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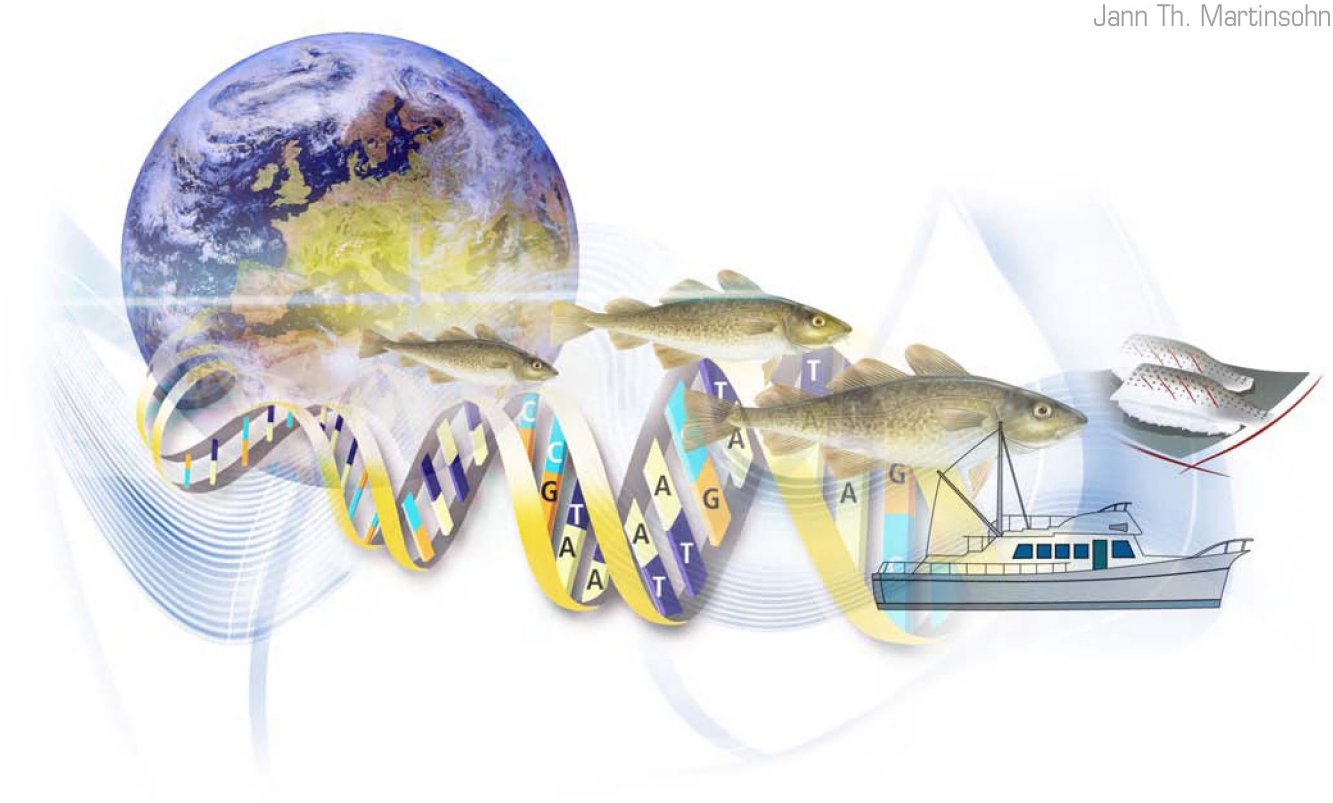
Deterring Illegal Activities in the Fisheries Sector

Genetics, Genomics, Chemistry and Forensics to Fight IUU Fishing and in Support of Fish Product Traceability

Jann Th. Martinsohn



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DETERRING ILLEGAL ACTIVITIES IN THE FISHERIES SECTOR

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Executive Summary

DETERRING ILLEGAL ACTIVITIES IN THE FISHERIES SECTOR

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Keywords: IUU fishing; traceability, origin assignment; fisheries control and enforcement; molecular techniques; genetics, genomics, forensics.

Marine fish are a precious natural resource and their exploitation for nutrition and income is deeply embedded in human culture. However, massive fishing activity, both legal and illegal, has had dramatic impacts, and poses a threat to the future of the fisheries sector. Virtually 70% of the world's fish stocks are fully exploited, overexploited or in a state of collapse. European waters are not exempt, with almost 90% of fish stocks being overexploited.

IUU fishing (Illegal, Unregulated and Unreported fishing) is vastly contributing to this situation. In 2010, the value of IUU fishing amounted to 10-20 billion Euros annually, with at least 1.1 billion Euros worth of illegal fish being imported into the European Union every year. Furthermore, fraud along the supply chain with fish products sold under false labels, such as low-cost catfish as valuable sole or cod fillets, poses additional challenges. These illegal activities have severe adverse effects, as they undermine sustainable fisheries, cause destruction of marine ecosystems, obstruct socioeconomic development, and impede consumer information and protection.

A number of nations have developed strategies to deter and fight illegal fishing activities, and numerous countries have adopted the International Plan of Action to prevent, deter and eliminate IUU Fishing (IPOAIUU), that has been developed in 2001 within the framework of the Code of Conduct for Responsible Fisheries by the FAO. The European Union has recently taken further initiatives and developped two major and complementing legal instruments: in January 2010, Council regulation (EC) No 1005/2008 ([1]), - the 'IUU regulation', entered into force, and in November 2009, Council regulation (EC) No 1224/2009[1] ([2]) - the new Control regulation- establishing a Community control system was adopted and is in the process of being implemented.

Both regulations place emphasis on detailed catch documentation and traceability for fishery products 'from ocean to fork', that is, covering all stages of the supply chain from catch, to landing, transport, processing, and the markets. Traceability is generally acknowledged as being a highly powerful tool in support of monitoring, control and enforcement in the fisheries sector. However, currently it is mainly based on certificates accompanying goods, and labelling of products, both measures which are vulnerable to falsification.

[1] Council Regulation (EC) No 1005/2008 of 29 September 2008 establishing a Community system to prevent, deter and eliminate illegal, unreported and unregulated fishing, amending Regulations (EEC) No 2847/93, (EC) No 1936/2001 and (EC) No 601/2004 and repealing Regulations (EC) No 1093/94 and (EC) No 1447/1999. Official Journal of the European Union 286, 29.10.2008, p. 1–32

[2] Council Regulation (EC) No 1224/2009 of 20 November 2009 establishing a Community control system for ensuring compliance with the rules of the common fisheries policy, amending Regulations (EC) No 847/96, (EC) No 2371/2002, (EC) No 811/2004, (EC) No 768/2005, (EC) No 2115/2005, (EC) No 2166/2005, (EC) No 388/2006, (EC) No 509/2007, (EC) No 676/2007, (EC) No 1098/2007, (EC) No 1300/2008, (EC) No 1342/2008 and repealing Regulations (EEC) No 2847/93, (EC) No 1627/94 and (EC) No 1966/2006. Official Journal of the European Union L 343, 22.12.2009, p. 1–50.

So how can inspectors and control and enforcement authorities validate and authenticate the information provided by documentation? How can the industry assure that the fish it is processing and selling is what it is supposed to be, e.g. the correct species and fished legally? And finally how can the consumer be certain that the information provided for fish products is correct?

A system is needed to effectively trace fish products throughout the food supply chain that is supported by independent control measures. Likewise control and enforcement authorities need efficient analytical tools for generating evidence in court trials. Molecular techniques based on genetics, genomics and chemistry, and embedded in a forensic framework, have great potential in this respect.

This JRC report describes available molecular techniques and technologies and discusses how these can be used for traceability and in support of fisheries control and enforcement. The report provides examples of cases where molecular techniques were employed to reveal fisheries fraud and to generate evidence in court cases. These examples clearly demonstrate the feasibility and operational potential of the techniques in real-world contexts. Furthermore, the report explores possibilities for translating forensic genetics and chemistry into a European fisheries control and enforcement framework, within the context of the current EU policies and legislation.

To be of value for enforcement and traceability, tools must be able to answer the following three key questions: i) What species is it?, ii) Where was it caught? and iii) Was the fish caught in the wild or does it derive from aquaculture? ([3]) This JRC report demonstrates, also through the provided examples, that techniques based on genetics, genomics and chemistry, can answer these questions, and therefore efficiently support traceability, control and enforcement. In this context it is interesting to note that the new control regulation, mentioned above, explicitly refers in Article 13 to "traceability tools such as genetic analysis" as having a potential to improve compliance with the rules of the Common Fisheries Policy.

Yet, despite enormous technological progress, particularly in the field of DNA analysis, the routine application of modern molecular techniques for fisheries control and traceability is far from being fully established. A number of obstacles impede capacity building and uptake of the techniques. There is a lack of the transfer of research results into practical applications for fisheries management and enforcement and insufficient information transfer between the relevant stakeholders. Also access to data, with standardised formats, needs to be established. It would be advantageous to create a central data and information dissemination hub, that is easily accessible to analytical laboratories, and that is in charge of compiling reference data needed for the analysis of fish and fish products. Addressing a similar problem, the EU initiative EMODNET is trying to tackle the fragmentation of marine knowledge, and the EU Data Collection Framework for the collection, management and use of data in the fisheries sector is another good example for how to render data accessible to stakeholders. A network of test laboratories should be set up, certified to carry out analysis for control and enforcement purposes, and sharing information and analytical protocols that are harmonised and validated. The Group of European Customs Laboratories (GCL) and the European Network of GMO Laboratories (ENGL) are existing examples for such networks. In fact, most EU member states already possess facilities with the necessary analytical capacity and it should not be very difficult to set up such a network. Finally, training of inspectors and laboratory staff for proper sample handling and analysis must be ensured.

Costs and benefits of the discussed molecular techniques and technologies are currently being assessed by the JRC. However, the current steep fall in costs of genetic and genomic technology, especially for DNA analysis, and the examples provided in the report indicate that the methods discussed are cost effective.

In conclusion, by discussing the state-of-the-art in the fields of genetics, genomics, chemistry and forensics this report demonstrates that molecular analytical technologies have operational potential in real-world contexts, and more specifically, potential to support fisheries control and enforcement and fish and fish product traceability 'from ocean to fork'. The ambition of this JRC report is to catalyse an informed dialogue among the various stakeholders, thereby contributing to effective capacity building and technology transfer.

[3] This question is becoming increasingly relevant in the light of rapidly rising aquaculture activity in the world.

Contents

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Many colleagues have contributed to this document by providing valuable information and sharing their expertise, experience and patience, and particular thanks go out to the consortium members of the Seventh Framework Programme (FP7) project FishPopTrace. I am grateful to Prof. Audrey Geffen and Prof. Filip Volckaert who reviewed this report, scrutinised the text, identified flaws and suggested important changes to improve the content. Many thanks to Thomas Barbas for his ceaseless interest and support and both to him and David Sampson for editing parts of the text. Thanks also to Claude Guillou for suggestions. I would like to express my gratitude to Alessandra Zampieri and my colleagues of the Joint Research Centre for support and encouragement throughout the writing of this reference report.

List of Abbreviations

BOL: Barcoding of Life Initiative

CBA: Cost Benefit Analysis

CFCA: Community Fisheries Control Agency

CFP: Common Fisheries Policy

COI: Mitochondrial cytochrome c oxidase I gene

DCF: Data Collection Framework

EBI: European Bioinformatics Institute

EC: European Community

EMODNET: European Marine Observation and Data Network

ENGL: European Network of GMO Laboratories

ERS: Electronic recording and reporting system

EURL-GMFF: European Union Reference Laboratory for GM Food and Feed

FAO: Food and Agriculture Organization

FDA: US Food and Drug Administration

FP7: Seventh Framework Programme

GCL: Group of European Customs Laboratories

GMO: Genetically Modified Organism

ICES: International Council for the Exploration of the Sea

IEF: Isoelectric Focussing

IUU: Illegal, Unreported and Unregulated fishing

mtDNA: mitochondrial DNA

MSY: Maximum Sustainable Yield

NGO: Non-Governmental Organisation

NOAA: US National Oceanic and Atmospheric Administration

OECD: Organisation for Economic Co-operation and Development

PCR: Polymerase Chain Reaction

RFLP: Restriction Fragment Length Polymorphism

SNP: Single Nucleotide Polymorphism

SOP: Standard Operating Procedure

STR: Short Tandem Repeat

TAC: Total Allowable Catch

VDS: Vessel Detection System

VMS: Vessel Monitoring System

WGAGFM: ICES Working Group on Applied Genetics for Fisheries and Mariculture

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By way of introduction

The exuberant fishing activity worldwide has led to a dramatic reduction of the natural and common resource, fish: according to the Food and Agriculture Organization (FAO), more than half of the stocks of marine fishes are fully exploited and therefore producing catches that are at their maximum sustainable limits, with no room for further expansion. One quarter are either overexploited, depleted or recovering from depletion ([1]). Owing to excess fishing pressure these stocks are dwindling and in need of rebuilding (FAO, 2009). European Community waters are not exempt from this dire situation: 88% of Community stocks are being fished above the Maximum Sustainable Yield (MSY[2]), meaning these stocks could increase and generate more economic output if fishing pressure was to be reduced. But even then, 30% of these stocks are being fished at rates above the safe biological limits, and a reduction in fishing pressure may not succeed in enabling the populations to recover (European Commission, 2009b).

The Common Fisheries Policy (CFP) is the principal instrument of the European Union for the management of fisheries and aquaculture. Its underlying rationale is to ensure sustainable exploitation of living aquatic resources. However, despite repeated attempts to overhaul and improve the CFP management scheme, European fish stocks have continuously been overfished for decades. This has resulted in a severe fisheries sector crisis, prompting a reform of the CFP, which is currently underway (European Commission, 2009b).

The volume and complexity of world trade activities in the fisheries sector reflect the importance and impact of global fishing activities, and the EU member countries – combined being worldwide the biggest net importer of seafood products – play a key role. World fish imports reached a new peak of more than EUR 60 billion in 2004, after a steep rise of 25% in only 4 years. Developed countries accounted for about 80% of the total value of imports and five countries of the EU ([3]) belong to the top importing countries worldwide (FAO, 2006). Meanwhile, to meet the demand, more than 60% of the EU's fish and fisheries products consumption have to be imported into the EU market. In 2008, a trade deficit of EUR 13.3 billion arose, with EUR 16 billion worth of fish and fishery products imported into the EU and exports amounting to EUR 2.7 billion (European Commission DG Trade, 2009).

These data illustrate the dynamic character of the international fish market and hint at its intricacy. Efforts to regulate fishing activities to maintain sustainable levels are greatly undermined by Illegal, Unreported and Unregulated (IUU) fishing and fraudulent activities along the supply chain, such as selling fish under a wrong label. The estimated annual value of IUU fishing of worldwide EUR 10 billion to EUR 20 billion (Agnew et al., 2009) is nearly twice the value of landings by the EU fleet (EUR 6.8 billion in 2006 (European Commission, 2008a)), and EU fisheries product imports derived from IUU catches have been conservatively estimated at EUR 1.1 billion in 2005 (European Commission, 2007a). This clearly shows that IUU fishing turned into a burning issue. The increasing dependence on fish product imports and complex marketing patterns hamper efforts to regulate and control the EU fisheries sector. Criminal activities do extend into the supply chain as shown by numerous recently revealed fraud cases in Europe and worldwide where fish has been sold under false labels. Such deceit erodes consumer confidence and may pose a threat to health and food safety, currently mainly assured by adherence to documentation and labelling schemes. The documented fraud cases show that the present systems are vulnerable to deceit, particularly if not supported by independent control measures.

IUU fishing has far-reaching and drastic consequences, since it causes unsustainable harvesting of fish stocks and other marine wildlife. It also results in the destruction of marine habitats and can lead to disturbances of whole ecosystems. In the long term, IUU fishing can deplete fish stocks to the point where they become commercially unviable or push species to the brink of extinction. It results in loss of fish for future harvest, loss of nutrition and loss of income and employment for legitimate fishers. Moreover, IUU fishing prohibits the fair and equitable sharing of a common resource, weakens labour standards, distorts markets of legally harvested fish and contributes to the loss of socioeconomic stability with consequences reaching far beyond mere fisheries activity (OECD, 2005).

In view of the actual status of most commercially exploited fish stocks, and the extent of illegal fishing, the provision of support to more efficient management strategies on various levels is

1 Of the 25%: 17% overexploited, 7% depleted and 1% recovering from depletion.

2 Terms printed in purple can be found in the glossary.

3 France, Germany, Italy, Spain and United Kingdom.

of utmost importance. In this context, species identification and tracing of fish back to their original source constitute a highly valuable tool for monitoring, control and surveillance (MCS)*.

Most fisheries' management schemes are mainly based on three broad types of tools, namely input controls, technical measures and output controls (Hoggarth et al., 2006). Output controls attempt to directly limit the amount of fish which can be caught and landed. An output control management regime is implemented by setting a Total Allowable Catch (TAC) for a particular species in a specific region, which is currently one of the main methods employed under the CFP, where TACs are set annually. TAC controls are usually applied only to landings and not directly to catch and they are generally regarded as good mechanisms to control the total catch in single species, high value fisheries with low discard rates. However, thorough monitoring schemes are required to ensure that individual quotas are not exceeded.

A strategy to circumvent catch limitations is to ignore such restrictions and to sell fish under a false label, e.g. a falsified geographic origin or different species name. Origin assignment and species identification are therefore important components of a legal framework underlying the fight against illegal activities as they support enforcement in the area of fisheries. However, traceability techniques also play a crucial role in consumer protection as they can be used to warrant tracking and surveillance throughout the food supply chain, from onboard samples to processed product ('ocean to fork'). Moreover, such techniques can be used to certify the origin of fish to support consumer confidence. This aspect has gained importance due to the increasing popularity of certificates attesting that fish products derive from sustainably exploited stocks, and more generally for eco-labelling schemes, which assure consumers that a product has been produced according to defined environmental standards (Brécard et al., 2009). However, unless carefully monitored, there is a substantial danger that eco-labelling schemes begin to fail. Also the risk of emerging free riders, i.e. vendors pretending to adhere to a eco-labelling scheme by using falsified labels, is considerable. This would ultimately damage credibility and undermine consumer confidence. Properly applied, molecular and chemical techniques can be used as powerful tools to control for compliance with rules established under eco-labelling schemes.

In the European Union legislative framework two important and related regulations addressing fisheries control and IUU fishing have recently been introduced; the IUU regulation (European Council, 2008b), introducing a catch certification scheme and supporting product traceability, and regulation (EC) No 1224/2009 which overhauls the preceding CFP control scheme (European Council, 2009). Modern molecular techniques can greatly contribute to control and improve compliance with both legal documents and it is interesting to note that regulation (EC) No 1224/2009 explicitly refers in Article 13 to 'genetic analysis and other fisheries control technologies' in the context of traceability.

Despite progress being made, clearly a coherent EU-wide approach to transfer technologies based on genetics, genomics, chemistry and forensics into applications readily available to European control and enforcement authorities is lacking. This Joint Research Centre (JRC) Reference Report explains and highlights available modern molecular technologies that are most promising for the support of fisheries control and enforcement as well as traceability schemes in the fisheries sector. It demonstrates how such technologies can effectively contribute to fight illicit activities in the EU fisheries sector and support sustainable fisheries management as well as consumer protection, by answering the following three crucial questions about fish and fish products:

- What species is it?
- Where was it caught?
- Is it a farm escapee?

In chapter 1, techniques and tools based on genetics, genomics and chemistry are introduced. Chapter 2 and 3 explain the application of these techniques for species identification and origin assignment. The requirements to effectively transfer (forensic) genetics and chemistry into a European fisheries control and enforcement framework are explored in chapter 4. Finally, the annex provides examples for successful applications of molecular technologies for control and enforcement in the fisheries sector, along with a summary of relevant EU legislation. Technical terms, highlighted in blue, are explained in a glossary.

* Terms highlighted in mauve are explained in the glossary.

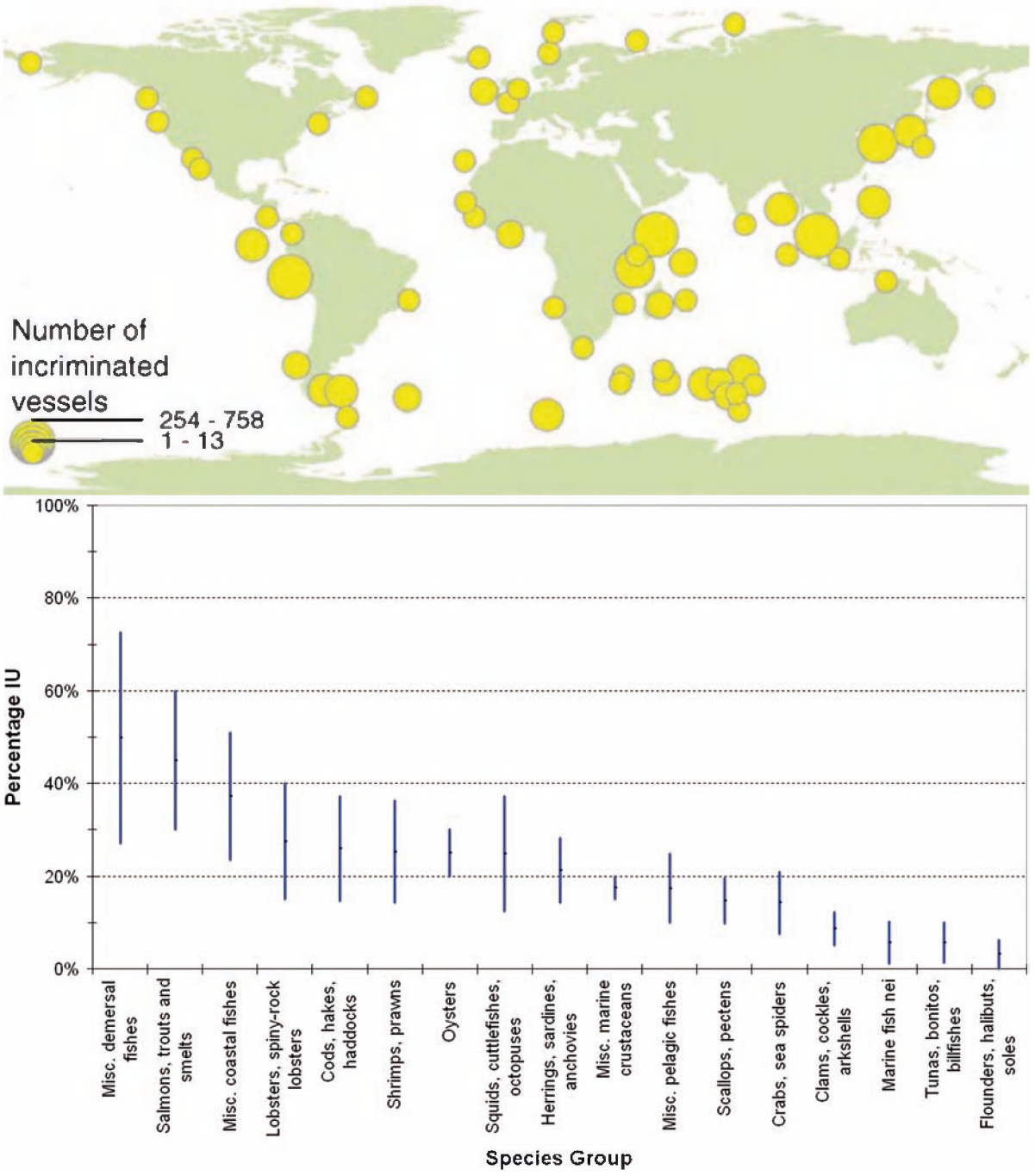


Figure 1. The global dimension of IUU fishing. *Left: Number of incriminated vessels for fishing illegally between 1980 and 2003. Reproduced with kind permission from Sumaila et al. (2006). Right: Illegal and unreported catch, expressed as a percentage of reported catch, and including upper and lower bounds, by species group in the period from 2000 to 2003. Reproduced with kind permission from Agnew et al. (2009).*

Certainly not covering the field in an exhaustive manner, the intention of this document lies in providing a solid basis to catalyse an informed discussion among relevant stakeholders with highly diverse backgrounds (regulators, inspectors, scientists, fisheries managers, industry representatives), thereby facilitating the uptake of genetics, genomics, chemistry and forensics into fisheries control and enforcement as well as fish and fish product traceability schemes.

1. The analyst's tool box: Available techniques and tools

Many diverse methods have been used to identify fish species or for stock identification. There are markers determined by morphology, such as meristics, morphometrics or the shape analysis of otoliths. On the other hand, markers determined by the environment can be used, such as parasite load (MacKenzie and Abaunza, 1998), fatty acid profiles (Budge et al., 2002, Grigorakis et al., 2002) and microchemistry (Cadrin et al., 2004).

With the advent of molecular biology and biotechnology, molecular and genetic markers are increasingly employed both for species identification and origin assignment.

For the purpose of this report only modern analytical techniques based on chemistry (section 1.1 and 1.2), genetic marker analysis (sections 1.3-1.6) and genomics (sections 1.8 and 1.9) will be discussed. The techniques reviewed have already been used for fish species identification and origin assignment and have the potential to be transferred into routine applications for fisheries control, enforcement and product traceability. Other methods used for fisheries management are described comprehensively elsewhere (Cadrin et al., 2004). This chapter also briefly introduces to state-of-the-art analytical technologies such as microarrays (section 1.7), DNA sequencing (section 1.8) and handheld analytical devices (section 1.10). Finally the role and importance of forensics for fisheries enforcement is discussed (section 1.11).

