Fisheries New Zealand
Tini a Tangaroa

Report of the Workshop on the Utility of Genetic Analyses for Addressing New Zealand Fisheries Questions

New Zealand Fisheries Science Review 2020/01

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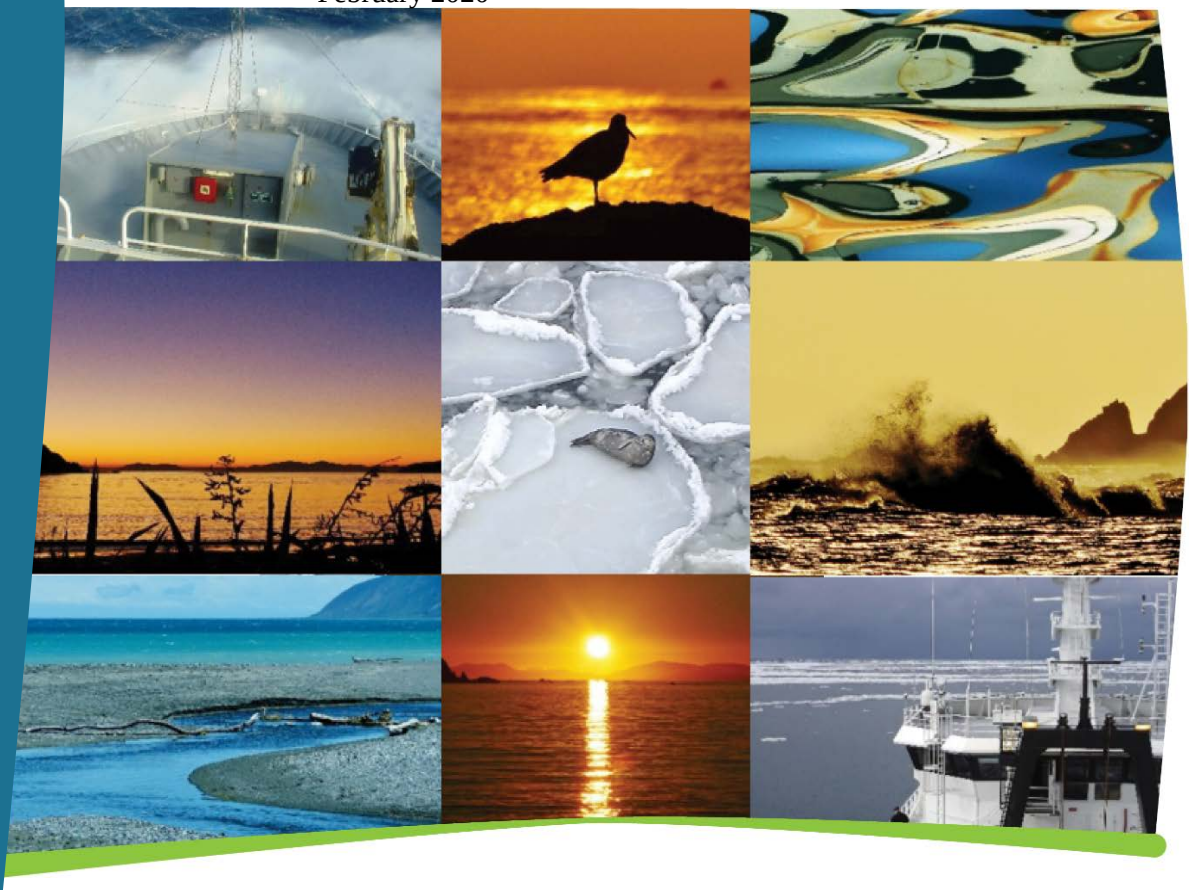
ISSN 2382-2406 (online)
ISBN 978-1-99-001732-2 (online)

February 2020



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Preface

The Ministry for Primary Industries and its predecessor, the Ministry of Fisheries, have conducted fully-independent expert reviews of stock assessments, research methodologies and research programmes since 1998. We also run specialist technical review workshops to further advance fisheries and other marine science methodologies and techniques. These fully-independent reviews and technical workshops are separate from, but complementary to, the annual Science Working Group processes that are used to ensure the objectivity and reliability of most of our scientific research and analyses.

A new publication series, Fisheries Science Reviews, was initiated in 2015 to ensure that reports from these reviews are readily accessible. The series will include all recent and new fully-independent reviews and technical workshop reports, and will also incorporate as many historical reports as possible, as time allows. In order to avoid confusion about when the reviews were actually conducted, all titles will include the year of the review. They may also include appendices containing the Terms of Reference, a list of participants, and a bibliography of supporting documents, where these have not previously been incorporated. Other than this, there will be no changes made to the original reports composed by the independent experts or workshop participants.

Fisheries Science Reviews (FSRs) contain a wealth of information that demonstrates the utility of the processes the Ministry uses to continually improve the scientific basis for managing New Zealand's fisheries.

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EXECUTIVE SUMMARY

Mace, P.; Ritchie, P.; Wellenreuther, M.; McKenzie, J.; Hupman, K.; Hillary, R.; Francis, M.; Bunce, M. (2020). Report of the Workshop on the Utility of Genetic Analyses for Addressing New Zealand Fisheries Questions. ***New Zealand Fisheries Science Review 2020/01.*** **28 p.**

A Specialist Technical Workshop was held on 18-22 March 2019, primarily to investigate the use of genetic methods for estimating population size and stock structure for i) fish species that are targets of marine fisheries, and ii) marine mammals, and also to discuss other genetic applications to a lesser extent. New developments in genetics and genomics have considerably advanced the field in recent decades and they are increasingly being used in specific fisheries-related applications such as estimating population size and movements, delineating stock structure and estimating genetic connectivity between stocks or stock components. Genetic methods and analyses used to be prohibitively expensive and it was not practical to apply them to many fisheries problems. While this may still be the case for many applications for most species, costs have been declining substantially as the technology has advanced, and Fisheries New Zealand considered it timely to review both the applicability of various techniques, their feasibility and their relative costs and benefits over other more conventional methodologies, or their utility in augmenting conventional methods.

In order to reduce costs, it is probably expeditious to focus initial consideration on fish and marine mammal species where the genome has already been mapped. However, the rapidly-developing progress in this area should continue to be encouraged, and the list of species with well-characterised genomes should be revisited in the future to determine whether there might be other candidates for consideration. It will also be important to identify and continue to research alternative sampling methodologies, and address issues such as species with complex reproductive strategies, species with very large or very small population sizes, and species with pronounced sub-population structuring.

There are also a number of potential future developments in the field of genetics that should be closely tracked. There was insufficient time to discuss all such developments in this workshop, but there are a number of novel uses of genetics already underway.

The output from this workshop will be evaluated to determine the priorities for current fisheries research involving genetics, and future workshops of this nature should be conducted periodically to summarise recent and emerging developments. In particular, the costs of gene sequencing have decreased substantially in recent years, and continue to decline. The easier it becomes to sequence large numbers of samples rapidly, the less expensive sequencing will become, and the easier it will be to incorporate genetic analyses into studies of population dynamics of fish and marine mammals.

1 INTRODUCTION

New developments in genetics and genomics have considerably advanced the field in recent decades and they are increasingly being used in specific fisheries-related applications such as estimating population size and movements, delineating stock structure and estimating genetic connectivity between stocks or stock components. Genetic methods and analyses used to be prohibitively expensive and it was not practical to apply them to many fisheries problems. While this may still be the case for many applications for most species, costs have been declining substantially as the technology has advanced, and Fisheries New Zealand considered it timely to review both the applicability of various techniques, their feasibility and their relative costs and benefits over other more conventional methodologies, or their utility in augmenting conventional methods. (It should be noted that conventional methods can also be very expensive).

Accordingly, a Specialist Technical Workshop was held on 18-22 March 2019, with the following Terms of Reference:

The workshops will address the following questions:

1. For each methodology for estimating population size identified for further exploration:

    - What are the assumptions that need to be met and the sources of uncertainty that need to be addressed?

    - What are the characteristics that would make a species, population, or stock of fish or marine mammal the most suitable for that technique? Apply these characteristics to the exemplar species of snapper, New Zealand common dolphins, or rock lobster, but also consider other species/stocks that may be better suited.

    - Are these likely to be cost-effective at present, or in the near future?

2. What are the most promising genetic methods and analyses for examining stock structure questions of relevance to fisheries science and management?

    - What are the assumptions that need to be met and the sources of uncertainty that need to be addressed?

    - What are the characteristics that would make a species, population, or stock of fish or marine mammal the most suitable for that technique? Apply these characteristics to the exemplar species of snapper and New Zealand common dolphins, but also consider other species/stocks that may be better suited.

    - Are these likely to be cost-effective at present, or in the near future?

3. Are there specific applications that have already been attempted that could be used to guide future studies in New Zealand?

