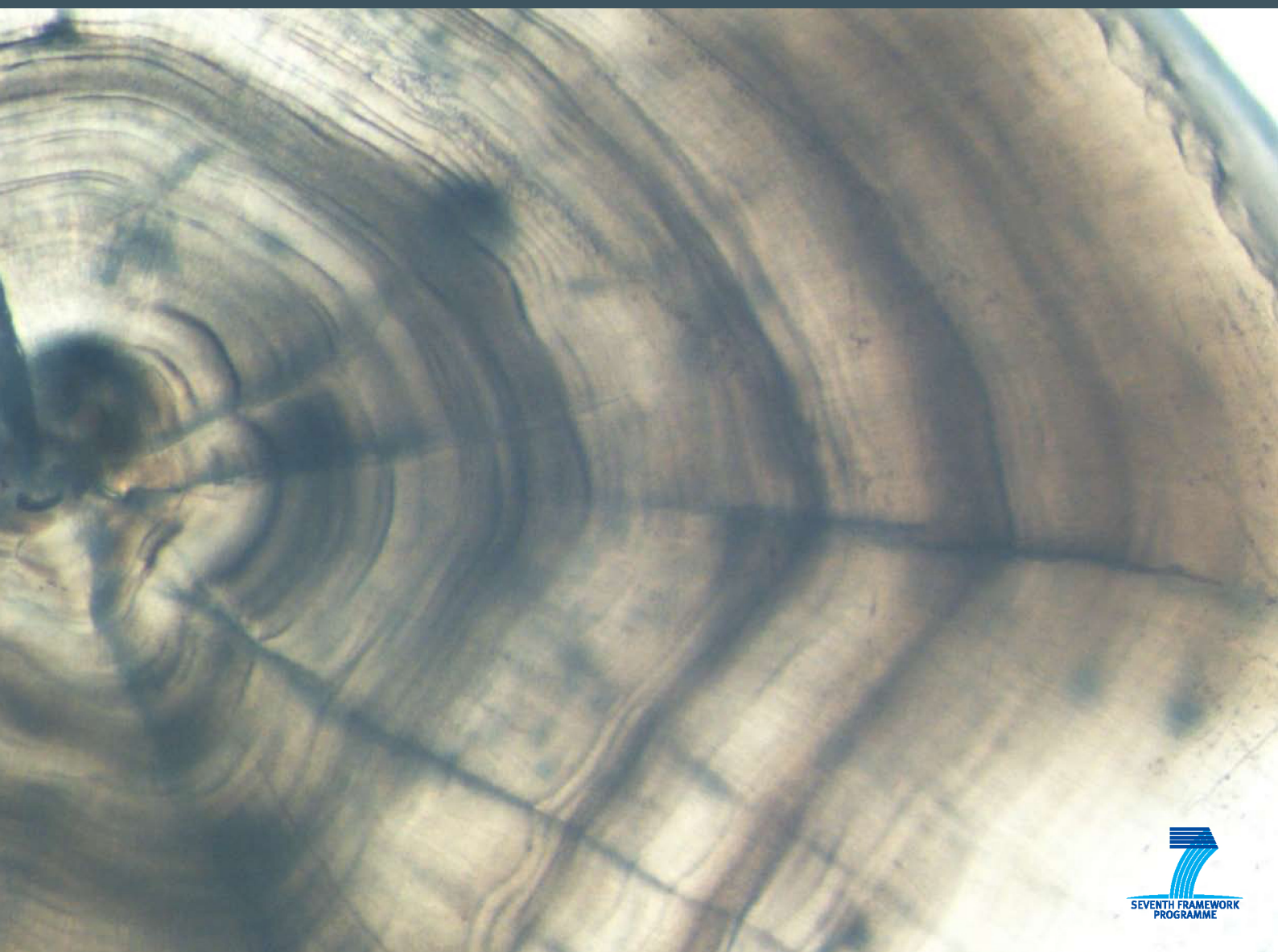


# Traceability of Fish Populations and Fish Products: Advances and Contribution to Sustainable Fisheries





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### Photos

All authors contributed photos and  
illustrations for the purposes of this brochure.

**The cover image** shows a slice of an otolith  
(fish ear stone). Otoliths have been used by  
[FishPopTrace](#) for analysis in support of fish  
population structure analysis. Thanks to  
Audrey Geffen

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FP7 Project FishPopTrace “The Structure of Fish  
Populations and Traceability of Fish Products”  
[KBBE 212399]

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Agriculture, forests, fisheries and  
aquaculture DG RTD, Timothy J. Hall 06

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## Message from **FishPopTrace** Coordinator, Gary Carvalho



When thinking about marine fish populations, two indisputable facts become apparent: first, we know that many **stocks** around the world are exploited beyond safe biological limits, and second, we have an avalanche of data on marine fish populations. Why then

“From the establishment of the FishPopTrace consortium, we highlighted the central role that population diversity plays in sustainable utilisation and conservation of exploited stocks.”

do we need more information, and is it not already too late to remedy the epidemic of overexploitation? The consortium, FishPopTrace, has spent the past three years examining these issues. The primary effort was focused on identifying, mapping and monitoring stocks in relation to **traceability** and **Illegal, Unreported and Unregulated Fishing (IUU)**. Despite the vast amount of existing data on marine fish, two limitations hinder its use in management: our ability to detect **population diversity** across local and regional scales, and the lack of precise population estimates. Such features not only compromise our ability to relate identifiable

assemblages to spatial scales of policy relevance, it is also difficult to achieve high levels of certainty in the origin and identity of fish products or individual fish. Within the consortium we have provided among the most comprehensive reviews of population diversity in four commercially representative species – cod, herring, common sole and hake – within European waters, based on a mix of “**genetic signatures**” from **spawning groups**, and “**environmental signatures**” from fish ear stones, or “**otoliths**”. Collectively we are now able to determine with high precision the origin of fish from specific locales, a key attribute for the use of output management tools (catch limits, **Total Allowable Catch (TAC)**, minimum landing sizes). Moreover, by comparing small genetic differences among populations, we can trace fish products throughout the food supply chain. FishPopTrace applied forensic standards to its analytical procedures, facilitating the production of evidence, in a way similar to human **forensics**. The ability to apply **population markers** within a strict forensic framework will enhance the governance of our fisheries through robust traceability of products and enforcement of regulations as an increased deterrent to illegal activities. It is the ability to create an informative and reproducible reference database within a legal structure across temporal and spatial scales relevant to management that renders FishPopTrace outputs as distinct from most other marine fish population data sets.

Returning to the issue of overexploitation and whether or not we are heading irrevocably towards the extinction of exploitable fish resources, numerous leaders in fisheries science highlight the need for improved governance of our oceans, especially in relation to IUU activities. The global impact of the ongoing and relentless loss of fish biomass, biodiversity and fisheries income adds considerable uncertainty to our forecasts of sustainability, as well as



threatening ecosystem function, food security and the socio-economic viability of fishing communities. To have routine or random testing of fish identity and provenance using independent tools such as those developed here, combined with **sustainable fishing** practices, can contribute significantly to our efforts in halting population declines. Such practices alone will not be sufficient, but when integrated with other actions such as reduced **by-catch**, co-management of policies and ecosystem-based approaches, it is possible to reduce the degradation of our fish resources.



Cod or pollack (around 1 mm in diameter). At this stage, it is difficult to identify the fish species from the eggs. Fish eggs. © Ifremer / Olivier DUGORNAY.

The outputs from FishPopTrace illustrate two key uses of population markers in fisheries management: their use as “tags” (traceability markers) to identify individuals and populations, and the rather more prosaic, but fundamental role that population diversity (**genetic resources**) plays in the resilience and recovery of exploited populations. It is only through the judicious identification and monitoring of population diversity, especially those features determined genetically, that it becomes possible to develop strategies to maximise and conserve genetic resources for adaption to environmental change. Our aim in FishPopTrace has been to move one step closer in support of that ideal. We hope that you find the highlights presented in this brochure of interest and occasionally provocative. While we have not aimed to provide a comprehensive overview of all we have done,

the brochure provides a flavour of our achievements and their relevance, as well as highlighting what remains to be done if we want to achieve what the EU Commissioner for Maritime Affairs and Fisheries, Maria Damanaki, has ambitiously stated, the aim of “bringing fishing activity into closer harmony with the ecosystem”.

“It is the ability to create an informative and reproducible reference database within a legal structure that separates FishPopTrace from other fish population studies”

# Message from Head of Unit E4: Agriculture, forests, fisheries and aquaculture DG RTD. Timothy J. Hall



The idea of uniting Europe has always been a dream in the minds of philosophers and visionaries. Victor Hugo, for example, imagined a peaceful 'United States of Europe' inspired by humanistic ideals. The dream was shattered by the terrible wars that ravaged the continent during the first half of the 20th century. These events however inspired the instigation of a genuine political solution to our divisions. The European Union (EU) was created to establish and guarantee long-lasting peace between its member states and its neighbours. Fifty years of European integration have shown that the EU has much more economic, social, technological, commercial and political weight as a whole, than if its member states act individually. The old saying 'unity is strength' is as relevant as ever to today's Europeans, while at the same time the EU uses its diversity as one of its key values.

Science has always been one of the most suitable fields to achieve this integration, mainly because cooperation is a key for achieving breakthroughs in knowledge and for transforming this into scientific advice, innovation, economic growth and general improvement of the quality of our lives. In this respect, research and technological development are not only two of the cornerstones of the political integration between EU member states, but they also constitute a bridge between Europe and the rest of the world through the promotion

of cooperation and partnership with third countries worldwide. However, European research suffers from insufficient funding, lack of an environment to stimulate research and exploit results, as well as the fragmented nature of activities and the dispersal of resources. It is the main objective of the developing European Research Area (ERA) to tackle these shortcomings and unlock the potential of research and innovation in Europe. The acknowledgement of the importance of Science in tackling present and future challenges has been recently renewed in the Innovation Union flagship initiative published recently by the European Commission, as an essential part of the 2020 strategy of the EU.

The EU's involvement in research goes back almost half a century. However, in the 1980s the EU took on a broader responsibility for stimulating and coordinating scientific research in its member states. To carry out this task, the EU set up a series of 'framework programmes' (FPs) which reflect the constantly changing nature of scientific and technological research and the Union's evolving priorities. The first framework programme covered the period 1984–87. The current one, the seventh (FP7), runs from 2007 until 2013 with an overall budget that exceeds 50 billion Euros. Today, the Union possesses three key funding instruments to support research and innovation: the first is the aforementioned Research Framework Programme, along with the Cohesion policy which is funded through the Structural Funds and Cohesion Fund; and the Competitiveness and Innovation Framework Programme.

The European fisheries sector is one of the many important socio-economic sectors covered by the framework programme. The European Union supports an integrated approach for fisheries research aiming at filling the gaps in knowledge in support to the scientific advice for fisheries management, building capacities and critical mass for research, supporting the industry and promoting international cooperation based



on the principle of mutual interest and benefit. The EU actions in this field intend to maximise synergies between Member States and Community efforts, to improve the dialogue between the scientific community, industry, policy makers and relevant stakeholders, to stimulate public and private investment in research technological development and innovation (RTDI) and to promote knowledge transfer and innovation.

FPs are implemented through various types of projects or “funding schemes” which, among others, aim at stimulating collaborative research, coordination, networking, dissemination, popularization and improvement of the take up of RTD results by end users. In particular, “Collaborative projects” are focused research projects with clearly defined scientific and technological objectives and specific expected results (such as developing new knowledge or technology to improve European competitiveness). They are carried out by consortia made up of participants from different countries, and from industry and academia.

FishPopTrace is a collaborative project (3 Million € EC contribution for 3 years, 15 participants from several European countries) funded under FP7, with the aim of contributing to more effective enforcement and conservation in fisheries through the identification and monitoring of wild fish populations and traceability of fish and fish products. The project has compiled and integrated data from previous and on-going European fish species traceability projects into a single compatible database. It has tested the utility of various traceability tools (including otoliths, fatty acid profiles, proteomics, gene expression, microarray platform for SNP genotyping) in widely distributed populations of cod, hake, herring and sole. It has produced outstanding outputs, including the development of a population monitoring system based mainly on genetic data and otoliths, that allow assessing population stability in a temporal and spatial framework. One of the most genuine features of the project was the emphasis put on the forensic validation of traceability tools in relation to enduser technology. This would be important for control in fisheries, as well as for traceability of seafood products based on forensic genetic applications. Last but not least, the project has interacted with services of the European Commission,

in particular those involved in the development and implementation of the Common Fisheries Policy (CFP), and has provided science-based input in relevant public consultations. Thus, it has contributed in shaping EU policies, mainly by supporting the introduction of genetic tools in the recently revised EU legal framework for fisheries control and enforcement.

