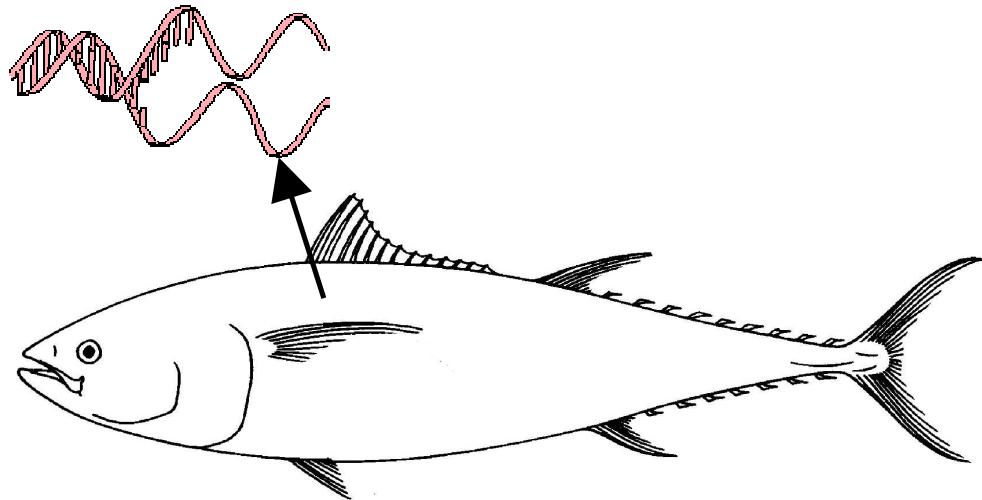


Watson Fish Consulting

PASS Contract JF0371: The potential for DNA-based methods to be used for the detection of illegal trade in fish from IUU



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Table of contents

	Page
Glossary	4
Executive summary	6
The problem of IUU fishing	7
Molecular biological methods for species and population detection	7
<i>Allozyme electrophoresis</i>	8
<i>The basis of DNA techniques</i>	8
<i>The different kinds of DNA</i>	10
Examples of the use of DNA-based methods in the detection of fish species and fish populations	11
Success and failure with DNA	15
How to use DNA to detect differences between fish populations	17
Detecting fish in mixed and degraded samples	19
Examples from wildlife	120
Examples of detection of illegal fishing by DNA	21
DNA as court evidence	21
DNA laboratories	24
The costs of using DNA evidence	25
Using DNA-based methods to combat IUU fishing	27
Conclusions	28
Appendix 1: references	30
Appendix 2: contact details for persons and organisations contacted	35

Glossary of terms

A more detailed explanation of the terms and associated techniques may be found by following the link at the end of each definition in the glossary.

AFLP – also sometimes AFLP-PCR. Amplified fragment length polymorphism works by targeting highly polymorphic DNA and as polymorphism is the basis of genetic differences, AFLP has potential to differentiate populations.

Allele – the DNA which occupies a particular position on a chromosome. Most alleles code for genes, but not all. More information can be found at [allele](#)

Base pairs – the DNA strand is built from base pairs, which are complementary nucleotides. In DNA, there are four nucleotides, adenine which pairs with thymine and guanine which pairs with cytosine. These are usually represented by letters as in:

AGAATC
TCTTAG

DNA barcoding – is a technique for the rapid identification of a species which is very similar to fingerprinting. Since all species contain at least some unique DNA, there are a number of target sequences which could be used. However, it is general practice to use mtDNA. There are a number of national and international projects to compile DNA barcodes for various species groups. Note that in a very small number of organisms, DNA barcoding does not provide reliable species identification. More information can be found at [DNA barcoding](#)

Genetic fingerprinting – is a technique using microsatellite DNA to identify an individual. Note that the technique does not “prove” that a sample comes from a particular individual; it provides a very high statistical probability that it comes from that individual. More information can be found at [genetic fingerprinting](#)

Haplotype – the genetic constitution of a single individual’s mtDNA, chromosome or part of it. Each half of a diploid chromosome may differ slightly at any individual locus. May be used in genotyping and genealogical studies. More information can be found at [haplotype](#)

Loci – the positions of genes on a chromosome.

Microsatellite – short fragments of nuclear DNA, consisting of 2-4 base pairs which repeat many times along a strand of DNA. They tend to be characteristic for a population and hence can be used in population studies. More information can be found at [microsatellite DNA](#)

mtDNA – mitochondrial DNA. A relatively short (compared to genomic DNA) loop of DNA which is, as the name indicates, is found in mitochondria, not in the nucleus. It used to be thought that mtDNA was 100% inherited from the mother, but there are cases where a small percentage is inherited from the father. As it tends to be passed without change from one generation to another with little change, it is especially

useful for phylogenetic studies. It may also be used in population studies. More information can be found at [mtDNA](#)

Nuclear DNA – DNA found in the nucleus. It is subject to rearrangement and mixing at reproduction. Nuclear DNA is often used to demonstrate parentage. More information can be found at [nuclear DNA](#)

Nucleotide – the basic building blocks of DNA and RNA. A dinucleotide is two nucleotides joined together (similarly trinucleotide, tetranucleotide, etc).

PCR – polymerase chain reaction. The process used to amplify the DNA sample to enable sufficient DNA to be replicated to be detected. PCR uses primers (see below) to multiply the target DNA selectively, so generally only a very specific section of DNA is amplified. As the amount of the target DNA is doubled with each cycle, the amount generated increases rapidly. More information can be found at [PCR](#)

Primer – the short strand of nucleic acid used in PCR to replicate the DNA. Primers are usually highly specific, only replicating a targeted section of DNA from a species, although there may also be general primers for wider taxa. Two primers are needed (forward and reverse), one for each of the opposing strands of DNA. More information can be found at [primers](#)

Restriction enzyme – an enzyme which is used to cut out a specific DNA sequence prior to PCR. Restriction enzymes, by linking to unique sequences of bases in DNA, can be used to cut out a segment of DNA unique to a species. More information can be found at [restriction enzymes](#)

RFLP – restriction fragment length polymorphism has two meanings. It may be used to describe the differences in sequences between two lengths of DNA or it may be used to describe the process of determining these differences. It is a key tool in genetic fingerprinting. More information can be found at [RFLP](#)

Sequencing – determining the sequence of nucleotides along a length of DNA. A powerful tool which has many uses, including the identification of species. More information can be found at [DNA sequencing](#)

SNP - single nucleotide polymorphism (generally called a “*snip*”) is where two sequences of DNA differ by a single nucleotide. SNPs account for a great deal of genetic variation and may be used to characterise populations. More information can be found at [SNP](#)

VNTR – variable number tandem repeat is a short sequence of nucleotides which is immediately repeated (hence tandem repeat). VNTRs are usually used on forensic studies to identify an individual. More information can be found at [VNTR](#)

Executive summary

The use of DNA-based methods for the identification of species and individuals is a well-proven technology to which there is widespread access. Much of the information relating to the use of this technology is freely available in the form of publications and on-line databases. A wide range of DNA sequences, primers and PCR reaction conditions is available which is applicable to the identification of fish species. This technology is already used to identify species in ecological and population studies and to identify fish species in food products. The methods are relatively robust, permitting the identification of fish species from processed food products, even those subject to heat treatment and to food products which contain mixtures of fish species.

The application of DNA technologies to the identification of populations of a fish species and the assignment of a sample to a particular population, stock or geographic area is less well-developed. Results currently show that for some species, with a low genetic diversity, either as a species or between populations, differentiation of populations and stocks by DNA is very difficult and results may have a high degree of uncertainty. Results from working with fish with a high level of genetic diversity and for populations which are genetically heterogeneous have been more promising and it is often possible to assign a sample to a particular population, stock or area with a high degree of confidence (95% or better). Thus, there does seem to be potential to use these techniques to monitor IUU fishing and to detect trade routes for fish derived from IUU fishing.

Costs for the development and use of DNA-based methods for detecting and/or prosecuting IUU fishing are difficult to estimate, but appear to be reasonable in comparison to the very high losses experienced by developing countries affected by IUU fishing. Preliminary screening using existing primers for one or two species could be carried out for as little as £10,000, excluding the cost of sample collection. Wider screening and screening for differences between populations would be more expensive, involving much higher numbers of samples and possibly much higher numbers of PCR reactions and/or sequencing. It should be possible to provide some meaningful data and practical tools for the detection of IUU on a major fishery for less than £100,000.

The level of confidence required for use of DNA evidence in prosecution of IUU fishing is far higher than that required for research and fisheries management. This does not rule out the use of DNA evidence in combating and prosecuting those involved in IUU fishing, it simply means that more stringent criteria must apply to the collecting of baseline data and the handling of forensic samples. DNA evidence has been used in court to prosecute IUU fishing, but not as stand-alone evidence.

The development of DNA baseline data for target fisheries is only one step required. For use in court evidence, the collection and processing of forensic evidence requires a laboratory to adopt special standards of operation, both for sample handling and for the DNA analysis. Ideally, laboratories performing such work would be accredited to ISO9001 and ISO17025 and would receive training in the proper collecting and processing of forensic evidence.

The problem of IUU fishing

Illegal, unreported and unregulated (IUU) fishing is a worldwide problem of substantial scale. It varies from the small-scale operations of migratory fishers who follow traditional fishing and trading routes to industrial operations operating in fleets. IUU fishing can vary in form from vessels of a coastal state's own fleet using illegal gear or exceeding quota to vessels fishing inside a coastal state's EEZ without any permits. By its very nature, IUU fishing is difficult to detect and thus to control. Control of IUU fishing has tended to be based on monitoring, inspection (or control) and surveillance (MIS), something which authorities in many developing countries have problems in funding to an adequate degree. MIS is labour-intensive and costly, often involving the commitment of substantial resources for fishery patrol vessels and planes. A further constraint to MIS is that it usually only serves to catch IUU fishing vessels in the act; it is currently more difficult to catch IUU vessels when not fishing or those trading in fish derived from IUU. Falsified log books, transshipping at sea, flags of convenience and authorities in receiving countries turning a blind eye all make the job of detection more difficult.

As a great deal of effort goes into disguising IUU fishing, estimating the scale and hence the impact of it gives rise to varying figures. EJF (2005) estimated the cost of IUU fishing to developing countries alone as being US\$2 – 15 billion annually. Even at the lower estimate, this represents a considerable loss of earnings. The impacts on food security and employment are harder to judge, but will be significant.

