SEAFISH
the authority on seafood

The Seafish Guide To
DNA Testing of Seafood

This is one of a series of guides in which Seafish explores topical issues affecting the UK seafood industry. Here we look at how DNA testing is being used by the seafood supply chain, what it can detect, where DNA analysis is carried out and the development of comprehensive DNA databases.

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A Definition

DNA testing is a way of identifying an organism as belonging to a particular species, or a subset of a species. In general terms (as there are differences in the exact methods) this technique uses the variations in short, standardised gene regions to identify known species. Through the innovative use of DNA testing, a specific segment of genetic material in a piece of fish can be used to determine exactly which species, and area of origin, it came from, providing reference samples are available for comparison. To identify the species DNA from the unknown sample is compared to DNA from known species using a reference library of DNA sequences[2,3].

DNA barcodes can be obtained from tiny amounts of tissue, in many cases even when it has been cooked and prepared. DNA testing can be used to identify the fish species of samples of whole fish, fillets, fins, fragments, eggs, or any properly preserved tissue available. The techniques have also been applied successfully to: processed fish; grilled or deep-fried cooked fillets; raw, frozen or dried tissue; fish scales; and even to samples with degraded DNA, caused by a combination of high pressure and temperature as used in canning, all using so-called mini-barcodes.

DNA testing has been used in the area of food labelling, where DNA testing has demonstrated that fish samples from markets, restaurants, supermarkets and fish and chip shops have been mislabelled or substituted. But equally it has important applications in the marine area to identify origin, and its use can be seen in the fields of fish conservation and management, such as quota, by-catch monitoring and assessing sustainable fisheries.

There are some key developments in this area with calls for an EU-wide approach to integrate DNA techniques into the Common Fisheries Policy. There are also moves to harmonise methodologies among the user laboratories, and to construct a Pan-European framework, built on advanced technologies, for product traceability and policy related monitoring, control and surveillance in the fisheries sector.

Media Spotlight

There have been numerous reports in the national and international press concerning the practice of ‘seafood substitution’, mislabelling of fish or concerns about stock status claims[4,5]. Seafood substitution occurs when a species is mislabelled and substituted in whole or in part for a different species. Some reports reveal obvious fraud where an expensive species is substituted with a cheaper one such as pangasius being sold as cod, or inadvertent substitution of species, where it is possible that the expensive species is sold as the cheap species, and vice versa.

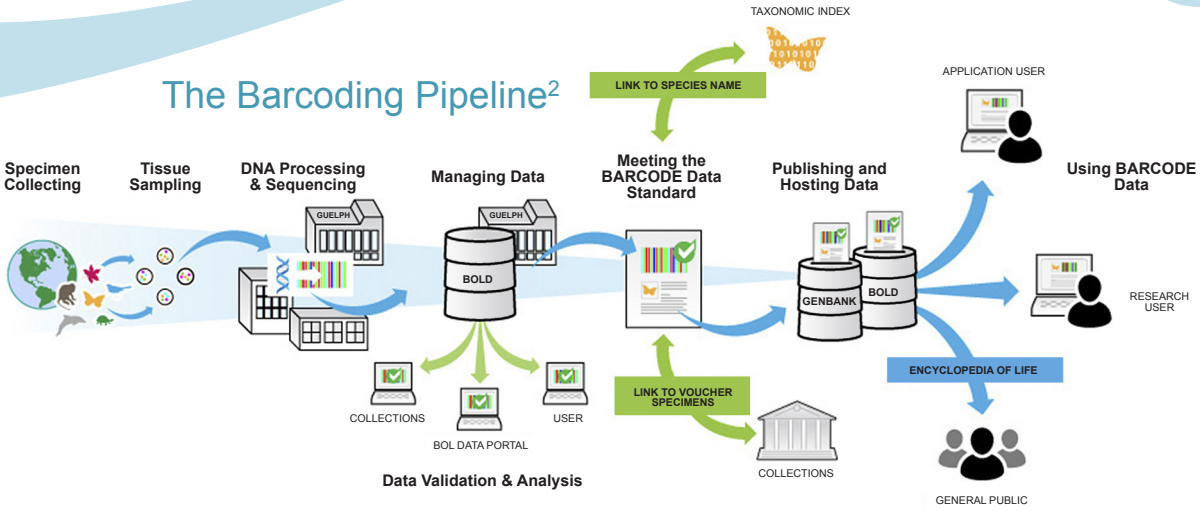
Seafood substitution or mislabelling happens for a variety of reasons, from a simple misunderstanding or lack of information to blatantly deceiving consumers to increase profits, or even worse, laundering illegally harvested seafood. Regardless of the reason, seafood substitution or mislabelling is illegal and can have serious consequences for the supply chain and consumers.