On behalf of my colleagues working in DG RTD Agriculture, forests, fisheries and aquaculture research unit, I would like to convey to the FishPopTrace consortium members our compliments for their commitment and the quality of their work, together with our appreciation for their contribution towards strengthening the fisheries-related European Research Area.

Timothy J. Hall

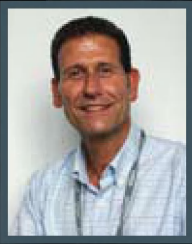


Head of Unit E4: Agriculture, forests, fisheries and aquaculture, DG RTD, European Commission





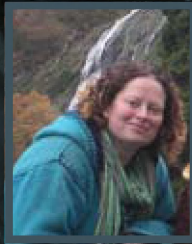
# FishPopFaces



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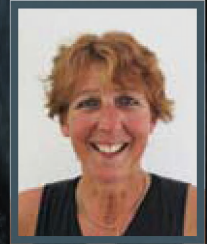
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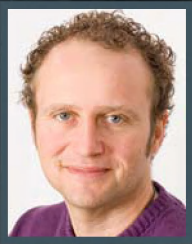
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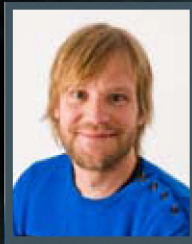
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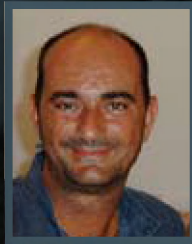
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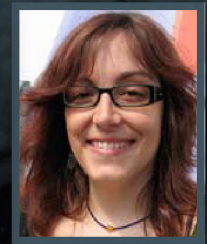
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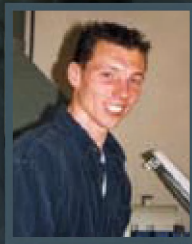
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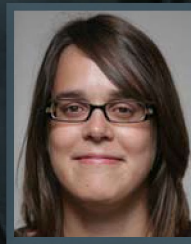
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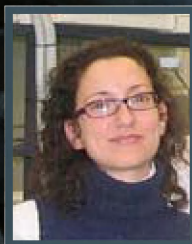
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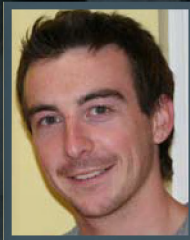


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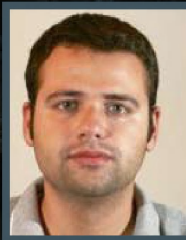




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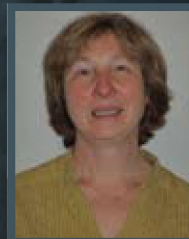
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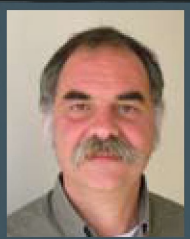
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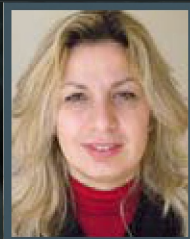
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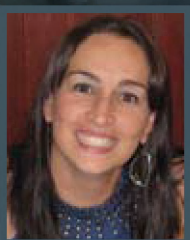
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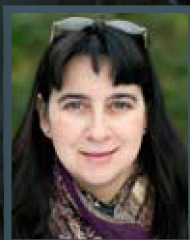
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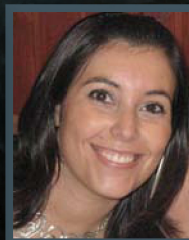
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## Section 1

# Sustainability in fisheries: the facts and challenge

### 1.1

#### Sustainability and the population-level approach

Recent global estimates based on the analysis of 1519 FAO world fisheries over the last 50 years reveals that 366 fisheries' collapses have occurred, representing nearly one fishery in four. More locally in European waters it is estimated that over 88% of stocks are overexploited. In combination with the high estimated levels of IUU activity (e.g. cod in North Sea 2009: reported landings 34,000 t vs. actual removal of 91,000 t), there is a major ongoing challenge to attaining sustainable yields, especially within the context of burgeoning climate change. There is thus an escalating need to develop an integrated and holistic approach to management of natural fish resources. From the establishment of FishPopTrace, we have highlighted the central role that population diversity plays in sustainable utilisation and conservation of exploited stocks. Consortium outputs will improve the traceability of fish and fish products through enhanced understanding of the dynamics, temporal stability and distribution of major populations of four key exploited fish species. Data and traceability tools will be integrated in a forensic framework to enable traceability within the context of enforcement and conservation policy. This in turn will facilitate implementation of effort limitation and regional allocation of quotas based on the relative abundance of respective stocks, not only in wild populations, but also through enforcement based on fish products. Traceability tools can also play a

prominent role in consumer protection both at the species and population level, especially in the face of frequent mislabelling, fraud and to sustain “eco-labelling” schemes of stocks certified in various ways. The framework provided by FishPopTrace will thereby enhance the **Common Fisheries Policy (CFP)** aim to promote sustainability through conservation of genetic resources, as well as in the protection of consumer interests. It is noteworthy that the International Union for the Conservation of Nature (IUCN) recognises **genes** as one of the three primary levels of biodiversity (along with species and ecosystems). Incorporation of population diversity into management instruments and policies will further underpin an **ecosystem-based approach to fisheries** through recovery of declining stocks and associated resilience in feeding interactions. Conservation of fish stocks has a positive effect not only on the economics and sustainability of the industry, but also on long-term sustainability of biodiversity.

**FishPopTrace highlights the central role that population diversity plays in sustainable utilisation and conservation of exploited stocks.**

### 1.2

#### Why the choice of **FishPopTrace** target species?

FishPopTrace employed three primary criteria in the choice of target species, relating to conservation status, traceability issues and representation of marine fish life-styles: Atlantic cod (*Gadus morhua* L.), Atlantic herring (*Clupea harengus* L.), European hake (*Merluccius merluccius* L.) and common sole (*Solea solea* L.). The selected species are all economically important, relatively widespread on a European scale, known to exhibit population structure and fall within EC priority species for enforcement and/or conservation. For example, as reported in the EC-compiled Fishing TACS and Quotas 2011, out of 17 fished zones for cod, only one is estimated to be “exploited at a rate that is consistent with producing the highest catch from the stock in the long-term (subdivision





25-32)", while eleven are either lacking information on stock status, or are fished outside of safe biological limits/advised to cease fishing completely. All target species are vulnerable to a varying degree to overfishing (e.g. IUU estimates of 35% for herring in the Baltic Sea). Finally, the four species have been selected to represent different life-styles ranging from a small **pelagic** (herring), coastal flatfish (sole), to **benthopelagic** (cod) and **demersal** "deep sea" (hake) species. Such a range in ecology is associated with variability in the extent and pattern of population structuring, thereby providing diverse material as proof-of-concept for other marine fish species. The FishPopTrace target species have also been chosen on the basis of their relative distribution, where cod and herring have a more northerly distribution than sole and hake. For the four species different levels of population genetic information are available, where cod has been studied genetically for decades, while relatively little is known about the population structure of hake. The multi-species approach encompasses different geographical scales for tracing individuals, representing a range of policy-led traceability scenarios.

**FishPopTrace focused on generating a new framework for identifying, mapping and monitoring the dynamics of fish stocks to tackle Illegal, Unreported and Unregulated Fishing (IUU).**

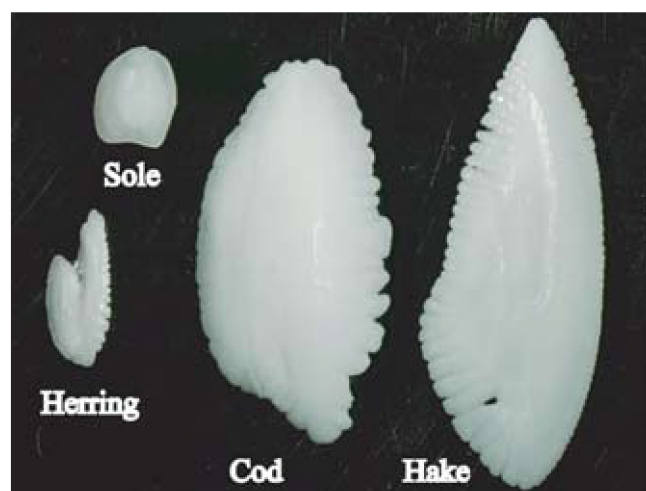
### 1.3 Choice of primary traceability tools: genetics and otoliths

Several tools are available to understand the extent to which fish populations interbreed and to trace back the geographic origin of landed fish. These include physical external tags (some of them logging the position and site characteristics), natural tags (such as size and shape characteristics of the body, chemical content and shape of the otoliths, and the composition of the parasite fauna) and **genetic markers** linked to **DNA**, the genetic material that makes up genes. However, once a fish enters the food supply chain, several tools become less suitable due to food processing for instance. Cooking excludes the use of external features as only the fillet in its processed state is available. Tools for monitoring natural populations and application to fisheries enforcement should therefore meet stringent criteria: they should mirror population identity and stability over an ecological (environmental isolation) and evolutionary (limited interbreeding) scale. Traceability tools should be available throughout the food supply chain from capture to a customer's plate (from ocean to fork) and should be amenable to forensic validation for use in a court of law. Currently available genetic tools for traceability are of limited utility in an international and forensic context. An emerging class of genetic markers are "SNPs" (Single Nucleotide Polymorphisms), representing sites in the **genome** with minute mutations (novel genetic differences) in

the DNA sequence. They are very abundant and widespread. Analyses of SNPs reach hitherto unprecedented levels of population identification, rendering them optimal tools in fundamental biology, conservation and traceability. Importantly, data from SNPs are especially amenable to archiving because they exhibit high reproducibility among different laboratories: newly collected data can then be readily compared with reference data with high reliability and ease. In addition, the identification of SNPs is not only responsive to changes unrelated to environmental differences, but also to **natural selection** ("survival of the fittest"), greatly improving the power to detect the distinct signatures of local and regional groupings.

**Traceability tools should be available throughout the food supply chain from capture to a customer's plate and should be validated for use in a court of law.**

On the other hand, **phenotypic** characteristics are also robust indicators of the origin of fish, though they are not available throughout the food supply chain. Whole fish can be characterised by their appearance, such as body shape, counts of body parts (e.g. scales and gill rakers), shape and composition of otoliths or so-called "ear-stones", and composition of muscle fat. Since the early 1900s fisheries scientists have made regular collections of commercial fish, to monitor abundance, growth and condition. The otoliths collected for aging have frequently been archived, thereby yielding a valuable legacy to set a historical base line.

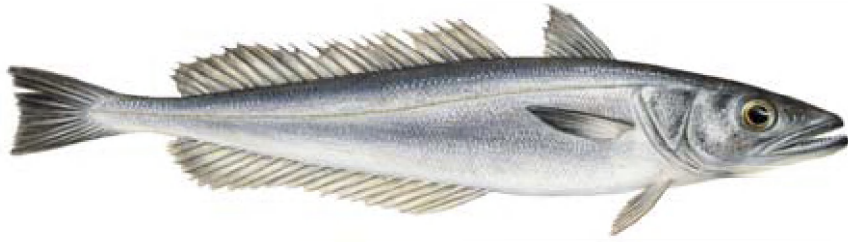


Otoliths or "ear-stones" of FishPopTrace target fish species.

These calcified structures grow in layers, similar to tree rings, throughout life. Seasonal and yearly increments are deposited, matching the age of the fish. But there is more. The width of each increment represents the growth history of each individual. The chemical composition of each increment reflects the local water mass, forming a permanent record, a kind of logbook, of where a fish was born, and where it has lived prior to capture. Chemical

## The *FishPopTrace* target species

reproduced with permission from the Scandinavian Fishing Yearbook



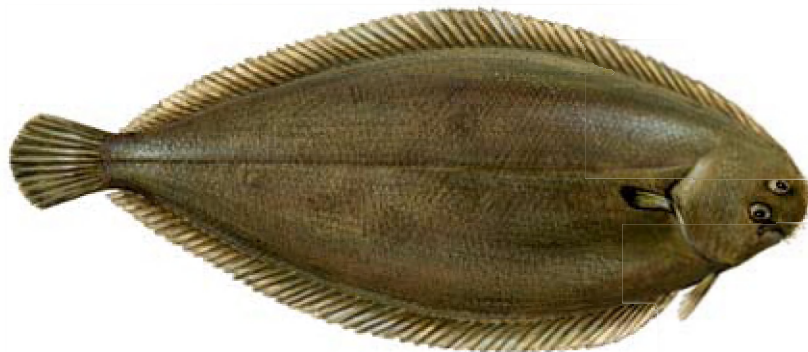
European hake (*Merluccius merluccius* L.)



Atlantic herring (*Clupea harengus* L.)



Atlantic cod (*Gadus morhua* L.)



