FishPopTrace Final Report

Fish Populations and Traceability- *FishPopTrace*: The Structure of Fish Populations and Traceability of Fish and Fish Products (*European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° KBBE-212399 (FishPopTrace)*

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4.1.1 Executive Summary

Marine fish species, in common with all living organisms, are fragmented to varying degrees, into a series of locally interbreeding populations. The extent to which such populations differ biologically ("population diversity") and their distribution in time and space are among the most important drivers of species' survival and persistence in the face of environmental change. Fisheries biologists and managers have emphasized such thinking since the early 20th century, though there typically remains a mismatch between biological populations and fisheries management units. Thus, it remains difficult to devise strategies to relate population diversity to variable harvesting regimes, and even more difficult to conserve overexploited stocks. Further uncertainty in striving for sustainability is the high level of illegal, unregulated and unreported fishing (IUU), estimated globally to cost the industry €10-20 billion, and prior to 2010, €1.1 billion worth of illegal fish was imported into the EU annually. FishPopTrace, a 15-partner EU Framework 7 project, aimed to address such challenges to sustainability within the context of the Common Fisheries Policy to: 1. Develop a range of cost-effective and reliable tools for identifying, monitoring and tracing marine fish populations in four representative European species (cod, herring, hake and common sole); 2. Promote fisheries governance by ensuring that the most effective tools can be applied to forensic standards, and thereby be legally supportive for prosecution and enforcement; 3. Foster technology transfer of outputs in relation to enforcement and conservation policies of the EU Common Fisheries Policy (CFP) and associated socio-economic consequences. We have applied our primary traceability tools: DNA SNP analysis (single genetic variants) and otolith ("calcareous ear bones") 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. 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 the Atlantic. 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. FishPopTrace outputs will thus 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.

4.1.2 A summary description of project context and objectives

4.1.2.1 Why is there a need for research on fish population structure and traceability?

Exploited fish resources, in common with all living organisms, are most often described and instantly recognisable by their species name - cod, herring, hake, or mackerel etc. Indeed, the species is a fundamental level of biological organisation that underpins our ability to understand, exploit and manage our natural biological resources. All species, however, whether on land or in the sea, are composed of groups of individuals that interbreed with each other more often than with other such groups, and these so-called, *populations*, share many characteristics, making them also recognisable, but at a regional or geographic level. In the management of fish resources it has been known for many decades that although it is important to recognise what species an individual belongs to, it is also crucial to identify and monitor the distribution and dynamics of populations. Ever since Charles Darwin first established the primary mechanism for evolutionary change by natural selection in the mid-19th century, there has been recognition that it is the nature and extent of *differences between local groups or populations* that determines the survival and persistence of species in the face of environmental change, through the process of *adaptation*. It is correspondingly a primary task of many fish biologists and managers to identify populations, and to design strategies to maximise their conservation: in general, the more populations that exist ("population diversity"), the more opportunity there is for fish resources to adapt to such changes as over-exploitation and climate change. FishPopTrace was among the first European consortiums to develop a new generation of tools to trace fish and fish products back to their original populations, region or *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. Why, then was there a need to undertake such work?

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 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.

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.

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 Illegal Unregulated and Unreported (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. 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. As recently stated by the European Commissioner for Maritime Affairs and Fisheries, Maria Damanaki (Press Conference for the Presentation of the JRC Reference Report: *Deterring Illegal Activities in the Fisheries Sector*: Slow Fish International Fair (Genoa), 27 May 2011): "Illegal fishing is a criminal activity which negatively affects the global economy, disrupts marine ecosystems, and damages fisheries communities and consumers. Without proper control and enforcement, our policy is toothless. Without respect for the rules in EU waters and beyond, there can be no sustainable fisheries". There is thus an urgent need not only to develop effective traceability tools, but to apply these in a way that can be legally enforceable, and serve as importantly an effective deterrent from engaging in such practices in the first place.

Genetic analysis and the use of some phenotypic (the observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences) markers can be used to detect, deter and prosecute against cases of IUU fishing, helping to promote fishery sustainability and socioeconomic 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 or other 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.

4.1.2.2 The aim and objectives of FishPopTrace

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. *FishPopTrace was aimed at improving our ability to trace fish and fish products through enhanced understanding of the dynamics, temporal stability and distribution of major populations of four key exploited fish species: European hake, herring, sole and cod*. Data and traceability tools have been 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 populations or stocks, not only in the wild, 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 "ecolabelling" schemes of stocks certified in various ways.

The FishPopTrace consortium consists of 15 partners with expertise in fish biology, population and conservation genetics, molecular biology, biochemistry, and wildlife forensics. Partners were drawn from a range of representative bodies including the food industry, the European Commission, Universities, Government Laboratories, and SMEs. Collectively, the objectives encompass: **1.** integrate recent and on-going data from European fish species traceability projects, and generate a single compatible database and tissue archive managed by the Joint Research Centre of the European Commission; **2.** develop and apply a new type of genetic marker, applied for the first time to traceability and population structure of marine fishes: Single Nucleotide Polymorphisms (SNPs) (a DNA sequence variation occurring when a single building block of DNA in the genome differs between members of a species), together with tools developed from otolith (one of small bones or particles of calcareous or other hard substances in the internal ear of fish, or "ear stone") microchemistry and shape variation in widely distributed populations of cod, hake, sole and herring, to discriminate biologically differentiated populations as a basis for traceability; **3.** undertake validation of traceability tools to maximise robustness, transferability and reliance for subsequent use by end-users; **4.** develop a population monitoring system based on otolith and genetic data that will assess the extent to which population traceability might change over time from various locations; **5.** test the utility of additional novel traceability systems (fatty acid profiles, proteomics, gene expression, microarray platform for SNP genotyping), as potential independent tools in traceability; **6.** foster technology transfer of FishPopTrace outputs in relation to enforcement and conservation policies of the EU Common Fisheries Policy (CFP) and associated socio-economic consequences.

4.1.2.3 FishPopTrace in the wider context of sustainability and conservation of fish resources

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.

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. There will remain, however, a need to take forward the recommendations from FishPopTrace, together with other actions under discussion in the revised Common Fisheries Policy, summarised eloquently by what the EU Commissioner for Maritime Affairs and Fisheries, Maria Damanaki, has ambitiously stated at the *Slow Fish International Fair* (Genoa), 27 May, 2011: The challenge now will be transferring these technologies (molecular technologies) into a day-to-day practice across Europe. I want to stress that this is a topical moment: we are making the ever-important first step into a new era. An era not so far away, in which molecular technology and genetics become the bread and butter of fisheries control and enforcement, just as they are common practice in law enforcement. It will reassure us that, once the product reaches the shops, it has been fished sustainably – which is all we need to know".

4.1.2.4 FishPopTrace – a European and global perspective

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. The target species are chosen to serve as illustrative European models that encompass species exhibiting different geographic distributions, life-style (e.g. pelagic vs. benthic) and population structuring, with each species requiring priority actions in enforcement and/or conservation. The consortium brings together recent and current expertise in fish traceability projects; Fish and Chips (GOCE-CT-2003-505491; http://www.fish-and-chips.uni-bremen.de/), FishTrace (QLRI-CT-2002-02755- http://www.fishtrace.org/), FISH-BOL (http://www.fishbol.org/), Consortium for the Barcode of Life (http://www.barcodeoflife.org/), to provide added value from past investment, and to promote an effective legacy after the completion of FishPopTrace activities. While a focus has been on European priorities, expertise and applications, impacts are likely to be global: first, through transfer to global fisheries fuelling European fish imports, and second through the necessarily global framework of international regulatory authorities such as ICES and the FAO, and organisations charged with monitoring and promoting compliance to sustainability practices such as the Marine Stewardship Council.

4.1.3 A description of the main S & T results/foregrounds

4.1.3.1 Scope of FishPopTrace outputs: overview

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, such as the DNA barcoding gene (Cytochromoe Oxidase I). 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.

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.

Before considering the outputs from FishPopTrace in more detail, we first highlight three fundamental aspects of the design of the project: the choice of fish species as targets for testing; second, the rationale for choice of main traceability tools developed, and finally, the role that fisheries forensics, the application of science to legal requirements in the fishery management context, has played in helping the consortium to address its objectives.