1.1 Fatty acid analysis

Fatty acids are important structural elements and sources of energy for all living organisms (Alberts, 2002). In marine animals about 20 different fatty acids are present at relative amounts greater than 1%. Although most of these fatty acids are found in all fish species, the quantitative composition of the fatty acids (fatty acid profile), i.e. the amount of each particular fatty acid in a fish tissue, may vary significantly at both the species and population levels. While there is a strong relationship between the diet of fish and fatty acid profiles, as well as with age and maturity along with environmental factors like water temperature and salinity (reviewed in (Grahl-Nielsen, 2005)), the fatty acid composition of fish also appears to be strongly determined genetically (Kwetegyeka et al., 2008).

Trophic conditions and the diet of marine fish are influenced by local environmental conditions and dietary components and their fatty acid composition can vary significantly between different geographic areas. Therefore, populations of a given fish species inhabiting different geographical areas, characterised by distinct trophic conditions, can potentially be distinguished by qualitative and quantitative analysis of their fatty acid composition.

Fatty acid composition has been used in combination with principal component analysis, a statistical multivariate treatment of analytical data, for stock identification in fisheries fraud investigations. An example of this application concerns the alleged false reporting of herring landed by a Norwegian purse seiner. Fatty acid analysis performed on the fish under question and compared to the fatty acid profile of fish of known geographic origin proved that the fisherman correctly reported the landings and consequently charges against him were dropped (Grahl-Nielsen, 2005). In a different type of approach, nuclear magnetic resonance (NMR) spectroscopy of lipids has been successfully used to distinguish farmed from wild Atlantic salmon, to discriminate between different geographical origins and to verify the origin of market samples (Aursand et al., 2009).

Although fatty acid profiling can be performed on fresh or frozen fish tissues, the application of this tool on processed fish products, such as smoked or canned, still needs to be evaluated. The potential of fatty acid profile analysis as a tool to identify the geographical origin of fish is also explored within the international project FishPopTrace ([4]), of which the JRC is a consortium partner.

1.2 Microchemistry and stable isotope analysis

Lately isotopic and elemental markers have gained importance in solving questions of natal origin, spawning site fidelity, connectivity and traceability (Campana, 2005). Because trace elements are

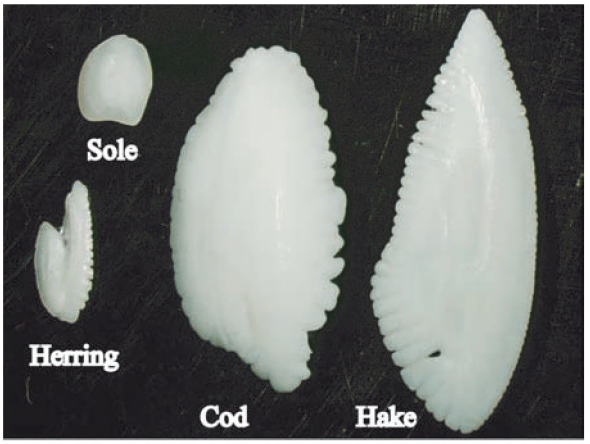
4 FishPopTrace is an international collaboration building a framework for sustainable fisheries management, conservation and fisheries control based on genetics, chemistry and forensics. It is funded by the European Community's Seventh Framework Programme from 2008 to 2011 MARTINSOHN, J. T. & OGDEN, R. (2009) FishPopTrace-Developing SNP-based population genetic assignment methods to investigate illegal fishing. *Forensic Science International: Genetics Supplement Series*. FishPopTrace is committed to technology transfer, providing applications to fisheries control, enforcement and fish product traceability. More information can be found at http://fishpoptrace.jrc.ec.europa.eu online.

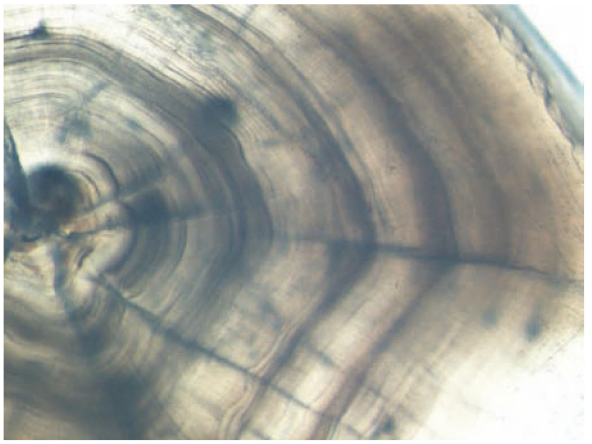
taken up from the surrounding waters, the chemical composition of hard tissues such as otoliths and scales reflect physical and chemical properties of the environment to which the fish have been exposed (geochemical signatures), which can be used to establish 'elemental fingerprints'. For this purpose the elemental concentrations from isotopes of various elements such as Sr, Ba, Mb, Fe and Pb are determined (Campana, 2004).

The specific characteristics of fish otoliths make them highly suitable for the identification and analysis of geochemical signatures: Otoliths grow throughout the life of the fish and the material deposited in annual growth increments (see ***Figure 2***) is neither reabsorbed nor altered. This is ideal to establish elemental fingerprints and the relation of those to migration and stock structure of fish (Campana, 2004). A wide variety of elemental analysis techniques are available, grouping into two main categories: bulk analysis, in which the otoliths are dissolved and assayed using chemical approaches; and probe analysis, where specific zones in the otolith may be targeted for analysis of specific life stages. Both approaches have been employed to examine population structures and stock tracing of various marine fish species (Campana et al., 2000, Geffen et al., 2003, Swan et al., 2006), including the study of trans-Atlantic movement and connectivity between populations of the heavily exploited and endangered bluefin tuna (*Thunnus thynnus*) from the Mediterranean Sea and the Gulf of Mexico (Rooker et al., 2008). The discrimination power of otolith microchemistry, even over relatively short geographical distances, has been demonstrated in a variety of studies. For example it could be shown that individual juvenile common soles (*Solea solea*) originating from two estuaries along the French coast, separated by a distance of about 200 km, could be discriminated by elemental otolith fingerprints (De Pontual et al., 2000). In a recent study on Atlantic cod (*Gadus morhua L.*) of the northeast Atlantic, otolith microchemistry analysis, in combination with otolith shape analysis, body morphometry, microbacterial assemblages, parasite load and DNA markers, allowed to distinguish wild from farmed fish and to determine the precise harvest origin of individuals (Higgins et al., 2010). Otolith trace element analysis for traceability is suitable for all stages along the supply chain where the heads remain with the fish. An asset of otoliths is that the elemental composition is resilient and does not degrade or change over time after death (Thresher, 1999).

Other hard tissues, such as scales or spines, can also be used to establish elemental fingerprints (Gillanders, 2001). For example, scale microchemistry has recently been employed as a tool to investigate the origin of wild and farmed Atlantic salmon (*Salmo salar*) (Adey et al., 2009). For inspection and enforcement this constitutes an interesting alternative to otoliths, as the isolation of scales for analytical purposes is less damaging to the individual fish than the extraction of otoliths. Finally, in a thorough study on pink ling (*Genypterus blacodes*) in New Zealand it was shown that trace element analysis on muscle tissue can be used to distinguish groupings of fish of different areas, thereby providing evidence in cases of marine fish with disputed origin (Graeme Bremner, Ministry of Fisheries, Dunedin, New Zealand: Personal communication).

Figure 2 Otoliths. *The left picture shows otoliths from different fish species illustrating the species specificity of the otolith forms. On the right hand side an enlarged detail of a sole (Solea solea) otolith is depicted, showing the 'tree-ring'–like structure of the growth increments. (Courtesy of A.J. Geffen; University of Bergen; Norway)*





1.3 Genetic markers

With the rapid advance of molecular biology the usage of molecular polymorphic markers in evolutionary and phylogenetic studies and also for the purpose of species and stock identification set in. Already in the 1950s molecular markers, namely blood group variants, were analysed to reveal the population structure of tunas, salmonids and cod. In the 1960s the focus shifted to the analysis of protein polymorphisms in fish by investigating haemoglobin variants in Atlantic cod (*Gadus morhua*) and whiting (*Merlangius merlangus*). Because they were easy to handle, results were reproducible and the method was reasonably inexpensive, later primarily allozymes were analysed (reviewed by Kochzius, 2008).

Especially protein analysis is still extensively used by control authorities for fish species identification (V. Verrez-Bagnis, H. Rehbein: Personal communication), which is however increasingly replaced by the analysis of species-specific genetic markers. In the following the analysis of protein and DNA markers is briefly discussed.

1.3.1. Protein markers

Genetic polymorphisms can be detected on the protein level since non-conservative codon changes in expressed genes will result in amino acid substitutions within the respective translated protein. To detect genetic variation, proteins can be examined by electrophoretic methods: Amino acids give a protein a characteristic net charge, depending on the pH of the surrounding medium. Therefore, proteins migrate in an electric field applied to a medium, at different rates in dependence of their physicochemical properties (net charge, size, shape) (reviewed in (Griffiths, 2000) and ***Figure 3***). Changes in single amino acids of a protein are often sufficient to change its migration behaviour in electrophoretic assays and this property is exploited to detect and analyse genetic polymorphisms, e.g. between different fish species or individual fish of the same species, but of different populations.

Probably the most widely used electrophoretic separation method of proteins in biochemistry, forensics, genetics and molecular biology is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In this technique proteins are separated according to their electrophoretic mobility as a function of length of polypeptide chain or molecular weight, since the binding of SDS to the proteins results in identical negative charge per unit mass (Berg et al., 2007).

Isoelectric focusing (IEF) is a particular electrophoretic technique for separating different proteins by their electric charge differences, still frequently used for fish species identification (Rehbein, 2003). It takes advantage of the fact that the charge of proteins changes with the pH of its surroundings. A protein that is in a pH region below its isoelectric point (pI) will be positively charged and migrates therefore towards the cathode. In IEF assays it migrates in a gel through a gradient of decreasing pH and the protein's overall charge decreases until the protein reaches the pH region that corresponds to its pI. At this point it has no net charge and so migration ceases. As a result, the proteins become focused into sharp stationary bands with each protein positioned at a point in the pH gradient corresponding to its pI. The technique is capable of extremely high resolution with proteins differing by a single charge being fractionated into separate bands (***Figure 4***).

In 2D-electrophoresis, SDS-PAGE and IEF can be applied to a sample of proteins one after the other (e.g. SDS-PAGE in the 'first dimension' followed by IEF as the 'second dimension'), thereby increasing the power to resolve and identify proteins.

By using monoclonal antibodies in a procedure called immuno- or Western blotting (Berg et al., 2007), basically any protein and at very low quantities can be analysed for polymorphisms by electrophoretic means. Alternatively, immunoassays not being dependent on electrophoresis, such as the enzyme-linked immunosorbent assay (ELISA), have been applied for fish species identification and food authenticity control (Taylor and Jones, 1992, Taylor et al., 1994).

The first molecular markers used in population genetics were allozymes. Allozymes are gene products of one of several alleles that have the same function but differ in their amino acid sequence and therefore in their physicochemical properties so that they migrate different distances in an electrophoretic assay (see above). Additionally, their enzyme activity can be exploited for detection. Following electrophoresis, visualisation of the allozyme under examination is carried out by applying a stain solution to the gel which contains a dye coupled to the substrate for the respective enzyme. The stain precipitates where the enzyme-catalysed reaction

takes place. This leads to a colour reaction revealing the enzyme position in the gel. Thereby allozymes are used as genetic markers to identify a genotype of an individual, i.e. the combination of two alleles at a particular gene locus (***Figure 4***). This approach was first used for population genetics in ground breaking work by Lewontin and Hubby (Hubby and Lewontin, 1966, Lewontin and Hubby, 1966) and has since been employed extensively for many organisms, including fish. By using several different polymorphic loci, a multilocus genetic profile can be established. The more loci used in this approach the higher the distinguishing power becomes, so that ultimately populations might be differentiated and/or individuals might be assigned to formerly identified and characterised fish populations and stocks. Allozyme variation has been exploited in numerous fish stock analyses (reviewed in (Koljonen and Wilmot, 2005)). A variety of software applications based on algorithms using multilocus genotype data to infer population structure and assign individuals to populations have been developed (Banks and Eichert, 2000, Cornuet et al., 1999, Pritchard et al., 2000). These can be used for traceability purposes.

Genetic analysis based on allozyme data is a highly established method. The costs are relatively low and the techniques used are not exceedingly complicated. Also, the analysis can be performed on otherwise unidentifiable fish products such as fillets. However, nowadays it has been replaced to a great extent by DNA-based techniques.

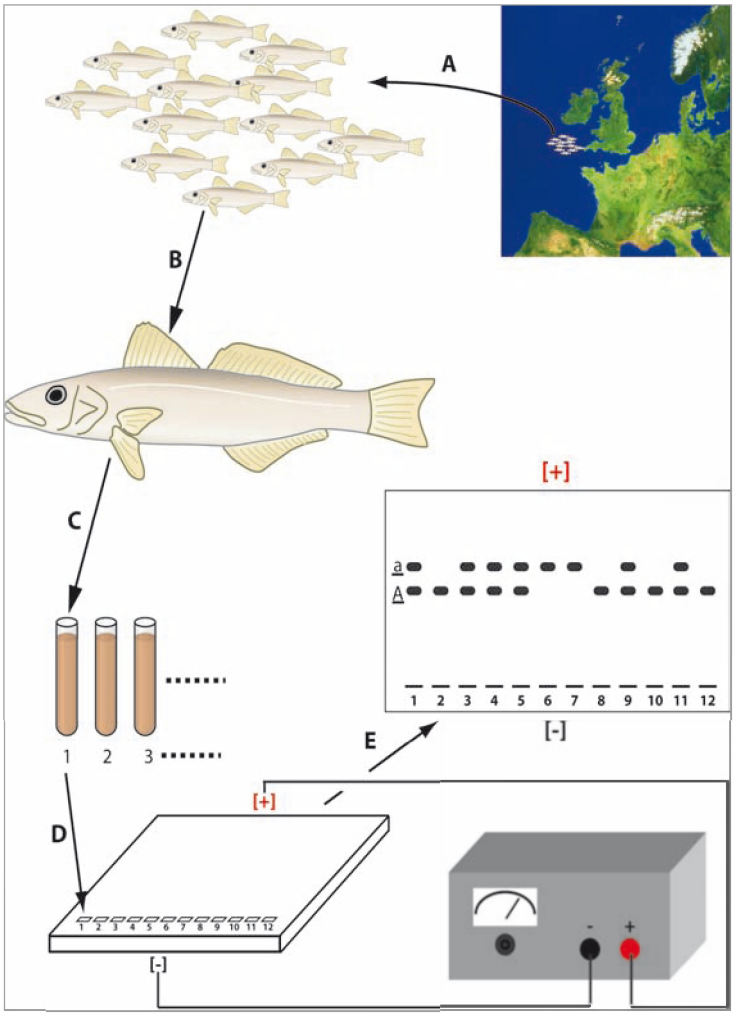


Figure 3. Schema depicting how protein phenotypes and genotypes are determined electrophoretically. *Fish are sampled (A) and proteins extracted from tissues of individuals (B,C). These extracts are placed into slots on gels (D). An electrical charge is applied to the gels, causing the proteins to migrate from the cathode [-] to the anode [+], leading to a separation according to size (D). Finally, the gel is stained to reveal the protein phenotype (E). In this example the stained gel reveals the phenotypes of 12 fish: Two bands appearing show that an individual is heterozygote, meaning it has two alleles of the protein (two forms of the same gene expressing the protein analysed), A and a, while one band means the individual is homozygote (just one form of gene expressing the protein analysed, A or a). The frequency of each allele is determined by adding the number of alleles that are revealed by the phenotypes (bands) and dividing that by the total number of alleles. If only one band is present, it is counted twice because that fish has two copies of the same allele (it is homozygotic). There are 12 fish, with a total of 24 alleles. In this example the frequency of the A allele is 14/24 or 0.6, and the frequency of the a allele is 10/24 or 0.4. Adapted from Utter, F., P. Aebersold, and G. Winans. 1988. Interpreting genetic variation detected by electrophoresis. Pages 21–45 in N. Ryman and F. Utter, eds. Population Genetics & Fishery Management. Washington Sea Grant Program, University of Washington Press, Seattle, Washington, USA. Fish symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science.*

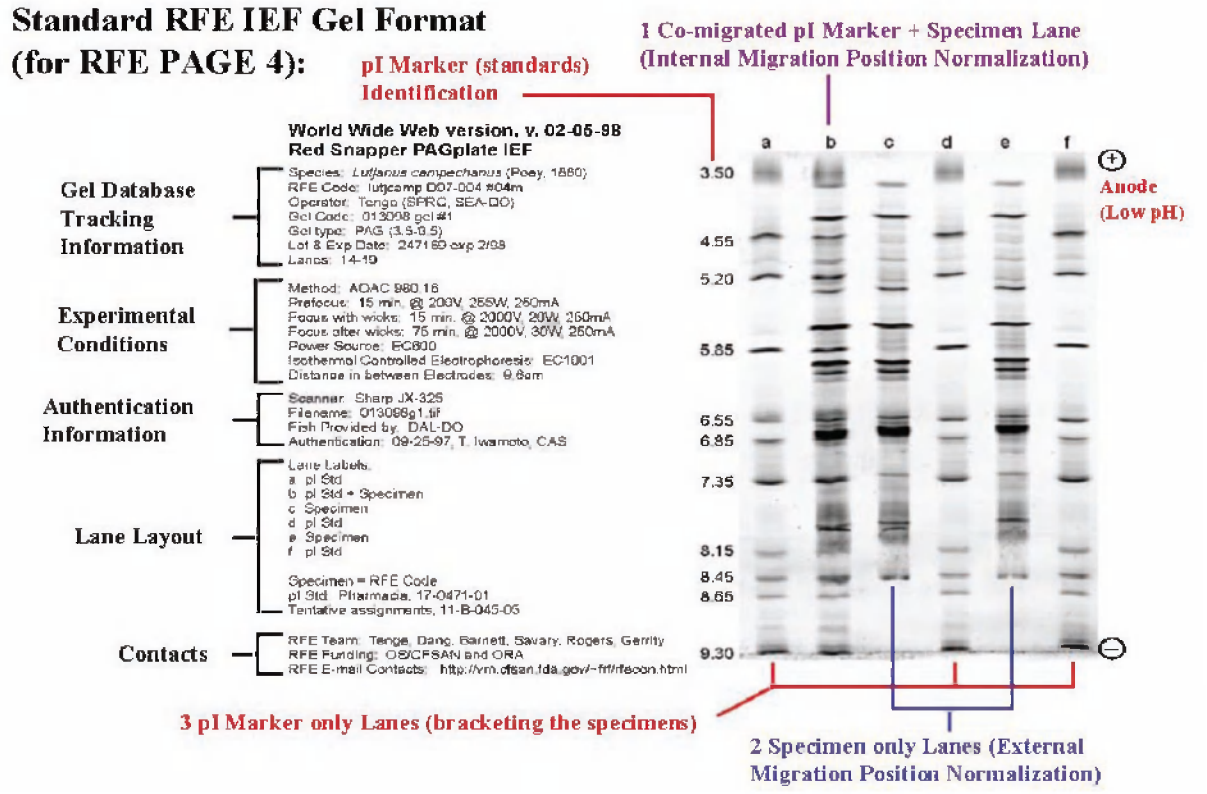


Figure 4. Example of an Isoelectric Focusing (IEF) Gel for fish species identification, used by the US Food and Drug Administration (FDA). *Lanes c and e are loaded with samples of Northern red snapper (Lutjanus campechanus (Poey, 1860)) and show a species-specific banding pattern. Lanes a and f are reference marker lanes and b and e are loaded both with protein marker and the species sample. With kind permission of Regulatory Fish Encyclopaedia, US Food and Drug Administration, 1993-2011.*

1.3.2. DNA markers

As outlined above, historically the first molecular markers used in population genetics were proteins. However, due to the enormous progress made in the field of DNA analysis and molecular biology, nowadays DNA markers are increasingly used and also applied to fisheries-related problems.

Deoxyribonucleic acid (DNA) encodes the genetic information that is decrypted during transcription (RNA synthesis) and translation (Protein synthesis). DNA is copied with an extraordinary high level of fidelity during the process of replication. However, errors do occur, albeit at a very low rate (for eukaryotes about once every 10^{10} nucleotides incorporated) (Alberts, 2002). Additionally, DNA is vulnerable to chemical and physical damage and despite cells harbouring a sophisticated DNA repair machine, errors can be introduced during such repair processes. These errors lead to mutations, which, when inherited, are the ultimate source of variation and novelty in Darwinian evolution. Mutations can lead to phenotypic variation, which is subject to natural selection, so that their frequency within a population will depend on the degree of adaptive advantage they confer to a given environment. Additionally, many mutations are introduced by a process called genetic drift, meaning they arise and persist without bearing any selective advantage or disadvantage (Graur and Li, 2000). These mutations are neutral from an evolutionary point of view, but since they are polymorphic they can be highly informative for species identification, population studies, stock analysis and origin assignment. Data for DNA variation has become a major focus for population geneticists who aim to discern which evolutionary processes have played important roles in nucleotide sequence evolution.

Due to the fast pace of technological development in the area of molecular biology and biotechnology, DNA-based analysis is applied routinely in numerous fields these days, including medical diagnosis and forensics (see below).

In many cases DNA has to be isolated from the tissue source to be analysed, mostly a routine step

for which commercial kits are available. DNA can be extracted from fresh, frozen, dried, ethanol- or histologically-preserved tissues, processed products, as well as dried scales (Rohland and Hofreiter, 2007, WGAGFM, 2007, Cuveliers et al., 2009). The possibility to perform DNA analysis even on processed fish products is of course a huge advantage for traceability, control and enforcement.

Next, for most analytical purposes, DNA has to be amplified by the Polymerase Chain Reaction (PCR), which, like a magnifying glass, brings DNA to detectable levels (***Figure 6***). In fact, constant further elaboration of PCR protocols made DNA extraction for many purposes obsolete: Often PCR can be directly performed on cells or tissues. Further analysis to reveal DNA sequence differences can for example be based on restriction analysis. Restriction endonucleases are enzymes that cleave DNA at specific short recognition sequences. DNA being incubated with endonucleases will be cut into specific fragments depending on the number of recognition sequences present. The number of generated fragments and their lengths can be determined after separation by gel electrophoresis, which leads to a particular 'restriction profile'. This is similar to the analytical procedure described above for proteins. Mutations in the DNA can lead to the elimination of restriction sites or their addition and a comparison of restriction fragment profiles of various individuals, sampled from different populations, can reveal such differences, so called Restriction Fragment Length Polymorphisms (RFLPs). RFLP analysis has been used extensively both for species identification and population analysis of marine fish. Nowadays DNA sequence differences between individuals are progressively more identified directly by sequencing, due to increasing cost effectiveness (see below). It should also be mentioned that many analytical protocols have been developed using a combination of PCR, RFLP and sequencing. However, their discussion would lead too far in the context of this report. They are delineated in a recent review by Kochzius (Kochzius, 2008).

In the meantime we entered the era of genomics: Entire genome sequences, even of higher animals, are published regularly and at a high frequency. The personal genome sequences of a number of humans are meanwhile being deciphered and while the sequencing power and throughput is constantly rising, the costs for DNA sequencing are dropping steadily. This ongoing fast progress in sequence technologies will beyond doubt have a considerable impact on future DNA analysis: Rather than using indirect assays, such as RFLP mapping, DNA sequences will directly be looked at for variation. This will be further elaborated below.

1.4 Mitochondrial DNA

Both for species identification and the study of population structure, the mitochondrial genome proved to be highly useful. Mitochondria are subcellular organelles, creating energy for cellular activity by aerobic respiration. Mitochondria contain their own genome, a single circular molecule of around 16 000 base pairs, separate and distinct from the nuclear genome. The mitochondrial genome of vertebrates contains 37 genes (compared to ca. 20 000 in the nuclear genome) plus a non-protein–coding control region (called 'D-loop'). Each mitochondrion contains numerous copies of its circular genome, and each cell contains hundreds of mitochondria in its cytoplasm. Therefore, since per cell thousands of mtDNA molecules are available, the mitochondrion offers an abundant source of DNA, greatly facilitating DNA isolation and analysis.

The nucleotide sequence of mitochondrial DNA (mtDNA) evolves rapidly (ca. 10 to 30 times faster when compared to the nuclear genome), is almost exclusively inherited maternally (maternal phylogeny) and does not recombine, making it ideal for interspecific or population (intraspecific) studies, as, in the absence of additional mutations, all offspring will have mtDNA haplotypes identical to their mothers. However, using mtDNA to characterise or identify a population will only take into account the matrilineal history of that population. This might not reflect the whole population in case gender-specific dispersal patterns exist.

Depending on whether mitotyping is used for species identification or population characterisation, different genomic regions are used, since the mutation rate of mitochondrial genes or regions is highly variable and dependent on genomic location (Rubinoff et al., 2006). Relatively slowly evolving genes like *cytochrome c oxidase subunit 1* (COI) and *cytochrome b* are more suitable for comparisons between species while rapidly evolving sequences like the D-loop, the most variable region of the mitochondrial genome, are useful for high-resolution analyses of population structure (***Figure 5***).

