

Population genetics: Basics and future applications to fisheries science in the Pacific

Giulia Anderson,¹ Jed Macdonald,² Joe Scutt Phillips³ and Simon Nicol⁴

Quantitative genetics and genomics provide an under-utilised avenue for monitoring capture fisheries. While most fisheries assessment tools use macro-scale observations as a proxy for the health, distribution and delineation of fish stocks, genetics directly measures the in situ molecular footprint of population health and structure. Genetics does not replace other forms of fisheries monitoring, rather it provides clarity and quantification of previously qualitative observations. As the Pacific region increasingly assimilates population genetics into its research toolkit, it is useful to briefly review population genetic analyses and their likely application in the fisheries science undertaken for member countries and territories of the Pacific Community.

Introduction

An individual's DNA⁵ serves as inherited genetic instructions for development, functioning, growth and reproduction. DNA very occasionally mutates, creating variation in these instructions. Each instance of variation contributes to genetic diversity at the individual and population scale. In turn, genetic diversity is the basis of most genetic inferences. Some types of variation produce differences in an individual's physical characteristics or fitness, while other types have no measurable consequences. Variations can be as small as the switch of a single nucleotide (the building block unit of DNA) in the genetic code, as complicated as the inversion of a DNA sequence, or involve the duplication (or deletion)

of entire segments of the genetic code. Because there are so many different ways for DNA to vary, the overall genetic code is unique per individual. There are also two different genomes contained within a cell, nuclear and mitochondrial, each with different patterns of variation. Genetic analyses take advantage of all of these characteristics. Moreover, the processes that generate and spread new mutations in DNA provide a basis for understanding how species evolve, and how populations develop differing traits and adapt to changing environments and stressors.

At its most conservative, DNA diagnostics can provide an accurate identification of a specimen's species, which is useful when other taxonomic features are missing (Fig. 1).

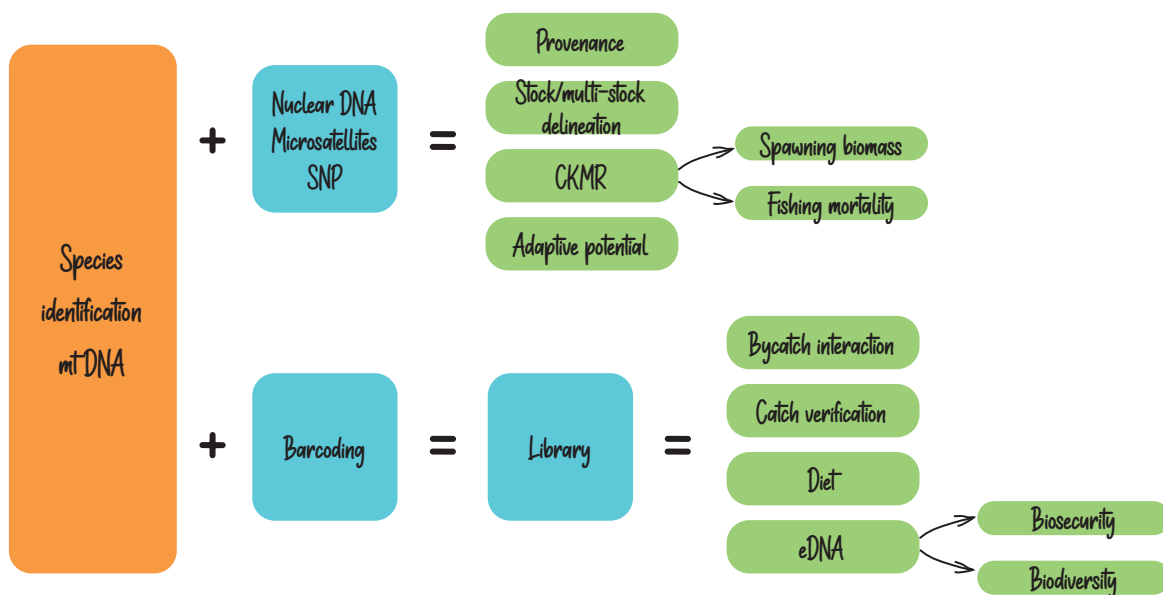


Figure 1. Schematic of fundamental applications of genetics.

¹ Fisheries Molecular Geneticist, SPC. Author for correspondence: GiuliaA@spc.int

² Senior Fisheries Scientist (Tuna Biology and Ecology), SPC

³ Senior Fisheries Scientist (Statistical Modelling), SPC

⁴ Principal Fisheries Scientist (Fisheries and Ecosystem Monitoring and Analysis), SPC

⁵ The definition of DNA and other words or acronyms specific to genetics can be found in the glossary at the end of this article.