Common sole (*Solea solea* L.)



methods (such as inductively-coupled mass-spectrometry (ICPMS)) measure otolith chemical composition, in order to determine the origin and fishing ground. The approach is particularly interesting as a tool to validate catch records. In this way, fish from different areas can be distinguished from each other. For example, herring in the Baltic have many separate spawning grounds and are fished by many fleets. It is important to be able to assign the catches to management stocks for assessment of the status of the fishery. Differences in the concentration of elements in the otolith can distinguish between some groupings of Baltic herring, especially between those caught in Bothnian Bay, the Gulf of Finland, and the south western Baltic (Rügen).



A comparison of the “average” otolith shape from different hake populations in European waters.

Otolith shape is another characteristic that distinguishes fish species and populations. Modern imaging techniques are used to photograph and analyse shape differences – such as the pattern of scalloped margins or notches. The example above compares the “average” otolith shape between different hake populations caught in the North-East Atlantic Ocean and Mediterranean Sea. In combination, otolith shape and chemical composition are valuable tools to trace fish to their spawning and fishing ground. Interestingly it is possible to trace back in time the pattern of fishing and fish populations over past decades in the archived collection of otoliths housed at fisheries institutes world-wide. In many cases, small traces of fish tissue with its DNA remains attached to these otoliths. DNA can then be isolated and characterised genetically, similarly to recently collected tissue – a practice followed in FishPopTrace. This provides us with a unique set of tools to look back in time at how commercial fish populations were distributed, and to see whether changes in policy and management have had an impact on the characteristics of fish populations.

## 1.4 Stability of primary traceability tools

Any traceability system needs to take into consideration whether the method is resilient to changes over time that may alter the accuracy and precision with which fish and fish products can be traced. We therefore need to establish the time scale over which the developed tools work, i.e. not just for the populations in specific areas as they are now, but also in the future. Conversely, it is of interest to establish whether or not temporal changes in populations can be assessed using the marker approaches developed in FishPopTrace.

Potential changes include long term changes in the sizes of local populations, e.g. in response to global change and exploitation. Are we for instance able to determine if global change leads to some populations gradually being replaced by others better suited for living at higher temperatures? Another type of change may be geographic, if individual populations change spatial distributions, for example in response to environmental changes to spawning or feeding grounds, or if their relative contribution to mixed-stock fisheries change. All major changes need to be taken into consideration for sustainable management of resources and for preservation of biodiversity. FishPopTrace has examined such issues. The approach is to assess the stability over time (temporal stability) of the developed population markers back in time based on analyses of a series of samples collected on different dates, and to model which levels of future change can be assessed using the markers at hand.

Time series of tissue samples analysed with SNP makers generally show stability, indicating that the genetic signatures of populations examined have changed little over two-to-ten year periods. Temporal stability is a basic assumption for the evolutionary models underlying the FishPopTrace traceability framework, and this result is therefore an important step. It also shows that the developed traceability tools are predicted to yield statistical power over future decades. The FishPopTrace traceability tools have also proved highly informative for addressing potential changes in migratory behaviour, both in terms of annual changes in where populations feed and whether fisheries exploit different stocks disproportionately during different times of the year, as well as for assessing the persistence, and dynamics, of local populations.

For otoliths, the temporal studies indicated overall stability, but also some differences between samples from different dates within locations that should be taken into consideration when developing tools for traceability and determination of management units.



The observed temporal stability, and conversely lack of short term change in population structure, is an important step in validating the traceability approach as reliable in time and space. Needless to say, future changes in population structure and the spatial distribution of individual management units are difficult to predict. Nonetheless, FishPopTrace has laid the basis for a monitoring framework

that can be developed and extended in years to come. Historical samples from fish are generally available from archived collections of otoliths and scales, offering a large potential for analyses on a population basis. Exploiting such opportunities to address population change is a priority – and we now have established the analytical framework for doing so. The technological advances are also progressing rapidly, enabling ever more detailed analyses of the functional properties associated with the observed genetic structures. Apart from the obvious potential for detailed monitoring of the origin of catches to maximise overall sustainable yield in management and to tackle IUU and consumer fraud, additional applications include monitoring genetic effects of fish farm escapees, population specific responses to climate change and monitoring and evaluating spatial planning activities, e.g. in connection with the potential effects of Marine Protected Areas and area closures.

...tests of fish and fish products will be implemented in certified laboratories close to main fish trading centres to provide information for fisheries departments, customs & excise and wildlife management.

## 1.5 Use of Traceability Tools in the Real World

The species-specific **panels of SNP markers** can be implemented by control and enforcement authorities for essentially two purposes: i) as a standard tool to identify source populations (assignment) and ii) to verify the claimed population of origin from landed fish through to processed fish products, in the 'ocean to fork' sense. Implementation may be broad. In the first case it is envisaged that fisheries management will take greater account of biological structure as opposed to the current arbitrary geographic structure. It is also expected that the subtle differences between adjacent stocks will be based on **adaptive markers**. Second, tests of fish and fish products will be implemented in certified laboratories close to the main fish trading centres to provide information to government agencies such as fisheries departments, customs & excise and wildlife management. Traders, processors and retailers will have access to technologies for self-certification. Finally court cases will have access to unambiguous scientific information. The otolith tools will enable traceability back to the catch trace back to the catch and spawning location of landed ungutted fish to complement available **molecular tools**; this is particularly useful when spatial scales of analysis are very small and hence the genetic differences between fish are limited.

...additional applications [of traceability tools] include monitoring the genetic effects of fish farm escapees, population specific responses to climate change and evaluation of spatial planning activities, e.g. Marine Protected Areas and area closures.



## Section 2

# Tackling fish piracy and fish fraud: scale and impact of the problem

### 2.1

#### Fish Piracy and Consumer Fraud

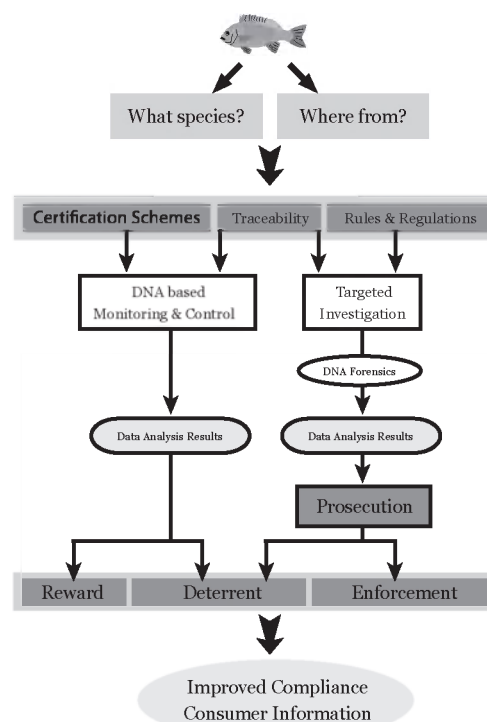
IUU fishing and fish related piracy undermines fisheries policy or management strategies and can have considerable ecological and socio-economic consequences. As an illegal industry, IUU fishing has an estimated annual global value of €10 – 20 billion, while legally conducted fishing has an estimated annual global value of €55 – 60 billion, and prior to 2010, €1.1bn worth of that illegal fish was imported into the EU every year. Such is the scale of IUU fishing that it can lead to the uncontained depletion of fish stocks. Genetic analysis and the use of some phenotypic markers can be used to detect, deter and prosecute against cases of IUU fishing, helping to promote fishery sustainability and socio-economic stability. Falsely labelled fish and fish products, falsely declared origin of catches, and distinctions between wild and cultured fish bred in aquaculture schemes can all be identified through the use of traceability tools. Such tools can support certification schemes and promote traceability. Existing regulations can be enforced through prosecution based on genetic evidence using forensically validated analysis. Application of traceability tools in this context has the effect of rewarding those in compliance with regulation through certification schemes, deterring potential fraudsters, and punishing regulation offenders. Thus an increased level of overall compliance is achieved and improved consumer information pervades through controlled product certification schemes.

### 2.2

#### The Need for a Legal Framework- Validation of Traceability Tools

The analysis produced by FishPopTrace helps us to understand how fish populations are structured and allows us to look for ways of identifying where fish come from. These techniques can be developed into a series of applied tools for forensic analysis and tracing fish and fish products from ocean to fork. However, before this can be achieved, **method validation** must be performed to demonstrate that laboratory testing is accurate and reliable. Each of the primary tools developed was considered, examined at each analytical stage and assessed for its suitability for testing fish and fish products on a routine basis in laboratories across Europe.

The method validation stage focused primarily on understanding the strengths and limitations of the DNA and otolith analysis techniques. Scientifically speaking, this involved assessing the level of variation among samples from the same population when tested under different conditions



Flow diagram for forensic analysis

and comparing these data with the level of variation among populations. This validation process allows us to have confidence in the accuracy and interpretation of test results in real life.

**The method validation stage focused primarily on understanding the strengths and limitations of the DNA and otolith analysis techniques.**

For example, one of the studies performed as part of the DNA validation was to consider whether fish DNA could still be tested after it had been processed along the food supply chain. To do this, we tried to analyse DNA markers in processed fish samples that had been frozen, salted, cooked and canned. DNA tends to become fragmented into small pieces if it is heavily processed which may prevent the DNA test from working. Different methods of recovering DNA from fish were also explored. We found that with certain DNA recovery methods, DNA from fish products could be

### Enforcing the law in fisheries:

1. A recent study found that 25% of products labelled for sale as cod or haddock in fishmongers and take-away restaurants were an entirely different species deliberately mis-labelled to achieve a higher market value.
2. A fisherman who declared his cod catch as originating in the Baltic Sea was suspected of a fraudulent declaration. Following a genetic analysis of specimens from the catch in question, it was proven that the catch had in fact originated in the North Sea. A fine of 50,000 Dkr was imposed and the catch was confiscated.
3. Escapees from aquaculture schemes pose a potential threat to the fitness of wild populations when they mate and reproduce with wild fish. Norwegian authorities in collaboration with the Institute of Marine Research at Bergen routinely conduct genetic sampling at salmon farms following a suspected fish escape. The farm of origin can be identified through the comparison of genetic material sampled from an escapee with the characteristics of the fish in each of the sampled farms.

analysed, regardless of the processing technique applied. This is an important result, as it allows us to extend the range of fish products that can be tested.

We found that with certain DNA recovery methods, DNA from fish products could be analysed, regardless of the processing technique applied.

In addition to having confidence in testing different types of sample, it is also important to know how test results may differ when generated by different members of staff within a laboratory, by different machines, or by different laboratories. The validation work also included a series of controlled tests in which the same samples were

analysed independently by different people. This involved sending otolith and DNA samples between laboratories in Norway, Spain, Denmark and the UK. The results have provided essential data on the reproducibility of different techniques across Europe.

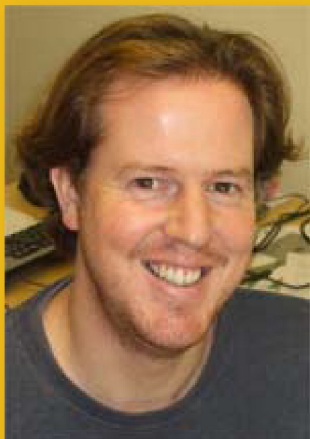
### 2.3 The Policy Relevance of a Forensic Framework

The results of the method validation allow the project to present findings within a forensic framework, delivering applied tools directly to stakeholders involved in the fight against IUU fishing. Inevitably it has not been possible to develop validated tests for every question relating to cod, sole, herring and hake in European waters, but by demonstrating the transfer of technology from primary research through to formal application, the method validation process within FishPopTrace provides a series of examples of how research tools can be converted into monitoring and enforcement applications. The challenges now are to extend the range of tests, both geographically and across additional species, to engage with accredited testing laboratories able to employ the tests around Europe and to encourage the use of geographic origin testing by enforcement and regulatory agencies.

Validation of methods allows presentation of findings within a forensic framework, delivering applied tools to stakeholders involved in tackling IUU fishing.



## Fisheries forensics - an interview with Dr Rob Ogden, TRACE wildlife forensics network



### 1. What is fisheries forensics?

Fisheries forensics is the use of scientific analysis to support investigations into illegal fishing and fish fraud. It usually involves the identification of fish species, or their place of origin.

### 2. Why do we need to have tools that comply with forensic standards?

Any scientific method that is used to provide evidence must have been tested to demonstrate that it produces accurate, reliable results, under a range of different conditions. Ultimately, people may go to prison based on the forensic evidence submitted, therefore just like human forensics, it is absolutely essential that the results are correct.

### 3. Isn't forensic testing just too expensive to use on fish?

Forensic testing does cost money, but there are ways to limit costs and it can actually save money too. Using the tests to simply monitor a fishery, rather than to provide forensic evidence, generates the same type of data for a fraction of the cost, allowing investigators to identify a problem before investing in forensic analysis. In cases where forensic evidence is produced, defendants often plead guilty, massively reducing the legal costs of running a full trial.