Clearly, while MIS will continue to play an important role in the detection and prevention of IUU fishing, the fact that it is effectively limited to use in a coastal state's own EEZ means that much of the activity relating to IUU fishing, such as landing, trading and processing will go undetected. Being able to detect fish which has been sourced illegally would enable the identification of vessels and ports involved in IUU fishing and the trade routes by which fish originating from IUU vessels reaches its ultimate markets (subject, of course to the cooperation of the relevant authorities). The ability to detect both the species of fish in trade and the area from which it was caught could be an important tool in designing interventions to prevent IUU fishing and may also prove to be robust evidence to be used in legal actions.

Molecular biological techniques for species and population detection

Traditional techniques for distinguishing species and populations relied mainly on morphometric and meristic variations. These are often difficult to use, are time-consuming and often fail to distinguish between populations. Methods which depend on physical characters have tended to be displaced by those based on molecular biology which have become increasingly powerful and capable of distinguishing at any taxonomic level, right down to the individual. These techniques are now used widely in genetic, genealogical, phylogenetic, taxonomic and population studies. Some of the techniques are sufficiently robust to be admitted as evidence in court.

Allozyme electrophoresis

Sometimes referred to as isozyme electrophoresis, this technique relies on enzyme polymorphism to distinguish populations and has been used widely to study fish. Enzymes may differ slightly (i.e. be polymorphic) while still retaining their metabolic functions. The small differences are sufficient to separate out the allozymes (different forms of the enzyme) by gel electrophoresis. The positions of the resulting bands on the gel can be used to differentiate between populations. Gels are usually run with a number of different enzymes in order to establish population differences. Statistical routines are used to separate out the different populations, depending on the enzyme polymorphisms observed. Enzyme polymorphism has a number of disadvantages:

- It requires large sample sizes in order to determine which enzyme polymorphisms exist and with what frequency they exist in each population;
- As enzymes are proteins, they are prone to degradation. Even such basic processing as salting and drying may be sufficient to degrade some enzymes and all are very sensitive to temperature so cooking generally destroys enzyme activity. Thus there are great limitations on the use of the technique in processed fish;
- Due to the large sample sizes involved and the number of gels to be run, allozyme electrophoresis is relatively labour-intensive and costly.

Notwithstanding the above reservations, allozyme electrophoresis has been used on many population studies on fish, notably with salmonids. However, DNA-based techniques generally prove to be more powerful at detecting species and population differences.

The basis of DNA techniques

There are a number of DNA techniques, each having its own specific uses and limitations. They can be used to identify a species (or higher taxonomic level), a population, a family, an individual or a specific fragment of DNA. They may rely on nuclear DNA, mitochondrial DNA (mtDNA) or indeed any other DNA or RNA in a cell, depending on the specific method used. Some of the most relevant techniques are outlined below. A more detailed review of DNA-based methods applicable to fisheries management can be found in Willock et. al. (2004).

PCR – the polymerase chain reaction is a general technique used in many DNA-based methods. Its function is to amplify the amount of DNA in a sample to a level at which other techniques may be applied for detection. PCR works by repeatedly amplifying a target fragment of DNA. First, the target DNA is cut out using restriction enzymes. It is then denatured by heat, resulting in the two halves of the DNA strand separating. Primers, which are short strands of nucleic acid, matched to the two DNA fragments that rebuild the opposite half of each of the separated strands, effectively reassembling a copy of the original, joined DNA fragment. Thus, the amount of DNA is doubled with each cycle. Usually 30 or more cycles will be performed before any further analysis is carried out. The PCR reaction is usually completely automated. Successful PCR relies on the identification of the optimum reaction conditions which include the temperature for denaturing, the temperature for reforming the DNA (annealing), and the concentration and composition of buffers.

Optimisation of all these conditions is necessary not only to ensure amplification of the target DNA, but also to avoid the production of unwanted reaction products.

While the term PCR strictly just refers to the amplification process outlined above, in general usage it usually refers to the complete process of extraction, clean-up, amplification and visualisation of products. Prior to PCR, it is necessary to carry out some additional procedures for extraction and clean-up of the DNA. These are generally straightforward for fish samples which mostly do not contain masking or inhibiting agents. These may however be found in processed fish products. After extraction, clean-up and amplification, visualisation of the reaction product is carried out. If the primers are specific and the PCR reaction conditions have been optimised, only a single reaction product should be seen. Most commonly, visualisation is carried out on gels, the position of the band associated with a particular product being related to the number of base pairs it contains. It may be found that the primers generate products from non-target DNA (usually from closely related species) in which case that primer may generally be considered unsuitable for population studies or where it is essential to identify a particular species with a high degree of confidence. Secondary products may also be generated which may be a faint band or as prominent as the band formed from the target DNA. It may be possible to remove the unwanted reaction product by optimising the reaction conditions; otherwise, it will be necessary to use a different primer pair. While some PCR procedures will require the development of specific reagents, a wide variety of commercially-produced kits for PCR is now available for extraction and clean-up of samples.

PCR is a relatively robust technique, aided by the fact that DNA is itself a relatively robust molecule, much less prone to breakdown than (say) proteins. Simple processing such as salting, drying or smoking will still allow DNA recovery and PCR. Even heat processing may still leave enough intact DNA to allow PCR to take place. It is also possible to carry out PCR where DNA has been degraded and fragmented, as long as some intact fragments of the target DNA remain. One major advantage of PCR is that it is possible to detect extremely small quantities of DNA (certainly down to picogramme amounts), enabling its use on very small samples (unlikely to apply in relation to IUU fishing) or on samples which only contain very small quantities of the target DNA.

PCR can be enhanced by the use of nested PCR which relies on a two-step process. This is used where unwanted reaction products have been found. By using one set of primers to amplify a length of DNA which includes the target fragment and then a second set of primers to amplify the target DNA, unwanted reaction products are eliminated. This is naturally a more complicated, more difficult and hence more expensive version of PCR.

DNA sequencing is used to identify the sequence of nucleotides on a fragment of DNA. Given that all species have some unique DNA, this can be used to identify species by cutting out, amplifying and sequencing a specific DNA fragment. Similarly, sequencing is also used to identify the sequence of nucleotides in genes. It is a widely used technique and there are several methods of sequencing. It was the technique used in the Human Genome Project. Generally sequencing relies on PCR to amplify the DNA although more recent developments rely on cloning. Sequencing has generally been regarded as relatively expensive, but costs have decreased to very

competitive levels. Some labs in S Korea are now able to offer sequencing for as little as US\$5 per sample. However, it needs to be kept in mind that this is for sequencing only; there will be costs associated in developing the techniques (including primers) to obtain the specific fragments of DNA prior to sequencing. Once a fragment of DNA has been sequenced, there are a number of statistical programs which can be used to compare that new sequence to those published in various DNA databases.

The different kinds of DNA

Each cell contains a number of different kinds of DNA, contained in different areas of the cell and each having its own characteristics. In addition, within the DNA molecule, there are fragments of DNA which also have specific characteristics. Brief explanations of the main kinds of DNA useful to the detection of IUU fishing are given below.

Nuclear DNA is found in the nucleus of the cell, contained in the chromosomes. It is subject to recombination at every generation and with a few exceptions, fish inherit their nuclear DNA from both parents, nuclear DNA is particularly useful for detecting parentage. The amount of DNA held in the nucleus varies considerably from one organism to another but, with large samples of fish this is not likely to be a significant factor. In addition to containing genetic material in the form of genes, nuclear DNA also contains non-coding DNA, often referred to as “junk-DNA”. Recent research shows that not all non-coding is “junk” and it is believed to play key functions in modifying or modulating the functions of genes. Coding DNA in genes contains the instructions for making other molecules, mainly proteins, but also RNA. Nuclear DNA is used in DNA fingerprinting.

mtDNA or mitochondrial DNA is very different from nuclear DNA. Unlike nuclear DNA, it is held in a varying number of loops containing far lower numbers of base pairs. mtDNA does contain genes which code for proteins, usually those involved in cellular respiration. It was thought that mtDNA was 100% inherited from the mother, but it is now thought that in some cases, a small percentage may be inherited from the father. Since mtDNA does not undergo recombination at reproduction, it is passed from one generation to another pretty much unchanged. As mtDNA is passed on largely unchanged for many generations, it is particularly valuable for use in phylogenetic or lineage studies. However, the mutation rate in mtDNA is about 5-10 times higher than for nuclear DNA which enables another use, that of a “clock” to estimate when species or populations diverged. It may also be used in population studies, but is not generally the DNA of first choice.

Microsatellites are short sequences, usually consisting of only a few base pairs which are repeated many times along a strand of nuclear DNA. They have a high mutation rate and so tend to be highly variable (polymorphic) in a population. Microsatellites are not necessarily species specific and the same microsatellite sequence may be found in related species. Microsatellite DNA may be amplified and visualised by PCR, although the need to develop multiple primers for population studies is a lengthy and costly process. Generally 6-10 microsatellite are selected to detect differences between populations. While 10 (or more) microsatellite may be used, the statistical programs used to identify whether populations differ can still establish

differences even if not all the microsatellite fragments are amplified (a frequent problem in the use of microsatellite). The use of microsatellite to detect population differences relies on the fact that the microsatellite will tend to become mixed within and characteristic of a population. The more reproductive isolation there is between populations, the more chance there is of successfully establishing that they differ by use of microsatellite. However, mixing of populations often means that such differences are not significant and thus it is not possible to distinguish or differentiate them. Establishing the differences between populations requires relatively large samples which again means that the use of microsatellite can be costly.

Examples of the use of DNA-based methods in the detection of fish species and fish populations

DNA-based methods have been used in studies on fish for a number of years to investigate phylogeny, to identify species and to identify different populations and stocks of fishes. In addition to this, there are a great many studies on the DNA of fishes which while not in themselves used for identification of species or populations, may prove to be of relevance (for example, studies on a specific gene). These studies have shown that while identification of species is relatively straightforward, there are some substantial barriers to the identification of stock or population due to variable levels of genetic variability. If two populations of a fish species have very little genetic difference between them, it will be very difficult to differentiate them using DNA-based techniques (or any other technique). Thus, the methods have their limitations and ultimately, the population biology of the species plays a very large part in determining whether stocks or populations can be differentiated. The problems and limitations may be illustrated by reference to some studies on fishes with different biological characteristics.