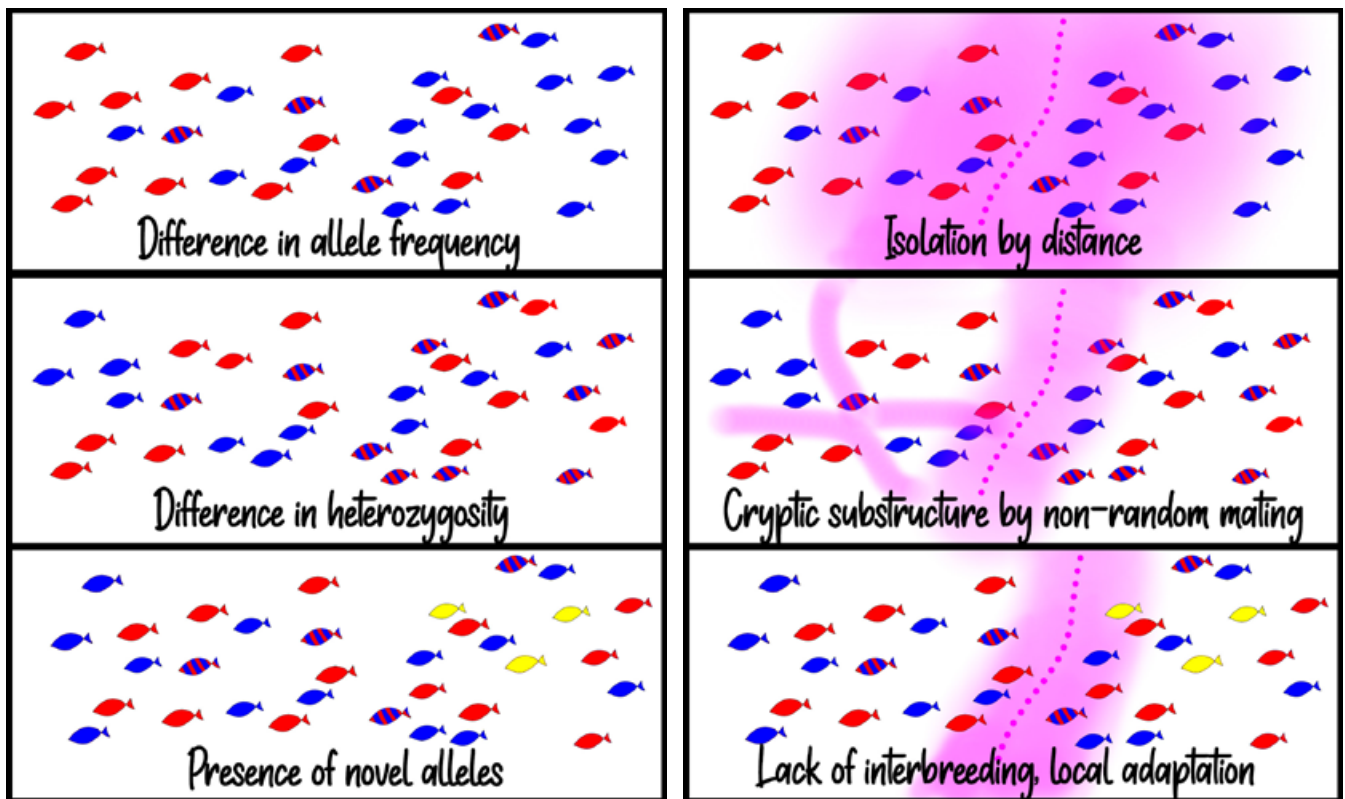


Figure 2. Examples of genetic patterns that indicate the presence of population structure (left panel), and how they might be interpreted (right panel). Different coloured fish represent the expression of different alleles. Dotted pink lines indicate proposed stock delineations; pink gradient indicates degree of uncertainty in where the actual divisions lie.

In more complex applications, genetic information can be used to evaluate the differences among individuals of the same species (Fig. 2), with implications for delineating the boundaries and estimating the degree of connectivity among populations. At its most powerful, the theory of population genetics provides an in-depth understanding of the relatedness of individuals and can directly estimate population size and mortality (Fig. 1).

The increased feasibility of incorporating a genetic perspective into fisheries monitoring, stems from the recent development and commercialisation of “high throughput” DNA sequencing technologies. The resulting increase in DNA processing capacity, reduction in cost, and greater quantities of data output make genetic assessment accessible, convenient and informative. An appealing argument for incorporating molecular approaches in fisheries science is the ability to avoid steps in data collection and analyses associated with other approaches that often introduce bias or weaken inference. However, molecular methods are also only effective if applied accurately and with appropriate expectations of how results can be interpreted.

Here we provide an introduction on genetic applications for fisheries management.

What are the steps in DNA analysis?

Studies that wish to incorporate DNA analyses have many protocols available, and these can be broadly classified into one of two methods of collecting information. *Traditional* methods rely on variations in the length of key fragments of DNA that indicate differences in the underlying genetic code. *Sequencing* methods (most notably next generation sequencing, or NGS) directly assess the genetic code itself. Some basic steps and details are common to both methods (see also Fig. 3).

- **Common Step 1.** Extract a sample of tissue that is not contaminated by foreign DNA. Because genetic analyses focus on genetic diversity, and the introduction of foreign DNA also introduces foreign genetic variation, analyses using contaminated samples will be seriously compromised. Contamination can easily occur by touching tissue samples taken from one organism with equipment that previously came in contact with other individuals of the same or similar species, or other, non-sterile surfaces.
- **Common Step 2.** Extract the DNA. This can be done with commercially available kits and a few specialised pieces of equipment, or can be outsourced to specialised facilities for automated, high-volume sample processing.