4.1.3.2 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 multispecies approach encompasses different geographical scales for tracing individuals, representing a range of policy-led traceability scenarios.

4.1.3.3 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 often 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.

On the other hand, phenotypic characteristics can also be robust indicators of the origin of fish, though they are not available for use 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. 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 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).

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.

4.1.3.4 Fisheries forensics: the policy relevance of a forensic framework

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. Evidence is sometimes required within the fisheries context ("fisheries forensics"), when regulations have been breached, such as illegal fishing activity, mislabelling of a fish product, or under-sized fish are captured. While there has been no shortage of policies and maritime laws to assist in the governance of our oceans, it has proven much more difficult to enforce maritime law, and to execute prosecutions. In part, such constraints are related to having an appropriate set of tools to yield sufficiently robust evidence in a court of law. As stated by Abraham Lincoln, "Law without enforcement is just good advice". The quote is as relevant to regulations governing the sustainable management and conservation of fish resources as to any other type of policies. As previously stated, the global level of IUU fishing and supply chain fraud offer financial gains on a scale that attracts organized criminal groups as well as unscrupulous individuals; the tools required to detect and tackle such activities and enforce fishing regulations need to be equally sophisticated.

The outputs produced by FishPopTrace help 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 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 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.

4.1.3.5 The application of FishPopTrace outputs – initial comments

It is also worth pointing out, prior to a more detailed consideration of FishPopTrace results and dissemination, that a parallel aim of the consortium was to identify and promote mechanisms that would engage stakeholders and end-users in fisheries. 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. Examples will be provided within the current document.

4.1.3.6 Highlights of FishPopTrace findings and outputs

Having provided some general comments on the overall aim, scope and design of FishPopTrace, here we consider some of the key findings arising from our research, as well as examples of how these have been communicated to a diverse community. Complementary information is also available from the consortium website (http://fishpoptrace.jrc.ec.europa.eu/), which is updated regularly, as well as remaining "active", with further development, after the conclusion of the project in May 2011. FishPopTrace was organised into a series of work packages (WPs), details of which can be obtained from other Report documentation, and also the FishPopTrace website. The WPs, which essentially follow a logical flow from establishing the state of art within the fish traceability field by accessing details of related European project, moves onto

sample acquisition, traceability tool development and validation, to their eventual full deployment across the many geographic and spawning populations of our target fish species. The flow of work throughout has utilised core principles of technology transfer, dissemination of activities and outputs, and management. Below, rather than following the practically useful, but often overlapping, and discrete nature of the WP structure, we organise our primary S and T results according to broad areas of context and activity, and pose a series of interrelated questions. Such a structure allows a more logical, easily accessible and meaningful flow of information. WPs were coordinated by specific work package leaders, though each feeds into an overall set of project objectives, and usefully represent tractable and cost-effective approaches to tackling the collective workload. Since the beginning of the project, however, our strategy has always been to ensure consortium-wide discussions and dissemination of each WP, highlighting links amongst them, thereby securing exploitation of complementarity and shared opportunities.

4.1.3.7 What was the state of art in European marine fish population structure and traceability at the outset of FishPopTrace?

Tremendous progress has been made in the fields of marine fish genetic improvement, genetic stock identification and genomics in recent years. The continuing development of new molecular genetics tools has provided high-resolution markers for assessing genetic population structure, for estimating demographic parameters and for providing insights into fish stock management. However, while part of this information is available in the primary scientific outputs (scientific journals, etc.), there is another kind of information, sometimes scattered, in the way of technical information, collections and databases held by individual experts or institutions, which are still very useful.

Having that in mind, a primary objective of FishPopTrace was to provide a summary review of existing information bases for marine fish genetic resources and to place the FishPopTrace project within the context of recent and on-going fish traceability programmes. To achieve this, three main tasks were fully accomplished during the first year of the project. First, the establishment of a common platform for accessing archived tissues for genetic analyses from past and current EU and national projects. By searching at http://fishpoptrace.jrc.ec.europa.eu/tools/projects it is possible to find the links to other existing projects related to genetic identification and traceability of marine fish species. Secondly, filtering of valuable data produced and stored in other fish traceability projects and compiled into the new FishPopTrace database, the "meta-crawler", accessible at http://fishpoptrace.jrc.ec.europa.eu/crawler. Finally, the establishment and the distribution of a tissue archive database of external samples that has been extensively used during the project, especially the historical samples. Links to collections of specific external samples with biogeographical information and source and distribution of samples external to the FishPopTrace general sampling scheme can be accessed at http://fishpoptrace.jrc.ec.europa.eu/data-access. The latter resource is an especially distinctive and valuable contribution to the community of scientists interested in traceability and population structure of marine fishes. First, it provides an integrated platform for accessing twelve EU-related projects associated with fish population structure and/or traceability (*FishTrace, FishBol, Fish and Chips, PescaBase, FinE, Hergen, CodTrace, UNCOVER, SeaFood Plus, TraceFish, Trace, SELSEA-Merge*), bringing together under a single umbrella, the recent collective effort and outputs

within the field. Second, it aims at addressing the obstacles and missed opportunities arising from the fragmented nature of many tissue archives – samples that have been collected, but which can be used again as new markers or questions emerge. The FishPopTrace resource provides a highly effective mechanism for enhancing cost-effectiveness of resources and project legacies. Finally, the design of the portal allows for ease of use and access, with minimal prior experience required in using the interfaces, thereby allowing a range of end-users to benefit from its use. Moreover, the design of the portal enables the timely development of new projects and consortia.

4.1.3.8 How did we ensure the collection of representative samples of cod, herring, hake and cod from European waters?

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. 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?

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 across two FAO Fishing Areas (27 Northeast Atlantic and 37 Mediterranean & Black Sea) and 29 Fishing Divisions, and catalogued for analytical purposes. A specific achievement of FishPopTrace was the species traceability of the common sole and of its cryptic species *Solea aegyptiaca*. The WP2 sole sampling coupled with the application of molecular tools suitable for species identification lead to unravel

a wider and more complex intermingling of the Egyptian sole in the Eastern, Central and Western Mediterranean than hitherto known.

The fish collected during the sampling phase were analysed in the laboratory. State-of-the-art technologies were employed, such as high throughput DNA-sequencing for the discovery of SNPs, analytical chemistry (inductively-coupled mass-spectrometry (ICPMS)) for the composition of otoliths, and so-called "novel tools" that are being tested for their potential use as traceability tools, proteomic and gene expression profiling, to generate data for population structure analysis. To examine population structure, sophisticated statistical software was applied on the data, and methods were also forensically validated, as previously described *(4.1.3.4).*

4.1.3.9 How can we develop a common set of traceability tools for identifying fish populations across different spatial scales, as well as tracing diverse natural and processed fish products?

A major objective of the consortium was to develop a set of traceability tools that could be applied comprehensively to key spawning populations of each of our target species. It was also important to develop tools that could be used at different stages of the food supply chain (e.g. on-board, port of delivery, market-samples, processed fish products), and that could also ideally reveal information about population structure and traceability at different levels (e.g. reproductive groups; sea areas of juvenile growth). The genetic markers, SNPs, and analysis of chemical signatures and shape variation in ear bones (otoliths) were chosen. As argued in Section *4.1.3.3*, SNPs can be retrieved throughout the food supply chain, even in highly processed products, salted, dried, fried, tinned, frozen, etc, whereas otoliths are available usually only at the early stages of processing soon after capture and when the fish is intact. However, in addition to using otoliths from new samples, there is a further opportunity of obtaining otolith samples: for many decades, fisheries and other laboratories have archived otoliths in connection with information on growth rates obtained from examining the otolith growth rings. Through our consortium extensive links, these archived and stored otoliths provided an additional set of samples from which DNA could be obtained, as well using them in the chemical and shape analyses.

Among the most striking scientific results is the provision of several hundred novel genetic markers in, hake, herring and sole. The genetic markers in cod were already largely available from another project (Canadian Cod Genome Project), with which FishPopTrace had an agreement. 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.