Swordfish (*Xiphias gladius*). Swordfish pose a number of problems in the identification of populations, reviewed in Ward et. al. (2001) which relate to the highly migratory nature of the species. Swordfish populations off the west and east coasts of Australia and from Reunion Island (Indian Ocean, off Madagascar) were studied, data from these populations being added to data from previous studies on swordfish for an overall analysis. Using data from mtDNA, the study could not find significant differences between the three swordfish populations. Microsatellite DNA revealed minor, but still significant differences between the three populations, the differentiation being largely down to differences detected at a single locus. When data from this study was combined with that of Reeb et.al. (2001), using mtDNA and unpublished work on nuclear DNA, three populations appeared to exist, although the differences between them were too small to be reliable indicators in practice. The authors concluded that the three probable populations found were northern Pacific (differentiated by mtDNA), southern Pacific (differentiated by mtDNA), which is similar to the Reunion Island stocks, despite the obvious geographic separation and the western Australian stock (differentiated by microsatellite DNA). Given the small, observed genetic differences between the populations and the fact that no one source of DNA proved to be wholly capable of differentiating the stocks, the authors concluded that more studies were needed to fully differentiate the populations of swordfish. This contrasts slightly with Reeb et.al. (2001) who found evidence to substantiate the existence of separate northern, southern and western populations of swordfish in the Pacific, but could not differentiate the eastern population.

The difficulty of separating swordfish into geographically localised populations is due to a number of factors. Firstly, the fish are highly migratory and thus stocks tend to be highly mixed, resulting in genetic homogeneity. The difference in DNA between populations tends to be masked by intermixing and interbreeding. Secondly, there are some very widespread populations of swordfish which would need to be identified and excluded from any attempt to identify local stock differences. Clade I has a worldwide distribution and so it is not possible to separate this into populations. Clade II is nominally found in the Mediterranean Sea and the Atlantic. However, it also showed up in the Ward (2001) study off western Australia, being found in two specimens. Subsequent work by Reed et.al. (2003) suggests that new microsatellite loci examined in the Mediterranean and Ecuadorian stocks of swordfish may be more useful in differentiating populations.

Bluefin tuna (*Thunnus thynnus*). Viñas et.al (undated a and undated b) examined the variability of bluefin tuna in the Mediterranean Sea using mtDNA and concluded that their data showed no significant difference between the stocks sampled. They noted that the Mediterranean stock of bluefin in the Mediterranean had always been treated as a single spawning stock and hence as a single population. Previous studies, including those using DNA had failed to differentiate stocks from around the Mediterranean and, despite the sampling of more populations, neither of the studies of Viñas et.al showed any significance differences in mtDNA. Their studies appear to support previous studies which indicate only two stocks of bluefin tuna, one on the western Atlantic and one in the eastern Atlantic, including the Mediterranean.

Bigeye tuna (*Thunnus obesus*). Grewe and Hampton (1998) looked for genetic differences between the Pacific populations of bigeye using both mtDNA and microsatellite. Despite the large sample sizes used (69-105 specimens from each of nine populations), and using 8 microsatellite loci, they were unable to demonstrate significant genetic differences between even the most geographically-separated populations (Ecuador and Philippines). Further examination of 664-806 fish (the number varies with microsatellite as not all microsatellite may be amplified by PCR for any one sample), using 4 microsatellite also failed to demonstrate genetic diversity. Evidence of some genetic differentiation was found with mtDNA, but it was not sufficient to conclude population differences. They concluded that the Pacific population of bigeye forms a single spawning stock. As a side effect from their study, they found that a significant proportion of tunas in the commercial catch were misidentified (from 1-5%, but sometimes as high as 30%) they suggested that much larger sample sizes would be needed to differentiate between any (putative) stocks of bigeye in the Pacific.

Yellowfin tuna (*Thunnus albacores*) and skipjack tuna (*Katsuwonus pelamis*). Ely et.al. (2005) looked for genetic differences between Pacific and Atlantic populations of yellowfin and skipjack tunas. It might be thought that two oceans, separated by a land barrier (North and South America) on one side and by a partial barrier (southern Africa and the Indian Ocean) on the other, might have become genetically distinct. However, using various regions of mtDNA, they found only small differences between the yellowfin populations in the Atlantic and Pacific and none between the populations of skipjack. The differences between yellowfin populations were lower than that found for other, large tunas. The failure to detect a difference was put down

to the very large population sizes of both species which tends to lead to low levels of genetic heterogeneity.

Large, pelagic and highly migratory fishes appear to pose a particular problem when it comes to genetic differentiation and thus the ability to assign any specimen or catch to have come from a particular area of population. Even using large numbers of microsatellite and large numbers of individuals for analysis, it may still not be possible to differentiate all populations from each other, or even to differentiate some populations from each other. The detection of IUU involving tunas poses special problems, but these are not insoluble.

Salmon represent an example of fishes with a biology which tends to lead to genetically distinct populations. Salmon are migratory fishes, but have a high fidelity for spawning sites, returning to more or less the exact part of a catchment in which they were spawned, with relatively little exchange within or between catchments. As many populations have been more or less isolated for thousands or tens of thousands of years, even neighbouring rivers may have genetically distinct populations. Wuttig et.al. (2004) studied rainbow trout and steelhead trout in the Copper River system in Alaska. They are respectively the resident and migratory forms of *Onchorhynchus mykiss*. Using existing, published sequences for 13 microsatellites, they were able to distinguish different populations within the catchment. Where rainbow and steelhead trout spawned at the same site, it was not possible to differentiate them using microsatellite. However, even short distances between populations (as little as 15km) resulted in clearly detected genetic diversity. Seeb et.al. (1997) reported on genetic variability in 10 putative populations of chum salmon (*O.keta*) a fish with a wide distribution around the northern Pacific Rim from Russia to the USA. Data from conventional allozyme analysis was compared with data from mtDNA. In the 6 populations in which data was compared, there was complete agreement in 5 cases between allozyme and mtDNA and in the 6th case, the results were concurrent, if not statistically identical. Seeb et.al. (2006) reviewed progress on the development of genetic markers for Chinook salmon (*O. tshawytscha*). The aim of the work was to develop markers for geographically and temporally separated stocks (stocks may run rivers at different times) in the Copper River system in Alaska. The study used microsatellite (work in progress and not complete) and SNPs using published and already know data from 25 SNPs. Based on a sample of 1272 salmon, five regions have been identified so far and any individual sample may be assigned to a particular region with 89-98% accuracy. Templin et.al. (2006) used similar techniques to look for differences between populations of Chinook salmon in the US part of the Yukon River in Alaska. 2646 salmon from 11 putative populations were examined using 13 standardised microsatellites for Pacific salmon. They achieved an average 83% accuracy in assigning individual salmon to its correct population (the exact geographical location of each fish was known) with most of the errors being accounted for by samples from a single locations, Anvik. This was put down to the poor state of the samples from Anvik. Smith et.al (2005a) used SNPs to examine the same Chinook fishery in the Yukon River. They examined 10 SNP loci of which 9 turned out to be polymorphic within the Yukon System. Using these SNPs, they were able to assign fish from known locations with a success rate of 64% to exact locality, 92-100% to region and 100% to country (remembering that the Yukon River lies in both US and Canada territories). Smith et.al (2005b) noted that SNPs offer

considerable advantages over other methods of determining stock of Chinook salmon, especially in terms of the number of samples which can be processed and the cost of processing samples.

Overall, it appears that the biology and especially the reproductive habits of salmon permit their differentiation into populations by a variety of means, using allozymes or various DNA methods. While it does take time and a substantial sampling effort, once a baseline of DNA data has been built up for the species, individual fish can usually be assigned to a particular population with a high degree of confidence. Even where the species is wide-ranging and highly migratory, such as Chinook, the identification of populations using DNA is not a great challenge.

Walleye pollock (*Theragra chalcogramma*). Walleye pollock form a substantial fishery from the US/Canadian coast across to Russia. Olsen et.al. (2002) tried to find evidence of genetically distinct stocks using allozymes, mtDNA and microsatellite. They looked at six stocks in three regions. Distinguishing stocks proved to be difficult and allozymes and mtDNA often gave different results. Microsatellites proved to be unable to identify and distinguish any populations. It was possible to identify the American and Asian populations as belonging to genetically different stocks, but there was less success on a smaller geographic scale. While the stocks in the Bering Sea and the Gulf of Alaska appeared to have some differences by allozymes, this was contradicted by the mtDNA-RFLP data and in addition, the mtDNA was different in each of the two years tested. At least part of the problem in differentiating stocks was thought to be due to the large population size of walleye pollock (c.f. yellowfin tuna and skipjack tuna).

European hake (*Merluccius merluccius*). Lundy et.al. (1999) tested Atlantic and Mediterranean populations of hake using 6 microsatellite loci. Four hundred and eighty three individuals were sampled from four Atlantic and two Mediterranean locations. The Atlantic and Mediterranean populations were clearly separated, but there was no significant difference between the two Mediterranean sites. Testing the Atlantic sites showed some difference from previously assumed populations. They used five published loci for hake and one for whiting (*Merlangus merlangus*), demonstrating that for DNA polymorphisms may be found in related species. All the populations were genetically diverse. The use of microsatellite showed that two populations currently treated as a single stock are actually genetically distinct and so should be managed separately.

Red snappers (*Lutjanus malabaricus* and *L. erythropterus*). Salini et. al. (2006) attempted to differentiate stocks of red snapper off northern Australia by using allozyme electrophoresis and mtDNA-RFLP. Two broad geographic stocks were identified for both species, but differentiation of more localised stocks was less clear, mainly due to contradictory results from allozymes and mtDNA. This was thought to be due to the fact that 13 allozyme loci were included in the analysis, giving a high chance of resolving differences between populations, whereas the single mtDNA control region examined may have not contained enough information on identify any genetic variation between most populations.

Patagonian toothfish (*Dissostichus eleginoides*) and Antarctic toothfish (*D. mawsoni*). Toothfish have been the subject of intense fishing activity since the

development of the fishery, and IUU fishing has been a particular problem due to the high value of the catch. Despite their similar biology, it has proved possible to differentiate stocks of Patagonian toothfish by genetic means, whereas the Antarctic toothfish stocks could not be differentiated by mtDNA-RFLP, sequencing or mtDNA control regions or by nuclear DNA (Smith and Gaffney, 2005). In general, research on Antarctic toothfish has tended to confirm that it has low genetic diversity. In contrast, Patagonian toothfish have high genetic diversity as measured by mtDNA-RFLP and microsatellite (Shaw et. al. 2004) and by microsatellite (but not by allozymes) (Smith and McVeagh, 2000) and several populations can be differentiated. This development in DNA appears to be very recent. For example, an extensive review of the Patagonian toothfish by Lack and Sant (2001) made no mention of the use of DNA in fisheries management or in detecting IUU fishing.