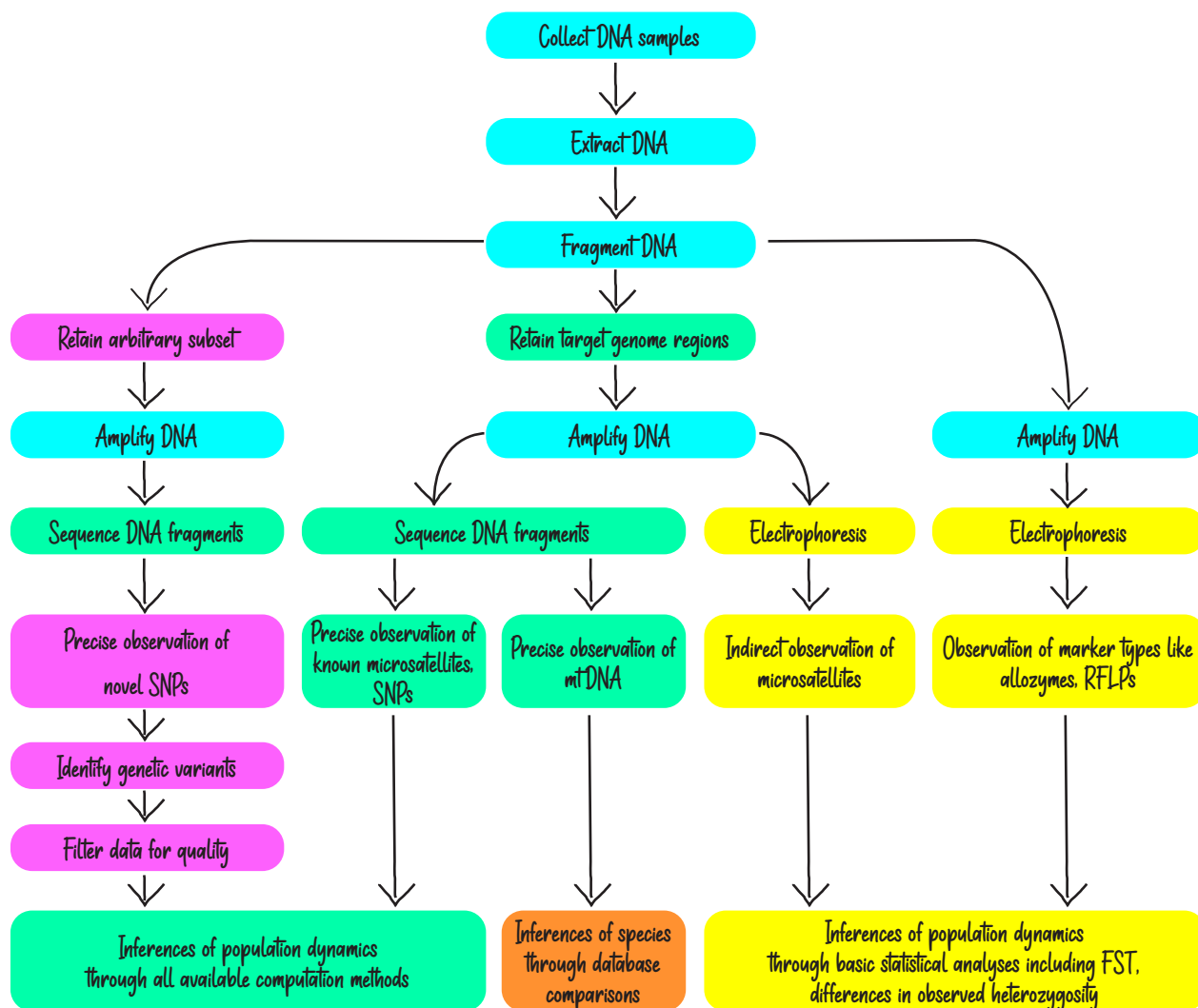


Figure 3. Basic steps in genetic analyses.

- Blue = common steps
- Yellow = traditional marker types/studies
- Green = sequencing marker types/studies
- Pink = NGS *de novo* sequencing (best method for producing maximum inference with limited existing information/initial investment)
- Orange = analyses specific to mitochondrial DNA (mtDNA) sequence data.

- **Common Step 3.** Fragment DNA. Traditional and NGS protocols use the same techniques to cut DNA into fragments, although for different reasons. Traditional methods directly infer the presence of genetic variation based on fragments produced in this step; next generation technology carries physical limits in the length of DNA strands that can be sequenced, and so requires that DNA be cut to a particular size. Both methods can also use fragmenting strategically to target (to either cut or not cut) particular genetic regions.
- **Optional Step 3b.** Select a subset of DNA for further analysis. This can be done by either targeting key regions of interest (especially in conjunction with targeted fragmenting), or filtering DNA fragments based

on arbitrary parameters (e.g. a particular length). Benefits of subsetting include discarding low-quality or uninformative DNA fragments that otherwise introduce “noise” into the data, and streamlining resources, effort and monetary investments associated with DNA processing.

- **Common Step 4.** Amplify the DNA. All protocols use some variation of polymerase chain reaction (PCR) to make many more copies of each DNA fragment. Although this is not an essential step in numerous traditional protocols, it makes it easier to identify genetic bands later. PCR is essential to NGS. In all cases, this amplification also reduces the need for large original volumes of tissue.

Traditional and NGS protocols begin to deviate at this point.

- **Traditional Step 5.** Visualise DNA fragments using gel electrophoresis. DNA will separate in isolated bands along the gel based on length and electric charge.
- **Traditional Step 6.** Call genotypes (often short-handed by turning “genotype” into a verb: e.g. “submit the raw data for genotyping”). Genetic variation among specimens can be inferred from the differences in distribution of DNA fragments across the gel. Each variable DNA fragment on the gel is interpreted as a version of that genetic segment (a variant or allele at that locus). It is also noted if each specimen carries one or two versions per segment, which determines if it is homozygous (one allele) or heterozygous (two alleles) at that locus.
- **Traditional Step 7.** Analyse data.

Because marker types associated with traditional methods produce limited data, they can only be analysed with simple statistics that produce low-resolution inferences. If using NGS methods, sequencing, genotyping and interpreting data are all much more involved.

- **NGS Step 5.** Sequence selected DNA fragments, nucleotide by nucleotide. This amounts to reading the very building blocks of the genetic code, brick by brick. Currently, the most popular protocol is genotyping-by-sequencing. The technology involved in this step is quintessential to the high-throughput sequencing revolution. Conveniently, it is standard to outsource NGS work to industrial laboratories.
- **NGS Step 6.** Identify variable genetic regions and call genotypes using automated software. The process requires tremendous computing power and is typically done using a “supercomputing” cluster as part of a sequencing laboratory’s standard services.
- **NGS Step 7.** Quality check and filter variant data. Datasets are subjected to a series of tests with thresholds determined by the available data quality and intended analyses.
- **NGS Step 8.** Analyse data.

NGS produces large volumes of data that can improve statistical power, and allows for in-depth analyses that capture the complex patterns that exist in DNA variation. Analyses that benefit from or depend on increased computational power include clustering algorithms, principle component analyses, multivariate analyses, analyses of variance tuned to molecular data (AMOVA), migration and bottleneck estimations, population assignment, and species identification.

What are the types of markers and analyses?

Variation in the genome occurs in multiple dimensions that are measured using different tools or marker types. For example, allozymes mark genetic variation based on the final structure of produced proteins. Microsatellites observe patterns of repeating nucleic sequences for variation in physical length. Single nucleotide polymorphisms (SNPs) identify nucleotide substitutions in the genetic code itself. Each type of variation, and the associated marker type, differs in characteristics such as mutation rate, evolutionary selective pressure, and the number of possible variants per locus. The differences determine what type of marker is useful to a research question. For example, microsatellites have a higher mutation rate than SNPs, making microsatellites useful indicators of recent demographic changes, and SNPs more useful for exploring historical patterns. Alternatively, many loci in mtDNA have such a high mutation rate that when tested in very large populations (typical of tunas), they predictably return an overload of genetic variation that is impossible to parse into meaningful patterns. However, mtDNA’s extreme variability, paired with its stepwise mutation pattern, makes it very useful in devising phylogenetic trees at the species level. Figure 4 describes the practical applications of the most popular traditional and NGS-relevant marker types, along with the relative investment needed and information gained from each.

Cost is almost always the limiting factor in genetic research. The protocols that produce each marker type require specialty equipment, human effort and expertise, which drive the associated expense. The number of loci to be genotyped per specimen, the number of specimens to be processed, and the number of tissue specimens to be collected, all impact the final cost, which also means that limited funding caps the volume of data that can be produced.

Inferential power per locus correlates with the number of possible genetic variants. A single microsatellite with 20 possible variants (alleles) carries roughly as much information as 10 bi-allelic SNPs. Studies using marker types with low information content can still produce statistical power if enough loci are observed, as is the case with NGS protocols that produce thousands to millions of SNPs. Ultimately, it is the total volume of data a study produces that determines what kind of analyses are appropriate to apply (Fig. 5).

