ICES WGAGFM Report 2009

ICES Mariculture Committee

ICES CM 2009/MCC:03

Ref. SCICOM

Report of the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM)

1–3 April 2009

Sopot, Poland

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CIEM Conseil International pour l'Exploration de la Mer

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Recommended format for purposes of citation:

ICES. 2009. Report of the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM), 1–3 April 2009, Sopot, Poland. ICES CM 2009/MCC:03. 74 pp.

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Executive summary

The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) met in Sopot, Poland 1–3 April 2009. The meeting was very well attended; with a total of 19 representatives present from 11 countries (12 national delegates and 7 experts appointed by the Chair)

Five Terms of Reference (ToRs) were on the agenda for 2009, However, due to the fact that several review papers have been published recently about genetic, genomic and biotechnology approaches of disease control in aquatic organisms, in addition to international and national projects targeting the development of genomic tools and resources for several aquaculture and fisheries species, we decided that ToR e) (To evaluate prospects for application of genetics/genomics to study and reduce the impact of fish and shellfish diseases in natural and cultured populations) should be postponed and modified into a new ToR in 2010 (ToR a) for 2010).

The WGAGFM also recognized the need to discuss the Science Plan 2009–2011 presented by SCICOM and added this to our schedule for this year's meeting (Section 7). We recognized two key areas where genetics studies can be carried out to make a significant and substantive contribution to advancing the ICES science objectives set out in the 2009–2013 plans. The first is with regard to increasing understanding of biodiversity. Biodiversity, in so far as it must be emphasized in the conservation context and in recruitment processes, is fundamentally genetic diversity. As such, genetic studies can be employed to understand how marine fish and shellfish species and stocks are structured into biologically distinct population units that are functionally relevant to the management of biodiversity. The second area where genetics can make a significant and substantive contribution is in relation to understanding how functionally relevant population units will respond to environmental change as regards their recruitment dynamics and stock character (e.g. age at maturity, species range, etc). However, these areas of study are necessarily connected as the second cannot be achieved unless the first is appropriately defined.

The first ToR was a progress and possible prospects report on the Meta-Database that the WG put on the agenda for the first time in 2007. In 2007 it was recommended that ICES should host a meta-database. The original idea to assemble primary data delivered by relevant research projects into one single all-embracing databank was temporarily abandoned in 2008 since it became obvious that such an approach would require management and financial capacities far beyond the available resources. Some more research into the subject and discussions with experts indicates that the optimal starting strategy to allow end-users gaining an overview of the current state-of-the-art in specific areas of interest, and to specifically search for available knowledge that resulted from research as well as datasets, is to develop a crawler tool. This crawler would periodically (e.g. each night) access selected project web pages and databases, and acquire as well as update available information, which would be stored on a designated server. End-user can query the information using a specifically designed user-menu. Under the current circumstances and with respect to available financial and personnel resources, we will pursue the crawler approach and discuss a joint or parallel and complementing activity of WGAGFM and the FishPopTrace consortium.

In ToR b) we summarized the potential and value of analytical technologies based on genetics for elaborate traceability schemes in support of Monitoring, Control and Surveillance (MCS) and enforcement in the fisheries sector. Traceability, the ability to

identify an item as well as to be able to track its origin, through all stages of the trade chain, as long as properly implemented, is highly valuable for MCS in the fisheries sector, as well as to fight fraud along the supply chain. However, this is only valid if traceability schemes are not solely based on documentation, certification and labelling, but are accompanied by powerful control and verification tools. Technologies for independent control of compliance with existing rules and supporting enforcement for non-compliance are urgently needed to fight the massive amount of illegal activities in the fisheries sector, which currently vastly escape control and add considerably to the precarious situation of world fisheries. The need for such technologies extends well into the fisheries (product) supply chain, where fraudulent activities, such as selling fish under false labels, hamper consumer protection. We discuss the application of such technologies for species identification and origin assignment of fish (products) as both are issue to fraud, but pose distinct challenges. We argue that due to the rapid ongoing progress in life science technologies there is a major opportunity to transfer results emanating from research to fisheries control applications and traceability.

However to guarantee the successful transfer of these technologies as applications for authorities, protocols should be standardized, and validated, preferably by applying forensic standards. Also awareness of existing legal and policy frameworks should be generated, including the identification of possible shortcomings. To this end an interdisciplinary dialogue, involving scientists as well as control authorities, the industry, and policy-makers should be established, as it would greatly enhance a mutual understanding about needs and challenges both from the scientific as from the fisheries management side thereby boosting such an approach. We believe that ICES, looking back at a long history of advisory activity at the interface of maritime science and policy-making, is well positioned to catalyse such a process and come forward with recommendations which could underpin such an endeavour.

The third ToR was an update on the EU project SALSEA-Merge on establishment of a large-scale genetic database. SALSEA-Merge is a collaborative project involving 14 research institutes across Europe as well as six conservation NGOs. The project aims to advance understanding of the factors affecting the marine mortality of European Atlantic salmon during their oceanic feeding migrations in the Northeast Atlantic, and although the specific oceanic factors responsible are as yet unknown, change in the oceanic environment associated with climate change is likely to be important. The SALSEA-Merge project demonstrates the potential to the development of useful molecular genetic tools for advancing not only understanding of mixed-stock fisheries on the European scale but also for advancing understanding of the marine ecology of species by allowing studies of the spatial and temporal distribution of stocks and their constituent populations to be undertaken. This potential in the Atlantic salmon was significantly enhanced by different research groups working on the species identifying a set of optimal markers for future work so that datasets collected by individual research groups could be integrated effectively and be used as the basis for the development of a trans-European baseline dataset.

In ToR d) we assessed the possibility for the development of an integrated global management model for Atlantic cod based on genetic information. Today the lack of conclusive evidence regarding the frequency of occurrence and evolutionary significance of micro-geographical population structure appear to be the largest impediment against implementing the use of genetic information for defining management units in cod. Studies should particularly focus on elucidating whether the observed genetic differentiation among spawning aggregations separated by a few tens of

kilometres are stable in time (across generations/decadal time-scale) and thus represent true semi-independent units. Since separation time among local populations is expected to be short, migration rates are expected to be high and effective population sizes relatively large, application of genetic markers subject to selection may prove valuable as genetic markers for Genetic stock identification (GSI) in conjunction with presumed neutral markers. Temporal sampling of spawning aggregations is a prerequisite for obtaining robust results, which can be used for defining management units. If current management units do not reflect the evolutionary relationships among populations, there is no excuse not to change current practice, and management should ensure conservation of biodiversity, including intraspecific genetic variation. New molecular genomic methods may provide evidence of ecological populations as well. It is also important to be aware of interspecific differences which are of great importance in multispecies approaches when focusing on area specific management.

1 Opening of the meeting

The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) met in Sopot, Poland from 1–3 April 2009. The ToRs were decided in the Council Resolutions adopted at the ICES Statutory meeting held in Halifax, Canada in 2008. Dr G. Dahle (Norway) chaired the meeting, which opened at 0900 h on Wednesday, 1 April and closed at 12.30, Friday, 3 April 2009.

1.1 Attendance

Nineteen persons from eleven countries (Belgium, Canada, Denmark, France, Germany, Ireland, Italy, Norway, Poland, UK, and USA) attended the meeting (Annex 2). Twelve were official members (or substitutes) for their countries and seven were appointed experts by the Chair for 2009. The latter were registered with ICES prior to the meeting.

1.2 Venue

The meeting was held at the Institute of Oceanology, Polish Academy of Science, Sopot, Poland. The WG wishes to express their appreciation to the local hosts Dr Roman Wenne and Dr Anna Was and the rest of the staff at the institute for their kind hospitality. The meeting venue was ideal with accommodation available in walking distance at the Haffner Hotel, and Wanda guest house in Sopot. The venue had a big conference room with projector and enough small meeting room for group meetings. The WG also wishes to extend their gratitude to the Sea Fisheries Institute in Gdynia who most generously hosted a spectacular dinner in the Aquarium at the Institute.

1.3 Meeting Format

WGAGFM has an established framework for completing its ToRs. Prior to the meeting, small *ad hoc* working groups, under the leadership of one person, are established to prepare position papers related to specific issues in the Terms of Reference. The leader of the ToR is responsible for presenting the position paper in plenary at the meeting and chairing the discussion. Thereafter, volunteers undertake the task of editing and updating position papers according to points raised in the plenary discussions. The ToR leader is responsible for preparing the final report text from their sessions. Prior to the meeting an agenda is circulated to all members.

2 ToR a) Establishment of a Meta-Database for Genetic Data on Fish and Shellfish Genetics covered under the ICES Remit – Progress and Prospects

E. Verspoor, L. Araudo, and J. Martinsohn

2.1 Rationale

This WGAGFM ToR was embarked on in 2007 pointing out that worldwide numerous studies have been carried out covering many aspects to fish and fisheries genetics[1]. These studies have produced a great wealth of data with potential value for future applications (such as in fisheries management), but could also serve as a fundament for new research projects. However, after the conclusion of research projects, the dispersal of generated data leads to a high risk of data-loss and greatly impedes a more coherent approach to genetic fish and fisheries research.

The WAGFM addressed this issue by proposing the development of a meta-database assembling and cataloguing existing data in the field of fish and shellfish genetics, and ensuring its accessibility to the research community. We argued that such an approach could efficiently counteract the current trend of data dispersal, thereby promoting research coherence, enhancing research progress, and facilitating the translation of results from fundamental research to applications in the fisheries and the aquaculture sector.

2.2 Progress since 2008

The 2008 WGAGFM report proposed an elaborate development and implementation strategy for an operational online version the Fish Genetic meta-database, although emphasizing at the same time that the implementation cannot be achieved *ad hoc* but rather by following a staged and progressive approach.

Originally it was considered to assemble primary data delivered by relevant research projects into one single all-embracing databank. This concept however was temporarily abandoned in 2008 since it became obvious that such an approach would require management and financial capacities far beyond the available resources. First and foremost ensuring data validity, quality and constant updating would be impossible to achieve with the existing resources. Also various difficulties of technical and other nature must be overcome. This concerns e.g. the vastly heterogeneous format of datasets (see below), but also other issues such as the clarification of property rights.

Following a WGAGFM internal consultation and discussion during the 2008 meeting, which confirmed the value and usefulness of a web based tool providing an overview over existing and concluded research projects and facilitating data access, the development of a public online portal (metadatabase) cataloguing existing genetic datasets and biological materials, as well as their location where they can be accessed, and which could catalogue historical and contemporary research projects for the species of interest, was proposed.

Although not providing direct access to primary data such an approach would allow researchers to gain a comprehensive overview of existing population genetic information for a given fish or shellfish species and enhance the capacity to carry out

[1] A first analysis of the current situation, and recommendations, were actually forwarded in the WGAGFM report 2006.

meaningful reviews to underpin advice and for developing new optimally targeted research programmes. Additionally, it would provide stakeholders, such as regulators or fisheries managers, with a one-stop location for rapidly identifying where information can be found which can be used to assess the state-of-the-art for a given species, or assess work carried out on the population genetics on species generally, as well as the extent of progress made with respect to applications of the research to fisheries and aquaculture.

It has to be emphasized though that an important set of data cannot be accommodated by such an approach: Fisheries genetics started well before it became routine to store data electronically, *i.e.* in IT-databases. An unknown but substantial set of such primary data resides on paper in academic institutions. The value of such data, also for future purposes, must not be neglected. It can *e.g.* be used in future projects as "standards" to be compared with new data and analytical results. Such datasets are also invaluable in time-series *e.g.* in the context of studies analysing genetic changes in populations over time and possible correlations with climate change. Therefore ways should be explored to save these data, and the only solution appears to be the transfer from paper into IT-database systems.

As one of the first steps it was foreseen to focus on using Atlantic salmon metadata which is being complied as part of the NASCO Salmon at Sea programme (SALSEA; http://www.nasco.int/sas/salseamerge.htm) under the EU funded FP7 SALSEA-MERGE project. The decision to use the SALSEA database as a starting point was taken as this should help to develop a portal prototype. Key information types should be identified to be included, to assess access and data presentation features needed, and allow the underlying IT requirements to be specified in detail and realistically tested. However the SALSEA Salmon database is not in place yet, and therefore it was not possible to proceed as originally planned.

However to pursue such a "step by step" procedure by initially focussing on one carefully selected project was in retrospect the right decision. Although exploring a panoply of research projects in the area of fish genetics, which are supported by websites, it became quickly evident that underlying databases (if available) and the data structure were vastly heterogeneous and at very different stages when it comes to database maintenance and management. This poses obviously a big challenge with respect to the development of an IT-tool supporting data access and mining. In fact this observation suggests rather returning to the original idea of developing a genuine Meta-database hosting primary data. This however, as discussed above, implies the availability of a fully dedicated staff and sufficient financial support, *i.e.* this should be done in the frame of a specifically designed project!

In 2008 the FP7 project FishPopTrace (https://fishpoptrace.jrc.ec.europa.eu) has started and it is one of the declared aims of FishPopTrace to support the coherence and integration of ongoing and concluded research projects in the area of fish genetics.

This will be achieved following two main paths (see FishPopTrace website on the page TOOLS under "Related Projects"):

Firstly links have been established to relevant projects. Each link is accompanied by introductory text, outlining content, scope and goals of the respective projects.

Secondly discussions with the IT-expert Luca Arnaudo (European Commission DG JRC) have lead to the conclusion that the optimal starting strategy to allow end-users gaining an overview of the current state-of-the-art in specific areas of interest, and to

specifically search for available knowledge that resulted from research as well as datasets, is to develop a crawler tool.

This crawler would periodically (e.g. each night) access selected project web pages and databases and acquire as well as update available information, which would be stored on a designated server. End-user can query the information using a specifically designed user-menu (see Figures 2.2.1 and 2.2.2).

The production phase of such a crawler tool has meanwhile been started under the responsibility of Luca Arnaudo. Currently the database of the EU project FishTrace (www.fishtrace.org) is used as a source database to develop a prototype. FishTrace was chosen as a starting point as the underlying database was developed and is hosted by the Joint Research Centre, greatly facilitating the prototyping.

Provided the unanimous consent of the FishPoptrace consortium it could be envisioned to make this platform available also to the WGAGFM. This would potentially increase the visibility and usage of this tool allowing better evaluating and improving during the developmental phase.

2.3 Imminent Future Strategy

During this years' WGAGFM meeting it was decided jointly and unanimously by the attending members, that the above outlined approach is suitable as a starting point. Under the current circumstances and with respect to available financial and personnel resources, it is possible to pursue the crawler approach and to discuss a joint or parallel and complementing activity of WGAGFM and the FishPopTrace consortium. This could be reciprocally beneficial in a variety of aspects concerning access to data, visibility, networking etc. Of course a condition would be the unanimous consent of the FishPopTrace consortium. First discussions about his option with the FishPopTrace coordinator were highly positive and currently no major obstacles are to be expected. However a more formal agreement involving the whole FishPopTrace consortium will be concluded very quickly.

After the agreement conclusion other databases will have to be integrated in the crawler approach and one possibility would be to use next the SalseaMerge database if available.

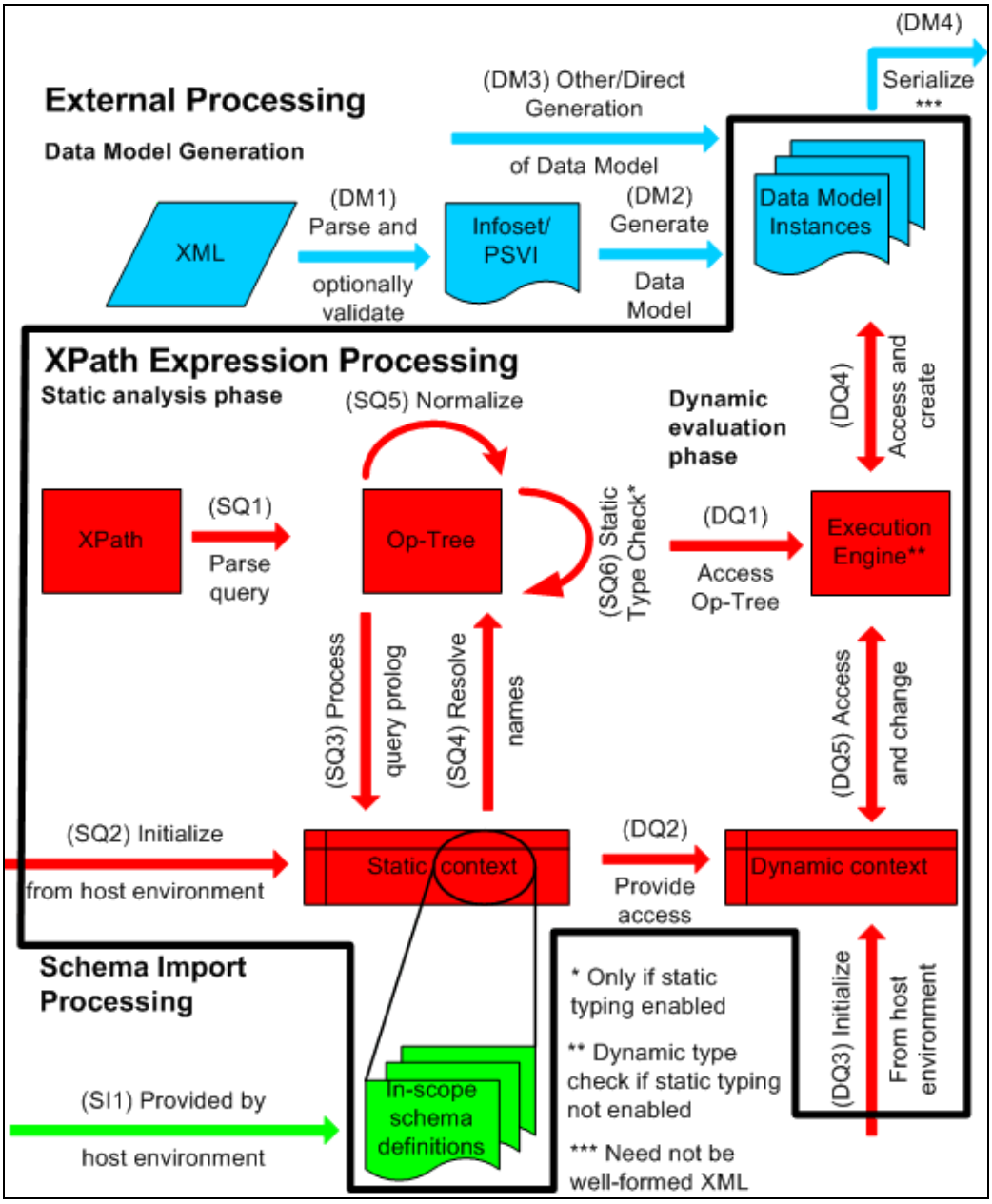


Figure 2.2.1. Diagram depicting the processes underlying the proposed crawler tool. Selected websites are crawled at determined intervals through Xpath and Xquery technologies. An index of accessed data are created automatically and stored in a database. Currently the following data are included: number of species stored; number of DNA sequences per species; number of tissue samples per species; number of bibliographic references per species. However data to be indexed can constantly be revised. Technologies adopted for the task: java 1.5 programming language; JPA and hibernate database communication automation; PostgreSQL database; WebHarvest libraries for web crawling APIs with Xquery. Courtesy of Luca Arnaudo; 2009.