Tiger prawn (*Penaeus monodon*). Work underway at Mangalore College of Fisheries, India has indicated that the use of RAPD and microsatellite will allow the identification of stocks of *P.monodon* around the Indian coast (Karunasagar, MCF *pers comm.*). Given that prawns have a planktonic larval form which would normally aid dispersal and thus in stock and genetic mixing, it is encouraging that they were able to differentiate stocks. It raises the possibility that it may be possible to identify stocks of prawns, lobster/crayfish/crawfish and crabs of commercial importance in the SFLP fisheries.

Lobster (*Jasus tristani*). Work being undertaken to detect IUU fishing of lobsters from the Tristan de Cunha EEZ also indicates that mtDNA is capable of differentiating between populations (Dick Beales, DfID *per comm.*). Surprisingly, even sample sizes of 40 lobsters were sufficient to detect differences in mtDNA between populations and thus to determine where a lobster had been caught. Further work is being undertaken to increase sample sizes for some populations and map out population differences. If lobsters (in the broadest sense) do have this characteristic of easily differentiated populations, they may make useful marker organisms for the detection of IUU fishing.

Success and failure with DNA

While the ease of recognising species from DNA is widely recognised, it is difficult to draw out the general principles that permit the differentiation of population. It is sometimes easier to recognise individuals than it is to identify which population a sample came from. There are no clear reasons for this, apart from the rather obvious conclusion that any species with low genetic heterogeneity *per se* will be very difficult to divide into recognisable and genetically distinct populations. Several factors may be identified which affect the potential for genetic differentiation:

Migration – highly migratory fishes tend to have much more mixing of stocks. However, this is not always the case. Salmon are highly migratory, with mixing of stocks at sea, but they then separate to a considerable degree prior to spawning and so maintain their genetic segregation. The worst scenario would be a highly migratory species which does not segregate at spawning, resulting in a constant remixing of the genes.

Egg and larval dispersal – even sedentary fish species with high site fidelity may have a prolonged pelagic egg and/or larval stage leading to dispersal by currents over a wide area, leading to constant genetic mixing. The impact of this is not always possible to predict. For example, Patagonian toothfish have a pelagic egg stage lasting 3 months, followed by a pelagic larval stage of unknown length. This might be thought to lead to extensive mixing between populations, but evidence from sampling adult fishes indicates that this is not the case. It is thought that physical barriers may exist to egg and larval dispersal or that current patterns may limit to where pelagic stages can spread.

Population size – as noted for skipjack tuna in particular, a very large population (strictly speaking, the *effective population size*) tends to lead to very low genetic diversity and hence it is very difficult to differentiate stocks, especially over relatively small distances. Conversely, small population size may lead to high genetic variability between stocks, but it should be noted that overfishing may in itself lead to a reduction of genetic variability within a stock.

Barriers to dispersion – these may affect the mixing of stocks in a number of ways. Physical barriers on the ocean floor, such as deep troughs may prevent migration of a species, even where ocean currents might allow some dispersal of eggs or larvae. The presence of a deep ocean trough is for example, thought to be the reason for the separation of French and Spanish stocks of European hake. Other barriers may include temperature barriers, such as cold currents or upwelling. While the temperature difference may in itself be sufficient obstacle for dispersion and hence mixing of stocks, the presence of a current which limit the dispersion of eggs and larvae will tend to reinforce this effect. Very strong currents, such as tidal races may be an obstacle to even the migration of adult fishes.

Phylogeny – the past, recent history of a species will affect its genetic diversity. The very low genetic diversity of some species or populations is thought to be due to genetic “bottlenecks”, which occurs where a species or population arises from a very small, founder population. The reasons for this are varied and include being shut off from the original population (e.g. by a rising land bridge), or by some cataclysmic event which lead to the destruction of most of the original population. It takes a very long time (on a geological scale), or further isolation of subsequent populations for genetic variability to re-emerge.

An extreme example of how these factors come together to influence population genetic heterogeneity can be seen in the cichlids of Lake Malawi. The *mbuna* genus *Pseudotropheus* has strong site fidelity, low larval dispersal and most species are unable to cross even quite small barriers, such as short stretches of sand between their rocky habitats. Van Oppen et.al. (1997) looked at genetic diversity of four species of *Pseudotropheus* along 3km of shore in Nkata Bay on Lake Malawi. Even on such a small scale, it was possible to find differences between populations of all four species using microsatellite DNA. Such differences were believed to have occurred within the last 200 years. Pereyra et.al. (2004) looked at *Protomelas*, a non-*mbuna* species which is not restricted to rocky shores and tends to migrate over distances of a few kilometres, including across sandy areas. In this genus, depending on species, genetic differentiation between populations was observed over distances of a few kilometres with one species showing differentiation only over the greatest distance tested

(280km). By contrast, the offshore, migratory genus *Diplotaxodon* is difficult to differentiate even over great distances ([George Turner](#) pers comm. and Turner et.al. 2001). The great majority of marine fishes are likely to follow the second two cases, especially the last and so will need relatively more sampling and more effort to differentiate populations. Even where marine fishes fall into the pattern of low adult migration and low larval dispersion, it is not certain that populations can be differentiated on a small geographic scale. For example, the seahorses *Hippocampus guttulatus* and *H hippocampus* can only be separated into Mediterranean and Atlantic populations using mtDNA and microsatellites (Lucy Woodall, Royal Holloway College, pers comm.).

How to use DNA to detect differences between fish populations

The use of DNA to detect differences between fish populations is included in reviews by Bloomer (2004) which is of particular reference to the DfID/FAO SFLP fisheries, dealing as it does with the BCLME (Benguela Current Large Marine Ecosystem) which covers Angola, Namibia and South Africa and Wilcock et.al. al. (2004). Reference should be made to those publications. Any researchers used to dealing with DNA-based methods should be able to devise suitable research program to determine the best means for differentiating stocks or populations of any fish species. The basic steps are outlined below:

Discovery of existing information. Many DNA sequences are published in short notes, often together with supporting methodology such as primer sequences, and optimisation of PCR reactions e.g. Chen et.al. (2004) *Dascyllus* VNTRs, Reeb et.al. (2003) swordfish microsatellite sequences and Brown et.al. (2005) *Pagellus* microsatellite sequences. However, the main source of DNA sequences is on the online database [GenBank](#). DNA sequences are held in the database of nucleotides. The database is searchable both by species and by the DNA type (e.g. search for “*Dissostichus eleginoides* mtDNA”), but results need to be treated with caution as the total number of results returned may include related subjects, such as DNA sequences for parasites found in that species. For example, the following searches returned the following results:

Dissostichus eleginoides – 17 sequences
Dissostichus eleginoides mtDNA or mitochondria – no sequences
Dissostichus eleginoides microsatellite - 5 sequences
Dissostichus eleginoides SNP – no sequences

Searches for some species will return hundreds of sequences, even where the search is restricted by (e.g.) microsatellite. Many of the sequences on GenBank are unpublished elsewhere. Some species searches result in results from other species being listed. For example, searches for sequences from *Pagellus* species may return sequences for species other than the one included in the search terms. GenBank contains a huge number of sequences from many species of fish, including those relevant to DfID SFLP areas of interest, such as:

Merluccius capanensis – 16 sequences, including those for the parasite *Kudoa*
M. paradoxus – 10 sequences
M. polli – 9 sequences

Trachurus trachurus – 36 sequences including parasites
T. capensis – 4 sequences
Sardinops sagax – 38 sequences including *S. caeruleus*
Sardinella aurita – 3 sequences
Dentex angolensis – no sequences
Dentex – 101 sequences

Review of biology – some factors relating to the biology of the species have a major impact on the detection of genetic differences. As mentioned above, the phylogeny of a species or of a population can have a large effect on its genetic diversity and must be taken into account. Sampling error must be avoided by ensuring that any putative populations are sampled at a time when they are segregated. Some species may aggregate for spawning and so need to be sampled outside the spawning season; others may segregate for spawning and so should be sampled during the spawning season. As noted for walleye pollock above, there may also be considerable temporal variation in stocks and their genetic variability and this must be taken into account.

Determine sampling size and location – sampling size and location depend on the biology of the species and its probably genetic variation. Put very simply, the less genetic diversity that exists, generally the more DNA sequences that must be examined (e.g. a higher number of microsatellite sequences) and the larger the sample size that must be taken to determine population genetic characteristics. Quite often hundreds or thousands of samples must be taken will need to be taken. There are methods for predicting the sample sizes which will need to be taken (often iterative, depending on results from preliminary surveys). The factors used to determine sample size and sample location are reviewed in Wilcock et.al. (2004) and, especially for the southern African fisheries in Bloomer (2004). It should be noted that the sample size required to produce a result which is useful to prosecution of illegal fishing as opposed to being useful for fishery management may be very much higher, involving greater number of individuals and a greater number of loci.

Select the right DNA – this is by no means simple and straightforward, nor is it that easy to predict. Reviewing the examples of use of DNA in the identification of stocks or populations, several types of DNA have been used, even for the same species. Most commonly, mtDNA and microsatellite have been used, but to some extent, this represents historical trends and more recent uses include SNPs. Alaska Fish and Game Department has now moved from microsatellite to SNPs for the genetic characterisation of fish stocks, citing reasons of cost, slow throughput and technical limitations when using mtDNA. SNPs are also viewed as provided the finest possible resolution for genetic characterisation (Jim Seeb, [Alaska Fish and Game](#)). The use of SNPs compared to mtDNA and microsatellite is reviewed by Morin et.al. (2004). The “right” DNA to use is affected by factors including the phylogeny of the species and population, its genetic diversity, and its spawning behaviour. Again, reference should be made to Wilcock et.al. (2004) and Bloomer (2004) for more details. In the absence of any relevant information on GenBank, it may not be possible to identify the optimum source of DNA, or even the right sequences or alleles (where appropriate) to use until some initial sample has been undertaken. It should be noted that for some species, especially for microsatellite, sequences may be common across a number of species, meaning that initial screening may be made using common sequences from related species.

Optimise PCR – PCR can be optimised to improve differentiation between species and populations. Pre-existing primers may be used from publications or GenBank, which may already have optimised conditions available. Given the large number of samples which need to be processed, methods which allow at least some degree of automation may be preferred.