4. Can the workshop recommend best practice in the study design and analysis of data for each case?

5. Are there other promising genetic methods and analyses on the horizon that Fisheries New Zealand should evaluate periodically?

In a New Zealand fisheries context, we are particularly interested in the use of genetic methods for estimating population size and stock structure, but other genetic applications were also considered to a

lesser extent. Workshop participants primarily considered species that are the target of fisheries, but also included marine mammals, which may be an inadvertent bycatch in fisheries. Some techniques may be appropriate for species or populations that number in the millions, although most are probably more appropriate for much smaller populations.

The technical workshop consisted of a group of core participants who are experts in either genetics, the application of genetics to fisheries, or fish population dynamics, and were from both within and outside New Zealand. Presentations on, and discussions about, genetic methods and analyses during the workshop were open to all interested parties.

The meeting was chaired by Pamela Mace (Fisheries New Zealand). A glossary is provided in Appendix A and a list of participants and their affiliations is given in Appendix C.

The remainder of this introductory section sets the scene for the questions and challenges that genetics might be able to address, first with respect to fisheries stock assessments, and second for marine mammals.

1.1 Fisheries Stock Assessments

The classic approach to single species fisheries management requires knowledge of four fundamental stock characteristics:

i) spatial stock structure;
ii) current biomass;
iii) current productivity dynamics; i.e. growth, recruitment and natural mortality;
iv) fishing mortality (the proportion of a fish stock removed each year).

Modern genetic methodologies, either applied at the population level or the individual level (e.g. as a marker technology), are potentially applicable to fisheries management across all four of these required stock assessment knowledge areas.

1.1.1 Understanding spatial stock structure

It is important to understand the geographical area over which a stock resides, as well as the nature and extent of movement dynamics within the stock (e.g. seasonal migrations or ontogenetic distributional shifts). Fisheries scientists separate stocks primarily on the basis of spatial separation of commercial catches, or spatial differences in age composition, and growth and the movement of tagged animals. Understanding of within-stock mixing and movement is commonly based on temporal changes in spatial abundance and age composition and tagged fish movements. To satisfy the requirement that a fish stock area is self-sustaining in terms of recruitment, fisheries scientists usually need evidence of spawning activity within the stock area as well as evidence of the presence of juveniles. Spatial uncertainty is a significant factor in the assessment of some of New Zealand's most important fish stocks including hoki, snapper, orange roughy, and more recently tarakihi.

Past applications of genetic methods for delineating New Zealand fish stocks have mostly failed to distinguish differences, largely because past methods have used very low resolution markers that were severely underpowered to detect changes. The increased genomic resolution of modern genetic methods have significantly improved the power of studies, and this means that it is now more feasible to delineate stocks on the basis of fine-scale genomic similarities and differences (see Bernatchez et al. 2017 for a comprehensive review).

Delineating stocks on the basis of individual fish movement observations (e.g. through tagging) is another way genetic approaches have been recently successfully applied in fisheries stock assessment. Single nucleotide polymorphism (SNP, Section 2.2) genetic methods have been used to identify and track

individual fish for some stocks. Genetic 'marking' of individual fish for the application of mark-recapture has been achieved both through direct tissue sampling and by close-kin inference analysis (Section 2.3).

1.1.2 Stock biomass and growth estimation

Fisheries scientists typically monitor relative stock abundance change using either fisheries-dependent (e.g. fisheries catch per unit effort), or fisheries-independent (e.g. trawl surveys) methods. Fisheries-independent surveys usually provide the more accurate abundance measure but are significantly more expensive. Conventional tagging is a common approach fisheries scientists use to estimate current stock biomass (Petersen mark-capture) and growth (growth increment analysis). The above mentioned SNP individual tagging approaches have high application utility for these estimation approaches.

1.1.3 Estimation of fishing mortality

Knowledge of how stock biomass is changing through time relative to catch removals is central to conducting stock assessments. Observations of the relative decay rates of individual age cohorts through time provide a measure of the level of total (both natural and fishing-related) mortality a stock is experiencing. Fishing mortality rates can be inferred from snap-shot stock age composition data, but can also be derived from tagged cohort decay analysis (e.g. Jolly-Seber tag survival analysis). Again genetic tags offer a viable alternative to physical tags for use in survival analysis.

1.2 Marine mammal population estimation

Effective approaches for the management and conservation of marine mammal populations require a sound knowledge of population demographics (including population size), and this is often only possible through capture mark-recapture (CMR) studies. For many species, such information is provided by studies that recognise individual animals so that their fate can be followed through time, thus allowing for the estimation of demographic rates such as survival.

One of the more popular techniques for identifying individual marine mammals is using photo-identification (photo-id) to capture the natural markings of an animal. Here animals are photographed over time and added to a catalogue of known individuals. This method is often preferred as it is non-invasive (no contact with the animal). Alternatively, genetic analysis can also be used to identify individual animals. The basic principle of this approach is that samples are genotyped at multiple loci and these genotypes are treated as molecular individual marks. Matching genotypes are considered to belong to the same individual and are classified as recaptures. This method is being applied increasingly due to its ability to capture additional individual- and population-level information (e.g., genetic diversity, parent-offspring relationships, sex ratios etc.). Despite this, it is sometimes avoided due to its invasive nature (e.g. using biopsy darts), or because it can be hard to gain permits. An alternative is to use scat samples which are considered non-invasive; however, there are often issues with DNA degradation and quality.

For both photo-identification and genetic mark-recapture, capture histories can be compiled. For example, for each sampling occasion, all individuals are determined to be either captured (coded as 1) or not captured (coded as 0), resulting in individual capture histories that are used for CMR analysis.

Both photo-id and genetic CMR must meet the basic assumptions of CMR analysis. These are that, for photo-marked individuals: i) marks are unique, ii) marks cannot be lost, and iii) all marks are correctly recorded and reported. In addition, CMR models make assumptions related to the behaviour of the animals or the researcher and include: i) marking does not affect future survival or catchability, and ii) animals must have an equal probability of being captured within each sampling occasion. The table in Appendix B addresses our ability to meet CMR assumptions using both methods.

2 OUTLINE OF POTENTIAL GENETIC APPROACHES FOR ESTIMATING POPULATION SIZE

2.1 Gene Tagging

The majority of population genetic studies that have been conducted for fisheries management purposes have used a population-based approach for their sampling design and analysis. A sample from a location is taken (e.g. 100 individuals per site) and a small part(s) of the genome is targeted for DNA sequencing or genotyping (e.g. mitochondrial DNA or microsatellite DNA, see below). The overall goal of these studies has been to determine the genetic stock structure for a fish population or complex (Ward 2000, Palsbøll et al. 2007). Information collected from a location has been pooled and used to estimate levels of genetic diversity and gene flow among sites (Waples & Naish 2009). Although these sorts of studies have produced useful information, the indirect approach that has been employed lacked the level of detail needed to accurately estimate stock size and movements of individuals. The population-level approach assesses the consequences of individuals moving and reproducing, whereas an individual-based approach can be used to generate direct information about individual fish. The latter has been considered as a replacement for conventional fish tagging methods (Hamazaki & DeCovich 2014, Miller et al. 2015).

The identification of individual fish for tag-recapture using DNA markers requires the development of a set of variable loci, which produce allelic combinations that are unique to each individual (Palsbøll 1999).

Genetic mark-recapture ("gene tagging") methods offer the benefit of being a permanent individual marker and are less likely to be influenced by human-biasing of reporting rates. Gene tagging methods are based on existing mark-recapture theory and methodology. The difference is that it requires a genetic profile of each sampled individual to be determined using multiple genomic loci. A multi-locus genotype is designed to be statistically unique and used to find a match to identify a recaptured individual. The probability of assigning an identical genotype to two separate individuals is negatively correlated with the level of variability within a population and the number of loci sampled.

Using genetic markers as an individual tag has previously been attempted using several different marker types (e.g. mitochondrial DNA and microsatellite DNA), but their statistical power has been limited because only a small number of genomic loci were available (Lukacs & Burnham 2005). However, Single-Nucleotide Polymorphism (SNP) markers derived from whole-genome sequencing are a relatively new tool that can be more easily scaled to produce a large number of loci (Choquet et al. 2019). This significant improvement in statistical power makes individual identification much more likely, especially for species with a large population size and variation in levels of reproductive success. Moreover, because SNP-based methods are able to generate a large number of markers, they can be designed to contain enough redundancy to reliably genotype DNA samples that are degraded (von Thaden 2017).