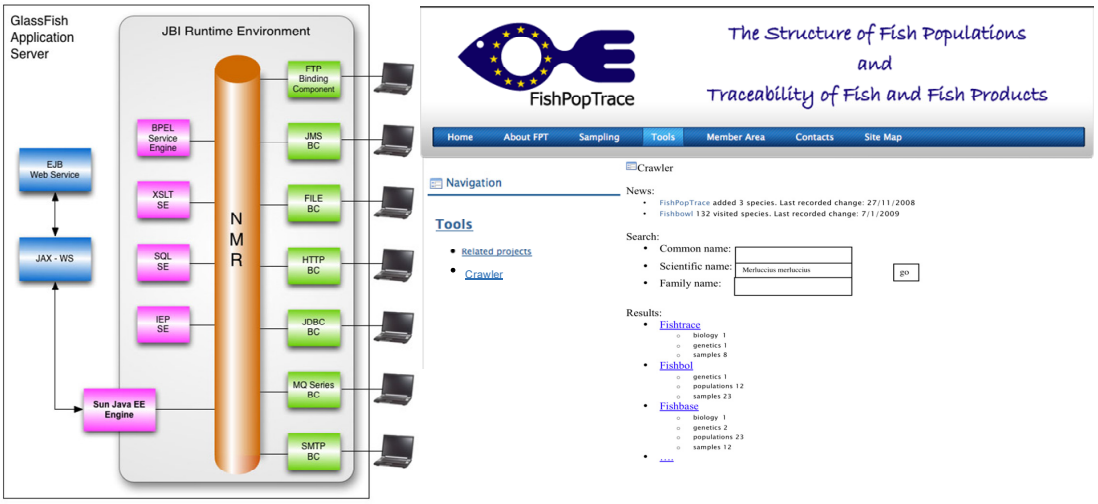


Figure 2.2.2. IT environment supporting the crawler tool and possible structure of the crawler interface (draft version). The website is supported by the technologies: Liferay® lightweight content portal; Glassfish® application server; Apache® web server. The website is developed in dynamic HTML, with use of CSS customization of the liferay portal, and JavaScript shortcuts for interaction speed. (Courtesy Luca Arnaudo; 2009).

2.4 Long-term Future Strategy

As pointed out above, a major challenge with respect to building a comprehensive meta-database cataloguing existing data in the field of fish and shellfish genetics, arises due to the lack of resources guaranteeing a proper management, *i.e.* constant upgrading, maintenance, versioning *etc.*. It was originally foreseen, that after development of such a database it would be hosted by ICES. However, rather than being a static entity, such a database will extend throughout its lifetime and it is not obvious how maintenance can be guaranteed without financial, personnel and expertise resources, specifically allocated to it.

Identifying past and present datasets and how to assemble those and to guarantee access to stakeholders is an important first step, but clearly an elaborate long-term future strategy is also needed.

The European Commission is currently investigating the possibilities of setting up a European Marine Observation and Data Network (EMODNET)[2]. Already in the WGAGFM 2008 report it was emphasized that it is worth exploring possibilities to ultimately integrate a fish genetic database into the Marine Observation and Data Network (EMODNET) or to tap on the underlying infrastructure.

EMODNET intends to provide a sustainable focus for improving systematic observation (*in situ* and from space), interoperability, and increasing access to data related to maritime affairs and fisheries, based on robust, open and generic ICT solutions. It should be explored to what extent the WGAGFM fish genetic Meta-Database might

[2] Commission of the European Communities (2009) "Staff Working Document - Building a European marine knowledge infrastructure: Roadmap for a European Marine Observation and Data Network". Brussels, 7.4.2009; SEC (2009) 499 final

fit into this large data network project to maximize its benefits to the research and resource management communities. This will be undertaken by Jann Martinsohn.

2.5 WGAGFM recommends:

1) Pursuing the development and implementation of a web-based fish population genetic meta-database, under the responsibility of WGAGFM, within the remit of ICES and in collaboration with the European Commission, as proposed in the WGAGFM reports of 2007 and 2008;

2) The meta-database should ultimately serve as a portal cataloguing relevant information on existing genetic data, primary and secondary reports on genetic research, and available biological samples for genetic analysis, indicating the repositories and contacts from which such data, samples and other relevant information can be obtained;

3) The Working Group or a subgroup thereof, reviews, by September 2009, the type of data to be included. If needed the currently incorporated data categories will be complemented and a comprehensive reference list of the selected data types be produced and presented at the WGAGFM meeting 2010;

4) A review be completed by April 2010 on the scope for including historical datasets that are not accessible by IT;

5) A web-based crawler tool, originally developed for the FP7 project FishPopTrace (https://fishpoptrace.jrc.ec.europa.eu) by the EC Joint Research Centre, be put at the disposal of the WGAGFM to catalogue relevant electronically available genetic data and make this accessible via a web interface to end-users; furthermore, together with ICES and the European Commission it be explored whether, and under which conditions, for this purpose a special website dedicated to ICES-WGAGFM, and implementing the crawler tool, can be developed;

6) The first projects used for Crawler development are the completed EU FishTrace project (www.fishtrace.org) and, if possible, the ongoing EU Salsea-Merge project (http://www.nasco.int/sas/salseamerge.htm)

7) Possibilities are explored to enhance and support the efforts underlying this ToR with respect to storing, managing and accessing relevant population genetic metadata, particularly where it is currently difficult to access through the web. If appropriate, and available, alternative resources and collaborations for database development and web-based tools that ensure accessibility to such data, should be considered;

8) The following tentative deadlines for delivery and reporting on progress for this ToR:

R3: Delivery September 2009; Report WGAGFM Meeting 2010;
R4: Delivery November 2009; Report WGAGFM Meeting 2010;
R5: Delivery of prototype Crawler foreseen April 2009; Implementation for WGAGFM after clarification of consent by the WGAGFM; Fishprace Consortium, ICES and the European Commission.

3 ToR b) Review the current status of traceability methods in the fisheries sector based on genetics

G. Carvalho, S. Helyar, D. Bekkevold, F. Volkert, R. Hanel, D. McPhee, M. Ford, J. Carlsson, J. Trautner, R. Ogden, and J. Martinsohn

3.1 Traceability in the context of Illegal, Unreported and Unregulated (IUU) - Fishing and the fisheries supply chain

3.1.1 Why is a traceability system required?

The fight against Illegal, Unreported and Unregulated (IUU) fishing plays a crucial role in the attempt to move towards sustainable fisheries. IUU fishing is a global problem that continues to be out of control. Its value has been assessed to amount worldwide to be between €10 to 20 billion (Agnew *et al.*, 2009), which is more than twice the value of annual landings by the EU fleet (€6.8 billion in 2004[3]). These estimates are probably rather conservative, but certainly IUU fishing represents the major source of fishing mortality (Figure 3.1.1.1). Such estimates are, however, probably very conservative, but nevertheless represent the major source of fishing mortality. Escaping control, IUU fishing threatens marine ecosystems, impedes management schemes for sustainable fisheries, and has a negative effect on socio-economic development. Moreover, globalisation has had major affects on the food supply chain. It has removed production from direct consumer control, increased competition, lengthened the food supply chain, and made it less transparent. There has been an associated increase in awareness in traceability issues to deal with food safety, quality assurance and animal welfare.

Illegal activities extend into the supply chain, as has become evident by fraud cases in the US and Europe where fish has been sold under false labels (for examples see Annex 1). Such practice leads to consumer misinformation and hampers efforts to ensure consumer protection. Consumer protection is currently mainly assured by documentation and labelling of products and such a system is prone to fraudulent activities. Increasing dependence on product imports and complex marketing patterns further impede efforts to regulate and control the fisheries sector. Increasingly, certification procedures that endorse sustainable fisheries, such as awarded by the Marine Stewardship Council (MSC) or consumer oriented websites describing fishery status, such as the NOAA Fishwatch program (http://www.nmfs.noaa.gov/fishwatch/), are employed to provide information on fishery products. However, such certification is also susceptible to fraud. Therefore, to fight illegal fishing activities and ensure sustainability, fairness and transparency in the fisheries sector, as well as for the information and protection of consumers, a traceability system is required. Traceability is defined by the CODEX Alimentarius Commision (CAC 2006) and according to ISO 22005:2007 as the "ability to follow the movement of a food through specified stages(s) of production, processing, and distribution and for the EU laid down in Regulation (EC) No. 178/2002.

[3] European Commission DG Mare Press Corner
http://ec.europa.eu/fisheries/press_corner/press_releases/archives/com07/com07_69_en.htm

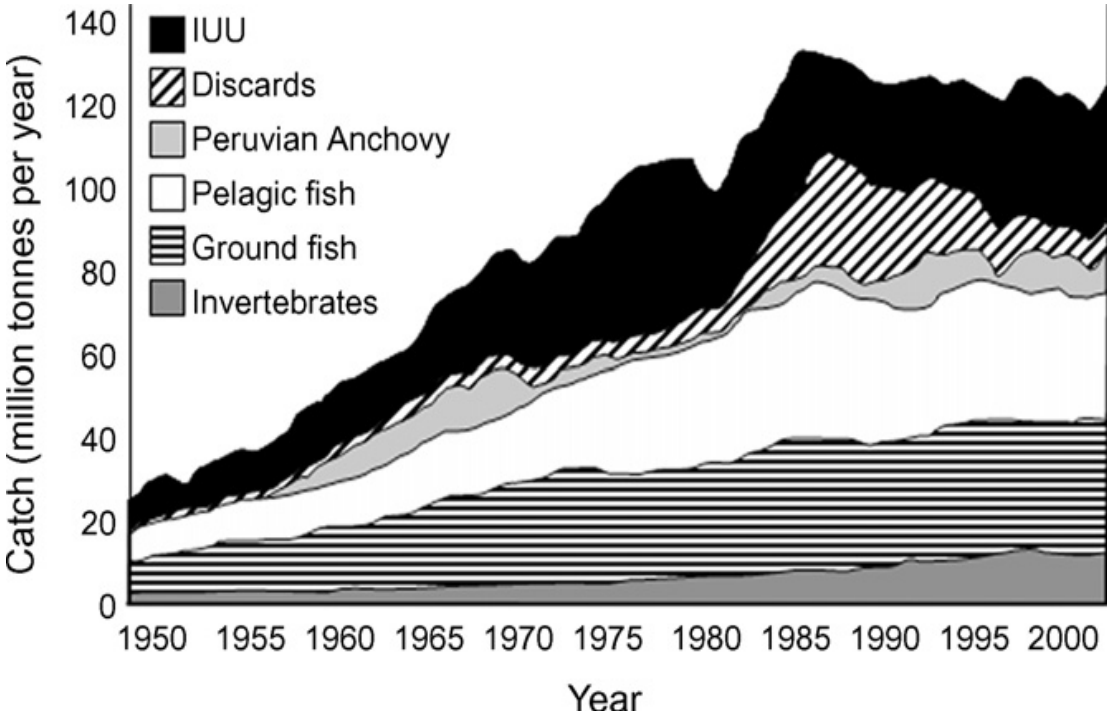


Figure 3.1.1.1. World marine fisheries catch, by major taxonomic groups and fishery. Shaded groups are based on landing statistics from the Food and Agriculture Organization of the United Nation. IUU, Illegal, Unreported, and Unregulated Fishing. With kind permission and updated from Pauly *et al.* (2002), and the Sea Around Us Project (http://www.seaaroundus.org/).

Any such system in the fisheries sector should be effective throughout the food supply chain ("from ocean to fork"), and be supported by independent control measures to verify the species and origins of fish and shellfish caught. Consequently there is an urgent need to identify traceability markers that can be used throughout the food supply chain, from on-board samples, to processed product, and which exhibit minimal variance. Furthermore, it is likely that traceability tools will in many cases need to be applied within a sufficiently robust forensic framework (Ogden 2008) to promote legal enforcement.

3.1.2 Traceability at the species and population levels

There are two broad biological levels at which a traceability systems are required: the species and population levels. The former is technically more tractable and with considerably more examples than the latter (Costa and Carvalho 2007; Hauser and Carvalho 2008), though to our knowledge a consistent approach, ensuring the identification of fish and shellfish species in fresh and processed seafood on a routine basis and with legal relevance is not yet established anywhere in the world. With the increase in cultured fish practices, tools to identify and distinguish cultured and wild seafood products are also of increasing importance (Dempson and Power, 2004; Chen *et al*., 2006). Identification of the population of origin of an individual or group of individuals is applied less often, as it poses significant challenges compared to species identification, where individuals are often assigned based on consistent phenotypic differences or diagnostic genetic differences. Such marked or fixed differentiation among populations within species is rare, since most populations are to some extent, connected by migration and gene flow. Instead, different marker variants have varying frequencies among populations, and traceability relies on probabilistic methods using a combination of markers to provide sufficient statistical

support (Pearse and Crandall, 2004; Ruzzante *et al*., 2006). Thus, there is a requirement to establish a marker-based framework that is sufficiently informative and robust to deliver evidence within a forensic framework, though absolute requirements will vary among organizations. Moreover, it must be emphasized to develop classes of markers that can be accessed throughout the food supply chain.

Three primary drivers demand information at the population as well as species levels. First, it is generally recognized that populations are the natural unit of evolutionary change, and as such, provide the genetic resources required for adaptive response to natural and man-made changes in the environment. It is therefore at the level of populations that genetic and ecological diversity should be described for conservation measures, which necessitates discrimination between populations and their distribution and abundance across regional waters.

Second, and following from above, it is at the population level, or an appropriate conspecific assemblage, that policy legislation and associated enforcement must take place. The nature of boundaries defining the units will, however, depend on the context and policy drivers (Waples and Gaggiotti, 2006). Additionally the design of efficient control schemes poses a challenge, since most fisheries management schemes are complex, consisting of a combination of output management tools (catch limits; catch quotas, minimal landing sizes) and input management tools (capacity and effort limitation (C.E.C. 2006)).

Third, there is an increasing requirement for traceability of seafood products, both for consumer protection and regulatory enforcement. To be successful such approaches rely on a sound underlying policy framework with a geographic context, which in turn depends on accurate information on the relative dynamics and abundance of populations from particular regions. Additionally, to ensure compliance with rules, powerful control and enforcement tools are indispensible, especially in the light of the widespread problem of IUU fishing (Gallic and Cox, 2006). Knowledge about genetically distinct populations permits identification and discrimination, and can provide invaluable support to fisheries control and enforcement (Ogden, 2008).

In the context of traceability at the within species-level, it is worthwhile emphasizing at the outset the distinction between units that may carry a geographic signature and those that are also biologically identifiable. Any traceability system may provide information that relates to geography ("population tag"), as well as providing regional signatures that indicate biological differentiation in relation to spawning identity. Both aspects are important for traceability, and are not mutually exclusive, because the former signals source of origin, whereas the latter yields information on biological variability that may underlie population resilience and evolutionary potential (Hauser and Carvalho 2008). Recognizing spawning (or interbreeding) groups therefore provides a baseline for conservation of genetic resources (Hauser and Carvalho, 2008). Despite the plethora of definitions for the term "stock" (Carvalho and Hauser, 1994), here we refer to a "population" as a spawning assemblage. For traceability purposes, however, regional identity that may, or may not, coincide with spawning groups and associated biological differentiation, is also a valid unit of recognition.

3.2 Existing structure and policy frameworks – and a global context

Most nations with direct access to marine environments have policies in place to manage the exploitation of oceanic resources which are under their jurisdiction. Worldwide the governance of oceanic areas and underlying laws are increasingly tailored to support sustainable fisheries, to monitor and preserve biodiversity and to

protect ecosystems. Compliance with existing fisheries laws by monitoring, control and enforcement forms an inherent part of this approach and is mostly carried out by government agencies. Here we provide a brief overview of existing policy frameworks in the US, Canada and the EU.

Fisheries and Oceans Canada (DFO), is responsible on behalf of the Canadian government for developing and implementing policies and programs that support Canada's scientific, ecological, social, and economic interests in oceans and freshwaters. The DFO has committed to: "*develop and promote the wise use of technology in order to ensure the long-term health of Canada's waters; conduct scientific research and related activities, which are vital to the understanding and sustainable management of Canada's oceans and aquatic resources; and study, conserve and protect aquatic ecosystems*" (www.dfo-mpo.gc.ca). The guiding legislation includes the Oceans Act, which charges the Ministry of Fisheries with responsibility for oceans management and providing coast guard and hydrographic services on behalf of the Government of Canada, and the Fisheries Act, which confers responsibility to the Minister for the management of fisheries, habitat and aquaculture. The DFO is also one of the three responsible authorities (Environment Canada and Parks Canada Agency (PCA)) under the Species at Risk Act.

In the USA, the National Marine Fisheries Service, federal agency which is part of the National Oceanic and Atmospheric Administration (NOAA), is assigned with administration of living marine resources and marine habitats under US jurisdiction. Responsibility includes management, conservation and protection of all living marine resources within the United States' Exclusive Economic Zone. The main underlying legislative document is the Magnuson-Stevens Act, which provides guidance for the National Marine Fisheries Service in fisheries management activities such as stock assessment. An important part of these management activities is control and monitoring, and also here the Magnuson-Stevens Act constitutes the key reference ensuring compliance with fisheries regulations.

Another important law that affects fisheries is the Endangered Species Act, which is similar to Canada's Species at Risk Act (see above). The National Marine Fisheries Service is responsible for applying this law to marine and anadromous species. Species that are identified as being 'endangered' or 'threatened' are subject to federal protection, and activities that harm such species are subject to review and restriction.

The Lacey Act covers the supply chain in that it sets rules for the labelling of fish and wildlife products. Originally introduced to fight illegal hunting at the beginning of the last century, its scope has been broadened considerably through numerous amendments. Nowadays it prohibits the selling of unlabelled fish and wildlife products and penalises mislabelling. The Lacey Act is an expansive law as under its remit any US citizen is liable if he breaks an underlying foreign fisheries or wildlife law and subsequently imports, exports, transports, sells, or receives that product into the US Any misdoing is regarded as a felony provided that the matter under investigation amounts in value to more than $350, and that the investigating authorities can prove that the defendants had knowledge of their wrongdoings that is, acted intentionally (if no knowledge can be proven the wrongdoing is regarded as a misdemeanour).

For the European Union, the Common Fisheries Policy (CFP) is the principal instrument for the management of fisheries and aquaculture and its underlying rationale is to ensure sustainable exploitation of living aquatic resources. The European Commission initiates legislation by preparing the legislative instruments adopted by the European Council and the European Parliament in connection with Community poli-

cies. After adoption, the Commission implements, manages and controls the policies. Several attempts have been made to review and improve the CFP management scheme. Nevertheless European fish stocks have been continuously overfished for decades and the EU fishing fleets remain too large in relation to available resources. Such mismatch has led to a continuous decrease in the amount of fish caught, and resulted in imminent collapse of several stocks (Pauly *et al*., 2002). As a consequence, approximately 60% of fisheries and aquaculture products have to be imported into the EU market to meet demand, and such dependency on imports is increasing (http://trade.ec.europa.eu/doclib/docs/2007/march/tradoc_133509.pdf). Monitoring, control and surveillance (MCS) is a central pillar of the CFP.

Since its establishment in 2005, the Community Fisheries Control Agency (CFCA) strives to improve compliance with the rules under the 2002 reform of the CFP. The Agency aims to strengthen the uniformity and effectiveness of enforcement by pooling EU and national means of fisheries control and monitoring resources and coordinating enforcement activities. Such operational coordination helps tackle the shortcomings in enforcement resulting from the disparities in the means and priorities of the control systems in the respective EU Member States. Its tasks and mandate are defined in close cooperation with the Member States in accordance with EU objectives and priorities.

Recently the EU Court of Auditors identified serious deficiencies of fisheries control inside the European Union (European Court of Auditors, 2007). The European Commission agreed with the Court's analysis and came forward with a CFP control reform proposal in November 2008, currently being discussed with Member States and the European Parliament (European Commission, 2008; further discussed below – Section 7.3).