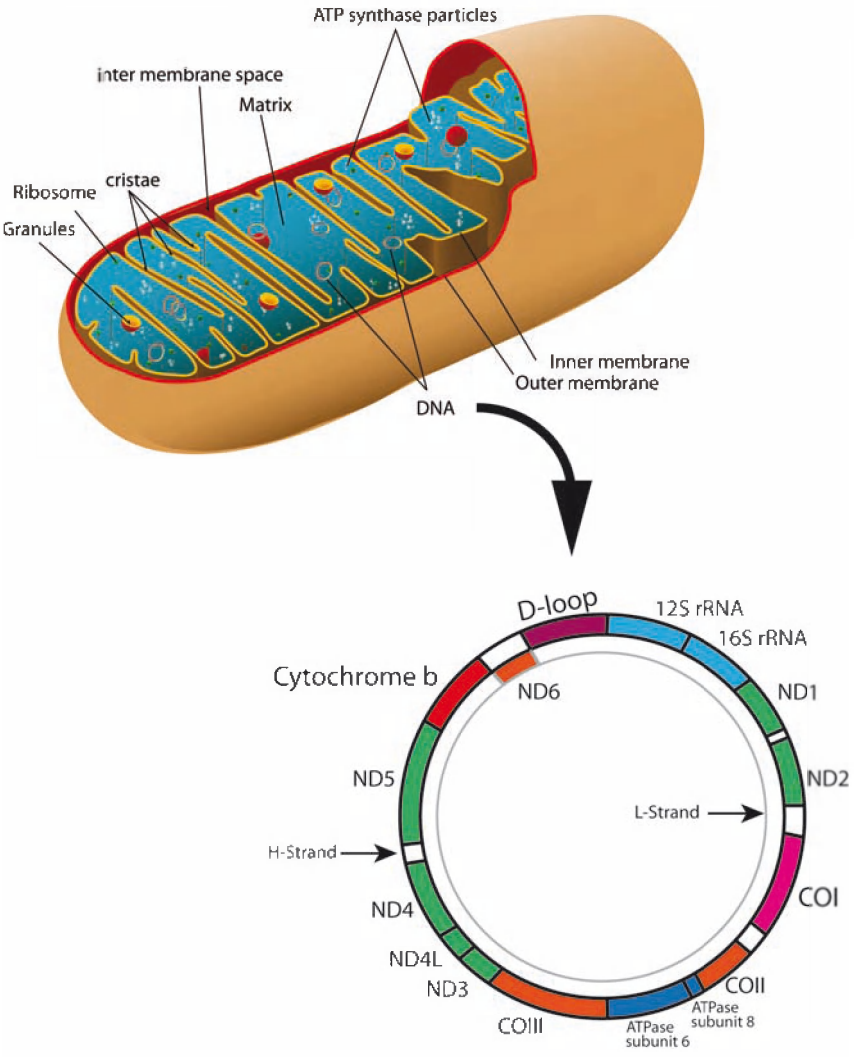


Figure 5. Schematic representation of a vertebrate mitochondrion and its genome. *Several mitochondrial genes and regions have been used for fish species identification and also fish population characterisation (e.g. 16S rRNA; Cytochrome b; D-loop; COI). Source of mitochondrion drawing: Wikimedia Foundation. The drawing is licensed under the Creative Commons Attribution. Author: Mariana Ruiz Villarreal.*

Basically, the usage of mtDNA (as with all DNA-based methods) for population or assignment studies relies on samples from individuals belonging to the same population carrying identical mtDNA (same mitotypes). If, on the contrary, differences are observed in the mitotype distribution between samples, this is evidence that these samples are derived from different populations.

Although primarily an academic issue in the context of this report, it should be mentioned that the above stated assumptions are not universally true. Instances of paternal inheritance of mitochondria have been observed, and recombination apparently also occurs (reviewed in (Barr et al., 2005, Piganeau et al., 2004)). In addition, cases of heteroplasmy, that is the presence of more than one type of mtDNA in an individual, have been detected (Rubinoff et al., 2006). Nevertheless, as will be discussed below, mtDNA analysis remains very valuable, especially for species identification, which is reflected by the use of the mitochondrial COI sequence for species identification by the 'DNA barcoding' approach (see above).

1.5 Repetitive DNA

Repetitive DNA sequences (designated as 'mini-' and 'microsatellites') are widespread throughout the eukaryotic genome and show sufficient variability (polymorphism) among individuals of a population that they have become important in several fields, including (human) identity testing.

'Minisatellites' consist of repetitive, variant repeats that range in length from 10 to over 100 bp. The discovery of their extreme polymorphism led Sir Alec Jeffreys, in a ground breaking proposal in the mid-1980s, to put forward that minisatellites can be

used to distinguish among individuals, a technique now commonly referred to as 'DNA fingerprinting' (Jeffreys, 2005). DNA fingerprinting has quickly found its way into forensic applications and as evidence material in courts of law.

Nowadays the use of minisatellites is basically substituted by microsatellites. In fact, microsatellites are probably the most widely used DNA marker in population genetics. They are also extensively used in forensic science, where they are referred to as Short Tandem Repeats (STRs). Microsatellites consist of tandem repeats of short sequence motifs of one to six nucleotides (e.g. 'tacgtacgtacgtacg', commonly represented as [tacg]4). Their polymorphism is characterised by a highly variable number of repeats (from 5 up to 100 repeats). To characterise microsatellite markers, they are amplified by PCR and the length of the resulting product corresponds to the number of repeats and can be determined by gel electrophoresis. The power of microsatellites for population structure or stock analysis depends on their diversity (number of alleles per locus) but more importantly on the number of microsatellites screened (Bernatchez and Duchesne, 2000).

Microsatellite primer sets for the amplification of specific microsatellites can be found on numerous websites, showing to what extent this method is meanwhile established. The highly varied information that microsatellites provide allows distinguishing populations but also, as discussed above, individuals. Web-based applications support individual distinction and assignment, e.g. to a fish population of origin. An example is WHICHRUN (http://www-bml.ucdavis.edu/whichrun.htm) that uses multilocus genotypic data to allocate individuals to their most likely source population (Banks and Eichert, 2000).

Despite the common application of microsatellites, there are inconveniences when using them for population genetic studies underlying control and enforcement purposes. Sometimes microsatellite alleles fail to amplify to detectable levels (null alleles and allele dropout) and also the mutation process of microsatellites renders traditional ways to measure genetic diversity difficult (Dewoody et al., 2006). Furthermore, as with all analytical techniques relying on PCR, the danger of sample contamination (allele drop-in) is not negligible. As microsatellites are scored as length polymorphisms, the estimated length of a microsatellite allele critically depends on the electrophoretic equipment and size standards used in individual laboratories as well as expert judgement. The general lack of calibration across laboratories poses considerable problems for creating comparable databases for traceability and impedes forensic applications to a certain extent.

However, these challenges have been addressed in the forensic DNA community, and STR analysis is meanwhile by far the most prominent and successful method for the genetic fingerprinting of individuals. The STRs in use today for forensic analysis are all tetra- or penta-nucleotide repeats (four or five repeat units), as these give a high degree of error-free data while being robust enough to survive degradation in non-ideal conditions. Shorter repeat sequences tend to suffer from artefacts such as stutter and preferential amplification, while longer repeat sequences are more prone to environmental damage (degradation) and do not amplify by PCR as well as shorter sequences.

The actual analysis is performed by extracting nuclear DNA from the cells of a forensic sample of interest (tissue such as hair or skin), then amplifying specific polymorphic regions of the extracted DNA by means of the polymerase chain reaction. Once these sequences have been amplified, they are resolved by electrophoretic methods, which will allow the analyst to determine how many repeats of the STR sequence in question there are.

The usage of microsatellites for fish population analysis is not as commonplace as that for human beings. However, numerous examples exist where microsatellite analysis is used for fish population analysis and management of Pacific salmon (Fisheries and Oceans Canada, DFO ([5])) and also for cod where microsatellites have even been used as evidence in a court case against a fisherman claiming a false origin of his catch (see examples in Annex).

5 DFO website: http://www.pac.dfo-mpo.gc.ca/science/facilities-installations/pbs-sbp/mgl-lgm/proj/index-eng.htm online.

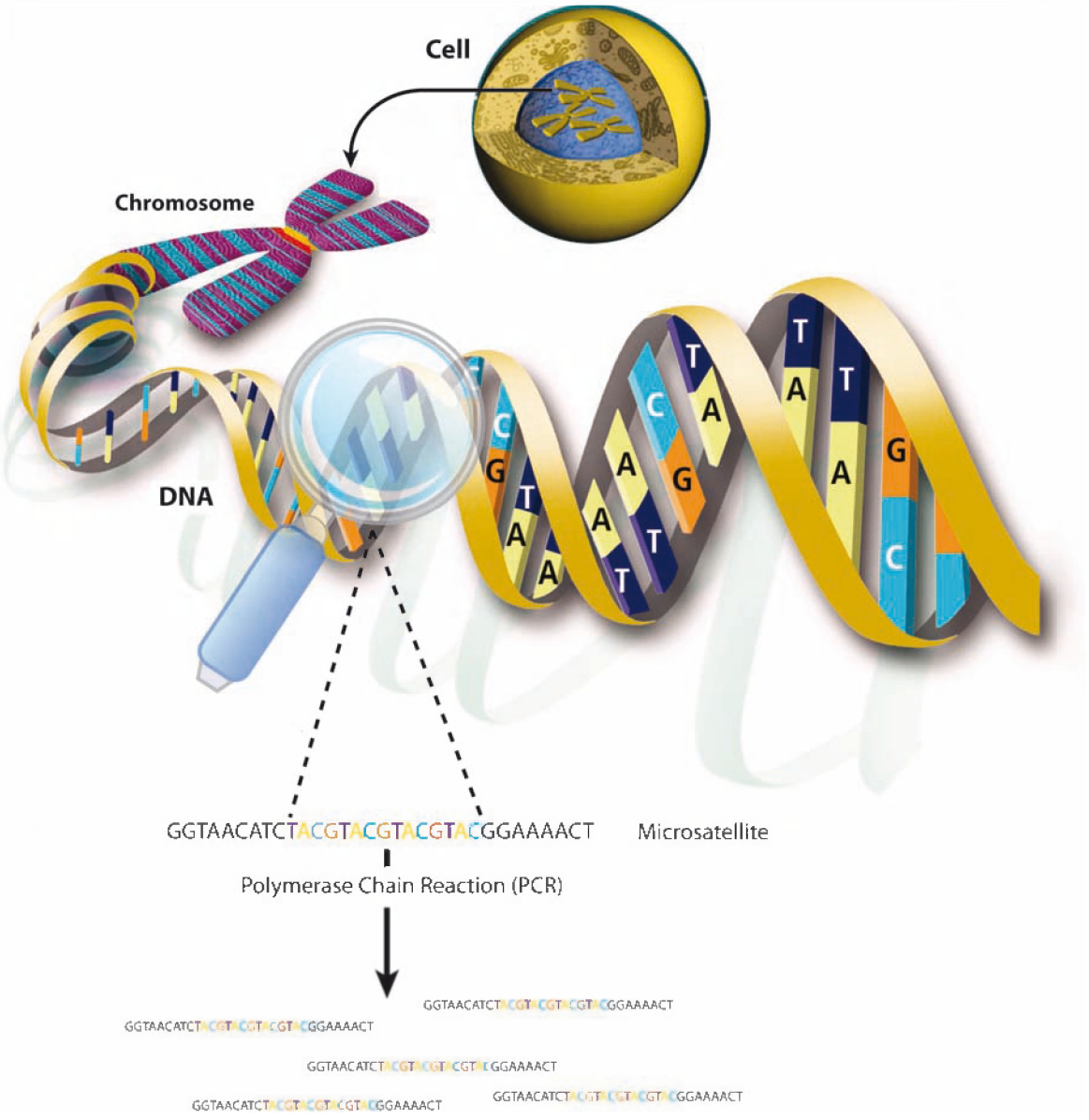


Figure 6. Illustration of PCR amplification of microsatellites. *See text for details. DNA molecule and cell with kind permission of Genome Management Information System, Oak Ridge National Laboratory; US Department of Energy Genome Program's Genome Management Information System (GMIS).*

1.6 Single Nucleotide Polymorphisms

Single Nucleotide Polymorphisms (SNPs) are nucleotide sites in the genome where more than one nucleotide (A, C, G or T) is present in a species (see ***Figure 7***). They are the most abundant polymorphism in the genome with one SNP occurring approximately every 500 bp in the genome of wild animals (Brumfield et al., 2003, Morin et al., 2004). However, per locus SNPs normally only exist in two alleles per locus (biallelic markers, i.e. either an A or a C at a given position but not an A, C, G or T), and are therefore by far less variable than the repetitive DNA markers described above, where often many alleles per locus can be found. The lack of resolution power for each SNP marker is outweighed by their

abundance in the genome. Also, SNP detection is easily adaptable to high-throughput screening methods (see below). SNPs have a great potential in the field of population biology, and also for origin assignment. In their study, Akey et al. described allele frequencies at more than 26 000 SNPs in three human populations. Two randomly chosen humans will differ from each other at up to seven million single nucleotide sites over their whole genome (Akey et al., 2002). SNPs are probably even more abundant in fish (see e.g. (Guryev et al., 2006)), because on evolutionary time scales humans (*Homo sapiens*) arose only recently (ca. 10^5 years ago (Futuyma, 1998) compared to teleost fish (ca. $1.5X10^6$ years ago (Volff, 2005)).

The analytical protocols based on SNPs are more easily transferred from one analytical laboratory to the other in contrast to microsatellite marker protocols. This constitutes an important asset for the establishment of a forensic control and enforcement framework. However, creating a baseline, that is identifying an appropriate number of SNP markers with sufficient (population) resolution power, is labour intensive and requires a fundamental research approach such as that currently undertaken by the Seventh Framework Programme (FP7) project FishPopTrace (http:\\fishpoptrace.jrc.ec.europa.eu). The introduction of ascertainment bias during the process of finding SNPs is a potential risk of this approach (Morin et al., 2004). Ascertainment bias is introduced if the selection of SNP loci derives from an unrepresentative sample of individuals, or it arises as a result of a particular analytical method used if the yield of loci is not representative of the spectrum of allele frequencies in a population. If too few individuals are used for SNP discovery, then SNP loci with rare alleles are likely to be underrepresented, and the following genotyping studies using those SNPs will reveal a (false) lack of rare alleles. Ascertainment bias has the potential to introduce a systematic bias in the estimates of variation within and among populations. Therefore, the protocol used to identify SNPs must be recorded in detail, including the number and origin of individuals screened, to enable ascertainment bias to be assessed and, if needed, corrected. Generally, SNP discovery is easier in model organisms, which are the subject of whole genome sequencing projects, compared to non-model organisms such as commercially exploited fish (see also below ***1.8 DNA sequencing and the dawning era of genomics***).

Even though a thorough knowledge about genomes resulting from whole-genome sequencing projects greatly facilitates the SNP discovery and genotyping process, there are alternative genomic resources to discover SNPs. These include expressed sequence tag (EST) libraries. Roughly speaking, ESTs are sequences of expressed genes, which have been identified from partial sequencing of a messenger RNA (mRNA) pool that has subsequently been reverse transcribed into cDNA. ESTs represent portions of expressed genes in a given tissue or cell line; their sequences are deposited in major international and public databases such as the European Bioinformatics Institute (EBI) database (Bouck and Vision, 2007). By fetching and analysing EST sequences, e.g. derived from a particular fish species, SNP markers can be identified using bioinformatics tools, a procedure coined '*in silico* SNP mining' (Guryev et al., 2005). In fact, for humans a great part of SNP discovery has been done *in silico*, (Ahren et al., 2004). However, even if polymorphisms cannot be identified directly from the databases, the sequences can be used to design PCR primers to screen for polymorphisms, for example, using an exon-primed intron-crossing (EPIC) approach (Primmer et al., 2002, Primmer et al., 2009).

In order to trace commercially important fish using SNPs, these markers would have to be found through laboratory screening. One possibility is the sequencing of genome segments from multiple individuals, but alternative methods for the identification of SNPs are also available (reviewed in (Brumfield et al., 2003)).

In a recent study the value of SNPs as markers to distinguish local stocks of Atlantic cod in US waters was assessed (Wirgin et al., 2007). SNPs occurred every 310 bp among the cod introns examined. The result of this study indicated that SNPs provide a high resolution power for stock identification, comparable to microsatellite loci analysis.

After the identification of candidate SNPs ('SNP discovery'), these have to be genotyped, i.e. the degree of genetic variation among individuals of a group under investigation is measured, to select a panel of SNPs with the highest resolution power for population analysis or origin assignment. For SNP genotyping many highly sophisticated high-throughput methods are available (Ragoussis, 2009). Additionally, most companies providing SNP detection and genotyping services also provide customers with software programmes facilitating further SNP analysis.

It has been estimated that three times more SNPs than microsatellite loci are needed to assess population genetic parameters with statistical confidence (Brumfield et al., 2003). However, this apparent disadvantage is by far outweighed by the ongoing progress in the genome analysis field and automation of analytical methods (see below).

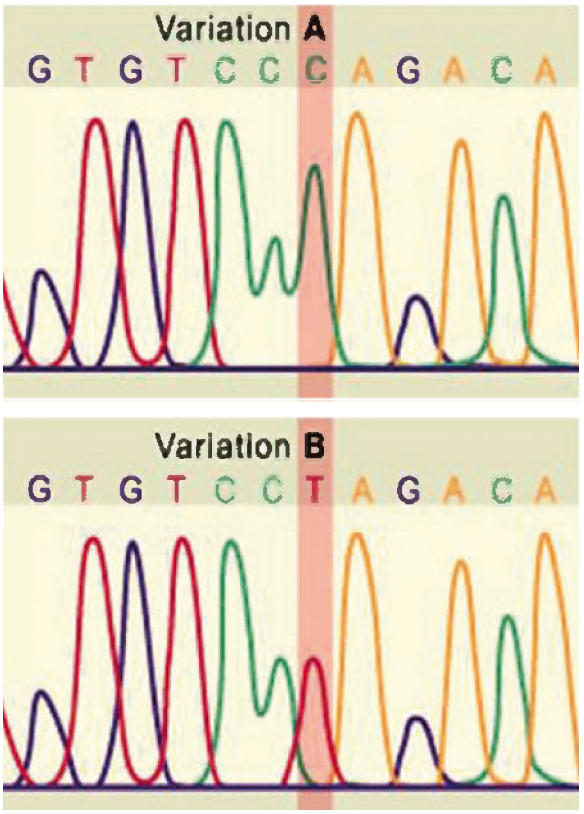


Figure 7. Illustration of SNPs revealed by DNA sequencing. *Depicted are two chromatograms showing the results of a DNA sequencing run on two individuals clearly showing a base alteration of the DNA four letter code ACGT at position 7 from C (the base Cytosine) in individual 1 to T (the base Thymine). Modified - Courtesy of R. Ogden, TRACE Wildlife Forensics Network, 2010.*

Due to their properties SNPs are increasingly the genetic marker of choice in many studies of ecology, population structure and also in fisheries conservation and management. The exchangeability of SNP data and underlying protocols facilitates multinational collaborations while the ease of data standardisation across laboratories and different genotyping platforms makes SNPs ideal for constructing species-wide data bases and for forensics (Sobrino et al., 2005). Examples are the North Pacific Anadromous Fish Commission (NPAFC) consortia of laboratories that are developing SNP arrays for studies of Pacific salmon (http://www.npafc.org), and the aforementioned project FishPopTrace, which develops tools to reveal the geographic origin of fish and fish products of cod, herring, hake and sole, using 1536-SNP arrays (Martinsohn and Ogden, 2009).

Table 1. Comparison of different genetic markers and their characteristics.

Comparison of different genetic markers

Marker	Usage	Advantage	Disadvantage
Protein	Population – Origin assignment	• Low costs • Simple protocols • Highly established	• Limited number of available markers • Material has to be fresh or frozen • Markers can be under selection (1)
Mitochondrial	Species identification Population – Origin assignment (D-Loop)	• Low costs • DNA easily obtainable at high quantities • Species identification supported by international project BOL (2)	• Population genetics meanwhile based on nuclear markers
STRs	Population – Origin assignment	• Large number of alleles per locus allows to obtain high resolution easily • Low ascertainment bias • Isolation easy • Supported by international expert community for human DNA forensics	• High mutation rate • Intricate mutation characteristics • Not highly abundant • Adaptation to automatic high-throughput analysis is possible (e.g. multiplexing) but not straight forward • Protocols are not easily transferable between laboratories
SNPs	Population – Origin assignment	• Stable/low mutation rate • High abundance • Typing is easy • Well suited for high-throughput analysis • Detection can be done 'in silico', i.e. by screening available DNA databases • Protocols are easily transferable between laboratories	• Discovery is demanding • Single SNPs, as being biallelic are not informative • Ascertainment bias • Might be under selection (2)

[1] *This does not necessarily constitute a disadvantage: A marker under selection might reflect the environment in which a given population is located, which can be exploited for origin assignment (an example is a SNP at the Pantophysin (Pan I) Locus in the Atlantic cod (Pogson, 2001).*

[2] *Barcoding Of Life at http://www.barcodinglife.org/views/login.php online.*

1.7 Microarrays

A DNA microarray (also called 'DNA-Chips') consists of a solid surface, made of glass or silicon, onto which thousands of DNA oligonucleotides are attached covalently. The oligonucleotides can be a short section of a gene or other DNA element that are used as probes to hybridise with a target DNA sequence under high-stringency conditions. For example, for fish species identification the probes can be short species-specific DNA sequences. The target sequence (e.g. DNA extracted from unidentified fish-fillets), is labelled with fluorophores. The target sequence will hybridise with its complement probe and remain stuck to it even after repeated washing steps. Subsequently, the microarray will be scanned and the target DNA that remained hybridised to the probe DNA after the washing procedure can be detected at a distinct coordinate on the microarray. The fluorescence-based detection also allows quantification to determine relative abundance of nucleic acid sequences in the target.

It is possible to monitor thousands of different (i.e. species identifying) DNA sequences simultaneously with one such chip (size about 1x1 cm). While the development of DNA microarrays is still considerably laborious, the running costs using this chip are moderate. Theoretically, using just one chip it would be possible to screen for all major economic fish species simultaneously. This could be a valuable asset when examining blocks of frozen imported fish of which the species composition is dubious. Microchip technology would allow rapid identification of the species contained in such fish admixtures and to validate the content and labelling specifications. As will be further elaborated in the chapter on identification of marine fish species, a comprehensible DNA chip for the identification of fish species from European seas has recently been developed (Kochzius et al., 2008), and there exist also commercially available microarrays used for food quality testing (bioMérieux, 2004, Agilent, 2010).

1.8 DNA sequencing and the dawning era of genomics

Development and innovation in the field of DNA sequencing technology has never been progressing at a faster pace than today (Flintoft, 2008) and has freed the way to enter the era of genomics, the study of genomes and their interacting elementary structures, through large-scale development of genomic tools, including whole genome sequencing. Mainly kicked off by the human genome project, these developments contribute immensely to medicine and the pharmaceutical industry where 'personal genomics' and 'personalised medicine' are on the rise (Blow, 2007). But also agriculture and farm animal production are profiting a great deal (de Koning et al., 2007).

New high-throughput sequencing technologies allowed for a dramatic decline in sequencing costs while speed and quality of analysis is boosted by orders of magnitude ([6]) (see ***Table 3***). Parallels have been drawn to the technology development in the information technology (IT) sector, for example, that the cost per reaction of DNA sequencing has fallen in line with Moore's Law (Mardis, 2008). In fact, high-throughput DNA sequencing relies heavily on recent advances in robotics, bioinformatics and computer databases. The swift emergence of these next generation sequence methodologies will and does already open new possibilities for genetic fish population analysis and consequently for fisheries management as well as monitoring, control and enforcement.

The present revolution in DNA sequencing is impressively illustrated by several recently started projects such as the Personal Genome Project at the University of Harvard, which aims to integrate data for genomics, environment and phenotype in more than 100 000 volunteers, and by the fact that sequencing a bacterial genome, a few years ago still a quite challenging approach, can nowadays be done in a matter of hours (Blow, 2007). Also, recently a group of genome and museum experts came forward with the 'Genome 10K' project which aims to sequence 10 000 vertebrate genomes within 5 years (Genome 10K Community of Scientists, 2009) (Genome, 2009) and at the writing of this document 10 human genomes have been sequenced for below USD 5 000 (see ***Table 3*** and ***(Anonymous, 2010)***).

This leap forward in DNA sequencing technologies has also had a strong impact on the progress being made for the deciphering of fish genomes. After focussing for many years on model organisms such

6 To this end the Archon X Foundation has advertised the ARCHON X PRIZE FOR GENOMICS (http://genomics.xprize.org/genomics/archon-x-prize-for-genomics), USD 10 million for the firm that manages first to sequence the complete human genome for a prize below USD 10 000. While the current costs are in the range of USD 300 000 to USD 400 000, the firm Complete Genomics announced to sequence 1 000 human genomes in 2009 for USD 5 000 each.

as the zebrafish (*Danio rerio*), progressively also commercially important marine fish are included on the vertebrate genome sequencing agenda (see ***Table 2***).

Sequencing entire genomes is invaluable to fisheries management in many respects. It will facilitate analyses of the distribution of genomic variation among fish species but also within and among populations of one species in time and space. Fundamental questions relating to phylogeny and local environmental adaptation (e.g. response to climate change) can more easily be tackled, helping to define management units and set conservation measures. Also traceability, control and enforcement will benefit from these developments. The positive effect of new generation sequencing technologies for the development of traceability and control tools in the fisheries sector is exemplified by the FP7 project FishPopTrace where in a few weeks for hake, herring and common sole over 100 million bases of sequence data have been generated to date. From these data approximately 7 500 candidate SNP markers per species were discovered that are assessed with respect to population identification power (Martinsohn and Ogden, 2009).

Table 2. Fish genomes being fully sequenced or genome sequencing in progress.

