala *et al.*, 2000; Gilbert & Phillips, 2000). Because of the importance of the fishery and the drastic decrease in stock abundance, research on snapper biology started soon after World War II, not only in the heavily fished northern populations, but also in hitherto less exploited stocks in the south, such as Tasman Bay in the north of the South Island. There are therefore time series of scale samples from Tasman Bay, beginning in 1950 just after the commencement of the fishery, when spawning stock biomass was essentially at natural levels, and covering the entire exploitation history of the stock, with a reduction in biomass by 87% and in numbers by 75%, and an estimated minimum population size of 3.3 million fish in 1985. These scale collections allow a temporal survey of genetic variability over more than 50 years and four generations, and are thus an invaluable resource for the proposed project.

While even such high levels of exploitation are unlikely to have reduced the population size to levels where population models predict the loss of genetic variability due to random genetic drift (Nei *et al.* 1975), biased reproductive success of the adult spawning stock and unpredictable environmental events may reduce the long-term genetically effective population size to levels far below census counts from the wild (Frankham 1995). Indeed,

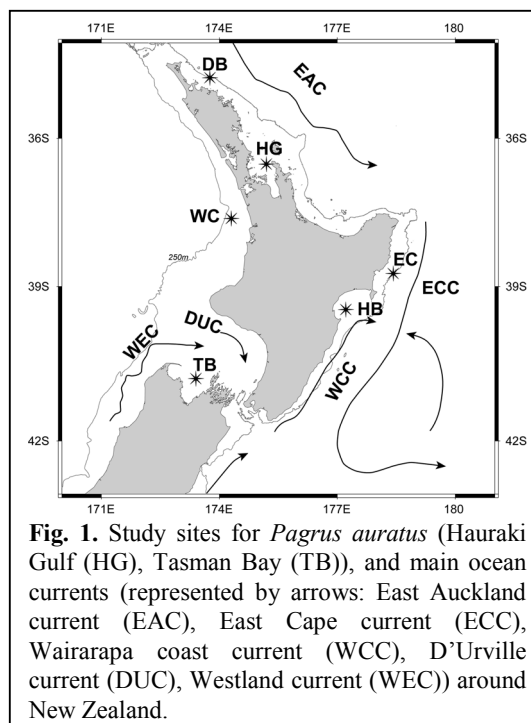


Fig. 1. Study sites for *Pagrus auratus* (Hauraki Gulf (HG), Tasman Bay (TB)), and main ocean currents (represented by arrows: East Auckland current (EAC), East Cape current (ECC), Wairarapa coast current (WCC), D'Urville current (DUC), Westland current (WEC)) around New Zealand.

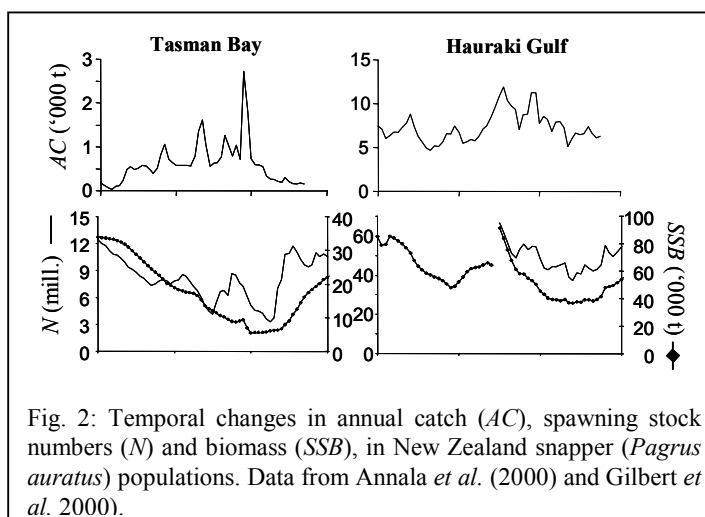


Fig. 2: Temporal changes in annual catch (AC), spawning stock numbers (N) and biomass (SSB), in New Zealand snapper (*Pagrus auratus*) populations. Data from Annala *et al.* (2000) and Gilbert *et al.* (2000).

length frequency distributions of juvenile snapper show that larval survival is both temporally and spatially extremely variable (Francis 1993), and the reproductive success of matings may depend largely on stochastic events. Furthermore, large-scale periodic changes of the climate known as El Niño have caused almost complete failure of certain year-classes (P. Smith, *pers. comm.*), so further reducing the long-term genetically effective population size. The combination of high fishing pressure, biased reproductive success and environmental events like El Niño may therefore have caused a loss of genetic diversity detectable with molecular methods and the scale samples.