“Anytime consumers or buyers purchase a seafood product that is not what they are paying for they are being misled. To address this the seafood industry, academia, and governments are proactively developing solutions to protect the integrity of the seafood supply chain.”

Key Facts

Approximately half of the most commonly traded fish species have been DNA barcoded[1]

The Barcoding Pipeline[2]



The process[2]

The process involves four clear stages:

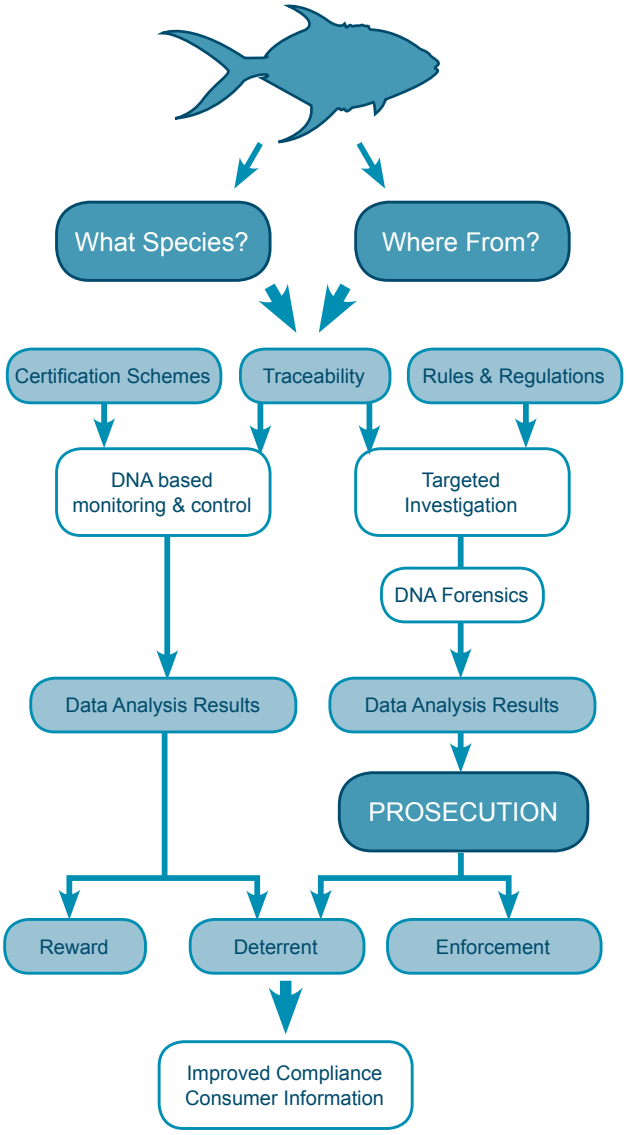
- **Samples:** The analyst acquires the fish sample and preserves it, as preserved tissue samples generally yield more accurate results. Then, in the lab, they remove a tiny piece of the sample for testing.
- **The Laboratory Analysis:** Laboratory protocols are followed to obtain DNA barcode sequences from the samples. DNA is extracted from the tissues, the fragments of interest are amplified using polymerase chain reaction (PCR), and the products obtained are sequenced to detect genetic identity. The data are entered in a database for analysis. The best equipped molecular biology labs can produce a DNA barcode sequence in a few hours.
- **The Database:** One of the most important components of the DNA testing process is the availability of a public reference library of species identifiers to be used to assign unknown specimens to known species.
- **The Data Analysis:** Specimens are identified by finding the closest matching reference record in the database.