### 4. Does anyone use fisheries forensics already?

There are a number of laboratories around the world where forensic techniques are being developed. In The USA and Australia, government authorities already use forensic species identification and routinely prosecute people suspected of illegal fishing. However, FishPopTrace is the first major project that has produced tests to identify the geographic origin of marine fish for fisheries enforcement.

### 5. How does fish forensics differ from human forensics?

Human forensics usually focuses on the identification of individual people, or linking people to trace evidence and crime scenes. In fisheries forensics, although the lab techniques are often similar, the issues are generally much broader and involve many different species. This presents challenges for forensic scientists who have limited resources to address the many different questions asked by enforcement agencies.

### 6. Is fisheries forensics the answer to IUU fishing?

Hopefully it will be one of the answers. Forensic sample analysis can provide extremely powerful evidence, but it is rarely used in isolation. Investigators need intelligence data to help identify potential issues, which is where trade monitoring can help, and there are many other technologies, such as vessel monitoring systems and digital forensics that support the detection and prosecution of illegal fishing. At the end of the day, enforcing compliance will always remain a last resort; deterring IUU fishing and encouraging compliance must be the way forward. The good thing about FishPopTrace is that many of the techniques can be also be used to demonstrate authenticity, promoting responsible fishing and supporting effective fisheries management.

## Section 3

# From the high seas to the laboratory: *FishPopTrace* in action

### 3.1 Sampling

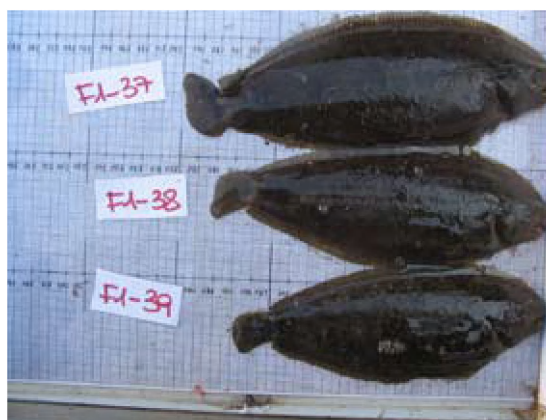
The ultimate goal of FishPopTrace was to provide applications in support of fishery management, including control, enforcement and traceability. However to get there, FishPopTrace started out as a research project, and asked the question: “Can we detect distinct populations of cod, hake, herring and sole in European waters?” Due to the apparent openness of the oceans, migration over long distances of many marine species, and dispersal of eggs and larvae over vast areas, the notion that marine fish tend to breed randomly across their geographic distribution, and do therefore not show population structure, prevailed for a long time.

The notion that marine fish do not show population structure prevailed for a long time.

If true, this would hinder our ability to delineate populations; FishPopTrace aimed to identify distinct features (genetic and phenotypic markers) of target fish populations. These markers should ultimately allow the assignment of fish to their population of origin. Fortunately, in recent years, research has shown that many marine fish are indeed subdivided, occasionally over small spatial scales. This sets a robust rationale for FishPopTrace, and our question can be refined to “can the population structure of cod, hake, herring and sole be determined at a scale that is useful for fishery managers and control authorities?”



On-board analysis of Sole, *Solea solea*



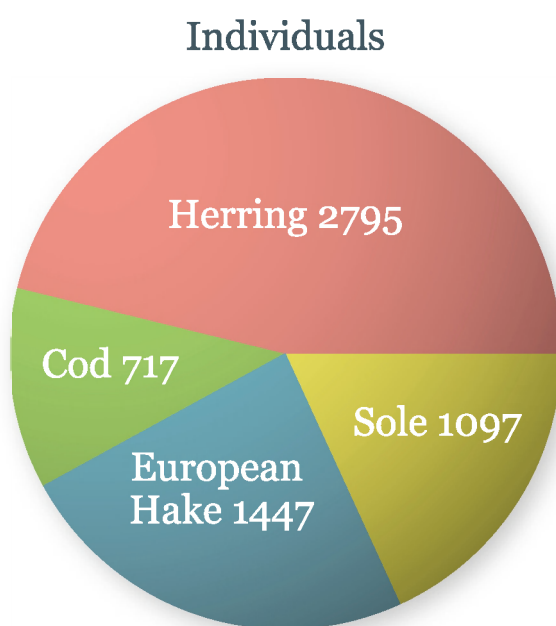
In recent years, research has shown that many marine fish are subdivided, occasionally over small spatial scales.

To answer this question scientifically, careful planning and consideration was required, starting with the collection of fish at sea before analysis in the laboratory. The collection, or correctly speaking the “sampling”, of fish for scientific purposes is not merely “fishing in the sea”! An elaborate sampling



strategy must be developed to maximise the probability that all potential populations of the target species are included across the area under investigation. To this end, the FishPopTrace sampling team designed species-specific sampling schemes covering the European seas, taking into account the species life-history traits (e.g. distribution, habitat preferences, migration behaviour, reproduction, age at maturity), population dynamics and structure (e.g. nursery and feeding areas, age classes) and environmental features (depth, bottom geomorphology, water mass dynamics, chemical and physical parameter variation). Standardisation procedures were developed from on-board species identification and tissue collection to documentation, storing, archiving and cataloguing in laboratories and in a central database system. In addition, sampling progress was monitored online on a publicly available sampling map. During the sampling effort more than 17,000 individual fish were sampled and catalogued for analytical purposes.

Standardisation procedures were developed from on-board species identification and tissue collection to documentation, storing, archiving and cataloguing, and on a central database.



Quantity of individual fish collected during the sampling phase.



## Sampling for tracing: keeping a file on a fish.

Interview with Diego Galafassi, a Brazilian MSc graduating student in Environmental Sciences.

Q 1. How do you keep a file for tracing a fish?

The FishPopTrace sampling tools can play an important role in advancing the tackling of IUU fishing in a context of increased access and connectivity of fish markets. The “sampling production process” of FishPopTrace started with the development of standardised methods and procedures to collect and keep track of samples uniformly across sampling partners and European seas. Following protocols created within the consortium, scientists went on board scientific and commercial fisheries boats for the collection of individual fish. The extraction of tissue samples from the individual fish was performed on board, in some cases minutes after being caught, due to special conditions required by subsequent analyses. Individual samples were then catalogued according to their species and their fishing area in the FishPopTrace online database.

Q 2. How do you assure the availability and reliability of trace data you catalogued?

The FishPopTrace online sampling database ensured that all sampling partners involved could merge in-depth information on each individual fish. Each partner independently uploaded sample details to the database that would then generate a unique ID for each sample. This in turn assisted the straightforward communication tasks among research groups and the sample end-users (“FishPopTracers”).







## An interview with Eveline Diopere, second year PhD student at the Laboratory of Animal Diversity and Systematics; Katholieke Universiteit Leuven.

Q1. What is your current involvement in the FishPopTrace project?

As a second year PhD student I have been developing and analysing SNP markers for sole. It's a fascinating task to look through all these mutations (small genetic differences) in the whole genome. There is no shortage of SNPs, but selecting the right ones is a challenge. We had previously obtained samples from all over Europe for inclusion in the development. Some of them I collected myself on a research cruise in the North Sea. Ultimately we genotyped some 30 sample sets from all over Europe and even from aquaculture.

Q2. What are the steps involved in the development of these genetic markers ?

There are a number of steps in the development of SNP markers. Firstly, messenger RNA was isolated from individual samples. Then the RNA was converted into a copy DNA library (cDNA library) using a special form of the polymerase chain reaction (PCR). Next the cDNA was sequenced using Roche 454 next generation sequencing (NGS) technology that generated 348,042 short DNA sequences. These sequences were then assembled into larger units representing full or partial genes and compared genetic variation among individuals by aligning the sequences together. Next we identified sites in the DNA alignments that differed among individuals or geographic localities and then we outsourced to an external company to prepare a SNP chip with 1536 SNPs and genotyped the 30 sample sets on an Illumina Golden Gate platform. Finally, we analysed the genotypes and performed validation.

Q3. What is Bioinformatics and why is it needed in fisheries research ?

The massive amount of data generated through new generation sequencing techniques requires appropriate analytical methods, which are implemented using specialist software applications. Initially I was overwhelmed by the sheer volume of data. Fortunately I've been learning a lot from colleagues here in Leuven and from other FishPopTrace partners. It's a challenge as the field is developing very fast but with time I'm gaining the expertise and becoming more confident.

## Section 4

# Key findings from *FishPopTrace*

### 4.1

## Genetic signatures and population mapping

### 4.1.1

## A new source of genetic markers for traceability tools

Among the most striking scientific results is the provision of several hundred novel genetic markers in, hake, herring and sole. Although these fish represent a major part of the European catch, many aspects of their biology remain unknown. This holds also for the number, location and independence of biological populations. The lack of high resolution genetic data has complicated sustainable management, which should rely on the basal biological independent units rather than geographically defined “stocks”. However, access to new genetic methods, the so-called **next generation sequencing**, has changed the picture in a matter of just a few years. From a dozen genetic markers a few years ago, we now have knowledge about thousands of small genetic differences (genetic variation) at numerous genes, allowing the design of hundreds to thousands of new genetic markers. The unique combinations of the variation make it feasible to assign the fish to specific populations and in some conditions to identify unique individuals.

Among the most valuable scientific results is the provision of several hundred novel genetic markers in hake, herring and sole.

### 4.1.2

## Application of traceability tools to real-life policy issues

We have applied our primary traceability tools: DNA SNP analysis and otolith **microchemistry** and **morphometrics** (form, shape) to test their power for tracing fish from the four species back to population/area of origin. Such information

provides a framework for a pan-European traceability database for fish and fish products to be applied for defining management areas, fighting IUU and for assuring safe, healthy and sustainable fish products for the European consumer. Our focus has been on the traceability of populations, but each test can also incorporate a species identity marker. The latter is important because over 60% of fish products consumed within Europe derive from imports outside the EU. Any traceability system that allows simultaneous testing of species and provenance will have significant global consequences.

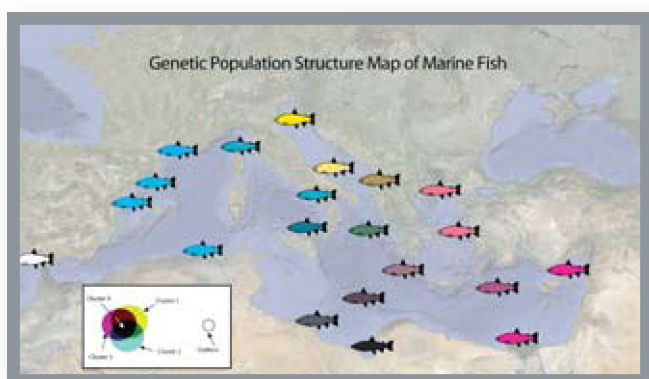


FishPopTrace Partner, PhD student, Morten Limborg from DTU-Aqua in the laboratory

We can now correctly assign fish to populations from more areas and with higher certainty than previously possible, reaching standards which can be used in a court of law. Based on use of the most highly distinct genes among populations we have already developed “minimum **assays** with maximum power” with from 10-30 SNPs. These assays have been developed to target some of the most pertinent needs for traceability tools in European fisheries management. For example, we now have fast, efficient and forensically robust tools to discriminate between cod from Canada, North Sea, Baltic Sea and Northeast Arctic populations, between North Sea and North Atlantic herring, between sole from the Irish Sea and Thames and between hake from the Mediterranean and Atlantic areas.

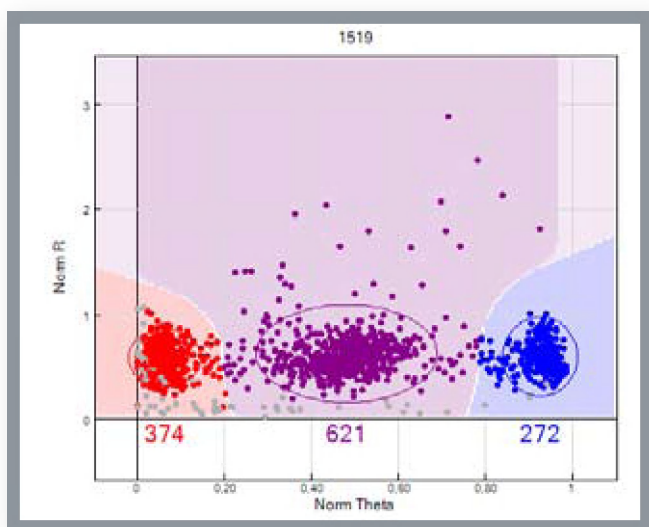


We can now correctly assign fish to populations from more areas and with higher certainty than previously possible, reaching standards which can be used in a court of law.