2. Objectives

Based on information on the biology and fishery of snapper (*Pagrus auratus*) we aim to use fresh specimen and scale collections:

- To measure levels of genetic diversity and population differentiation in present day populations in New Zealand.
- To assess temporal trends in molecular genetic diversity and to relate these to the level of exploitation and environmental factors.
- To compare the genetic effects of harvesting among populations with differing exploitation histories.

3. Materials and methods

3.1 Samples

Extensive time series of samples spanning more than 40 years are available for two sites (Hauraki Gulf, Tasman Bay, Figure 1) representing the two major stocks identified for fisheries management. It is not intended to analyze all the scales from Hauraki Gulf and Tasman Bay available (Table 1), rather we plan to select three years before and after the main exploitation phase. Since we intend to examine 50 scales per year in two populations, the total number of scales from these two sites to be analysed is 300 scales. In addition, samples of 50 individuals from present day populations in Hauraki Gulf and Tasman Bay will be collected during the 2004 fishing season with the assistance of Dr Peter Smith at NIWA in New Zealand. Following these initial analyses, sample sizes may be increased to resolve particular relationships or to determine the presence or absence of an allele of interest. From our experience in preliminary trials and in other projects, we feel that this work can be carried out within the time scale indicated.

3.2 Molecular Methods

3.2.1. DNA extraction

DNA will be extracted from single, dried scales using protocols previously applied to similar material (Nielsen et al., 1997, Purcell et al., 1996, Taggart *et al.* 1992, Whitmore

Table 1: Scale samples available at NIWA, New Zealand

<i>Year coll.</i>	Tasman Bay	Hauraki Gulf
1950	200	>400
1958	150	>400
1963	300	>400
1964	200	>400
1972	50	>400
1978	60	>400
1980	200	>400
1986	200	>400

et al., 1992), involving a long extraction phase using proteinase K and a detergent which liberates the DNA, followed by purification of the DNA using standard techniques. As seen in a range of archival tissue types, "ancient DNA" is degraded into low molecular weight fragments (for example see Hagelberg, 1994, Handt et al., 1994) and is probably chemically altered at particular sites (Pääbo et al., 1990, Pääbo, 1989). Nevertheless, the polymerase chain reaction (PCR) still allows the amplification of such degraded DNA and indeed, it was shown that about 90% of 60 year old scales, stored dry at room temperature yield enough DNA for many PCR amplifications of single-copy microsatellite DNA (Nielsen et al., 1997).

3.2.2. Molecular markers to be used

Genetic analyses will be carried out using variable microsatellite loci and mitochondrial DNA markers, which are both useful and complimentary markers for population studies (Awise, 1994) and are amenable to study using ancient DNA (Nielsen et al., 1997).

Microsatellite Analysis

Microsatellites consist of 1-5 base pair (bp) repeats that form tandem arrays up to 300 bp in length, and exhibit high levels of allelic variation in repeat number. Polymorphism exhibited by specific microsatellites is readily detected by amplification of the microsatellite through the use of oligonucleotide primers specific to the non-repetitive regions that flank the repeat array, in combination with the polymerase chain reaction (PCR). Allelic variation is scored by gel electrophoresis of the PCR products, most commonly on high throughput automated systems allowing the analysis of up to 300 genotypes in an hour. Microsatellites exhibit attributes that make them particularly suitable for the present study. First, their high variability results in a large number of rare alleles, which are more sensitive markers for reduction in population size than heterozygosity estimates (Nei *et al.* 1975). Second, they have been shown to be extremely powerful in the identification of population structure and the estimation of genetic diversity (e.g see Bowcock et al., 1994, Viard et al., 1996). Third, it is possible to keep amplification products short by appropriate design of specific primers, thus enhancing the chances of successful analysis of old material. The time-consuming process of isolating microsatellite primers is not necessary as species-specific primers for five variable microsatellite loci have already been designed for *P. auratus* (Takagi et al. 1997; N. Taniguchi, Kochi University, *pers. comm.*). These will be amplified using fluorescently labelled primers and run on the laboratory's MegaBace automated sequencer to determine allele sizes. We are therefore confident that the microsatellite analysis of dried scales will provide valuable results on both levels of genetic diversity and population structure of snapper.