Multiple algorithms are available within each analysis type that might be more or less applicable per species and study design. However, in general, studies using more and more informative marker types have access to more data-demanding analyses that also produce higher confidence and precise observations. Heterozygosity assessments are exempt from the trend because, while the nuance provided is very marginal, the information is the basis of almost all other analyses and is the gateway assessment to justify if further data exploration is worthwhile.

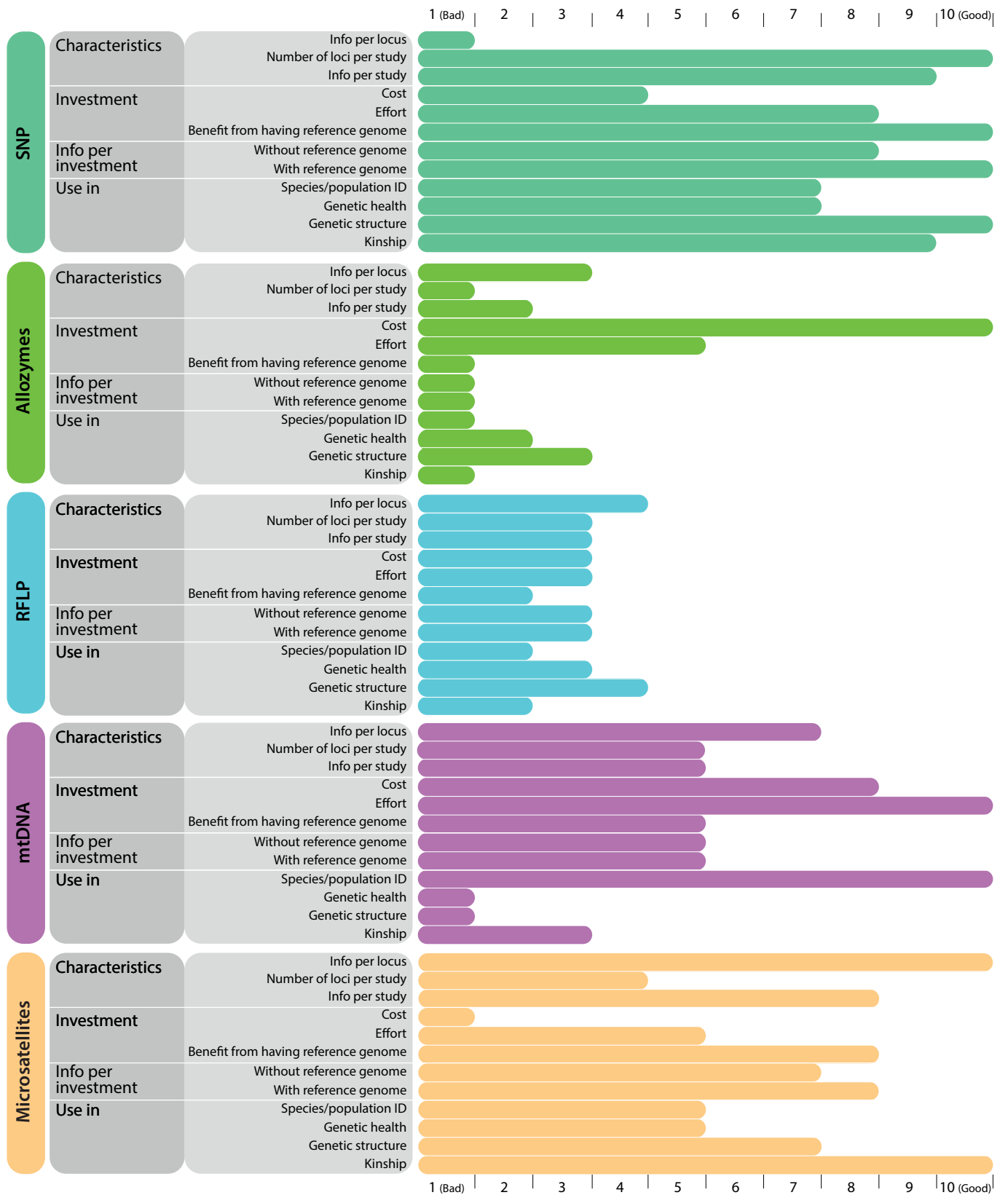


Figure 4. Comparative performance of the five most popular marker types across various parameters that commonly drive a researcher's choice of markers for a given study.

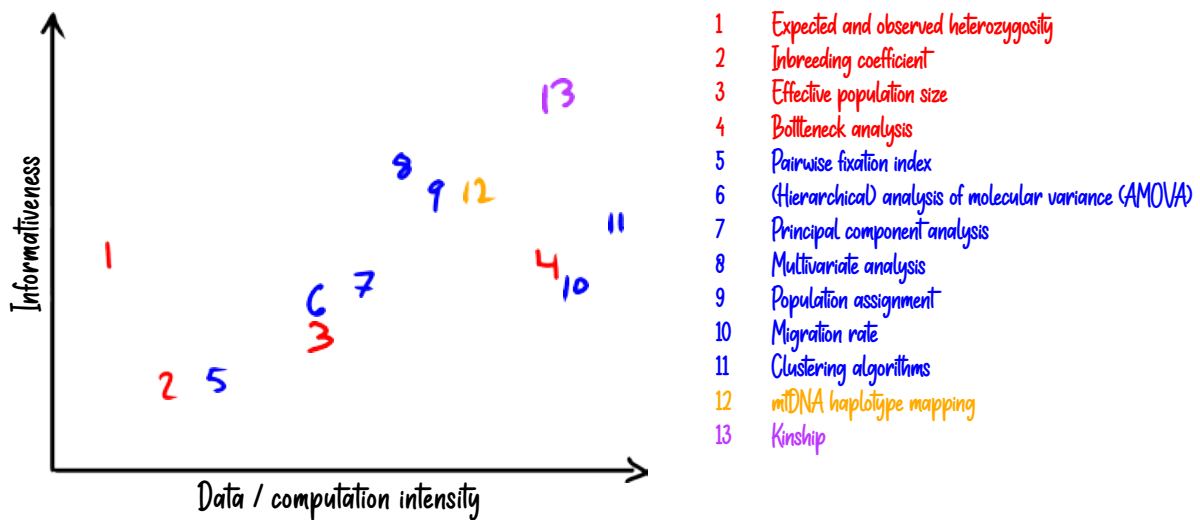


Figure 5. Information gained versus computational demands for a range of genetic data analyses. Analyses in red assess genetic diversity, those in blue assess population structure, those in orange assess species-level questions. Kinship is a unique bridge between diversity (when considered at the individual level) and structure (when zoomed out to the population level).