What DNA testing can be used for?

DNA testing is a tool for both basic and applied research. In addition to research issues, DNA testing is being used as a way to identify species involved in legal and regulatory matters. For enforcement and traceability requirements different types of DNA testing could be used to varify:

- **The species of fish.** There have been several cases in the UK, Europe and North America of reported mislabelling (whether deliberate or inadvertent) i.e. products labelled for sale as cod or haddock in fishmongers and take-away restaurants were an entirely different species.
- **Where it was caught.** Because different stocks can be in a different stock status condition, tracing the fish to a particular stock may be important in relation to sustainability claims and management control. As an example, DNA testing could be used to show that cod reportedly originating in the Baltic Sea originated in the North Sea, but further work is needed.
- **If the product is wild-caught or farmed.** In some locations escapees from aquaculture facilities pose a potential threat to the fitness of wild populations when they mate and reproduce with wild fish. The farm of origin could be identified through the comparison of genetic material sampled from an escapee, with the characteristics of the fish in each of the sampled farms but further work is needed.

Flow diagram for analysis[10]



"…even if it is proved criminality is behind the fraudulent adulteration of meat on the market, this does not relieve manufacturers and retailers of their legal duty and responsibility to produce and sell food that is safe and labelled accurately."
Trading Standards Institute (on horse meat). February 2013[11].

The legal framework

DNA testing is increasingly used as a way to identify species involved in legal and regulatory matters, effectively serving as a bridge linking scientific names with approved market names, and empowering regulatory bodies to enforce labelling standards.

- **European Regulation 1224/2009[6]** provides for inspections and traceability all along the production chain with genetic analysis explicitly mentioned as a tool to aid traceability.
- **European Regulation 178/2002[7]** covers food law and food safety. Within this regulation Article 18 covers traceability and states the requirement for food products on the market to be adequately labelled to accurately determine the species and origin.
- **The Fish Labelling Regulations 2013[8]** require the commercial designation of the species (ie an agreed common name for the species of fish); the production method (ie whether caught at sea, caught in inland waters or farmed); the catch area (ie either the ocean area, or in the case of freshwater fish, the country in which it was caught or farmed) to be clearly visible on the pack.
- **European Regulation 1005/2008[9]** established a new regulation to prevent, deter and eliminate the import of illegal, unreported and unregulated fishery products in the European Community.

Key Facts

All living organisms contain DNA in their tissues

In addition to species identification, for some species, it is also possible to use DNA approaches to identify geographic origin or stock location

How The Seafood Industry Is Using DNA Testing

A necessary part of the MSC process

The Marine Stewardship Council (MSC) certification and ecolabelling program for sustainable seafood states that products that carry the MSC ecolabel come from MSC certified sustainable fisheries, which are delivered through supply chains certified to the MSC Chain of Custody Standard.

In order to assure consumers and buyers that uncertified fish are not mixed with, or mislabelled as, MSC certified fish, oversight is an important and necessary part of the MSC process.

Genetic testing is one way the MSC does this. A sample of ecolabelled products are bought and tested periodically by the MSC, in order to accurately identify the species and, in some cases, the catch area. This allows the MSC to measure the effectiveness of its Chain of Custody certification and provides an opportunity to address any issues that may arise.

Key Facts

There are different techniques available to identify, or verify, a particular species, or a subset of a species – DNA testing is just one method that uses variations in DNA sequences

Identification is performed by either by DNA profiling or DNA sequence analysis of specific gene target sequences and results in a species specific ‘barcode’

Fish DNA sequence databases generated from authentic fish samples are available to enable reliable interpretation of the results

Young’s take DNA testing in-house

During 2013, with the acquisition of a new quantitative PCR machine, Young’s Seafood Limited has built in-house DNA testing capability into its Grimsby analytical laboratory, and the business now includes DNA testing as part of its raw material assessment processes. This is an evolution of the company’s approach to food integrity testing, in which it seeks to embrace new technologies as and when they become available, practical and affordable to industry at an operational level.