Gene or genetic tagging changes the way that we use population genetic data from an allele frequency-based framework, to an individual "DNA fingerprint" framework. The approach begins by considering all individual genotypes as potential direct matches (recaptures). Genotypes from the initial samples are then compared to individuals that are subsequently sampled. The proportion of matches in the second sample is the recapture rate that is used to estimate the population size. In a fisheries context, gene tagging can be used to estimate the absolute abundance of a cohort, for example for age two recruits of southern bluefin tuna (Preece et al. 2018). The success of a gene tagging approach is dependent on developing a marker system that can distinguish individuals in a large and genetically diverse population, repeatability of the genotyping with a range of sample types and qualities, the ability to process large number of samples quickly and cheaply (i.e. high-throughput multiplexing), and having computational and database space available to store and analyse the data. Permanent tag retention, no limitation on fish size for obtaining a sample, and use of the data for a wide range of other studies make gene tagging an attractive option for stock assessment purposes.

2.2 Genetic methods for gene tagging

Mitochondrial DNA (mtDNA) is the DNA found in mitochondria that is arranged as a circular genome which, in vertebrates, is approximately 16 kbp (kilo basepairs) long and is typically maternally inherited. This has been a popular marker for studying populations because the laboratory procedures are less demanding than those outlined below and the DNA sequencing methods have been easily transferred among species. Within a cell there is one nuclear genome and 10–1000 mtDNA genomes depending on the tissue type, meaning that mtDNA is several-fold more abundant than nuclear DNA. The numerical dominance of the mtDNA in a tissue sample makes it more reliable for isolating DNA from low quantity or degraded samples. Although it has limited value as a genetic tagging marker, it is often considered a relatively straight-forward and cost-effective approach for conducting a preliminary assessment of genetic stock structure (costs are about a quarter to a third of more sophisticated techniques) and may be adequate for some pilot stock structure projects. The science is well-established, considerably more species have been researched, and it is possible to detect mixed-species stocks. In most situations the detection of a significant level of mtDNA differentiation between two locations is strong evidence that they have been isolated for a long period of time.

Microsatellite DNA is a method that samples repetitive stretches of DNA, sometime called Simple Sequence Repeats (SSRs) or Short Tandem Repeats (STRs). The variation detected using these stretches is caused by differences in the number of repeat units within them. The instability of these genomic regions make them prone to mutation, and within a population polymorphic loci can have a large number of alleles. Studies that have used these bi-parentally inherited genomic markers usually develop a set of 10–20 loci specifically for a study species. The majority of microsatellite DNA loci are located in non-functional areas of the genome. Microsatellites have been common population genetic markers, but they have now been largely superseded by whole-genome sequencing.

Whole-genome sequencing represents the final major step in the development of DNA-based markers and if done thoroughly can result in complete genomic resolution. This approach typically identifies and utilises variation in **Single-Nucleotide Polymorphisms (SNPs)** which make up about 90% of genetic variation within a population in terms of frequency (but not the number of base pairs). Most SNPs only have two alleles. Large whole-genome data sets have been made possible because DNA sequencing technology has become so efficiently miniaturised that it has been scaled to conduct massively parallel sequencing. Large volumes of short read sequences are produced and can be assembled to reconstruct their order in an individual's genome. The overall cost per nucleotide of sequence for an individual's genome has been significantly reduced. However, contemporary DNA sequencing machines can only produce large volumes of sequences and cannot be easily downward scaled, which means sequencing runs appear to be expensive. The production of large volumes of genome sequence data can be challenging for the transfer, storage and analysis of datasets.

2.3 Pedigree analysis

A pedigree is a family tree describing the interrelationships of parents and offspring across generations (i.e. a genealogical study). Since the mating patterns of wild individuals are often unknown, indirect information such as genetic information needs to be used to reconstruct relatedness. Such genetic reconstruction of genealogical information is particularly useful in marine species such as teleost fishes, which are commonly characterised by external fertilisation and no post-hatching parental care, limiting the construction of pedigrees through observational means. The genetic reconstruction can be done with any molecular marker, with SNPs markers being likely to offer the most powerful reconstruction because they are densely distributed throughout the genome and can be relatively easily sampled with reduced representation libraries (e.g. RAD or GBS), whole genome sequencing or a SNP chip (Ellegren 2014).

Once the degree of genetic relatedness among individuals within and between generations is calculated, then it is possible to infer the survival and reproductive success of individuals. When extensive adult collection is not feasible, for example in species with high adult motility, sibship reconstruction can be an informative and suitable alternative to identify dispersal and connectivity patterns (Schunter et al. 2014).

Sibship reconstruction analysis is a common pedigree reconstruction method and has been used to study a variety of questions related to family structure and reproductive output in natural populations. Performing sibship analysis has the advantage that only one generation needs to be sampled, whereas parentage analysis requires sampling of both the adult and offspring generations. It should be noted that when one is working on species with large populations sizes (10–100 millions/species) then sampling needs to be conducted for a sufficiently large proportion of the population to ensure the successful capture of parent–offspring pairs, or a sufficient number of sibship pairs.

2.3.1 Close Kin Mark Recapture

Close Kin Mark Recapture (CKMR) is a form of pedigree analysis that focuses on the estimation of adult abundance and other key demographic parameters (e.g. mortality rates and effective reproductive output-at-age). In a stock assessment context, CKMR can be used to obtain absolute estimates of spawning stock biomass. In principle a number of different kinship linkages can be used, as their probabilities of occurrence are all (in different ways) expressed using estimates of adult abundance and demography (as well as using additional covariates from data collection). In practice, the most commonly used are POP (parent-offspring) and HSP (half-sibling) pairs. These are the easiest to distinguish genetically and the least complicated in terms of formulating the probabilities of detection (the mark-recapture side of CKMR). Considerations for CKMR are much the same as those for genetically-based conventional mark-recapture, but with the additional requirement of needing to sample both juveniles and adults for POP. HSP requires sampling of juveniles only, as was done for a recent white shark study (Hillary et al. 2018).

Previously, the genetic marker used most frequently for CKMR was microsatellites; this was followed by SNPs generated by reduced representation libraries such as GBS or RAD, and is now moving towards whole genome sequencing (which provides more SNPs). The general requirements, in terms of the genotyping method, are two-fold: i) the more distant the kinship relationship, the more detailed the genetic information has to be to identify related individuals and separate them out from other less-related linkages; and ii) the greater the number of animals that need to be sampled, based on the adult population size, the more detailed the genetic information needs to be as well. The second need for an increased number of SNPs is to keep false-positive rates down, to avoid lesser types of kin appearing in the true kin samples. It is important to identify loci that are informative for analysing relationships, and to discard less powerful ones, to keep the overall power of the analyses high.

For the now-routine sampling for southern bluefin tuna, approximately 7500 juveniles and 5500 adults have now been sampled; i.e. more juveniles than adults. The annual sampling rate is now set at 1500 juveniles and adults per year going forward. The decision to conduct close kin analyses on bluefin tuna was initially a substantial commitment with high risks. The sample size for the first year was about 3000 individuals and this was not sufficient to find adequate matches: only 7 were detected from the 3000. Thus, the initial financial investment may be high, and it is important to ensure that sufficient samples are collected to find a statistically-meaningful number of links. However, it is possible to reduce sampling in future years due to the ability to find matches from past samples. It may also be possible to expand to a situation where some juveniles from POP analysis then become adults themselves.

CKMR was initiated for southern bluefin tuna to provide an absolute spawning biomass estimate due to a revelation about misreporting, which made other sources of data suspect (Bravington et al. 2016a). There was also a good knowledge base of information about population structure and distribution, easy access to juveniles, and some (but harder) access to adults. Genetically-based conventional mark-recapture is now also conducted using non-lethal tagging with a dart (an arrow tip that pierces the skin behind the dorsal fin and shoots it into a vial) to monitor juveniles (which CKMR cannot do). The current levels of genetic mapping and linkages have also enabled estimation of the effective population size, which seems close to the total population size, although it is dependent on the method used to estimate the effective population size in numbers (N_e) or the effective number of breeders (N_b).

Obtaining mitochondrial DNA (mtDNA) sequences for identified kin pairs is vital to decomposing sexual dimorphism in the reproductive dynamics of adults. These data have been incorporated into CKMR models to estimate the sex ratio in adults, sexually dimorphic mortality and spatially reproductive dynamics, and multiple-partner mating relationships (particularly for juvenile half-siblings from the same year-class).

2.3.2 Pros and cons of HSPs and POPs

A combination of both HSPs and POPs is better because it means that more matches are possible. Additionally for teleosts (where reproductive success increases with age/size), having both POPs and HSPs can separate reproductive success from adult mortality. If genotyping resources are limited, POPs are probably preferable over HSPs because they are easier to identify genetically. HSPs are more difficult as the cost-per-sample to get usable matches may be higher. The HSP approach could be used if only adults are sampled, as long as their age is known accurately. Alternatively, adults could be sampled and both approaches used. The main limitations are the quality of the genotyping and the accuracy of key covariates such as the estimated or inferred age of the sampled animals.