3.3 Overview of available techniques

A range of techniques have been developed that are currently used for the identification and traceability of seafood products. These include techniques that allow both assignment to species, and assignment to stock or population of origin. There are four main groups of techniques (for details see Annex 2):

3.3.1 Morphological trait markers

Some morphological and meristic markers have been used to assess fish origin. However, most of these approaches appear to lack the statistical rigour for traceability use within a forensic framework. However, if used cautiously and in combination with other (e.g. genetic) data, such types of information may act to strengthen inference about origin (Cadrin *et al*., 2004).

3.3.2 Non-genetic analysis of soft tissues

There are several classes of assay used with soft tissues. Stable isotope analysis is the standard method for differentiation of farmed and wild fish, and combining this with fatty acid profiling has provided high analytical power (Thomas *et al*., 2008). Trace element analysis also has been successfully used, as has isoelectric focussing of proteins However, increasingly all the above techniques are being replaced by genetic and otolith-based methods. One technique that can be used *in situ*, providing instant results, is immunoassays based on monoclonal antibodies (ELISA), which can distinguish between some species and subspecies. A major limitation of all the above methods is that whereas they are generally reliable for fresh or frozen samples; intense heat or drying can destroy the required biochemical properties.

3.3.3 Otoliths: shape analysis, microstructure and microchemistry

There are four main techniques for the use of otoliths (a calcified and chemically inert ear bone) in population discrimination; these are univariate shape descriptors and elliptical Fourier analysis (EFA), otolith microstructure, micro-chemistry trace element analysis and stable isotope analysis. Otolith composition and morphology are suitable for all stages of the food chain where the heads remain with the fish. More importantly, otolith composition and shape are resilient characters, and do not degrade or change over time after death (Thresher 1999). The main limiting factor of these methods is that they can only be used for teleost species, and are not applicable to products that have been processed and no longer incorporate the head of the fish.

3.3.4 Genetic analysis of associated organisms

A recent interesting approach to determining the provenance of samples involves the genetic testing of a bio-indicator linked with the organism, for example by analysing of the rDNA profiles of the bacterial community of Vietnamese Pangasius fish. The resulting profile could distinguish between farmed and wild samples (Le Nguyen *et al*., 2008). Also the EU Framework Program 5 project, WESTHER, examined intestinal parasites associated with herring. Early results showed that fish from different nursery areas carry unique parasitological identities based on differences in the mtDNA (ITS and COI) (herring, http://www.clupea.net/westher/, also European eel, Wielgoss *et al*., 2008).

3.3.5 Genetic markers

The use of DNA based techniques has several benefits (and some limitations) over the above methods, including increased sensitivity and reliability particularly with highly processed samples. DNA is more thermostable than protein, and does not vary with the tissue type, age. Unlike otoliths, DNA is present in all tissue types, and can still be recovered from even highly processed samples. For these reasons DNA markers are widely used in fisheries, both for species and population level identification (e.g. to identify mislabelled products, Genetic Stock Identification (GSI) and Mixed Stock Analysis (MSA); Hauser and Carvalho 2008). Either mitochondrial or nuclear DNA can be used, and there are many techniques available. Details on the advantages and disadvantages associated with each method are given in Annex 3. Several recent reviews highlight the range of techniques and applications currently in use for trade monitoring (Bossier, 1999; Gil, 2007; Baker, 2008; Kochzius, 2008; Rasmussen and Morrissey, 2008).

3.4 Overview of genetic approaches- with a focus on conceptual aspects and a critique of how such techniques match the requirements of a traceability tool(s)

The genetic approach to trace marine organisms depends on the question posed. If a species (taxonomic unit) has to be identified, a specific DNA fragment has to be characterized and matched with a database of voucher sequences for identification. If the stock origin of an individual has to be identified, the allelic composition of several specific DNA markers of an individual will be determined and compared to the allelic variation predetermined for the species in question from different geographical areas (the baseline data). Both scenarios are addressed below.

3.4.1 Species level identification

Most seafood products lose their defining morphological features during the early stages of processing, making them impossible to identify with traditional taxonomic approaches. The use of genetic methods for taxonomy has provided an alternative tool that can be used at all stages both in the life cycle and the fisheries chain, and is becoming routine in fisheries legislation. The applications range from the investigation of illegal trade, for example, caviar (DeSalle and Birstein, 1996) and shark fins (Abercrombie *et al*., 2005; Blanco *et al*., 2008), through to issues of consumer protection and fraud (Marko *et al*., 2004; Wong and Hanner, 2008). Many of the methods for discrimination of species are based on amplification of mtDNA and large databases of reference voucher sequences (e.g. FishBol and FishTrace) permit samples to be identified without prior species knowledge (Kyle and Wilson, 2007). Recent developments have added multispecies array-based techniques to the tool kit (Kochzius *et al*., 2008; Teletchea *et al*., 2008). However, when faced with samples containing multiple species, or fragmented DNA due to degradation or processing, the scope for sequencing is limited, and assays based on shorter DNA regions are required, making SNPs an ideal marker. Techniques to characterize degraded DNA have been continuously benefiting from developments in the characterization of ancient (aDNA) (Millar *et al*., 2008).

More recently, a revolution in sequencing technologies has reshaped the field of molecular genotyping (Shendure and Ji, 2008). Various technologies are used, but all rely on massively parallel sequencing and miniaturisation. They sample large fractions of the genome, and hence are much more representative than the short DNA fragments of just a few marker loci used thus far. Such techniques are fast, have high throughput, work on partial or even whole genomes, and have a relatively low cost (on a per nucleotide basis). Throughput volume of DNA sequencing has increased several orders of magnitude, regardless of whether samples comprise discrete individuals or are mixtures. A consequence is that SNP loci are discovered in large numbers (Sobrino and Carracedo, 2005). When combining the markers on a single "chip" (Kochzius *et al*., 2008), it is possible to identify routinely, reliably and economically many fish taxa simultaneously. Such tools have exciting applications in natural populations, including fisheries, and come close to the vision of Paul Hebert to identify taxa on site.

3.4.2 Population level identification–identification of stock origin

Population genetic research has demonstrated that many marine organisms are separated into more or less genetically distinct populations (recent reviews in Hauser and Carvalho 2008; Reiss *et al*., in press) allowing genetic traceability. As most populations have relatively shallow histories on an evolutionary time-scale and experience gene flow through migration, it is necessary to apply methods which use the combined information for allele frequency differences at a number of genetic markers for Genetic Stock Identification (GSI). If the numbers of populations and their genetic relationships can be established, genetic marker based traceability can be applied, allowing the assignment of individuals of unknown origin to their natal population within a probabilistic framework. Individual assignment tests have been applied demonstrating traceability of for example Atlantic cod (Nielsen *et al*., 2001a; and below). In addition, Genetic Assignment methods have been used to trace effects of enhancing local populations with individuals from exogenous sources (e.g. Hansen *et al*., 2002; Larsen *et al*., 2005; Nielsen *et al*., 2001b). Methods have also been developed that permit the estimation of the proportional contribution of individual populations

to mixed-population samples. Such genetic mixed-stock analyses are routinely applied for real time tracing of fisheries pressure on individual populations in a number of Pacific salmonid species, safeguarding against overexploitation of small and vulnerable populations (e.g. Smith *et al*., 2005). Despite the clear potential to such methods, they have rarely been applied to tracing marine fish (but see for example Koljonen *et al*., 2005; Ruzzante *et al*., 2006; Wennevik *et al*., 2008). Stock identification of individual fish caught at sea is now being used as a consumer awareness and marketing tool. For example, collaboration between Oregon State University, Oregon fishers and NOAA Fisheries is testing an application of physical barcoding, combined with genetic identification of stock of origin to allow consumers to identify the origin of salmon bought in the marketplace on line (http://www.pacificfishtrax.org/).

The potential to using genetic methods to trace individuals to natal population relies heavily on the completeness of the baseline information, which ideally encompasses representative diversity from all alternative populations. Such a requirement is obviously a limiting factor for all traceability techniques; however, with genetic approaches, problems with missing baseline information can potentially be remedied using statistical modelling and extrapolation procedures (e.g. Pritchard *et al*., 2000; Pella and Masuda, 2006). A potential shortcoming of genetic traceability techniques is the typically weak structuring of many marine fish populations (e.g. Anderson *et al*., 2008). The limitation can however, potentially be addressed by increasing the numbers and types of markers employed and/or integration of other independent marker types influenced by different evolutionary processes, such as neutral marker information combined with information for markers associated with candidate genes under divergent selection (e.g. Hauser *et al*., 2006), and the integration of genetic and phenotypic traits (Ruzzante *et al*., 2006).

3.5 Traceability at the species and population levels–past and present projects

Projects related to the traceability of fish and shellfish at the species level in Europe have been funded to a large extent by the European Union Research Framework Programs (FP). Historically, government agencies committed to fisheries enforcement limited themselves to morphological identification. It was only in the late twentieth century that databanks with protein profiles became available (Bossier and Cooreman, 2000). However, practical and operational limitations were such that attention was shifted to the more promising DNA approaches. The first two European projects to systematically adapt species identification to high throughput approaches were both funded under FP5. The project "Fishtrace–Genetic catalogue, biological reference collections, online database of European marine fish" (http://www.fishtrace.org) aimed to facilitate cooperation and the pooling of data and material for the genetic identification (sequencing of *Cyt B* and *rhodopsin* genes) and characterization of marine fish species from European waters and markets (Sevilla *et al*., 2007). A taxonomic database was established with species descriptions and DNA data, and linked to voucher specimens stored and catalogued in natural history museums. The project "Fish and Chips" (http://www.fish-and-chips.uni-bremen.de/PostNuke/html/index.php) examined the potential to DNA chips in the identification of marine organisms (fish, phytoplankton, and invertebrates). Research led to a mitochondrial 16S rDNA oligo DNA prototype microarray for the identification of eleven fish species. An advanced "Fish chip" of 50 fish species is planned (Kochzius *et al*., 2008). Elements of traceability were also integrated in the project on seafood safety SeaFoodPlus (http://www.seafoodplus.org) and the affect of escapees in aquaculture on natural populations–Genimpact (http://genimpact.imr.no). Cur-

rently, species identification of fish is implemented through the Fish Barcode of Life Initiative, FishBOL (http://www.fishbol.org/partners.php). The initiative targets a single locus cytochrome oxidase I (COI) for species-specific identification. As of March 2009, an impressive 6309 species have been DNA barcoded within the FishBOL programme.

Although species-focused projects (e.g. on Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), and herring (*Clupea harengus*)) have addressed stock traceability with increasing success over the past 10 years (Compare Borsa *et al*., 1997 with; Hemmer-Hansen *et al*., 2007 for European flounder), it is only recently that the tracing of stocks has been addressed systematically on a large-scale. At the time of writing, the EU Framework Programme 7 project "Fish Populations and Traceability – FishPopTrace" (https://fishpoptrace.jrc.ec.europa.eu) is underway. The project aims to construct a Pan-European framework, built on advanced technologies, for product traceability and policy related monitoring, control and surveillance (MCS) in the fisheries sector. The partners combine up-to-date array-based SNP screens to select informative population genetic markers and otolith data as proxies for ecological and life-history population structuring. Four model species have been chosen: cod (*Gadus morhua*), hake (*Merluccius merluccius*), herring (*Clupea harengus*) and sole (*Solea solea*) (Martinsohn and Ogden, 2008). A series of forensically validated marker systems will be developed to trace geographic origin for application to fisheries enforcement.

3.6 Sampling and design issues

One important issue to consider in any traceability program is the plan for obtaining appropriate samples. The specific sampling plan will vary depending on the questions addressed. In some cases, development and implementation of an appropriate sampling plan will be the most costly and logistically challenging aspect of the tracing program. Opportunities for obtaining samples occur at multiple points along the chain from fishing boat to final consumption, and the inferences that can be drawn from such samples vary, depending on the point in the chain they are obtained. For example, samples obtained from restaurants or retail markets will be useful in estimating the degree of mislabelling (Logan *et al*., 2008), but may not be very useful for quantifying the level of unreported or illegal catch if there are significant uncertainties in the supply chain going back from final consumption to the original catch. Samples obtained in ports may allow for inferences to be made about a specific fleet or fishery, but would not provide the spatially explicit information about catch location that would be available for samples taken at sea. In general, non-random or opportunistic sampling will be useful for determining if potential problems exist, but the use of tracing technology to quantify the level of IUU will depend upon a sampling plan developed to specifically address the question of interest.

3.7 Forensic validation and statistics

The conversion of molecular markers from a research tools into applied technologies to support MCS and enforcement measures requires validation of the analytical process. Validation studies are implemented to examine the accuracy, reproducibility and robustness of a method and its component parts in order to demonstrate the reliability and limitations of results when applied across a range of variables and real-life situations (Ogden, 2008). In the context of fisheries traceability and forensic identification, genetic analyses must be validated with respect to DNA extraction, amplification, detection and data interpretation. A typical validation protocol would therefore include tests of sample type, extraction method, individual molecular marker charac-

teristics (inheritance, linkage, PCR conditions, detection limits and specificity) and any subsequent statistical approaches to data analysis.

The overall aims of validation are to test assumptions implicit in the analytical process and to establish operational parameters that allow the successful dissemination of validated techniques to other testing laboratories. Specific guidelines for the validation of forensic genetic identification systems and food authenticity assays have been established by the human forensic and food safety communities respectively, allowing validation studies for fisheries genetic identification methods to be designed and implemented in accordance with best scientific practice and international testing standards. The production and peer-reviewed publication of validation data provides the wider scientific community and legal system with the information required to accept novel techniques as applied traceability and enforcement tools.

The application of biological markers to enforcement in fisheries management is a relatively new field (see Ogden 2008 for a review), and has been generally limited to species identification techniques. Consequently, validation studies have focused on methods associated with differentiating species using different sample types (Jerome *et al*., 2003). Techniques such as DNA nucleotide sequencing have been fully validated, from the specific gene region analysed, to the algorithm used to match sequences (Dawnay *et al*., 2007). Whereas much academic research has been undertaken to investigate population structure across many species, population identification is not commonly used in enforcement, and therefore the relevant techniques have usually not been sufficiently validated for forensic application.

Recent research focussing on population identification of fish stocks has utilized genetic markers (microsatellites and SNPs Schwenke *et al*., 2006), otolith markers (Belchier *et al*., 2004), and a variety of statistical algorithms (Hauser *et al*., 2006) to identify the geographic origin of individual fish. Such approaches provide a framework to tackle the problems of population identification in global fisheries; however they are often species-specific in nature and remain products of academic study, rather than for forensic application, though there are some examples of forensic identification to the stock level (e.g. Schwenke *et al*., 2006). The most robust marker systems and statistical analyses available are those produced for human forensic identification. The transfer of their associated validation techniques to non-human systems has begun (Dawnay *et al*., 2007), and should form the benchmark for the validation and quality-assurance of population markers developed in fisheries control and enforcement.

3.8 Technology transfer

For a traceability scheme based on genetic or other complementary methods to provide a potentially independent control mechanism, it is likely that it will start out as a fundamental research project. However it is crucially important that the results obtained from academic research are taken up by the end-users, for example fisheries protection agencies, and certification bodies (e.g. MSC), and as such, the techniques must be fast and cost-effective. Furthermore, as results may ultimately be admitted as evidence of prosecution or defence in court trials, they must be robust, reliable, and reproducible.

For species identification, there is the requirement for an easily applied genetic marker, preferably the same to be used across all species and suited for unambiguous identification on whole organisms or processed seafood products. As outlined in 4.1, such an approach is already standard practice for some species. However despite its

principal availability, the efficient uptake by stakeholders of standardized protocols is still lacking in general. Evidence of engagement for traceability in relation to origin assignment, with the exception of salmonids (Smith *et al*., 2005) and whales (Ross *et al*., 2003; Baker *et al*., 2007), is even more limited (see Section 4.2). Whereas isolated examples of DNA technologies used to reveal fraud and their use as evidence in court cases (see Annex 1) exist, an effective and coherent transfer of population structure analytical methods, traceability platforms, and tools for conservation of fisheries resources and enforcement to end-users remains to be tackled. End-users could be research institutions, test laboratories, the industry, (including fishers, aquaculturists, and/or their organizations for example if seeking exoneration from accusation of fraud), media, control authorities, courts, international (non-governmental) organizations, as well as international and national bodies involved in policy-making and enforcement. During the process of technology transfer an emphasis should be placed on the relevance, balance of costs and benefits (CBA) and potential to uptake. Such an assessment is of crucial importance as it allows policy-makers to judge how relevant and worthwhile the implementation of technologies into a policy framework will be. It is also important for the developers of methods to engage with appropriate stakeholders and end-users within the context. Methodologies must also be adjusted to be applicable to the nationally-based policy frameworks in relation to conservation, consumer protection and enforcement.

3.9 Broader perspectives of traceability and genetics

3.9.1 Conservation of genetic resources

The occurrence of significant population structure at various geographical levels in marine taxa has several consequences for conservation of biodiversity. To preserve the evolutionary legacy and future evolutionary potential to a given species, it must be emphasized to secure viable populations covering the full geographical and environmental range (Nielsen and Kenchington, 2001). The existence of biologically differentiated populations, so-called "biocomplexity" (Hilborn *et al*., 2003), even in marine pelagic fish (Ruzzante *et al*., 2006), has been credited with a major role in conferring resilience and in buffering overall productivity of fish population complexes. Thus, a key aim of sustainable fisheries management is to develop an unambiguous species identification system then describe the spatial and temporal scale of population structuring. Tools need to be developed to monitor the dynamics and contribution of discrete components to overall fisheries production. Even apparently small genetic differences among populations of marine fish assessed using presumed neutral genetic markers could translate into important adaptive variation distributed among populations (Waples 1989). It is therefore important that tools exist not only to detect such biodiversity and to monitor its dynamics in response to global climate, altered harvesting strategies and establishment of marine protected areas, but also importantly that such tools can yield an appropriate enforcement framework for its conservation.

3.9.2 Integration with Ecosystem-based approach to fisheries management

During the twentieth century fisheries management focused on a species-specific approach; although helpful in some cases, it largely failed to varying degrees (e.g. discards, bycatch, trophic modification, mixed fisheries, high-diversity fisheries, and spatio-temporal dynamics). Consensus is growing that an ecosystem-based approach (EBA) to the management of marine natural resources provides the optimum strategy to achieve sustainable management of natural resources. EBA requires five steps:

setting objectives, monitoring and research, assessment, advice and adaptive management (ICES 2001, 2007). Although obvious in principle to scientists and managers, acceptance of EBA fisheries management by the public and politicians, and implementation in the management strategy remains contentious. A crucial element in the implementation of EBA is assessment, for example through unambiguous and high-throughput identification of species and geographical assignment of catch. Presently, scientific advice on fisheries regulation is mainly channelled into recommendations on biologically safe levels of catches for individually regulated stocks, based on catch reported via logbooks and landing declarations. However, such information is not entirely reliable, because stakeholders (fishers) may feel tempted to provide falsified data on the landings to escape sanctions imposed by the relevant framework. Accordingly, independent data such as that acquired by traceability, are critical to stock assessment and management. Here a starting point for the effective and sustainable management of fish stocks is their identification, biological characterization and the monitoring of compositional changes at appropriate sub-specific levels. In addition, GSI and other stock identification methods can be used to directly gather information, for example by providing information on habitat use (Van Doornik *et al*., 2007) or prey composition (Purcell *et al*., 2004).

3.9.3 Future policy developments

In 2001 the FAO adopted the international plan of action to prevent, deter and eliminate illegal, unreported and unregulated fishing (IPOA-IUU; http://www.fao.org/docrep/003/y1224e/y1224e00.HTM). The IPOA-IUU was developed as a voluntary instrument, within the framework of the Code of Conduct for Responsible Fisheries. It applies to all States and entities and to all fishers. The document also discusses the implementation of measures to prevent, deter and eliminate IUU fishing. Measures focus on all State responsibilities, flag State responsibilities, coastal State measures, port State measures, internationally agreed market-related measures, research and regional fisheries management organizations.