Common name	Scientific name	Environment (http://www.fishbase.org)	Model Organism/ Commercial	Genome size	Status
Zebrafish	Danio rerio	benthopelagic; freshwater	Model	1 700 Mbp	Fully sequenced (http://www.ensembl.org/)
Three-spined stickleback	Gasterosteus aculeatus	benthopelagic; anadromous; freshwater; brackish; marine; depth range 0-100 m	Model	460 Mbp	Fully sequenced (http://www.ensembl.org/)
Medaka (Japanese ricefish)	Oryzias latipes	Benthopelagic; amphidromous; freshwater; brackish	Model	700 Mbp	Fully sequenced (http://www.ensembl.org/)
Japanese pufferfish	Takifugu rubripes	Demersal; freshwater; brackish; marine	Model/ Commercial	390 Mbp	Fully sequenced (http://www.ensembl.org/) (http://www.fugu-sg.org/)
Spotted green pufferfish	Tetraodon nigroviridis	Demersal; freshwater; brackish	Model	390 Mbp	Fully sequenced (http://www.ensembl.org/)
Atlantic salmon	Salmo salar	Benthopelagic; anadromous; freshwater; brackish; marine; depth range 0-210 m	Commercial	1 500 Mbp	In progress (http://www.bcgsc.ca/project/salmon) (Ng et al., 2005)
Rainbow trout	Oncorhynchus mykiss		Commercial	2 400 Mbp	In progress (Rexroad et al., 2008)
Atlantic cod	Gadus morhua	Benthopelagic; oceanodromous; brackish; marine; depth range 0-600 m, usually 150-200 m	Commercial	900 Mbp	First draft of full sequence (Oct. 2009) (http://codgenome.no/)
European sea bass	Dicentrarchus labrax	Benthopelagic	Commercial	600 Mbp	http://www.molgen.mpg.de (Kuhl et al., 2010)

While for the FishPopTrace approach whole-genome sequences were not available, future genome sequencing of marine fish will further facilitate the discovery of polymorphic DNA markers such as SNPs (see above ***1.6 Single Nucleotide Polymorphisms***). Recently the cod genome project consortium announced the first ever draft sequence and assembly of the Atlantic cod genome (http://codgenome.no). Cod is one of the most important exploited fish species and also an emerging aquaculture species. The cod genome was revealed by exclusive use of new generation high-throughput sequencing technology combined with state-of-the art bioinformatics algorithms. It took just several months, two sequencing machines and approximately USD 500 000 to yield a 30-time coverage of the 750-million-base cod genome sequence (Pennisi, 2009).

Table 3: The human genome sequencing progress. The table lists the four instances in which the human genome (three billion bases (3 gigabases – 3 000 Mbp)) was sequenced until today. The project costs dropped by a factor 10^4 from the Human Genome Project to the two anonymous individuals sequenced in 2008, and the time needed from beginning to the end of the project decreased by a factor of 80. During the writing of this report approximately 20 other human genomes have been sequenced (Anonymous, 2010).

Project	Duration	Costs [USD]	Quality*
Human Genome Project	1996-2003	3 billion	Assembly
Craig Venter	2007	100 million	7X
James Watson	ca. 4 months (2008)	2 million	7.5X
Anonymous (Yoruba/Nigeria)	ca. 8 weeks (2008)	100 000	30X
Anonymous (Han-Chinese)	ca. 8 weeks (2008)	100 000	36X
Anonymous (Three Human Genomes)	Few weeks (2009)	4 400	45-87X

* *Quality: While during the Human Genome Project a composite sequence from the DNA of several anonymous volunteers was compiled and assembled, the four other projects revealed the genome sequence of one individual each. For quality assurance the sequence of Craig Venter was, during the sequencing project, in fact sequenced 7 times, of James D. Watson, 7.5 times, of the two anonymous individuals 30 and 36 times, respectively. If this is taken into account the decrease in costs and time needed is even more impressive.*

Sources:

a) US National Institute of Health (http://www.genome.gov/11006943).

b) PLoS Biology (2007) 5(10): e254; Nature News (2008) 452: 788.

c) Technology Review (June 2007; http://www.technologyreview.com); Nature News (2008) 452: 788.

d) Nature (2008) 456(7218): 53; Nature Editorial (2008) Vol 456 (7218) November 2008.

e) Nature (2008) 456(7218): 60; Nature Editorial (2008) Vol 456 (7218) November 2008.

f) Science (2009) Published Online November 5, 2009; Science DOI: 10.1126/science.1181498.

And Mardis, E.R. (2008) 'The impact of next-generation sequencing technology on genetics' TIGS 24(3): 133.

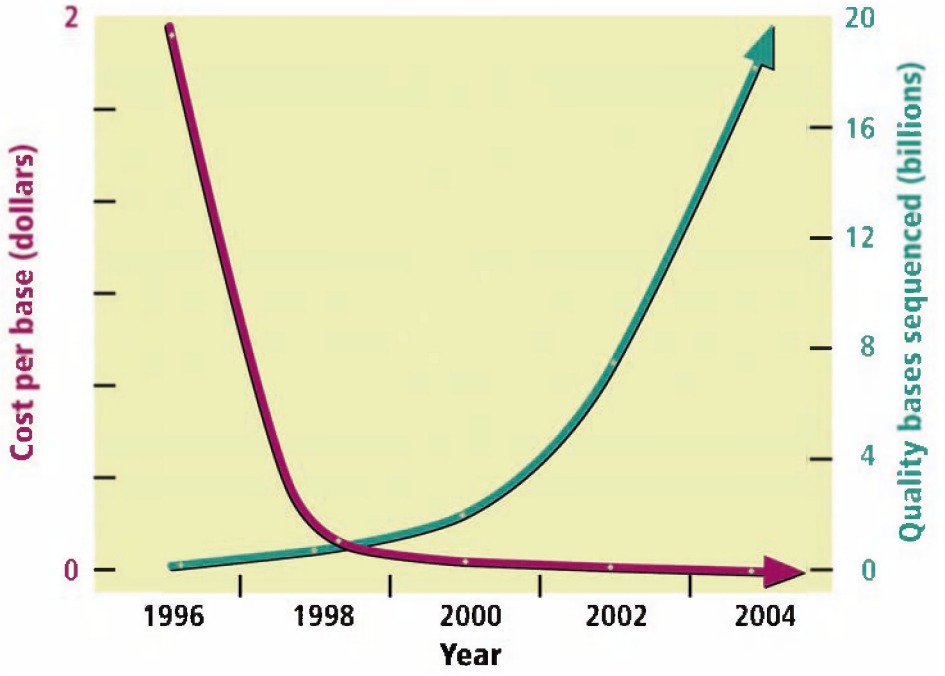


Figure 8. The exponential decrease in DNA sequencing costs is accompanied by a likewise increase in sequencing quality. *The graph plots the costs per base (in red) and the number of bases sequenced against the time interval ranging from 1996 to 2004. This trend of a radical cost-reduction for DNA sequencing is still ongoing (Anonymous, 2010). Reprinted with kind permission of the US Department of Energy Genome Programs.*

1.9 Gene expression, functional genomics and proteomics

Gene expression, the process by which information encoded by a gene is used in the synthesis of a gene product (DNA to mRNA, or other RNA products, by transcription, and mRNA to proteins by translation), can differ between species (interspecies level). Gene expression also changes in response to the environment, and is subject to adaptive evolution on an intraspecies level. For the past 10 years microarrays have been used to analyse gene expression in fish to investigate questions related to ecology, evolution and environment. Gene expression variation has also been assessed in natural populations of marine fish (Larsen et al., 2007), to study speciation and to examine host-pathogen interactions (Goetz and MacKenzie, 2008). Microarrays used in the study of population differences in gene expression target thousands of genes simultaneously ('transcriptomics' – reviewed in (Nielsen et al., 2009)). Such microarrays have now been developed for a large number of marine fishes, e.g. gilthead sea bream (*Sparus aurata*) (Ferraresso et al., 2008), Atlantic halibut (*Hippoglossus hippoglossus*) (Douglas et al., 2008) and Senegalese sole (*Solea senegalensis*) (Cerda et al., 2008). They are presently particularly used for aquaculture species as they help to elucidate transcriptional changes under specific farming conditions or during infections. This can improve knowledge about reproduction, development, nutrition and immunity thereby supporting the optimisation of production under culture conditions.

Proteomics, the investigation into the sets of proteins expressed by the genome of an organism under given environmental conditions, has recently been employed to understand protein diversity across and within human populations (Biron et al., 2006).

Analysis of gene expression, both at the RNA (transcriptomics) and protein (proteomics) levels, is in principle applicable to develop suitable markers for traceability, be it for origin assignment (if different expression patterns between populations of fish occupying different regions/environments can be established, this can be used for traceability) or to distinguish between wild and farmed fish. This has recently been demonstrated in a study on European hake (*Merluccius merluccius*), where

proteomics have been used to establish differential protein expression patterns in hake from the Mediterranean Sea, the Cantabrian Sea and the Atlantic Ocean (Gonzalez et al., 2010). However, while assessing the potential of such novel tools is also part of explorative research of projects such as FishPopTrace, they are not yet at an applicable stage for control and enforcement.

1.10 Handheld analytical devices

A crucial aspect in the fisheries control and enforcement sector is the 'response time', i.e. the lapse of time between the sampling of a suspicious fish (product) lot (e.g. by biopsy of presumably falsely labelled fish) and the reception of analytical results. Ideally inspectors would be able to perform an analysis on the spot, at least as a first measure to produce evidence sustaining raised suspicion, and as support when a decision about confiscation of a lot has to be taken without delay. Thanks to the rapid progress in technology development based on the PCR and also microchip development this has moved into reach. Especially the need for rapid identification of pathogens responsible for disease outbreaks and epidemics are a driver for the invention of portable analysis devices. However, engineering for such machines is also carried out in support of forensic genetic analysis at crime scenes (Liu et al., 2008). While various recent peer reviewed publications and announcements show that major progress has been made in this area (Arnaud, 2008), currently no cost-effective handheld analytical device supporting fisheries control and enforcement of traceability is available.

1.11 Wildlife forensic science in support of fisheries enforcement

Forensic science (often shortened to 'forensics') is the application of a broad spectrum of sciences to answer questions of interest to the legal system, in relation to a crime or to civil action. Evidence produced by forensic science can support investigations and deter illegal fishing activities or fraud along the food supply chain. This has been demonstrated in the fisheries sector, where forensic genetic or chemical analyses provided important contributions to the body of evidence during recent cases, as illustrated by examples depicted in the annex. To assure the admissibility of analytical results as evidence in court, it is crucial that strict standards and guidelines are applied. This requires rigorous validation of the techniques, together with appropriate sampling, evidence handling and statistical evaluation of analytical results before criminal courts will routinely accept and apply the power of DNA testing, or chemical analysis, in cases involving animals.

The introduction of DNA technology, namely genotyping, is certainly one of the most significant advancements in the history of forensic science (Jobling and Gill, 2004), and human DNA profiling systems have evolved immensely since their first introduction about 20 years ago (Jobling and Gill, 2004).

A major challenge is to present evidence that is robust, lucid and unequivocal, since all elements such as sample collection and transfer, data compilation and analysis, as well as reference data sets are liable to be scrutinised for flaws during court trials (Ogden, 2008). This is why forensic science has to fulfil certain legal standards to be admissible as evidence before courts of law.

In the US, basically two different standards are applied to assure that evidence is based on methods and reasoning that lead to correct conclusions (Cassidy and Gonzales, 2005). The Frye Standard (Frye, 1923) requires that scientific evidence must have gained general acceptance in the field to which it belongs. The technology employed must have left the experimental stage and be fully established, as shown by peer reviewed publications of designed, controlled studies. The Daubert Standard (Daubert, 1993) goes further since it requires an independent judicial review and reliability assessment of the methods used. In addition to the general acceptance principle, the techniques used to produce evidence must have been scrutinised by thorough statistical evaluation.

However, rather than focusing on the scientific approach itself, challenges to forensic evidence are more likely to arise from perceived sample processing or reporting errors. The DNA evidence is worthless without the ability to demonstrate that samples are handled according to approved protocols. When collecting the evidence – and this is especially true for material which will ultimately serve for DNA analysis – contamination control and prevention of cross-contamination at the scene are essential. The handling of physical evidence is one of the most important factors in an investigation. Samples must be collected, inventoried, preserved, transported and submitted for testing without

compromising the evidential chain of custody. In this respect, guidelines established for processing human crime scenes, for example those written and approved by the Technical Working Group on Crime Scene Investigation (National Criminal Justice Reference Service 2000), may be transferred into fisheries enforcement, to help prevent errors that might result in inadmissibility of evidence. Quality assurance is also obtained through Standard Operating Procedures (SOPs), documents containing instructions that forensic scientists follow to perform procedures that are routine, standardised and for which no *ad hoc* modification is acceptable. They help to ensure the quality and integrity of data and provide a basis for guidance, uniformity and accountability (Bowker et al., 2006).

Evidence based on genetics or chemistry generated to forensic standards will normally constitute an element of an 'evidence body'. In fisheries crime cases the evidence body could also include logbook records, VMS/VDS data and circumstantial evidence provided by inspectors.

In fact, as pointed out recently by the wildlife forensic expert Rob Ogden, in practical terms many forensic geneticists favour the formulation of a likelihood ratio that compares the probability of observing the evidence (sample profile) under the prosecution and defence hypotheses. This allows the forensic scientist to account for uncertainty, circumstance and alternative claims and, importantly, does not place any quantitative restriction on how significant the result must be. A quantitative estimate of the relative likelihood of the evidence is produced: the larger the number, the more probable the evidence given the allegation. This approach is widely employed to present human and non-human individual DNA profile matches and is also recommended for cases involving the assignment of fish catches to their population of origin (Ogden, 2008).

Forensic genetics and chemistry can already greatly support the fight against the international phenomenon of IUU fishing (see ***Figure 12***, p.38). The current acceleration of genomic data production in non-model animals like marine fish (see ***Table 2***), promises to further increase the development of validated forensic methods available to enforcement officers. At present, techniques for species identification are at hand, thanks to the international endeavour of the Fish Barcoding of Life project (see ***2. Fish Species Identification***). The ability to undertake origin assignment of marine fish depends on the species and geographic region in question, with applications to salmon (an anadromous species) being most advanced followed by cod (see ***example 15 in Annex***). A more general transfer of modern technologies into a forensic framework for fisheries control and enforcement will depend on specifically tailored research projects, such as FishPopTrace, but also on combined efforts for a technology transfer involving all stakeholders, as recently stressed during a FAO workshop on forensic technologies for fisheries control and enforcement ([7]).

7 Informal Workshop on the Use of Forensic Technologies in Fisheries Monitoring, Control and Surveillance; FAO; Rome, Italy; 9-10 December 2009.

2. Fish species identification

Renaming and mislabelling of seafood occurs globally to a significant extent and undermines conservation efforts and fisheries management, adversely affects the fisheries economy and deceives the consumer (Jacquet and Pauly, 2008). For example, a study on food fish in the US revealed that 75% of fish sold as 'red snapper' were mislabelled and were actually other species (Marko et al., 2004). Also, recently an analysis of fish samples from markets and restaurants in North America, based on DNA barcoding (see below), revealed that about 25% of analysed specimens were mislabelled (Wong and Hanner, 2008). In addition, the examples depicted in the Annexes highlight the frequent occurrence of mislabelling in the fisheries sector. As outlined in the introduction, at the core of the EU legislation dealing with traceability is Regulation (EC) 178/2002 (European Parliament and European Council, 2002). This regulation is complemented by Regulations (EC) 104/2000 (European Council, 2000) and (EC) 2065/2001 (European Commission, 2001), which specify that labels must clearly establish commercial name, production method and geographic origin. This labelling information has to be given at every step of the production and retailing chain. Moreover, in Article 28 of the Community fisheries control system regulation (European Council, 2009), explicit reference is made to the establishment of a comprehensive control regime, covering the whole chain of production and marketing in line with the above mentioned regulations. However, to control for compliance and enforce such laws, clearly efficient analytical tools are needed.

As long as the fish is intact, the species can be determined by visual inspection of external features. In this case a background in fish species identification is sufficient, and only if there is a high degree of resemblance between species will expert taxonomic knowledge be necessary. Other methods based on visual inspection, like species identification by otoliths, exist but they require specialised knowledge and the availability of the feature to be examined.

To incorporate fish species identification fully and on a routine basis into a traceability scheme, it should be applicable to processed fish (fillets, canned and cured fish etc.), an asset that molecular methods provide.

Protein analysis has routinely been used for authentication of seafood by control authorities, in particular isoelectric focusing (IEF) of proteins, and immunological methods (reviewed by (Rehbein, 2003)). DNA-based methods are also commonly used and with increasing frequency to reveal species substitution in fish and seafood products. For this purpose both mitochondrial and nuclear markers have been analysed by numerous highly efficient analytical methods, which are discussed in detail elsewhere (Rasmussen and Morrissey, 2008, Rasmussen and Morrissey, 2009). DNA-based methods have several advantages over their protein-based analysis: DNA is less sensitive to degradation; it can be extracted at all stages from egg to adult, from processed products and even historical or museum samples (Nielsen and Hansen, 2008), synonymous mutations can be revealed by sequencing, Polymerase chain reaction (PCR) amplification makes it possible to analyse minute amounts of tissue and DNA sequence data are easier to replicate and interpret across laboratories (Ward et al., 2009). The latter point is particularly crucial for forensic applications.

A valuable genetic marker for species identification exhibits low intraspecific but high interspecific polymorphism. These conditions are met by the mitochondrial *cytochrome c oxidase subunit I* (COI) gene (see ***Figure 5***), of which a 648-nueclotide stretch of is employed as a DNA marker in the Barcode of Life initiative (CBOL - http://barcoding.si.edu/index.htm) (Waugh, 2007). CBOL is devoted to developing DNA barcoding as a global standard for identifying species. The COI sequence, also referred to as 'DNA-barcode', can distinguish between closely related species and even classify new species from identical appearing ones.

The Fish Barcode of Life Initiative (FISH-BOL) is part of CBOL and a global effort to coordinate an assembly of a standardised reference sequence library for all fish species, one that is derived from voucher specimens with authoritative taxonomic identifications. Meanwhile, following this approach, more than 7 000 fish species have been barcoded (February 2010) (www.fishbol.org). DNA barcoding has already successfully been applied to reveal mislabelling of seafood in North America ((Wong and Hanner, 2008) and see examples in the Annex), and the Food and Drug Administration (FDA) in the US considers to use it as a replacement for the technique of protein IEF for fish and fish product identification (Yancy et al., 2008). For the Project FishTrace the *cytochrome b* gene (see ***Figure 5***) has been used for species determination (http://www.fishtrace.org). In the FishTrace database, hosted by the Joint Research Centre (JRC), more than 200

commercial marine fish species are recorded in a genetic catalogue, and moreover all compiled DNA sequences are linked to a voucher stored in a natural museum. The *cytochrome b* gene has been employed in a recent study to reveal mislabelling of fish products (Logan et al., 2008). Both the FISH-BOL and FishTrace databases are public and can therefore easily be used by control and enforcement authorities to access reference DNA sequences during the identification of potentially mislabelled seafood products.

As outlined above, microarrays provide the possibility to screen for a number of fish species simultaneously, a potential asset for the analysis of admixtures. In recent studies techniques from DNA barcoding and microarrays have been combined, using the mitochondrial genes 16S rRNA, cytochrome b and cytochrome c oxidase subunit I (COI). It was shown that this approach is suitable to identify and differentiate 30 fish species, among them commercially highly important species such as Atlantic mackerel (*Scomber scombrus*) and Atlantic horse mackerel (*Trachurus trachurus*) (Kochzius et al., 2008, Kochzius et al., 2010).

Thanks to the efficiency of modern high-throughput DNA sequencing technologies, and the resulting relative ease with which Single Nucleotide Polymorphims (SNPs) can be discovered, SNPs can also be used as powerful species identification markers. This is currently tested by the JRC in collaboration with partners ([8]) for the identification of caviar, a product notorious for being an issue in illegal trade (European Commission, 2006).

8 TRACE Wildlife Forensics Network (UK); University of Edinburgh (UK); University of Padova (IT); The Centre of Molecular Genetic Identification (VNIRO) (RU); The Iranian Fisheries Research Organisation (IR).

3. Origin assignment of fish

Origin assignment, the ability to determine the geographical origin of fish and fish products at every stage along the supply chain, is essential for any traceability framework in the fisheries sector, as well as to control and enforcement. This includes increasingly also the need to answer the question whether a fish originates from the wild or from aquaculture (see also examples in the Annex).

Traceability in the 'ocean to fork' sense is currently mainly supported by the implementation of product information rules. Labels on fish products marketed in the European Union have to indicate clearly species and origin along with other information. However, to reveal fraud and for enforcement purposes independent control technologies are required. To enable traceability of fish or fish products back to their origin in the sense of the 'fork to ocean', it has to be ensured that individuals of different exploited stocks are distinguishable and identifiable by some means.

Many of the techniques described in this report can be employed for fisheries management and conservation schemes, and to maintain or improve marine resources and their utilisation. To this end assessments have to establish the status of stocks and to determine the level to which exploitation is sustainable. However, prior to such analysis it is essential to identify and discriminate the stocks of commercially exploited fish species. In recent years, the field of fish population genetics and, related to this field, genetic stock identification (GSI), has experienced major progress, from

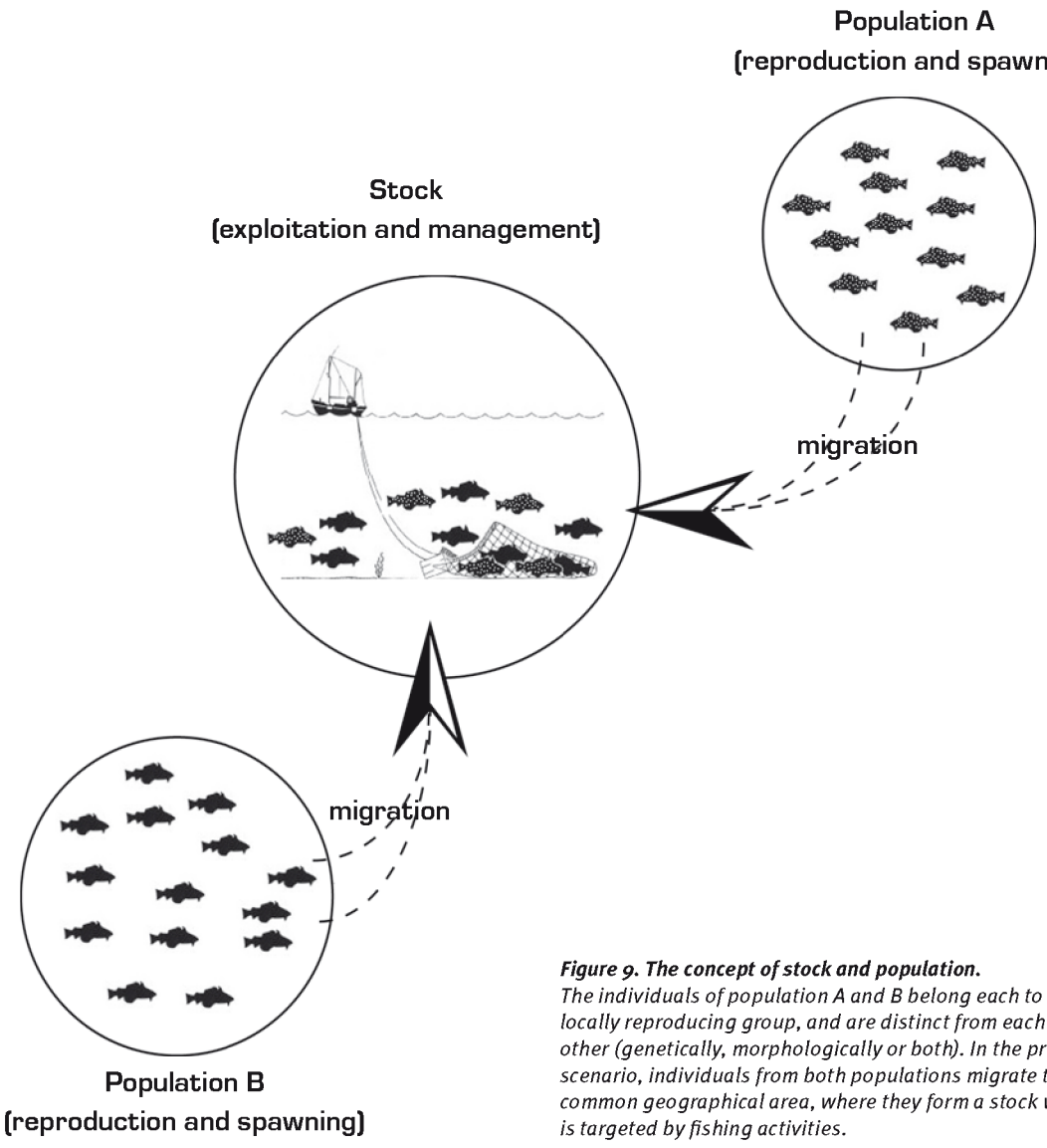


Figure 9. The concept of stock and population.
The individuals of population A and B belong each to a locally reproducing group, and are distinct from each other (genetically, morphologically or both). In the present scenario, individuals from both populations migrate to a common geographical area, where they form a stock which is targeted by fishing activities.

which fisheries management including control and enforcement can greatly benefit. However, marine fish, especially pelagic species, challenge both fisheries managers and scientists due to their highly variable life history traits, variable population sizes and schooling habits. Many species move across several management areas. They may comprise one or more stocks that do not necessarily match imposed stock boundaries. Also, many species are highly migratory. Indeed, the identification of stock units and the design of matching management regimes for marine fishes are far from trivial. They rarely display the highly distinct stock divisions of freshwater and anadromous species and might be caught far away from their spawning grounds (Begg and Waldman, 1999).