Data analysis in population genetic assessments has evolved in line with genotyping protocols. Originally, studies observing a handful of allozymes, each with a few alleles, used a simple statistical test to quantify differences in the proportion of each allele and the proportion of heterozygotes per marker per population. More informative analyses compared proportions of heterozygotes and homozygotes (statistics like the inbreeding coefficient F_{IS} , and the fixation index F_{ST}). As improved DNA sequencing protocols enabled studies to produce orders of magnitude more data-points, it became feasible, if not essential, to synthesise data based on variance among loci, averaged trends, and probability assignments, among others, in order to recognise useful patterns.

Although high-volume datasets still pay homage to baseline statistics such as F_{IS} and F_{ST} , they invariably take advantage of the improved nuance of more computationally intensive analyses such as clustering algorithms, which trial thousands of iterations of potential specimen groupings in order to recommend the most parsimonious network among observed individuals.

To summarise the generic relationships driving study design, a study's chosen genetic marker type and analysis type(s) build off one another. Choice in marker type predicts the amount of data that will be produced, which in turn influences the types of analyses that can be performed. Anticipating the statistical power necessary to capture biological patterns of interest determines the type of analyses that should be applied and the type of markers that can provide the required volume of data. External considerations, such as cost of DNA processing for the various marker types, often dictate that a compromise is reached between the most informative and most economical research methods.

The unique characteristics of each marker type, which extend beyond the amount of genetic information they commonly generate, influence their utility for particular research questions. In short:

- mtDNA sequence interpretation is best suited for identifying species;
- microsatellites are adequate for assessing kinship and recent population structure;
- SNPs are the king/queen of marker types, and match or out-perform all other marker types in most types of analyses (with the exception of species ID) given a large enough number of markers; and
- Allozymes and RFLPs remain relevant as options when NGS is not feasible.

Applications to fisheries

Genetic analyses can be applied to a wide range of fisheries-related questions (Table 1). Some basic rules can help to navigate how genetics terminology translates to such questions. Concerns related to population sustainability, including overfishing, appropriate fishing quotas, or stock robustness in the face of climate change, can be addressed by studies of population genetic health. Questions related to fish distribution, movement, intermixing, schooling and population dynamics, can be addressed through analyses of population genetic structure.

In reality, the division between genetic health and genetic structure analyses is very fine. Based on the accepted rule that large, highly connected populations tend to be more genetically robust, studies of genetic health can validate

Table 1. Summary of common fisheries topics and the related information provided by genetic studies.

	Fisheries applicable topics	Possible inferences	Limiting factors
Genetic diversity/health	Absolute stock abundance	Quantitative estimation of the number of adults in a population	Depends on close-kin mark-recapture (CKMR), a new technique that has been tested in a limited number of species with only a moderate number of spawning adults.
	Fishery sustainability Overfishing and recovery status Need for fishing quota adjustments	Presence of past or current reductions in population size, whether due to overfishing or natural causes Presence and degree of inbreeding and loss of population-wide genetic diversity	Ability to sense historical reduction events varies with type of genetic marker Potential for confounding factors to replicate patterns associated with overfishing Not possible to isolate fishing mortality from natural mortality
	Adaptive capacity Future sustainability	Likelihood of population stability in the face of climate change and other environmental variability	Genomic adaptive capacity is fairly straightforward to quantify, but wholistic adaptive capacity also needs to consider ecological input, behaviour, phenotypic plasticity, etc
Population structure	Stock number and boundaries	Presence and strength of population structure, with implications for identifying underlying drivers	Depends on effective sampling to capture spatial and temporal differentiation; cannot make confident inferences about locations and times that were not sampled
	Stock sub-structuring	Presence and strength of more subtle, dynamic, or small-scale population structure Different kinds of potential patterns include isolation by distance, metapopulations, temporally variable structure due to migration, etc Drivers could include moderately strong site fidelity, other reductions in mobility, non-random mating, environmental selection, behaviour, etc	Extremely diverse calculations are available, with subtle differences in assumptions; selecting inappropriate equations/models can produce erroneous results As structure gets more subtle, differentiating biologically significant patterns from noise in the data is more challenging and subjective; likewise, isolating drivers with similar patterns becomes more complex.
	Mobility, connectivity	Degree of reproductive mixing between groups Potential underestimation of stock separation reported by other genetic tools, based on the disproportionate moderating effects a few migrants can have on genetic structure	Significant diversity exists among algorithms intended to infer migration rates, which often introduce assumptions about stock structure. Inappropriate model selection can significantly impact results
	Specimen provenance	Extent of admixture between different stocks Individual mobility when calibrated with catch location	Confidence of population assignment depends on extent of differentiation between populations
Conservation/Management	Species identification	Confirmation of species that are difficult to identify morphologically Hybridisation between species	Requires pre-existing data in global databases for comparison; can be limited for non-model species
	Recognising conservation concern	Intensity of necessary response based on reduction of adaptive capacity, presence of inbreeding, low genetic diversity, population fragmentation, etc	Acquiring enough specimens can be difficult in rare species
	Recommendation of appropriate action	Best practices to avoid introducing outbreeding depression or other negative consequences through conservation initiatives	Genetic analyses can easily recognise when population differentiation exists or not, but require much greater investment to predict if mixing two differentiated groups will produce maladapted offspring
	Effectiveness of ongoing conservation and management policies	Change over time in depth of genetic diversity, heterozygosity, and other measures of adaptive capacity	Depleted populations often violate assumptions that underly genetic assessment algorithms, skewing and introducing subjectivity into results
Behaviour	Migration patterns	Can confirm if fish sampled on and off an expected migration route are from the appropriate stock	Genetics does not provide indicators of previous travel; better assessed using tagging and otolith chemistry and confirmed genetically if appropriate
	Spawning locations Spawning times	Presence and number of distinct migratory spawning sites in species that are known to undertake spawning migrations Presence of non-random mating in mixed stock spawning grounds	Genetics only provides inferences based on patterns of population structure; spawning locations and times are better assessed using tagging, catch data, etc
	Future responses to unprecedented stress (especially climate change)	Extent of genetic adaptive capacity Presence of potentially advantageous genetic variations that would allow individuals to withstand new stress	Inappropriate to rely on genetic features to predict individual behaviour in complex organisms such as teleost fish; better assessed using modelling

Confidence levels:

Green High confidence assessment using predominantly genetic tools

Yellow Moderate confidence from genetic assessments; high stakes decisions should incorporate external research tools, but genetic inferences are sufficient for basic understanding

Grey Marginal confidence from genetic assessments; strongly recommended to incorporate external techniques before drawing conclusions

results against known population structure. Conversely, observations of unexpectedly low genetic diversity (and consequently health) in a large population can indicate the presence of previously unrecognised population substructure. Especially when a research question carries high stakes, such as informing conservation of a vulnerable species or recommending management changes to an important fishery, it is common to incorporate as many analytical approaches as possible to maximise confidence in the results.