For SNP development, we first sequenced a high number of so-called Expressed Sequence Tags (ESTs: a short sub-sequence of DNA known to produce a protein) from the muscle of sole, hake and herring. After assembly, a large number of SNPs were identified using a range of approaches. We first performed a visual inspection of 1536 SNPs per species and along a pipe line of detailed analyses followed by validation, we chose the best panel for traceability and forensic analyses to be used in WPs 5 and 6. A total of 1200 to 1300 samples per species were analysed during the validation-genotyping step and initial genetic analyses were performed to define the final analysis panel. It was important to ensure that any SNP markers chosen were easily scored using our high throughput methods, where many hundreds of SNPs can be typed simultaneously, as well as ensuring that they did represent accurately the underlying genetic variation in individuals; it is the cumulative sum of genetic variation across hundreds of SNPs from a population that ultimately determines to what extent we can distinguish it from other populations – a co-called "population signature". The following number of reliable SNPs was defined: 426 SNPs for sole, 281 SNPs for herring and 395 SNPs for hake. In conjunction with the previously-mentioned cod project, we were able to type 1290 SNPs!

Procedures for the analysis of otolith microchemistry have been standardized and optimized to provide the most robust and clear signal for otolith core information (representing the early life) and the narrowest otolith edge signal (representing the most recent life of a fish). Additionally, the methodology for otolith shape analysis was standardized to produce reliable digital photographs. Protocols for image analysis have been developed to automate the processing of the images and the statistical analysis of shape.

4.3.1.10 What about forensic validation of our primary traceability tools?

The remit for FishPopTrace was to take the RTD outputs across work packages and evaluate their suitability and readiness for application to fish traceability by performing a series of validation studies. These validation studies would generate the data necessary to assess the robustness, reliability and reproducibility of tools developed within FishPopTrace for monitoring or enforcement purposes. Briefly we wanted: 1) To establish a set of criteria for the validation of traceability tools; 2) To undertake developmental validation of each identification technique; 3) To develop statistical methods for the identification of source populations using multiple markers; 4) To implement internal validation studies and inter-laboratory calibration of techniques. 5) To collate all validation data to enable the construction of Standard Operating Procedures. Work towards these objectives was conducted across multiple techniques (the primary tools: molecular markers, otolith microchemistry, otolith shape analysis; and the novel tools: proteomics, fatty acid profiling, gene expression, genotyping arrays) and for several different aspects within a single technique; for example, DNA extraction, marker validation, genotyping method and reference data were all investigated within the molecular marker validation work. As such, the work conducted was broad in nature and required a high level of integration with other work packages, as well as the direct involvement of multiple partners with diverse areas of expertise.

A large amount of effort was put into the otolith validation work and many advances were made, as the basic technologies used for both the microchemistry analysis (LA-

ICPMS) and shape analysis (comparative imaging software) were essentially specific to individual laboratories and were not routinely used for the forensic identification of biological material. The largest component of validation work however focused on the validation of molecular markers. In order to deliver a validated assay, the marker validation work expanded to include a range of tests. For example, aside from considering the different sample types and extraction processes suitable for subsequent screening with SNP markers, the validation of SNP markers and their application covered an evaluation of the marker itself within the genome, an assessment of genotyping method and validation of the reference data used to assign the genotype (the specific genetic variants, in this case, as defined by the two forms of that gene or SNP marker ("alleles") - of an unknown sample to its population of origin. Inter-laboratory calibration studies were also designed and implemented as part of the validation studies for otolith microchemistry, otolith shape analysis and SNP genotyping panels. In each case, a second laboratory was used to test the analytical protocols and ensure that comparable data were generated using the same method. For otolith analyses, control samples were prepared by using the matching otolith pair to ensure that testing was based on a like-for-like comparison. In addition, the otolith microchemistry analysis uses commercially available elemental calibration standards to ensure that the analytical baseline is identical in each laboratory. For the genetic markers, standard control samples representing each genotype for each SNP marker were prepared (e.g. samples designated by their genotypes as defined at the respective SNP marker: AA, AB & BB genotypes). These control samples are available alongside the standard protocols generated for transfer into testing laboratories beyond the lifetime of FishPopTrace. More specifically, results for the SNP genotyping demonstrate the consistency of using the so-called "Taqman© genotyping assay", designed to quickly and accurately designate the genotypes of each SNP marker, across laboratories with no genotype scoring variation observed for the hake assay. Our data support the use of Taqman© as a genotyping chemistry suitable for the production of forensic evidence, though this is only feasible where a relatively small panel of SNP markers (<50) are examined. For the otolith microchemistry and morphometric analyses, variation among laboratories was observed, as expected, though the magnitude of the measurement differences was larger than anticipated, particularly for microchemistry. These results were one of the main reasons why the otolith microchemistry analysis method is not yet considered to be suitable for routine use in the production of forensic evidence.

The results of from the forensic studies are documented in a series of validation protocols, reports and analytical methods. As the validation process is designed to be entirely objective, the fact that several techniques were not considered to have performed sufficiently well to pass through to formal application is not seen as a failure; on the contrary, the ability to critically evaluate each technique based on quantitative, comparative data demonstrates the success of outputs in terms of its intended function. In the final analysis, the molecular (SNP) markers were the only technique demonstrably suitable for application within a forensic framework; the other markers, while often extremely informative, are not yet applied in a format that is likely to withstand the scrutiny of legal challenge.

4.3.1.11 *How informative were the SNP and otolith traceability tools when applied to fish populations and processed products?*

It was important for FishPopTrace not only to provide informative data on the structuring and dynamics of main spawning populations of cod, herring, hake and sole, but also to demonstrate the feasibility of obtaining large quantities of robust traceability data in a timely and cost-effective manner. Thus, there was considerable discussion about the most appropriate method for genotyping the SNPs, that is, the routine determination of the genetic variants across many SNP loci in each population. A large number of genotyping methods and platforms were evaluated resulting in the selection of an Illumina 1536 Golden Gate array© as our general genotyping method when exploring many hundreds of SNP markers simultaneously. This array was used for a single step validation and genotyping approach for putative SNPs and all population samples of the targeted species. Analysis of otolith microchemistry and morphometrics were conducted in parallel for a large subset of the samples used for genetic analysis. Based on the SNP genotyping, genetic maps illustrating genetic relationships among population samples were created (figure 1).

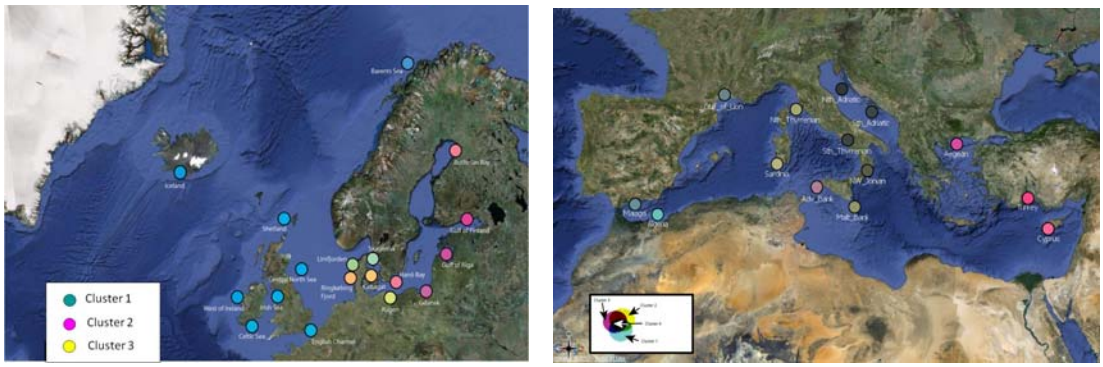

Figure 1: Maps illustrating the genetic relationships among population samples for a) herring and b) hake.