Schematic representation of the genetic population structure in a marine fish inhabiting the Mediterranean. Genetic similarity is elucidated through colour similarity. Major genetic groupings are assigned their own basal colour. Mixed genetic groupings are shown as a mix of the appropriate colours.

The core approach was the development of a “**SNP chip**” for each of three species: sole, hake, and herring. (Canadian researchers had already created a SNP chip for cod to assist in aquaculture research). These DNA-covered microchip-like devices enabled testing the identity of 1536 possible SNPs for each group of individuals from a specific population. Once all individual fish were characterised using the , the frequency of each SNP variant was determined, creating a potentially diagnostic pattern, or “population signature”.



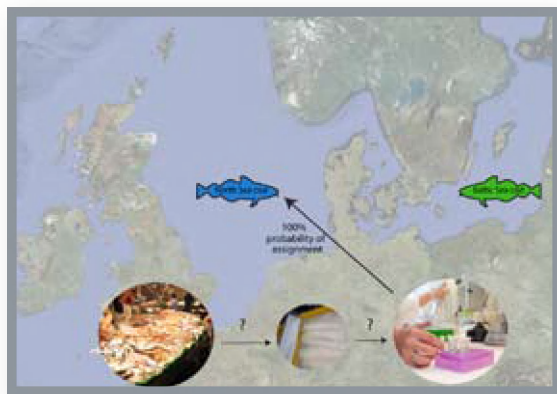
Example of a successful SNP genotyping with the Illumina GoldenGate assay, in which the three clusters of data points identifying the two **homozygote** and the **heterozygote** genotypes are well separated and clearly identified.

Once all individual fish were characterised, the frequency of each SNP variant was determined, generating a potentially diagnostic pattern, or “population signature”.

The FishPopTrace consortium has investigated questions relevant to fisheries and to European consumers. A common concern for the latter is the source of Atlantic cod. Fish from the Baltic are worth less because they tend to have lower quality flesh and higher levels of contaminants. The cod team used its SNP chip to examine, without knowing the source, samples from both locations. By looking at 20 SNPs, the researchers correctly identified the origin of each individual fish. With just 10 SNPs, 96% of the unknown samples were still correctly identified. The SNP chip for sole (*Solea solea*) also performed well. This flatfish attracts the highest price of the four species and is severely overfished in Europe. Only two of the twelve fishery areas within European waters are considered to be fished within safe biological limits. A key question is whether sole from the North Sea can be distinguished from populations in the Mediterranean, which are considered to be of higher quality. Just one SNP could reveal which sole was which with 96% accuracy.

The European hake (*Merluccius merluccius*) is a species managed by differing regulations. For example, Atlantic hake must be 27 cm long to be legally landed, while in the Mediterranean, vessels can catch hake that are only 20 cm in length. Fishing vessels in the Bay of Biscay are known to occasionally catch smaller fish, which are then misreported as originating in the Mediterranean. Since the removal of pre-reproductive small individuals may undermine efforts to promote sustainability, it is of considerable value to distinguish hake from the Atlantic and Mediterranean. Our findings showed that just 10 SNPs could reveal the origin of hake with near-perfect accuracy.

Long-considered a sentinel of studies on marine fish population structure, the most challenging test case was perhaps Atlantic herring, (*Clupea harengus*). A geographically wide-spread and abundant species, with complex seasonal migratory behaviour, herring within European waters typically display only minor and sometimes transient genetic differences among populations. By applying the SNP chip to herring, however, it was possible to accurately distinguish many populations, including those in the northeast Atlantic and North Sea, a goal important to a joint EU - Norwegian fishery management plan. The flexibility of combining differing numbers of SNPs on a allowed the identification of some herring populations at smaller scales, even around the United Kingdom, where there is substantial misreporting of catches.



To illustrate the concept of population assignment- the act of tracing an individual back to the most likely source. An unidentified fish specimen is routinely sampled at a fishmonger stall. A genetic analysis reveals that the sample is cod and can be assigned with certainty as originating in the North Sea.

Thus as far as the new SNP markers are concerned, it has been possible by varying the numbers used on a SNP-chip, to assign individuals back to their source population across different geographic scales with high levels of certainty and reproducibility. Such outputs are especially significant since previous types of genetic markers either detect levels of population differences that are too low, or there are inherent difficulties in comparing data generated from different laboratories. The use of a marker system such as SNPs, which is essentially based on the presence or absence of large numbers of single genetic variants means that data can be compiled from sources in a much more reliable and high throughput way. The approach thereby enables the generation of baseline and ongoing additions for subsequent genetic monitoring. Moreover it is imperative that any such tools can be used in a legal context, necessitating forensic validation. This has been achieved for SNP markers within FishPopTrace across a range of policy-driven IUU scenarios.

By varying the numbers of SNPs used on a chip it was possible to trace individuals across different geographic scales with high certainty and reproducibility.

#### 4.1.3 New tools for understanding impacts of environmental change

In addition to improved traceability of fish populations, the DNA code of thousands of genes now makes it possible to link the function of genes with the **phenotype**/external characteristics of

each fish: We have zoomed in on genes that are involved in adaptation to the local environment (under natural selection), which also has vastly increased our ability to identify populations. The long-awaited link between natural selection and the genetic blueprint has now become possible in natural marine fish populations. Such an advance is significant because scientists are poised to move beyond the mere detection of genetic differences among marine fish populations, to the identification of how and why such differences relate to their survival in stressful environments, so-called local adaptation. Such information can inform managers on the ability of populations from specific localities to adapt to natural and man-made changes, including over-fishing, contaminants and climate change.

In addition to improved traceability, the DNA code of thousands of genes now makes it possible to link gene function to biological characteristics of each fish.

The long-awaited link between natural selection and the genetic blueprint has now become possible in natural marine fish populations.

#### 4.1.4 SNPs and future priorities within IUU and fisheries management

The tools developed and tested within FishPopTrace provide a giant leap forward in terms of tracing fish and fish products. Here we provide new, more powerful, fast and cost-effective tools as well as the baseline data on a pan-European scale for four economically and culturally highly valuable species. We are now able to trace the fish from the four species from ocean to fork in all types of products. In some areas, however, fish from different populations meet and mix. Establishing mixture signatures for these areas is a high priority. Likewise, although our sampling effort has been substantial there may be populations which have not been fully sampled. However, due to the flexibility of our approach, more data can be added to the database with ease, as there is little need for calibration of SNPs compared to previously applied



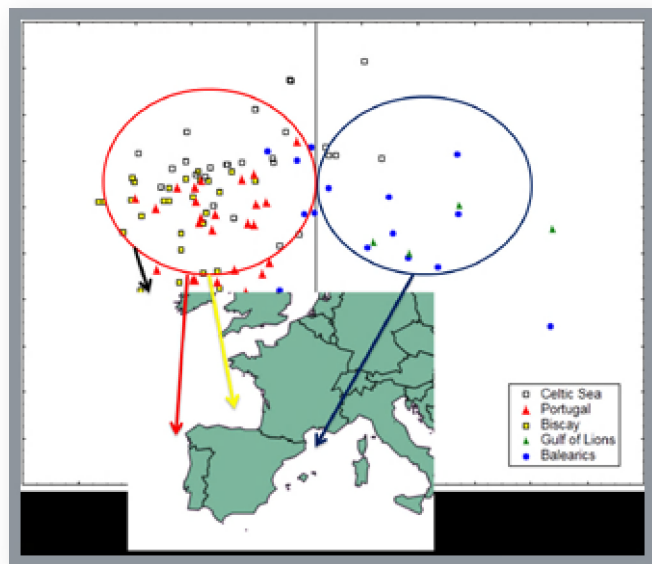
genetic markers. Finally the implementation of the methods into practical fisheries management and control and enforcement should be a priority for the project members and for the EU.

We are now able to trace the fish from the four species from ocean to fork in all types of processed products.

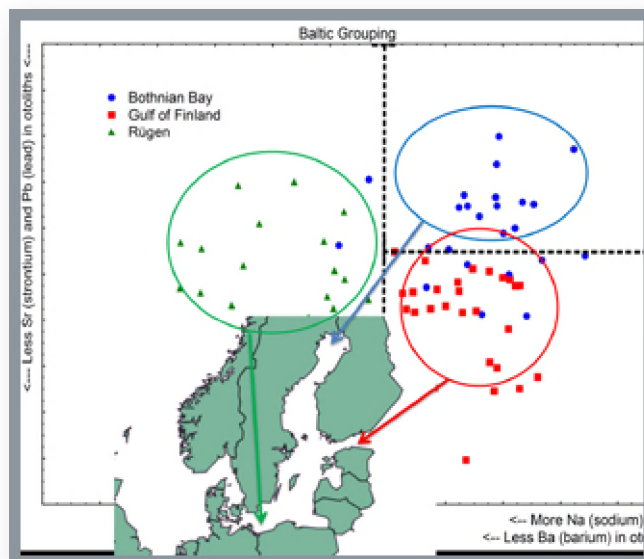
#### 4.2

### Otoliths: The fish ear stone as a precious gem for traceability

Sole and hake are high value species, fished intensively in the Atlantic and Mediterranean. The fishing regulations are complex and diverse for these areas. In particular there is a difference in the legal landing size for Atlantic and Mediterranean sole and hake. So how can we be sure that the fish presented are legal? This is a difficult question for those places where there is a good chance that fish from either sea can be available. In this scenario, we found that the otoliths of sole from the Mediterranean were higher in the chemical elements barium and strontium than sole from the Atlantic, likewise for the otoliths of hake. Using these features, we were able to distinguish between fish from potential sources in the Atlantic and Mediterranean with nearly 90% accuracy.



Identifying where hake come from using otolith microchemistry : Separation of fish from different populations, based on the otolith elemental “signature”. The symbols represent individual fish. Fish with more similarity in the chemical composition of their otoliths are grouped closer together, and those with very different otolith compositions are grouped further away from each other. In the case of Atlantic and Mediterranean populations of hake, the position of individual fish along the horizontal axis is mostly determined by otolith sodium (Na), strontium (Sr), and zinc (Zn). The position of individuals along the vertical axis is mostly determined by the amount of lithium (Li), barium (Ba), and copper (Cu) in the otoliths.



Identifying where herring come from using otolith microchemistry: Separation of fish from different populations, based on the otolith elemental “signature”. The symbols represent individual fish. Fish with more similarity in the chemical composition of their otoliths are grouped closer together, and those with very different otolith compositions are grouped further away from each other. In the case of herring in the Baltic, the position of individual fish along the horizontal axis is mostly determined by otolith sodium (Na) and barium (Ba). The position of individuals along the vertical axis is mostly determined by the amount of strontium (Sr) and lead (Pb) in the otoliths.

Using otolith chemical analysis, we could distinguish between fish from the Atlantic and Mediterranean with nearly 90% accuracy.

Otolith shape is another characteristic that differs between fish species, and between the populations within each species. Modern image analysis techniques are used to photograph, digitise, and analyse subtle shape differences – such as the pattern of scalloped margins or notches.

Together, otolith shape and composition are valuable tools that can be used to trace fish back to their home area.

## Section 5

# Into the Future: Exploring the Value of Novel Traceability Tools

### 5.1

#### Testing Proof of Concept with New Approaches to Traceability

Besides developing applications based on genetics and otolith analysis for fishery management, FishPopTrace also explored the value of “novel tools”. Novel tools are new ways to analyse fish products that could complement more established methods (genetics, otolith shape and microchemistry) and help to trace individual fish to the place where they have been fished. To this end, two major goals were set. The first one was to develop a DNA chip, a miniaturised tool with improved sensitivity, speed, and costs, based on a sophisticated technology that uses a special glass slide with DNA “sensors” spotted onto it. A pilot study on cod individuals selected from different geographic regions showed that this new technology is very promising but requires further optimisation.

Besides developing applications based on genetics and otolith analysis for fishery management, FishPopTrace explored the value of “novel tools”.

The second goal was to find additional traceability markers. The idea is that environmental differences, such as water temperature, or the nutrients taken up by fish, might influence specific features locally, making it possible to trace where the animal was living. The effects of local food or climate we experience ourselves: Even in today’s globalised world, human diets vary according to locally available products. Who has not heard of the benefits of a Mediterranean diet? Similarly, we might be more tolerant of cold or heat depending on the time we had to adapt to the environment, and this is only partially determined by our genes. Following this line, we explored the environmental effects on fish and their possible use as traceability markers in three directions.