Trust in inferences based on genetic data is generally high, but still not unequivocal. Genetic health is directly dependent on genetic diversity, which in turn is directly quantified using genetics tools; therefore, confidence in these types of analyses can be very high. However, structure analyses are more involved assessments of these same data, and are used to draw conclusions about a broader diversity of topics. For example, it often falls on the data interpreter to determine if heterozygosity in a sample group is lower than expected because of the presence of cryptic population structure, an abrupt isolation event in the distant past, migration patterns, or inadvertent biases in sampling of a mixed stock, among other options. Context usually provides clues to elevate one explanation over others, but brings its own potential for misinterpretation. In short, structure analyses carry relatively more possibility of inaccurate representation than baseline genetic health assessments, yet it is important to note that all genetic analyses depend on collecting samples that are both representative of the larger population being studied, and free of contamination.

The single greatest determining factor in the success of population-level genetic research is appropriate sample collection. Genetic analyses present snapshots of population parameters based on where and when individuals were sampled. Even when sample groups are accurate representations of the population, if they are not taken in a pattern that captures the phenomena of interest, results will be inconclusive. This is especially relevant for highly mobile pelagic species. For example, such species might comprise multiple populations that use the same spawning ground asynchronously, each occupying the same location at different times of year. If samples are taken annually but not seasonally, there will be no evidence of sequential habitation. Likewise, inferences made using a specific sample set may not apply to unobserved locations, times, life stages, or parts of the genome. Sample groups of mature adults consist of the tiny fraction of multiple cohorts that survived to that point, whereas juvenile sample groups contain more individuals from only a few cohorts and have not yet experienced the full range of environmental pressures. Applying patterns derived for one group to the other will likely produce inappropriate interpretations. The onus lies with the research team to collect samples in a pattern that can produce informative and accurate conclusions regarding the question that they wish to ask.

There are also blind spots in the types of inferences that are safe to derive from genetics. Unlike otolith chemistry

or other biomarkers, DNA remains stable across an individual's life and, therefore, carries no information about the behavioural or movement history of the individual. However creative applications are possible. For example, at a group level, the shared spawning location of related individuals can be inferred from DNA. Thus, genetics does have the capacity to verify pre-existing hypotheses concerning behaviour and migration.

When it doesn't go smoothly . . .

The biggest challenge to the success of genetic studies occurs when there is insufficient capacity or funds to collect the appropriate number and distribution of specimens, and to extract enough genetic data from each to answer questions with confidence. However, other hiccups can also occur that threaten to compromise a study's integrity and success.

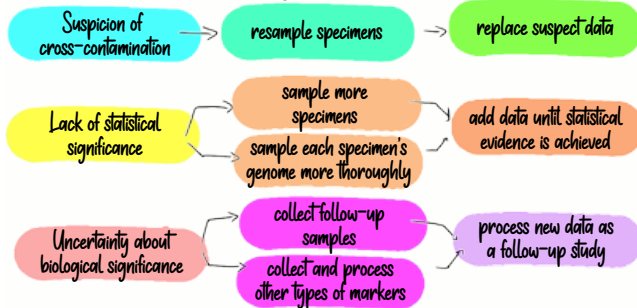
Examples include a study design that fails to sample in a pattern that captures the true pattern of genetic variability. Even given an appropriate study design, non-random or inadequate collection of specimens will result in sample groups that do not represent the larger population. Further, poor sample collection and storage protocols, and mishandling during DNA processing can allow DNA to degrade or be contaminated, leading to information loss or, in the case of unidentified contamination, the risk of interference with biologically relevant trends in the data. Even given successful extraction of genetic data, use of inappropriate statistical analyses, or failure to recognise the limits of such analyses, can lead to misinterpretation and inaccurate conclusions.

Fortunately, most issues can be avoided or corrected. Figure 6 provides potential avenues around some of the most common complications.

Some basic awareness during the study design and implementation phases can help avoid many obstacles.

- Knowing a species' life history first informs expectations of results, allowing researchers to anticipate the statistical power (and, therefore, the number of specimens and amount of genetic data per specimen) needed to generate accurate and conclusive results.
- Second, life history drives the sample collection strategy. In a classic example of non-random sampling, if a study intends to address broad-scale population dynamics but accidentally samples kin groups or other forms of local population substructure, it will produce inferences that are appropriate to the sample subgroup but distorted when projected onto a larger population. Anticipating the extent to which such substructure exists helps a researcher select an appropriate specimen collection strategy and avoid introducing noise into datasets focused on a specific question.

Problem solving with adequate funds



Problem solving without adequate funds



Figure 6. An incomplete list of examples of how studies might respond to common complications. The exact method of recourse depends on available resources, and not all methods succeed equally in regaining confidence in conclusions.

- Life history also informs the selection of the most appropriate downstream analyses. Complex analyses like clustering algorithms carry assumptions about the underlying model of population structure (e.g. metapopulation versus isolation by distance) upon which all other projections are built. Inappropriate assumptions will bias results and produce artefacts from an otherwise good-quality dataset.
- Comparing new data with existing data is standard practice to provide global context to current observations, and can improve inference power by incorporating compatible, published information into the new dataset. However, it is best to premeditate major comparisons and build study designs around that compatibility, otherwise the probability of two separate studies incidentally inventing parallel designs is low to non-existent. Incongruencies between any part of study design can introduce artificial differences in results and reduce confidence in the biological significance of observations.
- Access to a reference genome, which consists of the published, nucleotide-by-nucleotide sequence of the entire genome of a species, potentially adds tremendous value to a study because it improves confidence in the selection of informative loci and can validate otherwise blind assumptions about selective pressure on a locus or its association with functional traits. Lack of a reference genome means conclusions depend exclusively on the accuracy of general population genetic theory, and especially limits confidence in assessments of local adaptation.
- A reference genome also aids in efficient budgeting. Just as sampled specimens only provide information from those locations and times, sampled genomic regions

only demonstrate patterns relevant to those exact loci. Knowing what parts of the genome will be informative to a research question (perhaps a particular gene, or a sex chromosome) allows for targeted genetic sampling, which limits costs from extraneous genetic sampling and reduces noise in the data.