HSP and POP are essentially two independent datasets meaning that they can be used together in stock assessment models much as one might use two alternative abundance indices. They are both included in the southern bluefin tuna integrated stock assessment used by the Commission for the Conservation of Southern Bluefin Tuna (CCSBT).[1]

Finding HSPs and POPs may also inform within-stock movements. With multiple spawning sites and sampling access across sites, the relative importance of, or preference for, adults from different sites is contained within the data sets (both POPs and HSPs). The CKMR model just needs to be adjusted accordingly with those processes parameterised. This has been done for a shark species in Northern Australia where adults migrate between rivers, and those reproductive spatial preferences were estimated to be different for males and females.

Genetically-based conventional mark-recapture could be used to find HSP and POP matches, but there may be a high risk of not finding sufficient matches. It also may not be cost-effective for genotyping to a CKMR standard for conventional genetic mark-recapture experiments. Potential advantages of CKMR over genetically-based conventional mark-recapture may include:

i) Situations where juveniles are easier to sample, or where there is a high likelihood of missing a large proportion of the adult population;
ii) Cases where all samples are from dead specimens, or where sampling is lethal;
iii) For genetically-based conventional mark-recapture, at least two periods of sampling must be undertaken; for CKMR one may suffice. In either case, an ongoing tagging programme gives a large increase in statistical power for the same *N*.

Appropriate sample size design is a key and evolving issue. When only POPs were used in the first CKMR work on southern bluefin tuna, the general rule of thumb was that $10\sqrt{N}$ **total** samples (evenly split between juveniles and adults) would be quasi-optimal and result in around 50 POPs. Since HSPs (and other close-kin relationships) have been used, the tools for design work have been changing to reflect these developments. Work has been done to develop efficient tools to design a sampling strategy (number of samples, years/ages/sites of sampling) that would be expected to meet pre-defined objectives (e.g. "target" CVs in abundance/mortality estimates) given the current best available information on the population in question (e.g. from a stock assessment).

[1] https://www.ccsbt.org/en/system/files/ESC23_14_AU_CloseKinProjectReport_0.pdf

2.3.3 CKMR analysis for marine mammals

The feasibility of using CKMR (Bravington et al. 2016b) to estimate *Nc*, for marine mammal populations particularly, is based on i) the population structure of the species, ii) the availability of life-history data for the available samples, and iii) potential biases inherent to the nature of samples (e.g. kin-bias due to multiple samples from a single stranding or by-catch event; non-random distribution of samples, i.e. many samples from a few localities) (Attard et al. in review).

A recently-refined broadly applicable simulation-based approach has been implemented to conduct robust relatedness estimates in non-model organisms using genome-wide data, and this approach has been used to identify parent–offspring pairs in a cetacean species (Attard et al. in review). If CKMR is deemed a feasible method for the species under examination, this new simulation approach will be extremely useful for inferring parent–offspring pairs within the CKMR framework (Attard et al. in review).

2.4 eDNA

As opposed to traditional ways to survey populations (microsatellites and SNPs), eDNA is completely untested as a means by which to survey population sizes of fishes. Traditional population genetic methods use tissue samples and can easily infer alleles and their frequency in a population. In contrast eDNA methods use environmental DNA samples such as water, plankton tows and sediments. Fish DNA in such substrates will typically be low in quantity and therefore difficult to genotype. Some researchers are currently investigating the ability to genotype from water by, for example, enriching the cellular fractions of water in an attempt to generate sufficient copies for genotyping. The value of this approach if it does work is that samples could be taken non-invasively. A scoping paper on this area was recently published Adams et al. (2019).

While traditional population genetics might be out of reach of eDNA technologies in their current form, there are two other uses that might prove fruitful:

i) First, it is entirely feasible to determine mtDNA haplotype data from eDNA if it is carefully filtered for errors. While not as precise as nuclear markers, mtDNA has been used to infer (effective) population size. It can, in other words, provide a high level overview of genetic diversity.

ii) Second, via the use of qPCR or digital PCR, researchers are increasingly exploring how the amount of DNA in the water column relates to biomass. A number of experiments in mesocosms have shown meaningful relationships to fish biomass. Likewise there are correlations between eDNA in the water and catch data (e.g. from trawls). The salient point with regards to eDNA concentrations is that there is unlikely to be a tight 1:1 ratio of a fish to its eDNA biomass, meaning that it will not be possible to estimate numbers of fish from eDNA samples. A number of studies have reported large variation in the shedding rates of DNA. It is theoretically possible via spike-in of other DNA (adding a known quantity of DNA to a sample) to generate a relative and an absolute measure of a species within a biological sample.

In sum, while eDNA might not currently be a position to inform the estimation of population size, it is worth considering the collection of environmental samples for later calibration against future advances.

3 DELINEATING STOCK STRUCTURE

Genetic markers have been used to identify fish stock structure, which is typically defined as groups of randomly mating individuals with temporal and spatial integrity. Panmixia is the null model for population genetic studies. Genetic data are gathered from multiple locations, and statistical tests, such as the F-statistics, are used in an attempt to reject panmixia. Traditional genetic markers (mitochondrial DNA and microsatellite DNA) have often failed to detect population differences in marine species even between apparently geographically isolated subpopulations for which there is evidence of some reproductive isolation (Waples 1998). Theory and empirical studies tell us that even very low levels of genetic exchange

among stocks will be sufficient to eliminate most of the genetic evidence for stock differentiation (Palsbøll et al. 2007). This is a particular problem for marine species because of their large population sizes and potentially high gene flow among areas. Mitochondrial and microsatellite DNA marker types are limited to assessing only one side of the ecological and evolutionary processes (genetic drift and gene flow) and almost always miss the impact of differential survival and reproduction caused by natural selection (adaptation). They almost always fail to detect adaptive differences among populations. This is because: i) they only sample a small fraction of the genome; ii) neutral and adaptive variation are typically uncoupled, and iii) even very low migration among otherwise demographically isolated populations will be sufficient to eliminate genetic evidence of differentiation in non-selected gene regions. The techniques have been useful for obtaining general estimates of genetic diversity levels and detecting strong levels of genetic differentiation. However, there is not much hope that traditional genetic markers will provide much more in-depth insight into stock structure. Nor is there a requirement for them to, given more extensive SNP development.

Failure to detect population genetic sub-division does not infer immediate acceptance of the null hypothesis that populations are panmictic; it simply means that it cannot be rejected with the available evidence. Marine species typically have low levels of genetic differentiation among populations, which means it is important for studies to have large sample sizes and if possible to sample at more than one point in time (Waples 1998). In addition, commonly used genetic markers might not sample enough loci to detect the subtle patterns of genetic difference or be able to detect specific gene regions that code for important adaptive differences between populations. A major gap in our understanding is the lack of information on adaptive genetic variation – genes under the influence of natural selection – because these play a key role in determining reproductive success, migration and recruitment within and between populations. Using whole-genome sequencing to detect SNP markers enables an enormous number of independent genetic loci to be sampled simultaneously, greatly increasing the statistical power of data sets. These high-resolution data sets provide a more complete understanding of population structure and the evolutionary process. For example, SNPs have been developed for *Gadus morhua* (Atlantic cod) and numerous adaptive gene regions have been detected and found to be associated with temperature, depth and latitudinal differences (Pampoulie et al. 2006, Nielsen et al. 2009, Bradbury et al. 2010). These studies have provided a new perspective on stock structure.

Whole-genome sequencing techniques have advanced us well beyond the 'needle in a haystack' capability of traditional genetic techniques and can be used to detect significantly more instances of population differentiation and cryptic adaptive diversity. The advantage of high-resolution marker sets is that they detect both genome-wide (neutral) and allele-specific (adaptive) patterns of diversity. Genomic data produced from gene tagging and close-kin analyses for mark-recapture studies are easily transferable and can be used to conduct genetic stock structure analyses of fisheries. The continued use of a population-level approach to understanding genetic variation will provide helpful information about the long-term patterns of stock movements and provide an effective means to monitor levels of genetic diversity within and among fish stocks.

4 ASSUMPTIONS TO BE ADDRESSED / UNCERTAINTIES TO BE MET

Three basic assumptions are required for most forms of population genetics analysis: random mating, a sufficient sample size in terms of the number of individuals sampled from each population or site, and a sufficient number of loci used to differentiate populations. These are relevant both for stock structure and population size studies.

Generally, random mating is assumed. If mating is non-random (e.g. harems for some marine mammals), this needs to be accounted for. Random mating is generally assumed for commercially-harvested fish, but this may not always be the case.

A lack of adequate numbers of individuals sampled for commercially-exploited marine species has been highlighted in at least one recent study, particularly relating to calculations of effective population size

(Marandel et al. 2019). They suggested that approximately 1% of a population may need to be sampled to obtain adequate estimates of N_e.

Close kin mark recapture requires some different assumptions. Random mating is a central assumption for CKMR. While the requirements for the genetic analysis are slightly different relative to gene tagging, it is still necessary to have sufficient loci to be able to identify the close-kin. In addition, the method cannot be used if there are subsets of the adult population that cannot be sampled (if using POPs) and are not accounted for. The year of birth of the juveniles is key, which requires either a good understanding of growth or, preferably, direct ages.