### 5.2

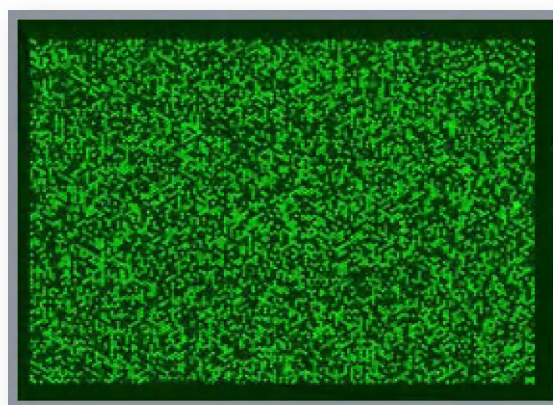
#### Fatty Acid Profiles

First, we looked at the composition in fatty acids of fish flesh. Two teams analysed lipid profiles of muscle and heart tissues in fish samples collected at different geographic locations for four species. Over 600 hake and sole from different Atlantic and Mediterranean fishing areas were examined and over 220 specimens were processed for cod and herring, originating from the English Channel, North Sea, Baltic Sea, and Barents Sea. Statistical analysis of the fatty acid composition in the tissues showed a clear differentiation of geographical populations, both within the Atlantic or the Mediterranean as well as between these two basins.

### 5.3

#### Gene expression and microarrays

Secondly, we examined which genes are switched on or off, that is expressed (active) or not, in the muscle tissue of hake and sole living in different areas. A DNA microarray was developed to analyse 14,898 genes in the muscle of hake collected from six different areas, ranging from the North Atlantic to the Mediterranean. We demonstrated that for certain areas (North Sea, Aegean Sea) it might be possible to trace back fish to their geographic origin measuring the expression of few marker genes.



Laser-scanning image of hake gene expression DNA-microarray. Each spot corresponds to a different gene. Approximately 15,000 genes are represented on this array. The brighter the spot, the more expressed (activated) is the corresponding gene.



## 5.4

### Protein Expression – Proteomics

Thirdly, we looked at protein expression. Proteomics is the analysis of the entire set of proteins expressed by the genome of a living cell, tissues, organs or whole organism. Recent advances in molecular biology have greatly facilitated the development of powerful tools for proteomic analysis. A proteome scan approach based on 2-dimensional fluorescence difference gel electrophoresis (DIGE) technology generated thousands of protein markers that allow the identification of different hake populations. Among the identified proteins, some that reliably assigned individuals to their population of origin have been selected to be used as biomarkers for traceability of hake fish products. Currently an antibody array specific for hake protein markers is under development, to make the assignment of individuals to the original stock feasible as a diagnostic routine.

“Antibody arrays” specific for hake protein markers is under development, to allow assignment of fish to the original stock as a diagnostic routine by end-users.



2D “Coomassie” stained gel that corresponds to the proteome map for liver hake tissue. Circled and numbered spots are proteins that are differentially expressed between two population samples

## 5.5

### Expanding the Traceability Tool-Box

The results obtained exploring novel tools for traceability are very exciting and hold promise to further advance our ability to identify the stock/population of origin for individual fish. These new methods have the potential to complement, and in some cases, even supplement more established approaches, as they rapidly respond to the environment where the fish was living just prior to capture, and therefore provide information on geographic origin. It should be noted, however, that this was an exploratory exercise in FishPopTrace, and these novel tools need to be validated fully before being used for control and enforcement purposes.

Dr Elena Gonzalez, working in FPT as a post-doctoral scientist at the Department of Biochemistry and Molecular Biology IV, Facultad de Veterinaria, Universidad Complutense de Madrid

How was your experience as a young scientist in FishPopTrace?

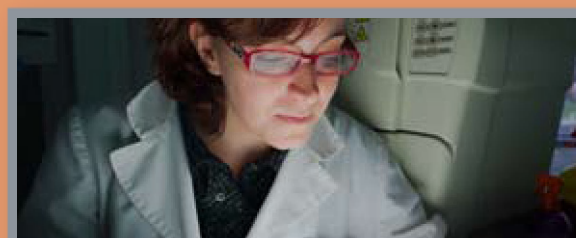
I did my masters degree and doctorate in population genetics of marine fishes at the Natural History Museum of Madrid, so when I was offered to collaborate in this European project I just jumped onto it without hesitation! - I thought it would be a good opportunity to promote and expand my scientific career in different ways...and I was right. Within the project, there were researchers and other postdocs and students that come from many backgrounds that helped us (and me) to approach scientific challenges with combined expertise. For me it has been a great way to learn and expand myself, both in science and the way you approach solving scientific problems. I personally would advise to also take chances when surrounded by others with knowledge beyond your expertise. This project has achieved great results that could not have been achieved otherwise outside of a project of this magnitude, so my experience within the project has been really productive and satisfactory.

What was your work in FPT focused on?

My main role within the project was to find protein biomarkers of the target marine species for traceability. That is, to find some proteins (supposedly under directional selection) that show differential expression between populations. In other words, to be able to find proteins that can define a population. We are developing antibody arrays specific for hake, to analyse protein mixtures and assign individuals to the original stock based in their protein expression profile.

How do you think proteomics could help in food traceability?

Population proteomics is still in its infancy but it could be considered the next step in the study of the biological systems, so in many ways it could complement other traceability tools already developed for fish food. Just a few years ago, proteomics constituted an expensive technique, but recent advances in genome sequencing and instrumentation have helped in the analyses of the proteins in a more affordable way. Moreover, proteins are easy to obtain directly from almost any kind of tissue, so I really believe it could be a promising technique for tracing food through the production and distribution chain in the future.



## Section 6

# From the laboratory to the end-user: Towards the application of science

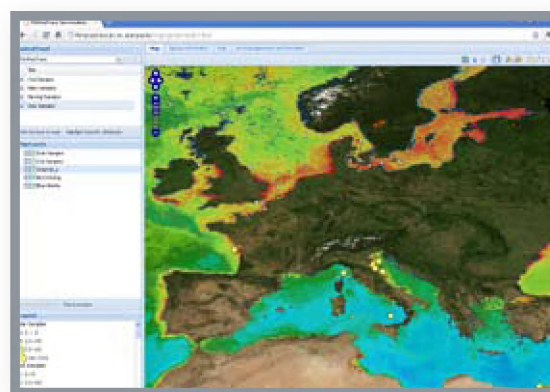
While starting out as a research project, FishPopTrace set the ambitious goal to move beyond, and bridge the often prevailing gap between academic science, policy making and end-users. To this end, a technology transfer strategy was developed which included pro-active engagement with the Common Fisheries Policy and communication with stakeholders. Such an approach is crucial to ensure that results and information generated by FishPopTrace do not remain exclusively in the academic realm but are accessible to end-users, particularly control and enforcement authorities and fishery management bodies. Several features have been developed and activities followed by FishPopTrace to facilitate the accessibility of results and recommendations.

dispersal or loss, and therefore contributes to continuous data accessibility in support of future scientific endeavours which can feed into fishery management. The web-based platform disseminates results and information emerging from FishPopTrace in a format comprehensible to stakeholders, including control and enforcement authorities, and integrates scientific information with environmental parameters. This approach is highly valuable for fishery management and conservation in a broader sense.

FishPopTrace aimed to bridge the gap between academic science, policy making and end-users.



Typical processed fish products where most identifying features (except the genetic material, DNA) have been removed.



Web-based geovisualization browser showing cod and sole samples collected against a monthly average of Chlorophyll A concentration.

The FishPopTrace central data repository contributes to continuous data accessibility in support of future scientific endeavours for fisheries management.

### 6.1

#### The *FishPopTrace* database and Geo-visualisation platform

Essential components of the FishPopTrace technology transfer activity are the web-based interface (<http://fishpoptrace.jrc.ec.europa.eu>), its database compiling all sampling and experimental data beyond the projects active phase, and a public geo-visualisation platform. The central data repository avoids data

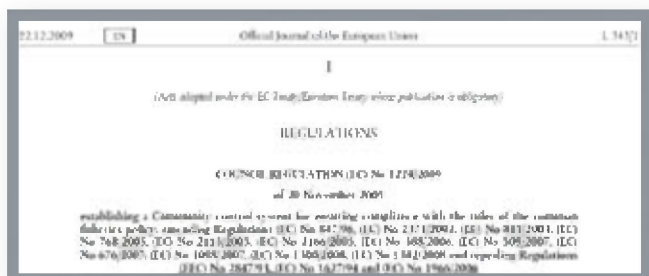
### 6.2

#### Production of policy relevant documents:

FishPopTrace generated a concise document [*Traceability in the EU Fisheries Sector - Rationale and implementation in the EU and the international context*], downloadable from our webpage (<http://fishpoptrace.jrc.ec.europa.eu/publicdocs>), which



analyses the current policy framework relevant for traceability. While emphasis is put on the European Union, a view on other nations is included. Moreover, in line with its commitment to engage with the EU Common Fishery Policy, FishPopTrace submitted two contributions to the public consultations launched by the European Commission for the CFP Control Reform and the CFP reform. Both contributions are downloadable from the FishPopTrace webpage (<http://fishpoptrace.jrc.ec.europa.eu/fisheries-policy-contribution>).



Council regulation (EC) No 1224/2009 establishing a Community control system for ensuring compliance with the rules of the common fisheries policy. Article 13 'New technologies' refers explicitly to "traceability tools such as genetic analysis" having a potential to improve compliance with the rules of the Common Fisheries Policy. FishPopTrace participated actively through advice and communication with the European Commission during the preparation phase of this regulation. The CFP control regulation No 1224/2009 is tightly linked to the Council regulation (EC) No 1005/2008, the so called 'IUU regulation' to deter and eliminate IUU fishing, the implementation of which can also be supported by molecular tools.

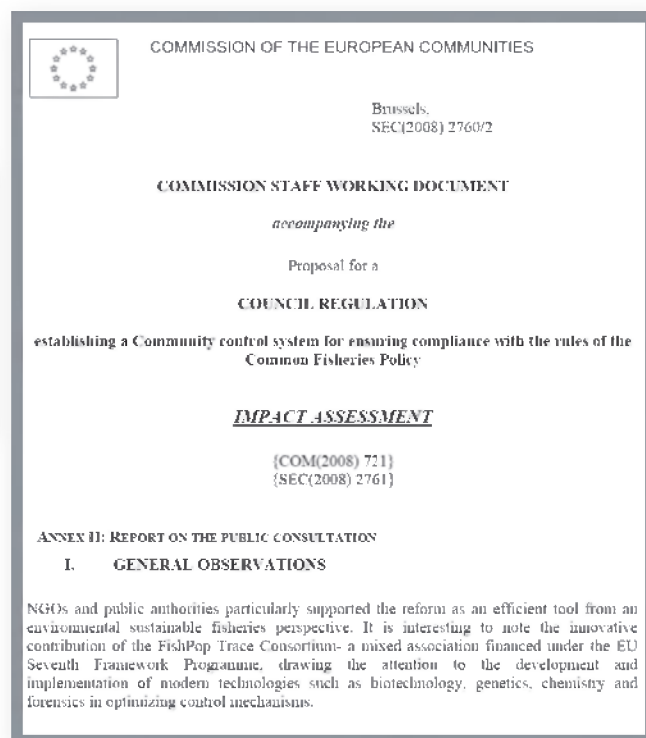
### 6.3 Interaction with the European Commission and the Community Fishery Control Agency

In 2008 the EC Joint Research Centre organised a workshop on advanced technologies based on biotechnology, genetics, chemistry and forensics for traceability and control in the fishery sector. This workshop was held at the DG Mare premises in the context of the preparation of the CFP control reform. Several members of the FishPopTrace consortium participated in this highly successful assembly, which also contributed to a reference to 'genetics' in the final CFP control regulation [(EC) No 1224/2009]. Also in 2008 FishPopTrace presented the scope and aims to a panel of Community Fishery Control Agency staff, in the presence of the Executive Director Mr. Harm Koster. In 2009 FishPopTrace was invited for a presentation to DG Mare and DG RTD 'New technologies applied to control and enforcement in fisheries. Results from recently ended (FP6) and ongoing RTD projects.'

### 6.4 Assessing Costs and Benefits of DNA-based technologies for fishery control and enforcement

An extensive assessment of benefits and costs for the use of DNA-technology for fishery control and enforcement was launched by FishPopTrace. To this end a questionnaire was elaborated in cooperation with the fishery economist

Jordi Guillen (JRC; Italy) and sent out to 65 ministries as well as control and enforcement authorities worldwide. The responses are currently being analysed and a report will be finalised in summer 2011.



Extract of the impact assessment by the European Commission accompanying the Commission 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 Annex II under 'General Observations' the public consultation contribution of FishPopTrace has been explicitly mentioned.

### 6.5 Transfer of results and protocols into a forensic framework:

Detailed Standard Operating Procedures (SOPs), that is documents containing instructions for forensic scientists to perform procedures that are routine, standardised and for which no *ad hoc* modification is acceptable will be produced. SOPs are indispensable for forensic applications to criminal investigations, as they help to ensure the quality and integrity of data, and provide a basis for guidance, uniformity and accountability. The SOPs developed by FishPopTrace will enable the dissemination and reproduction of individual assays throughout European laboratories.