- Cross-contamination among samples is a perpetual concern in genetic analyses and, once it occurs, it is difficult to correct. Vigilance in following sterilisation protocols from sample collection to sequencing is an essential point, and minimises the risk of needing to discard hard-earned data later.

Pacific resources

Because of the significant bioinformatics focus of modern genetic assessments, the global genetics research community has developed robust support networks that stem from or mirror other computer science-associated fields. Many genetic assessment software programs exist on free platforms like R and Github. Online chatrooms such as Bio-stars, Stacks and ResearchGate have a wealth of discussion fora for troubleshooting specific software and interpreting results. Software developers are often very responsive to direct email contact regarding the performance of their published code.

It is also standard practice to outsource next generation DNA sequencing work to specialised, commercial laboratories. International shipment of tissue and preliminarily processed DNA is, therefore, common.

That said, working within the region generally reduces logistics, and potentially cost. The MOANA Labs at the University of the South Pacific in Suva, Fiji, has in-house capacity for small-scale projects using basic DNA extraction and traditional protocols. New Zealand Genomics Limited, a collaborative effort of several universities with government support, is Illumina-Propel certified to conduct NGS protocols at its University of Otago campus. Diversity Arrays Technology in Australia has developed an independent and highly popular NGS protocol, DArTseq. Many other, similarly equipped labs exist across the Pacific region.

The Pacific Community in focus

The Pacific Community (SPC) has supported collaborations with other Pacific organisations, including the University of the South Pacific and the Commonwealth Scientific and Industrial Research Organisation (CSIRO), to conduct preliminary population genetic structure and genetic health assessments of albacore, bigeye, skipjack and yellowfin tunas. These applications have identified the need for further research to investigate substructure driven by uneven reproductive success, the presence of kin in schools, and local adaptation. Other collaborations are developing and expanding new bioinformatic applications of genetic data, such as the close-kin mark-recapture concept, which infers absolute population size. Metabarcoding methods have also been applied for identifying tuna and seabird diets (with the University of Canberra and the Institute of Research for Development).

To progress these projects, SPC has recently commenced work on a design study to define the analytical tools, sampling coverage and investment required to resolve long-standing ecological questions for tropical tuna species in the western and central Pacific Ocean. To assist with this design study, SPC has also initiated the establishment of an informal advisory panel. While the initial role of this panel is to advise on the question of tuna stock structure, it is expected to evolve in scope to comment on the implementation of molecular genetics studies undertaken by SPC. Panel participants include experts in the application of molecular genetics and sample collection in the Pacific region. While the advisory panel is informal, SPC is utilising it as a first step in ensuring science quality and appropriate peer-review for molecular genetics applications in the region.

Here, we have aimed to demystify some of the key aspects of genetic analyses in the context of fisheries research. In developing genetics research capacity for the region, including logistical support for other Pacific research groups and in-house technical expertise for data assessment and interpretation, SPC hopes to foster knowledge exchange and encourage the wider application of genetic tools within Pacific fisheries management frameworks.

Glossary

- allele:** An allele is a variant form of a given gene, meaning it is one of two or more versions of a known mutation at the same place on a chromosome. Source: <https://en.wikipedia.org/wiki/Allele>
- allozyme:** Allozymes are variant forms of an enzyme that differ structurally but not functionally from other allozymes coded for by different alleles at the same locus. Source: <https://www.genome.gov/genetics-glossary/r#glossary>
- CKMR:** Close-kin mark-recapture (CKMR) is a recently developed method for estimating abundance and demographic parameters (e.g. population trends, survival) from kinship relationships determined from genetic samples. Source: <http://afstws2019.org/sessions/>
- DNA:** Deoxyribonucleic acid (DNA), is a complex molecule that contains all the information necessary to build and maintain an organism. All living things have DNA within their cells. In fact, nearly every cell in a multicellular organism possesses the full set of DNA required for that organism. Source: <https://www.nature.com/scitable/topicpage/introduction-what-is-dna-6579978/>
- eDNA:** Environmental DNA (eDNA) is DNA obtained from environmental samples (e.g. soil, seawater, air), rather than directly from the organisms themselves. When organisms interact with their environment, DNA from, for example, sloughed tissue or scales (in fish), body mucus, or blood is expelled, accumulates and can be measured by sampling that environment. Source(s): <https://www.sciencedirect.com/science/article/pii/S0006320714004443?via%3Dihub>
<https://onlinelibrary.wiley.com/doi/full/10.1002/edn3.132>
- heterozygosity:** Heterozygosity is the condition of having two different alleles at a locus. Fundamental to the study of genetic variation in populations. Source: <https://www.oxfordbibliographies.com/view/document/obo-9780199941728/obo-9780199941728-0039.xml>
- locus (loci):** A locus is the specific physical location of a gene or other DNA sequence on a chromosome, like a genetic street address. The plural of locus is "loci". Source: <https://www.genome.gov/genetics-glossary/r#glossary>
- microsatellite:** A microsatellite is a tract of repetitive DNA in which certain DNA motifs (ranging in length from one to six or more base pairs) are repeated, typically 5–50 times. Source: <https://en.wikipedia.org/wiki/Microsatellite>
- mtDNA:** Mitochondrial DNA (mtDNA) is the DNA located in mitochondria, cellular organelles within eukaryotic cells that convert chemical energy from food into a form that cells can use. Source: https://en.wikipedia.org/wiki/Mitochondrial_DNA
- NGS:** Next generation sequencing (NGS) is any of several high-throughput approaches to DNA sequencing using the concept of massively parallel processing. Source: https://en.wikipedia.org/wiki/Massive_parallel_sequencing
- RFLP:** Restriction fragment length polymorphism (RFLP) is a type of polymorphism that results from variation in the DNA sequence recognised by restriction enzymes. These are bacterial enzymes used by scientists to cut DNA molecules at known locations. Source: <https://www.genome.gov/genetics-glossary/r#glossary>
- SNP:** A single-nucleotide polymorphism (SNP) is a substitution of a single nucleotide at a specific position in the genome, that is present in a sufficiently large fraction of the population (e.g. 1% or more). Source: https://en.wikipedia.org/wiki/Single-nucleotide_polymorphism