Mitochondrial DNA (mtDNA) analysis

The mitochondrial D-loop region is highly variable and is therefore suitable for providing an independent measure of population variation to microsatellites. The maternal inheritance and haploidy of mtDNA reduce its genetically effective population size to about a quarter of that of nuclear DNA, thereby accentuating genetic drift effects (Hauser *et al.* 1995). Furthermore, the presence of many copies of mtDNA in each cell means that preserved samples usually contain much more amplifiable mtDNA than nuclear DNA (microsatellites), and it thus has been extensively used in ancient DNA studies (Hagelberg, 1994). In addition, mtDNA sequence variation can be used to reconstruct population phylogenies, and thus provide further information elucidating differences in genetic diversity.

Data from previous sequence studies will be used to design two sets of primers amplifying a total of about 350bp in the hypervariable 5' region (Donnellan and McGlennon, 1997; Tabata, 1997). These primers will be used to amplify short sections of the mitochondrial DNA for analysis using SSCP (single strand conformational polymorphism, Hayashi 1992). SSCP detects sequence variation from differences in the electrophoretic mobility of single-stranded DNA. While the mobility of double-stranded DNA depends on fragment length, single stranded DNA forms intrastrand base pairs and thus complex 3D structures dependent on the base sequence. Even a single nucleotide change can dramatically affect the strand's mobility and may thus be detectable by SSCP. Variants detected by SSCP will be sequenced on the MegaBace automated sequencer to estimate genetic divergence among haplotypes.

4. Significance

The project is in two main aspects of great significance: (i) an applied aspect is to improve the understanding of the genetic effects of exploitation, not only of New Zealand snapper, but of harvested fish species worldwide; and (ii) a more fundamental aspect is the opportunity to verify interpretations based on present day genetic diversity by investigating temporal trends in the population history.

Conclusive evidence for a loss of genetic variability or of genetically distinct populations from the time-series of scale samples would demonstrate the importance of long-term considerations for fisheries management. While such long-term concerns about the maintenance of diversity in exploited populations have frequently been published in the scientific literature, the lack of any unequivocal empirical evidence has hindered its recognition by fisheries managers. Such recognition would present a critical first step in the incorporation of genetic issues in the management of exploited species, and thus be of vital importance to the conservation and sustainable utilization of genetic resources.

On a more fundamental level, the project will investigate the use of ancient DNA to reconstruct past population events. Usually such events in a population's history are inferred from observations on extant samples, often allowing alternative explanations. For example, differences in genetic variability between populations is often seen as evidence for population bottlenecks, even if there is no indication for reduced population sizes from other data and other factors such as selection, gene flow and molecular genetic processes may cause equally high differences. The snapper scale samples will here allow the verification of results and interpretations obtained from the present day population survey with data from time series of scales.

5. Timeline

<i>Year</i>	<i>Course Week</i>						<i>Subsequent work</i>											
<i>Month</i>	5	6	7	8	9	10	A	M	J	J	A	S	O	N	D			
DNA extraction from fresh samples	*						*											
Microsatellite primer optimization	*	*																
DNA extraction from scales				*	*		*	*										
Microsatellite analysis					*			*	*	*								
Mitochondrial primer optimization						*												
Mitochondrial DNA SSCP					*	*					*	*	*					
Detailed data interpretation						*									*	*		
Report and Presentation						*										*		

The main aim of the coursework is to learn and optimize techniques which will be used in MMBL later on. DNA will be extracted from a few fresh samples and will be used for microsatellite primer optimization. Once these primers are optimized, DNA will be extracted from a representative set of scales and microsatellites will be amplified. Week 9 and 10 will be used for initial optimization of mtDNA SSCP, and the final report and presentation will be prepared in the final week of the quarter.

Population screening of all samples will be carried out after the course, with the first two months spent on DNA extraction from fresh samples and scales. The subsequent six months will be used for microsatellite and mtDNA SSCP screening, leaving two months for data interpretation and preparation of thesis and publications.

6. Budget

Item	Cost
Course Fee	450
Bench fee in MMBL subsequent to course (\$450 x 9 month)	4,050
Primers 7 pairs unlabeled (5 microsatellites, 2 mtDNA) a \$25 5 labeled a \$60	175 300
Extraction (assuming N = 50 individuals) 100 fresh samples (2 populations of 50 each) 300 scales (2 populations, 3 temporal samples)	200 600
Taq	175
MegaBace Runs (microsatellites) 400 samples x 5 loci = 2000 genotypes; approx 25 runs (20.8 runs and some spare)	2,917
Size standard (\$ 20 x 25 runs)	500
Total	9,367

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