Some methods, such as genetic tagging, may ideally require obtaining the length or age of the tagged fish at the time of tagging. If a hook biopsy method is used, approximate length frequencies of the tagged fish may be able to be inferred by interspersing biopsy and conventional hooks; it may also be possible to do this for age frequencies. At time of recapture, the length (and age) of every individual, both tagged and untagged, should ideally be recorded and compared with each biopsy sample.

5 CRITERIA FOR IDENTIFYING SPECIES OR STOCKS FOR GENETIC STUDIES

For a small number of New Zealand species, an appropriate reference genome or other genetic tools to identify individuals and/or populations has already been developed. These species include:

- Commercial fish species:
    - snapper and trevally already completed;
    - tarakihi and blue cod will soon have reference genomes (sequenced but not yet assembled);
- Protected fish species: giant grouper, white shark, whale shark, spinetail devil ray, oceanic whitetip and basking shark;
- Marine mammals: bottlenose dolphin and potentially others (using work done overseas).

Criteria for further identifying and prioritising species or stocks to sequence include that the stock structure, densities and distributions of such species are largely known, or can be elucidated using genetic studies. Other criteria include the economic value of a stock or species and/or sustainability concerns. To consider conducting genetic tagging studies for individual species, a method for initially tagging fish must also be developed (i.e. a method for taking tissue biopsies). Some methods have already been developed and are understood: e.g. an in-situ longline biopsy method for fish and land-based biopsy methods for pinnipeds. This method is not however suitable for all species (e.g. rig and orange roughy) which rarely interact with longlines. Ling, bluenose, hapuku, bass, snapper, some highly migratory species, and some sharks may be good candidates. Other methods using trawls and pots are expensive. For certain species, in situ tagging is not necessary because the species experiences little trauma when it is brought to the surface; e.g. rock lobsters.

Sampling requirements differ for species that are highly mobile and/or migratory, or sedentary or semi-sedentary. There is a spectrum of mobility characteristics.

Potential candidate species:

- Deepwater species: it is likely to only be possible to use indirect methods for population size; for example, CKMR may be suitable for small populations. Stock structure analyses may be more feasible.
- Inshore species: snapper, tarakihi, blue cod, bluenose, trevally, rock lobster and paua are all potential candidates.
- Protected species: selected shark, marine mammal and seabird species could be investigated.

Species where CKMR may and may not be useful
CKMR may be particularly useful in the case where it is easier to sample juveniles than adults (e.g. large sharks such as the white shark, where only juveniles were genotyped in a previous study (Hillary et al. 2018). This will not be the case for most cetaceans, such as common dolphins. For pinnipeds it may be equally easy to sample adult females and juveniles, but possibly not adult males. Pinnipeds may have fewer reproductively successful males than females; for some sharks the opposite may be true. A preponderance of hyper-successful reproductive adults will bias estimates down. CKMR is currently being used for school shark in Australia, and also Pacific bluefin and Atlantic bluefin.

Genetically-based conventional mark-recapture (i.e. tagging that is similar to conventional non-genetic tagging using physical tags) needs to be non-lethal, whereas CKMR can be either.

Marine mammals that wash up on shore or strand or are caught in trawls usually have small tissue samples taken and these are stored by various New Zealand institutions, such as the University of Auckland, the University of Otago and Massey University. However, some samples may be compromised due to poor handling and processing. Also, animals that strand may not be fully representative of the genetics of the population.

10 million adults is the upper limit in terms of what current SNPs could probably handle both in terms of the cost of genotyping and false-positive rates for kin identification given the number of samples involved. That being said, full genome sequencing will be an invaluable tool in ameliorating the problem of false positives effectively creating an upper bound on "workable" population sizes.

CKMR is also not suitable for very small populations (fewer than 500 adults) due to violations of assumptions of the independence of sampled individuals.

6 COSTS AND COST-EFFECTIVENESS

Population genetics/genomics studies have large upfront costs. However, once the genome is largely sequenced, costs will decline and data recovery will be greater and more accurate.

A possible order of the sequence of operations for developing new genetic/genomic studies is:

1. Develop a reference genome. Costs will vary depending on the genome size; i.e. the number of megabases (Mb, a measure of the length (number of base pairs) of a genome segment, its variability and whether a closely related genome is already available and can be used as a reference during the assembly process). Many of the most common inshore fish species have "smallish" genomes; i.e. a size that is under a gigabase.
2. Conduct a population genomics survey by sub-sampling the population well to capture the existing diversity (this requires about 50–100 samples per location), select polymorphic loci (with a Minor Allele Frequency (MAF) level that is appropriate), and assess the levels of genetic diversity or variation.
3. Develop a Single Nucleotide Polymorphism (SNP) chip, which will require deciding on the number of polymorphic SNPs that should be in the chip; it is likely to vary from 10 000–1 million SNPs/chip.
4. Conduct routine genotyping (repeated long-term on a periodic basis), ensuring that a sufficient number of individual fish are sampled.

    - Note that sequencing costs are less than the analytics for steps 1, 2 and possibly step 3.
    - It may be best to utilise different providers for different steps. There is a need to ensure continuity of providers and that any methodologies developed are transferable between providers.

These processes and costs for comparing conventional and genetic approaches for tagging experiments, and their associated costs, are outlined in Table 1.

Table 1: Comparison of conventional vs genetic tagging approaches for population estimation, including costs

Process	Conventional tag Petersen mark-recapture	Genetic tag Petersen mark-recapture	CKMR population estimation
Developing sample design	Tagging design goal is to achieve homogeneous (random) distribution of tags in population. Estimated precision is determined largely by the number of tag recoveries the design achieves. Design will need to balance release and recovery targets to optimise cost. One-off	Tagging design goal is to achieve homogeneous (random) distribution of tags in population. Estimated precision is determined largely by the number of tag recoveries the design achieves. Design will need to balance release and recovery targets to optimise cost. One-off	Sample design is based on the number of individuals that are targeted to get the needed number of POPs, and/or HSP pairs. One-off
Costs:	*Setup design costs more or less equivalent across all three approaches*		
Tagging method development	There are likely to be costs associated with choosing an appropriate conventional tagging technology for a new species. However, there are a broad range of proven tagging technologies to choose from for which performance criteria are generally well established. One-off	Setup cost for developing genetic markers, most likely SNPs, via GBS or WGS. Depending on the species' genome size and resolution needed, several thousand SNPs will be targeted for each individual. Costs are likely to be far higher than those associated with a conventional physical tagging technology. See text immediately above the table for a possible order of the sequence of operations for developing new genetic/genomic studies One-off	Live release is not necessary, so a wider range of sampling platforms can be used. Again, several thousand molecular markers will need to be generated, most likely SNPs, and thus the setup costs are the same as for the genetic tag method. One-off
Costs:	*Minimal costs, due to availability of proven technologies.*	*1000 samples at 35 NZD per fish for GBS (1% of genome, typically generating 20 000–40 000 SNPs)* *1000 samples 50–60 NZD per fish for WGS (typically generating more than 40 000 SNPs);* *100 000 samples to develop SNP chips would cost about 20–30 NZD per fish* *Note: if protocols for generating SNPs are already developed for the species, then*	*For genotyping sufficient to identify HSPs, about 1500–3000 loci would need to be used at a cost of 20–25 NZD per sample*

Process	Conventional tag Petersen mark-recapture	Genetic tag Petersen mark-recapture	CKMR population estimation
		the setup costs are minimal (e.g. snapper, trevally).	
Population tag release	Most conventional tagging methods require bringing fish to the surface to tag. Although surface tagging allows biometric information (e.g. length) to be collected for each fish at the time of tagging there is typically a significant level of mortality associated with the surface tag-release process which needs to be factored into the release design (e.g. by over-tagging).	Genetic tags offer a significant advantage over conventional tags if genetic samples can be collected at-depth (e.g. through the use of biopsy hooks) such that the tag-release mortality is negligible. This will be particularly important for deep water species. Gene tagging hooks at depth can be in intersected with conventional hooks to get biometric length data of fish caught at the same time, and then lengths of gene-tagged fish at depth can be estimated based on that.	Live release is not required, so the tagging methodology can be much simpler. Fish can be brought to the surface and sampled, and then either released or kept.
Costs:		*There are potentially additional logistical and validation costs associated with biopsy tagging, which will mean the release cost per unit tag is likely to be higher than conventional tag release, but this cost is likely to be offset by not needing to release additional genetic tags to off-set surface release mortality.*	
	The unit cost of a typical conventional tag (e.g. PIT tag) is typically 2–5 NZD.	*Gene hooks 1–2 NZD* *Initial sample storage and preservation cost 1–3 NZD per sample.* *DNA extraction followed by sequencing. This should ideally be done via reduced representation libraries, a SNP chip or whole genome sequencing.* *20–60 NZD per sample depending on throughput*	
	Vessel and other logistical costs per unit tag release 30-35 NZD	*Vessel and other logistical costs per unit tag release 35-40 NZD*	
	Cost of an additional study to estimate release mortality	*Tag survival study potentially not required*	*Tag survival study not required*

