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# Genetic identification of SBT

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## Abstract

Genetic techniques could provide a powerful tool to complement other monitoring, control and surveillance (MCS) techniques for SBT. Genetic techniques (both species discriminating and DNA fingerprinting) have already been used to monitor trade flows and labelling of fish (incl. caviar) and whale products. Genetic sampling can be used to discriminate SBT from other tuna species and/or discriminate 'legal' and 'illegal' SBT at any point in the supply chain including retail 'point-of-sale'. Genetic testing to discriminate SBT from other species of tuna for quota decrementation purposes has been in use in Australia since 2000. Audit-based DNA fingerprinting linked to a DNA register for legally caught SBT could greatly assist compliance measures within the CCSBT. The cost-effectiveness of genetic tools as part of a CCSBT MCS scheme remains a question for the CCSBT Compliance Committee.

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## Introduction

While genetic techniques are now widely used within fisheries and aquaculture science, the use of genetics in these areas has typically been focussed on 'stock' discrimination, selective breeding and transgenic applications.

Responding to concerns about mislabelling of seafood, genetic techniques have been successfully applied to questions about the species composition of fish and whale products. There are a wide-array of genetic techniques that can reliably discriminate which species is in, or not in, a sample. Such techniques have already been widely used by both Governments and private enterprise. Many commercial and semi-commercial laboratories (such as those linked to Universities) offer species testing services using genetic techniques at relatively low cost.

One well known application of genetic testing of fish species involved the implementation of genetic testing of sturgeon caviar. While species substitution (i.e. mislabelling) of sturgeon caviar products had long been suspected, genetic testing subsequently showed that there were indeed high levels of mislabelling and/or mixing of sturgeon caviar. Mislabelling of eggs involved substitution or mixing with eggs from cheaper or protected species of sturgeon and eggs of other species such as herring (*Clupea harengus*). In one case herring eggs had been coloured and reshaped and mixed with sturgeon caviar but genetic testing showed that more than one-third of the shipment was indeed herring eggs.

Genetic sampling has also been used to discriminate between cetacean species from various samples including purchases from retail outlets. Genetic detection of mislabelling of whale meat has been reported in both peer-reviewed journals and International Whaling Commission reports.

DNA fingerprinting of fish has typically focussed on selective breeding for aquaculture (Anon. 1998). However, DNA fingerprinting that is linked to a DNA register for legally sourced fish could provide a powerful compliance tool that could be applied at any point in the supply chain.

## Discussion

*Why use genetic testing to discriminate SBT from other species?*

While most experienced fishers and trained fisheries officers can readily discriminate between whole SBT (*Thunnus maccoyii*) and other 'bluefin' tuna (i.e. Atlantic bluefin, *Thunnus thynnus* and Pacific bluefin *Thunnus orientalis*) from other members of the same genus, discriminating between these 'bluefin' tuna species is more difficult.

Bluefin tuna in excess of 300kg can be identified as being either Atlantic or Pacific bluefin as such fish would exceed the maximum recorded size of SBT. Experienced fish biologists can morphologically discriminate between the three species of bluefin tuna at all sizes (with some uncertainty) while fish are unprocessed (i.e. whole with gut and gills *in situ*).

However, once the tuna is processed, species identification becomes increasingly uncertain as the level of processing progresses. For example, two of the key features used to discriminate SBT from the other bluefin tuna species are the colour patterns of the liver and the number of gill rakers. Once the fish is gilled and gutted these two

diagnostic features are no longer available. Once tuna are filleted/loined, reliable and legally-defensible species discrimination becomes almost impossible without the use of genetic techniques.

Genetic markers to discriminate SBT from other tunas have been available for some time. Since 2000, Australia has considered all bluefin to be SBT for the purposes of compliance with individually transferable quotas unless genetic tests are undertaken (at the expense of the fisher) to verify that fact that the fish is not an SBT (i.e. quota is decremented for all bluefin unless the genetic test shows that the specimen is not SBT).