The European Community has endorsed the IPOA-IUU plan of action, and it is supported by the Council Regulation (EC) No. 1005/2008, to prevent, deter and eliminate IUU fishing which was adopted September 2008. The scope of the regulation is extensive in that it encompasses all catches and derived products, worldwide, of any vessel or fleet, and import as well as export. Until 1 January 2010, when the regulation comes into force, the European Community will establish rules to ensure its efficient implementation, and provide assistance to third and lesser developed countries.

Furthermore two other components will support the effort to fight IUU fishing. Firstly the European Community catch certification scheme (Chapter III of Council Regulation (EC) No. 1005/2008) which aims to improve traceability of all fishery products traded with the Community and facilitate their control within conservation and management rules, in cooperation with third countries. Once implemented, fishery products can only be imported into the Community if accompanied by a catch certificate. Through this instrument, the competent authorities of flag state of the vessel catching the fish will certify that catches have been made in accordance with applicable laws, regulations and international conservation and management measures.

Secondly the aforementioned proposal for the reform of the current control system of the CFP (European-Commission 2008), submitted by the European Commission to the Council and Parliament suggests measures to significantly improve. Fisheries

control in the EU. Importantly in the context of this ToR, Article 13 of this document refers explicitly to new technologies and traceability tools such as genetic analysis to be considered to achieve this goal. The proposal is currently under review and discussion by the Council.

3.10 Recommendations

1) The development and application of traceability tools that can be applied throughout the food supply chain ("ocean-to-fork"). Here, DNA-based methods are ideal as they support traceability ranging from whole organism, blood and tissue remains to processed product.

2) We further recommend integration of the DNA approach with other independent techniques, such as analyses of otolith microchemistry, fatty acid, stable isotopes, pigments, etc.

3) In view of recent technical advances, additional investment should be made in the development of appropriate tools to detect and monitor populations (or other identifiably significant sub-specific units).

4) The establishment of a statistically rigorous sampling scheme, allowing assessment of spatio-temporal variation

5) The application of marker information, such as Single Nucleotide Polymorphism data, that is fully transferable across analyses and laboratories.

6) Sample and data repositories are established within a statistically rigorous framework.

7) Technical and statistical tools and procedures are fully validated to internationally recognize forensic standards.

8) Engagement with programmes at the global level such as the DNA barcoding enterprise coordinated by the Consortium for the Barcode of Life (FishBOL).

9) Traceability systems are developed that recognize units that may carry both a geographic signature and those that are also biologically identifiable.

10) The application of traceability tools should be extended to include methods for conservation of marine genetic resources and ecosystem-based management.

11) An appropriate strategy to promote the uptake of traceability tools by international authorities through focussing on a few methods with the highest discriminatory

12) Power, greatest reproducibility, simplest validation and most flexibility with respect to the type of tissue and degree of processing.

13) The development of the above mentioned technologies should be accompanied by a sound technology transfer strategy, engaging relevant stakeholders, such as managers, consumers, wholesalers, enforcement authorities and policy-makers.

3.11 References

Abercrombie, D. L., S. C. Clarke and M. S. Shivji 2005. "Global-scale genetic identification of hammerhead sharks: Application to assessment of the international fin trade and law enforcement." Conservation Genetics 6: 775–788.

Anderson, E. C., R. S. Waples and S. T. Kalinowski 2008. "An improved method for predicting the accuracy of genetic stock identification." Canadian Journal of Fisheries and Aquatic Sciences 65(7): 1475–1486.

Baker, C. S. 2008. "A truer measure of the market: The molecular ecology of fisheries and wildlife trade." Molecular Ecology 17(18): 3985–3998.

Baker, C. S., J. G. Cooke, S. Lavery, M. L. Dalebout, Y. U. Ma, N. Funahashi, C. Carraher and R. L. Brownell 2007. "Estimating the number of whales entering trade using DNA profiling and capture-recapture analysis of market products." Molecular Ecology 16(13): 2617–2626.

Blanco, M., R. I. Perez-Martin and C. G. Sotelo 2008. "Identification of shark species in seafood products by forensically informative nucleotide sequencing (fins)." Journal of Agricultural and Food Chemistry 56(21): 9868–9874.

Borsa, P., A. Blanquer and P. Berrebi 1997. "Genetic structure of the flounders platichthys flesus and p-stellatus at different geographic scales." Marine Biology 129(2): 233–246.

Bossier, P. 1999. "Authentication of seafood products by DNA patterns." Journal of Food Science 64(2): 189–193.

Bossier, P. and K. Cooreman 2000. "A databank able to be used for identifying and authenticating commercial flatfish (*pleuronectiformes)* products at the species level using isoelectric focusing of native muscle proteins." International Journal of Food Science and Technology 35(6): 563–568.

Cadrin, S., K. Friedland and J. R. Waldman 2004. Stock identification methods: Applications in fishery science, Elsvier.

Carvalho, G. R. and L. Hauser 1994. "Molecular-genetics and the stock concept in fisheries." Reviews in Fish Biology and Fisheries 4(3): 326–350.

Chen, I.-C., F. Chapman, Wei C-I, Portier KM and S. O'Keefe 2006. "Differentiation of cultured and wild sturgeon (*acipenser oxyrinchus desotoi*) based on fatty acid composition." Journal of Food Science 60: 631−635.

CODEX-Alimentarius-Commission 2006. "Principles for traceability / product tracing as a tool within food inspection and certification system." CAC/GL: 60–2006.

Costa, F. and G. Carvalho 2007. "The barcode of life initiative: Synopsis and prospective societal impacts of DNA barcoding of fish." Genomics, Society and Policy 3: 29–40.

Dawnay, N., R. Ogden, R. McEwing, G. R. Carvalho and R. S. Thorpe 2007. "Validation of the barcoding gene coi for use in forensic genetic species identification." Forensic Science International 173: 1–6.

Dempson, J. and M. Power 2004. "Use of stable isotopes to distinguish farmed from wild Atlantic *salmon, salmo salar*." Ecology of Freshwater Fish 13: 176−184.

DeSalle, R. and V. J. Birstein 1996. "Prc identification of black caviar." Nature 381: 197–198.

European-Commission 2008. "Proposal for a council regulation establishing a community control system for ensuring compliance with the rules of the common fisheries policy." COM2008: 721 final.

European-Commission. 2006. "Communication from the commission to the council: Fishing opportunities for 2007 policy−statement from the European commission." COM 499: final.

European Court of Auditors 2007. "Special Report No 7/2007 pursuant to Article 248(4) second paragraph, EC, on the control, inspection and sanction systems relating to the rules on conservation of Community fisheries resources." Special Report No 7/2007.

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

down procedures in matters of food safety." Official Journal of the European Communities L 31/1 (01.02.2002): 1–24.

Gallic, B. L. and A. Cox 2006. "An economic analysis of illegal unreported and unregulated (iuu) fishing: Key drivers and possible solutions." Marine Policy 30: 689–695.

Gil, L. A. 2007. "Pcr-based methods for fish and fishery products authentication." Trends in Food Science & Technology 18(11): 558–566.

Hansen, M. M., D. E. Ruzzante, E. E. Nielsen, D. Bekkevold and K. L. D. Mensberg 2002. "Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations." Molecular Ecology 11(12): 2523–2535.

Hauser, L. and G. R. Carvalho 2008. "Paradigm shifts in marine fisheries genetics: Ugly hypotheses slain by beautiful facts." Fish and Fisheries 9(4): 333–362.

Hauser, L., T. R. Seamons, M. Dauer, K. A. Naish and T. P. Quinn 2006. "An empirical verification of population assignment methods by marking and parentage data: Hatchery and wild steelhead (*Oncorhynchus mykiss*) in forks creek, Washington, USA." Molecular Ecology 15(11): 3157–3173.

Hemmer-Hansen, J., E. E. Nielsen, J. Frydenberg and V. Loeschcke 2007. "Adaptive divergence in a high gene flow environment: Hsc70 variation in the European flounder (*Platichthys flesus* l.)." Heredity 99: 592–600.

Hilborn, R., T. P. Quinn, D. E. Schindler and D. E. Rogers 2003. "Biocomplexity and fisheries sustainability." Proceedings of the National Academy of Sciences of the United States of America 100(11): 6564–6568.

Jerome, M., C. Lemaire, W. Verrez-Bagnis and M. Etienne 2003. "Direct sequencing method for species identification of canned sardine and sardine-type products." Journal of Agricultural and Food Chemistry 51(25): 7326–7332.

Kochzius, M. 2008. Trends in fisheries genetics. The future of fisheries sciences in North America (proceedings of the 50th anniversary symposium of the American Institute of Fishery Research Biologists. Seattle, February 13–15 2007). R. Beanmish and B. Rothschild. Seattle, Springer.

Kochzius, M., M. Nolte, H. Weber, N. Silkenbeumer, S. Hjorleifsdottir, G. O. Hreggvidsson, V. Marteinsson, K. Kappel, S. Planes, F. Tinti, A. Magoulas, E. G. Vazquez, C. Turan, C. Hervet, D. C. Falgueras, A. Antoniou, M. Landi and D. Blohm 2008. "DNA microarrays for identifying fishes." Marine Biotechnology 10(2): 207–217.

Koljonen, M. L., J. J. Pella and M. Masuda 2005. "Classical individual assignments versus mixture modeling to estimate stock proportions in Atlantic salmon (*Salmo salar*) catches from DNA microsatellite data." Canadian Journal of Fisheries and Aquatic Sciences 62(9): 2143–2158.

Kyle, C. J. and C. C. Wilson 2007. "Mitochondrial DNA identification of game and harvested freshwater fish species." Forensic Science International 166(1): 68–76.

Larsen, P. F., M. M. Hansen, E. E. Nielsen, L. F. Jensen and V. Loeschcke 2005. "Stocking impact and temporal stability of genetic composition in a brackish northern pike population (*Esox lucius l.*), assessed using microsatellite DNA analysis of historical and contemporary samples." Heredity 95(2): 136–143.

Le Nguyen, D. D., H. H. Ngoc, D. Dijoux, G. Loiseau and D. Montet 2008. "Determination of fish origin by using 16s rdna fingerprinting of bacterial communities by pcr-dgge: An application on Pangasius fish from Vietnam." Food Control 19(5): 454–460.

Logan, C. A., S. E. Alter, A. J. Haupt, K. Tomalty and S. R. Palumbi 2008. "An impediment to consumer choice: Overfished species are sold as pacific red snapper." Biological Conservation 141(6): 1591–1599.

Marko, P. B., S. C. Lee, A. M. Rice, J. M. Gramling, T. M. Fitzhenry and J. S. McAlister 2004. "Fisheries: Mislabeling of a depleted reef fish." Nature 430: 309–310.

Martinsohn, J. T. and R. Ogden 2008. "A forensic genetic approach to European fisheries enforcement." Forensic Science International: Genetics Supplement Series 1: 610–611.

Millar, C. D., L. Huynen, S. Subramanian, E. Mohandesan and D. M. Lambert 2008. "New developments in ancient genomics." Trends in Ecology & Evolution 23(7): 386–393.

Nielsen, E.E., Hansen, M.M., Schmidt, C., Meldrup, D., Grønkjær, P. 2001a. Population of origin of Atlantic cod. Nature 413:272.

Nielsen, E.E., Hansen, M.M., Bach, L.A. 2001b. Looking for a needle in a haystack: Discovery of indigenous Atlantic salmon (*Salmo salar* L.) in stocked populations. Conservation Genetics 2(3): 219–232.

Nielsen, E. E. and E. Kenchington 2001. "A new approach to prioritizing marine fish and shellfish populations for conservation." Fish and Fisheries 2: 328–343.

Ogden, R. 2008. "Fisheries forensics: The use of DNA tools for improving compliance, traceability and enforcement in the fishing industry." Fish and Fisheries 9(4): 462–472.

Pauly, D., V. Christensen, S. Guenette, T. J. Pitcher, U. R. Sumaila, C. J. Walters, R. Watson and D. Zeller 2002. "Towards sustainability in world fisheries." Nature 4186898: 689–695.

Pearse, D. and K. Crandall 2004. "Beyond fst: Analysis of population genetic data for conservation." Conservation Genetics 5: 582–602.

Pella, J. and M. Masuda 2006. "The gibbs and split-merge sampler for population mixture analysis from genetic data with incomplete baselines." Canadian Journal of Fisheries and Aquatic Sciences 63(3): 576–596.

Pritchard, J. K., M. Stephens, N. A. Rosenberg and P. Donnelly 2000. "Association mapping in structured populations." American Journal of Human Genetics 67(1): 170–181.

Purcell, M., G. Mackey, E. LaHood, H. Huber and L. Park 2004. "Molecular methods for the genetic identification of salmonid prey from pacific harbour seal (*phoca vitulina richardsi*) scat." Fishery Bulletin 102(1): 213–220.

Rasmussen, R. S. and M. T. Morrissey 2008. "DNA-based methods for the identification of commercial fish and seafood species." Comprehensive Reviews in Food Science and Food Safety 7(3): 280–295.

Reiss, H., G. Hoarau, M. Dickey-Collas and W. J. Wolff (in press). "Genetic population structure of marine fish: Mismatch between biological and fisheries management units." Fish and Fisheries.

Ross, H. A., G. M. Lento, M. L. Dalebout, M. Goode, G. Ewing, P. McLaren, A. G. Rodrigo, S. Lavery and C. S. Baker 2003. "DNA surveillance: Web-based molecular identification of whales, dolphins, and porpoises." Journal of Heredity 94(2): 111–114.

Ruzzante, D. E., S. Mariani, D. Bekkevold, C. Andre, H. Mosegaard, L. A. W. Clausen, T. G. Dahlgren, W. F. Hutchinson, E. M. C. Hatfield, E. Torstensen, J. Brigham, E. J. Simmonds, L. Laikre, L. C. Larsson, R. J. M. Stet, N. Ryman and G. R. Carvalho 2006. "Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring." Proceedings of the Royal Society B-Biological Sciences 2731593: 1459–1464.

Schwenke, P. L., J. G. Rhydderch, M. J. Ford, A. R. Marshall and L. K. Park 2006. "Forensic identification of endangered chinook salmon (*oncorhynchus tshawytscha*) using a *multilocus* snp assay." Conservation Genetics 7(6): 983–989.

Sevilla, R. G., A. Diez, M. Norén, O. Mouchel, M. Jérôme, V. Verrez-Bagnis, H. Van Pelt, L. Favre-Krey, G. Krey, The Fishtrace Consortium and J. M. Bautista 2007. "Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome b and nuclear rhodopsin genes." Molecular Ecology Notes 7(5): 730–734.

Shendure, J. and H. L. Ji 2008. "Next-generation DNA sequencing." Nature Biotechnology 26(10): 1135–1145.

Smith, C. T., W. D. Templin, J. E. Seeb and U. W. Seeb 2005. "Single nucleotide polymorphisms provide rapid and accurate estimates of the proportions of us and Canadian chinook salmon caught in Yukon River fisheries." North American Journal of Fisheries Management 25(3): 944–953.

Sobrino, B. and A. Carracedo 2005. Snp typing in forensic genetics−a review. Methods in molecular biology, HUMANA PRESS INC: 107–126.

Teletchea, F., J. Bernillon, M. Duffraisse, V. Laudet and C. Ha¨nni 2008. "Molecular identification of vertebrate species by oligonucleotide microarray in food and forensic samples." Journal of Applied Ecology 45: 967–975

Thomas, F., Jamin, E., Wietzerbin, K., Guerin, R., Lees, M., Morvan, E., Billault, I., Derrien, S., Rojas, J.M.M, Serra, F., Guillou, C., Aursand, M., McEvoy, L., Prael, A., and R. RJ 2008. "Determination of origin of Atlantic salmon (*salmo salar*): The use of multiprobe and multielement isotopic analyses in combination with fatty acid composition to assess wild or farmed origin." Journal of Agricultural and Food Chemistry 56: 989–997.

Thresher, R. E. 1999. "Elemental composition of otoliths as a stock delineator in fishes." Fisheries Research 43(1–3): 165–204.

Van Doornik, D.M., Teel, D.J., Kuligowski, D.R., Morgan, C.A., and C. E. 2007. "Genetic analyses provide insight into the early ocean stock distribution and survival of juvenile coho salmon off the coasts of Washington and Oregon." North American Journal of Fisheries Management 27: 220–237.

Waples, R. S. 1989. "A generalized-approach for estimating effective population-size from temporal changes in allele frequency." Genetics 121(2): 379–391.

Waples, R. S. and O. Gaggiotti 2006. "What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity." Molecular Ecology 15(6): 1419–1439.

Wennevik, V., K. E. Jorstad, G. Dahle and S. E. Fevolden 2008. "Mixed stock analysis and the power of different classes of molecular markers in discriminating coastal and oceanic Atlantic cod (*gadus morhua* l.) on the Lofoten spawning grounds, northern Norway." Hydrobiologia 606: 7–25.

Wielgoss, S., H. Taraschewski, A. Meyer and T. Wirth 2008. "Population structure of the parasitic nematode *anguillicola crassus*, an invader of declining North-Atlantic eel stocks." Molecular Ecology 17: 3478–3495.

Wong, E. H. K. and R. H. Hanner 2008. "DNA barcoding detects market substitution in north American seafood." Food Research International 41(8): 828–837.

3.12 Annex 1: Examples demonstrating the feasibility of DNA-based methods for fisheries MCS and Enforcement"

3.12.1 Illegal importation and sale of over ten million pounds of falsely labelled catfish

Between 2002 and 2005 a group of US and Vietnamese fish food companies engaged into a scheme to intentionally mislabel frozen farm raised catfish (*Pangasius* spp.) fillets, which were imported into the US from Vietnam. The companies attempted thereby to evade duties (anti-dumping tariff) imposed by the US Department of Commerce on those imports, and to sell the product as being derived from a higher value species. Suspicion was raised because US Customs documents indicated that Vietnam had increased export of grouper, sole, conger eel, whisker fish, pango etc. significantly.

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

This large-scale conspiracy was revealed and investigated in cooperation between diverse US federal agencies such as the NOAA Office of Law Enforcement (OLE), the Customs Border Protection (CBP), the Immigration and Customs Enforcement (ICE) and others. Species identification was undertaken with DNA testing carried out by scientific laboratories such as the NOAA Marine Forensics Department. The conspiracy consists, as a whole, of several cases some of which are still under trial and documented by the US Department of Justice. Just recently (October 2008) two implied suspects were convicted facing a maximum of five years in jail and fines of up to US$ 250,000. The suspects are scheduled to be sentenced in February 2009.

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

Sources: NOAA Office of Law Enforcement; Fish Worldnews 31/10/08; Paul Raymond (NOAA Office of Law Enforcement); Linda Park (NOAA National Marine Fisheries Service).

3.12.2 Illegal shark fin trade

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

In the USA. DNA tests are used to uncover and prosecute illegal shark fin traders. In many cases traders violate strict laws protecting shark species (e.g. Endangered Species Act). Whereas, until recently, identifying a shark species the fins came from was time consuming, meanwhile a genetic test, developed by scientists of the Harvey Research Institute at the Nova South Eastern University in Florida greatly facilitates the analytical process.

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

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

The tests positively identified a total of 230 pounds of fins that came from seven different prohibited species including dusky sharks, basking sharks and the great white shark. NOAA later announced that the seafood dealer had agreed to a settlement of

$750,000 in the case. It was emphasized by the NOAA attorney Charles Juliand that the settlement was possible in great part due to the strength of the DNA evidence.

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

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

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

Sources: US Department of Justice – United States Attorney's Office; Los Angeles Times; The Ledger; Paul Raymond (NOAA Office of Law Enforcement).