Separate maps of so-called "neutral" (subject to chance fluctuations) and loci "subject to selection" (directional changes in SNP frequency resulting from adaptation) were created as well as maps including all loci. Distinct regional groups were apparent using both classes of markers, where Atlantic and Mediterranean basins were the most distinct. However, also on a smaller geographical scale distinct groups were obvious, such as minor basins within the Mediterranean and the Baltic Sea. On a very fine scale, markers under selection revealed previously unrecognised population structure (e.g. within the North Sea), highlighting the concept of investigating variation in genes as very powerful for resolving fine scale population structure. The exact number of loci subject to selection varied according to species, geographical region studied and statistical method employed. Overall the outlier loci appeared to be associated with major climatic differences among populations. In total the high number of markers and those with highly elevated levels of genetic differentiation provided unprecedented high power for assignment back to population of origin. At large and medium geographical scales, minimum panels including only a subset of loci could provide close to 100% correct assignment to population of origin. On a small geographical scale more loci were needed, however in most cases highly informative results could be provided.

The core approach for the genetic component of the work was the development of a "SNP chip" (a collection of SNPs that allow simultaneous investigation of several or many SNP variants in target DNA) 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 chip, the frequency of each SNP variant was determined, creating a potentially diagnostic pattern, or "population signature".

More specifically, 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. *Gadus morhua*. 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 over fished 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 allowed the identification of some herring populations at smaller scales, even around the United Kingdom, where there is substantial misreporting of catches.

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.

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.

The results of the otolith analysis provided independent insights into the population structure and ecology of the targeted species (Figure 2). The information generally corroborated the genetic results, but also in some cases provided complementary information with higher resolution power, in particular on a small geographical scale.

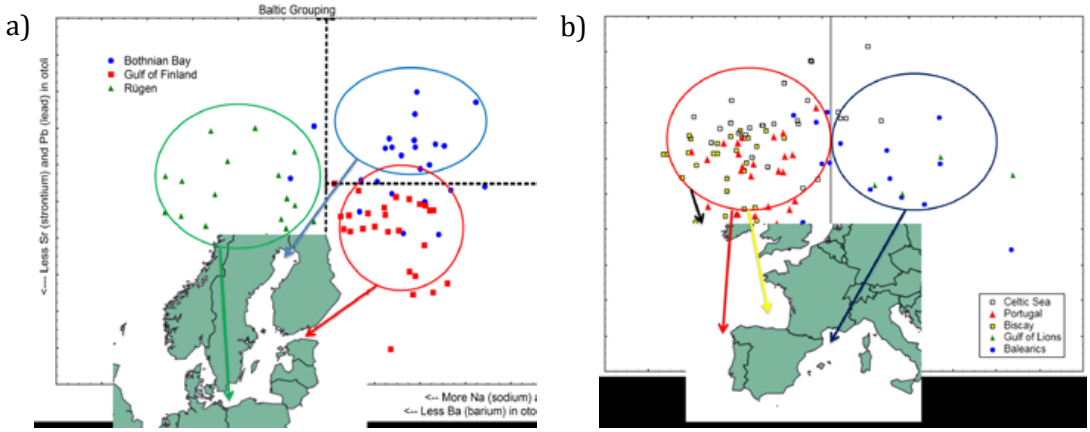

Figure 2: Identifying where fish come from using otolith microchemistry: Separation of fish from different populations, based on the otolith elemental "signature". The symbols represent individual fish. Fish with more similarity in the chemical composition of their otoliths are grouped closer together, and those with very different otolith compositions are grouped further away from each other. In the case of a) 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. In the case of b) 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.

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. 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. For example, Figure 3 shows 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. Together, otolith shape and composition are valuable tools that can be used to trace fish back to their home area.

Figure 3: A comparison of the "average" otolith shape from different hake populations in European waters.

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.

In summary, we 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 application.

4.3.1.12 How stable are our traceability tools over time?

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 needed 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. It is of interest, therefore, 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 conservation 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.

Due to the biological differences among both species and populations within species, as well as the management and conservation issue arising, the scopes for monitoring temporal change will vary on a case-by-case basis and will operate on different time scales. We specifically targeted our work to reflect and represent different types of monitoring objectives. 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 (figure 4). 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.

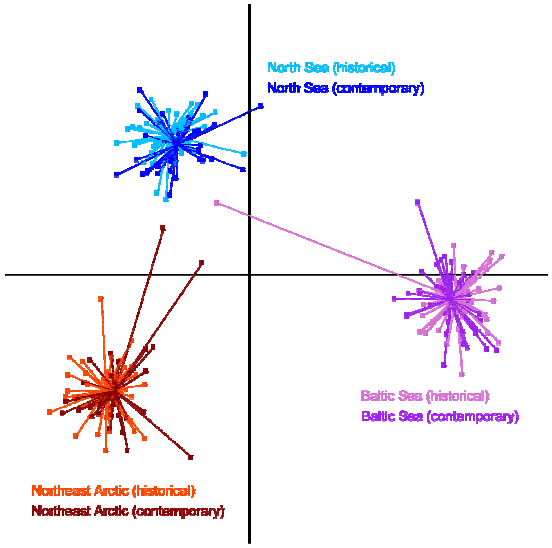

Figure 4: Spatio-temporal principle component analysis of 872 unlinked markers showing temporal stability in three populations of Atlantic cod

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 genetic marker analyses in all cases demonstrated large potential for genetic monitoring. Such tests over time for stability coupled with analysis of population structuring demonstrate a set of valuable tools for tracing the locations and movements of individuals in space and time. This was shown, for example, by the herring mixed-stock analysis, where temporal changes in stock composition could be determined with previously unobtainable precision (figure 5). Analyses can now be targeted to real-time monitoring of seasonal or annual changes in population exploitation rates. The genetic analyses of longer time series (hake, cod) conveyed novel information on other aspects of genetic monitoring. First, we corroborated the value of archived samples (e.g. DNA extracted from historical otoliths) for comparing genetic profiles over time. This was exemplified by our study of Atlantic cod, where analyses of SNPs associated with functional gene variation showed profound temporal changes coinciding with increased fisheries exploitation rates. Such methodologies can also be targeted to monitoring population changes in adaptive responses to climate change. Second, we demonstrated the SNP-based method's power for monitoring population origin of individuals in data poor areas. This was exemplified by the temporal analyses of hake, where a sample presumably representing local fish was found to be mainly non-native. Without this information, assessments of local stock strength and dynamics would be biased. Other types of markers, such as otoliths also exhibited temporal stability although temporal differences were also evident. Such differences underline the care that needs be taken if applying these types of tools in monitoring programmes.

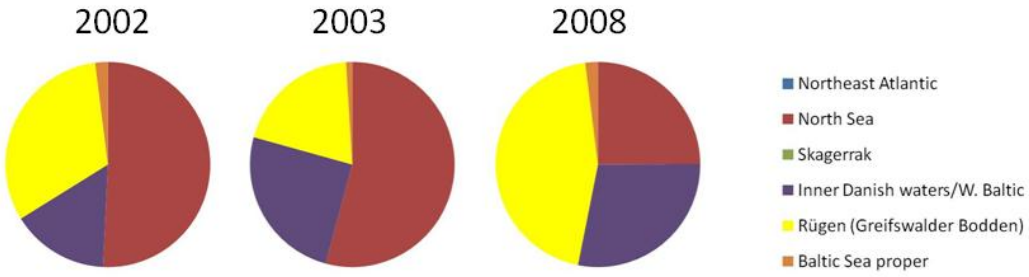

Figure 5: The results of mixed-stock analyses for three samples of feeding aggregations in the Skagerrak.

In summary, we show that population-specific traits vary little over time across species, demonstrating that the developed tools are reliable for insights across shorter and longer time scales. 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.

4.3.1.13 In addition to SNPs and otoliths, what other traceability tools might be developed for future application?

Besides developing applications based on genetics and otolith analysis for traceability of fish and fish products, 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. Two different versions of a prototype platform for SNP genotyping were developed and tested on a test panel of cod SNPs and samples. Both are based on a so-called "padlock probe technology", and a second version, with a smaller number of targeted SNPs, showed very promising results, opening the possibility of routine genotyping of an informative SNP panel (10-100 loci) at a reduced cost.

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.

First, we looked at the composition in fatty acids (FA) of fish flesh. FA analysis was performed on all four target species (334, 292, 132, 98 samples respectively for sole, hake, cod, and herring), exploring spatial (5-7 geographic sites were surveyed per species) and temporal variation, demonstrating that geographic populations can be effectively differentiated according to FA composition. 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. 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. 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.

