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Gene-tagging for fisheries sustainability

Gene-tagging is an innovative application of state-of-the-art biotechnology in an unusual field: fisheries management. It uses the latest techniques of genetic marker technology to assist Australia's fishing industry to exploit marine resources in a sustainable and economically efficient way. Previously used for large land animals such as bears and wolves that are rare and hard to capture, we are now implementing this innovative approach on a fish species.

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Australians are fortunate to enjoy access to rich tropical and temperate fisheries resources. Commercial harvesting provides income for fishermen and workers in related industries such as fish processing and boat-building, and provides valuable export dollars. Similarly, recreational fishermen contribute to the income of regional communities by purchasing accommodation, gear, fuel and other services. Sustainable management is therefore essential, for economic, social and environmental reasons. A comprehensive set of resource sustainability standards are being developed by the industry under the Wildlife Protection (Regulation of Exports and Imports) Act 1982 and the Environment Protection and Biodiversity Conservation Act 1999. These will ensure persistence of the resources into the future. A vital component of any management strategy is that it be informed by accurate data. This review describes a gene tagging approach for the management of mackerel.

Australian spanish mackerel

Commercial catches of Australian spanish mackerel (*Scomberomorus commerson*) are about 1500 tonnes annually and are valued at around $9 million. The recreational catch is similar and growing. Spanish mackerel are large (Figure 1), and their flesh is excellent for the table, not being too dry or too oily. A fast swimming pelagic predator, *S. commerson* is principally found in schools near reefs and shoals, and the populations are not amenable to survey by trawl, gill net or by air. They are favourite targets for both commercial and recreational fishermen [1]. Recent research has shown that the spanish mackerel maybe susceptible to overfishing as populations are localised and unlikely to be replenished by movement of individuals from surrounding areas.

After this - if you don't know how many fish there are swimming around, how do you know how to set an allowable catch? If fishermen are allowed to catch too many, the resource may decline. If catch limits are set too low, the resource may be under-utilised. Australian fisheries managers often have to rely on estimates of the number of fish calculated from CPUE data from records kept by commercial fishermen. CPUE is 'catch per unit effort' where 'catch' is the weight of fish caught, and 'effort' is a standardised measure of the time taken to catch those fish. CPUE is high if the fish are abundant and low if the fish are scarce. But CPUE has to be constantly standardised when fishermen become more efficient at harvesting fish, for example by using spotter planes to locate fish schools and mobile phones for communication.

DNA fingerprinting of fish

A more accurate method to determine the resource size is to tag some fish individually, and let them go. The numbers of tagged fish caught by the end of the fishing season gives an estimate of the proportion of

Figure 1. Narrow-barred Spanish mackerel held by Jonathon Staunton-Smith and Michelle Sellin of the Southern Fisheries Centre.

the resource that was harvested. For instance, if 100 fish are tagged, and ten are caught over the fishing season, then about 10% of the available resource has been removed. For most species of fish, we know what the optimum harvest rate should be, and adjustments to the harvest rate can be made next fishing season; for example by reducing its duration. But plastic tags have many problems. They fall off, or affect the survival of the fish. Many fish don't survive the tagging process, especially Spanish mackerel. Their vigorous attack and fight, yet relative fragility, mean that Spanish mackerel are difficult to economically catch and tag in sufficient numbers for one-off or monitoring estimation of the harvest proportion. For a variety of reasons, reporting rates, by fishermen returning tagged fish to researchers, are often not as good as they should be.

Fisheries researchers have puzzled over more suitable tagging methods for the last decade. Finally we might have an answer. If a small piece of tissue can be taken from a fish remotely - eg. by having it bite on a specially-designed lure - then that fish can be identified with a DNA-fingerprint (Figure 2). In this way, a population of genetically tagged fish that are still swimming around can be identified. The tagged fish have no visible tag, but they are identifiable if caught later. When a future catch is screened, fish that provide a match with the DNA fingerprint of a previously-tagged fish are recaptured and the harvest rate measure at the end, or even during, the fishing season can be calculated.

The specially designed fishing lure has hooks that take a tissue sample from inside the mouth of the fish. Hooks on the lure automatically release the fish at depth and return the tissue sample to the research vessel. It is then a relatively simple process to match genotypes of fish from the commercial catch to fish whose genotypes were previously determined.

Genotyping

Genotyping with robotic PCR facilities and genotyping services of the Australian Genome Research Facility (AGRF, Melbourne) is an essential part of this work. DNA is extracted from tissue sampled with the special lure, as well as from a random sample of fins collected from the Spanish mackerel caught commercially. The DNA is PCR amplified for a panel of custom markers and genotyped. To control genotyping costs, several fish can be genotyped in each gel separation lane by taking advantage of the variety of fluorescent dyes available. For example, fish one will be genotyped with the marker panel labelled with dye one, fish two will be labelled with dye two and fish three with dye three. In this way the different fluorescent dyes enables the genotype for each fish to be deciphered on the one gel.