3.12.3 Conviction of a fisherman claiming a false origin of cod in Europe

More than 7 tonnes of large cod were landed in the Baltic Sea region by a North Sea fisherman. According to the declaration of the logbook, the cod had been caught in the Baltic Sea but Danish Inspectors from the Danish Directorate of Fisheries suspected upon visual examination that the fish looked like North Sea cod rather than Baltic Sea cod. Also the poor quality of the fish did not match well with the reported time of catch. Therefore the inspectors delivered five cod specimen to the Danish Institute for Fisheries Research (DIFRES) for genetic analysis. The scientists performed a genetic origin assignment test on the delivered samples and all five cod were assigned to the North Sea.

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

This example shows that as well in Europe Advances Technologies (ATs) are used for control and enforcement in the fisheries sector and that evidence produced by ATs is admitted in court trials in EU member countries. More importantly, it also shows that ATs cannot not only be used efficiently for species identification but in addition for the more challenging question of origin assignment.

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

3.12.4 Individual origin assignment in a case of European fishing competition fraud

In June 1999, a 5.5 kg salmon was presented to the judges of a local fishing competition in Finland. Based on visual inspection, suspicion arose that the salmon may not have been caught in Lake Saimaa, as claimed by the fisherman. In order to set a precedent for future competitions, the organizers were interested in conclusively ascertaining that the suspect fish did not come from the claimed geographical origin. To press criminal charges against the alleged offender, tissue samples were submitted

for genetic analysis. Genetic origin assignment showed that the probability of the suspect salmon originating in one of the regions that supply most of Finland's fish markets was found to be over 600 times higher than it originating in the declared origin. When confronted with this evidence, the offender confessed that he had purchased the salmon at a local shop.

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

Sources: Primmer *et al*. (2000) Proceedings of the Royal Society of London Series B.

3.12.5 Uncovering false labelling of fish in Germany

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

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

Officials from the District Office took samples of a batch of more than 4 tonnes of fish fillets, declared as "tropical turbot caught in West African waters" (Spottail spiny turbot (*Psettodes belcheri*) and the Channel flounder (*Syacium micrurum*)). The IHU department for food security analysed the samples by DNA sequencing and comparison to reference sequences of the international DNA sequence database GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html). This analysis revealed that of 22 samples only 5 were correctly labelled, a major part were in fact pangasius fillets, others fillets of perch subspecies from the Indopacific Ocean.

Based on these results the State Consumer Protection Authorities urged the involved firms (a Belgian wholesaler along with two firms from Hamburg) to rectify the goods. Because this request was not followed, the authorities confiscated and destroyed the merchandise. Additionally the affair was passed to the prosecution of Hamburg, and Belgian authorities were informed. The outcome of this case is still pending (December 2008).

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

The above cited examples show that, at least for some countries, DNA based technologies are already successfully employed to reveal fraud and even as evidence in court cases. This clearly proves the feasibility and applicability of such technologies. However, the challenge of integrating these technologies homogenously into national and international policy and legal frameworks remains.

Annex 2: Comparison of the major non-genetic methods used in fisheries forensics.

Marker	Species level	Population level	Advantages	Disadvantages	References
Morphological trait markers					
e.g. vertebrae number, scale pattern, parasite prevalence etc.	Yes	Yes	No longer commonly used, but may act to strengthen inference about origin.	Appear to lack the statistical rigour use within a forensic framework	Cadrin *et al.*
Soft tissue					
Stable isotope analysis	No	Mainly used for differentiation of farmed and wild fish	Well established	Complex protocols	Molkentin *et al.* 2006 Dempson *et al.* 2004
Fatty acid profiling	Yes	Mainly used for differentiation of farmed and wild fish	Well established	Only applicable for fresh or frozen samples	Orban, 2003 Thomas *et al.* 2008 Joensen *et al.* 2001, Standal *et al.* 2008
Trace element analysis	No	Yes		Only applicable for fresh or frozen samples	Rodushkin *et al.* 2007 Yamashita *et al.* 2007
Isoelectric focussing of proteins	Yes	No	Low costs Simple protocols Well established	Only applicable for fresh or frozen samples Limited number of available markers	Bossier and Cooreman 2000
Immunoassays	Yes	No	Instant results	Hard to develop Species specific Only applicable for fresh or frozen samples	Lopata *et al.* 2002
Marker	**Species level**	**Population level**	**Advantages**	**Disadvantages**	**References**
Otoliths					
elliptical Fourier analysis	Yes	Yes	Do not degrade.	Not applicable if head is not available. Teleosts only	Stransky 2008, Duarte-Neto 2008 Tuset 2006 Burke et al. 2007
otolith microstructure	No	Yes	Do not degrade.	Not applicable if head is not available. Teleosts only Complex protocols	Clausen *et al.* 2007

Marker	Species level	Population level	Advantages	Disadvantages	References
micro-chemistry	No	Yes	Do not degrade.	Not applicable if head is not available. Teleosts only Complex protocols	Thresher 2007 Jonsdottir 2006
Stable isotope	No	Yes	Do not degrade.	Not if head is not available. Teleosts only Complex protocols	Gao *et al*. 2004, Gao and Bean, 2008

Annex 3: Comparison of the major genetic methods used in fisheries forensics.

Marker	Species level	Population level	Advantages	Disadvantages	References
FINS[1]	Yes	No	• Operational and commonly used in fish product and forensic labs.	• Requires the development of species-specific primers • Application required under stringent conditions to avoid specific amplification.	Bartlett *et al.*1992 Jerome *et al.*2003 Gil 2007
RFLP[2]	Yes	Yes	• Limited skill and investment needed for application.	• Requires extensive preparatory and validation research,	Colombo *et al.* 2002 Lin *et al.*2007 Larmuseau *et al.*2008 Michelini *et al.* 2007 Scwenke *et al.* 2006
SSCP[3] **and RSCA**[4]	Yes	Yes	• Some reproducibility issues	• Highly sensitive after extensive preparatory work as can detect single base changes.	Fernandez *et al.*2001 Comi *et al.*2005 Van Houdt *et al.*2006
RAPD[5]	Yes	Yes	• Simple, low cost and low amount of DNA needed	• Largely abandoned due to poor reproducibility	Gil 2007 Rasmussen and Morrissey 2008
AFLP[6]	Yes	Yes	• Highly reproducible	• Labour intensive and may pose quality problems. • large amounts of high quality DNA are required	Vos *et al.* 1995 Rasmussen and Morrissey 2008
Microsatellites	No	Yes	• Large number of alleles per locus • Supported by international expert community for human DNA forensics. • Reliable characterization, reproducibility and resolution	• High mutation rate • Not highly abundant • Adaptation to automatic high-throughput analysis is difficult • High initial development costs • Difficult to standardize between laboratories	Seeb et al. 2007 Ruzzante *et al.*2006 Bekkevold *et al.*2005
SNPs[7]	Yes	Yes	• Stable/low mutation rate • High abundance • Well suited for high-throughput analysis • Detection can occur "*in silico*" • Protocols are easily transferable between laboratories	• Large numbers need to be screened for initial identification of highly informative loci • High initial costs	Apostolidis et al. 2008 Sprowles *et al.*2006 Moen *et al.*2008 Narum 2008
RT-PCR[8]	Yes	No	• Quick and robust • Suitable for the detection of species-specific DNA in small amounts of degraded material in admixtures.	• Expensive • Requires skill	Holland *et al.* 1991 Trotta *et al.* 2005 Dalmasso *et al.* 2007

Marker	Species level	Population level	Advantages	Disadvantages	References
Multispecific micro-array (LAB-ON-A-CHIP)	Yes	No	• Multiple fish species can be simultaneously identified • Reliable and economically. • It can be used for small amounts of DNA, admixture samples and degraded DNA. • High throughput • Only operational high-throughput method currently on the market	• High initial development costs • Likely to be superseded by high throughput SNP genotyping.	Dooley *et al*.2005 Kochzius 2008 Gil 2008
PCR kits	Yes	No	• Have been commercialized for a number of fish taxa of economic importance	• Limited range of species	Bionostra fishID kit; Biomérieux GeneChip; Biotools; Tepnel Biosystems

1. FINS: Forensically Identifiable Nucleotide Sequencing
2. RFLP: Restriction Fragment Length Polymorphism
3. SSCP: Single Strand Conformational Polymorphism
4. RSCA: Reference Strand Conformation Analysis
5. RAPD: Randomly Amplified Polymorphic DNA
6. AFLP: Amplified Fragment Length Polymorphism
7. SNPs: Single Nucleotide Polymorphisms
8. RT-PCR: Real –Time PCR

4 ToR c) Update and insights from the EU project SALSEA-Merge on establishment of a large-scale genetic database for assigning individual to population of origin

Phil McGinnity and Eric Verspoor

4.1 Project Overview

SALSEA-Merge is a Collaborative Project (small or medium-scale focused research project) funded under EU FP7, within SUB-ACTIVITY 6.2.2. MANAGEMENT OF MARINE ENVIRONMENTS; Area 6.2.2.1 Marine resources; ENV.2007.2.2.1.2. Ecology of important marine species. The project is sponsored by NASCO under the International Salmon Research Board SALmon at SEA (SALSEA) research programme and coordinated by the Norwegian Institute of Marine Research in Bergen. It involves 14 research institutes across Europe as well as six conservation NGOs. The full project title is "*Advancing understanding of Atlantic Salmon at Sea: Merging Genetics and Ecology to Resolve Stock-specific Migration and Distribution patterns.*" The project participants are listed in the table below:

Particpant No.	Participant organisation Name (*non contracting partners)	Country
1 (Coordinator)	Institute of Marine Research (IMR)	Norway
2	Marine Institute (MI)	Ireland
3	Fisheries Research Services (FRS)	UK
4	Norwegian Institute for Nature Research (NINA)	Norway
5	University of Exeter (UE)	UK
6	National University of Ireland, Cork (NUIC)	Ireland
7	Queen's University Belfast (QUB)	UK
8	University of Wales, Swansea (UWS)	UK
9	Danish Institute for Fisheries Research (DIFRES)	Denmark
10	Institute of Freshwater Fisheries (IFL)	Iceland
11	University of Turku (UT)	Finland
12	University of Oviedo (UO)	Spain
13	Geneindex (GENI)	France
14	Finnish Game and Fisheries Research Institute (FGFRI)	Finland
15	*Faroese Fisheries Laboratory (FFL)	Faroes
16	*Atlantic Salmon Trust (AST)	UK
17	*International Atlantic Salmon Research Board - NASCO (IASRB)	UK
18	*Total Foundation (TOTAL)	France
19	*Conservatoire du Saumon Sauvage (CSS)	France
20	*Loughs Agency (LA)	UK

The project aims to advance understanding of the factors affecting the marine mortality of European Atlantic salmon during their oceanic feeding migrations in the Northeast Atlantic. Increased marine mortality over the last two decades underlies the declines seen in numbers of adults returning to rivers to spawn. In some rivers in the southern part of the species range, wild salmon now face extinction despite unprecedented management measures to halt this decline. The specific oceanic factors responsible are as yet unknown though change in the oceanic environment associated with climate change is likely to be important. Arguably the greatest challenge in salmon conservation is to gain insight into the spatial and ecological use of the marine environment by different regional and river stocks, which are known to show variation in marine growth, condition, and survival. The study represents the first pan European study in the marine sector to exploit genetic tags to identify the origin of individuals collected at sea to provide specific insights into the marine ecology of regional stock components and, potentially, of individual breeding populations.

To date it has been impossible to both sample as well as identify the origin of sufficient numbers of wild salmon at sea to permit this type of specific understanding to be gained. Yet such knowledge is fundamental to refining the management of human impacts on European Atlantic salmon stocks within overall marine ecosystem management paradigm to ensure they are sustainable. The programme prescribed set out to deliver innovation in the areas of genetic stock identification techniques, genetic stock marker development, as well as fine scale estimates of growth on a weekly and monthly basis, the use of novel high seas pelagic trawling technology, and individual stock linked estimates of food and feeding patterns. In addition, the use of the three-dimensional Regional Ocean Modelling System, merging hydrography, oceanographic, genetic and ecological data, will deliver novel stock specific migration and distribution insights. It is expected that the project will provide an important model for understanding the factors affecting survival of many other important marine species.

The project is composed of five work packages:

WP 1: Development of Genetic Identification Methodology

WP 2: Marine Data Acquisition

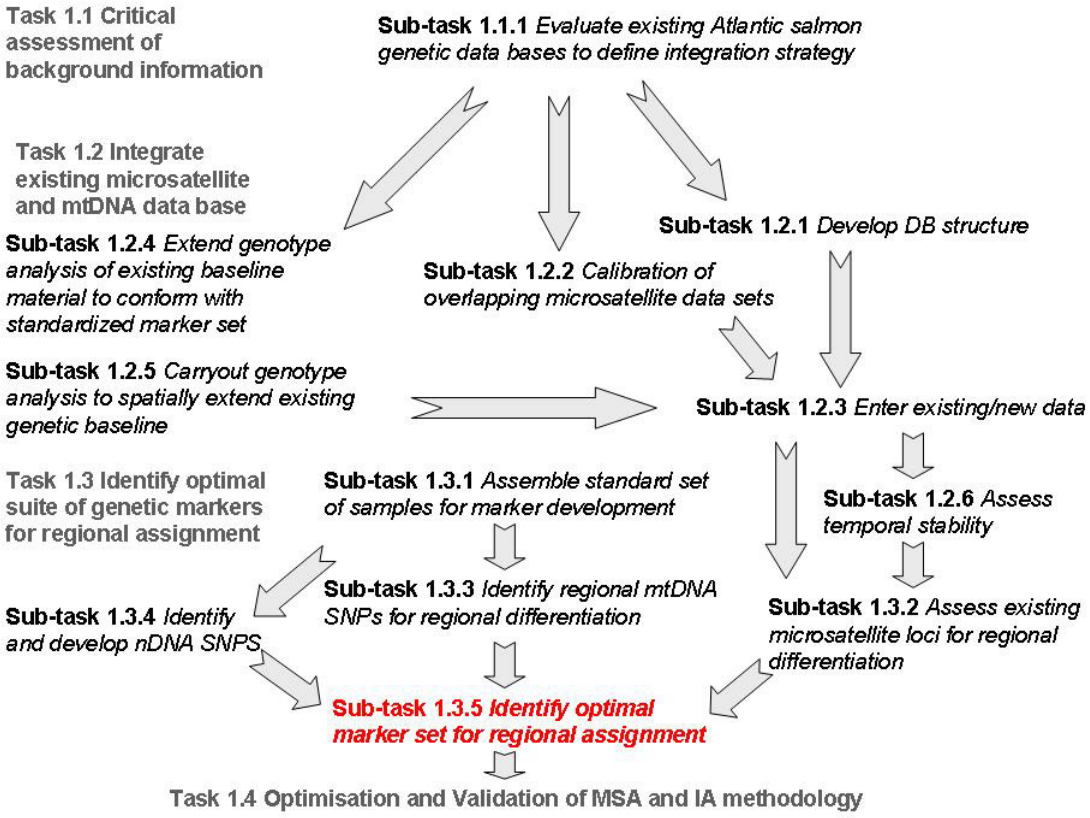
WP 3: Genetic Identification of Stock Origin of Samples

WP 4: Biological Analysis of Samples

WP 5: Merge and analyse data

4.2 Progress to Date

The genetic components of the project lie within WP1, WP3 and WP5. Work to date has been confined to WP1. This encompasses a number of tasks and subtasks as set out in the figure below. These encompass three basic development issues relating to developing a sufficient 1) suite of cost-effective molecular markers, 2) baseline dataset for the markers, and 3) methodology for mixed-stock analysis (MSA), mixed population analysis (MPA) and individual assignment (IA) to allow accurate regional assignment of salmon sampled at sea. Work under WP1 has so far been confined to the first two areas.



4.2.1 Development of a suite of cost-effective molecular markers

A review was undertaken of existing genetic markers and available marker information on European salmon stocks, lead by Carlos Garcia de Leaniz (Swansea University). This identified that a large body of information already existed for a large number of microsatellite loci that spanned stocks across much of the salmon distribution in Europe. For a large number of stocks already studied, the available data encompassed a suite of loci of largely tetranucleotide loci that would be optimal for cost-effective screening and integration of datasets across research groups. Furthermore, a preliminary analysis of the MSA and IA capacity of the loci lead by John Gilbey (Marine Scotland), Jamie Coughlan (Cork University) and Paulo Prodhol (Queen's University) indicated that they would be sufficient as the basis for developing the genetic tool, though further work is underway to identify additional markers for increasing the resolution of regional assignment. Agreement was reached by the participating groups at a meeting held in spring of 2008 to develop a basic tool using a modified "Virginia Panel", the set of microsatellites recommended by salmon genetics researchers at the Atlantic Salmon Microsatellite Analysis Network (SALMAN) meeting in Virginia in 2005 as the focus for future work. This panel is shown in the table below. The modification made was to exclude SsaD486 which is largely invariant in Europe and so is largely uninformative with regards to the regional origin of European salmon. Some groups will be extending their analysis with further loci on a regional basis to allow a more fine scale regional assignment e.g. with SsaD48 and SsaD71.

Locus name	No. repeat bases	Max Size Range*	Max no. Alleles*	Source	No. Rivers Screened*
Ssa197	4	135–279	30	(O'Reilly *et al.*, 1996)	186
Ssa 202	4	200–320	18	(O'Reilly *et al.*, 1996)	166
Ssa171	4	193–285	32	(O'Reilly *et al.*, 1996)	126
SSsp2210	4	104–164	15	(Paterson *et al.*, 2004)	98
SSsp2216	4	202–305	18	(Paterson *et al.*, 2004)	98
SSsp1605	4	222–254	14	(Paterson *et al.*, 2004)	97
Ssa 14	2	138–152	4	(McConnell *et al.*, 1995)	80
Ssa 289	2	107–132	8	(McConnell *et al.*, 1995)	80
SsaF43	2	99–131	13	(Sánchez *et al.*, 1996)	71
SSsp2201	4	259–371	21	(Paterson *et al.*, 2004)	68
SSspG7	4	112–214	24	(Paterson *et al.*, 2004)	57
SsaD486**	4	162–210	7	(King *et al.*, 2005)	35
SsaD144	4	112–298	35	(King *et al.*, 2005)	34
SsaD157	4	316–346	23	(King *et al.*, 2005)	2
SSsp3016	4	70–130		Paterson *et al.*in press	

***This is based on all available information from across the species range – Garcia de Leaniz *et al.* in prep.**

****locus excluded from panel used for SALSEA-Merge tool.**

4.2.2 Development of baseline dataset for the markers

Work on the development of a baseline dataset for the agreed marker set that will be sufficient for the regional assignment of salmon caught at sea has three main components – 1) calibration of genotyping across laboratories, 2) development of a common, integrated database of genetic information, and 3) populating the database with information.

The calibration exercise has now been completed by Jon Ellis (Exeter University). This is based on the typing by all participating laboratories for the agreed suite of microsatellite loci of the Matis Prokaria reference plates that encompass DNA from ~180 salmon representative of the salmon's transatlantic distributional range. The results show that error rates, following correction for systematic errors arising from the use of different screening platforms or different primer sets, ranged from <0.5% to <4.5%, with the final levels expected to be overall <1% after avoidable error sources have been corrected.

A database for holding genetic baseline information on the chosen suite of markers has been developed by Bernt Drange (Norwegian Institute of Marine Research) and John Gilbey (Marine Scotland). Data entry will be by submitted spreadsheets which will be quality controlled and converted to a standardized allele nomenclature for each locus using appropriate laboratory specific correction algorithms based on the calibration exercise.

Work is currently underway to enter existing data and all laboratories are now engaged in collecting further samples and carrying out additional screening to complete the baseline dataset to a level sufficient for regional assignment. It is anticipated that the final dataset will encompass 300+ rivers representing >90% of European salmon production.