Assignment techniques rely on matching an individual fish to one of several groups of fish with similar or the same characteristics. These characteristics are due to common environmental factors, to common genetic material or both. For the discussion of traceability and assignment techniques it is important to be aware that the terms 'stock' and 'population' are not necessarily interchangeable (see ***Figure 9***). In both cases the membership of individuals in a distinct group is measured by a census. However, 'stock' is a technical term describing a group of individuals that is under consideration for exploitation and management purposes. The definition of a fish stock generally includes elements of congruency among individuals such as demographic and phenotypic features. Some stocks might not be genetically distinct groups of fish, but simply reflect differences in phenotypic life history parameters in response to environmental variation and fishing pressure. Normally, a group of individuals identified as a stock occupies a well defined spatial range (fishing area) independent of other stocks of the same species. Nevertheless, random dispersal or directed migration due to seasonal or reproductive activity can occur. The term population takes into account the biology of organisms. It describes the collection of individuals of a particular species, which can be defined as a local interbreeding (panmictic) group ([9]). This group has reduced genetic exchange (gene flow) with other groups of the same species, meaning that mating between individuals of different groups only rarely occurs. This leads to sufficient isolation from other groups of individuals from the same species for some level of genetic differentiation to be established (Waples et al., 2008).

The assignment of individuals to their origin (Individual Assignment – IA) relies on the probability estimation of encountering a combination of certain characteristic features (e.g. genotype or trace element composition in tissue) found in these individuals in a number of potential source populations (see ***Figure 10***). It follows that IA can only work if the feature composition of the potential source populations has formerly been determined by sampling, i.e. that a baseline has been created. The latter point is highly relevant as an incomplete baseline can compromise traceability, and thus seriously impede control, enforcement and traceability: While the analysis of individuals, be it genetically or by other means, might be straight forward, assigning the individual to its source population becomes difficult if the reference data baseline is incomplete. However, even in the case of incomplete baselines, lack of information can to some extent be compensated for by appropriate statistical tests and simulations (Hansen et al., 2001).

Assignment techniques result in a probability ('likelihood') of geographic origin or group membership. This has to be considered, especially if applications are integrated into forensic approaches with relevance to court cases (see ***1.11 Wildlife forensic science in support of fisheries enforcement***).

For a long time, the prevailing notion that marine fish have a negligible level of population structuring, and limited genetic diversity, caused an apparent lack of barriers in the marine environment and a high level of dispersal. This, of course, would be a major obstacle to origin assignment. However, this picture has changed since recent studies have shown that for several highly commercial marine fish species, including herring and cod, population structuring is evident even at small geographic scales (Hauser and Carvalho, 2008; and see ***Figure 11***). This is a major step forward for methods in support of fisheries control and enforcement, and is mainly due to the use of new genetic markers and their analysis with new statistical methods. Molecular markers are inherited and the genetic structure of populations remains ba-

9 This definition is simplified for the purpose of this report. The meaning of the term 'population' in a scientific and genetic context, together with its theoretical framework, has recently been discussed by Waples & Gaggiotti WAPLES, R. S. & GAGGIOTTI, O. E. (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, 15, 1419.

sically stable over generations (Waples, 1990), which is an important asset for the identification of populations. Furthermore genetic loci can be regarded as uncorrelated, independent variables, simplifying statistical analysis of patterns. Also, in an approach coined 'landscape genetics', genetic data is integrated with an array of potential explanatory variables representing the surrounding environment, further supporting the creation of baseline populations. Different statistical methods and software packages have been developed for clustering individuals from large data sets into subgroups or populations (Guillot et al., 2009). Since marine populations are often exposed to highly heterogeneous environments, formed by oceanic current regimes and local conditions such as temperature and salinity, along with geographical barriers such as islands, straits etc. the same approach can successfully be applied to marine animals ('seascape genetics', (Hansen and Hemmer-Hansen, 2007)).

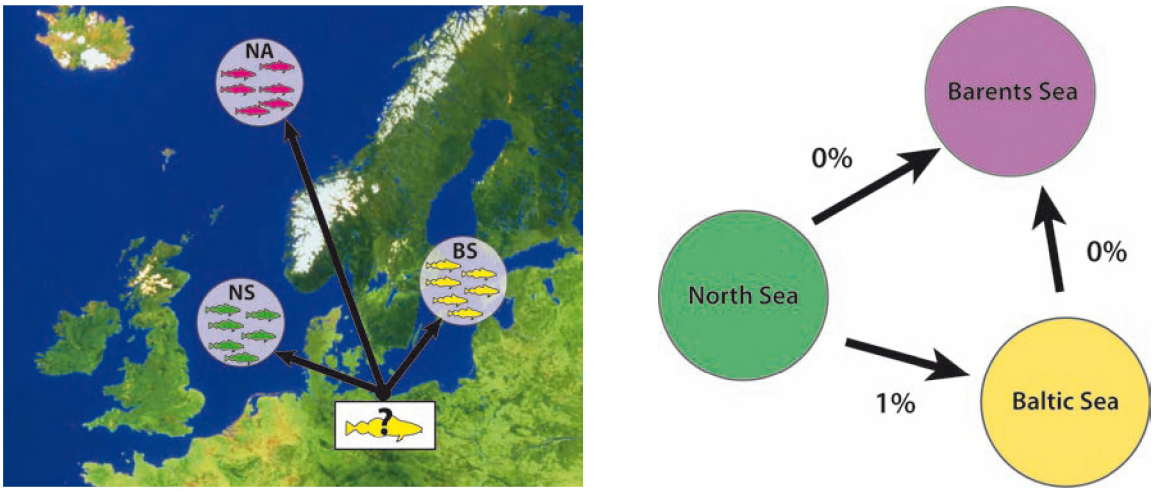


Figure 10. The principle of Individual Assignment (IA). *a) Individual fish of unknown origin are assigned to a set of baseline stocks or populations. In this scenario, using the three baseline populations North Atlantic (NA), North Sea (NS) and Baltic Sea (BS) the fish in question will be assigned to BS. (Map: © European Union, 2010). b) Percent misidentification of cod from each of three geographic areas based on individual-based assignment. Each individual of unknown origin is assigned probabilistically to a number of known baseline populations based on its multi-locus genotype (here for cod and using nine microsatellites; courtesy of E.E. Nielsen).*

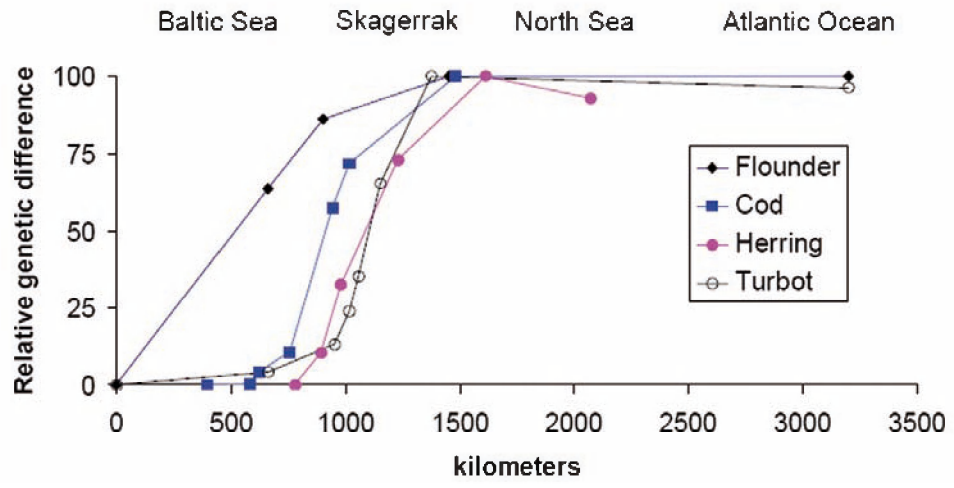


Figure 11. Effect of geographic distance on genetic difference and principle of individual assignment. *The graph shows the effect of geographic distance on genetic difference from the Baltic Sea to the open Atlantic Ocean, in a number of marine fish species (courtesy of E.E. Nielsen; SIMWG, 2006).*

While IA of marine fish, of primary value for monitoring, inspection and enforcement, remains challenging when compared to freshwater and anadromous species with higher genetic differentiation, the feasibility has been proven. Nielsen et al. (2001) were able to assign Atlantic cod individuals to a North Sea, Baltic Sea or northeast Arctic Ocean population with almost 100% certainty, using microsatellite analysis and the algorithm STRUCTURE, a Bayesian method (Pritchard et al., 2000). Genetic data has even been used as evidence in court during a case where a fisherman claimed the wrong origin for his catch of Atlantic cod (see ***Example 15 in the Annex***).

As shown by the examples for applications provided in the Annex, DNA-based technologies for origin assignment are for various reasons most prevalently used, particularly in a legal context. It is worth nothing that other analytical methods can complement genetic analysis. Fatty acid analysis has been employed to distinguish between marine fish of wild and aquaculture origin (Seaborn et al., 2000), and in a thorough study on pink ling (*Genypterus blacodes*) in New Zealand it was shown that trace element analysis of muscle tissue, applying forensic standards, can be used to distinguish groupings of fish from different areas (Bremner, 2009). This is distinct from a genetic approach in that demographic relationships are revealed rather than reproductive relatedness. Both approaches are complementary, and assist fisheries management on several levels, including control and enforcement. Finally, in the case of origin assignment based on genetic methods, recent research shows that the use of new genetic markers (e.g. Single Nucleotide Polymorphims (SNPs)) and new statistical approaches (land/seascape genetics) can reveal marine fish population structure at progressively finer levels of resolution. This provides an opportunity for fisheries management including control/enforcement and traceability applications but calls at the same time for a dialogue between scientists and end-users (authorities and industry) to determine which level of population structure characterisation is needed.

4. Towards a coherent fisheries control, enforcement and traceability framework for Europe: Capacity building and technology transfer

Modern applications based on DNA technology, chemistry and forensics are not only available in theory but have already found their way into the practice of fisheries control, enforcement and traceability, including in court cases as shown through the examples provided in the annex. Figure 12 summarizes how DNA based analysis of fish and fish products, can improve compliance and also support consumer information, either in the context of monitoring and control or in the context of a targeted investigation by applying forensic standards.

However, these success stories are rather scarce and not generally known. Still lacking is a coherent, consistent and homogeneous EU-wide approach fully integrating such technologies under the Common Fisheries Policy (CFP), also for market traceability along the supply chain. Presently, no reference to these technologies exists in the legal CFP framework ([10]). There are other modern technologies, such as the Vessel Monitoring System (VMS), the Vessel Detection System (VDS) and Electronic Recording and reporting System (ERS), which are backed up by EU legislation and where the EU played a pioneering role that had global impact.

In the fields of molecular biology, chemistry and DNA analysis there is a substantial gap between the scientific domain and the transfer of research results into practical applications for fisheries management. This is due to a variety of reasons, extensively discussed in a recent review (Waples et al., 2008). Yet there are numerous examples of successful applications showing that DNA analysis, chemistry and forensics have enormous potential for the support of fisheries control and enforcement (see annex). This is particularly true for species identification, including in processed products. Also, origin assignment (e.g. whether a fish is from a prohibited stock, or is cultured or wild) will increasingly become applicable thanks to scientific efforts which have progressively refined the identification of levels of marine fish population structure.

This is manifested by a recent collaboration between the Danish Fisheries Inspectorate and the Danish Technical University (DTU). Based on DNA evidence admitted in a court case, a fisherman, who had claimed a false origin for his catch of cod, was convicted. In another successful case, fishers had exceeded their catch quota for Baltic sprat (*Sprattus sprattus*) and invented a trip in the logbook to declare that the excess fish came from the North Sea. DNA evidence showed that the claimed North Sea origin of the catch was highly unlikely. Confronted with the evidence, which had been combined with satellite tracking analysis, the fishers accepted a fine and the confiscation of the catch without going to court (Lars Bonde Erikson; Officer of the Danish Fisheries Inspectorate: personal communication). This outcome also confirms the often observed high deterrent effect of DNA evidence: apparently quite often, defendants plead guilty and accept

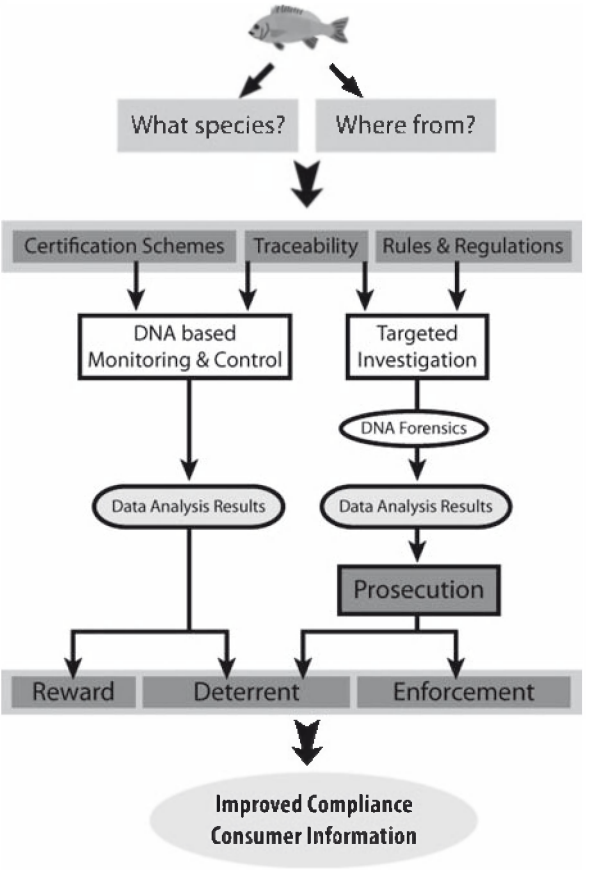


Figure 12 Flow diagram depicting how genetics can support control and enforcement in the fisheries sector and along the supply chain to improve compliance with rules and consumer information and protection. (Modified, based on Martinsohn & Ogden; 2009)

10 However, in the recent Council Regulation establishing a Community control system for ensuring compliance with the rules of the Common Fisheries Policy (EC) No 1224/2009, genetics and other modern technologies are explicitly referred to.

charges and fines, when confronted with evidence based on DNA analysis (Ruth Withler, DFO Canada and Rob Ogden, Trace Wildlife Forensics Network – personal communication). This of course has a considerable cost-saving effect, as lengthy court trials are avoided.

A regular cooperation between the Danish Fisheries Inspectorate and the DTU has meanwhile been established: the inspectors are equipped with tissue sampling kits and can send samples for genetic analysis to a laboratory of DTU (Lars Bonde Erikson; Danish Fisheries Inspectorate: personal communication).

While these examples constitute proof of feasibility, the coherent EU-wide transfer of modern analytical technologies to fisheries control and enforcement as well as traceability schemes, needs the involvement and combined effort of all stakeholders.

One considerable impediment is the current lack of a central data hub, where DNA and chemical data relevant to fisheries control and enforcement are centrally stored, professionally managed and easily accessible. In today's world of genomics, transcriptomics and proteomics, with the advent of ultra high throughput analytical technologies, massive amounts of raw and aggregated information are generated. As a result, data storage, following strict guidelines to assure proper management and accessibility, is indispensible. For general DNA data this has been jointly addressed through the creation of the European Bioinformatics Institutes (EBI) database (http://www.ebi.ac.uk) and its two counterparts, the US National Center for Biotechnology Information GenBank (http://www.ncbi.nlm.nih.gov/Genbank) and the DNA Database of Japan (DDBJ) (http://www.ddbj.nig.ac.jp). The EBI database is produced under an international collaboration with GenBank and DDBJ. In an exemplary international approach each of the three entities collect a portion of the total sequence data reported worldwide, and all new and updated database entries are shared regularly as part of the International Nucleotide Sequence Database Collaboration (INSDC).

The EBI is a non-profit academic organisation, based in Hinxton (UK) that forms part of the European Molecular Biology Laboratory (EMBL) in Heidelberg (DE). This database can be used by scientists to upload generated data, be they DNA sequences, genetic data or proteomic data. The EBI defines its mission as:

- to provide freely available data and bioinformatics services to all facets of the scientific community in ways that promote scientific progress;
- to contribute to the advancement of biology through basic investigator-driven research in bioinformatics;
- to provide advanced bioinformatics training to scientists at all levels;
- to help disseminate cutting-edge technologies to industry.

[http://www.ebi.ac.uk/Information/; accessed May 2011]

The author believes that a similar infrastructure, for compiling genetic and other data generated by fish biology research and tailored to the needs of fisheries managers, control authorities and the industry, would constitute an invaluable asset. While this is largely resolved for species identification with the DNA-barcoding approach (FISH-BOL), a comprehensive strategy including population baseline data for origin assignment is not yet available. Recognising this shortcoming, in 2007 the ICES Working Group on Applied Genetics in Fisheries and Mariculture (WGAGFM) came forward with the proposal to create a meta database cataloguing existing data in the field of fish and shellfish genetics (WGAGFM, 2007). It reasoned that the current lack of such an information technology (IT) infrastructure leads to dispersal and loss of highly valuable information for fishery management including control and enforcement as well as fish and fish product traceability.

Indeed, one eminent dilemma is that genetic and (bio)chemical studies on fish, being still almost exclusively embedded in the academic research realm, are financed by time-limited funding schemes. Researchers, after having concluded a project, must move on and have neither the means nor the time to put the results of their studies into a more sustainable context. In the frame of the Seventh Framework Programme (FP7) project FishPopTrace, the Joint Research Centre (JRC) is currently exploring how to improve information and data acquisition in the area (http://fishpoptrace.jrc.ec.europa.eu/crawler). The JRC team observed

that many, if not most, projects in the field might have local databases, which are however often not supported by a web interface, highlighting the urgent need for a common professionally managed infrastructure.

The fragmentation of marine data is not restricted to biological information. The European Commission is aware of this problem and proposed a new European Marine Observation and Data Network (EMODNET) in its Green Paper on maritime policy (Commission, 2007b). Adopted in its EU's maritime policy blue book (Commission, 2007a), the Commission undertook steps towards EMODNET in order to improve availability of high quality data, which included in 2009 an EU action plan to make progress in this area on the basis of a road map (Commission, 2009). The basic principles underlying EMODNET, which have been formulated by the Commission together with an expert group, include the development of standards across disciplines, data validation and quality control, building on existing efforts (see also Annex: Existing Databases). There is no doubt that this initiative is well suited to help resolve the issue of data fragmentation in the maritime sector, and inclusion of genetic and chemical data relevant to fish species and population structure would therefore also support fisheries control, enforcement and traceability.

Of importance in this respect is also the Data Collection Framework (DCF) under Council Regulation (EC) No 199/2008, establishing a Community framework for the collection, management and use of data in the fisheries sector in support of scientific advice regarding the CFP (European Council, 2008a). This regulation clearly establishes a basis for scientific analyses of fisheries data and provides for the formulation of sound scientific advice for the implementation of the Common Fisheries Policy (see also Annex: Policy Framework). While general biological data are referred to under this regulation, molecular data with relevance to fisheries management are currently not actively collected and compiled by any of the EU member states.

To put the technologies presented in this report on a solid basis for routine use in the context of the European Communities and under the CFP, certain requirements will have to be fulfilled. Firstly, as discussed above, access to data, with standardised formats, must be ensured. Second, a network should be set up, of test laboratories, certified to carry out analysis for control and enforcement purposes, and sharing information, harmonised and validated protocols, Standard Operating Procedures (SOPs), as well as expertise. This does not necessarily mean that new laboratories have to be created: most EU member countries do already have facilities with the necessary capacity. However, at present these laboratories work rather in isolation and on an *ad hoc* basis, upon request by the authorities. Probably, the collaboration between the Danish Technical University (DTU) and the Danish Fisheries Inspectorate, mentioned above, constitutes best practice in the EU today.

Examples for networks of analytical laboratories supporting authorities or legislation exist in the European Union: the Group of European Customs Laboratories (GCL) and the European Network of GMO Laboratories (ENGL).

The GCL provides scientific support to the European Customs Union. This association, to which about 80 European Customs Laboratories adhere, provides the structure for the co-ordination of the EU Member State Customs Laboratories. Several actions support networking amongst the laboratories and aim to rationalise, coordinate and optimise the use of human and technical resources. This also includes inter-laboratory comparison, harmonisation and validation of methods and protocols used by the laboratories. Based on these activities, ultimately an integrated Network of European Customs Laboratories should be built (http://ec.europa.eu/taxation_customs; accessed April 2011).

Another example is ENGL, the European Network of GMO Laboratories. ENGL provides a platform of EU experts involved in the development, harmonisation and standardisation of means and methods for sampling, detection, identification and quantification of Genetically Modified Organisms (GMOs) or derived products in a wide variety of matrices, covering seeds, grains, food, feed and environmental samples. This network of laboratories exists since 2002 and consists of more than 100 national enforcement laboratories, representing all 27 EU Member States plus Norway and Switzerland (http://ihcp.jrc.ec.europa.eu/; accessed April 2011). Moreover ENGL collaborates closely with the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF), which was established by Regulation (EC) No 1829/2003 on GM Food and Feed. The core task of the EURL-GMFF is the scientific assessment and validation of detection methods for GM Food and

Feed as part of the EU authorisation procedure. The European Commission Directorate General Joint Research Centre (JRC) has been given the mandate for the operation of the EURL-GMFF (http://gmo-crl.jrc.ec.europa.eu; accessed April 2011).

The GCL and ENGL networks could serve as paradigms for the building of a similar network for the fisheries sector. Importantly both networks are supported by a sound EU legislative framework, which is crucial for a swift and solid introduction in multinational laboratory networks. A first step is identifying and listing laboratories of all EU member countries having the capacity to carry out such analyses even when applying forensic standards. Some of them may already be supporting control agencies.

Another crucial component of capacity building is training. This is true both for inspectors working in the field who will have to be familiarised with how to take tissue samples for analyses, and also for laboratory personnel and enforcement officers.

Furthermore, information dissemination activities have to be part of capacity building, so that national authorities know where to go and whom to contact to receive expert advice and to carry out analytical work. A central hub for the EU, endowed with this assignment and liaising among the stakeholders would greatly support such an effort. The Community Fisheries Control Agency (CFCA) could potentially be well positioned to assume this role (European Council, 2005).

Also, costs and benefits should be weighted, to objectively assess the value of the discussed techniques and technologies for fishery control and enforcement. Generally speaking, a Cost Benefit Analysis (CBA) is a technique designed to determine the feasibility of a project, plan or policy, by carefully weighing its costs and benefits using a common monetary unit (Pearce et al., 2006). It should provide a valuable reference and tool for stakeholders and policy analysts. Interestingly CBA is an integral part of policies at EU level in the Environmental Protection (European Commission, 2008b), and according to the consolidated version of the treaty establishing the European Community, in preparing its policy on the environment, the Community shall also take account of the potential benefits and costs of action or lack of action (Title XIX – Environment; Art. 174 (European Union, 2006)).

The current steep fall in costs for genetic and genomic technology, especially for DNA analysis, and the examples provided below strongly indicate that the methods discussed in this report are indeed cost effective. The JRC is currently performing a CBA on the use of DNA based technologies for fishery control and enforcement to be published in summer 2011. It contacted more than 80 control authorities and fishery ministries worldwide, and is currently analysing the received data on cases which have been investigated and resolved by using (forensic) DNA analysis. Preliminary results indicate that indeed the benefits of using DNA based analytical technologies for fisheries MCS overcome the operational costs, further emphasizing the value of the technologies presented in this report. Moreover, these technologies feature an added value in that they provide benefits to fisheries management that go beyond control, enforcement and traceability (Waples et al., 2008).

By discussing the state-of-the-art in the fields of genetics, genomics, chemistry and forensics this report illustrates that molecular analytical technologies and forensics can greatly support fisheries control and enforcement as well as the verification of authenticity and origin of seafood in line with traceability 'from ocean to fork'. While many of the technologies are already applied successfully, there does not yet exist a coherent EU-wide and international effort for technology transfer, involving all stakeholders, including control and enforcement bodies, regulators as well as the industry. By providing this reference report, the JRC wishes to initiate and catalyse a focussed and informed dialogue thereby contributing to sustainable fisheries, healthy ecosystems in our oceans and a thriving fisheries industry.

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6. Annexes

6.1 Activities of the Joint Research Centre in the area of control, enforcement and traceability in the fisheries sector

The mission of the Joint Research Centre (JRC) is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology (S&T) for the Union. Close to the policymaking process, it serves the common interest of the Member States, while being independent of special interests, whether private or national. In agreement with its mission, in recent times the JRC has pursued a number of activities in support of improving control, enforcement and traceability in the fisheries sector under the Common Fisheries Policy (CFP) remit and on the international stage:

- During 2008/2009 the JRC assisted the European Commission Directorate-General for Maritime Affairs and Fisheries (DG MARE) to assess (forensic) chemistry and genetics for fisheries control and enforcement and organised and chaired an interdisciplinary workshop (June 2008), at the premises of DG MARE during the preparation phase of the new CFP control regulation No 1224/2009 of 20 November 2009.

- The JRC is Scientific Steering Committee member of the FP7 project *FishPopTrace* (http://fishpoptrace.jrc.ec.europa.eu/), which develops tools based on (forensic) chemistry and genetics for fisheries management, traceability, control and enforcement. The consortium is composed of 16 members from the EU, Norway, Russia and the US and consortium meetings were attended by staff from the European Commission ([11]), the Food and Agriculture Organization (FAO) and of non-governmental organisations (NGOs).

- The JRC pursues a number of projects in support of genetic species identification of fish and fish products and origin assignment, which are supported by public databases. An example is FishTrace (http://www.fishtrace.org), which is exceptional in that a genetic catalogue is associated to biological reference collections of more than 200 commercial marine fish.