### 6.6 Participation in major dissemination events

Despite being presented at various major scientific conferences, FishPopTrace pursued participation in key international dissemination events, such as 'Research Connection 09' organised by the European Commission. The FishPopTrace consortium is also involved in the organisation

of a Food Chain Integrity Conference, a major stakeholder event launched by the European Commission. This conference will be a 'showcase' for Framework Programme projects related to traceability, to be held in September 2011.

## 6.7

### Networking with relevant international organisations and advisory bodies

To build awareness of the potential and value of technologies and tools developed by FishPopTrace it is essential to engage with international organisations which have a broad overview on fishery issues worldwide. Several FishPopTrace consortium members established contacts with the FAO (December 2009), and provided advice and information on the use of forensic genetics for fishery control and enforcement in the fight against the global phenomenon of IUU fishing. Members also presented the application of forensics at the Sea Food Summit (Paris, 2009) and the Global Fishery Enforcement Training workshop (Maputo; 2011) organised by the international Monitoring, Control and Surveillance network (iMCS), which is endorsed by the FAO. Through our consortium member, Robin Waples, FishPopTrace has built a link to the US federal agency National Oceanic and Atmospheric Administration (NOAA), a key scientific advisory body for fishery management, including control and enforcement. Several members of the FishPopTrace consortium are also members of the ICES Working Group on Applied Genetics for Fisheries and Mariculture (WGAGFM), building a permanent link between FishPopTrace and ICES. Furthermore, primarily through the Joint Research Centre, which constitutes the STECF secretariat, ways to better include modern molecular technologies in fishery management decisions under the CFP remit are explored.

### Towards 'on-the-spot' analysis: Proteomics go local.

On the development of an immunological assay to characterise origin of European hake (*Merluccius merluccius*) populations.

For fisheries control and enforcement the 'response time', i.e. the lapse of time between the sampling of suspicious fish (products) and to the analysis of samples should be minimised. Ideally inspectors would be able to perform an analysis on the spot, at least as a first measure to produce evidence sustaining suspicion.

Based on quantitative differential proteomic analyses of hake populations, several protein markers were selected to discriminate the origin of hake. The generation of natural chemicals, called "polyclonal antibodies", were developed. These purified chemicals have been used to generate different formats of immunological *in vitro* tests for quick and functional discrimination of hake populations, an assay similar to pregnancy tests. Currently this technology is taken further to develop for Enzyme-Linked Immunosorbent Assays (ELISA) high-throughput systems (HTS) for fishery stock management at ports.









## NEWSFOCUS



## To Fight Illegal Fishing, Forensic DNA Gets Local

A new generation of genetic tests could give authorities a much better idea of exactly where fish have been caught

IN 2003, GENETICIST FINN NIELSEN OF the Technical University of Denmark got an unexpected phone call from Danish fisheries inspectors. They suspected that a fishing vessel had violated its quota by catching too many cod from the North Sea. But the captain claimed that he had caught the fish legally in the Baltic Sea. The difficulty for the authorities was that the fish from both places were the same species, the Atlantic cod (*Gadus morhua*), and they look alike.

Nielsen had been studying the genetics of the Atlantic cod, however, and he used DNA markers called microsatellites to show that the fish in question were very likely from the North Sea. A judge agreed, fining the captain \$8500 and confiscating his \$44,000 catch.

It was a rare victory against the massive problem of illegal fishing. For technical reasons, however, microsatellite tests for identifying the local origins of caught fish haven't been widely adopted. A €3.9-million European research project, called *fishPopTrace*, aims to solve the problem by developing a different kind of test that could be used to track not only the breeding fisheries regulations but also for catching fraudulent labeling of

fish in supermarkets. The consortium, which began in 2006, is exploring a range of possible techniques, such as protein patterns in fish tissue and the composition and shape of ear bones called otoliths. But the group is betting heavily on genetic variants called single-nucleotide polymorphisms (SNPs). That's because SNPs should be tests that are faster, cheaper, and easier to scale up than those based on microsatellites.

The ultimate payoff is the advent of reliable and widely used tests to determine not just which species of fish has been caught but which particular local population it came from. "Being able to take [information] to the population level is a big step forward," says Michael Hildebrand, chief scientist of the advisory group Oceana, based in Washington, D.C., who is not a member of the consortium. At a meeting last month in Madeira, Portugal, the *fishPopTrace* consortium discussed its results, and although most of its work is unpublished, other fisheries experts

Illegal catch. Fisheries inspectors such as these can't tell all violators, but genetic tests may prove another deterrent.

these say the use of SNPs is promising. "This is going to explode," predicts Kevin Glover of the Institute of Marine Research in Bergen, Norway. "They've shown it's feasible." In February, the consortium will present final results to policymakers, environmental groups, and others at a meeting in Brussels.

### Fishing fraud

Although it's difficult to get reliable information on the extent of illegal fishing, researchers estimate that this illicit business is worth as much as \$2.3 billion worldwide each year. Sometimes the violations consist of boats catching more fish than allowed. More egregious is the taking of fish from areas that have been closed to recover from overfishing. Another problem is consumer fraud: the intentional mislabeling of fish as a more valuable species or as coming from a more desirable location.

Government agencies fight illegal fishing using a variety of tools. Some place observers on larger vessels to keep an eye on what's loaded, checking that only fish of the right size are caught, for example. In the European Union, larger boats must have GPS units to make sure they're not fishing in off-limits areas. But observers can't follow all fishing vessels, and monitoring systems can be bypassed. So when it comes to catching scoff-laws, forensic genetic approaches that distinguish between species have been critical. For more than 20 years, DNA tests have been used to identify species that have been illegally caught or mislabeled.

It is much harder to track a fish to its native population. DNA tests look for unique markers, and there are fewer genetic differences between populations of the same species than between species. This is particularly true in the oceans, where populations tend to be large and overlap, which can wash out genetic differences between localities. The microsatellite approach, used by Nielsen for cod, can find markers unique to populations, but it isn't particularly efficient or always feasible. And the technique hasn't been scaled up to an efficient tool, in part because of calibration issues between laboratories.

In December 2006, the European Commission put out a call for proposals to develop tools to better characterize populations of marine fish and improve the traceability of fish products. *fishPopTrace*, coordinated by Gary Carvalho of Bangor University in the United Kingdom, won the competition.

### Online

sciencemag.org  
Fish interview with author Finn Nielsen

Downloaded from www.sciencemag.org on December 9, 2010

1468

10 DECEMBER 2010 VOL 330 SCIENCE www.sciencemag.org

Published by AAS

News Focus, Science, December 2010

Independent Newspaper UK, January 2011

## There's something fishy going on

**Environment** The majority of shoppers want to buy seafood that's sustainably caught – and supermarkets claim to provide it.

Reporting of FishPopTrace in article: "The tools we are developing in the EU-funded programme, FishPopTrace, will be used throughout the supply chain, at sea, right through to when they are dunked in batter and put on a plate", explains Professor Gary Carvalho, Bangor University, UK

### Cracking down on illegal fishing

The International Fishing Industry has taken big steps to ensure years of the improving the sustainability of fish stocks. Despite this, many... unregulated and unregulated (IUU) fishing and consumer fraud

Professor Gary Carvalho of Bangor University is working on a technique – as part of an international project – that will allow him to be traced back to where they were caught.

By combining genetic and chemical analysis the scientists will be able to tell exactly what type of fish is in a fish finger as well as where it comes from.

But we're not just talking fish fingers. The technique is so powerful that scientists will be able to identify marked, stolen, stolen or poached fish.

And because it's based on genetic information, it's accurate enough to stand up in court of law. The plan is to use it to tell the world where illegal fishing can continue unchecked.

Science writer and broadcaster Michael Parkinson joins Gary and his colleagues Dr David Hughes to find out more.

Download (10.7 MB)

Click the play button above to listen now.

Planet Earth Podcast, Natural Environment Research Council (UK), June 2009

## Tools for uncovering fishy business

Gary Carvalho, Bangor University, UK describes efforts to tackle illegal, unreported and unregulated (IUU) fishing and consumer fraud

**What is the need?**  
Up to 25% of the global marine fisheries catch is either illegal, unreported or unregulated – which is in violation of international obligations, or which take place in regions without clear conservation and regulatory measures. Representing up to an estimated US \$25 billion each activity pose a major obstacle not only to the sustainability and conservation of fish resources, but importantly also to the socio-economic stability of many communities that depend upon fish products for food and employment. For example, the Baltic Fisheries Assessment Working Group reported that in the East Baltic, up to 25-40% more cod is landed than is reported. The effects of such activity are amplified by apparent consumer fraud: a recent study using DNA barcoding found that a quarter of fish products labelled as "cod" or "haddock" in various outlets in Dublin, Ireland, were in fact completely different species. Fish consumers are being misled by incorrect identification, which may further compromise efforts to achieve sustainability and public perception of stock status. The added fact that up to 80% of fish products sold in Europe originate from outside the area, has caused that matters relating to IUU are high on the agenda of the EU Common Fisheries Policy (<http://ec.europa.eu/fisheries/cfp/>).

While there is already a highly effective framework in place for testing species identity, based on the cytochrome c oxidase I (COI) barcoding approach of FISH-BOL (<http://www.fishbol.org/>), determining where fish come from in the oceans is more challenging, but crucially important.

The ability to trace fish back to population or geographic area underpins attempts to achieve sustainability for these marine resources. First, it is recognised that populations are the natural unit of evolutionary change, and as such, provide the genetic resources required for adaptive response to natural and man-made changes in the environment. Second, it is at the population level that policy legislation and associated enforcement must take place. In addition to vessel management tools such as catch limits, TACs, minimum landing sizes, there is a growing need to develop control by input management tools through capacity and effort limitation. Such a policy requires information on the relative dynamics and abundance of fish stocks from particular regions. Third, there is an increasing requirement for traceability of fish and fish products, both for consumer protection and for regulatory enforcement, in particular with respect to IUU fishing. For example, in the UK, the Marine Stewardship Council encourages consumers to eat cod from 'stocks maintained within safe limits'. A traceability system based on regional stocks is necessary to prevent fraudulent allocation.

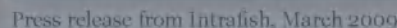
Despite the long-term notion of sub-specific fishery units and effects of phenotypic and genetic data, effective incorporation of such thinking into fisheries management remains impeded. Discriminating between marine fish populations is often problematic, and owing to low levels of differentiation and high variance in phenotypic characters, there is no single test to trace cod in the collection of information on population boundaries and dynamics of populations. Moreover, globalisation has led

Continued on page 2

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Fisheries Society of the British Isles Newsletter, Spring 2011





## Section 8

# The FPT Legacy

## A Life Beyond the Consortium

### 8.1

#### The need for continuity

It is generally acknowledged, and anchored in the EU legislation, that scientific advice is indispensable for sound fishery management. Many of the techniques and technologies used for the scientific studies carried out by FishPopTrace have experienced a technological revolution in recent years with analytical power steeply rising and costs sharply dropping. This is particularly true for DNA-sequencing technologies and genome analysis. This boost in analytical power has also led to major advances in fisheries science and has had, in some instances, a positive impact on marine fishery management. However we observe that the great potential offered by modern state-of-the art scientific approaches is generally far from being routinely exploited to answer questions relevant to the management of marine species or their conservation. This regrettable lack of scientific information uptake has a variety of reasons. Central to the problem is the disruption of output and dissemination of research projects after their funding has ended.

The great potential offered by modern state-of-the art scientific approaches is generally far from being routinely used in management of marine species or their conservation.

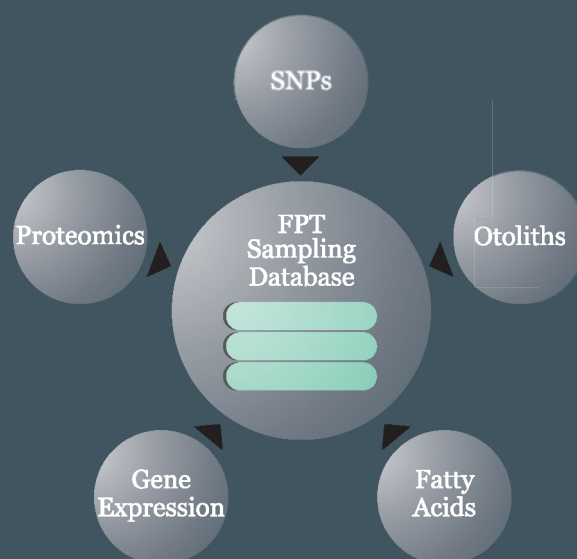
### 8.2

#### The *FishPopTrace* website and database

While large collections of information are created during the sampling and analysis phases of projects such as FishPopTrace, usually only a small subset of this information is published as primary scientific outputs. The body of data generated during the lifetime of the project remains vastly inaccessible, as collections and databases are maintained by individual experts or institutions. Such data sources may have a high potential value for future questions, but might get lost or forgotten as new projects begin or objectives change. To avoid such a scenario, a flexible database structure was designed and implemented for FishPopTrace. The database is housed at the Joint Research Centre of the European Commission and was designed to grant access to data and further addition of data beyond the

project duration. Access to the database is enabled through a website portal, currently hosted on the FishPopTrace Web page. The database structure is independent of the portal. Access can be granted to the database through any number of portals placed on different web pages. In this way, the information collection in the database initiated under FishPopTrace can be continuously accessed and built on by encompassing other marine species or further information.