4.3 Conclusions

The SALSEA-Merge project demonstrates the potential of the development of useful molecular genetic tools for advancing not only understanding of mixed-stock fisheries on the European scale but also for advancing understanding of the marine ecology of species by allowing studies of the spatial and temporal distribution of stocks and their constituent populations to be undertaken. This potential in the Atlantic salmon was significantly enhanced by different research groups working on the species identifying a set of optimal markers for future work so that datasets collected by individual research groups could be integrated effectively and be used as the basis for the development of a trans-European baseline dataset.

4.4 Recommendations

ICES, based on the success of the SALSEA-Merge initiative, should:

- Promote and actively encourage research at national and international (e.g. EU) levels into the identification and optimization of molecular genetic marker suites that can resolve population structuring in other species under the ICES remit
- Encourage the development of integrated, transnational molecular genetic databases on marine species under the ICES remit, using the SALSEA-Merge genetic database as a prototype/model
- Make provision to host and curate trans-national genetic databases on marine species covered by its remit
- Support and promote extension of the SALSEA-merge database for European Atlantic salmon stocks to encompass stocks in the Western Atlantic
- support endeavours to extend work on the use of genetic markers to advance understanding of the marine ecology of Atlantic salmon beyond the life of the existing EU SALSEA-Merge project
- Review the potential of use molecular genetic markers in other marine species under ICES remit for monitoring spatial and temporal movements of individuals, populations and stocks to advance understanding of their marine ecology

5 ToR d) Assess the possibility for the development of an integrated global management model for Atlantic cod based on genetic information

Jakob Hemmer-Hansen, Torild Johansen, Einar Eg Nielsen, Morten Limborg, Ellen Kenchington, Martha O'Sullivan, Jens Carlsson

5.1 Using genetic information to define management units in marine fishes

The use of genetic information for defining management units and protecting biodiversity has a long tradition in conservation genetics (e.g. Moritz 1994; Crandall *et al*., 2000; van Tienderen *et al*., 2002). However, conservation genetics has traditionally been applied to terrestrial species (Avise, 1998), and management of marine fish has made very limited use of genetic information for gaining information about the population structure of managed species. This may be surprising, given that fisheries managers and scientists have long been aware of discordance between biological population structures and the management units applied in many fisheries. Many management units were set decades ago for political or other non-biological reasons and their legacy continues to this day despite clear evidence of migration and multiple populations confounding assessment results. Recently, this failure to recognize and manage biological complexity has been linked to population collapses in marine fish (e.g. Hutchinson 2008, Reiss *et al*., 2009).

Most commonly, current management units are too large and reconciliation with biological data on the stocks results in smaller management units. For example, in 2009, ICES, in providing advice to a NEAFC request, reviewed the stock structure of *Sebastes mentella* in the Irminger Sea and adjacent areas. One, two and three-stock scenarios were examined based on a suite of scientific information including geographic distribution, genetic variation (e.g. allozymes, mitochondrial DNA, and nuclear DNA), phenotypic variation (e.g. life-history traits, morphology, and fatty acid composition), and connectivity (e.g. larval dispersal, natural tags (parasites, and otolith structure and chemistry) and artificial tags). Based primarily on genetic information; i.e. microsatellite information, and supported by analyses of allozymes, fatty acids, as well as some parasite patterns, ICES concluded that there are three biological stocks of *S. mentella* in the Irminger Sea and adjacent waters and proposed management units that would best reflect the biological stocks (ICES, 2009).

The North Atlantic cod is distributed throughout the North Atlantic on the continental shelf and slope. It is found from 40°N to 65°N in the northwest and from 45°N to 80°N in the Northeast Atlantic. It spawns from the inner fjords to the coasts and on-banks along the coast. The time of spawning depends on the region. As an economically important species, cod were among the first fish species to be studied by molecular genetic methods (see e.g. Sick 1961, Møller 1966). Since the early days of haemoglobin and allozyme studies, numerous genetic studies have been conducted using a variety of genetic markers, such as mitochondrial DNA (e.g. Árnason, 2004, Carr and Marshall 2008), nuclear RFLP (e.g. Pogson *et al*., 1995), microsatellites (e.g. Nielsen *et al*., 2001; Nielsen *et al*., 2003; O'Leary *et al*., 2007; Skarstein *et al*., 2007, Wennevik *et al*., 2008) and more recently also Single Nucleotide Polymorphisms (SNPs, Moen *et al*., 2008). Besides presumably neutral genetic markers, DNA based studies have also targeted genes supposedly under selection in natural populations of cod (e.g. PanI and haemoglobin, see e.g. Case *et al*., 2005; Pogson and Fevolden 2003; Skarstein *et al*., 2007 and Andersen *et al*., 2008). Genetic information has also been

used to investigate the phylogeographical history of cod, which has been affected by multiple glacial cycles (Bigg *et al*., 2008; Carr and Marshall 2008). Thus, among species of importance for fisheries management, cod is the species with the most detailed population genetic data on broad as well as local geographical scales. This information could be integrated into fisheries management. However, with a few exceptions, genetic information is rarely directly used for the management of cod today. Thus, the purpose of this paper is to use cod as a model species to examine if available population genetic data can be used for a global integrated management model for the species, i.e. do we have the quantity and quality of genetic data needed in order for genetics to be usefully integrated into fisheries management. Firstly, we briefly review current management schemes for cod. Secondly, we evaluate current knowledge of population structure gained through population genetic studies and assess to which extend these data conflict with current management areas. Finally, we look at the future potentials for the application of genetic data for the management of cod and other marine fish, and we point to research areas in need of improvements in order for genetics to be efficiently integrated with current management strategies.

5.2 Current management of cod

5.2.1 Cod fisheries management

Despite recent population crashes and relatively strict fishery regulations in some areas, cod is still among the most important species with about 800.000 tonnes landed in the Northeastern Atlantic in 2005 (Reiss *et al*., 2009). Many of the cod stocks show spawning-stock biomass below safe biological limits and recruitment levels are extremely low. As for many other marine fish, the definition of cod management areas is based on political and economical rather than biological criteria. Reiss *et al*. (2009) provides an overview of the management of cod, with particular focus on the Northeastern Atlantic. Here, we highlight some of these areas of relevance to our aim, i.e. to identify cases where management and genetics may conflict.

5.2.2 Management in the Northwestern Atlantic

Cod in the Northwestern Atlantic (primarily Canada and western Greenland) is managed according to the Northwest Atlantic Fisheries Organization (NAFO) statistical divisions and subdivisions. Thus, Canadian cod is managed in two main regions. One is the northern cod, collectively managed in NAFO regions 2J, 3K and 3L, which cover a vast geographical area (from app. 46°N to 56°N along the Canadian coast). The remaining Canadian management areas are subdivided into smaller geographical regions which have been used since the early 1970s for fishery management purposes. These management areas are the Southern Gulf of St. Lawrence (region 4T), Sydney Bight (region 4Vn), Eastern Scotian Shelf (regions 4W and 4Vs), Southwestern Nova Scotia (region 4 X) and the Canadian part of Georges Bank (region 5Ze). In US waters, cod is managed in two main areas, i.e. the Gulf of Maine (region 5Y) and the American part of Georges Bank (region 5Ze) and southwards. Generally, these management areas of Northwestern Atlantic cod are every large, but they do encompass many of the known main spawning grounds at the continental shelf banks.

5.2.3 Management in the Northeastern Atlantic

Cod in the Northeastern parts of its distribution are also managed on rather coarse geographical scales. Thus, cod in Icelandic waters are all managed collectively and separately from neighbouring areas in eastern Greenland and the Faroe Islands. Cod

at the Faroe Islands are managed in a large region collectively with eastern Greenland and areas north of the British Isles and west of Scotland. Furthermore, whereas the Irish Sea is managed as a single area, most ICES areas to the west and south of Ireland and Great Britain (e.g. Celtic Sea and English Channel) are managed as one area. Similarly, the entire North Sea is managed as one area along with large areas in the Norwegian Sea. Currently, Baltic Sea cod is managed separately from North Sea cod, and the Skagerrak, the Kattegat and the western Baltic Sea in the transition zone between the North Sea and the Baltic Sea are also managed separately.

Cod in Norwegian waters have traditionally been divided into North East Arctic Cod (NEAC) and Norwegian Coastal Cod (NCC). NCC is a more stationary cod compared to NEAC (Jakobsen 1987). The highly migratory NEAC stock migrates from the feeding grounds in the Barents Sea to the coast of Norway to spawn during March to May. Most of the fishery for NCC is in the spawning period, which overlaps in time with the migration and spawning of the NEAC. Because of the overlap in spawning times and areas, a part of the Norwegian fishery for cod (particularly around Lofoten) is targeting two different populations, and mixed-stock analyses have been used to determine the contribution from each population to the fishery (Wennevik *et al*. 2008). Thus, this example illustrates one of the few cases where genetic data has been integrated into current management of cod. The coastal cod in Norwegian waters is further divided into three management units. The NCC is defined from the Russian boarder along the coast to 62°N. Coastal cod is managed as part of the North Sea cod south of 62°N, whereas from Lindesnes in southern Norway to the Swedish boarder coastal cod is kept out of the quota system for the North Sea/Skagerrak areas.

5.3 Evaluation of genetics for defining management units in cod

As reviewed by Reiss *et al*. (2009), Atlantic cod is by far the marine fish species which has received the most attention in population genetic studies. As such, this species should be a good candidate for applying genetic data for defining management units.

Reiss *et al*. (2009) argue that there is currently a mis-match between genetic population structure and management units in cod (see Table 5.3.1). The most observed cases of temporally stable genetic population structure in cod concern the major break between the eastern and western Atlantic and the marked isolation of the Baltic Sea and Northeast Arctic Cod (NEAC; O'Leray *et al*., 2007; Pogson *et al*., 1995; Nielsen *et al*., 2001; Nielsen *et al*., 2003; Westgaard and Fevolden, 2007, see also Table 5.3.1). These results support current management schemes for the species. However, mismatches do seem to occur on micro-geographical scales, where genetic differences have been described for inshore vs. offshore cod populations in the western Atlantic (e.g. Ruzzante *et al*., 1996; Ruzzante *et al*., 1997). For the eastern Atlantic, differences have been found for cod around Iceland (e.g. Pampoulie *et al*., 2006), within the North Sea (Hutchinson *et al*., 2001) and between Norwegian fjords (e.g. Knutsen *et al*., 2003; Skarstein *et al*., 2007). Hence, in addition to information on macro-geographical scales, population genetics has provided very important data which indicates that population structure in cod is much more complicated than hitherto believed. Consequently, according to Reiss *et al*. (2009), management schemes would need to be refined in these regions.

Ultimately, the added value from using population genetics for cod management should be to increase our understanding of the evolution and biology of the species. This should ideally form the basis for setting management units and assuring sustainable management. Merely describing low but statistically significant cases of ge-

netic structure will be of limited use for management unless genetic data (in conjunction with other data) improve our understanding of the biology of the species (see also Waples *et al*., 2008; ICES, 2009). For cod, most of the reported cases where genetics conflict with current management schemes have not been confirmed by temporal replication. Temporal replication is as an important criterion to be met in order for genetic data to be useful for defining management units, because temporal stability would support that patterns do indeed reflect biologically relevant separation between samples (Waples, 1998).

Consequently, although genetic data do indicate fine scale structure of cod populations, it is currently largely uncertain if these results truly reflect genetically and biologically unique populations. From a management point of view (and maybe also from a population geneticist's), this may be seen as a somewhat disappointing outcome after years of research. However, cod is one of the marine fish where genetic recourses are building up rapidly these years. For instance, Moen *et al*. (2008) recently published 318 Single Nucleotide Pylymorphisms (SNPs) developed from EST libraries. In addition, with the ongoing sequencing of the cod genome (http://codgenome.no/wiki/index.php/Main_Page) and other cod sequencing projects (e.g. www.codgene.ca, http://www.codgen.olsvik.info); it is likely that the nearest future will bring both the quantity and quality of genetic markers needed to provide detailed information on micro-geographical scales.

As pointed out by Reiss *et al*. (2009), there is often a dichotomy between the time-scales on which fisheries management and population genetics operate. Thus, whereas fisheries are managed on ecological time-scales and concerned with demographic cohesiveness of groups of individuals (i.e. governed by migration), population genetics traditionally operate on evolutionary time-scales and is concerned with evolutionary cohesiveness of groups of individuals (i.e. governed by gene flow; Waples and Gaggiotti, 2006). Hence, groups of fish might be demographically independent, but not genetically unique, for instance because the groups were isolated relatively recently, or because population structure is ephemeral on evolutionary time-scales. These potential problems also apply to cod. However, with the fast increase in the availability of genomic resources in cod, it is likely that it will indeed be possible to move from evolutionary to ecological time-scales in studies of genetic structure of cod populations, for example by turning to genes subject to local selection pressures in combination with selectively neutral markers. In fact, a number of gene associated genetic markers likely to be under adaptive evolution in cod have recently been identified (Moen *et al*., 2008). Such markers may be useful for improving our understanding of the mechanisms shaping population structure in cod and might thus also serve as population specific markers in relation to setting management units for the species.

5.4 Conclusions and perspectives

The lack of conclusive evidence regarding the frequency of occurrence and evolutionary significance of micro-geographical population structure appear to be the largest impediment against implementing the use of genetic information for defining management units in cod. In general, there is very little discrepancy between major evolutionary units and current management units, although there are still a few "black spots" on the map requiring further investigation. Accordingly, population genetic studies of the species on a small geographical scale should be encouraged (see e.g. Hutchinson *et al*., 2001; Knutsen *et al*., 2003; Nielsen *et al*., 2009). Studies should particularly focus on elucidating whether the observed genetic differentiation among

spawning aggregations separated by a few tens of kilometres are stable in time (across generations/decadal time-scale) and thus represent true semi-independent units. Archived material in the form of ethanol preserved tissue and historical otoliths should be available in many areas for these types of investigations. Because separation time among local populations is expected to be short, migration rates are expected to be high and effective population sizes relatively large, application of genetic markers subject to selection may prove valuable as genetic markers for Genetic stock identification (GSI) in conjunction with presumed neutral markers (see section above). One note of caution is that natural selection may alter allele frequencies within a cohort, so tests for temporal stability from egg-to-adult should be conducted.

As illustrated by Reiss *et al*. (2009), the knowledge of the genetic population structure is not nearly as advanced for the majority of other exploited species in the North Atlantic, which represents one of the best studied areas for population genetics of marine fish. There is an immediate need for more studies merely identifying the broad scale population structure of those species if genetic methods should play a role for defining management units. Again temporal sampling of spawning aggregations is a prerequisite for obtaining robust results, which can be used for defining management units.

Even if we are able to unambiguously identify each semi-independent evolutionary unit in each of the managed species, there will still be arguments relating to ecological/evolutionary populations (see above). This debate may be relevant, but should not be used as an excuse not to use genetics for defining management units. First of all, if current management units do not reflect the evolutionary relationships among populations, there is no excuse not to change current practice. Secondly, the first priority of management should be to ensure conservation of biodiversity, including intraspecific genetic variation. Accordingly, evolutionary units should be a management priority. Finally, new molecular genomic methods may provide evidence of ecological populations as well. One thing to note is that it is quite likely that there are differences in patterns of population structure for different species managed in the same areas. For instance, some species exhibit temporally stable and highly significant population structure in areas where other, seemingly ecologically similar, species do not (e.g. sardines and anchovy; Gaggiotti and Vetter, 1999, herring and sprat; Jørgensen *et al*., 2005 and Limborg *et al*., 2009; see also Table 1 in Reiss *et al*., 2009 and discussion in Limborg *et al*., 2009). The realization of these interspecific differences is of great importance in multispecies approaches when focusing on area specific management. Unfortunately, this does not make genetically based scientific advice to management less complicated, but stresses the importance of acquiring genetic data for more marine fish species, because it may be difficult to predict patterns of genetic structuring based on knowledge of, for instance, physiology, migration patterns and life-history characters for individual species (see also discussion in Hemmer-Hansen *et al*., 2007).

Given that it is difficult to base global management of cod on genetic data at present, there is a clear need for careful consideration of how we can proceed from here. Genetic data has certainly got the potential of facilitate sustainable fisheries management, but we need a validation framework for determining when there is sufficient genetic evidence of changing current management practice. Various types of hard (e.g. highly significant temporally stable DNA based evidence) and soft evidence (e.g. differences in morphology, growth patterns) for population structuring is available, and a framework would ideally encompass both types of information. At the same time, however, the framework should rank the various types of evidence in an effort to evaluate where management decisions are required. In some respects this process

is similar to the prioritization of population schemes developed for salmonids (Allendorf *et al*., 1997) and marine fish (Nielsen and Kenchington 2001), and we suggest consulting these approaches for defining management units. Finally, the framework should also include what should be done in particular difficult cases such as population mixing and global change, potentially shifting the distribution of evolutionary populations and, accordingly, management units (see Cheung *et al*., 2009). Therefore, the framework needs to be dynamic and possibly include genetic monitoring (Schwartz *et al*., 2007). When such a validation framework is in place we expect that genetic methods will play a strong role for future definitions of management units in exploited marine organisms.

5.5 Recommendations:

1) Given the evidence of broad scale population structure in Atlantic cod between regions (e.g. Baltic Sea vs. North Sea, northeast Arctic cod vs. Norwegian coastal cod), further studies investigating micro-geographical structure within regions should be encouraged.

2) The development of a standardized framework for the integration of genetic data across research groups to allow a more robust definition of management units in cod should be promoted.

3) Given the ongoing development of genomic resources in cod, genetic markers subject to selection should be developed to be used in association with neutral markers to resolve finer scale population structure and provide new insights in relation to management units.

4) Cross disciplinary approaches combining genetics with life-history characters, physiology, ecology and modelling should be encouraged to improve our understanding of population structure in cod.

5) Where current management units contradict identified genetic populations, management should be reviewed to assure conservation of intraspecific biodiversity.

5.6 References

Allendorf, F.W., Bayles D., Bottom, D.L., Currens, K.P., Frissell, C.A., Hankin, D., Lichatowich, J.A., Nehlsen, W., Trotter, P.C., Williams, T.H. 1997. Prioritizing Pacific salmon stocks for conservation. Conservation Biology, 11: 140–152.

Andersen, O., Wetten, O.F., De Rosa, M.C., Andre, C., Alinovi, C.C., Colafranceschi, M., Brix, O., Colosimo, A. 2009. Haemoglobin polymorphisms affect the oxygen-binding properties in Atlantic cod populations. Proceedings of the Royal Society B-Biological Sciences, 276, 833–841.

Árnason, E. 2004. Mitochondrial cytochrome b DNA variation in the high-fecundity Atlantic cod: Trans-Atlantic clines and shallow gene genealogy. Genetics, 166, 1871–1885.

Árnason, E., Pálsson, S. 1996. Mitochondrial cytochrome b DNA sequence variation of Atlantic cod Gadus morhua, from Norway. Molecular Ecology, 5, 715–724.

Árnason, E., Pálsson, S., Arason, A. 1992. Gene flow and lack of population differentiation in Atlantic cod, Gadus morhua L, from Iceland, and comparison of cod from Norway and Newfoundland. Journal of Fish Biology, 40, 751–770.

Árnason, E., Petersen, P.H., Kristinsson, K., Sigurgíslason, H., Pálsson, S. 2000. Mitochondrial cytochrome b DNA sequence variation of Atlantic cod from Iceland and Greenland. Journal of Fish Biology, 56, 409–430.

Avise, J.C. 1998. Conservation genetics in the marine realm. Journal of Heredity, 89, 377–382.

Beacham, T.D., Brattey, J., Miller, K.M., Le, K.D., Withler, R.E. 2002. Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. ICES Journal of Marine Science, 59, 650–665.