The Molecular Fisheries Lab at the Southern Fisheries Centre in Queensland has developed appropriate marker panels from Spanish mackerel genomic libraries. A panel consists of three to four microsatellite loci that have non-overlapping allele sizes. For example, locus one may have alleles in the population ranging from 100 to 150 base pairs. The alleles of loci two and three may range from 175–215 and 250–300 base pairs. This allows the alleles of several loci from each individual to be resolved in a single gel separation lane, following appropriate multiplexing either at the PCR or gel-loading stage. Ideally, PCR multiplexing will be used to minimise PCR costs per fish. In addition, loci have to meet other stringent criteria; for example loci must:

- Have the same PCR annealing temperature and magnesium chloride concentration to facilitate PCR multiplexing;

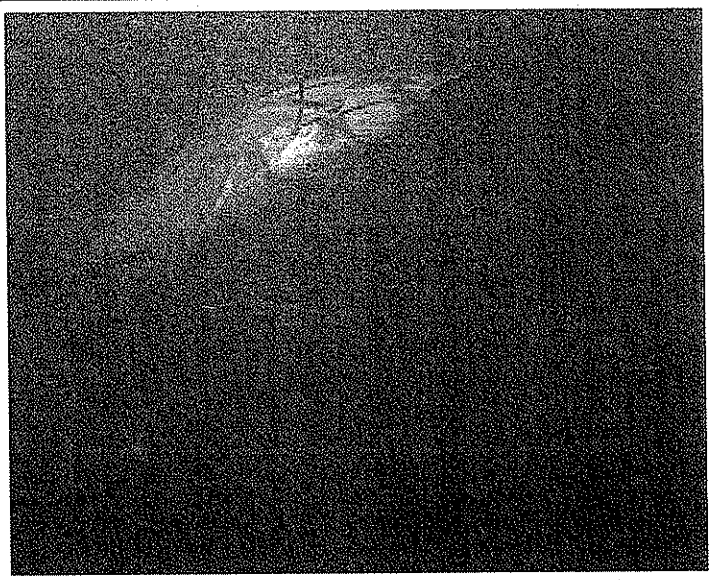

Figure 2. Genetic samples from Spanish mackerel blood are obtained using a fishing lure. In this image a Spanish mackerel is striking the remote-sampling fishing lure at approximately 5m during a 0.75 second interval.

• have minimal stuttering, and robust amplification even from DNA extracted using 'quick and dirty' techniques; and
• be highly polymorphic.

The shadow effect

The development of microsatellite marker panels does not stop there, however. The panel needs to be designed to minimise the 'shadow' effect, which haunts all large scale genotyping projects [2]. This shadow effect describes the number of false recaptures that occur when a finite number of loci are used on large numbers of individuals. Recall that the aim of gene-tag is to find those individuals among the commercial catch that were tissue-sampled using the specially designed fishing lure. If the genotype of a commercial fish is identical to a tissue-sampled fish, then it is assumed to be re-capture. But how accurate is this assumption? Could the commercial fish be identical to the tissue-sampled fish by chance (false match), rather than by being re-captured (true match)? As the number of commercially-caught fish that are genotyped increases, then the chance of encountering a false match increases. This is a real problem for this project as 5–10,000 fish will be genotyped over the three years of the study.

To quantify the strength of the 'shadow' effect for the Spanish mackerel loci, and deal with the 'false match' problem, we have developed custom software that calculates the efficiency of each locus for individual identification (probability of identity). The software also calculates all possible combinations of the loci that could be combined in a single lane given their allele sizes. Then for each combination of non-overlapping loci, it calculates the number of false matches for a given number of tagged, screened and recaptured individuals. This powerful tool allows us to design the best genotyping strategy. For example, a staged approach may be the most cost-efficient. All commercial fish could be screened for a few loci, and potential 'matches', both false and real, are identified. The remaining loci could be used to genotype the potential 'matches' and to identify the real recaptures.

Our gene-tag approach to harvest rate estimation for fish builds on research by Dave Paetkau [3], Lisette Waits [4], Pierre Taberlet [5] and colleagues on large terrestrial animals such as bears and wolves. They had a different problem; their animals were so rare (wolves) or hard to capture (bears) that conventional tagging programs were expensive, time-consuming and dangerous. However, the animals could be individually genotyped from minute hair or skin samples collected from field sites such as scratching posts or even from faeces samples. Over time, the accumulated genotype data was used to estimate parameters important to the design of conservation strategies, such as population size, size of individual territories and patterns of movement during breeding and feeding seasons. Most recently, in Australia the same technology has been applied to the endangered northern hairy nosed wombat from hair samples harvested with sticky tape at burrow entrances.

Tagging other marine species

Our gene-tag project on Spanish mackerel will lay the ground-rules for the application of this marker technology to other species of fisheries. Crab and lobster populations are notoriously difficult to tag, as they shed their exoskeleton and tag at moulting. Some species such as trepang or beche-de-mer, cannot be tagged at all. In other fish species, the affect of plastic tags on fish survival can only be measured under experimental conditions in the lab, not in the wild where it really matters.

A comparison of gene-tag and plastic tag re-capture data should quantify the rate of tag loss or tag mortality in the wild and allow more accurate re-analysis of existing plastic tag data. For each new species however, a new panel of microsatellite markers needs to be developed. In future, this process may be facilitated by electronic catalogues of microsatellite primers for a large range of species, and the ability of the better loci to cross species boundaries (eg. [6]). Finally, the feasibility of the gene-tag approach is a product of the rapid rate at which new molecular genetics techniques are evolving and the ability of research service providers, such as AGRF, to keep pace with these changes.

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