Process	Conventional tag Petersen mark-recapture	Genetic tag Petersen mark-recapture	CKMR population estimation
	0.5–1 million NZD		
Tag recovery	Tag recovery requires scanning the requisite number of fish for tags within a designated timeframe (sample units typically being commercial and recreational catches). Scanning typically requires a logistically complex dedicated recovery programme, and for cryptic tags may require the use of complex and costly scanning equipment.	Tag recovery requires scanning the requisite number of fish for tags within a designated timeframe (sample units typically being commercial and recreational catches). Scanning typically requires a logistically complex dedicated recovery programme. A tissue sample will be collected from each fish examined for genetic assay. Biopsies via harpoons operated from smaller boats may also be used for marine mammals.	Samples can be taken from commercial fishing operations so there is a wider array of options, relative to conventional or genetic tagging.
Costs:	*Typical one-off scanner unit cost: 5–50k NZD* *Unit cost of scanning one fish as part of a dedicated recovery programme: 1.0 NZD*	*Unit cost of collecting one fish tissue sample as part of a dedicated recovery programme: 1.0 NZD* *Initial sample storage and preservation cost (as above) NZD 1–3 per sample* *DNA extraction followed by sequencing (as above)* *NZD 20–60 per sample, throughput dependent*	
Analyses and reporting	Analysis report writing, presentation	Analysis report writing, presentation	Analysis report writing, presentation
Costs:	*Costs likely to be similar across all three approaches*		
Costed example tagging programme designs Design goal: recover 500 marks from a population of 55 million adult fish	Typically lower catch examination costs associated with conventional tags favour a high catch examination to number of tag releases ratio. Release target: 33 000 to achieve effective release of 28 000 Scanning target: 1 000 000	Typically higher catch examination costs associated with genetic tags favour a lower catch examination to number of tag releases ratio. Release target: 110 000 (assumes 100% survival) Scanning target: 250 000	Initial sampling should be designed to obtain 50–100 kin pairs; more detailed design studies may be needed to reach specific goals or accuracy thresholds.
Costs:	*Survival study:* *1 000 000 NZD*	*Initial SNP sequence development includes cost of at-sea tissue collection programme:*	*Sample size depends on adult population size and demography so total cost will vary from case to*

Process	Conventional tag Petersen mark-recapture	Genetic tag Petersen mark-recapture	CKMR population estimation
	Tag scanners (50k NZD): 200 000 NZD *Tag releases (35 NZD tag): 1 155 000 NZD* *Tag recovery (1 NZD tag): 1 000 000 NZD* ***Total: 3.36 million NZD***	*1 000 000 NZD* *Tag releases (25 NZD tag): 2 750 000 NZD* *Tag recovery (25 NZD tag): 6 250 000 NZD* ***Total: 10 million NZD***	*case. A population size of ~10 000 000 would cost about 1 million NZD to process.* *Unit numbers are comparable the genetic sampling method.*

Notes:

i) Initial SNP sequencing has already been completed for New Zealand snapper (and some other species).
ii) For CKMR, genetic sequencing costs are probably no more time consuming or expensive, but the costs of analysing the data are likely to be higher. The quality control process and kin identification are difficult to completely automate and require of the order of 0.15–0.2 FTE of a qualified statistician/bioinformatician's time to fully process to the CKMR modelling stage, with the same amount of time to do the CKMR abundance and demography modelling.

7 RISKS AND RISK MITIGATION

Risks
Projects may fail at any stage. It is therefore important to design studies so that they can produce usable results, even if the overall objective is unable to be met.

Genetic analysis processes require considerable quality assurance and quality control (QA/QC) to ensure that results can be obtained and are valid. In particular, handling and operational procedures to collect and process samples need to be considered as these can affect the ability to extract viable genetic material.
Sample size (number of individuals sampled) seems to be particularly problematic for many studies involving marine fish species. In Australia, getting good samples in sufficient numbers to enable genetically-based conventional mark-recapture was an issue for some species such as Spanish mackerel.

The same applies for CKMR studies. Some types of analysis will require larger sample sizes; this is case-dependent. For example, larger sample sizes will be needed in order to ensure a sufficient number of matches if only juveniles are sampled.

Risk mitigation
Feasibility studies should be conducted for completely unmapped species. The first step is to determine the size of the genome, as well as the levels of genetic variability. If there is little variability, the study is unlikely to yield useful results. If there is considerable variability, costs will be high.

For close kin analysis it may not be necessary to have a reference genome; instead a reduced representation GBS method can be used. Less sequencing is needed for small populations; e.g. sharks or bluefin tuna. The required sample size is roughly proportional to population size.

Existing genetic resources makes it cheaper to conduct whole genome sequencing rather than partial genome sequencing going forward.

8 EFFECTIVE POPULATION SIZE

The genetically effective population size (N_e) is the equivalent number of breeding adults, and is typically much smaller than the census population size. The strength of genetic drift – a constant process that eliminates genetic diversity from a population – is determined by the N_e. A low N_e can lead to loss of genetic diversity or variation. It has been suggested that if N_e falls below 50 individuals, it would be

difficult for a species to recover, and that a population's N_e should be rebuilt to at least 500 (Frankham et al. 2017). However, these models have been developed for threatened terrestrial species and it is unclear whether they are appropriate N_e thresholds for marine fish species. A number of papers have indicated that N_e/N is very low for commercially-fished marine species (Hauser & Carvalho 2008 and references therein). However, such estimates have been called into question in recent studies. For example, Waples et al. (2018) compared CKMR true adult abundance with various estimates of N_e for southern bluefin tuna, and found that N_e/N was at least 0.1, and possibly as high as 0.5. Marandel et al. (2019) submitted that most estimates to date are likely to be highly negatively biased, due to low sample sizes. They suggested that approximately 1% of a population may need to be sampled to obtain adequate estimates of N_e – thereby probably making it infeasible to estimate N_e for fish populations that range in the millions or billions.

More work needs to be done to understand how a fishery's N_e relates to the "soft limit" of 20% of the unfished stock size (B_0), which is used as a biomass limit in fisheries management. Similarly, does a reduction in population to a level above 20% B_0 cause a significant loss of diversity? To address these questions it will be necessary to determine the average number of successful breeders per generation in a stock. This could be achieved using genetic information obtained from a comprehensive genetic tagging study that sampled both adults and juvenile offspring. A parentage analysis would enable a direct estimate of N_e and the population genetic data from a stock could be used as an indirect estimate of N_e. This type of analysis could be used to correct indirect N_e estimates obtained from other populations.

Other considerations to note are first, that a reduction in population size also has the potential to disrupt population genetic structure that may have built up over millennia. Second, it has been posited that intensive size-selective fishing could cause directional selective changes that result in populations evolving into smaller fish that reproduce at younger ages (Allendorf et al. 2008). Overall from a fisheries management perspective it is not particularly harmful to over-split a genetically panmictic stock. In fact, there could be a more significant loss of genetic diversity if distinct genetic stocks are treated as a single panmictic stock. Fisheries management should aim to maintain genetically distinct local populations at a size that does not cause a high rate of genetic diversity loss.

Estimating the effective population size for marine mammals using genetic methods

Abundance estimation remains a challenge for the conservation management of wildlife populations, particularly for widely distributed populations with highly mobile individuals that are difficult to observe such as marine mammals (Bravington et al. 2016b). As an alternative to traditional and expensive methods to estimate abundance (i.e. distance sampling and mark-recapture), genetic methods are increasingly being used.

The two key variables to be estimated when deriving an abundance estimate from genetic methods include the effective population size (N_e) and the census size (N_c). Here N_e is defined as the size of an idealised population which would lose genetic variation or become inbred at the same rate as the actual population (Fisher 1930, Wright 1931). N_c is defined as the total number of individuals in the population.

Traditionally, estimating contemporary N_e was based on small genetic datasets, such as microsatellites (Luikart et al. 2003, Barbato et al. 2015). Due to the recent availability of high-resolution SNP datasets and refinement of several genetic estimators, the accuracy of contemporary N_e estimates of have substantially improved (Luikart et al. 2003, Barbato et al. 2015).