Bentzen, P., Taggart, C.T., Ruzzante, D.E., Cook, D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences, 53, 2706–2721.

Berg, E., Sarvas, T.H., Harbitz, A., Fevolden, S.E., Salberg, A.B. 2005. Accuracy and precision in stock separation of north-east Arctic and Norwegian coastal cod by otoliths−comparing readings, image analyses and a genetic method. Marine and Freshwater Research, 56, 753–762.

Bigg, G.R., Cunningham, C.W., Ottersen, G., Pogson, G.H., Wadley, M.R., Williamson, P. 2008. Ice-age survival of Atlantic cod: agreement between palaeoecology models and genetics. Proceedings of the Royal Society B-Biological Sciences, 275, 163–172.

Carr, S.M., Marshall, H.D. 2008. Intraspecific phylogeographic genomics from multiple complete mtDNA Genomes in Atlantic cod (*Gadus morhua*): Origins of the "Codmother," trans-atlantic vicariance and midglacial population expansion. Genetics, 180, 381–389.

Carr, S.M., Snellen, A.J., Howse, K.A., Wroblewski, J.S. 1995. Mitochondrial DNA sequence variation and gentic stock structure of Atlantic cod (*Gadus morhua*) from bay and offshore locations on the Newfoundland continental shelf. Molecular Ecology, 4, 79–88.

Case, R.A.J., Hutchinson, W.F., Hauser, L., van Oosterhout, C., Carvalho, G.R. 2005. Macro- and micro-geographic variation in pantophysin (PanI) allele frequencies in the NE Atlantic cod Gadus morhua. Marine Ecology Progress Series, 301, 267–278.

Cheung, W.W.L., Close, C., Lam, V., Watson, R., Pauly, D. 2008. Application of macroecological theory to predict effects of climate change on global fisheries potential. Marine Ecology-Progress Series, 365, 187–197.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M., Wayne, R.K. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution, 15, 290–m–n295.

Dahle, G. 1991. Cod, Gadus morhua L., populations identified by mitochondrial DNA. Journal of Fish Biology, 38, 295–303.

Dahle, G., Joerstad, K.E., Rusaas, H.E., Ottera, H. 2006. Genetic characteristics of broodstock collected from four Norwegian coastal cod (*Gadus morhua*) populations. ICES Journal of Marine Science, 63, 209–215.

Dahle, G., Jørstad, K.E. 1993. Hemoglobin variation in cod−a reliable marker for Arctic cod (*Gadus morhua* L.). Fisheries Research, 16, 301–311.

Fevolden, S.E., Pogson, G.H. 1997. Genetic divergence at the synaptophysin (Syp I) locus among Norwegian coastal and north-east Arctic populations of Atlantic cod. Journal of Fish Biology, 51, 895–908.

Gaggiotti, O.E., Vetter, R.D. 1999. Effect of life history strategy, environmental variability, and overexploitation on the genetic diversity of pelagic fish populations. Canadian Journal of Fisheries and Aquatic Sciences, 56, 1376–1388.

Galvin, P., Sadusky, T., McGregor, D., Cross, T. 1995. Population genetics of Atlantic cod using amplified single locus minisatellite VNTR analysis. Journal of Fish Biology, 47, 200–208.

Gjøsæter, J., Jørstad, K., Nævdal, G., Thorkildsen, S. 1992. Genotype distributions of cod from the Norwegian Skagerrak coast. Sarsia, 76, 255–259.

Hemmer-Hansen, J., Nielsen, E.E., Grønkjær, P., Loeschcke, V. 2007. Evolutionary mechanisms shaping the genetic population structure of marine fishes; lessons from the European flounder (*Platichthys flesus* L). Molecular Ecology, 16, 3104–3118.

Husebø, Å., Imsland, A.K., Nævdal, G. 2004. Haemoglobin variation in cod: a description of new variants and their geographical distribution. Sarsia, 89, 369–378.

Hutchinson, W.F. 2008. The dangers of ignoring stock complexity in fishery management: the case of the North Sea cod. Biology Letters, 4, 693–695.

Hutchinson, W.F., Carvalho, G.R., Rogers, S.I. 2001. Marked genetic structuring in localised spawning populations of cod *Gadus morhua* in the North Sea and adjoining waters, as revealed by microsatellites. Marine Ecology-Progress Series, 223, 251–260.

ICES. 2009. Report of the Workshop on Redfish Stock Structure (WKREDS). 22–23 January 2009. ICES Headquarters, Copenhagen. ICES CM 2009/ACOM:37.

Imsland, A.K., Jónsdóttir, Ó.D.B., Daníelsdóttir, A.K. 2004. Nuclear DNA RFLP variation among Atlantic cod in south and south-east Icelandic waters. Fisheries Research, 67, 227–233.

Jakobsen, T. 1987. Coastal cod in northern Norway, Fisheries Research, 5, 223–234.

Jónsdóttir, Ó.D.B., Daníelsdóttir, A.K., Nævdal, G. 2001. Genetic differentiation among Atlantic cod (*Gadus morhua* L.) in Icelandic waters: temporal stability. ICES Journal of Marine Science, 58, 114–122.

Jónsdóttir, Ó.D.B., Imsland, A.K., Atladóttir, Ó.Ý., Daníelsdóttir, A.K. 2003. Nuclear DNA RFLP variation of Atlantic cod in the North Atlantic Ocean. Fisheries Research, 63, 429–436.

Jónsdóttir, Ó.D.B., Imsland, A.K., Daníelsdóttir, A.K., Marteinsdóttir, G. 2002. Genetic heterogeneity and growth properties of different genotypes of Atlantic cod (*Gadus morhua* L.) at two spawning sites off south Iceland. Fisheries Research, 55, 37–47.

Jónsdóttir, Ó.D.B., Imsland, A.K., Daníelsdóttir, A.K., Thorsteinsson, V., Nævdal, G. 1999. Genetic differentiation among Atlantic cod in south and south-east Icelandic waters: synaptophysin (Syp I) and haemoglobin (HbI) variation. Journal of Fish Biology, 54, 1259–1274.

Jorde, P.E., Knutsen, H., Espeland, S.H., Stenseth, N.C. 2007. Spatial scale of genetic structuring in coastal cod *Gadus morhua* and geographic extent of local populations. Marine Ecology Progress Series, 343, 229–237.

Jørgensen, H.B.H., Hansen, M.M., Bekkevold, D., Ruzzante, D.E., Loeschcke, V. 2005. Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. Molecular Ecology, 14, 3219–3234.

Jørstad, K.E., Nævdal, G. 1989. Genetic variation and population structure of cod, *Gadus morhua* L., in some fjords in northern Norway. Journal of Fish Biology, 35, 245–252.

Knutsen, H., Jorde, P.E., Andre, C., Stenseth, N.C. 2003. Fine-scaled geographical population structuring in highly mobile marine species: the Atlantic cod. Molecular Ecology, 12, 385–394.

Lage, C., Kuhn, K., Kornfield, I. 2004. Genetic differentiation among Atlantic cod (*Gadus morhua*) from Browns Bank, Georges Bank, and Nantucket Shoals. Fishery Bulletin, 102, 289–297.

Limborg, M.T., Pedersen, J.S., Hemmer-Hansen, J., Tomkiewicz, J., Bekkevold, D. 2009. Genetic population structure of European sprat *Sprattus sprattus*: differentiation across a steep environmental gradient in a small pelagic fish. Marine Ecology-Progress Series, 379, 213–224.

Moen, T., Hayes, B., Nilsen, F., Delghandi, M., Fjalestad, K.T., Fevolden, S.E., Berg, P.R., Lien, S. 2008. Identification and characterization of novel SNP markers in Atlantic cod: Evidence for directional selection. BMC Genetics, 9, Article Number: 18.

Moritz, C. 1994. Defining 'evolutionary significant units' for conservation. Trends in Ecology and Evolution, 9, 373–375.

Mork, J., Giæver, M. 1999. Genetic structure of cod along the coast of Norway: Results from isozyme studies. Sarsia, 84, 157–168.

Mork, J., Ryman, N., Stahl, G., Utter, F., Sundnes, G. 1985. Genetic variation in Atlantic cod (*Gadus morhua*) throughout its range. Canadian Journal of Fisheries and Aquatic Sciences, 42, 1580–1587.

Møller, D. 1966. Genetic differences between cod groups in the Lofoten area. Nature, 212, 824.

Nielsen, E.E., Hansen, M.M., Ruzzante, D.E., Meldrup, D., Grønkjær, P. 2003. Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. Molecular Ecology, 12, 1497–1508.

Nielsen, E.E., Hansen, M.M., Schmidt, C., Meldrup, D., Grønkjær, P. 2001. Fisheries–Population of origin of Atlantic cod. Nature, 413, 272–272.

Nielsen, E.E., Kenchington, E. 2001. A new approach to prioritizing marine fish and shellfish populations for conservation. Fish and Fisheries, 2, 328–343.

Nielsen, E.E., MacKenzie, B.R., Magnussen, E., Meldrup, D. 2007. Historical analysis of Pan I in Atlantic cod (*Gadus morhua*): temporal stability of allele frequencies in the southeastern part of the species distribution. Canadian Journal of Fisheries and Aquatic Sciences, 64, 1448–1455.

Nielsen, E.E., Wright, P.J., Hemmer-Hansen, J., Poulsen, N.A., Gibb, L.M., Meldrup, D. 2009. Micro geographical population structure of cod *Gadus morhua* in the North Sea and west of Scotland: the role of sampling loci and individuals. Marine Ecology-Progress Series, 376, 213–225.

Nordeide, J.T. 1998. Coastal cod and north-east Arctic cod–Do they mingle at the spawning grounds in Lofoten? Sarsia, 83, 373–379.

O'Leary, D.B., Coughlan, J., Dillane, E., McCarthy, T.V., Cross, T.F. 2007. Microsatellite variation in cod *Gadus morhua* throughout its geographic range. Journal of Fish Biology, 70, 310–335.

Pampoulie, C., Jakobsdóttir, K.B., Marteinsdóttir, G., Thorsteinsson, V. 2008. Are vertical behaviour patterns related to the pantophysin locus in the Atlantic cod (*Gadus morhua* L.)? Behavior Genetics, 38, 76–81.

Pampoulie, C., Ruzzante, D.E., Chosson, V., Jorundsdottir, T.D., Taylor, L., Thorsteinsson, V., Danielsdottir, A.K., Marteinsdottir, G. 2006. The genetic structure of Atlantic cod (*Gadus morhua*) around Iceland: insight from microsatellites, the Pan I locus, and tagging experiments. Canadian Journal of Fisheries and Aquatic Sciences, 63, 2660–2674.

Pepin, P., Carr, S.M. 1993. Morphological, meristic, and genetic analysis of stock structure in juvenile Atlantic cod (*Gadus morhua*) from the Newfoundland Shelf. Canadian Journal of Fisheries and Aquatic Sciences, 50, 1924–1933.

Pogson, G.H. 2001. Nucleotide polymorphism and natural selection at the pantophysin (Pan I) locus in the Atlantic cod, *Gadus morhua* (L.). Genetics, 157, 317–330.

Pogson, G.H., Fevolden, S.E. 2003. Natural selection and the genetic differentiation of coastal and Arctic populations of the Atlantic cod in northern Norway: a test involving nucleotide sequence variation at the pantophysin (PanI) locus. Molecular Ecology, 12, 63–74.

Pogson, G.H., Mesa, K.A., Boutilier, R.G. 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: A comparison of allozyme and nuclear RFLP loci. Genetics, 139, 375–385.

Poulsen, N.A., Nielsen, E.E., Schierup, M.H., Loeschcke, V., Groenkjaer, P. 2006. Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). Molecular Ecology, 15, 321–331.

Reiss, H., Hoarau, G., Dickey-Collas, M., Wolff, W.J. 2009. Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish and Fisheries, available online, DOI: 10.1111/j.1467–2979.2008.00324.x.

Ruzzante, D.E., Taggart, C.T., Cook, D. 1998. A nuclear DNA basis for shelf and bank-scale population structure in NW Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. Molecular Ecology, 7, 1663–1680.

Ruzzante, D.E., Taggart, C.T., Cook, D., Goddard, S.V. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua* L.) off Newfoundland: microsatellite DNA variation and antifreeze level. Canadian Journal of Fisheries and Aquatic Sciences, 53, 634–645.

Ruzzante, D.E., Taggart, C.T., Cook, D., Goddard, S.V. 1997. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: a test and evidence of temporal stability. Canadian Journal of Fisheries and Aquatic Sciences, 54, 2700–2708.

Ruzzante, D.E., Taggert, C.T., Lang, S., Cook, D. 2000a. Mixed-stock analysis of Atlantic cod near the Gulf of St. Lawrence based on microsatellite DNA. Ecological Applications, 10, 1090–1109.

Sarvas, T.H., Fevolden, S.E. 2005a. Pantophysin (Pan I) locus divergence between inshore v. offshore and northern v. southern populations of Atlantic cod in the north-east Atlantic. Journal of Fish Biology, 67, 444–469.

Sarvas, T.H., Fevolden, S.E. 2005b. The scnDNA locus Pan I reveals concurrent presence of different populations of Atlantic cod (*Gadus morhua* L.) within a single fjord. Fisheries Research, 76, 307–316.

Schwartz, M.K., Luikart, G., Waples, R.S. 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution, 22, 25–33.

Sick, K. 1961. Haemoglobin polymorphism in fishes. Nature, 192, 894–896.

Sigurgíslason, H., Árnason, E. 2003. Extent of mitochondrial DNA sequence variation in Atlantic cod from the Faroe Islands: a resolution of gene genealogy. Heredity, 91, 557–564.

Skarstein, T.H., Westgaard, J.I., Fevolden, S.E. 2007. Comparing microsatellite variation in north-east Atlantic cod (*Gadus morhua* L.) to genetic structuring as revealed by the pantophysin (Pan I) locus. Journal of Fish Biology, 70 (Suppl. C), 271–290.

Smith, P.J., Birley, A.J., Jamieson, B.A., Bishop, C.A. 1989. Mitochondrial DNA in the Atlantic cod, *Gadus morhua*: lack of genetic divergence between eastern and western populations. Journal of Fish Biology, 34, 369–373.

van Tienderen, P.H., de Haan, A.A., van der Linden, C.G., Vosman, B. 2002. Biodiversity assessment using markers for ecologically important traits. Trends in Ecology & Evolution, 17, 577–582.

Waples, R.S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Journal of Heredity, 89, 439–450.

Waples, R.S., Gaggiotti, O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology, 15, 1419–1439.

Waples, R.S., Punt, A.E., Cope, J.M. 2008. Integrating genetic data into management of marine resources: how can we do it better? Fish and Fisheries, 9, 423–449.

Wennevik, V., Jorstad, K.E., Dahle, G., Fevolden, S.E. 2008. Mixed stock analysis and the power of different classes of molecular markers in discriminating coastal and oceanic Atlantic cod (*Gadus morhua* L.) on the Lofoten spawning grounds, Northern Norway. Hydrobiologia, 606, 7–25.

Westgaard, J.I., Fevolden, S.E. 2007. Atlantic cod (*Gadus morhua* L.) in inner and outer coastal zones of northern Norway display divergent genetic signature at non-neutral loci. Fisheries Research, 85, 306–315.

Table 5.3.1. Genetic population structure studies in cod throughout its distribution listed by a regional scale (i.e. Entire Atlantic, Northeast Atlantic and Northwest Atlantic). Location details the no. of sites and the respective areas studied (see explanation of abbreviations below). Genetic differentiation refers to whether genetically significant population structure was identified by the respective study. The mismatch column points out if reported population structure is incongruent with current management units (extracted from Reiss *et al.* 2009).

Region	Location	Genetic differentiation	Mismatch	Source
Entire Atlantic	NS (6), IS (1), CSs (2), Channel (2), BS (1), Scot (1)	Yes	Yes	Hutchinson *et al.* (2001)
Entire Atlantic	Newf (5), Nov (1), Labrador (2), BS (1)	Yes	Yes	Bentzen *et al.* (1996)
Entire Atlantic	Norw n (1), IS (1), Nov (1), Newf (1)	Yes	No	Galvin *et al.* (1995)
Entire Atlantic	Baltic (1), BS (1), Ice (3); Greenl (3), Scot (1)	Yes	No	O'Leary *et al.* (2007)
Entire Atlantic	NS (1), Newf (1)	No	No	Smith *et al.* (1989)
Entire Atlantic	Ice (1), NS (1), BS (2), Nov (1), Newf (1)	Yes	No	Pogson *et al.* (1995)
Entire Atlantic	Scot (2), BS (1), Trondheimfjord (1), CS (1), NS (4), Ice (1)	Yes	No	Jónsdóttir *et al.* (2003)
Entire Atlantic	Newf (4), Nov (4), Ice (1), NS (1) Balsfjord (1), BS (1)	Yes	No	Pogson (2001)
Entire Atlantic	NS (1), Greenl (1), Nov (1), Ice (1), BS (1) Norw (2), Baltic (2)	No	No	Mork *et al.* (1985)
NE Atlantic	Skagerrak (5)	Yes	Yes	Jorde *et al.* (2007)
NE Atlantic	Norw (11), BS (1), Sptz (1)	Yes	Yes	Westgaard and Fevolden (2007)
NE Atlantic	Faroe (5), NS (2), Baltic (2)	Yes	No	Nielsen *et al.* (2007)
NE Atlantic	Baltic (1), NS (1)	Yes	No	Poulsen *et al.* (2006)
NE Atlantic	Norw (8)	Yes	Yes	Wennevik *et al.* (2008)
NE Atlantic	Ice (5), Faroe (2)	Yes	No	Pampoulie *et al.* (2008)
NE Atlantic	Ice (22)	Yes	Yes	Pampoulie *et al.* (2006)
NE Atlantic	Skagerrak (6)	Yes	Yes	Knutsen *et al.* (2003)
NE Atlantic	Baltic (12), NS (2)	Yes	No	Nielsen *et al.* (2003)
NE Atlantic	NS (1), Baltic (1), BS (1)	Yes	No	Nielsen *et al.* (2001)
NE Atlantic	NS (1), Norw and BS (10), Sptz (1)	Yes	Yes	Skarstein *et al.* (2007)
NE Atlantic	Greenl (9), Ice (42)	No	No	Árnason *et al.* (2000)
NE Atlantic	Faroe (5)	No	No	Sigurgíslason and Árnason (2003)
NE Atlantic	Norw n (5), Norw s (2), BS (2)	No	No	Árnason and Pálsson (1996)
NE Atlantic	Norw (6) and BS (3)	Yes	Yes	Dahle (1991)
NE Atlantic	Ice (12)	No	No	Árnason *et al.* (1992)
NE Atlantic	NS (6), Ice (8), CS (3), Baltic (1), BS (2), Norw (2)	Yes	No	Case *et al.* (2005)

Region	Location	Genetic differentiation	Mismatch	Source
NE Atlantic	Ice (2)	Yes	Yes	Jónsdóttir *et al.* (2002)
NE Atlantic	Ice (6)	Yes	Yes	Jónsdóttir *et al.* (1999)
NE Atlantic	Ice (5)	Yes	Yes	Imsland *et al.* (2004)
NE Atlantic	Norw n (8)	Yes	Yes	Pogson and Fevolden (2003)
NE Atlantic	Norw n (22)	Yes	Yes	Fevolden and Pogson (1997)
NE Atlantic	Ullsfjord (7)	Yes	Yes	Sarvas and Fevolden (2005b)
NE Atlantic	Ice (2)	Yes	Yes	Jónsdóttir *et al.* (2001)
NE Atlantic	Norw (35), BS (5), Sptz (5),NS (1)	Yes	Yes	Sarvas and Fevolden (2005a)
NE Atlantic	Norw s (3), Norw n (2)	Yes	Yes	Berg *et al.* (2005)
NE Atlantic	BS (1), Norw (5)	Yes	No	Dahle and Jørstad (1993)
NE Atlantic	Skagerrak (4)	No	No	Gjøsæter *et al.* (1992)
NE Atlantic	Norw (8)	Yes	Yes	Nordeide (1998)
NE Atlantic	Norw (8)	No	No	Mork and Giæver (1999)
NE Atlantic	Norw (28)	Yes	Yes	Jørstad and Nævdal (1989)
NE Atlantic	Norw (3)	Yes	Yes	Dahle *et al.* (2006)
NE Atlantic	Norw (7), Danish Belt Sea (2)	Yes	Yes	Husebø *et al.* (2004)
NW Atlantic	Newf (1), Grand Bank (1)	Yes	Yes	Ruzzante *et al.* (1997)
NW Atlantic	Newf (10), Nov (6), Labrador (3)	Yes	Yes	Ruzzante *et al.* (1998)
NW Atlantic	Nantucket Shoals (1), GeorgesBank (1), Browns Bank (1)	Yes	Yes	Lage *et al.* (2004)
NW Atlantic	NW Atlantic (19)	Yes	No	Beacham *et al.* (2002)
NW Atlantic	Newf (6), Grand Bank (3)	Yes	Yes	Ruzzante *et al.* (1996)
NW Atlantic	Newf (10) and Trinity Bay (3),Labrador Gilbert Bay (4)	Yes	Yes	Ruzzante *et al.* (2000a)
NW Atlantic	Newf (5)	No	No	Carr *et al.* (1995)
NW Atlantic	Grand Banks (10)	No	No	Pepin and Carr (1993)

Diff. = differentiation among population on the studied scale; Mis. = indications for a mismatch with management units.