Overall, our quest to test the potential of additional traceability tools yielded very exciting results and opened the way for the use of novel technologies to complement more established tools (genetic markers, otoliths shape and composition) in traceability studies. 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. Importantly, any traceability system needs to have sufficient flexibility such that constraints imposed by the specific requirements of any specific technique (e.g. necessity to store proteins in super-cooled conditions using liquid nitrogen) can be matched with available resources and opportunities. Additionally, where required, information on spawning population (a group of spawning fish in a particular area and at a particular time which do not interbreed to any substantial degree with any other spawning group) as revealed by genetic tools, might be usefully complemented by knowing what water body the fish was captured from, such as otolith microchemistry, lipids or gene expression for example. The genetic data typically generates population-level signatures across a longer-term evolutionary scale (e.g. many hundreds to thousands of years) based on the average breeding behaviour of fish populations across generations, whereas the other marker types have potential to reveal provenance across a shorter-term, ecological time-scale (e.g. weekly).

4.3.1.14 Once all the traceability results have been generated, how can these be archived, retrieved and made accessible to others?

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 FishPopTrace web pages (http://fishpoptrace.jrc.ec.europa.eu/). 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 website was constructed and was operational from the early stages of the project acting as a source of information about the project and similar projects. A member section of the website was constructed as a file sharing resource. This store has accumulated a wealth of project derived data, articles, presentations, reports and related documents over the project lifetime.

The FishPopTrace database was designed to be as data independent and flexible as possible through strong collaboration from all consortium partners, including a database-specific meeting hosted by the JRC. Following its construction it has been hosted and maintained by the JRC, including backups, versioning and modifications/bug fixes. An Excel template was agreed on and used for uploading the characteristics of collected specimens to the database. A web-based interface, deployed in the website, was developed to allow consortium members to query and view the contents of the database. Additional functionality was added to allow the uploading of samples to the database through the interface, using the Excel template. Furthermore, the results of user-specified queries through the interface can be downloaded and saved in the Excel template format. These Excel sheets can be edited and uploaded again through the web interface. The interface automatically handles updates to samples, deletion of samples and the insertion of new samples. A crawler tool was developed as part of our initiative to act as a data hub. This crawler automatically searches for news and articles related to FishPopTrace by matching a set of key words. The results are collated and updated daily on our website.

As analysis data became available from our partners, a series of experimental databases were constructed to house this data. Excel templates were agreed for each type of analysis. A series of scripts were produced to extract data from the templates and insert it into the respective databases. A web-based interface is available to query the information in the experimental databases. The results of each analysis type can be cross-referenced for an individual fish or group of fish.

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 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 (figure 6). 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.

a) b)

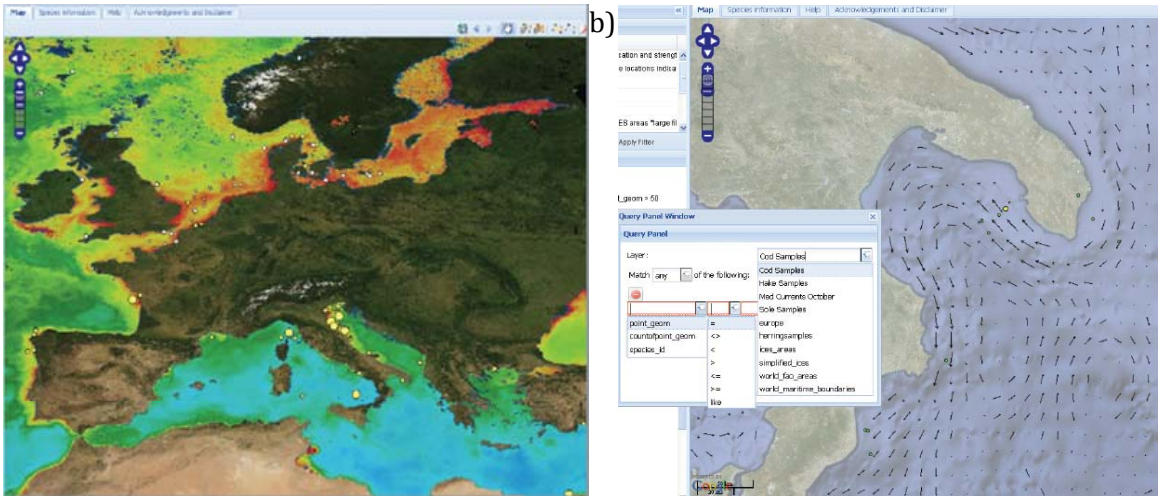

Figure 6: Geobrowser showing a) chlorophyll B concentrations and cod and hake sample distribution (white and yellow circles respectively) and b).showing marine current direction overlay

4.1.4 The potential impact (including socio-economic impact and the wider societal implications so far) and the main dissemination activities and exploitation of results

FishPopTrace is designed to integrate information on the distribution, extent and dynamics of fish population structuring in four target species, as a basis for conservation in relation to sustainable utilisation of bioresources, and in the provision of population markers for traceability of fish and fish products. As previously mentioned, a key strategy of FishPopTrace was to bridge the often prevailing gap between academic science, policy making and end-users. To this end a technology transfer strategy was developed, which consisted of intensive and targeted networking and communication with policy institutions, authorities and stakeholders, engagement with the Common Fisheries Policy and a Cost Benefit Analysis. Here we present salient highlights, rather than an exhaustive account, of how we aimed to facilitate uptake of outputs by end-users.

4.1.4.1 Context, objectives and brief overview in relation to potential impact

The basis of all traceability systems is to identify items that move along the supply chain. Characteristics of traceability systems (i.e. identification, information and the links between supply chain participants) are common, irrespective of the nature of product being traced. In addition to identifying the product, which in the case of FishPopTrace, necessitates information on species and population identity, additional information can be associated with the product, such as date and GPS location of capture, biological data relating to size or reproductive condition, identifier of fishing vessel, etc. Hence, to achieve basic traceability, each fish or product requires two fundamental components: (i) an "identifier" (species and population level) and (ii) a record of its history, which may include data about where, how and when it was produced at various stages throughout the food supply chain. Within FishPopTrace, the DNA barcoding gene, *COI*, which allows robust identification of most species, can be, and was run in parallel with genetic samples, and where required for other tools. Accordingly, with a potentially large set of disparate data, any traceability system will require an efficient and standardised database, with high quality assurance, for data storage and retrieval, with cross-referencing where necessary to other major portals. Moreover, plans need to be put into place to provide the possibility for continuation and extension of the traceability database after completion of the project. Such resources were established during the project, and a mechanism is in place via the EC's Joint Research Centre, to maintain and develop the FishPopTrace data base and working platform. A key component for the rationale of FishPopTrace was to ensure reference and integration where appropriate to past EU-related initiatives including FishTrace, Fish and Chips and TraceFish and the TRACE project (http://www.trace.eu.org/fa/mbm/index.php). FishPopTrace set out to contribute to formulation of policies in relation to traceability in several ways:

(1) ***To generate validated traceability tools that can be used to enforce management policies on effort limitation, quotas and marine protected areas, as well as in consumer protection.*** Such tools are now available, and have been fully validated for the genetic and otolith markers, though variance with otolith microchemistry and shape data, render it unlikely at present to be adopted for routine forensic applications. Significant advances have also been made in the so-called novel tools (fatty acids, proteomics and gene expression), with examples of a population-level signature of fish from specific locales. Again, however, additional focused effort is required to advance the use of these tools for subsequent traceability studies; they are likely to be most useful where complementary environmental information on recent origin rather than breeding relationships is required.