- The JRC is member of the ICES ([12]) Working Group for Applied Genetics in Fisheries and Mariculture (WGAGFM), which recently published recommendations for improved traceability in the fisheries sector and for the creation of an EU data hub hosting genetic and other data relevant to fisheries management.

- At the end of 2009 the FAO Fisheries & Aquaculture Department asked the JRC to advise the FAO on the organisation of an international expert workshop on the subject, which was held in December 2009 and chaired by the JRC. The JRC is member of the FAO Forensics Working Group.

6.2 Examples for applications of molecular technologies for control and enforcement in the fisheries sector

The cases depicted in the following cover all crucial aspects related to fisheries control, enforcement and also traceability: species identification, origin assignment and the identification of fish farm escapees.

The examples cover a wide geographical range and various nations, which are Denmark, Germany, Ireland, Norway, Spain, the UK, and the US. They were gathered from sources like press releases, newspaper articles, websites, scientific communications, but also and foremost, through personal communication with stakeholders such as industry representatives, scientists, forensic experts, staff of control and enforcement authorities and fishery ministries.

It is remarkable to what extent genetics are already used and also how efficient applications for fisheries control and enforcement prove to be! Interestingly, a recurrent theme in the evaluation of such technologies is their high cost effectiveness and strong deterrent effect. Repeatedly it was noted by stakeholders using such technologies that defendants, confronted with evidence based on (forensic) genetics in court cases, tend to renounce contesting the charges.

11 Directorate-General for Maritime Affairs and Fisheries (DG MARE) and Directorate-General for Research (DG RTD)

12 International Council for the Exploration of the Sea, one of the major scientific advisory bodies to the European Commission EUROPEAN, C. (2003) Communication from the Commission: Improving scientific and technical advice for Community fisheries management. Official Journal of the European Union C, 47, 5.

While it was foreseen to include other new technologies, such as chemistry and biochemistry, the examples focus exclusively on (forensic) genetics. Applications derived from chemistry and biochemistry have beyond a doubt merit for fisheries control and enforcement. However, during the research it turned out that they seem to be employed much less frequently. This is probably above all due to the swift progress being made in the area of DNA technology, as well as their comparatively straightforward transfer into applications.

6.2.1. Species identification

The unambiguous species identification of fish and fish products is indispensible for any powerful traceability scheme, covering the supply chain from 'ocean to fork' in the fisheries sector (see also ***2. Identification of fish species***). In fact, at least in the scientific realm, species identification, based on DNA analysis, is fully established. The following examples stress on one hand the urgent need for efficient independent control tools on a routine basis in the fisheries sector, and on the other hand that, especially DNA-based technologies, are already widely used for this purpose, even as evidence in court trials.

[1] United States of America
Illegal import and sale of over 10 million pounds of falsely labelled catfish

Between 2002 and 2005 a group of US and Vietnamese fish food companies engaged in a scheme of illegally importing over 10 million pounds of frozen farm-raised catfish (*Pangasius hypophthalmus (Panga Basa)*), worth USD 15.5 million, from Vietnam. The culprits intentionally mislabelled frozen pangasius fillets as sole, grouper, flounder, snakehead, channa and conger pike. The companies attempted thereby to evade duties (anti-dumping tariff) imposed by the US Department of Commerce on those imports, and to sell the product as being derived from a higher value species. Suspicion was raised since US Customs documents indicated that Vietnam had abruptly and significantly increased the export of the above mentioned high value species.

Conviction of the defendants, being accused of re-selling of falsely labelled fish products, was based on the violation of the Lacey Act, according to which the receipt, acquisition or purchase of fish that was taken, possessed, transported or sold in violation of US laws or regulations, is prohibited.

This large scale conspiracy was revealed and investigated in cooperation between diverse US federal agencies such as the NOAA Office of Law Enforcement (OLE), the Customs Border Protection (CBP), the Immigration and Customs Enforcement (ICE) and others. Species identification was undertaken with forensic DNA testing carried out by scientific laboratories such as the NOAA Marine Forensics Department ([a]). The test results were admitted as evidence during the court trials.

The conspiracy consists, as a whole, of several cases, some of which are still under trial and documented by the US Department of Justice. In October 2008 two implied suspects were convicted facing a maximum of 5 years in jail and fines of up to USD 250 000. In May 2009 one of the convicts was sentenced to five years and three months in federal prison – one of the longest sentences ever imposed for this type of crime.

The successful disclosure of this conspiracy illustrates the potential of advanced technologies, when properly integrated into a legal framework and applied in cooperation between control/ enforcement bodies and scientists.

Sources: NOAA Office of Law Enforcement; Fis Worldnews 31/10/08;

Personal communication: Paul Raymond (Special Agent – NOAA Office of Law Enforcement); Linda Park (NOAA National Marine Fisheries Service).

[2] United Kingdom
Vietnamese catfish sold as cod

The striped catfish (*Pangasius hypophthalmus*) is one of 20 types of catfish produced in Vietnam and exported to Britain, where it is also sold in supermarkets as well as fish-and-chip shops. Trading standards officers have recently reported increasing instances of the low value river cobbler being sold as cod.

[a] Charleston Laboratory; NCCOS' Center for Coastal Environmental Health and Biomolecular Research (CCEHBR); NOAA's National Centers for Coastal Ocean Science (NCCOS).

In 2009 such fraud was revealed at a fish bar in the district of Worcestershire. Charges were brought, under the Food Safety Act under which the maximum penalty is GBP 20 000 and/or 6 months in prison. The owner was convicted and had to pay a fine. More prosecutions are in the pipeline in the West Midlands and checks are being carried out at other fish-and-chip shops.

According to Mr John Dell, Head of the Worcestershire Trading Standards Enforcement Department, the true fish species identity was determined by DNA profiling. A second prosecution for a similar offence will shortly go to court and also for this case DNA analysis will be the basis of evidence.

Ms Elisabeth Moran, public analyst of the Worcestershire Scientific Services, which has carried out the analysis in these cases, further explained that the fish species identification was indeed entirely DNA-based. The method employed is accredited to ISO:17025 by the United Kingdom Accreditation Service (UKAS) and generally forensic standards are applied to official samples.

Sources: The Times (13 July 2009)

Personal Communication: Ms E. Moran (Worcestershire Scientific Services; Bewdley Road Stourport-on-Severn; Worcestershire DY13 8QR)

Mr J. Dell (Head of Enforcement Department Worcestershire Trading Standards; Worcestershire County Council County Hall Worcester WR5 2NP UK)

[3] Germany
Uncovering false labelling of fish

Overfishing of traditional commercial fish species in the northern hemisphere has led to a strong increase in the import volume from markets in Africa and Asia. Many species, like Nile tilapia (*Oreochromis niloticus niloticus*) or catfish (*Pangasius spp.*), derive from aquacultures, others from wild fish landed along the West African coastline, North Pacific or the Indian Ocean.

Correct identification and naming of such fish species proves difficult both for wholesalers and for customers, since these exotic species were not part of the traditional fish trade in northern Europe. This, together with the intentional selling of low-value as high-value fish to increase gains, provides a high incentive for mislabelling. Proper declarations are further hampered since in many cases the imported fish has been processed to fish fillets in the country of origin. That these issues are relevant to EU Member States was recently shown in an investigation carried out by the District Office of Hamburg in collaboration with the Institute for Hygiene and Environment (IHU) of the State Hamburg.

Officials from the District Office took samples of a batch of more than four tonnes of fish fillets, declared as 'tropical turbot caught in West African waters' (Spottail spiny turbot (*Psettodes belcheri*) and the Channel flounder (*Syacium micrurum*)). The IHU department for food security analysed the samples by DNA sequencing and comparison to reference sequences of the international DNA sequence database GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html). This analysis revealed that of 22 samples only 5 were correctly labelled; a major part were in fact pangasius fillets and others fillets of perch subspecies from the Indopacific Ocean.

Based on these results the State Consumer Protection Authorities urged the involved firms (a Belgian wholesaler along with two firms from Hamburg) to rectify the goods. Since this request was not followed, the authorities confiscated and destroyed the merchandise. Additionally the affair was transmitted to the prosecution of Hamburg, and Belgian authorities were informed. The outcome of this case is still pending (June 2009).

Sources: Jahresbericht 2007/08 Institut für Hygiene und Umwelt Hamburg Germany; Dr Norbert Hess, Department of Gene-Technology, Institut für Hygiene und Umwelt Hamburg, Germany.

[4] Europe
Added note on catfish imports

In the light of the above mentioned fraud cases in the US and Europe, it is interesting to note that Europe imports significant and increasing amounts of catfish (*Pangasius spp.*), also known as Vietnamese basa or pangasius. Currently, around 225 000 tonnes of catfish per year are consumed in the EU, which implies a consumption of 500 g per inhabitant, equivalent to 2.5% of the total per capita consumption of Community seafood products (19 kg per inhabitant/year). Spain is the main import market in the EU for Vietnamese catfish, with an annual consumption per capita of 858 g.

Spain imported 46 236 tonnes of Vietnamese catfish in 2008, a 28% increase compared to 2007. According to statistics of the Vietnamese Association of Marine Product Producers and Exporters (VASEP), these imports were valued at USD 122 million in 2008, against USD 101 million the previous year.

This caused some concern raised by the Spanish Fisheries Confederation (CEPESCA), because pangasius is displacing more traditional wild and farmed species in the Spanish market like Greenland halibut, pollock, hake, sole, anglerfish, croaker, whiting, trout, seabream and seabass.

Sources: FIS Worldnews (17 June 2009)

[5] United States of America
Illegal shark fin trade

Due to an extensive market for shark fin soup, there is worldwide a great demand for shark fins. 'Shark finning', the process of cutting off the fins of a shark and discarding the body, is a global problem contributing considerably to unsustainable exploitation of shark stocks, thereby putting numerous shark species at risk. Shark finning by vessels in maritime waters under the sovereignty or the jurisdiction of EU Member States, or by vessels flying the flag or registered in EU Member States in other maritime waters, is prohibited by EU law.

In the US, DNA tests are used to uncover and prosecute illegal shark fin traders. In many cases traders violate strict laws protecting shark species (e.g. Endangered Species Act). While, until recently, identifying the shark species that the fins came from was time consuming, a genetic test developed by scientists at the Nova Southeastern University in Florida now greatly facilitates the analytical process.

In late 2003, agents from the NOAA Office of Law Enforcement confiscated about one ton of dried shark fins that a New York City seafood dealer was planning to ship to Asian markets.

Scientists from the laboratory of Dr M. Shivji (Guy Harvey Research Institute at Nova Southeastern University in Florida), working with federal agents, took tiny samples from 21 sets of fins using a quick identification method that uses DNA markers. The test was run after noticing that one of the confiscated bags of fins was labelled 'porbeagle', a shark species that, under federal law, must not be killed in US waters, and another label read 'blanco'. It was suspected that 'blanco' labelled a batch of fins from the Great White shark, another species protected under US law.

The tests positively identified a total of about 230 pounds of fins that came from 7 different prohibited species including dusky sharks, basking sharks and the Great White Shark. NOAA later announced that the seafood dealer had agreed to a settlement of USD 750 000 in the case. It was stressed by the NOAA attorney Charles Juliand that the settlement was possible in great part due to the strength of the DNA evidence.

According to Mr Paul Raymond (Special Agent – NOAA OLE), key to the success of the shark DNA tests is the speed with which enforcement officers can get results. Before the genetic tests, NOAA officials had to send shark samples to the NOAA forensic lab in Charleston, SC, where scientists ran a lipid analysis on the sample of meat for species identification purposes. Getting results could take a month or more and consequently investigators only reluctantly used this option.

With the DNA test, investigators can take a sliver of dried fin, place it into a vial of ethanol and mail it to the analytical laboratory. Results can be ready in about 4 hours, and 2 people can process between 80 and 100 samples in an 8-hour day.

This constitutes a good example of cooperation between academic institutions and control/enforcement bodies. It also stresses the considerable shortening in the 'response time' between inspection/sample acquisition and analytical result, when using DNA-based techniques.

Sources: US Department of Justice – United States Attorney's Office; Los Angeles Times (16.08.2006); The Ledger (13.08.2006).

Personal Communication: Mr Paul Raymond (NOAA Office of Law Enforcement).

[6] Canada
Forensic genetic identification of abalone

Abalone species (*Haliotis spp.*) are exploited worldwide as gastronomic delicacies and are subject to lucrative international trade. Due to its low

reproduction rate and partly excessive exploitation, abalone is nowadays in many areas of the world subject to conservation measures supported by elaborate management schemes which are underpinned by laws and enforcement measures. However, illegal harvesting of abalone frequently occurs and abalone is transported for sale to distant locations. Abalone may thus enter legal marketing channels long after harvesting and distant from the time and place of harvest. Often, product processing impedes morphological species identification and also detection of protected indigenous abalone in local markets may be hindered by mixing them in with imported species of dubious provenance. Dried and frozen abalone meats are sufficiently valuable to merit their use as currency in organised international drug trafficking with the result that much of the illegal product may never enter legal retail trade.

The inability to visually identify abalone meat to species in the absence of the shell impedes enforcement efforts to reduce the illegal exploitation of the world's abalone. Thus, the importation of abalone out of the shell provides an opportunity to 'launder' illegally caught indigenous abalone. Here, as shown in Canada, forensic genetic species identification can be of great value. In Canada, the pinto or Northern abalone (*Helios kamtschatkana*) is listed as threatened under the Canadian Species at Risk Act (SARA), meaning that legal harvest or possession of this species in Canada does simply not exist. Nevertheless, illegal harvest has persisted and enforcement personnel of federal fishery and provincial conservation officers as well as the federal Royal Canadian Mounted Police requested development of genetic methods to enable identification of pinto abalone once the shell had been removed.

With a forensic genetic species identification test, developed by laboratories of Fisheries and Ocean Canada (DFO), the detection of *H. kamtschatkana* among abalone samples collected by British Columbian enforcement officers in investigations was carried out during 2007 in 7 instances.

Interestingly, genetic identification of abalone has not only assisted fishery officers in detecting the species when intermingled with other abalone species, but also provided an incentive to offenders to plead guilty to charges rather than contesting them in court proceedings. This has (with respect to investigation and enforcement efforts) a cost-saving effect and stresses the high deterring potential of modern forensic technologies since the perceived likelihood of detection and conviction is one of the factors influencing the successful use of enforcement as a deterrent in illegal fishing. In one of the investigations conducted in 2007 for which DNA evidence was used, the offenders have pled guilty in lieu of a trial and a fine of CA$ 25 000 was imposed.

In October 2009 the Conservation and Protection Intelligence and Investigation Services Unit from Fisheries and Oceans Canada (DFO) successfully concluded a 3-year multi-country, multi-agency operation involving the illegal sale and possession of Northern abalone, a threatened species under the SARA. In Canada, harvesting this species is strictly prohibited and possession is illegal under the federal Fisheries Act and SARA.

In August 2008 in Richmond Provincial Court, the manager of a Richmond-based seafood company appeared before court for the illegal possession of an unspecified quantity of frozen Northern abalone found mixed with other species of abalone. The offender has pled guilty in lieu of a trial and a fine of CA$ 25 000 was imposed.

In a related case, in June 2009 another company appeared in Richmond Provincial Court. The Judge Jane accepted a guilty plea and ordered a fine of CA$ 35 500, directing CA$ 34 500 of that amount to DFO to promote conservation and protection of Northern abalone through scientific research. The company, which was found in possession of approximately 120 pounds of Northern abalone, was also prohibited from possessing any species of abalone for the next 2 years. These 2 companies were successfully brought to trial following a 3-year multi-country, multi-agency investigation triggered in 2007, in part by an alert regarding suspicious activity supplied by a Canada Border Services Agency (CBSA) inspector. A team of DFO fishery officers spent months unravelling the complex trail of illegally harvested and illegally trafficked Northern abalone. Members of DFO's Conservation and Protection Intelligence and Investigation Services Unit travelled to the United States and Mexico as part of the investigation, which also uncovered a related abalone smuggling operation at the US-Mexico border near Tijuana. The case subsequently involved not only Canadian enforcement agencies – including the CBSA, the Canadian Food Inspection Agency, the Royal Canadian Mounted Police, the Province of British Columbia and DFO – but also led to international collaboration with the National Marine Fisheries Service (NMFS) in California and Washington

State, the State of California Department of Fish and Game, the US Fish and Wildlife Service in California and Washington State, US Custom and authorities in Mexico.

In addition to the above prosecutions and abalone seizures, the investigation led to the discovery of a significant quantity of Northern abalone (around 750 pounds) in the Lower Mainland of British Columbia. The animals were seized and taken out of circulation by DFO Intelligence and Investigation fishery officers and forfeited under court order. Molecular Genetics Research Scientists from DFO's Pacific Biological Station in Nanaimo provided conclusive forensic DNA evidence for the Court that aided in successful prosecutions or guilty pleas on both sides of the border. With the assistance of the DFO Intelligence and Investigation unit and DNA evidence, the NMFS in San Diego successfully obtained a conviction in this operation in September 2009. An individual and a company pleaded guilty to misdemeanour counts under the Lacey Act for the illegal possession of White abalone under the US Endangered Species Act. The individual received a USD 50 000 fine, USD 10 000 of which went into abalone research and education in the United States.

Sources: Personal Communication: Ruth E. Withler. Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC V9T 6N7, Canada.

Supernault et al. (2009). Forensic genetic identification of abalone (Haliotis spp.) of the northeastern Pacific Ocean. Conservation Genetics DOI 10.1007/s10592-009-9925-x.

DFO News Release (30 October 2009) 'A Three Year Multi-Country DFO Investigation Around Threatened Northern Abalone Concludes Successfully'. http://www.dfo-mpo.gc.ca/media/npress-communique/2009/pr23-eng.htm

[7] United Kingdom
Belgian fisherman convicted by DNA test

A Belgian fisherman has been unmasked as a poacher in England after a DNA test of his landing. The Belgian fishing boat moored in the port of Liverpool, when inspectors were controlling the catch of 270 kg of fish. The catch was already cleaned and filleted, so it was impossible to determine immediately which fish species exactly it was. The fisherman claimed it was French or sand sole (*Pegusa lasciaris*), which is comparably cheap and which he was allowed to fish. However, the inspectors doubted this allegation and ordered a DNA test, which proved that it was in fact the much more expensive common sole (*Solea solea*), which is also subject to strict fishing quotas in EU waters. Based on this evidence the fisherman was convicted at the court of Liverpool to pay a fine of EUR 15 000.

Sources: LBActualiteiten; Wekelijkse informatie van de LNV-vertegenwoordiging

Buitenland; Jaargang 14, 23 april 2010; nummer 14 Netherlands.

[8] Ireland
Mislabelling of fish products revealed by DNA analysis

A study carried out by scientists of the University College Dublin revealed a high level of renaming and mislabelling of seafood in Ireland. DNA tests showed that 39 out of 156 (25%) cod and haddock products, randomly sampled from supermarkets, fishmongers' shops and take-away restaurants throughout Dublin, were genetically identified as entirely different species from that indicated on the product labels, and therefore were considered mislabelled under EU regulations. More significantly, 28 out of 34 (82.4%) smoked fish samples were found to be mislabelled.

Sources: Miller, D.D. and S. Mariani (2010). 'Smoke, mirrors, and mislabelled cod: poor transparency in the European seafood industry'. Frontiers in Ecology and the Environment doi:10.1890/090212: 14.

[9] Fish or Fake?
DNA test to reveal authenticity of restaurant chain product

On behalf of the newspaper Miami Herald the laboratory of Professor Mahmood Shivji (Guy Harvey Research Institute at the Nova Southeastern University in Florida – see also 'Illegal shark fin trade'), set out to apply a DNA test so as to examine the authenticity of the contents of a fish sandwich sold by the restaurant chain McDonald's. Motivation for this initiative was apparently ongoing rumours that the product in question did not contain any fish at all.

However, DNA extraction and sequencing revealed unambiguously that the sandwich was topped with fish, namely Alaska Pollock (*Theragra chalcogramma*). According to Prof. Shivji no traces of any other animal species, such as pig, could be found.

Sources: The Miami Herald 24.08.2009.

[10] DNA Barcoding used to reveal mislabelling I: USA

DNA-barcoding is used to identify unambiguously species by analysis of a very short genetic sequence from a standard part of the genome the way a supermarket scanner distinguishes products using the black stripes of the Universal Product Code.

The gene region that is being used as the standard barcode for almost all animal groups, including fish, is a 648 base-pair region in the mitochondrial *cytochrome c oxidase I* gene ('COI'). DNA-barcoding can easily be applied, even on processed fish products.

This approach was recently used by graduate students of the Trinity School in Manhattan to test 60 samples of seafood taken from New York sushi restaurants and seafood markets, for their authenticity.

They sent the samples off to the University of Guelph in Ontario, where a laboratory participating in the Fish Barcode of Life project (Fish-BOL; http://www.fishbol.org) carried out the genetic analysis. The samples were compared to the global barcoding library, meanwhile containing 44 237 barcodes representing nearly 7 000 fish species (July 2009).

The finding was that one fourth of the fish samples with identifiable DNA were mislabelled. For example, a piece of sushi was sold as the highly priced white tuna (*Gymnosarda unicolor*) but turned out to be Mozambique tilapia (*Oreochromis mossambicus*), a much cheaper fish that is often raised by farming. Roe supposedly from flying fish (*Exocoetus spp.*) was actually from smelt (*Osmerus spp.*). Seven of nine samples that were called red snapper (*Lutjanus purpureus*) were mislabelled, and they turned out to be anything from Atlantic cod (*Gadus morhua*) to Acadian redfish (*Sebastes fasciatus*), an endangered species.

This example is included here since, apart from showing how widespread false labelling of fish products apparently is, it clearly illustrates the ease with which revealing DNA tests can today be accomplished (here by students, who did not even specialise in biology!). Although the testing technique is at the forefront of research, anyone can take advantage of it by sending samples off to a laboratory, making it an ideal, and due to the low DNA sequencing costs, highly cost-effective tool for fisheries control authorities!

Additionally, there are plans to develop a hand-held barcode reader that will 'read' the species identifying DNA sequence from any tiny piece of tissue. An inspector onboard a vessel, or at a seaport, could insert a tissue sample containing DNA – a snippet of a fin – into the device, which would detect the sequence of nucleic acids in the barcode segment. This information would be relayed remotely and instantly to the reference database, a public library of DNA barcodes, which would respond with the specimen's name, photograph and description.

Sources: Scientific American Magazine 02.10.2008; The New York Times 22.08.2008; FIS Worldnews 12.05.2009.

[11] DNA Barcoding used to reveal mislabelling II: Canada

During 2008/2009 an extensive study was carried out at the University of Guelph, Canada, under the supervision of Professor R. Hanner, Associate Director of the Canadian Barcode of Life Network, on 500 samples supposed to be derived from highly priced fish. About one fourth of the samples taken from restaurants, supermarkets and fish markets from every region tested was either of a cheaper species, such as tilapia or farmed Atlantic salmon, or mislabelled as pricier species like white tuna, red snapper or wild Pacific salmon. Fillets labelled as Mediterranean red mullet could be mislabelled spotted goatfish and fish marketed as Alaskan halibut might be the endangered Atlantic halibut.

Sources: FIS Worldnews, 13 November 2009; Montreal Gazette, 21 October 2009.

[12] DNA analysis and barcoding used to reveal false labelling of tuna sushi

In an attempt to compare DNA analytical approaches for species identification and to improve DNA

barcoding for the identification of all tuna species of the genus *Thunnus*, a study was carried out in a collaboration of the American Museum of Natural History and Columbia University, New York. Sixty-eight samples of tuna sushi were purchased from 31 restaurants in Manhattan (New York City) and Denver, Colorado. According to the experimenters a piece of tuna sushi has the potential to derive from an endangered species, a fraudulent activity, or can pose a health hazard and all three of these cases were uncovered in this study. Nineteen restaurant establishments were unable to clarify or misrepresented what species they sold. Five out of nine samples sold as a variant of 'white tuna' were not albacore (*T. alalunga*), but escolar (*Lepidocybium flavorunneum*), a gempylid species banned for sale in Italy and Japan due to health concerns. Nineteen samples were northern bluefin tuna (*T. thynnus*) or the critically endangered southern bluefin tuna (*T. maccoyii*), though nine restaurants that sold these species did not state these species on their menus.

Sources: Lowenstein, J.H., Amato, G. and Kolokotronis, S.-O. (2009) 'The Real maccoyii: Identifying Tuna Sushi with DNA Barcodes - Contrasting Characteristic Attributes and Genetic Distances'. PLoSOne 4(11) e7866.

[13] Spain
Fisheries control and enforcement based on DNA technology

According to the Spanish General Secretariat of the Sea (Secretaria General del Mar), molecular genetic techniques will be applied for fisheries control in the near future, in particular to control for correct labelling of fish products. Currently, pilot projects are launched with voluntarily participating fishing companies, which supply fish specimens for analytical purposes. These samples are sent to commercial laboratories for DNA sequencing and analysis.

Also, the technical and analytical service department of ANFACO-CECOPESCA (the Spanish National Association of Fish and Fish Product Manufacturers) receives samples of fish products from companies of the fish and seafood processing industry for authenticating purposes of raw material or final products. This service is also consulted by the Frontier Inspection Point (PIFs – Puestos de Inspección Fronteriza), which delivers samples for genetic analysis. However, currently these analyses requested by PIFs are not carried out in a routine way. Only certain batches of fish or other groups of marine species are analysed. The authentication is carried out using DNA analysis.

For analytical purposes, and as a depository for reference DNA sequences, the Department of Molecular Biology and Biotechnology of ANFACO-CECOPESCA hosts a DNA database covering a wide range of marine species (fishes, cephalopods, bivalves ...). This database is only used internally by personnel of this department.