Detecting fish in mixed and degraded samples

The detection of DNA in foods, even complex and highly processed foods is relatively straightforward. For example, tests have been developed and tested for the detection of GM soya and maize in food products, for example by [Food Standards Agency](#) and commercial kits are available for this such as the [Warnex](#) kits. Even the detection of fish species in complex mixes of fish and processed fish products is not problematic and a number of patents for this purpose are listed on GenBank. Examples in publications are based on the detection of “cod fish” in Japanese fish markets and fish products. The term “cod fish” in Japan refers to a wide range of fish species, not just those in the genus *Gadus*. Aranishi et.al. (2005) reported on the use of PCR-RFLP to identify gadoid species based on mtDNA. The technique was able to identify Alaskan pollock (*Theragra chalcogramma*), Pacific cod (*Gadus macrocephalus*) and Atlantic cod (*G. morhua*) in frozen fish samples. Akashi et.al. (2006) examined 13 species of fish traded as “cod”, including *Gadus* spp., *Merluccius* spp. and *Theragra* spp using PCR-RFLP and direct sequencing of mtDNA. They managed to identify species of these genera in frozen samples and even in dried fish samples which contained degraded DNA. The authors recommended PCR-RFLP as the preferred method for testing, based on the fact that it is a simple, robust technique which lends itself to handling large sample sizes. In theory, there is no reason why this should not also apply to the detection of origin of fish in even processed fish products, but this remains to be proven in practice.

DNA is a robust molecule in comparison to proteins and can withstand normal processing techniques used in food processing. Such simple processing as drying will degrade DNA to some extent, but Shivji et.al. (2001) managed to develop a multiplex PCR based on genomic DNA to identify six species of shark from dried shark fins (although they were not able to test for cross-reaction of primers with some closely related species). Processing such as cooking will tend to fragment DNA into short sections, but even so, enough products of over 100 base pairs may survive to enable PCR and hence identification of fish species. Even in such complex and highly processed products as canned petfood, it is still possible to identify the DNA of specific animals. One widely reported example of this was the detection of cetacean DNA in petfoods in Japan, (see for example [American Cetacean Society](#)). There is no reason why this cannot apply to at least the detection of fish species in highly processed foods.

In addition to being able to withstand some processing, DNA can still remain intact, or in sufficiently large fragments to enable PCR after being subject to sample deterioration. Samples used by Templin et.al (2006), included scales taken from the carcasses of dead, post-spawning salmon and although there was a higher failure rate than from samples taken from live or fresh salmon (fin clips, heart muscle, etc), it was still possible to amplify DNA from as few as 2 scales. In addition, the techniques

have now been extended to extract and amplify DNA from stored samples, such as cod otoliths (Hutchinson et.al, 2003). This suggests that the techniques could be applied even to smoked, salted or dried fish as often produced by the artisanal sector.

Examples from wildlife

DNA-based techniques have been used widely in tracking the wildlife trade and in providing evidence for use in court. Some of the uses are of limited relevance to the trade from IUU fishing, being mainly related to the proof of the parentage of animals or proving that an individual human or animal has been the source of a sample. For example, out of 26 cases where DNA evidence was used in the prosecution of wildlife crime in the UK, in 7 cases the DNA was used to prove a person had been present at a crime scene and in 19 cases the DNA was used to prove that the provenance and parentage of birds had been claimed falsely (Jon Wetton, Forensic Science Service, *pers. comm.*). Other examples mainly relate to the identification of species, the trade of which is illegal such as:

- The detection of shahtoosh wool in shawls, the detection of endangered species such as tiger and bear in Oriental traditional medicines (PAW, 2005, TRAFFIC Bulletin 18(2):54-55)
- The identification of tiger DNA in samples of Oriental traditional medicines supposedly containing tiger bone (Wetton et.al. 2003)

Nonetheless, the majority of wildlife crime prosecutions rely on more conventional evidence such as morphology to identify the animals in question.

The use of DNA evidence in the detection and prosecution of the illegal wildlife trade may increase as the database of DNA profiles of species increases. This will permit better discrimination of species and of populations such as:

- Wasser et.al. (2004) developed improved DNA methods for the identification of African elephant populations which can be used to identify the probable source of ivory. Ivory contains relatively little DNA and so it remains to be seen how widely this technique can be applied and especially whether it can be applied to worked ivory. Nonetheless, this information is being used by INTERPOL to monitor the international ivory trade and to target countries for investigation. A test case involving the use of DNA evidence to show that elephant ivory was not sourced from the country claimed on documentation is being prepared (Wasser, University of Washington, *pers comm.*).
- Dizon et.al. (2000) reported on developments by the International Whaling Commission to develop DNA methods for the identification of whale species and to assign an area of origin to whale samples. With the exception of one species group of uncertain taxonomy, it was shown to be possible to identify whale species with a high degree of confidence. However, being able to assign a whale sample even to the ocean or origin, let alone to a particular stock proved to be difficult and with results varying considerably from species to species. This is a far higher level of assignment than would be required for fish as it is not necessary to assign an individual to its origin, rather this would be carried out on a sample of fish, comprising a number of individuals. It

should be noted that the social nature of some whale species and particularly the dominance of matrilineal inheritance in some species means that their population genetics has significant differences from fish.

It should be noted that WDNAS is currently (September 2006) carrying out a survey of bushmeat imports (all are illegal) to the UK on behalf of DEFRA (Ross McEwing WDNAS, *pers comm.*). Details of this work cannot be released at present, but as it may provide information relevant to the detection of fish traded from IUU fishing, DfID and DEFRA should examine it once released to see what lessons are transferable to the detection of IUU fishing.

Examples of detection of illegal fishing by DNA

As most examples are in grey literature, unreported or present in court records, tracking down specific examples where DNA has been used to detect and prosecute successfully IUU fishing is difficult. This may to some extent represent the relative novelty of its use in fisheries law and may also reflect the relatively small number of commercial fishery species for which adequate genetic data are available. Some examples of the use of DNA evidence are given below:

Peter Smith ([NIWA](#)) *pers comm.* reported how NIWA has used the DNA barcoding approach for bluefin tuna identification, having in the past used RFLP DNA and that this method was accepted by the law courts in New Zealand. Any court case considers a whole range of evidence, but the genetic data have been the key method and evidence for specimen identification. DNA evidence has also been used to identify fish fillets in court cases.

Sharon Appleyard ([CSIRO](#)) *pers comm* outlined how DNA methods are used in Australian fisheries management. The lab regularly uses molecular markers (microsatellites, mtDNA-RFLPs, sequencing) for fisheries stock assessment and species identifications (e.g., in toothfish, yellowfin & bigeye tuna, southern & northern bluefin tuna, shark species), although only in several cases have the results impacted on management plans (i.e., toothfish, bluefin tuna domestic fishing in Australia) and the prosecution of IUU fishers (no further details were provided). DNA evidence appears to not have been used so far in Australian courts to identify the area of origin of fish.

DNA as court evidence

The use of DNA for fisheries management is very different from that for court evidence. The level of proof required in court is far higher than that for management purposes. Fisheries managers may accept an 85-95% probability that a fish sample can be assigned to a particular population, but courts generally require very much higher levels of probability, usually as close to 100% as possible. In “headline” terms, fisheries management can accept a 1:20 chance that an individual has been assigned to the wrong populations, whereas a court assessing human DNA would expect a 1:3,000,000 chance of DNA having been wrongly assigned to an individual. In part, this represents differences in the type of DNA evidence produced in court and the size of the databases on which that evidence is based. Enough is known about the worldwide variation in human genes that it is now possible to provide evidence that

DNA could only come from one individual in 6 billion, a number approximately equal to the current human population.

Typically DNA evidence presented in court will be presented to prove beyond reasonable doubt that either an individual can be linked to the scene of a crime (for example, badger hairs found on clothing may link a person to an individual badger killed by dogs) or to prove that an animal cannot have been from the source claimed. Typically, this would be through a parentage test to show that an animal supplier's breeding stock could not have been the parents of a particular individual as claimed. Evidence that a sample comes from a particular species is also possible with a very high degree of certainty, assuming standard precautions have been followed (e.g. checking primers for cross-reaction with related species). In such cases, the levels of confidence are very high, typically presented as headline figures as a "one in three million chance" of the match not being correct.

The databases used in support of human DNA evidence are vast, typically being comprised of tens of thousands, if not hundreds of thousands of items of data. Databases for human DNA gain from worldwide contributions and worldwide access. Using such large databases with which to compare sample DNA lends a very high level of confidence to fit; it is simply very unlikely that the sample will be wrongly assigned. By contrast, fishery management DNA databases are often based on samples of a few hundred samples at most often spread across a number of populations. Only in a few fisheries, such as the Alaskan Chinook fishery, does the database extend to thousands of individuals.

However, the lack of robust DNA evidence does not mean that there is no role for it in the prosecution of IUU fishing as there are a number of strategies which can be adopted to overcome the lack of confidence. Based on advice from Jon Wetton (FSS, *pers comm.*) and Ross McEwing (WDNAS, *pers comm.*), there are ways in which conventional, fisheries management DNA evidence can be presented in court as proof of IUU fishing:

- The typical 85-95% probability of assigning an individual is far lower than that currently accepted by courts. Therefore, this should not be represented as "proof", and the shortcomings of the evidence should be recognised. For example some DNA evidence has been presented in UK courts with even lower levels of confidence, but as long as the way in which the evidence has been derived and how the level of confidence has been derived is explained, the court can simply take this into account in considering DNA as part of the total evidence. Failing to do this and presenting the DNA evidence as without recognising its limitations will mean the evidence will be discredited.
- Do not try to prove the unprovable. Given a sample of fish, believed to have originated from IUU fishing, there are two approaches which could be taken. The approach of "proving" that the fish sample was caught from a particular stock and thus came from a particular is very high risk. Unless a baseline database of DNA from all populations of a species is available, defence can easily claim that the fish sample simply came from one of the populations which has not been sampled. Conversely, requiring the vessel owner to state where the fish had been caught makes the proof much easier. All that needs to

be done is to compare the sample against the genetic profile in the area where it is claimed the fish was caught. If the profile does not match, clearly the vessel owner is providing misleading information. That places the burden of proof on the vessel operator, not on the prosecuting authority.

- DNA is not presented as the sole evidence. It must be backed up by other evidence of IUU fishing from monitoring, inspection/control and surveillance (MIS). MIS is often weak in developing countries and may not be reliable. Other supporting evidence may be provided, such as incomplete or falsified documentation.