(2) ***The establishment of a forensic framework and associated Standard Operating Procedures – SOPs that can be transferred across the fishing industry***. A central component of the FishPopTrace technology approach was the forensic framework. Forensics is the application of scientific analysis, while employing strict operational standards, to inform criminal investigations. For FishPopTrace this meant to forensically validate genetic and chemical data and underlying protocols to allow assignment of fish (products) to their geographical origin, and to produce Standard Operating Procedures (SOPs), laboratory

instruction documents for performing analytical procedures that are routine, standardised and for which no *ad hoc* modification is acceptable. Our strategy, (detailed here), was to choose some policy-driven traceability scenarios for each of our target fish species, and to generate SOPs to enable illustrative and critical outlines of the stringent procedures to follow in order to produce legally-enforceable recommendations. The SOPs summarised the analytical process from DNA extraction through to statistical assignment of the test sample to its geographic origin. The analytical steps all involve the use of standard molecular genetic protocols and kits; the data analysis uses Microsoft excel and one freely available population genetic software programme. The SOPs are designed so that they can be easily adapted to changes in SNP panels, reference data sets or even target species.

(3) ***The establishment of a public database containing the "identifier" (standardised repository of reference species and population identifiers) and a record of its history (source, date, fleet details, etc) for comparison with samples ranging from wild-caught fish to fully processed products***. The resource is now available and described within this report (Section *4.3.1.13*)

(4) ***The establishment of a functional interface (FishPopTrace database) between consortium outputs and past and current actions in traceability, especially with reference to recommendations from TraceFish***. The resource is now available and described within this report (Section *4.3.1.12*)

(5) ***A common database with spatially and temporally characterised population variability of four target species, providing a base-line map to formulate spatially-defined models of stock assessment***. The full data have been deposited within the FishPopTrace website, and examples of the population maps have been included within this report.

Below we provide a summary, with illustrative examples, of the range of activities and dissemination modes to promote the impact of FishPopTrace, and also importantly, include current evidence of impact of consortium activities.

4.1.4.2 FishPopTrace website, documents, publications, consultations etc.

The approach followed by FishPopTrace has generated a paradigm of a holistic framework for fisheries control and enforcement. Peer reviewed scientific publications have already, and will continue to ensure that the results of FishPopTrace are scrutinized and disseminated in the research area. Such dissemination of outputs, however, is unlikely to be sufficient to reach out to end-users, which is why FishPopTrace stressed the importance of inter-laboratory trials of the protocols developed and used by FishPopTrace. The consortium also stressed the central role of the FishPopTrace database and interface. The experimental FishPopTrace data has been validated by the consortium members and submitted to the JRC, which acts as the host of the databases. The consortium spent considerable effort on finding ways how best to use the FishPopTrace website to generate maximum benefit for stakeholders and end-users. This is well reflected in the development of the web-based project crawler tool developed in the early stages of the project. Ultimately it was agreed that a geo-visualisation (also called Geographic Information System – GIS) platform would generate a practical opportunity to support the combination of experimental data and

results with environmental parameters, in a so-called “seascape genetics” approach (integration of genetic data on population mapping and various environmental parameters, such as temperature, salinity and depth profiles), on one side and to demonstrate experimental outcomes and their value for applications to stakeholders and end-users. Such environmental parameters will be enlarged progressively in collaboration with the JRC Institute for Environmental Sustainability (IES). Importantly it is also possible to visualize and overlay fisheries management sections such as ICES or FAO subdivisions. Thus, the findings from FishPopTrace open the door to various valuable applications for fisheries management, especially when displayed and accessible in the context of a Geographic Information Systems (any system that captures, stores, analyzes, manages, and presents data that are linked to location; GIS) environment. Such applications range from fundamental questions such as whether stock units match biological (population) units and to the extent to which issues relating to control, enforcement ad traceability can be investigated or applied.

A key component of technology transfer is to ensure that strictly designed protocols of traceability can be generated for practical uptake; so-called Standard Operating Procedures (SOPs). SOPs are documents containing instructions that forensic scientists and laboratory staff follow to perform procedures that are routine, standardised and for which no *ad hoc* modification is acceptable. They are indispensible when supporting crime investigations scientifically as they help to ensure the quality and integrity of data and provide a basis for guidance, uniformity and accountability. SOPs for selected analytical scenarios have been developed under the lead of partner by 16 (TRACE). SOPs have been developed for the four case studies, as follows:

Hake	*Merluccius merluccius*	Atlantic vs Mediterranean
Sole	*Solea solea*	Southern North Sea vs Irish Sea
Cod	*Gadus morhua*	North Sea vs North East Atlantic vs Baltic
Herring	*Clupea harengus*	North Sea vs North East Atlantic

The SOP for hake has been completely validated. SOPs for the other three species have not been tested using the Taqman© genotyping chemistry, but have been demonstrated using Kaspar©. Taqman© chemistry is recommended for use due to the appropriateness of the proprietary software. The primary target group of the transfer of these developed tools are test laboratories, accredited to perform analysis for control and enforcement purposes.

To ensure effective and targeted dissemination, SOPs will be placed on the public area of the FishPopTrace website, and, more importantly, the Community Fisheries Control Agency will be contacted to explore whether the JRC can expect any support for dissemination purposes (establishing contact of EU member state control and enforcement authorities). It was also important to ensure the proper execution of experiments carried out by the FishPopTrace consortium members, to facilitate the comparison of analytical results and to guarantee transparency within and externally to the consortium. The efforts of FishPopTrace to this end started with the sampling of fish and continued throughout the project through meetings and discussion of the respective experimental subgroups and during the FishPopTrace consortium meetings.

The sampling protocols' are collected in the FishPopTrace web-member area and accessible to all consortium members and upon request also to other scientists. The sampling itself has been rendered transparent from very early on with the public web-display of the FishPopTrace sampling effort. To our knowledge this is a unique approach among projects concerned with marine or anadromous fish where sampling is a prerequisite. For the experimental phase the respective subgroups (otoliths; proteomics; SNPs; fatty acids) streamlined their strategies and protocols. For example the SNP subgroup organised a 2-day meeting at the premises of P02 (DTU Aqua) in February 2009 to discuss a common and optimised experimental approach. This led later even to a publication in the peer-reviewed journal *Molecular Ecology Resources* (Helyar, S.J., Hemmer-Hansen, J., Bekkevold, D., Taylor, M.I., Ogden, R., Limborg, M.T., Cariani, A., Maes, G.E., Diopere, E., Carvalho, G.R., Nielsen, E.E. (2011) Application of SNPs for population genetics of non-model organisms: New opportunities and challenges *Molecular Ecology Resources*, 11 (SUPPL. 1), pp. 123-136.).

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 website (http://fishpoptrace.jrc.ec.europa.eu/fisheries-policy-contribution)

Most recently, Jann Martinsohn (Partner 8), from the EC Joint Research Centre published on line a JRC Reference Report (2011), entitled: *Deterring Illegal Activities in the Fisheries Sector: Genetics, Genomics, Chemistry and Forensics to Fight IUU Fishing and in Support of Fish Product Traceability* (available here). The JRC report advocates a coherent and practical EU-wide approach towards making new molecular technologies available to European control and enforcement authorities. Among various recommendations, it aims to encourage informed dialogue among the various stakeholders and proposes the following concrete measures:

- Enhancing dissemination of relevant information and advice to all stakeholders;
- Providing laboratories in Member States access to standardised repositories of reference data and other relevant knowledge for the analysis of fish and fish products. These repositories could be similar to the FishPopTrace database that was profiled, and hosted by the JRC;
- Establishing a network of certified test laboratories to undertake analysis for control and enforcement purposes and to share robust and standardised validated analytical protocols, similar to the SOPs generated by FishPopTrace;
- Securing full training of inspectors and laboratory staff for cost-effective and reliable sample handling, processing and archiving.

A significant dissemination opportunity was provided by the European Commission DG RTD as they invited the FishPopTrace consortium to join the organising steering committee for the *Food Chain Integrity Conference*, a major dissemination event on traceability to be held in September 2011 in Brussels; a book chapter has resulted from the original invitation to participate, to be presented officially during the Food Chain integrity conference (Martinsohn, J.T., Geffen, A.J. Maes, G.E., Nielsen, E.E., Ogden, R., Waples, R.S. and Carvalho, G.R. (2011) Tracing fish and fish products from ocean to fork using advanced molecular technologies; In: *Food chain integrity: A holistic approach to food traceability, safety, quality and authenticity*; Edited by J Hoorfar, National Food Institute, Denmark, K Jordan, Teagasc, F Butler, University College Dublin, Ireland and R Prugger, Technoalimenti S.C.p.A., Italy; Woodhead Publishing Series in Food Science, Technology and Nutrition No. 212).