Until recently, most estimates of N_e have used the two-sample (temporal method), which depends on random changes in allele frequency over time (Do et al. 2014). However, recently several new single-sample estimators have been developed and are being widely used (Tallmon et al. 2008, Do et al. 2014). One single-sample estimator which is often used is the the linkage disequilibrium (LD) method in NeEstimator (Do et al. 2014). The LD method is based on the assumption that genetic drift is responsible for the linkage disequilibrium at independently segregating loci in a finite population (Waples & Do 2010). Another single-sample estimator which is often used is the sibship frequency (SF) method (Wang 2009) in Colony (Jones & Wang 2010). The SF method was proposed to infer N_e from the sibship frequencies estimated from a sibship assignment analysis (using the multi-locus genotypes of a sample of offspring

taken at random from a single cohort in a population) (Wang 2009). This estimator is more flexible than the LD method as it can be applied to populations with non-overlapping generations of both diploid and haplo-diploid species under random or non-random mating, using either codominant or dominant markers (Wang 2009). It can also be applied to the estimation of N_e for a subpopulation with immigration (Wang 2009). Despite this, the LD method is considered simpler to calculate than the SF method (Wang 2016). A recent study used extensive simulations to compare the biases and accuracies of multiple single-sample estimators (including the LD and SF methods) for different population properties and showed that the LD and SF methods are much more accurate than other methods available (Wang 2016). However, as pointed out by Waples (2016), the methods used by Wang (2016) still require further development, as they do not account for overlapping generations and are connected to other populations by migration.

9 eDNA AND POTENTIAL APPLICATIONS

eDNA is defined as all the genetic material that can be recovered from an environmental sample. eDNA can originate from multiple species and from a variety of biological sources including whole organisms, biological secretions, reproductive propagules, shed skin, degrading tissue or free DNA molecules. Once extracted, the information embedded within the DNA provides a lens through which to study the organisms that were present within that environment. Accordingly, the dependence of traditional biological surveys on retrieving whole organisms and the complexities associated with morphological identification (e.g. time-consuming microscopy, difficulties identifying different life stages and sexes, and cryptic species), is largely overcome. The application of eDNA in marine research is expanding rapidly on the international stage and is particularly well suited to marine ecosystems.

The use of eDNA was discussed in Section 2 only as it related to estimates of population size. However, the applications of eDNA extend into numerous other areas of fisheries as it relates to a wider understanding of diet, species assemblage and networks. This section will speak to how eDNA might be used in this capacity as it relates to fisheries management in its broadest sense. While some long-term persistence is likely in sediments, to date the degradation of eDNA in seawater is very rapid and eDNA is detectable for hours rather than days, but will be very dependent on the environmental conditions.

1) Species detection: eDNA of fish has been compared to numerous other survey methods including cameras, trawls and electro-fishing. Like all existing methods, eDNA has its 'blind-spots' – for example, it is imperative that the correct PCR assays are used to detect target taxa. As a method it seems to be very good at detecting cryptic taxa, juveniles and bait fish which are difficult to identify using other methods. In addition to fish, eDNA has been applied in the detection of elasmobranchs and marine mammals.

2) Assemblages: related to the area of species detection is the ability to build a wider assemblage data for any given site. The co-occurrence of fish or their wider prey groups (e.g. coral, zoo plankton) can provide insights into the wider ecological matrix that might surround focal areas (e.g. on/off a seamount, or in nursery areas).

3) Diet. DNA-based metabarcoding approaches have been used to study the diet of a number of fish and mammal taxa. Building up a picture of what taxa eat and how this varies spatially and temporally is increasingly being used to for example i) model how juvenile fish use their habitat, ii) identify the food items that might be used in aquaculture and iii) determine whether diets are changing with a changing climate or disease.

4) Marine Protection: Marine protected areas (MPAs) are an integral part of global efforts to preserve ocean biodiversity. It is well recognised that there is an urgent need for more effective whole-ecosystem biomonitoring tools to better manage and understand the benefits of MPAs relative to designated fishing areas. It is likely that within a decade eDNA technologies will develop to the point where they offer a powerful new lens on species diversity and (with the aid of time-stamped data) will generate data that has a predictive function. Like existing methods to study marine protection, eDNA will become even more

powerful once multiple data streams (e.g. abiotic data) are integrated. These insights will provide the evidence needed for more informed decision making surrounding ocean resources.

The value of eDNA for addressing many areas of fish and marine ecology means that there is an increasing awareness of the need to collect and store environmental samples. Autonomous water sampling/filtering devices are being deployed and these have extended onto ROVs and remotely operated gliders. It is important to recognise that even in its current state of development eDNA is delivering meaningful data in many areas of marine management including invasive species detections, disease detections and biotic surveys. With further development, eDNA has the potential to contribute to the areas of stock assessment, spawning estimates, prioritising conservation areas and quantifying impacts of fishing. Importantly, as genetic databases continue to grow, the resolving power of eDNA will continue to grow with it.

10 GENETIC ANALYSIS CAPABILITY WITHIN NEW ZEALAND

In general, the Crown Research Institutes (CRIs) in New Zealand have solid expertise in genetic analysis and pedigree reconstructions based on relatedness values, though most of this expertise is currently being applied to breeding programmes in the primary industry sectors focussed on horticulture (e.g. Plant and Food Research (PFR) breeding programmes on kiwifruit and apple), terrestrial livestock (e.g. AgResearch breeding programme on sheep) and forestry (e.g. Scion breeding programme on pine). In recent years, some of this has also been applied to fisheries species or aquaculture species (PFR: snapper, trevally, blue cod; NIWA: kingfish and hapuku; AgResearch: Chinook salmon; Cawthron Institute: aquaculture species). In addition, all New Zealand universities have staff experienced in the use of genetic analyses to investigate species demographics and related patterns. For example, Victoria University of Wellington has a fisheries genetics research group, which collaborates with scientists in PFR and NIWA and Massey, Auckland, Waikato and Otago Universities all conduct marine genetics research. Some universities, for example The University of Auckland, have dedicated institutes focussed on marine science (e.g. the Leigh Marine Laboratory and the Institute for Marine Science).

Some of the CRIs and universities have sequencing facilities in-house to support small scale projects, but samples are commonly sent overseas, particularly for larger projects (e.g. over 500 samples) due to time and costs savings. The national infrastructure platform Genomics Aotearoa (https://www.genomics-aotearoa.org.nz/) supports a collaborative national network to ensure that New Zealand is internationally participating and leading in the rapidly developing fields of genomics and associated bioinformatic analyses.

11 FUTURE CONSIDERATIONS

Species to be considered for genetic analysis now and in future
In order to reduce costs, it is probably expeditious to focus initial consideration on fish and marine mammal species where the genome has already been mapped. However, we should also encourage the rapidly-developing progress in this area, and revisit the list of species with well-characterised genomes in the future to determine whether there might be other candidates for consideration. It will also be important to identify and continue to research alternative sampling methodologies, and address issues such as species with complex reproductive strategies, species with very large or very small population sizes, and species with pronounced sub-population structuring.

On the horizon
There are a number of potential future developments in the field of genetics that should be closely tracked. A comprehensive review on the power of genomic techniques to inform the management of fisheries resources was provided by Bernatchez et al. (2017), and provides a good overview of the state of the art and the various genomic applications that can be carried out. Ovenden et al. (2015) has also provided a comprehensive review of the uses of genetics for a variety of fisheries issues. There was insufficient time to discuss all such developments in this workshop, but there are a number of novel uses of genetics already

underway. For example, age-associated changes in DNA are well-documented. It is possible that these "methylation sites" could eventually be used to determine the chronological age of individuals. This would greatly augment the utility of genetic mark-recapture experiments as the initial biopsy samples could also be used for ageing.

Methods for quantifying eDNA are also rapidly developing and, while a number of issues with obtaining quantitative estimates suitable for estimating population size from eDNA samples were identified in this workshop, future progress should be closely tracked.

Sampling methods for obtaining biopsies should also be further developed. For example, this workshop only identified one way of obtaining tissue in genetic tagging experiments; namely, the use of biopsy hooks. Are other techniques possible, particularly for fish species or fish sizes that are not well-sampled using longlines? Trawls with an open cod end and a tunnel in it that funnels fish through one at a time and either inserts a tag or takes a biopsy have already been tested for some specific uses.

Conclusion

The output from this workshop should be evaluated to determine the priorities for current fisheries research involving genetics, and future workshops of this nature should be conducted periodically to summarise recent and emerging developments. In particular, the costs of gene sequencing have decreased substantially in recent years, and continue to decline. The easier it becomes to sequence large numbers of samples rapidly, the less expensive sequencing will become, and the easier it will be to incorporate genetic analyses into studies of population dynamics of fish and marine mammals.

12 ACKNOWLEDGEMENTS

We would like to thank Gretchen Skea for assisting greatly with the logistics of this workshop and Lyndsey Holland for helpful comments on the report and compiling most of the glossary. We also thank all workshop participants for their valuable insights.

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APPENDIX A. GLOSSARY

[Adapted and expanded from Ovenden et al. 2015]

Allozymes – Genetic markers based on visualisation of allelic variants of enzymes (a type of protein) encoded by DNA.

Allele – Alleles are variants at specific loci (see below). Diploid individuals have two copies of an allele at each locus, one from each parent. The alleles can be identical (the individual is homozygous) or they can differ (heterozygous).