Appendix 1. Some publications related to the use of genetics in fisheries

	Fisheries applicable topics	Examples		
Genetic diversity /health	Absolute stock abundance	Bravington M., Grewe P. and Davies C. 2016. Absolute abundance of southern bluefin tuna estimated by close-kin mark-recapture. <i>Nature Communications</i> 7:1–8. Hillary R. et al. 2018. Genetic relatedness reveals total population size of white sharks in eastern Australia and New Zealand. <i>Scientific Reports</i> 8:1–9.		
	Fishery sustainability	Pinsky M.L. and Palumbi, S.R. 2014. Meta-analysis reveals lower genetic diversity in overfished populations. <i>Molecular Ecology</i> 23:29–39.		
	Adaptive capacity Future sustainability	Ehlers A., Worm B. and Reusch T.B.H. 2008. Importance of genetic diversity in eelgrass <i>Zostera marina</i> for its resilience to global warming. <i>Marine Ecology Progress Series</i> 355:1–7. Nicotra A.B., Beever E.A., Robertson A.L., Hofmann G.E. and O’Leary J. 2015. Assessing the components of adaptive capacity to improve conservation and management efforts under global change. <i>Conservation Biology</i> 29:1268–1278. Foo S.A. and Byrne M. 2016. Acclimatization and adaptive capacity of marine species in a changing ocean. <i>Advances in Marine Biology</i> 74:69–116.		
Population structure	Stock number and boundaries	Pecoraro C. et al. 2018. The population genomics of yellowfin tuna (<i>Thunnus albacares</i>) at global geographic scale challenges current stock delineation. <i>Scientific Reports</i> 8:13890.		
	Stock substructuring	Knutsen H. et al. 2011. Are low but statistically significant levels of genetic differentiation in marine fishes ‘biologically meaningful’? A case study of coastal Atlantic cod. <i>Molecular Ecology</i> 20:768–783. Selkoe K.A. et al. 2010. Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. <i>Molecular Ecology</i> 19:3708–3726. Liu B.J., Zhang B.D., Xue D.X., Gao T.X. and Liu J.X. 2016. Population structure and adaptive divergence in a high gene flow marine fish: The small yellow croaker (<i>Larimichthys polyactis</i>). <i>PLoS One</i> 11:1–16. Eldon B., Riquet F., Yearsley J., Jollivet D. and Broquet T. 2016. Current hypotheses to explain genetic chaos under the sea. <i>Current Zoology</i> 62:551–566. Anderson G., Hampton J., Smith N. and Rico C. 2019. Indications of strong adaptive population genetic structure in albacore tuna (<i>Thunnus alalunga</i>) in the southwest and central Pacific Ocean. <i>Ecology Evolution</i> doi:10.1002/ece3.5554 Hoey J.A. and Pinsky M.L. 2018. Genomic signatures of environmental selection despite near-panmixia in summer flounder. <i>Evolutionary Applications</i> 11: 1732–1747. Grewe, P. et al. 2015. Evidence of discrete yellowfin tuna (<i>Thunnus albacares</i>) populations demands rethink of management for this globally important resource. <i>Scientific Reports</i> 5:1–9.		
		Mobility, connectivity	Hedgecock D., Barber P.H. and Edmands S. 2007. Genetic approaches to measuring connectivity. <i>Oceanography</i> 20:70–79. Manel S. et al. 2007. A new individual-based spatial approach for identifying genetic discontinuities in natural populations. <i>Molecular Ecology</i> 16:2031–2043.	
		Specimen provenance	Benestan L. et al. 2015. RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (<i>Homarus americanus</i>). <i>Molecular Ecology</i> 24:3299–3315. Kerr Q., Fuentes-Pardo A.P., Kho J., McDermid J.L. and Ruzzante D.E. 2019. Temporal stability and assignment power of adaptively divergent genomic regions between herring (<i>Clupea harengus</i>) seasonal spawning aggregations. <i>Ecology Evolution</i> 9: 500–510.	
		Conservation/management	Species Identification	Amaral C.R.L. et al. 2017. Tuna fish identification using mtDNA markers. <i>Forensic Science International: Genetics Supplement Series</i> 6, e471–e473.
			Recognising conservation concern	von der Heyden S. 2009. Why do we need to integrate population genetics into South African marine protected area planning? <i>African Journal of Marine Science</i> 31:263–269.
			Recommendation for action	Flanagan S. and Jones A.G. 2017. Constraints on the F ST – Heterozygosity Outlier Approach. <i>Journal of Heredity</i> 561–573. doi:10.1093/jhered/esx048
Behaviour	Effectiveness of ongoing policies	Flanagan S. and Jones A.G. 2017. Constraints on the F ST – Heterozygosity Outlier Approach. <i>Journal of Heredity</i> 561–573. doi:10.1093/jhered/esx048 Dann T.H., Habicht C., Baker T.T. and Seeb J.E. 2013. Exploiting genetic diversity to balance conservation and harvest of migratory salmon. <i>Canadian Journal of Fisheries and Aquatic Sciences</i> 793:785–793.		
	Migration patterns	Arai T., Kotake A. and Kayama S. 2005. Movements and life history patterns of the skipjack tuna <i>Katsuwonus pelamis</i> in the western Pacific, as revealed by otolith Sr: Ca ratios. <i>Journal of the Marine Biological Association of the United Kingdom</i> 1211–1216. doi:10.1017/S0025315405012336		
	Spawning activity	Richardson D.E. et al. 2016. Discovery of a spawning ground reveals diverse migration strategies in Atlantic bluefin tuna (<i>Thunnus thynnus</i>). <i>Proceedings of the National Academy of Sciences</i> 113:3299–3304.		
	Future responses to stress	Beever E.A. et al. 2016. Improving Conservation Outcomes with a New Paradigm for Understanding Species’ Fundamental and Realized Adaptive Capacity. <i>Conservation Letters</i> 9:131–137. Nicotra A.B., Beever E.A., Robertson A.L., Hofmann G.E. and O’Leary J. 2015. Assessing the components of adaptive capacity to improve conservation and management efforts under global change. <i>Conservation Biology</i> 29:1268–1278.		