A notable highlight in terms of international exposure of FishPopTrace was the publication of a two-page News Focus article in *Science*, in December 2010, entitled: "*To Fight Illegal Fishing, Forensic DNA Gets Local*" (available here), in which Erik Stokstad, Environment and Sustainability Editor for *Science,* described how FishPopTrace had produced a new generation of genetic tests that could provide authorities a much better idea of exactly where fish have been caught. Profiling of FishPopTrace in *Science* not only acknowledges the state-of-art approach employed by the consortium, but importantly also provided detailed examples of how the SNP-based tools in particular had been employed to tackle various policy-led case European traceability case studies for each of the target fish species. Dr Stokstad was in attendance for the fifth consortium workshop in Madeira, allowing his participation in open scientific sessions, together with targeted partner interviews on methods, key findings and impact of FishPopTrace outputs in the context of IUU and fish sustainability.

An amendment to the FishPopTrace Technical Annex, associated with the granted three month no-cost extension, was the production of a stakeholder pamphlet, published (May 2011) in conjunction with the Brussels stakeholder workshop, May 11, 2011. The remit of the brochure (*Traceability of Fish Populations and Fish Products: Advances and Contribution to Sustainable Fisheries* (available for downloading at the FishPopTrace website (http://fishpoptrace.jrc.ec.europa.eu/), was to present the context, key findings and policy relevance of FishPopTrace activities and outputs, presented in an attractive, accessible and concise format. In addition to a contribution from Mr Timothy Hall, Head of Unit E4: Agriculture, forests, fisheries and aquaculture DG RTD, the brochure presented details of consortium membership, the role of traceability and population structure in sustainable management of fish resources, the challenges of IUU and fish fraud, key methods and findings from the project, as well as the extent of exposure of the project in the media. The brochure concluded with a consideration of mechanisms and opportunities for securing a legacy to FishPopTrace, and a call for need to extend the impact of FP7 science projects beyond the natural life of the funded period. In addition to numerous schematics and bulleted headlines to attract interest, the text included several "interviews" with members of the consortium, both in relation to science (e.g. Fisheries Forensics by Rob Ogden, TRACE, P16), and in terms of experience and impact on career development of early-stage researchers (e.g. Elena Gonzalez, University of Madrid, P04). Assistance was provided by a graphic designer, and local publishers allowing us to meet the strict deadlines. One-thousand copies of the brochure were produced, and distributed throughout the consortium (multiple copies per partner), to participants of the Stakeholder meeting held in DG

Mare (see delegate list here, plus many members of DG-Mare and DG-RTD), and to an extensive and representative list of stakeholders, end-users, scientists and the media.

4.1.4.3 Meetings, conferences etc

A major dissemination opportunity for FishPopTrace was the participation of various partners (P01, Gary Carvalho, Sarah Helyar), P02, Dr Einar Nielsen, PhD student, Morten Limborg) P16, Dr R Ogden), and co-organisation (Gary Carvalho) of a major international symposium held in Seattle, USA, in March 2010, *SNP Symposium III Applications of SNP Genotyping in Non-Model Organisms.* FishPopTrace was profiled as among the first major efforts to develop SNP markers in marine fish: the Editorial of the special issue of *Molecular Ecology Resources*, published in January 2011, *SNP Discovery and Applications of SNP Genotyping in Non-model Organisms* (edited by Seeb, J, Carvalho, GR, Hauser, L, Naish, K, Roberts, S & Seeb, L:), states: "*FishPopTrace* is an international project funded by the European Union (EU) to generate panels of SNP markers for geographic assignment of four commercially important marine species (Atlantic cod, European hake *Merluccius merluccius*, common sole *Solea solea*, and Atlantic herring *Clupea harengus*; Martinsohn & Ogden 2009). Fifteen research groups from the EU, Norway, and Russia are collaborating to discover and validate SNPs and to create a standard set of operating procedures for use by each (http://fishpoptrace.jrc.ec.europa.eu/). Their plan incorporates both the ability to modify the number of SNPs in relation to the level of population differentiation and associated geographic scale, and the inclusion of SNPs under selection that collectively allows unprecedented levels of population assignment in commercial fish. Moreover, the essentially binary nature of SNP variation facilitates their forensic validation and use in global datasets to fight illegal fishing and promote consumer protection".

Members of FishPopTrace 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), thereby building a permanent link between FishPopTrace and ICES. Furthermore, primarily through the Joint Research Centre, which constitutes the STECF secretariat, contributions from Jann Martinsohn have promoted ways to better include modern molecular technologies in fishery management decisions under the CFP remit. Particularly noteworthy is also that FishPopTrace has been invited in April 2011 to present project and results at the plenary session of the Scientific, Technical, Economic Committee for Fisheries (STECF), which resulted in a highly positive assessment of the potential of genetics for fisheries management in the plenary report which will be submitted to the EU member states (available here).

Martin Taylor and Gary Carvalho (Partner 1, Bangor University) organized a DNA barcoding workshop at the University of Penang, Malaysia, in November 2009, with a focus on the application of barcoding technologies to fish biology and fisheries management. A key application of the barcoding pipeline was traceability in fisheries

forensics, and there was also the opportunity to present aspects of the FishPopTrace project to workshop participants, as well as seminars in the Fisheries Research Institute, Department of Fisheries, Malaysia, the University of Malaya in Kuala Lumpur and the Centre of Research for Computational Science and Informatics in Biology, based at the University of Malaya and the Malaysian Fisheries Department in Penang. The format of the workshop included seminars and hands-on experience of the BOLD database, which also forms a component of the FishPopTrace traceability framework.

Figure 7: Participants at the DNA barcoding workshop at the University of Penang, Malaysia, in November 2009,

A meeting was held at the FAO, Rome in December 2009 to discuss the use of forensic approaches in the context of MCS and IUU fishing. An invitation was received from Michelle Kuruc, who attended the FishPopTrace Madeira workshop, for four members of FishPopTrace to participate: Jann Martinsohn (P06; subsequently elected as Chair of the workshop), Rob Ogden, (P16; who delivered an introductory lecture on forensic technology in monitoring, control and surveillance), Einar Nielsen (P02) and Gary Carvalho (P01), as participants. The aim of the workshop was to identify the most appropriate traceability tools for use within a forensic framework, and to generate a set of recommendations for taking the approach forward within a global context. The workshop included broad representation from a variety of nations and backgrounds, including forensic biologists, fisheries compliance officers, Directorates for fish inspection quality control, and various research organizations and Universities with experience in the development and application of advanced tools, as well as their implementation to fisheries. The workshop provided a key opportunity for profiling the objectives and progress of FishPopTrace, which was deemed a significant international effort in the quest to tackle IUU and promote consumer protection. An output from the workshop was to provide support for the establishment of an FAO Expert Group, in Fisheries Forensics.

4.1.4.4 Cost-benefit analysis of FishPopTrace traceability tools

In line with its goal to fully engage with end-users and stakeholders, as well as the Common Fisheries Policy (CFP), and to facilitate a technology transfer, FishPopTrace carried out an assessment of costs and benefits arising from the use of DNA technology and forensics in support of fisheries control and enforcement and traceability. A questionnaire on costs of DNA analysis for fishery control purposes was developed and sent out to 91 institutions in 32 countries. These countries included 24 EU member states and 8 third countries. Addressees included EU institutions, fishery ministries, customs offices, non-governmental organisations, fisheries control agencies, academic institutions, the industry and governmental departments. Based on evaluation of responses received this report shows that a number of authorities in several countries do use DNA testing on fish and fish products for control and enforcement purposes. In all cases where the available data allowed a cost benefit analysis in detail, the benefits significantly outweighed the costs. Moreover general observations lead to the conclusion that enhanced capacity building enabling the routine application of DNA-testing would significantly lower the costs. Additionally several experts independently pointed out that (forensic) DNA-testing in the frame of criminal investigations has a highly deterrent effect further increasing the cost-effectiveness. Based on the results obtained, the authors conclude that the routine application of DNA-testing could greatly support fishery control and enforcement as well as traceability and certification schemes in a cost-efficient manner, particularly if a coordinated effort by all stakeholders leads to a substantial building of capacity.