DNA testing has been added to the suite of internal product integrity tests available to the business and these will complement the existing testing regime of c.2,000 traditional gel-electrophoresis fish speciation tests carried out per annum. The aim is to increase this capability over time, to investigate incoming raw materials and ensure they are able to develop more comprehensive DNA surveillance of their fish products. The company is also conducting extensive field trials of a novel technology for fish speciation testing with a real-time application.

External Testing Facilities For SMEs

Seafish has been contacted by increasing numbers of small to medium sized enterprises (SMEs), wanting guidance and information on how to verify the species of fish they have purchased. This is for various reasons, either they have started purchasing from a new supplier and want to verify the source, or their customers/major buyers have started to ask for species verification as a condition of trading. At a basic level most of these businesses want to know what tests they should be using, and where they can have their products analysed. They are using the results to provide reassurance for themselves, and their customers, that the species they are buying are exactly as expected.

Seafish has provided information about the different types of tests available and a list of laboratories/testing facilities which can help. This size of business would find it too expensive to invest in in-house testing, so external testing facilities are providing an invaluable service.

Issues to consider

In the past couple of years fish species identification and verification has become more apparent at a commercial level and DNA testing is a tool that can help in that process, but there are a number of different types of species identification methods and it is important that the right method is used for the right purpose. Businesses with robust traceability systems will have methods in place which will identify risks and DNA testing can be used to assist in this process when necessary, but there are some limitations and considerations about DNA testing which need to be addressed.

There are issues with products. Approximately half of the most commonly traded fish species have been DNA barcoded so there is still a lot more to do. You can't always use all the different tests on all types of products so tests need to be specifically tailored to the product and the identification or verification need, also geographical origin assignment is occasionally a problem.

Protocols need to be in place to help determine which test to use and how to understand the results. This will involve standardisation of sampling, transport and storage, a more joined-up approach across laboratories, and more robust quantitative methods. There are also still some uncertainties about the test methods themselves, the level of accuracy, the interpretation of results and being able to understand the results.

There are also issues about how to differentiate between low level cross-contamination caused by the inadvertent transfer of DNA onto the production line or factory, and fraudulent substitution.

Next steps

The Joint Research Centre of the European Commission report[12] 'Deterring Illegal Activities in the Fisheries Sector' calls for an EU-wide approach to integrate DNA techniques into the Common Fisheries Policy, and to provide evidence of general traceability. There is a need to harmonise methodologies among the user laboratories, and also to update databases of reference samples and genetic profiles of commercially relevant samples, to provide a standardised response regardless of the country or laboratory where the analyses are performed.

This has already happened In the United States where the Food and Drug Administration approved a new protocol that standardises the method of DNA analysis used to identify fish species in seafood in 2011[13].

In Europe LABELFISH[14], the Atlantic Network on Genetic Control of Fish and Seafood Labelling and Traceability, is part of this process. It aims to set up a network of laboratories and national bodies to develop a common strategy and a proposed standard European methodology, and use harmonised analytical techniques to control genetic traceability and labelling of seafood products sold on the Atlantic Area market. http://labelfish.eu/

Using DNA Testing

The Seafish fact sheet[15] 'Recent advances in DNA and other techniques for identifying seafood provenance in the supply chain' explains in detail some of the techniques currently used to accurately identify seafood and seafood products, and reviews some of the benefits and limitations of the latest DNA techniques, as well as other techniques, used for identification purposes within commercial, enforcement and fisheries science.

DNA analysis allows both suppliers and consumers to have more confidence in product authenticity and justify market prices, and can be used as a tool to protect endangered fish species, and to ensure the correct labelling of seafood and seafood products. These techniques can be used by processors to improve assurance that the raw materials are the species described. Secondary processors and retailers can also use them to check on semi-processed and fully processed product.