The FishPopTrace database structure. Characteristics of over 7,500 collected fish specimen are documented in a central database. This information includes precise location of catch, depth, length, age, sex, and reproductive characteristics. Analysis performed on any of these documented specimens is referenced in a series of satellite databases. Thus, the results of each analytical technique applied can be compared for the same specimen. Additional databases can be added to this structure to store analyses performed using other analytical techniques beyond the scope of FishPopTrace.



The database is housed at the Joint Research Centre of the European Commission to grant access and allow further addition of data beyond the end of FishPopTrace

To avoid data loss and dispersal, a flexible web-based database structure was designed and implemented for *FishPopTrace*.

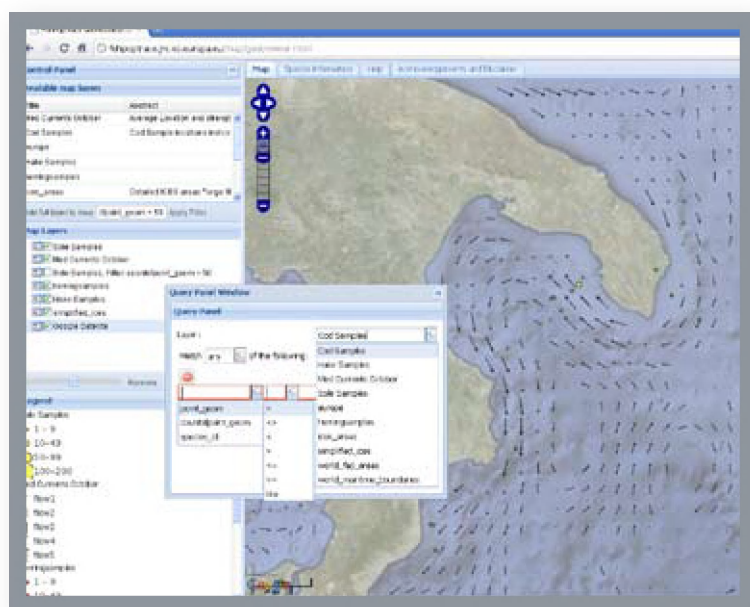
### 8.3 The *FishPopTrace* Geographic Visualisation Platform

The information stored in the database structures can be accessed in two ways through the FishPopTrace website, the information dissemination portal of the consortium. Selected genetic characteristic information can be publicly accessed through a map-based interface, a Geographic Visualisation Platform, designed to highlight biological (genetic; chemical etc.) characteristics of the target species in relation to their environment (ocean currents, temperature, salinity, etc.) in a geographic context. Members of the consortium can also login and query the database, view the information online or download the information in a spreadsheet to apply changes 'at home' before uploading the edited data sets.

The geographic visualisation platform disseminates information in a transparent and comprehensible way for use as a management and regulation decision support tool.

The purpose of the geographic visualisation platform is to disseminate information, also to non-experts, in a transparent and easily understood way for use as a management and regulation decision support tool. Data are provided in a geographic and environmental context. Aspects such as ocean currents, surface temperature and salinity value variations and their potential relationship with the development of features, such as genetic differences in the target species, can be visualised. This provides valuable support to research, but constitutes also a useful communication tool for population/stock structure information to stakeholders involved in fishery management and policy development.

Population and/or stock relevant information characterising a species may be accessible from a research laboratory, but without an effective means of communication to the appropriate stakeholders, insights provided by genetic analysis cannot be considered in the development of informed policy decisions. The FishPopTrace database and website, with its map browser and direct data access interface provides information relevant to fishery management and policy decisions in a format highly accessible to stakeholders. FishPopTrace will thereby contribute to efficient fishing activities within an economically viable and competitive fisheries industry, and hence contribute to the Common Fisheries Policy's aim of providing a fair standard of living for those who depend on fishing activities as well as taking into account the interests of consumers.



Web-based geovisualization browser showing major current circulations south of Italy. The interactive browser can be queried and manipulated to show information matching user defined criteria

The **FishPopTrace** database and website, with its map-browser and direct data access interface provides information relevant to fishery management and policy decisions in a format highly accessible to stakeholders.



## Comment:

### On the importance of extending the impact of science beyond the natural life of research projects.

Worldwide, a wide range of studies have been carried out on the structure of fish stocks, genetic populations, the effect of selective pressures such as fisheries exploitation and environmental change. Moreover, research has addressed the genomic architecture of heritable performance traits important to local adaptation and aquaculture economics. The results of these studies represent a treasure trove, being a tremendously valuable resource of biological understanding with high relevance not only to fishery management, conservation, aquaculture, but also to fisheries control and enforcement issues and fish and fish product traceability. The output from such work is continually expanding, driven by rapid technological advances, particularly in the field of molecular genetics and genomics. Yet, in contrast, the capacity to access and integrate the increasing body of information has advanced relatively slowly, if at all. Primary data sets generated tend to be highly dispersed and are at significant risk of being lost after the conclusion of research projects. This might also explain why, while the collection, management and use of data, including biological, in the fisheries sector for scientific advice regarding the Common Fisheries Policy is anchored in the EU legislation (Regulation (EC) 199/2008), genetic data has not yet been considered at all.

The increasing discrepancy between data generation and data storage, management and accessibility means that maximal benefits from the scientific work carried out are unlikely to be gained by the research community and fisheries managers, and also that valuable information is out of reach for policy makers.

The FishPopTrace consortium member Joint Research Centre, as an institution working at the interface of science and policy making, specifically addresses this issue. On one hand, as for the FP5 project FishTrace ([www.fishtrace.org](http://www.fishtrace.org)), it provides a web-linked dissemination and data storage platform sustained beyond the project funding period. On the other hand, it strives to help build a bridge between the academic and policy realm. This has been reflected in various activities pursued by FishPopTrace. We hope that one legacy of FishPopTrace will be an improved uptake of scientific information to contribute to the enrichment of marine knowledge, supporting sustainable fisheries and conservation efforts and leading to a future with a socio-economically healthy fishery sector in the sense of the Marine Knowledge 2020 strategy\*.

\* Communication from the Commission to the European Parliament and the Council of 8 September 2010 – Marine knowledge 2020 marine data and observation for smart and sustainable growth [COM(2010) 461 final].

**FishPopTrace** will thereby contribute to efficient fishing activities within an economically viable and competitive fisheries industry, and hence contribute to the Common Fisheries Policy's aim of providing a fair standard of living for those who depend on fishing activities as well as taking into account the interests of consumers.

## Section 9

# Glossary

**Adaptive markers** – genetic markers that are linked to genes involved in adaptation to the local environment (i.e. they are subject to natural selection)

**Assays** – A process to examine and determine the characteristics of, in this case, several SNP genotypes

**Benthopelagic** - Living and feeding near the bottom as well as in mid-waters or near the surface. Feeding on benthic as well as free swimming organisms

**Bycatch** - Catch of species other than the intended target species in a fishing operation. Bycatch can either be discarded or landed

**Common Fisheries Policy (CFP)** - The European Union's principle instrument for the management of fisheries and aquaculture. The CFP aims at assuring the sustainable exploitation of fish stocks and fish farms in a healthy marine environment and a prosperous EU-wide socioeconomic setting

**Demersal** - Describing a fish that lives close to the floor of the sea

**DNA** - Short for *deoxyribonucleic acid*. The genetic material determining the makeup of all living cells

**ecosystem-based approach to fisheries (EAF)**- the main purpose of the EAF is to plan, develop and manage fisheries in a manner that addresses the multiple needs and desires of societies, without jeopardising the options for future generations to benefit from the full range of goods and services provided by marine ecosystems

**Fisheries forensics** - The application of science to legal requirements in the fishery management context

**Forensics** - Forensics is a field of science dedicated to the methodical gathering and analysis of evidence to establish facts that can be presented in a legal proceeding

**Genes** - A gene is the basic unit of heredity in a living organism. Genes hold the information to build and maintain their cells and pass genetic traits to offspring

**Genetic marker** - Phenotypic (i.e. expression of certain genes, e.g. proteins) or genotypic (e.g. DNA sequence) characteristics that can be used to infer the genotype (genetic constitution) of an individual

**Genetic resources** – the collective extent to which individuals and populations differ in their genes and associated biological characteristics

**Genome** - The total amount of genetic information in the chromosomes of an organism, including its genes and DNA sequences

**Heterozygote** - The presence of two dissimilar alleles at a genetic locus

**Homozygote** - Two identical alleles at a genetic locus

**Illegal, Unreported and Unregulated Fishing (IUU)** - The only internationally agreed definition of “IUU fishing” is to be found in the FAO’s IUU Action Plan. In line with this definition, and for the sake of clarity, the Commission considers that the scope of the EU policy to deter, prevent and eliminate IUU fishing should cover:

- behaviours infringing applicable rules on the management and conservation of fisheries resources, occurring in waters subject to or beyond the jurisdiction of a State;
- fishing activities carried out in a high seas area and subject to a Regional Fisheries Management Organisation (RFMO) by fishing vessels without flag or flying the flag of States not party to the RFMO and in a manner contravening the rules issued by this organisation;
- and fishing activities carried out in a high seas area not subject to any conservation and management measures in a manner inconsistent with State responsibilities for the conservation of fisheries resources under international law

**Method validation** - Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice

**Microchemistry** - Chemistry that deals with minute quantities of materials, frequently less than one milligram in mass or one milliliter in volume

**Molecular tools** - tools that are based on the analysis of biological macromolecules, such as nucleic acids and proteins

**Morphometrics** – Morphometrics is a field concerned with studying variation and change in the form (size and shape) of organisms or objects. There are several methods for extracting data from shapes, including measurement of lengths and angles, and outline analysis

**Natural selection** - The process in nature by which, according to Darwin's theory of evolution, only the organisms best adapted to their environment tend to survive and transmit their genetic characteristics in increasing numbers to succeeding generations while those less adapted tend to be eliminated

**Next generation sequencing** - A high throughput sequencing method that allows production of thousands or millions of sequences at once

**Otoliths** - One of the small bones or particles of calcareous or other hard substance in the internal ear of fish, an ear stone

**Panels of SNP markers** – A collection of SNPs are used simultaneously to estimate genetic variation across several to many regions in an individual's genome

**Pelagic**- Fish that spend most of their life swimming in the water column as opposed to resting on the bottom are known as pelagic species

**Phenotype** - The observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences

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**Population diversity** – the extent to which populations differ in various biological characteristics, some of which at least will be controlled genetically

**Population marker** – Any feature that characterises a population of individuals and helps to separate it from other populations of the same species

**Pyrosequencing** - a method of DNA sequencing based on the “sequencing by synthesis” principle. It differs from Sanger sequencing, in that it relies on the detection of pyrophosphate release on nucleotide incorporation, rather than chain termination with dideoxynucleotides. The technique was developed by Pål Nyrén and Mostafa Ronaghi at the Royal Institute of Technology in Stockholm in 1996, and is the basis of a variety of next generation sequencing technologies

**Single nucleotide polymorphisms (SNPs)** - A single-nucleotide polymorphism (SNP, pronounced snip) is a DNA sequence variation occurring when a single building block of DNA (nucleotide -- A, T, C, or G) -- in the genome (or other shared sequence) differs between members of a species. Individuals in populations may share many SNPs, that together create a unique DNA pattern for that population

**SNP chip** – a collection of SNPs that allow simultaneous investigation of several or many SNP variants in target DNA

**Spawning group** – A group of fish spawning in a particular area at a particular time which do not interbreed to any substantial degree with any other group spawning in a different area or in the same area at a different time

**Sustainable fishing** - The number or weight of fish in a stock that can be taken by fishing without reducing the stock biomass from year to year, assuming that environmental conditions remain the same

**Stocks** - Fish stocks are subpopulations of a particular species of fish, for which intrinsic factors (growth, recruitment, mortality and fishing mortality) are the only significant factors in determining population dynamics, while extrinsic factors (immigration and emigration) are considered to be insignificant

**Total Allowable Catch (TAC)** - An output management tool to limit the catch (yield) for the entire stock. TACs are typically partitioned into quotas.

**Traceability** - 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



## The *FishPopTrace* Consortium members



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Wildlife DNA Services



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TRACE Wildlife Forensics Network



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