Clearly the above relies on having reliable evidence in the first place and assumes that due diligence has been observed in the collecting and compilation of the DNA baseline data in the first place. Acceptable levels of due diligence are not always followed in fisheries research; indeed it is not always needed if research is being carried out on (e.g.) variations in a particular gene sequence to determine its links to enzyme polymorphism. A number of areas where the data to be collected for use as court evidence may differ from that to being used in fisheries management include:

- Rigorous testing of primers for absence of cross-reaction. While it may be adequate for fisheries management purposes to do this for species found in the same area, for court use, it may be necessary to extend this to a wider range of species as, while they may not occur together in the seas, they may occur together in trade and in fish products;
- A greater number of loci may need to be included in the analysis. Thus in use of microsatellite, it may be necessary to use 15-20 loci, rather than the more common 5-15 which are generally adequate for research purposes;
- A much larger number of individuals may need to be sampled, over a wider geographic area. There are cost implications to this and it may need international cooperation;
- Rigorous testing of data to determine the level of confidence which can be achieved. This would need to include testing of the chances of an individual being wrongly assigned to a population. This is a particular problem with rare genes – they are more likely to result in a sample being assigned to a large population rather than a small population;
- Comparing methods (e.g. mtDNA, microsatellite, RFLP) to see which provides the most robust data.
- A fundamental difference between sampling for research and management purposes and sampling to provide forensic evidence is the way in which the data are collected. Data for research and management is usually collected and then analysed to see if it matches a hypothesis (e.g. that the fish comprise a single, genetically undifferentiated population). This is quite different in approach from that required to provide forensic DNA evidence which must work back from what is an acceptable level of proof required by the court which is a far more stringent requirement.

An example of how fish samples were handled for court evidence is provided by Peter Smith ([NIWA](#)) *pers comm*. “The courts in New Zealand ask for a “brief of evidence”, which has a different structure to a scientific paper. I imagine that law courts in the UK have a similar system. Obviously we use the same DNA techniques in either a scientific paper or a brief of evidence. One of the key differences is in the collection and handling of the samples. We have to establish an evidence trail, for a brief of evidence. Samples taken by a Fishery Officer must be passed directly to the person undertaking the lab analyses, and the samples kept in a locked laboratory (the samples cannot be sent as unaccompanied freight). The person undertaking the lab analyses must also prepare and present the brief of evidence. A record sheet accompanies the samples and each time the samples are passed from collector to lab, the movement is recorded”. This is not a difficult requirement to observe, but does require some procedures to be adopted which differ from those for the collection and handling of scientific data.

Further guidance on the collection of DNA-base evidence in wildlife crime can be found in PAW (2005). The guidance goes far wider than just DNA and includes the collection and documentation of evidence and laboratory analysis. Comparison can be made with the summary of the use of DNA evidence in wildlife crime by [NAWEG](#), as in their information bulletin (NAWEG, 2001). Much more detailed information on the taking, handling and use of DNA evidence (albeit for humans) can be found at [APRI](#), which includes Kreeger and Weiss (2003). Further and wider guidance on wildlife crime evidence can be found at [Trent University](#) (but note that the wildlife DNA laboratory link was down on 31/08/06 and 07/09/06 due to laboratory relocation). Obviously, the rigorous standards applied to the collection of human DNA for evidence would not apply to the collection of fish samples for detecting or prosecuting IUU fishing, but they will provide an indication of some of the precautions which will need to be taken.

It is as well to be aware of the kinds of arguments which can be used to counter evidence from DNA. No examples were found with direct relevance to IUU fishing or wildlife crime, but examples can be found for human DNA evidence such as at [William Thompson](#) home page at University of California, Irvine and see Thompson et.al. (undated). Much of the defence against DNA evidence relies on the limitations of techniques such as PCR and on the statistical assumptions which underlie conclusions drawn from DNA sampling. With evidence of IUU fishing being based on less rigorous data and evidence than for human DNA, it is as well to recognise the limitations of the techniques used and to state these clearly in the presentation of any evidence, even if not related to prosecution.

All the above does rely on the baseline data and the sample testing being carried out at a laboratory operating to a high quality standard.

DNA laboratories

There are many laboratories around the world which would be capable to carrying out the work required for the differentiation of species or of populations/stocks by DNA. Certainly in S Asia and SE Asia, there are many private and public laboratories capable of providing such services. Options are likely to be more limited in Africa.

Regardless of where a lab is situated, it is vital that it operates to a very high and verifiable standard. The two standards generally in operating are ISO9001 and ISO17025, the latter specifically applying to laboratory operation. Certification to ISO17025 must be carried out by a recognised accreditation body. These do not exist in all countries, but there are arrangements in place for accreditation bodies to work on a regional or international basis. In the last resort, even if a laboratory does not have ISO17025 accreditation, they may be audited by another laboratory which is accredited. This is less satisfactory than being accredited directly.

There is a further requirement for the operation of laboratories which differs from that required for fisheries research and that is that the collection of forensic evidence is totally different from the collection of survey data for scientific and management purposes. From the moment the sample is taken, very strict standards of sample handling, storage, processing (including DNA analysis) and record keeping must be applied. These will be far more stringent than those normally applied to research and specific training may need to be delivered to laboratories with no experience of handling forensic samples (Jon Wetton, FSS *pers comm.*).

The equipment required for the creation of a DNA laboratory (should it be required) is now standard and can be sourced “off the shelf”. No particular problems are anticipated in that respect. The exact equipment to be supplied would be defined by the actual sampling and DNA analysis to be performed.

The nomination of a laboratory to carry out forensic testing is largely a matter for individual governments to resolve. However, where there is no suitable laboratory available in-country, or even regionally, the question then arises of where to locate such a laboratory. One option is simply to contract out the entire sample processing to a nominated laboratory which can provide the service at best price and best standard. Given the ease of sending samples worldwide and the existence of service laboratories in many countries (e.g., Korea), this is a feasible option. The development of a regional laboratory does raise some policy problems notably that of providing evidence that could lead to the prosecution of vessel owners from coastal states within the region or even the country in which the laboratory is based. This should not be an insurmountable problem, especially where the laboratory is managed and operates to ISO9001 and ISO17025, both of which rely on external accreditation and auditing.

The costs of using DNA evidence

This is very difficult to estimate as most of the data generated has been for research and management purposes, and the cost of doing this is likely to be less than that of generating data for use as forensic evidence on court. Much of the cost of using DNA lies in the reagents and consumables (which may be available in commercial kit form) and, as these are supplied mainly by international suppliers, costs will be broadly similar wherever they are used. The main variable cost is likely to be labour which will be far cheaper in developing countries than in (e.g.) Western Europe. Cost estimations were given in Wilcock et.al. (2004) which can be summarised:

mtDNA RFLP

Costs based on the analysis of 750 individuals across 15 sites

Labour 12 pm of time excluding overheads £24,600 (88% of total costs)

Consumables £3,240 (12% of total costs)

Total cost £27,840 equivalent to £36 per individual

mtDNA sequencing

Costs based on sequencing 20 individuals across 15 sites, 300 individuals in total

Labour 3 pm of time excluding overheads £6,150 (65% of total costs)

Consumables £3,240 (35% of total costs)

Total cost £9,390 equivalent to £31 per individual

Microsatellites

Costs based on the analysis of 1500 individuals across 15 sites for 10 loci

Microsatellite isolation

Labour 6 pm of time excluding overheads £12,300 (87% of isolation costs, 31% of total costs)

Consumables £1,800 (13% of isolation costs, 5% of total costs)

Total cost of isolation £14,100 (£9 per individual)

Sample screening

Labour 6 pm of time excluding overheads £12,300 (49% of screening costs, 31% of total costs)

Consumables £12,600 (51% of screening costs, 32% of total costs)

Total cost of screening £24,900 (£16 per sample)

Total cost for microsatellite £39,000 (£25 per individual)

Based on the above costs (recognising that overheads could add substantially to labour costs), the costs of establishing a baseline database from scratch are reasonable in comparison to the losses incurred by coastal states from IUU fishing. For example, to establish baseline data on the basis of analysis of DNA from 10,000 individuals of a species would cost £360,000 for mtDNA RFLP and £160,000 for microsatellite (recognising that a greater number of loci may need to be screened for forensic evidence use). These costs are reasonable compared to the estimated losses to IUU fishing of US\$2-15 billion annually. Obviously, these costs are only a very rough estimate of the costs in practice, but they do provide an indication of the scale of costs. It would not be necessary to develop DNA detection methods for all species and realistically, only those species which are prominent in the international trade from IUU fishing would be targeted.

The costs of using SNPs was compared to that of microsatellite by Morin et.al. (2004) and was reported to be considerably cheaper for use in compiling a database of genotypes. However, no information was provided on uses beyond this and further work would be needed to provide accurate information on costs. Smith et.al. (2005) also noted cost savings to be made from SNP genotype by using SNPs from other species.

Using DNA-based methods to combat IUU fishing

There are two options for combating IUU fishing. Firstly, the less confrontational and lower cost route would be to use it to monitor the trade and identify the routes by which fish IUU reaches markets and to use this information to quantify the trade. This could be done with data used for research and management, adopting the same criteria as currently used for identifying and monitoring fish stocks. Much of this could be done using existing data. However, the opportunities for using such data for putting real pressure on countries which turn a blind eye to IUU fishing are limited. “Naming and shaming” persistent offending vessels might work, as would denying them access to port facilities in countries which have committed themselves to ending IUU fishing. It may further be possible to use such data to limit IUU fishing by closing access to markets, such as by including the need to verify source of origin in documentation accompanying fish. Commercial pressure may even be enough to ensure this – a company may not be able to afford the bad publicity from being found to be dealing in product from IUU fishing and so may include this in the list of due diligence to be carried out by suppliers. Nonetheless, the over impact on IUU fishing may not be great as material may simply be diverted to alternative markets. To have maximum impact, it may be necessary to adopt the highest level of proof and use this for legal enforcement.

Assuming suitable data could be compiled to meet the standards of evidence required for prosecution, other supporting evidence would be needed (e.g. from MIS, falsified log books). Having assembled evidence, the question then arises of where prosecution should take place and by whom? One obvious area for intervention would be in major fish importing countries where regular inspection to confirm that the species and area of origin of fish is as declared on accompanying documentation and consequent prosecution of offenders could help to close off the entry of IUU fish. This is one area where the UK could take a lead. The cooperation of countries involved in EU fishing licence agreements would assist greatly in this.

Catching vessels involved in IUU fishing would need the active involvement of the coastal states so affected. In such cases, and where they do not have the resources to develop the DNA baseline data and use it to generate forensic evidence, some external assistance would need to be provided, either through direct government to government cooperation, or by contracting a commercial laboratory to examine and analyse samples. This may be an appropriate role for DEFRA and/or DfID. The involvement and backing of the European Commission DG Fisheries would also be helpful in tackling the issue EU-wide.