Candidate gene – Gene that is suspected to have a direct functional relationship to a given trait.

Close Kin Mark Recapture (CKMR) – Close-kin mark-recapture is a recently developed method for estimating abundance and demographic parameters (e.g. population trend, survival) from kinship relationships determined from genetic samples.

Conspecifics – Two or more individuals belonging to the same species.

Cryptic species – Closely related but genetically dissimilar species that are morphologically difficult to distinguish.

DArT Seq – Diversity Array Technology Sequencing (a form of reduced representation sequencing).

eDNA – environmental DNA; residual DNA collected from environmental samples (e.g., soil, seawater), as opposed to being directly sampled from the tissue of an individual organism.

Fitness – A measure of the relative ability of an individual to survive and reproduce. Normally measured as the number of offspring contributed to the next generation.

FST – Measure of genetic population sub-division, ranging between 0 and 1. Generally, FST < 0.03 indicates little genetic sub-division whereas FST > 0.15 indicate large genetic sub-division.

GBS – Genotyping By Sequencing (a form of reduced representation sequencing).

Genetics – the study of specific and/or a limited number of genes or gene regions.

Genetic tagging – Using individual genotypes as tags to be recaptured, but otherwise analysing the data in much the same way as conventional mark-recapture models.

Genetic diversity – The amount of allelic variation at one or more genetic loci. It may be calculated for an individual, a population or a species.

Genetic drift – evolutionary process resulting from changes of allele frequencies in a population caused by random sampling of alleles between generations. Genetic drift may result in the loss of some alleles (including beneficial ones) and the fixation (increase to 100% frequency) of others, and is most pronounced in small populations, where it typically leads to loss of genetic diversity.

Genome – The complete DNA sequence contained in an organism.

Genomic resources – The available DNA sequences for a particular species in the public domain, from single gene regions to entire genomes.

Genomics – The study of the entirety or a large proportion of an organisms genes. Mitogenomics refers to the study of the complete mitochondrial genome specifically.

Genotyping – A laboratory procedure to determine the genetic composition of an individual at specified genetic loci.

IBD – Identical By Descent, in genealogical studies referring to a matching DNA sequence resulting from inheritance from a common ancestor, without recombination. May also refer to Isolation By Distance in genetic connectivity studies.

kbp – thousand base pairs.

Locus (plural loci) – the location in the genome of specific genes.

MAF – Minor Allele Frequency.

Microarray – A high-throughput platform for measuring genetic diversity and gene activity. Short pieces of DNA (probes) representing genes of interest are attached to a substrate. The probes hybridise and fluoresce as a sample containing DNA of interest is washed over the slide. This provides information about the genes that are present in the sample.

Microsatellites – A type of genetic marker consisting of short repetitive sequences such as ACACA... . Alleles differ in the number of repeats, which are detectable as length variation.

mtDNA (mitochondrial DNA) – A circular genome external to the cell nucleus within mitochondrion organelles in the cell cytoplasm. mtDNA is maternally inherited, haploid, and multiple copies are present in each cell.

Mutation rate – The instantaneous rate at which nucleotide changes occur in the genome.

N_b – Effective number of breeders.

N_c **or** N – Census population size in numbers.

N_e – Effective population size in numbers: the number of individuals in a population contributing offspring (and thus genetic material) to the next generation.

Next-generation DNA sequencing – Novel technologies for rapidly revealing the DNA sequence of large components of genomes.

Nuclear DNA (nDNA) – DNA occurring in the cell nucleus in the form of chromosomes, as opposed to mtDNA that occurs outside the nucleus in the mitochondrion.

Nucleotide – Basic building block of DNA that exists as A, T, G or C (Adenine, Thymine, Guanine and Cytosine). In RNA, U (Uracil) replaces Thymine.

Parentage analysis – The process of identifying the pedigree relationships among individuals based on observed genotypes and the principles of Mendelian inheritance.

PCR (polymerase chain reaction) – Laboratory technique that uses a polymerase enzyme to make multiple copies of a target DNA sequence from a small amount of starting material.

Phenotype – The physical characteristics of an animal, which are a determined by its genotype and the environment.

Phylogenetics – The study of evolutionary relationships among organisms (typically species). Molecular phylogenetics uses genetic sequence data to infer relationships, phylogenomics uses entire genomes to reconstruct evolutionary relationships.

PIT tag – Passive integrated transponder tag capable of short distance radio broadcast of unique digital id to a radio receiver.

Polymorphism – A gene is said to be polymorphic if more than one allele occupies that gene's locus within a population.

PoP – Parent offspring Pair. The ability to genetically link parents and offspring enables the possibility of estimating population numbers based on the number of successful pairings between a random sample of adult (spawner fish) drawn from a population and second random sample of 1+ juveniles.

Population Genetics – The study of genetic variation between and within populations, typically by comparing allele frequency variation at multiple loci.

RAD-Seq – Restriction site Associated DNA Sequencing (a form of reduced representation sequencing).

Real time PCR (real time quantitative PCR) – Fluorescence detection is used to measure the progress of a PCR reaction as the targeted DNA molecule increases in abundance. The technique is used to estimate the amount of DNA or RNA initially present in a sample.

Reduced representation sequencing – partial genome sequencing; produces a sample of short, unconnected fragments, scattered at random across the genome.

Sibship reconstruction – method used to determine full-sib and half sib familes

SNP (single nucleotide polymorphism) – Variation in the DNA sequence (i.e., the nucleotide identity, typically A, T, C or G) at a single nucleotide position.

SNP chip – a type of DNA microarray which is used to detect SNP polymorphisms within a population.

Stock – Specific to fisheries management, a stock is recognised as a sub-set of a biological species population for which the processes of reproduction, somatic growth and death are homogeneous and relatively contiguous in space and time. Two or more spatial populations of fish, belonging to the same species, may be considered to constitute separate stocks if there is evidence to suggest that the level of movement (diffusion) between the areas is slight such that the life processes observed within each area (i.e. recruitment, growth and mortality) are significantly distinct. The term ‘stock’ should be regarded as a management definition rather than a classical biological definition. Certainly the term `stock’ should not be assumed to imply genetic isolation.

WGS – Whole Genome Sequencing.

APPENDIX B. PROS AND CONS OF PHYSICAL, GENETIC AND PHOTO-ID TAGGING FOR FISH AND MARINE MAMMALS.

Assumption	Physical tagging	Genetic tagging	Photo-id tagging
The size of tagged fish or mammal is known	Length of tagged fish typically known	Direct measurement of biopsied fish difficult unless individuals are brought on board a vessel in very good condition and can be measured alive. Application of indirect measurement methods likely to be required	N/A: Size not relevant to marine mammals
The survival probability of each tagged fish or mammal is known with high certainty	Initial tag survival often not known and difficult to estimate	*In situ* or indirect marking offered by genetic methods eliminates this source of uncertainty	N/A: Not relevant to photo-id studies
Fish do not lose their marks; marine mammal marks are not lost or missed	Often an issue with physical tags: needs to be estimated	Tag retention 100% certain - except that some samples may be degraded; this can be corrected for by assuming random distribution between tagged and untagged fish	Often an issue for photo-id - mark might change; dependant on photo-quality and distinctiveness criteria
All recaptured tagged fish or marine mammals are recognised and reported	Physical tag scanning systems are seldom 100% effective - easy to introduce bias	Scanning success of genetic markers potentially superior but unknown for snapper - bias unlikely	Might not recognise or catalogue all individuals
Tagged and untagged animals behave in the same way and have the same survival probability	Behavioural modification and ongoing health effects associated with conventional tags and tagging procedure are a significant source of uncertainty	Genetic tagging potentially 100% satisfies this requirement	Photo-id potentially 100% satisfies this requirement
Mark ratio (recapture probability) is homogeneous across the entire spatial stock range	This assumption may be violated because of slow mixing, non-random distribution of tags, and partial coverage of the available habitat (e.g. where depth-related trauma may limit the areas where physical tagging can occur)	Significantly fewer restrictions on where tagging can occur if in situ and depending on the species, but other issues still apply	Slow mixing, non-random distribution of tags, and partial coverage of the available habitat are all issues
Tagged and untagged individuals observed during a given sampling occasion have the same probability of recapture on the next occasion	Can be violated using physical tags if individuals become 'trap-happy' or 'trap-shy'	Less likely to be violated, but individuals biopsied by hook may become hook-shy	Can be violated using photo-id if individuals become trap-happy or trap-shy
Tagged and untagged individuals have equal probability of survival between sampling intervals	Natural mortality may vary with age, so older age classes may have lower probability of survival	Capture probability can affect survival (i.e. animals only captured once may not have survived)	Capture probability can affect survival (i.e. animals only captured once may not have survived)
Invasiveness	Yes	Possibly	No
Lethal/non-lethal	Potentially lethal	Non-lethal	Non-lethal

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