*Can DNA fingerprinting be used to tell if an SBT was caught 'legally'?*
Beyond discriminating between species, forensic genetic techniques have the ability to discriminate between individuals of the same species. The order of DNA base pairs varies between individuals and every individual has a different sequence. Using these complete DNA sequences, every individual could be identified (i.e. 'DNA finger-printed') however, full sequencing is impractical. Instead, a small number of sequences of DNA that are known to vary among individuals a great deal are used to derive a certain probability of telling individuals apart or to match previous samples (i.e. such patterns do not give an individual "fingerprint," but rather they give a probability of being the same or different from other samples).

After the discovery of DNA fingerprinting 1985, the use of such techniques to distinguish 'legal' and 'illegal' tuna was proposed only a few years later by Cone (1989) and others. However, there has been little progress in this area.

There are now a wide array of DNA fingerprinting techniques that have been used successfully on a number of fish species. These include isozyme markers, restriction fragment length polymorphic (RFLP) markers (e.g. Southern blot), random amplified polymorphic DNA (RAPD) markers, amplified fragment length polymorphic (AFLP or AmpFLP) markers, polymorphic expressed sequence tag (EST) markers, microsatellites or simple sequence repeat (SSR) markers, short tandem repeats (STR incl. Y-chromosome STR) and single nucleotide polymorphic (SNP) markers.

A few grams of muscle is more than sufficient to provide enough DNA for sequencing. Small, transportable 'genetics labs in a suitcase' have also been used to eliminate the problem of quarantine retrictions preventing the export of samples. Hence, it is now possible to use DNA fingerprinting at any point between capture and retail sale.

The use of DNA fingerprinting to discriminate 'legal' from 'illegal' SBT would require a DNA Registry of all legally caught SBT (in excess of 400,000 individuals per annum in recent years). While DNA registries are maintained by a number of countries for several cetacean species (e.g. Norwegian minke whale DNA registry) the much larger number of individuals on a DNA Register for SBT would incur a significant cost using currently available methods.

*What does genetic sampling cost?*

Like many scientific techniques, genetics tools are not without associated uncertainty and while modern techniques continue to reduce uncertainty and/or the analysis costs, the costs-effectiveness of genetic tools needs to be carefully considered. Costs include both development and/or implementation costs and such costs remain relatively high for some sophisticated genetic techniques (e.g. DNA fingerprinting).

The cost of genetic techniques depends greatly on the questions to be answered and the level of certainty required. Before a genetic sampling regime can be costed, it is essential to understand what questions the testing is required to answer. In the case of using genetics to assist MCS within the CCSBT it is the role of the Compliance Committee and Commission to specify the questions to be answered and appropriate sampling regime.

By way of example of some current costs for genetic testing, CSIRO testing to discriminate SBT from other tuna species costs around A$250 per sample (paid by fishers) however it is expected that a species specific probe-based approach would reduce this cost to around $20 per sample after the initial development phase. The costs of human STR analysis has fallen to around US$50-100 per sample and given sufficient volume for testing it could be expected that SBT testing could fall to similar levels.

*Are there ancillary benefits from compliance –based genetic testing of SBT?*
A given individuals' *variable number tandem repeats* (VNTRs) within the DNA sequence come from either one or both of the parents but the individual will not have a VNTR that is not shared with at least one parent. Hence, genetic population analysis can use this relationship information to describe populations and monitor changes over time.

## Conclusions

1. Genetic discrimination of SBT from other species of tuna is already in operation in Australia as part of the quota monitoring system. These tests could be readily extended to the CCSBT and applied at any point in the supply chain as part of a broader MCS scheme including in non-member countries.
2. Whilst it is clear that DNA fingerprinting linked to a DNA Register is capable of discriminating 'legal' and 'illegal' SBT, the cost of such a system is currently high and the cost-effectiveness of such a regime is a question for the CCSBT Compliance Sub-Committee.

## References

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