4.1.4.5 Other modes of dissemination

In addition to extended interest from the popular press and media (including *Science, New Scientist* and the *Economist*), FishPopTrace has enjoyed enhanced profiling from within EC instruments. For example, FishPopTrace was profiled on the CORDIS Technology Marketplace, as an example of cutting-edge technologies and applications to environmental management, "*So our fish do not disappear without trace*", as well as being chosen as a 'success story' for the European Commission's CORDIS website, *Project Success Stories - A sustainable future for fishing*, and for inclusion in association with EURESIN (ygaethofs@euresin.eu), a new, on-line database that will cover all life science projects funded under the KBBE programme in FP7 (yet to be published).

Bangor University submitted a Press Release on FishPopTrace in the early spring which resulted in an article in Fishing News (10 April, p. 8, www.fishingnews.co.uk, entitled "Protecting the Fishing Industry- new £3.6m programme to safeguard fish stocks for industry and consumers". The Press Release also stimulated a response from the UK body charged with the management and conservation of marine resources, the Department for Food and Rural Affairs (DEFRA): Tim Bostock, international fisheries policy adviser to DEFRA's sustainable fisheries team emphasises that the UK has taken an international lead in the fight against illegal fishing. "As part of our work under the ministerial high seas task force, we have been working with like-minded countries in developing approaches intended to deter illegal operations by hitting them where it hurts- in their wallets. Forensic tools such as those being developed [in FishPopTrace] will provide a powerful deterrent and help protect those who wish to operate legitimately. Tackling illegal fishing is an important facet of more effective fishery management and the monitoring, control and surveillance operations. A successful

completion of this project will certainly be welcomed by agencies charged with the enforcement of conservation management" he said.

In addition, articles appeared in the local press and the Coordinator, Gary Carvalho, was interviewed about the project on BBC Radio Wales, for the science programme, "Science Café". FPT has been publicised on the Bangor University homepage under "Latest News". FishPopTrace also enjoyed additional exposure on the UK Natural Environmental Research Council, *Planet Earth on-line*, which profiles via podcasts, timely and significant studies in environmental sciences. The podcast, *Cracking down on illegal fishing*, presented an interview with Sarah Helyar and Gary Carvalho from Bangor University (P01), in which they described the context and impact of IUU fishing at European and global levels, and the use of molecular approaches, including those developed within FishPopTrace, in tracing fish products for monitoring, control and enforcement.

FishPopTrace fully acknowledged that in order to transfer results and tools to stakeholders beyond the scientific realm, major efforts have to be put into achieving maximal visibility, while not overselling potential benefits (impartial dissemination). Obviously the publication of articles in peer reviewed journals had to remain one major dissemination component (detailed in Section 4.2). Dissemination of results has also been achieved through pro-active engagement with stakeholders during the reporting period. This includes invitation of various external 'observing' scientists such as Dr Petra Spaniol (DG Fisheries) to our kick-off meeting in April 2008; Dr M Kuruk, FAO, Fisheries Forensics in Monitoring, Control and Surveillance (workshop 2); Dr Jan van Aken, EU Marine Programme, Pew Foundation (workshop 2); Dr Harald Barne, DNV Research & Innovation / Biorisk Management (workshop 2); Dr. K. Glover (Institute for Marine Research; Norway), a renowned fisheries geneticists working, among others, in the field of traceability of fish farm escapees, and monitoring of genetic impacts on native fish populations, to the 5th FishPopTrace consortium meeting.

4.1.4.6 Concluding remarks

Worldwide, a broad 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), 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 socioeconomically healthy fishery sector in the sense of the Marine Knowledge 2020 strategy[1].

In addition to the key FishPopTrace outputs, a prominent mechanism for underpinning a legacy from FishPopTrace has been the production of a *FishPopTrace Policy Document* (available here), in which the context of European policy and legislation relating to traceability of fish products and IUU activities is considered specifically in relation to FishPopTrace. Despite the provision of validated traceability tools, detailed population maps of target species, and an extensive reference data base across primary and novel tools, it remains a considerable challenge to secure the momentum and applications generated. The Policy Document presents the consortium's attempt to facilitate the continuation of its efforts, which is based on three pillars:

1. Ongoing collaboration between FishPopTrace consortium members
2. A sustained FishPopTrace website, database and geo-visualisation portal;
3. Representation of FishPopTrace consortium members in relevant initiatives and organisations.

Members of the FishPopTrace consortium members are collaborating in sub-groups on a number of research projects and plan to do so in future. We expect this to have a positive impact on the continuation of dissemination of FishPopTrace outcomes and continued engagement with stakeholders. For example, several FishPopTrace partners, together with some partners from existing (AQUA-Gen) and past (GEN-IMPACT) European projects, plan to submit a proposal to the imminent KBBE call (*Providing molecular tools for monitoring the potential genetic impact of aquaculture on native populations*). Experience in traceability and forensics, together with established reference data bases for population mapping of some native species, will be especially valuable in this context. The FishPopTrace website will be central to the attempt to engage with the scientific community and stakeholders beyond the funding period of FishPopTrace. It is planned to maintain the website and database properly managed and updated in regular intervals and also to update the FishPopTrace member area. The strength of the FishPopTrace consortium was also enhanced by the engagement of various partners in internationally renowned working groups and initiatives, relevant to fisheries management. For example the FishPopTrace Coordinator was, until recently, Chair of the European Fish Barcoding of Life initiative (FISH-BOL), and Dorte Bekkevold (P02), is the Chair-elect of the ICES Expert Group on Application of Genetics to Fisheries and Mariculture. Links to the FAO have been built (e.g. FishPopTrace

[1] 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

consortium members have been invited to the FAO Fisheries Forensics Expert Group Meeting in 2009, and contact to the FAO is maintained) as well as links to the European Commission, in particular DG MARE. The FishPopTrace Policy Document descibes a strategy for future engagement of FishPopTrace consortium members with the Common Fisheries Policy as well as with international fisheries management frameworks. It should be noted however, that the success of this approach will greatly depend on the response of policy makers, fisheries managers and other stakeholders. Only a common effort can ensure that the legacy of FishPopTrace will be preserved and can contribute to an improved fisheries management framework under the remit of the Common Fisheries Policy.

Within the life-time of FishPopTrace, there have been various significant policy developments in relation to fish traceability and the quest to reduce IUU activities, some of which have been noted: for example the Slow Fish International Fair (Genoa), 27 May 2011). An important policy earlier development was the first meeting of the new European Parliament Fisheries Committee on 1 September 2009. Dr Joe Borg, Member of the European Commission Responsible for Fisheries and Maritime Affairs delivered a speech to the new Committee, "A new era of cooperation" (full text available here), in which he outlined his vision for the reform of the Common Fisheries Policy (CFP). Among the issues to address is what he referred to as "the three pillars": to re-order the priorities on which the CFP is based; to end the chronic over-capacity of the European fishing fleet, and to redistribute responsibility to ensure delivery of goals. Emphasis on imaginative solutions to over-capacity was highlighted, including the introduction of transferrable fishing rights, or to manage stocks with effort only, replacing the traditional TACs and quotas. An interesting corollary of the latter is that it might reduce the incidence of under declaring or falsely declaring catches- though detection of such illegal activity and subsequent enforcement of spatially-based fishing effort will remain important. It is in the context of such developments that the contributions from FishPopTrace were especially pertinent: the need to enhance detection rates, the need to deter illegal activity, and the need to prosecute when required. All such requirements necessitate advanced technologies to yield reliable, accessible and forensically-validated tools – the overall aim of FishPopTrace and other activities relating to traceability of fish and fish products.

4.1.4.7 Address of project public website, and relevant contact details

The FishPopTrace project website can be found at;

http://fishpoptrace.jrc.ec.europa.eu/home

The contact details of all partners are available at;

http://fishpoptrace.jrc.ec.europa.eu/contacts

All additional documents referred to within this report can be found at the FishPopTrace website, and by then following: **Data access** > **fileshare portlet** > **documents** > **FPT documents** > **FPT Final Report.**