ANFACO-CECOPESCA was involved in court cases where DNA analysis was used to authenticate seafood products but reports referring to the trials are confidential and cannot be disclosed.

Sources: Personal Communication: M. Isabel Parra Sánchez, Jefe de Area Gestión Actividad Pesquera, S. G. de Asuntos Pesqueros Comunitarios, Secretaria General del Mar, Madrid, Spain; Montserrat Espiñeira Fernández, Área de Biología Molecular y Biotecnología, ANFACO-CECOPESCA, 36310 VIGO, Spain.

[14] United Kingdom
Forensic Genetics in support of fisheries management

In the UK the forensic genetic analysis provider TRACE Wildlife Forensics Network (http://www.tracenetwork.org) is consulted to analyse samples of fish (products) genetically with increasing frequency.

Clients include organisations providing sustainable fishery certificates and 'eco-labels'. Such certificates confirm that a given fishery adheres to defined and internationally recognised standards ensuring the sustainable exploitation of stocks. A fishery is only endowed with a certificate and label after an elaborate assessment scheme has been applied. As these certificates confer a positive image with respect to product quality and environmental friendliness, they add value to the products on the market and they currently increase rapidly in popularity. On the downside, such an approach incites for fake imitations, which is why any such certification scheme relies on efficient and independent control methods.

Forensic genetics are very valuable in this respect. Existing DNA analytical technologies (such as DNA barcoding – see above) are applied following

forensic criteria and Standard Operating Procedures (SOPs). This approach ensures the authenticity of the certificates and facilitates the recognition by national authorities or as evidence in court cases, in case of non-compliance.

Sources: Personal Communication: Dr Rob Ogden, Director, TRACE Wildlife Forensics Network, Edinburgh, UK; Marine Stewardship Council at http://www.msc.org.

6.2.2. Origin assignment

As discussed in ***3. Origin assignment of fish***, the assessment of where fish and fish products come from is a substantial component of any traceability framework in the fisheries sector. This is much more challenging than species identification (see above) since it has to be ensured that exploited stocks are identifiable by some means. Only then traceability in the sense of the 'ocean to fork' approach can be realised. Therefore, first a baseline has to be created, meaning that for any species of interest in the context of fisheries control and enforcement, genetic or other data has to be available that reveals the existing population structure. This will allow performing origin assignment tests. It is probably rather these more complex requirements explaining the small amount of examples for origin assignment than less frequent fraud cases linked to false origin declarations. However, despite these challenges, there exist already impressive examples where marine fish (as well as *cetaceans* and *bivalves* – see below) have been traced back to their origin.

[15] Denmark
Conviction of a fisherman claiming a false origin of cod

More than seven tonnes of large cod were landed in the Baltic Sea region by a North Sea fisherman. According to the declaration of the logbook, the cod had been caught in the Baltic Sea but Danish Inspectors from the Danish Directorate of Fisheries suspected upon visual examination that the fish looked like North Sea cod rather than Baltic Sea cod. Also, the poor quality of the fish did not match well with the reported time of catch.

Therefore, the inspectors delivered five cod specimen to the Danish Institute for Fisheries Research (DIFRES) for genetic analysis.

The scientists performed a genetic origin assignment test on the delivered samples and all five cod were assigned to the North Sea.

In the following court trial the fisherman was convicted in 2006 to pay a fine of DKK 50 000 and the catch, having a value of DKK 155 400 was confiscated. The DNA test was considered an important element of the evidence and scientists gave testimony as expert witnesses.

This example shows that in Europe advanced techniques, in this case genetic marker analysis, are used for control and enforcement in the fisheries sector and that evidence produced by these techniques is admitted in court trials in EU member countries. More importantly, it also shows that advanced techniques cannot only be used efficiently for species identification but in addition for the more challenging question of origin assignment.

According to Mr Bonde Erikson, from the Danish Directorate of Fisheries, presently there are similar new cases subject to court proceedings, such as sprat caught in the North Sea, but claimed to be caught in the Baltic Sea. He confirmed to update the JRC on this case as soon as there are new developments (Personal communication, December 2008).

Sources: Personal Communication: Lars Bonde Erikson (Danish Directorate of Fisheries; Inspectorate of Fisheries); Dr Einar Eg Nielsen (Technical University of Denmark).

[16] Denmark
A paradigm for collaboration between national authorities and academic institutions

As illustrated by the above depicted example, Denmark is, compared to most other EU Member States, in an advanced state with respect to the use of DNA-based technologies for fisheries control. Remarkably, the Technical University of Denmark (DTU) and the Danish Fisheries Inspectorate have meanwhile established a close collaboration aiming at fully integrating molecular genetics into the Danish fisheries control scheme.

The DTU supports the inspectorate with training sessions for inspectors, providing introductions to genetic analysis for species and origin deter-

mination, as well as instructions on how to take and handle tissue samples. Since 2009 inspectors are equipped with small tool boxes, containing the necessary equipment to operate with tissue samples for DNA analysis, as well as an instruction manual. In case inspectors suspect illegal activities, and do rely on evidence based on DNA analysis, they take tissue samples and send them for further analysis to the DTU National Institute of Aquatic Resources.

Moreover, if it comes to criminal prosecution, scientists of the Institute of Aquatic Resources can appear as expert witnesses before the court.

Sources: Personal Communication: Mr Lars Bonde Erikson (Danish Directorate of Fisheries; Inspectorate of Fisheries); Dr Einar Eg Nielsen (Technical University of Denmark).

[17] Finland
Individual origin assignment in a fishing competition fraud

In June 1999, a 5.5 kg salmon was presented to the judges of a local fishing competition in Finland. Based on visual inspection, suspicion arose that the salmon may not have been caught in Lake Saimaa, as claimed by the fisherman. In order to set a precedent for future competitions, the organisers were interested in conclusively ascertaining that the suspect fish did not come from the claimed geographical origin. To press criminal charges against the alleged offender, tissue samples were submitted for genetic analysis. Genetic origin assignment showed that the probability of the suspect salmon originating from one of the regions that supply most of Finland's fish markets was found to be over 600 times higher than it originating from the declared origin. When confronted with this evidence, the offender confessed that he had purchased the salmon at a local shop.

As in the former Danish case this example emphasises the potential practical application of an origin assignment procedure. It shows that such a strategy can be used, for example, in suspected cases of illegal poaching, in order to assign or exclude individuals from originating from a claimed population.

Sources: Primmer C.R., Koskinen M.T., Piironen J. (2000) The one that did not get away: Individual assignment using microsatellite data detects a case of fishing competition fraud. Proceedings of the Royal Society - Biological Sciences (Series B) 267: 1699.

[18] Canada
Support to fisheries control and enforcement based on genetics

The molecular genetics laboratory at the Pacific Biological Station of Fisheries and Oceans Canada (DFO), a governmental institution, processes thousands of salmon samples each year for Pacific salmon conservation and management issues. Canada now manages many of its mixed-stock domestic and international Pacific salmon fisheries on a real-time or post-season basis using microsatellite DNA estimates of stock composition of the catch.

As a 'by-product' of this remarkable management scheme, the laboratory also performs genetic species and stock origin identification on Pacific salmon tissue samples for enforcement officers pursuing charges of illegal harvest or sales of salmon. Dr Ruth Withler, the laboratory head, has been qualified as an expert witness and testified in provincial court (British Columbia) for these cases.

The laboratory has provided genetic analyses for several hundred investigations conducted by fishery officers since about 1998. Interestingly, in most cases, the defendants plead guilty or at least 'admit' the DNA evidence (meaning that they accept the results and waive their right to cross examination on that topic). The fishery officers claim that having the DNA evidence in their favour greatly increases the number of cases in which a guilty plea is entered to charges – saving the government the costs of trials. In addition, it saves the fishery officers a lot of time that they would otherwise have to spend in court appearances.

Sources: Personal Communication: Ruth E. Withler. Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC V9T 6N7, Canada.

[19] Norway
Traceability scheme based on genetics covering the Mink whale fishery

Introduction and statement

This example is included as 'proof of principle' as it demonstrates convincingly the power of genetics when integrated into a stringent traceability scheme in the sense of 'ocean to fork'. The example is solely provided to exemplify the use of DNA technology, regardless of the animal species it is applied to. However, due to the ongoing political (and scientific) debate surrounding the whale fisheries, the following introductory paragraph is indicated so as to avoid any misunderstanding.

The European Commission's position on whaling is unambiguous as are the regulations for EU waters. Whaling in the European Union is not allowed in EU waters. Under EU environmental law all whale species are protected within EU waters. Exceptions are aboriginal peoples whaling for their subsistence – as allowed under the International Whaling Commission (IWC) Convention – provided it falls within the confines of catch limits based on scientific advice. The Commission calls on the members of the IWC to fully adhere to the 1986 whaling moratorium. All whaling operations such as scientific whaling by members of the IWC must remain in the control of the IWC. The Commission asks all EU Member States to uphold their strong stance against whale hunting and forge a common front to counter attempts by some countries to undermine conservation efforts.

Norwegian whalers exploit minke whales (*Balaenoptera acutorostrata*) in three stock areas in the North Atlantic: off West Greenland, in the Central Atlantic (Denmark Strait, Iceland, Jan Mayen) and in the Northeast Atlantic, the latter having been the most important one. Norwegian commercial whaling was halted after the 1987 season to wait for an assessment by the IWC to be undertaken by 1990. However, in 1993 commercial whaling for minke whale was re-established by the Norwegian government based on a Revised Management Procedure (RMP) developed by the International Whaling Commission (IWC) Scientific Committee. Over the period from 1993 to 2007, the annual catch of minke whale has varied from 218 to 647 animals (about 0.5% of the total estimated population size).

The management of minke whale is based on the application of the RMP. The RMP is a rule for setting catch quotas based on historical catches and abundance estimates. Quotas are set as five-year block quotas. The abundance estimates are based on dedicated sightings surveys and a methodology accepted by the IWC Scientific Committee. In 1995, the total estimate of minke whale abundance in the areas where Norwegian whaling takes place was 118 300 whales. From 1996 to 2001, the Northeast Atlantic was covered by annual surveys, producing a total estimate for the covered area of 107 200 minke whales. A new abundance estimate was scheduled for 2008.

Genetic analysis for Norwegian minke whale fishery monitoring and control

The Norwegian whaling activity is monitored and controlled by using a combination of specific genetic markers to identify every single whale captured under the Norwegian quota since 1996. Each individual whale is analysed independently twice and recorded in a national database managed by the Institute of Marine Research (IMR). This approach allows total control over all individuals captured in the last 12 years. Inspectors can go to a local market place and identify where a piece of meat was captured, the sex of the animal, date and time of capture, boat, GPS position etc. Any illegal attempt to catch whales is thereby easily revealed, which results in very strong determent.

The IMR (an academic research institution) controls the whale samples and the capture data, and carries out all genetic analysis to produce data for the database which is formally held by the Norwegian Directorate of Fisheries, the authority responsible for fishery implementation and enforcement in Norway.

This database is also very important considering that Norway exports small amounts of minke whale to Japan. Each package of meat dedicated to export is actually genotyped both in Norway and Japan. All meat for the Norwegian domestic market is genotyped at the end of the catch season, and control checks can be carried out by the authorities in the markets to ensure compliance with the rules. Interestingly, the database not only supports a control and enforcement scheme, but also a mark recapture population estimate approach (additional to genetic mark recapture from biopsy samples collected during research cruises). This can be used for detailed population genetic studies in support of conservation efforts.

Sources: Personal Communication: Dr Kevin Glover, Institute of Marine Research (IMR), Bergen, Norway; European Commission Press Release IP/08/896 Brussels, 5.06.2008; Norwegian Ministry of Fisheries and Coastal Affairs at http://www.fisheries.no/marine_stocks/mammals/whales/marine_stocks_marine_mammals_whales.htm.

6.2.3. Identification of farm escapees

According to the latest Food and Agriculture Organization (FAO) report on the state of world fisheries and aquaculture, aquaculture continues to be the fastest growing animal food-producing sector, with a per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.8 kg in 2006, an average annual growth rate of 7%. It is set to overtake capture fisheries as a source of food fish. From a production of less than 1 million tonnes per year in the early 1950s, production in 2006 was reported to be 51.7 million tonnes with a value of USD 78.8 billion.

A major challenge with cage-rearing fish in the marine environment is containment. Farm escapees pose a significant threat to the surrounding ecosystem, since they can reproduce with wild populations thereby potentially diminishing the overall fitness. Such damaging genetic introgression from domesticated fish is also a problem in Europe as shown by examples in Norway and Ireland.

According to official statistics from the Norwegian Directorate of Fisheries, the number of farmed escaped salmon in Norway has varied between 260 000 and 715 000 fish per year in the period from 2001 to 2005 (http://www.statistics.no) – probably a rather conservative estimate. The following examples show that DNA analysis can be used to monitor farm escapees, but also to support enforcement measures in case of non-compliance with existing laws concerned with fish farming.

[20] Norway
Genetic assignment used to identify farm escapees

Recently in Norway genetic assignment was being used to identify the farm of origin for escaped Atlantic salmon (*Salmon salar*).

Following reports by local fishers of escaped farmed salmon in Romsdalfjord, western Norway, samples were collected from 16 cages located on 7 operational farms (see ***Figure 1***). These baseline samples, in addition to 29 sampled farm escapees, were screened for 15 genetic markers (microsatellite loci), to be used to distinguish farmed from wild animals.

Assignment based on this genetic data indicated that 21 of the 29 escapees originated from a single farm in Romsdalfjord. In addition, the data were used to acquit the other six farms operating in the fjord from being the source of the escapement. This study can provide governing bodies with a framework upon which to develop a management tool to trace farmed escaped salmon.

While in Norway it is not an offence for farmers to lose fish from an aquaculture installation unless the farm fails to comply with regulations, farmers are legally obliged to immediately file a report of escapement, or even suspicion of escapement, to the Norwegian Directorate of Fisheries (FKD, 2004). In this case, no reports were filed despite the Directorate contacting all fish farms operating in the area. Upon completion of the analyses, genetic data were presented to the Norwegian Directorate of Fisheries and the Norwegian National Authority for Investigation and Prosecution of Economic and Environmental Crime. However, staff from the company operating the farm acknowledged, before the conclusion of the DNA analysis, that there had been an incident with the cage to which most of the escapees were traced, which is consistent with the results of the study.

Recently in Norway there had been an additional legal investigation on farm escapees, which resulted in a NOK 450 000 fine imposed by the Norwegian police as a direct result of genetic analyses. Interestingly, in this case a part of the fine was used as economical compensation for the genetic analyses conducted by the laboratory facilities. This is an important point with regards to costs and benefits inherent to the use of new

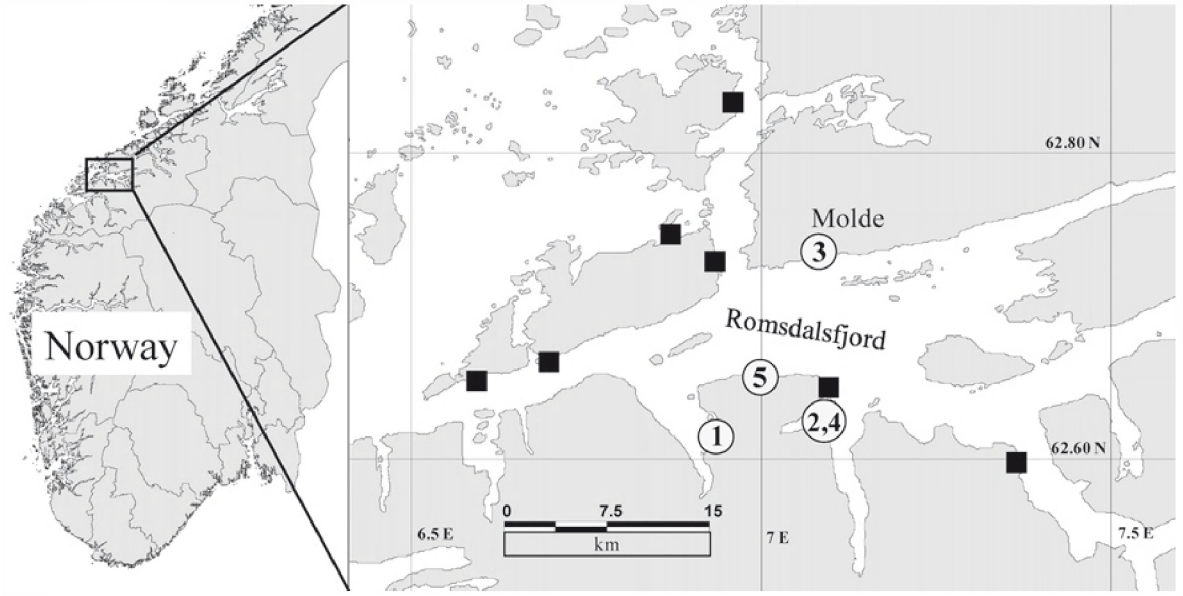

Figure 1. Identification of salmon farm escapees with genetic analysis in Norway. *Location of the farms (black squares) from which baselines samples were collected, and location of the five fishers who recaptured the escapees (the fisher numbers in circles). (With kind permission of K.A. Glover et al. (2008))*

technologies for fisheries monitoring, control and surveillance (MCS). The above described genetic assignment method is also established for rainbow trout and cod, and it appears that the approach applied is universally applicable (and is likely to be so worldwide).

Sources: Glover, K.A., Skilbrei, O.T., Skaala, Ø. Genetic assignment identifies farm of origin for Atlantic salmon Salmo salar escapees in a Norwegian fjord (2008) ICES Journal of Marine Science, 65 (6), pp. 912-920.

Personal Communication: Dr Kevin A. Glover. Institute of Marine Research, PO Box 1870, Nordnes N–5817, Bergen, Norway.

[21] Ireland
Genetic distinction of 'farmed' versus 'wild' salmon used as evidence during a court trial

The Food Safety Authority of Ireland (FSAI) recently brought a seafood company to court for alleged breaches of the Food Safety Authority of Ireland Act, 1998 (www.irishstatutebook.ie/1998/en/act/pub/0029/index.html). At the core of the charges was the claim that the company had misleadingly labelled salmon as 'Irish smoked wild salmon', while it had been bred and raised in cages to the point of harvesting.

Dr Eileen Dillane and Dr Phil McGinnity, experts in fish genetics from the University College Cork (UCC), were invited as expert witnesses during the court trial. Dr Dillane carried out DNA tests on samples from the fish randomly taken by FSAI compliance officers. The test revealed that 22 of the 24 packets examined contained individual fish of the Fanad farm strain (one of the other two packets containing a replicate of the 22 individuals, the other yielding no DNA), a salmon derived from Norwegian salmon and very different genetically to wild Irish salmon.

Dr McGinnity gave evidence that fish could escape from a fish-farm into the wild but regular inspections of commercial catches show between about 4 to 8 farmed fish per 1 000 wild are appearing in hauls. The chances of every one of the 22 fish being of the Fanad strain if they had been caught at sea were 'one in many millions'. Asked about a possible 'catastrophic event' where many thousands of fish might escape from a farm just as a trawler was passing and then caught at sea, he said no major escape had been reported in recent years and farms were obliged to report such escapes to renew their licences.

The Judge was satisfied that the company in question had offered for sale packets of salmon labelled 'Irish smoked wild salmon' which contained in fact salmon that had been bred and raised in cages to the point of harvesting and had been owned by someone.

However, despite the genetic evidence provided, ultimately the charges against the company where dismissed due to doubts whether the salmon was indeed farmed according to the definitions laid down in the Act. Additionally, referring to European Council Regulation 1198/2006 on the European Fishery Fund, the judge said the FSAI had not proved salmon bred and grown 'generally' in Irish 'fish farms' were cultivated using techniques that would identify them as 'farms' under the terms of this regulation.

Sources: Personal Communication: Dr Phil McGinnity, Department of Zoology, University College Cork, Ireland. Irish Times 25.03.2009 (www.irishtimes.com/newspaper/ireland/2009/0325/1224243368353.html)

FIS Worldnews 26.03.2009.

6.3 The existing policy framework with relevance to fisheries control, enforcement and traceability

1. COUNCIL REGULATION (EC) No 1224/2009 of 20 November 2009 establishing a Community control system for ensuring compliance with the rules of the common fisheries policy, amending Regulations (EC) No 847/96, (EC) No 2371/2002, (EC) No 811/2004, (EC) No 768/2005, (EC) No 2115/2005, (EC) No 2166/2005, (EC) No 388/2006, (EC) No 509/2007, (EC) No 676/2007, (EC) No 1098/2007, (EC) No 1300/2008, (EC) No 1342/2008 and repealing Regulations (EEC) No 2847/93, (EC) No 1627/94 and (EC) No 1966/2006.

Official Journal of the European Union L 343 (22.12.2009)

In 2008 the European Commission and the Court of Auditors had identified major weaknesses in the existing control system applicable to the Common Fisheries Policy (CFP) and, in response, the Commission set out to prepare a proposal for a CFP control reform which was accompanied by a public consultation open to all interested stakeholders. End of 2008 the European Commission published the 'Proposal for a Council Regulation establishing a Community control system for ensuring compliance with the rules of the Common Fisheries Policy' [COM(2008) 721 final]. In this document 'genetic analysis' as a tool in support of traceability was explicitly mentioned (Article 35). Moreover, in the accompanying Impact Assessment [Brussels, Sec(2008) 2760/2] the project FishPopTrace, in which the Joint Research Centre (JRC) participates as steering committee member, was mentioned as a valuable project:

'*It is interesting to note the innovative contribution of the FishPopTrace Consortium, a mixed association financed under the EU Seventh Framework Programme, drawing the attention to the development and implementation of modern technologies such as biotechnology, genetics, chemistry and forensics in optimizing control mechanisms*'. [p.57]

'*Another interesting proposal on this objective was made by the above mentioned mixed FishPopTrace consortium. They suggested the development of framework incorporating strict forensic validation based on molecular biology to complement the Monitoring, Control and Surveillance in the fisheries sector. It was underlined that these applications are available but there is still a lack of cooperation between scientific institutions, control authorities and policy makers. An example of a project working in the area was given- The Global Fish Barcode of Life Initiative for identification of fish species from sample of tissue or fish products on the basis of DNA sequence library*'. [p.62]

Meanwhile the new regulation is implemented. It proposes a restructuring of the Community fisheries control system. The new system provides for inspections all along the production chain and in Article 16 an explicit reference to 'traceability tools such as genetic analysis' is made. Moreover, the use of the satellite-based vessel monitoring system, electronic logbooks and electronic notification of catch data for control is part of the regulation. The powers of the national fisheries inspectors will be extended and dissuasive sanctions will be harmonised. The new Regulation provides for sanctions with regard to Member States that do not comply with the rules of the CFP and the introduction of a fishing permit with a penalty point system for infringements committed. This Regulation also proposes an improvement in cooperation among Member States for the management and communication of control data by means of secure national websites providing remote access for the Commission.

2. Council Regulation (EC) No 1005/2008 of 29 September 2008 establishing a Community system to prevent, deter and eliminate illegal, unreported and unregulated fishing, amending Regulations (EEC) No 2847/93, (EC) No 1936/2001 and (EC) No 601/2004 and repealing Regulations (EC) No 1093/94 and (EC) No 1447/1999.

The 'IUU Regulation'; Official Journal of the European Union L 286, 29.10.2008, p. 1–32

As described in the introduction, illegal, unreported and unregulated (IUU) fishing constitutes an imminent threat to the sustainable exploitation of living aquatic resources. It therefore jeopardises the foundation of the Common Fisheries Policy and impedes international efforts to improve ocean governance. To counteract IUU fishing, the 'IUU regulation' provides for the reinforcement of surveillance of activities at sea, to identify IUU operators, to enhance the implementation of fisheries legislation and to improve the application of sanctions in the event of infringements. This Regulation represents one of the first steps towards an integrated maritime policy. It is part of the Community Action Plan against IUU fishing adopted in 2002 and in line with action carried out at an international level (FAO IPOA-IUU (Food and Agriculture Organization International Plan of Action to Prevent, deter and Eliminate Illegal, Unreported and Unregulated Fishing) – see below) intended to prevent, discourage and eradicate IUU fishing.

One of the central features, however, and with acute relevance to this document is that the regulation foresees to allow access to the European Union (EU) market only to fishery products that comply with the rules, as attested by a compulsory accompanying catch certificate. The catch certificate, issued by the flag State, guarantees that products imported into the EU do not originate from IUU fishing, and accompany fishery products throughout the supply chain to facilitate checks. Obviously the technologies described in this report can be applied as independent control tools to verify and validate the catch certificates. This would help to uncover infringements, support prosecution and also have a deterring effect.

3. International Plan of Action to Prevent, Deter and Eliminate Illegal, Unreported and Unregulated Fishing

IPOA-IUU, FAO 2001

The IPOA-IUU was developed as a voluntary instrument, within the framework of the Code of Conduct for Responsible Fisheries, in response to a call from the Twenty-third Session of the Committee on Fisheries (COFI). A draft text for an IPOA-IUU was elaborated at an Expert Consultation in Sydney, Australia, in May 2000. This document formed the basis for negotiations at Technical Consultations that were held at FAO Headquarters, Rome, in October 2000 and February 2001. The IPOA-IUU was adopted by consensus at the Twenty-fourth Session of COFI on 2 March 2001 and endorsed, also by the EU, by the Hundred and Twentieth Session of the FAO Council on 23 June 2001.

4. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

Official Journal of the European Union L 286, 29.10.2008, p. 1–32

Regulation (EC) 178/2002 is currently the core EU legislative document with respect to food safety and traceability. It lays down general principles and requirements of food law, and establishes the European Food Safety Authority (EFSA). This regulation sets procedures regarding food safety and refers explicitly to traceability as a means to ensure safety of food and consumer protection. In Article 3 traceability is defined as ***'the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution***'.