Conclusions

There is definitely potential for existing knowledge to be used to enable DNA evidence to be used to demonstrate IUU fishing of species. Such knowledge is available widely and openly and should not present any particular barriers to use in practice. Identification of population and stock is far more problematic and may simply not be possible or highly unreliable for some species. This remains the greatest challenge as most IUU fishing takes place by raiding stocks to which the vessels have no legitimate access, rather than taking species for which they have no permits. The problems lie less in the DNA techniques which can be applied to stock identification and which are improving all the time, than in the intrinsic biology of species. Species with highly migratory adults, high dispersal of larvae, mixing of stocks during spawning and with a large population size will continue to pose challenges. Costs for the set-up of a system to generate DNA evidence to be used in the detection of IUU fishing are reasonable, and small in comparison to the loss of income to developing countries. The costs and difficulties of establishing laboratories capable of delivering DNA evidence which can be used in court are greater, but still reasonable, especially if the spin-offs are taken into account (such a laboratory could provide a wide range of forensic DNA services as they do in UK).

Costs for the detection of many species will be low as useable DNA sequences, primers and reaction conditions can be had more or less off the shelf from sources such as GenBank. There may be some development work testing which primers work best and optimising PCR conditions, but this would be limited. It is debatable whether a test kit as such would need to be developed and whether it would be cost effective. As fish samples are unlikely to require complex DNA extraction and clean-up procedures, the tests could be carried out using standard laboratory reagents. Based on costs given in Wilcock et. al. (2004) it can be estimated that the cost of developing a PCR method for identifying a single species could be as low as £10,000 per species, allowing for testing of cross-reaction with other, closely related species. Costs would be even lower than this if a fully validated, published primer is available. Costs are likely to be appreciably lower outside the UK due to lower labour costs (the cost of consumables will not be lower outside the UK).

By contrast, costs for developing DNA-based methods of differentiating populations are higher and uncertain. It is quite possible to carry out extensive research and conclude that it is not possible to differentiate populations. The costs estimated in Wilcock et.al. (2004) are applicable to this and, depending on the analysis used, would be in the range of £27-39,000 for initial screening of each species. Additional costs beyond this may be incurred if the first-round screening shows the need for more sampling. Realistically, it might be wise to budget for costs in the order of £100,000 to screen populations of 4-5 species of key importance in (for example) one of the fisheries covered by the SFLP project off West Africa. As indicated earlier, costs would be substantially higher if “off the shelf” methods and primers could not be used and if it were necessary to use large sample sizes.

Once developed, there might be some prospects for cost recovery, especially if methods were to be based on test kits, if a licensing or manufacturing agreement could be made with a company producing DNA reagents. Such an option will only be attractive to a manufacturer if volume production can be achieved (probably in the

low thousands of units). The use of any DNA-based method to detect or prosecute IUU fishing directly would generate a payback to coastal states whose fisheries are affected by IUU fishing in terms of preventing or recovering lost income, fines and seizures.

There are still problems to be overcome in using the methods to limit, eliminate or prosecute IUU fishing. These mainly relate to the use of the data and the evidence it generates (who “owns” it for example) and in generating the political will to pursue those who turn a blind eye to IUU fishing. However, using DNA evidence of the existence of IUU fishing and the routes by which fish from IUU fishing enter trade may in itself generate enough political pressure to get reluctant authorities to take action.

Appendix 1: references

- Akasaki T, T Yanagimoto, K Yamakami, H Tomononaga, and S Sato (2006) Species Identification and PCR-RFLP Analysis of Cytochrome *b* Gene in Cod Fish (Order *Gadiformes*) Products Journal of Food Science **71**(3): 91-95
- Aranishi F, T Okimoto and, S Izumi (2005) Identification of gadoid species (Pisces, Gadidae) by PCR-RFLP analysis. Journal of Applied Genetics **46**(1): 69-73
- Bloomer P (2004) Feasibility study into the application of genetic techniques for determining fish stock identity of transboundary populations in the BCLME region. Project LMR/CF/03/04, Molecular Ecology & Evolution Programme Department of Genetics University of Pretoria 0002 Pretoria South Africa
- Brown RC, M. Tsalavouta, V. Terzoglou, A. Magoulas and B. J. McAndrew (2005) Additional microsatellites for *Sparus aurata* and cross-species amplification within the Sparidae family. Molecular Ecology Notes **5**: 605-607
- Chen CA, M C Anonuevo Ablan, J Williams McManus, J D Bell, V S Tuan, A S Cabanban, and K-T Shao (2004) Variable Numbers of Tandem Repeats (VNTRs), heteroplasmy, and Sequence Variation of the Mitochondrial Control Region in the Threespot Dascyllus, *Dascyllus trimaculatus* Perciformes: Pomacentridae). Zoological Studies **43** (4): 803-812
- Dizon, A., C. S. Baker, F. Cipriano, G. Lento, P. Palsbøll, and R. Reeves. (eds.) 2000. Molecular Genetic Identification of Whales, Dolphins, and Porpoises: Proceedings of a Workshop on the Forensic Use of Molecular Techniques to Identify Wildlife Products in the Marketplace. La Jolla, CA, USA, 14-16 June 1999. U.S. Department of Commerce, NOAA Technical Memorandum, NOAA-TM-NMFS-SWFSC-286. 52pp. + xi.
- Ely B, J Viñas, J R Alvarado Bremer, D Black, L Lucas, K Covello, A V Labrie and E Thelen (2005) Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonus pelamis*). BMC Evolutionary Biology **5**: 19. This article is available from: <http://www.biomedcentral.com/1471-2148/5/19>
- Grewe P and Hampton J (1998) An assessment of bigeye (*Thunnus obesus*) population structure in the Pacific Ocean, based on mitochondrial DNA and DNA microsatellite analysis. Report to the Forum Fisheries Agency. CSIRO, Australia
- Hutchinson WF, C van Oosterhout, SI Rogers and GR Carvalho (2003) Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). Proceedings of the Royal Society of London B **270**: 2125-2132
- IWC (2005) Report of the Working Group on DNA. Unpublished report.

Kreeger, LR and DM Weiss (2003) Forensic DNA Fundamentals for the Prosecutor: Be Not Afraid. APRI http://www.ndaa-apri.org/pdf/forensic_dna_fundamentals.pdf

Lundy CJ, P Moran, C Rico, R. Milner and G M Hewitt (1999) Macrogeographical population differentiation in oceanic environments: a case study of European hake (*Merluccius merluccius*), a commercially important fish. *Molecular Ecology* **8**: 1889-1898

Morin PA, G Luikart, R K. Wayne and the SNP workshop group (2004) SNPs in ecology, evolution and conservation. *Trends in Evolution and Ecology* **19**(4): 208-216

Oolse TB, SE Merkouris and JE Seeb (2004) An examination of spatial and temporal genetic variation in walleye pollock (*Theragra chalcogramma*) using allozyme, mitochondrial DNA, and microsatellite data. *Fisheries Bulletin* **100**: 752-764

NAWEG (2000) DNA Analysis in Wildlife Forensics. http://www.cec.org/programs_projects/law_policy/regional_enforce_forum/naweg/dna-e.pdf

PAW (2005) Wildlife Crime: a guide to the use of forensic and specialist techniques in the investigation of wildlife crime. Partnership for Action Against Wildlife Crime, UK.

Pereyra, R, Taylor, MI, Turner, GF, and Rico, C (2004) Variation in habitat preference and population structure among three species of the Lake Malawi cichlid genus *Protomelas* *Molecular Ecology* **13**: 2691-2607

Reeb CA, L Archangeli and BA Block (2000) Structure and migration corridors in Pacific population of the Swordfish *Xiphias gladius* as inferred through analysis of mitochondrial DNA. *Marine Biology* **136**: 1123-1132

Reeb CA, L Archangeli and B A Block (2003) Development of 11 microsatellite loci for population studies in the swordfish, *Xiphias gladius* (Teleostei: Scombridae). *Molecular Ecology Notes* **3**:147-149

Salini JP, J R Ovenden, R Streer, R Pendry, Haryanti AND Ngurah (2006) Genetic population structure of red snappers (*Lutjanus malabaricus* Bloch & Schneider, 1801 and *Lutjanus erythropterus* Bloch, 1790) in central and eastern Indonesia and northern Australia *Journal of Fish Biology* **68** Supplement **B**; 217-234

Seeb, LW, PA Crane and EM Debevec (1997) Genetic analysis of chum salmon harvested in the South Unimak and Shumagin Islands June Fisheries, 1993-1996. Alaska Department of Fish and Game, Regional Information Report No 5797-17, Anchorage, USA

Seeb LW, D Moore, CT Smith and WD Templin (2006) Progress in development of a DNA baseline for genetic identification of Chinook salmon of the Copper River

Basin, Alaska. Alaska Department of Fish and Game, Fishery Data Series No 06-20, Anchorage, USA

Shaw, PW, AI Arkipkin and H Al-Khairulla (2004) Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep-water troughs as barriers to genetic exchange. *Molecular Ecology* **13**(11):3293-3003. Abstract only.

Shivji M, S Clarke, M Park, L Natanson, M Kohler and M Stanhope (2002) Genetic identification of pelagic shark body parts for conservation and trade monitoring. *Conservation Biology* **16**(4): 1036-1047

Smith CT, WD Templin, J E Seeb, and L W Seeb (2003) Single Nucleotide Polymorphisms Provide Rapid and Accurate Estimates of the Proportions of U.S. and Canadian Chinook Salmon Caught in Yukon River Fisheries *North American Journal of Fisheries Management* **25**: 944-953

Smith CT, CM. Elfstrom, LW Seeb and JE Seeb (2005) Use of sequence data from rainbow trout and Atlantic salmon for SNP detection in Pacific salmon. **14**; 4193-4203

Smith CT, JE Seeb, P Schwenke and LW Seeb (2005) Use of the 5'-Nuclease reaction for single nucleotide polymorphism genotyping in Chinook salmon. *Transactions of the American Fisheries Society* **134**(1): 207-217 Abstract only.