Some guidelines on how to go about using the technology:

- Consider what is an appropriate sampling strategy to adopt i.e. periodic checks, when a new supplier is appointed, or when there are concerns about a particular supply chain, as part of a defined contract.
- Determine exactly what the testing needs to demonstrate i.e. identify a species, verify a species, prove geographical area, origin (i.e. farmed or wild-caught).
- Consider which technique will be most appropriate and cost effective for the specific purpose.
- Decide on a sampling strategy that addresses the areas of highest risk in a supply chain and build a sampling programme which will address this risk.
- Develop a relationship with an appropriate supplier of analytical services. Such a supplier should be able to advise on the specific techniques that need to be used and on the costs and benefits, especially where a routine programme is being considered. Samples taken on an ad hoc basis are almost certain to cost more per sample than routine work, whichever technique is chosen.
- Make sure analytical laboratories offering commercial services demonstrate their competence. ISO 17025 is the standard appropriate to testing and calibration laboratories and laboratories should be accredited for the methods offered.
- Give the laboratory a list of which species are suspected in the samples so they can choose the appropriate techniques. There may be some closely related species for which the genetic markers do not give sufficient discrimination.

Where to go

Seafish has compiled a list of laboratories/ services in the UK which offer fish DNA testing:

- **Eurofins** – www.eurofins.co.uk/food-testing/food-authenticity/fish-speciation-testing.aspx
- **Campden BRI** – www.campdenbri.co.uk/case/authenticity-testing.php
- **Public Analysts Laboratories** – www.publicanalystservices.co.uk/news/launch-comprehensive-fish-testing-services.aspx
- **Agilent** – www.genomics.agilent.com/en/home.jsp
- **Neogen** – www.neogeneurope.com/LabServices/SI_Testing.html
- **Trace** – www.tracenetwork.org/

This list is not necessarily comprehensive and is provided for information. It does not imply any endorsement by Seafish of the services provided.

DNA Databases

To identify the species most techniques depend on using a reference library of DNA sequences for a reliable interpretation of the results. DNA reference databases for fish species are well established, but obviously need maintaining and updating.

National Centre for Biotechnology Information includes GenBank, a genetic sequence database, with an annotated collection of all publicly available DNA sequences. www.ncbi.nlm.nih.gov/genbank/index.html

FISH-BOL, the global Fish Barcode of Life Initiative, is a concerted global research project launched in 2005, with the goal to collect and assemble standardised DNA barcode sequences to aid the molecular identification of at least five representatives of all 30,000+ species of marine and freshwater fish. www.fishbol.org/enabling_tools.php

FishTrace is a genetic catalogue, biological reference collection and online database of European marine fishes. Its main aim is to catalyse the co-operation and the pooling of data and material corresponding to the genetic identification and characterisation of marine fish species from European waters and/or marketed in Europe. https://fishreg.jrc.ec.europa.eu/fishtrace_int/

FishPopTrace (The Structure of Fish Populations and Traceability of Fish and Fish Products) delivered in July 2011. It aimed to construct a Pan-European framework, built on advanced technologies, for product traceability and policy related monitoring, control and surveillance in the fisheries sector. https://fishpoptrace.jrc.ec.europa.eu/

This has led to genetic traceability tools for the origin assignment of commercial fish and fish products, launched in April 2012 by UK government to directly utilise the outputs of FishPopTrace. The research is funded by the Department for Environment Food and Rural Affairs (Defra) and led by TRACE Wildlife Forensics Network, in partnership with LGC, a laboratory testing company in the UK, and in collaboration with a broad panel of stakeholder groups. It will focus on cod, hake, herring and sole in the UK. https://fishpoptrace.jrc.ec.europa.eu/fpt-legacy

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12. http://publications.jrc.ec.europa.eu/repository/handle/111111111/16295

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15.www.seafish.org/media/publications/SeafishFactSheet_RecentAdvancesintheuseofDNAintheSeafoodSupplyChain_201109.pdf

About Seafish

Seafish was founded in 1981 by an Act of Parliament and aims to support all sectors of the seafood industry for a sustainable, profitable future. It is the only pan-industry body offering services to all parts of the industry, from the start of the supply chain at catching and aquaculture; through processing, importers, exporters and distributors of seafood right through to restaurants and retailers.

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