Article 18 further delineates how traceability should be ensured, which is summarised in the following:

1. Traceability must be established at all stages of production, processing and distribution.

2. Business operators must be capable of identifying any person from whom they have been supplied and have to have systems in place which allow for this information to be made available to authorities on demand.

3. Business operators must be capable to identify to which business entity their products have been supplied and provide this information to authorities on demand.

4. Point 2 and 3 support and ensure traceability throughout the market chain by the so called 'one-step-back one-step-forward' approach.

5. Food or feed which enters the market chain within the Community must be adequately labelled or identified to facilitate its traceability. This should be ensured through relevant documentation or information in accordance with specifications provided in Article 58(2).

While not explicitly referring to fish and fish products, these are covered by Regulation 178 since it implements rules about food and feed in general. Importantly it intends to ensure traceability by enacting every operator along the market chain to identify the predecessor and successor.

In Article 17 this regulation defines the distribution of responsibilities to ensure the proper implementation of the rules laid down:

Firstly, food and feed business operators are obliged to ensure at all stages of production, that food or feed satisfy the requirements of food law that are relevant to their activities.

Secondly, the EU Member States monitor, control and enforce the food law, and survey that the business operators fulfil their respective obligations.

For that purpose, they must maintain a system of official controls and food and feed safety surveillance and other monitoring activities covering all stages of production, processing and distribution.

Additionally, Member States lay down the rules on measures and penalties applicable to infringements of food and feed law, which have to be effective, proportionate and dissuasive. Interestingly these latter specifications are analogous to the Common Fisheries control and enforcement scheme, where infringement penalty measures are also introduced at Member State level, leading to a highly heterogeneous enforcement landscape in the European fisheries sector.

The legal document complementing Regulation 178 is Regulation (EC) 104/2000, which establishes that commercial name, production method and catch area or country of production on each product are required for labelling. Regulation 104/2000 was subsequently extended by Regulation (EC) 2065/2001, adding more specific details to the final information given to the customer in terms of production method and geographic origin. The labelling information has to be given at every step of the production and retailing chain.

The current legal framework for traceability in the European Union relies essentially on labelling of goods and the accompanying documents serving as certificates. It is therefore to a vast extent dependent on primary information provided by the producer and other stakeholders involved in the production and supply chain.

This is why, to ascertain reliable and proper control and enforcement schemes, independent validation methods are indispensable. Labels do provide authorities and consumers with information about the product, but as shown in numerous examples this information is prone to counterfeit. There DNA Analysis, Chemistry and Forensics for Species Identification, Origin Assignment and Traceability of Fish and Fish Products can provide invaluable support.

5. COUNCIL REGULATION (EC) No 199/2008 of 25 February 2008 concerning the establishment of a Community framework for the collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy.

Official Journal of the European Union L 60, 5.3.2008, p. 1–12

A centralised data-hub, hosting or at least linking to the available data necessary to support an EU-wide approach for fisheries control, enforcement and traceability along the supply chain is currently not available. Rather,

such data emerges from fundamental research projects, is published in aggregated form in peer reviewed journals, or in local unaccessible databases, is highly dispersed, prone to loss and therefore not useful to control authorities. The ICES WGAGFM ([13]) has recently proposed measures to create a EU metadatabase to host scientific data relevant to fisheries management, but there is no short-term solution in sight (WGAGFM, 2007). It should in the meanwhile be explored whether and to what extent the upcoming data collection framework directive could help to fight the dispersal and loss of scientific data for fisheries control and enforcement.

This Data Collection Framework Directive establishes a Community framework for the collection, management and use of data in the fisheries sector also to support the necessary scientific advice for the implementation of the Common Fisheries Policy (CFP). This Community framework is the result of an extensive consultation with Member States, national scientific institutes responsible for data collection and key end-users, such as the International Council for the Exploration of the Sea (ICES). It is part of the EU's integrated maritime policy and replaces the Community framework implemented in 2000 with effect from 1 January 2009.

The biological, technical, environmental and socioeconomic data relates to fleets and their activities as well as catches and the impact of fishing activities on the marine ecosystem. The biological data concerns all fishing activities, such as commercial fisheries, recreational fisheries, economic data relating to fishing and aquaculture activities and industries processing fisheries products. Multi-annual national and Community programmes, adopted for a period of three years, constitute the framework for the collection, management and use of data. The European Commission, assisted by the Committee for Fisheries and Aquaculture, establishes the Community programmes, which constitute the basis for the EU Member States to develop national programmes, consisting of:

- Multi-annual sampling programmes which enable evaluation of the fisheries sector and activities based on biological, ecosystem and socioeconomic data. Collection relates to vessels and companies in the sector, at landing locations or by consulting registers and economic data;
- A data collection programme on board commercial and recreational fishing vessels, if necessary. Observation activities at sea are undertaken on board the vessels by scientists or, for practical or security reasons, by the vessel's crew for a self-sampling programme;
- A programme of research surveys at sea in order to measure the impact of fishing on the environment and the abundance and distribution of fisheries resources;
- A programme for managing and using the data for scientific purposes.

The Member States provide protocols and methods for the collection and analysis of data in their national programmes. Also, the Member States cooperate with each other and with third-party countries if they are in the same marine region. For this reason, Member States coordinate their national programmes, particularly by Regional Coordination Meetings organised by the Commission, so as to avoid duplication of data collection.

The Commission approves the national programmes and monitors their implementation. Both are based on assessments by the Scientific, Technical and Economic Committee for Fisheries (STECF) as regards compliance and scientific and technical execution of national programmes. The Commission also estimates the associated costs. In cases of non-compliance, Member States amend their national programmes when requested by the Commission.

The EU contributes 50% of the budget allocated for data collection. A maximum sum of EUR 300 million is earmarked for the period from 2007 to 2013, within the framework of Community financial measures for the implementation of the CFP (Regulation (EC) No 861/2006).

The Commission may suspend or recover financial assistance if the execution of the national programme does not comply with the stipulated rules, such as compliance with deadlines, control of quality, and validation and transmission of data collected. A reduction of aid is also stipulated under certain conditions, but this is proportionate to the degree of non-compliance and must not exceed 25% of the annual cost of the national programme.

13 Working Group on Applied Genetics in Fisheries and Mariculture of the International Council for the Exploration of the Sea.

Management and use of data within the framework of the CFP:

The data collected are stored in secure computerised national databases. These data, whose quality is controlled by Member States, are primary data as well as aggregated data which results from primary data analysis. The data transmission to end-users for scientific analysis is regulated. These data may also be used to support discussions in Regional Advisory Councils within the framework of the CFP for policy development and for scientific publications by researchers. Data processing methods can be provided.

The deadline for data transmission depends on the type of use, which needs to be specified in the request. Member States may refuse to provide data in certain cases, in which case the European Commission is entitled to examine the refusals. If not justified, the Member State must provide the data to the end-user within one month. Should they fail to do this, the refusal may constitute a reason for reducing financial assistance. End-users may also have their access to data restricted or prohibited if they do not comply with certain obligations.

The data collected as part of research surveys at sea are transmitted to the international scientific organisations and to scientific committees for the relevant Regional Fisheries Management Organisation (RFMO).

Support for scientific advice:

National experts are encouraged to participate in RFMO and international scientific authorities' meetings in which the Community participates.

For this purpose, Member States and the Commission work together to improve the reliability of scientific advice and the quality of RFMO programmes and working methods within a context of openness and impartiality.

6.4 Existing databases with relevance to fisheries control and enforcement and seafood traceability

There exist projects that are sustained by databases, publicly accessible through a Web-interface, some of which could potentially support traceability and fisheries control and enforcement. These are briefly outlined:

FISH-BOL (http://www.fishbol.org): The Fish Barcode of Life Initiative (FISH-BOL) is a global effort to coordinate an assembly of a standardised reference sequence library for all fish species, one that is derived from voucher specimens with authoritative taxonomic identifications. A public electronic database containing DNA barcodes, images, and geospatial coordinates of examined specimens is available. The reference DNA sequence used is a stretch of the mitochondrial *COI* gene. The database contains linkages to voucher specimens, information on species distributions, nomenclature, authoritative taxonomic information, collateral natural history information and literature citations. Meanwhile, 7 276 fish species, including all major commercial ones, are barcoded (March 2010). This project is ideally suited to support species identification on fish and fish products, in the context of control, enforcement and traceability and has already repeatedly been used for this purpose.

FishTrace (http://www.fishtrace.org): This project was financed under FP5, and aimed at the development of a tool for identification of fish species and traceability of fish products. It is a genetic catalogue associated to biological reference collections (voucher samples deposited in a network of natural museums) from more than 200 commercial marine fish. Genetic, taxonomic and biological information is compiled in a public online database, curated by the JRC, which ensured the continuation of this project beyond its 'lifetime' fixed by the funding scheme. As for FISH-BOL this project is ideally suited to support species identification on fish and fish products, in the context of control, enforcement and traceability.

FishPopTrace (http://fishpoptrace.jrc.ec.europa.eu/home): This FP7-funded project started in 2008 and is run by an international consortium of 16 members, and aims at building a framework for sustainable fisheries management and conservation based on genetics, chemistry and forensics by:

- developing traceability tools fully supporting a 'from ocean to fork' approach;
- integrating new and established technologies based on molecular genetics, otolith microchemistry and morphometrics;
- applying forensic standards to technology development for fisheries control, enforcement and conservation;
- focusing on four fish species that differ in life style and distribution: cod, hake, common sole and herring;
- tailoring newly developed tools to the needs of end-users and stakeholders;
- engaging with priorities of the European Common Fisheries Policy;
- enhancing awareness of IUU issues within the industry, academics, policymakers and consumers.

A Web-based database developed and managed by the JRC will host the complete set of experimental data, and serve, together with an interface as a platform for dissemination and tool implementation in support of fisheries control, enforcement and fish product traceability.

SalSea Merge (http://www.nasco.int/sas/salseamerge.htm): Salmon at Sea Merge is a project supported by the International Atlantic Salmon Research Board to investigate the migration and distribution of salmon in the northeast Atlantic. The aim is to deliver innovation in the areas of: genetic stock identification techniques; new genetic marker development; fine scale estimates of growth on a weekly and monthly basis; the use of novel high seas pelagic trawling technology; individual stock-linked estimates of food and feeding patterns; and novel stock specific migration and distribution models. By merging genetic and ecological investigations, to advance understanding of stock specific migration and distribution patterns and overall ecology of the marine life of Atlantic salmon and gain an insight into the factors resulting in recent increases in marine mortality. Origin assignment in salmon is highly advanced and even though this is also largely due to its very specific reproductive behaviour as an anadromous fish species, which renders it highly accessible to population studies and limits its value as a 'paradigm' for truly marine species.

EMODNET (http://ec.europa.eu/maritimeaffairs/emodnet_en.html): In the light of the highly fragmented nature of marine data, the European Commission proposed a new European Marine Observation and Data Network (EMODNET) in its Green Paper on maritime policy (Commission, 2007b). Adopted in its EU's Maritime Policy Blue Book (Commission, 2007a), the Commission undertook steps towards EMODNET in order to improve availability of high quality data, which included in 2009 an EU action plan to make progress in this area on the basis of a road map (Commission, 2009).

The basic principles underlying EMODNET that have been formulated by the Commission together with a specially-constituted Expert Group are as follows:

- collect data once and use it many times;
- develop standards across disciplines as well as within them;
- process and validate data at different levels. Structures are already developing at national level but infrastructure at sea-basin and European level is needed;
- provide sustainable financing at an EU level so as to extract maximum value from the efforts of individual Member States;
- build on existing efforts where data communities have already organised themselves;
- develop a decision making process for priorities that is user-driven;
- accompany data with statements on ownership, accuracy and precision and;
- recognise that marine data is a public good and discourage cost-recovery pricing from public bodies.

In the meantime, EMODNET is being tested through preparatory actions, by using available portals for a number of maritime basins set up for hydrographic, geological, biological and chemical data as well as functional habitat maps.

An impact assessment conducted during 2009 will assess options for moving towards a definitive EMODNET, both in the intermediate period from 2011 to 2013 and in the long term after 2014. The

assessment was also accompanied by a consultation open to the public, from April to June 2009and which received 300 replies.

While there is no doubt that this initiative is ideally suited to resolve the issue of data fragmentation in the maritime sector, and would therefore also support fisheries control, enforcement and traceability, there are still open questions surrounding the funding. Also, strategies will have to be developed on how to integrate existing genetic or similar databases into EMODNET. Moves will begin to integrate EMODNET with initiatives under the EU's Research Infrastructure actions and the Common Fisheries Policy Data Collection Regulation (Council, 2008).

7. Glossary

Accuracy: Degree of closeness of a measured or calculated quantity to its actual (true) value. Or Accuracy is the degree of conformity of a measured quantity to its actual (true) value.

Allele: One of a series of different forms of a gene at a locus. In diploid organisms alleles can be homozygous (same gene form at one locus) or heterozygous (two different forms at one locus).

Allozyme: An enzyme encoded by different alleles of a gene. The allelic forms can be revealed by electrophoresis which has been used in many genetic applications including stock identification.

Anadromous fish: Anadromous fish live in the sea and migrate to fresh water to breed. Their adaptations to conditions of different habitats are precise, particularly with regard to salinity of the water. The most prominent example are Salmonids (Salmonidae) such as the Atlantic salmon (Salmo salar), most of which spend part of their life at sea, but return to freshwater where all species spawn in a gravel bed in rivers or streams where they themselves originate from (homing behaviour). This feature makes salmon particularly accessible to genetic studies revealing population structures, such as in the Irish National Atlantic Salmon Genetic Stock Identification Project 2008 (University College Cork; Central & Regional Fisheries Boards; Irish Marine Institute).

Ascertainment Bias: Introduced bias if the selection of genetic loci derives from an unrepresentative sample of individuals, or it arises as a result of a particular analytical method used if the yield of loci is not representative of the spectrum of allele frequencies in a population. Ascertainment bias has the potential to introduce a systematic bias in the estimates of variation within and among populations. This challenge arises particularly when using Single Nucleotide Polymorphisms (SNPs).

cDNA: Complementary DNA synthesised from mature mRNA templates in a reaction catalysed by the enzyme reverse transcriptase. Used for cloning purposes but also to create expressed sequence tag (EST) libraries, which contain short sub-sequences of transcribed cDNA sequence derived from a specific cell or tissue type. Useful for SNP discovery of animals for which a whole genome sequence is not yet available.

Chain of custody: A legal term that refers to the ability to guarantee the identity and integrity of evidence material from collection through to reporting of the test results.

Electronic recording and reporting system (ERS): The electronic reporting system (ERS) is used to record, report, process, store and send fishing activity data (e.g. catches, landings, transhipments, sales) and to report them to fisheries authorities in the Member States. The system is compulsory for vessels above 15 m (as from 1 January 2012 – vessels above 12 m). It replaces paper logbooks and is therefore often referred to as an electronic logbook or "e-logbook". It also replaces sales notes. Commission Regulation (EC) No 1077/2008 of 3 November 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 1966/2006 on electronic recording and reporting of fishing activities and on means of remote sensing and repealing Regulation (EC) No 1566/2007.

ELISA: The Enzyme-Linked Immunosorbent Assay is a highly sensitive technique based on the specific interaction between antigen and antibody. It can be used for testing individual samples, but also in fully automated systems for high-throughput screening. Recent adaptations by diagnostic industries for dipstick ('immunosticks') convenience and portability have enhanced the usefulness of these immunology-based technologies in the field. The principle is based on the immobilisation of the reagent to be tested (e.g. a protein of a tissue

sample) on a plastic surface. Secondly, a test-antibody specific for the reagent is added, followed by washing steps, and addition of an enzyme-conjugated secondary antibody. The addition of a substrate leads to a colour reaction, allows identification and quantification of the reagent even at very low levels down to sub-picogram levels per millilitre. ELISA has been routinely used in forensic laboratories for many years and also in the context of fish species identification (Taylor and Jones, 1992, Taylor et al., 1994, Levine, 2010)

Gene Flow: Exchange of genetic information between populations through migration and reproduction.

Illegal, Unreported and Unregulated (IUU) Fishing: According to the International Plan of Action to Prevent, Deter and Eliminate Illegal, Unreported and Unregulated Fishing (IPOA-IUU) the following definitions apply:

Illegal fishing refers to activities:

- conducted by national or foreign vessels in waters under the jurisdiction of a State, without the permission of that State, or in contravention of its laws and regulations;
- conducted by vessels flying the flag of States that are parties to a relevant regional fisheries management organization but operate in contravention of the conservation and management measures adopted by that organization and by which the States are bound, or relevant provisions of the applicable international law; or
- in violation of national laws or international obligations, including those undertaken by cooperating States to a relevant regional fisheries management organization.

Unreported fishing refers to fishing activities:

- which have not been reported, or have been misreported, to the relevant national authority, in contravention of national laws and regulations; or
- undertaken in the area of competence of a relevant regional fisheries management organization which have not been reported or have been misreported, in contravention of the reporting procedures of that organization.

Unregulated fishing refers to fishing activities:

- in the area of application of a relevant regional fisheries management organization that are conducted by vessels without nationality, or by those flying the flag of a State not party to that organization, or by a fishing entity, in a manner that is not consistent with or contravenes the conservation and management measures of that organization; or
- in areas or for fish stocks in relation to which there are no applicable conservation or management measures and where such fishing activities are conducted in a manner inconsistent with State responsibilities for the conservation of living marine resources under international law.

Source: FAO. International Plan of Action to prevent, deter and eliminate illegal, unreported and unregulated fishing. Rome, FAO. 2001. 24p.

Otoliths: The otoliths of teleost fish form part of the inner ear (the membranous labyrinth), located in the brain cavity. The ear is part of a sensory system, which detects position with respect to external stimuli generated by gravity, acceleration and sound. Otoliths are polycrystalline bodies, composed of calcium carbonate crystals in the form of aragonite. They radiate outwards in three dimensions from a centrally located nucleus and pass through a network of fibrous collagen-like protein called otolin (Northcutt et al., 1983). The largest of the three otoliths, called sagitta, is generally used in studies for bony fish (teleosts).

Precision: Degree to which further measurements or calculations show the same or similar results. Or Precision characterises the degree of mutual agreement among a series of individual measurements, values and/or results.

Reproducibility: Ability to obtain the same result when the test or experiment is repeated.

Meristics: Method used in fisheries science to identify stocks, which is based on the counting of discrete morphological elements (number of vertebrae, fin rays etc.).

Microchemistry: Chemistry that deals with minute quantities of chemical materials. Includes trace element analysis and element composition of otoliths or tissue as natural markers to identify fish stocks or populations.

Mitotyping: Analysis of mitochondrial DNA to characterise and identify individuals or affiliations to groups or populations. Often used in a forensic context and for law enforcement purposes.

Monitoring, control and surveillance (MCS): A key component of the fisheries management process. The International Plan of Action to Prevent, Deter and Eliminate Illegal, Unreported and Unregulated Fishing (IPOA-IUU, 2001) identifies many tools states can employ to combat illegal fishing and urge strengthened MCS capacity.

Definitions of MCS:

- Monitoring the collection, measurement and analysis of fishing activity including, but not limited to: catch, species composition, fishing effort, bycatch, discards, area of operations etc. This information is primary data that fisheries managers use to arrive at management decisions. If this information is unavailable, inaccurate or incomplete, managers will be handicapped in developing and implementing management measures.

- Control involves the specification of the terms and conditions under which resources can be harvested. These specifications are normally contained in national fisheries legislation and other arrangements that might be nationally, subregionally or regionally agreed. The legislation provides the basis for which fisheries management arrangements, via MCS, are implemented.

- Surveillance involves the regulation and supervision of fishing activity to ensure that national legislation and terms, conditions of access and management measures are observed. This activity is critical to ensure that resources are not over exploited, poaching is minimised and management arrangements are implemented.

Source: FAO. © 2005-2010. Fisheries Topics: Governance. Monitoring, Control and Surveillance. Text by G.V. Everett. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 27 May 2005. http://www.fao.org/fishery/topic/3021/en#container.

Monoclonal antibodies: Monospecific antibodies (recognise one specific antigen) produced by one type of immune cell that are all clones of a unique parent cell. Given almost any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance (for example in ELISAs). Monoclonal antibodies are important tools in biochemistry, molecular biology and medicine.

Morphometrics: Quantitative characterisation, analysis and comparison of biological form, shape and size variation. Morphometry is essential to evolutionary biology and other disciplines and belongs to the classical methods for fish stock characterisation.

mRNA: Messenger ribonucleic acid. RNA transcribed from the DNA template, which is read by ribosomes and translated into proteins.

MSY: Maximum Sustainable Yield – the largest average yield (catch) that can be taken in the long term from a stock.

Neutral genes: Genes that are not under selection. None of the protein products of different alleles do lead to an increase in fitness.

NMR spectroscopy: Nuclear magnetic resonance spectroscopy is a technique that exploits the magnetic properties of certain nuclei. This analytical chemistry technique is used in quality control and research for determining the content and purity of a sample as well as its molecular structure. For example, NMR can quantitatively analyse mixtures containing known compounds. For unknown compounds, NMR can either be used to match against spectral libraries or to infer the basic structure directly.

Polymerase Chain Reaction (PCR): Technique to amplify in a test tube a single or few copies of a piece of DNA exponentially over many orders of magnitude, thereby generating thousands to millions of copies of a particular DNA sequence. This greatly facilitates the analysis of DNA. The method relies on the in vitro use of DNA polymerases during thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short synthesised DNA fragments) containing sequences complementary to the area framing the target sequence allow the DNA polymerase to selectively amplify.

Polymorphic Markers: The presence of more than one allele (form) at one gene locus.

Restriction Endonucleases (Restriction Enzymes): Enzyme that cuts DNA at specific recognition nucleotide sequences known as restriction sites. Extensively used in molecular biology for analytical purposes and for cloning. In population genetics utilised to reveal restriction fragment length polymorphisms (RFLP), which are due to mutations changing recognition sites and therefore the length of cut DNA fragments.

Small interfering RNA (siRNA): Sometimes known as short interfering RNA or silencing RNA, it is a class of double-stranded RNA molecules, 20 to 25 nucleotides in length, that play a variety of roles in biology. Most notably, siRNA is involved in the RNA interference (RNAi) pathway, where it interferes with the expression of a specific gene. In addition to their role in the RNAi pathway, siRNAs also act in RNAi-related pathways, e.g. as an antiviral mechanism or in shaping the chromatin structure of a genome; the complexity of these pathways is only now being elucidated.

SOPs – Standard Operating Procedures: Documents to ensure the quality and integrity of data and provide a basis for guidance, uniformity and accountability in a forensic context. SOPs contain instructions that forensic scientists follow to perform procedures that are routine, standardised, and for which no ad hoc modification is acceptable.

Short Tandem Repeats (STRs): DNA regions with short repeat units (usually 2 to 6 bp in length). STRs are found surrounding the chromosomal centromere (the structural centre of chromosomes). STRs have become popular DNA markers in human forensic genetics because they are easily amplified by PCR. Any individual inherits one copy of an STR from each parent, which may or may not have similar repeat sizes. The number of repeats in STR markers can be highly variable among individuals, which renders these STRs effective for identification purposes.

Vessel Detection System (VDS): The Vessel detection System a satellite-based technology (satellite imaging of sea areas) which may help to locate and identify fishing vessels at sea. The basic function of VDS is to provide fishing vessel position reports at regular intervals to fisheries authorities. According to EU legislation (Regulation 1224/2009), fisheries control authorities shall have a technical capacity to use VDS.

Vessel Monitoring System (VMS): The vessel monitoring system is a satellite-based monitoring system which at regular intervals provides data to the fisheries authorities on the location, course and speed of vessels. The system is compulsory for EU vessels above 15 m (as from 1 January 2012 – vessels above 12 m). Non-EU vessels of the same size are obliged to have an operational satellite tracking device installed on board whenever they are in Community waters. VMS is nowadays a standard tool of fisheries monitoring and control worldwide, but it was the EU which led the way, becoming the first part of the world to introduce compulsory VMS tracking for all the larger boats in its fleet. The EU legislation requires that all coastal EU countries should set up systems that are compatible with each other, so that countries can share data and the Commission can monitor that the rules are respected. EU funding is available for Member States to acquire state-of-the-art equipment and to train their people to use it. VMS is anchored in the following EU legislations: Commission Regulation (EC) No 2244/2003 of 18 December 2003 laying down detailed provisions regarding satellite-based Vessel Monitoring Systems and Commission Regulation (EC) No 1077/2008 of 3 November 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 1966/2006 on electronic recording and reporting of fishing activities and on means of remote sensing and repealing Regulation (EC) No 1566/2007.

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Abstract

Illegal, Unreported and Unregulated (IUU) fishing and fraud along the fish product supply chain impede sustainable fisheries worldwide and pose a major challenge to regulators, as well as control and enforcement authorities. In this context the ability to determine authenticity and provenance of fish and fish products throughout the fish product supply chain constitutes a key asset.

This JRC reference report describes existing techniques based on genetics, genomics and chemistry that are suitable for fisheries control and enforcement and traceability of fish products. It argues that despite their value these techniques are currently underutilised, and discusses how they can be implemented by control and enforcement authorities. To demonstrate feasibility, examples are provided of cases where such techniques have already been successfully employed to reveal fisheries fraud and to generate evidence in court cases. Furthermore, strategies of how (forensic) genetics and chemistry could be translated into a routine European fisheries control and enforcement framework — in the context of EU policy and legislation — are explored.

The purpose of this report is to catalyse an informed dialogue amongst stakeholders and to contribute to a more efficient technology transfer process.

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