Smith PJ and PM Gaffney (2005) Low genetic diversity in the Antarctic Toothfish (*Dissostichus mawsoni*) observed with mitochondrial and intron DNA markers. *CCALMR Science* **12**: 1-9

Smith PJ and M McVeagh (2000) Allozyme and microsatellite DNA markers of toothfish *Dissostichus eleginoides* population structure in the Southern Ocean *Journal of Fish Biology* **57**: 72-83

Templin WD, WA Decovich and LW Seeb (2006) Yukon River Chinook salmon genetic baseline: survey of Pacific Salmon Commission loci for US populations. Alaska Department of Fish and Game, Fishery Data Series No 06-46, Anchorage, USA

Thompson, WC, S Ford, T Doom, M Raymer and D Krane (undated) Evaluating Forensic DNA Evidence: Essential Elements of a Competent Defense Review <http://www.cs.wright.edu/itri/EVENTS/SUMMER-INST-2003/SIAC03-Krane2.PDF>

Turner, GF, RL Robinson, BP Ngatunga, PW Shaw and GR Carvalho (2001) Pelagic cichlid fishes of Lake Malawi/Nyassa. *Journal of Aquaculture and Aquatic Sciences. Cichlid Research: State of the Art.* **IX**: 287-302

Van Oppen, MJH, GF Turner, C Rico, CJC Deutsch, KM Ibrahim, RL Robinson, and GM Hewitt (1997) Unusually fine-scale genetic structuring found in rapidly speciating Malawi cichlid fishes *Proceedings of the Royal Society of London B.* **264**: 1803-1812

Viñas J, M. El Tawil and C Pla (undated) Preliminary genetic analysis of Mediterranean Bluefin tuna caught in Libyan waters. Publication not attributed.

Viñasz J, C. Pla, MY Tawil, A. Hattour; AF Farrugia and JM de la Serna (undated). Mitochondrial genetic characterization of Bluefin tuna (*Thunnus thynnus*) from three Mediterranean (Libya, Malta, Tunisia); and one Atlantic locations (Gulf of Cadiz). Publication not attributed.

Ward, R.D., Reeb, C.A. and Block, B.A. (2001) Population structure of Australian swordfish, *Xiphias gladius*. Final Report to the Australian Fisheries Management Authority, Canberra. Published by CSIRO Marine Research.

Wasser SK, A M. Shedlock, K Comstock, E A Ostrander, B Mutayoba, and Matthew Stephens (2004) Assigning African elephant DNA to geographic region of origin: Applications to the ivory trade. PNAS **101**(41); 14847-14852. This article is published online at: www.pnas.org/cgi/doi/10.1073/pnas.0403170101

Wetton JH, C SF Tsang, C A Roney, A C Spriggs (2004) An extremely sensitive species-specific ARMs PCR test for the presence of tiger bone DNA. Forensic Science International **140**(1): 139-145

Wilcock HR, W F. Hutchinson and G R. Carvalho (2004) A review of the potential applications of population genetics in fisheries management. Report to the Department for Environment, Food and Rural Affairs. Molecular Ecology & Fisheries Genetics Laboratory University of Hull.

Wuttig K, Olsen J and Fleming D (2004) Stock status and population biology of the Copper River steelhead. Alaska Department of Fish and Game, Fishery Data Series No 04-18, Anchorage, USA

Appendix 2: contact details for persons and organisations contacted

Contact Name	Contact Details	Date Contacted	Reply Received
<p>Note : some attempts to contact researchers and practitioners lead to a dead end. In most case, this appeared to be due to staff moving on, having completed e.g. PhD or post-doc studies. Dead end queries are not included in this list. Only date of original contact is listed, not the dates of all subsequent contacts.</p>			
UK contacts			
Prof George Turner	<p>Prof G.F.Turner, Professor of Evolutionary Biology and Biodiversity Department of Biological Sciences, University of Hull, HU6 7RX Phone 01482 466425 Fax 01482 465458 <i>e-mail:</i> g.f.turner@hull.ac.uk Turner research web page: http://www.hull.ac.uk/cichlids Biological Science Dept web page: http://www.hull.ac.uk/biosci/ Postgraduate Study at Hull: http://www.hull.ac.uk/05/courses/prospectus/index.html Hull Environment Research Institute: http://www.hull.ac.uk/HERI/</p>	2 August 2006	02/08/06 – further info requested.. Received 03/08/06.
Dr Martin Taylor	<p>Dr Martin Taylor, School of Biological Sciences University of Wales - Bangor Deiniol Road Bangor Gwynedd LL57 2UW</p>	2 August 2006	Out of office reply 02/08/06 Reminder sent 24/08/06 Reply received 24/08/06

	<i>e-mail:</i> m.taylor@bangor.ac.uk Molecular Ecology and Evolution Group: http://biology.bangor.ac.uk/research/evolevol		
Prof Gary Carvalho	Gary R. Carvalho Professor of Molecular Ecology School of Biological Sciences University of Wales Bangor Brambell Building Bangor Gwynedd LL57 2UW <i>e-mail:</i> g.r.carvalho@bangor.ac.uk Molecular Ecology and Evolution Group: http://biology.bangor.ac.uk/research/evolevol	8 August 2006	Out of office reply 08/08/06 Reminder sent 24/08/06 – out of office reply. Reply received 24/08/06
Dr David Agnew	Dr David Agnew, MRAG Ltd. 18 Queen Street, London W1J 5PN <i>e-mail:</i> david.agnew@mrag.co.uk	2 August 2006	Out of office reply 02/08/06
CEFAS general enquiries	Cefas Weymouth Laboratory The Nothe, Barrack Road Weymouth Dorset DT4 8UB <i>e-mail:</i> n/a via web form	2 August 2006	No reply

Chris Kerr	National Wildlife Crime Intelligence Unit, National Criminal Intelligence Service	2 August 2006	02/08/06 – autoreply that NWCIU has been closed 08/08/06 reply with new contact details for PAW and TRAFFIC
Ape Alliance	Enquiry through website	2 August 2006	No reply
Press office	Forensic Science Service, web query to press office	2 August 2006	02/08/06 – reply from press office saying enquiry had been forwarded. 07/08/06 phone conversation with Jon Whetton 0121 329 5428 <i>e-mail:</i> Jon.Wetton@fss.pnn.police.uk
General enquiries	TRAFFIC International, 219a Huntingdon Road, Cambridge CB3 0DL, United Kingdom <i>e-mail:</i> traffic@trafficint.org	2 August 2006	08/08/06 reply received through Chris Kerr from: Stephanie Pendry UK Enforcement Officer TRAFFIC International 219a Huntingdon Road Cambridge, CB3 0DL Tel: 01223 277427 <i>e-mail:</i> Stephanie.Pendry@trafficint.org
Alison Rosser	DICE, Durrell Institute of Conservation and Ecology, Department of Anthropology, Marlowe Building, University of Kent, Canterbury, Kent CT2 7NR <i>e-mail:</i> A.M.Rosser@kent.ac.uk	2 August 2006	03/08/06 – leads to WDNS, PAWS, CCAMLR supplied
Rod Ogden	Wildlife DNA Services 9th Floor, Alun Roberts Building, University of Wales, Deiniol Road, Bangor. LL57 2UW	3 August 2006	Reply received from Ross McEwing 25/08/06 Email:

	<i>e-mail:</i> rob-ogden@wdnas.com		rossmcewing@wdnas.com
PAWS general enquiries	PAW Secretariat, Zone 1/11, Temple Quay House, 2 The Square, Temple Quay, Bristol, BS1 6EB <i>e-mail:</i> paw.secretariat@defra.gsi.gov.uk	3 August 2006	PAWS contact is via FSS and TRAFFIC
Non-UK contacts			
CCAMLR general enquiries	CCAMLR, Commission for the Conservation of Antarctic Marine Living Resources, Pox Box 213, North Hobart 7002, Tasmania, Australia <i>E-mail:</i> ccamlr@ccamlr.org	3 August 2006	04/08/06 advised to contact CSIRO
Sharon Appleyard	Dr Sharon Appleyard, Molecular Geneticist, CSIRO Marine and Atmospheric Research, GPO Box 1538, Hobart TAS 7001 Australia (Ph: + 61 3 6232 5458, Fax: +61 6232 5000)(Email: Sharon.Appleyard@csiro.au).	4 August 2006	Reply received 11/08/06. Follow-up from 11/08/06.
C Galazzi	IFAW <i>e-mail:</i> cgalazzi@ifaw.org	7 August 2006	Out of office reply 07/08/06 Reminder sent 24/08/06 Passed to IFAW UK office. Nikki Kelly recommended contacting Ross McEwing, WDNAS
Sam Wasser	Samuel K. Wasser, Ph.D Endowed Chair in Conservation Biology Research Professor, Department of Biology, Box 351800 Director, Center for Conservation Biology University of Washington, Seattle, WA 98195-1800 Ph: 206-543-1669; FAX: 206-616-2011 wassers@u.washington.edu	7 August 2006	07/08/06 reply plus clarifications

	http://depts.washington.edu/conserv/		
General queries	<i>e-mail:</i> dna-surveillance@auckland.ac.nz	8 August 2006	Reply received 08/08/06 from Howard Ross, Lecturer Bioinformatics Institute, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand phone: +64 (9) 373-7599 ext 86160 fax: +64 (9) 367-7136 E-mail: h.ross@auckland.ac.nz Follow-up email to Scott Baker sent 24/08/06
Prof Terry Bradley	College of the Environment and Life Sciences Department of Fisheries, Animal & Veterinary Science 20A Woodward Hall, 9 East Alumni Avenue, Kingston, RI 02881 Phone: 401-874-2477 Fax: 401-874-7575 http://www.uri.edu/cels/favs/tb.html Email: tbradley@uriacc.uri.edu	14 August 2006	Reply received – out of office. Advised to contact Arpita Choudry at NOAA. Email sent to Arpita.Choudhury@noaa.gov 21/08/06. Information requested not received by 15/09/06
Prof Mike Bruford	Cardiff School of Biosciences Cardiff University CARDIFF CF10 3US Tell 029 208 74312	14 August 2006	Out of office message. No reply.

	e-mail: BrufordMW@cf.ac.uk		
Prof Paulette Bloomer	Molecular Ecology & Evolution Programme Department of Genetics University of Pretoria 0002 Pretoria South Africa E-mail: paulette.bloomer@up.ac.za http://www.up.ac.za/academic/genetics/	16 August 2006	Reply received 01/08/06. Was on field work. No information received by 15/09/06
Peter Smith	NIWA Private Bag 14901, Wellington 301 Evans Bay Parade, Greta Point, Wellington, New Zealand Phone: +64 4 386 0855 Fax: +64 4 386 0574 p.smith@niwa.co.nz http://www.niwascience.co.nz/ncfa/	16 August 2006	Reply received 16/08/06.
Prof Barbara Block	Hopkins Marine Station Of Stanford University Oceanview Boulevard, Pacific Grove, CA 93950-3094, USA Tel (831) 655-6200, FAX (831) 375-0793 http://www-marine.stanford.edu/HMSweb/block.html http://www-marine.stanford.edu/	16 August 2006	Reply received 16/08/06 and subsequent follow-up
Jim Seeb	Genetics Laboratory, Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, Alaska 99518-1599 USA Jim Seeb Program Director/Scientist Tel (907) 267-2385	16 August 2006	Reply received 16/08/06

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