Locations: NS = North Sea; CS = Celtic Sea; IS = Irish Sea; BS = Barents Sea; Ice = Iceland; Norw s/n = Norwegian coast, north and south; Scot = Scotian Shelf; Sptz = Spitsbergen; Greenl = Greenland; Nov = Nova Scotia; Newf = Newfoundland.

6 ToR e) to evaluate prospects for application of genetics/genomics to study and reduce the impact of fish and shellfish diseases in natural and cultured populations

R. Wenne, P. Boudry and A. Wąs

6.1 Current situation regarding ToR e)

Several review papers have been published recently about genetic, genomic and biotechnology approaches of disease control in aquatic organisms (e.g. Adams and Thompson, 2006; Quillet *et al.*, 2007; Dios *et al.*, 2008, Davis *et al.*, 2009). Additionally, several EU (e.g. AQUAFUNC and AQUAGENOME: http://genomics.aquaculture-europe.org/; AQUAFIRST: http://aquafirst.vitamib.com/), international (e.g. cGRASP: http://www.cgrasp.org/) or national funded projects (e.g. NAGRP Aquaculture Genome Projects in USA, FUGE in Norway) have been very recently completed or are currently in progress regarding the development of genomic tools and resources for several aquaculture and fisheries species. Some of these projects are directly targeting disease or emphasize resistance and are therefore likely to provide novel information and techniques regarding the control of diseases. This information needs to be presented in a broader context, because pathogens are one of many factors impacting fish and shellfish natural and cultured populations. Additionally, issues directly related to pathology of marine fish and shellfish are handled by WGPDMO (Working Group on Pathology and Diseases of Marine Organisms). As a result, it is proposed to postpone and modify ToR e) in the following way:

Revised justification and title: **Genomic approaches to the study of adaptation of marine organisms in changing environments: what can populations tell us about genes underlying phenotypic changes and what can genes tell us about adaptive evolution of populations?**

Genomics of marine organisms can contribute to better understand how they can adapt to variation of environmental factors in the wild or under aquaculture conditions. In the wild, environmental variation can result from climate change, acidification of oceans, increasing levels of pollutants or fisheries. In aquaculture, they can be due to novel rearing practices or to the extension of new pathogens. Adaptive responses can have phenotypic and genetic components that must be disentangled to model the evolutionary response of species.

Firstly, genetically-based phenotypic differences between wild or culture populations have been demonstrated in many marine species. In these cases, genome scans, based on large numbers of genetic markers using high throughput genotyping technology, can identify regions of the genome associated with these differences and therefore resulting from response to differential selection pressures. When mapped on the genome, these markers contribute to identify QTLs and the genetic architecture of the concerned traits. Secondly, analysis of sequence variation of coding and non-coding parts of the genome can be used to infer the role of selection on the shaping of the observed molecular diversity. Thirdly, transcriptome sequencing, revolutionized by the new generation of sequencing technologies, strongly facilitate the identification of genes differentially expressed in organisms exposed to different environmental conditions, or resulting from divergent selection in the wild or under aquaculture conditions. Candidate genes should then be validated using functional genomics approaches (i.e. reverse genetics, mutagenesis, RNAi). They can be used for gene assisted selection or for population management purposes. Finally, both approaches

(i.e. genome scans and transcriptome studies) can be combined through eQTL and genetical genomics studies. These allow inferring genetic and environmental variance components associated with transcriptional abundances underlying adaptive traits. Such approaches provide further links between adaptation of marine organisms and the molecular bases of the concerned traits.

Novel genomics approaches aiming to better describe and understand these processes will be reviewed in the present ToR and study cases concerning fish and shellfish will be presented. Current developments will be described, highlighting the potentials and limitations of these approaches to contribute to better manage marine biodiversity.

New ToR leader: P. Boudry.

6.2 References

Adams, A., Thompson, K.D. 2006. Biotechnology offers revolution to fish health management. Trends in Biotechnology 24(5): 201–205.

Davies, G., Genini, S., Bishop, S.C., Giuffra, E. 2009. An assessment of opportunities to dissect host genetic variation in resistance to infectious diseases in livestock. Animal, 3 (3): 15–436

Dios, S., Novoa, B., Buonocore, F., Scapigliati, G., Figueras, A. 2008. Genomic Resources for Immunology and Disease of Salmonid and Non-Salmonid Fish. Reviews in Fisheries Science, 16: 119–132, suppl. 1

Quillet, E., Boudry, P., Lapegue, S. 2007. Genetic variability of response to pathogens: a tool to improve health of farmed fish and molluscs. Productions animales, 20: 239–251

7 WG response to the new Science plan

ICES has developed a new science plan to guide its activities in support of fish conservation and fisheries management from 2009–2013. This plan focuses on developing and informing an ecosystem approach to management (EAM) in support of promoting and encouraging science for the sustainable use of the ocean. This requires the integration of work under a broad set of disciplines to understand physical drivers as well as biological processes and their variability, requires.

Genetic studies, through the biological understanding they can deliver, will be critical to achieving the ICES science objectives. These are important to understanding ecosystem processes. The interaction of genes and gene variation with the environment defines the physiology and behaviour of individuals and populations and, through these, individual survival and reproductive success, and overall recruitment and abundance. Therefore an understanding of genetics is essential to be able to understand population responses to environmental change and can provide insight into the impact of different management approaches on the response of populations to specific environmental changes. Analysis of the distribution of genetic variation within and among individuals in space and time can be used to delineate population structuring and as a tool to track population behaviour to help understand demographic dynamics and ecological processes.

Taking genetics into account has presented particular problems in marine fish due to the difficulties of working in the ocean environment. However, while still a major challenge, it is increasingly possible to address genetic issues in marine fish and shell fish species. Advances in understanding gained from model species from terrestrial and freshwater ecosystems have identified key areas where genetics is particularly relevant in biodiversity management and exploitation. At the same time, advances in molecular technologies have significantly enhanced our ability to undertake genetic studies of marine species and to exploit molecular genetic variation as biodiversity markers and individual tags. A sufficient body of theoretical population genetics, as well as an increasing body of empirical evidence, now exists to underpin the importance of genetics in biological population processes and the potential of using molecular genetic variation as biological markers as applied management tools.

Two key areas exist where genetics studies can now be carried out to make a significant and substantive contribution to advancing the ICES science objectives set out in the 2009–2013 plans. The first is with regard to increasing understanding of biodiversity. Biodiversity, in so far as it must be emphasized in the conservation context and in recruitment processes, is fundamentally genetic diversity. As such, genetic studies can be employed to understand how marine fish and shellfish species and stocks are structured into biologically distinct population units that are functionally relevant to the management of biodiversity. The second area where genetics can make a significant and substantive contribution is in relation to understanding how functionally relevant population units will respond to environmental change as regards their recruitment dynamics and stock character (e.g. age at maturity, species range, etc). However, these areas of study are necessarily connected as the second cannot be achieved unless the first is appropriately defined.

To advance their science objectives, the WGAGFM strongly recommends that ICES encourage and support specific national and international collaborations and research programmes on the genetics of marine fish; species-specific local knowledge is

needed for genetic insights to be effectively integrated into existing management programmes. In particular, this should include work to:

Develop optimal suites of molecular genetic markers for resolving functionally important intraspecific phylogenetic and adaptive population structuring

Identify genetic polymorphisms relevant to recruitment processes such as selective density-dependent and independent mortality, age of maturation, fecundity, disease resistance.

Identify optimal experimental approaches/ designs capable of increasing understanding of adaptive processes in the wild in marine fish, with particular attention to factors during key life-history stages where the scope for selective change is greatest e.g. embryonic and early juvenile stages where genetic factors might be expected to be particularly important

Compile collections of material for genetic analysis (e.g. under the Data Collection Regulations) to provide material for studies of population structuring and genetic processes underlying recruitment dynamics and for assessing spatial and temporal changes in the genetic character of populations in response to environmental and ecosystem change

A particular challenge, that involves understanding genetic processes, relates to the issue of the impact of climate change. In this regard, it must be emphasized to assess the nature and extent of recruitment depression associated with genetic maladaptation caused by climate change. This will have implications for species distribution, productivity and ecosystem structure and function and involve the direct effects of temperature change on ecosystems as well as effects such as ocean acidification associated with increased atmospheric CO_2; early life-history stages particularly vulnerable and key to recruitment – slight change can affect behaviour, directly or indirectly.

Recent advances in molecular genetic techniques make it relatively easy and inexpensive in principal to quantify temporal changes in the genetics of populations over tens or even hundreds of years. However, the optimal design of monitoring programmes and data needs need to be specified and field studies implemented. This will require the identification of potential candidate polymorphic loci that are involved in controlling recruitment processes, particularly during important early life-history stages, and likely to be influenced by climate change. It will also testing and validating these candidate genes to identify those most useful for monitoring important genetic responses. Finally, the work required will be the design of genetic monitoring strategies to increase understanding of climate induced population maladaptation and, taking into account adaptive evolutionary processes, identify optimal management responses where substantive, undesirable impacts occur. The insights gained will provide a new generation of predictive management tools that can be used to collect data to monitor change and develop and validate predictive impact models to guide impact management processes.

7.1 Recommendations

ICES should

1) Include specific provisions within their new science plan to deal with the issues associated with impacts of population maladaptation (leading to a loss of genetic fitness, productivity) on fish recruitment processes and spe-

cies distributions caused by environmental changes, such as those induced by climate shifts.

2) Encourage the EU to put out a call under FP7 for research projects to explore the impact of population maladaptation on fish recruitment processes and species distributions caused by environmental changes, such as those induced by climate shifts, to help develop overall predictive models of effects on ecosystem and population productivity

3) Support the routine collection of data/ material that will allow for monitoring of genetic changes over time under the Data Collection Regulation

4) Increase awareness of genetic issues related to environmental change in other international forums such as EFARO, etc

5) Host a Special theme session on genetic processes underlying population responses to climate change

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Annex 2: Agenda

Wednesday 1 April:

9.00	Welcome by local hosts
9.15	Welcome and updates from WG Chair
9.30–12.30	Presentation and discussion of position papers for TOR's a) – e):

a) Report on progress with the establishment of a meta-database for genetic data on fish and shellfish genetics covered under the ICES remit.

b) Review current status of the application of traceability methods in the fisheries sector based on genetics.

c) Update and insights from the EU project SALSEA-Merge on establishment of a large-scale genetic database for assigning individual to population of origin.

d) Assess the possibility for the development of an integrated global management model for Atlantic cod based on genetic information.

e) To evaluate prospects for application of genetics/genomics to study and reduce the impact of fish and shellfish diseases in natural and cultured populations.

12.30–14.00	Lunch
14.00–16.00	Presentation and discussion of position paper for TOR's a) – e) (continued)
16.30–17.00	Status and formation of TOR working groups
17.00–18.00	Open session. (Present results, projects, management problems) Discussion of Science Plan 2009–2011

Thursday 2 April:

09.00–12.30	Work in groups on TOR's a) – e)
12.30–14.00	Lunch
14.00–16.30	Presentation of revised TOR reports
17.00–18.00	Open session. (Present results, projects, management problems)
19.00–	Dinner at the Aquarium of the Sea Fisheries Institute in Gdynia, hosted by the Sea Fisheries Institute.

Friday 3 April

09.00–11.00	Final adjustments of TOR reports–Recommendations
11.00–12.15	Suggestions for new TORs for 2010 and future meeting venue
12.15–12.30	Evaluation and closing of meeting.

Annex 3: WGAGFM terms of reference for the next meeting

The Working Group on the Application of Genetics in Fisheries and Mariculture [WGAGFM] (Chair: G. Dahle, Norway) will meet in Cork, Ireland from 5–7 May 2010 to:

a) genomic approaches to the study of adaptation of marine organisms in changing environments: what can populations tell us about genes underlying phenotypic and demographic changes and what can genes tell us about adaptive evolution of populations?

b) defining genetic data needs and explore opportunities and requirements for the integration of genetic data resulting from the implementation of the EU data collection regulation (DCR 199/2008);

c) review the issues and challenges associated with the utilization of SNPs as markers in population genetic studies with special attention to data handling and statistical tools;

d) pursuing the establishment of a meta-database cataloguing existing data in the field of fish and shellfish genetics;

e) review the genetic effects of exploitation on deep-sea fish.

WGAGFM will report by 30 May 2010to the attention of SCICOM.

Supporting Information

Priority:	The current activities of this Group will lead ICES into issues related to the ecosystem affects of fisheries, especially with regard to the application of the Precautionary Approach. Consequently, these activities are considered to have a very high priority.
Scientific justification and relation to action plan:	**Term of Reference a)** Genomics of aquatic organisms can contribute to reduce reduction of impact of fish and shellfish diseases in several ways. Firstly, Genetically-based differences between wild or culture populations have been demonstrated in many cases. Genome scans, using microarray-based SNP genotyping technology or alternative approaches, aims at identifying regions of the genome associated with these differences in resistance/susceptibility. Secondly, genomes and transcriptome sequencing contribute to the characterization of genes involved in immune and defence systems that will help to identify genetic bases of innate and acquired resistance to pathogens. At the transcriptome level, differential gene expression of fish or shellfish exposed to pathogens, or is genetically resistant/susceptible to pathogens, can also be used to identify genes involved in response to disease. Candidate genes can then be validated using functional genomics (i.e. reverse genetics, mutagenesis, RNAi.) and/or used for marker assisted selection. Such approaches can be combined with QTL through the mapping of eQTLs, providing further links between variation for disease resistance and its molecular bases. (Lead: Pierre Bodry) **Term of Reference b)** The WGAGFM has repeatedly emphasized the need to base the management of fish stocks on population units. Unfortunately the distribution and potential migration routes of populations rarely correspond to ICES or NAFO designated management areas. Hence management units can potentially cover the distribution range of more than one population. Information on the genetic diversity, structure and stability of exploited fish stocks is essential to a

sustainable exploitation and the traceability of catches and fish products.
To be prepared to answer questions on the response of marine genetic diversity in times of global climate change and heavy fishing pressure, genetic sampling on a regular and systematic basis is required.

Therefore ICES should propose that the European Commission integrate genetic monitoring of marine (fish) stocks into the data collection regulation (DCR). This will provide a broad and reliable baseline for management, conservation and traceability purposes. A priority list of species and recommendations for sampling, storage and molecular markers to be applied should be suggested by ICES (WGAGFM and other Expert groups.) taking into account the current genetic knowledge for the species and the availability of marker systems.

To do:
Priority list of species

- Definition of sampling and storage protocols
- Select a certain set of markers for each species
- Calibration of methods between laboratories

(Lead: Jochen Trautner)

Term of Reference c)

Over the past two decades, exceptional advances in molecular analytical methodologies have resulted in a myriad of new types of genetic markers. Single Nucleotide Polymorphisms (SNPs) have been one of the latest additions to the molecular toolbox. SNPs have greatly benefited from the recent development of high-throughput and relatively cost-effective genotyping platforms (e.g. Affymetrix, SNPstream, TaqMan, Sequenom, Illumina). The unprecedented amount of genetic information provided by SNPs, make them the marker of choice for studies ranging from individual, family and population identification, to the discovery of genes and genomic regions affecting adaptive phenotypic variation. While the potential usefulness of SNPs is unquestionable, they are not without problems. For instance, to deal with the often abundant SNP genotype data (varying from a few hundred to several thousand loci at the time), generated from distinct screening platforms, quality control to ensure accuracy of allele call is a critical issue. Where data are available, there is evidence of considerable amount of genotyping error. These have been shown to potentially bias the estimation of population demographic parameters, as well as, to affect linkage analysis, measures of linkage disequilibrium, and subsequent genomic wide association studies. In addition to genotyping error, missing calls also appear to be a common feature of high-throughput genotyping. While a number of independent investigations have elaborated on these and other related relevant issues, comparatively few published studies addressing the potential caveats of SNP screening and subsequent data analysis. Given the increasing number of research groups working on fish genetics considering embracing this new molecular methodology, a review of the current state-of-the-art focusing on technical challenges, good laboratory practices, data handling and analysis would be extremely useful as a guide to users.

(Lead: Paulo Prodöhl and Phil McGinnity)

Term of Reference d)

This ToR was first elaborated in 2008 and reviewed and continued in 2009. We suggest further pursuing this effort as its underlying rationale (counteracting the dispersion and loss of valuable genetic data) is as relevant as ever, and as we also expect important steps to be made during 2009.

Despite a formal analysis of costs and benefits of creating a fish genetic meta-database not being available, the benefits are as obvious as considerable, justifying a continuation of this ToR:

- Loss of data will be avoided;
- Existing data will be assembled and is available for recurring usage;
- Superfluous efforts and costs will be reduced;
- Research coordination and collaboration will be catalysed;
- Outreach to (non-scientific) stakeholders will be improved;
- The transfer of applications based on genetics, emanating from the research realm, into fisheries management schemes will be facilitated.

Future development activities should bear some important aspects in mind such as:

- Data standards should be developed;
- (Meta)data validation and quality checks should be established, possibly through accredited laboratories;
- At some stage it a sustainable management structure has to be established;
- Compatibility with the EMODNET activity and progress of the EMODNET initiative should be monitored.

Initiatives will be taken during 2009 following the strategy outlined in this years' report, and resulting progress will be reported to the WGAGFM panel in 2010.

(Lead: Eric Verspoor, Luca Arnaudo, Jann Th. Martinsohn)

Term of Reference e)

Over-exploitation of traditional coastal stocks and a rising demand for seafood have resulted in the shift of commercial fishing towards less-known, deep-sea species in many parts of the world; by 2000, 40% of the world's trawling grounds were classed as deep sea. However the deep-sea is a cold, low nutrient environment with a slow turnover, and deep-sea species tend to be slow growing and reach sexual maturity much later than fish found in shallower, more nutrient rich waters. These properties make deep-sea fish unsuitable candidates for fishing, because stocks are highly vulnerable, and show very slow recovery after depletion. As a result, dramatic declines have been seen in many targeted species, with many stocks collapsing to <20% of their pre-exploitation abundance in just a few years.

Catches of grenadiers, for example, peaked at 83,800 tonnes annually in the early 1970s and have been in decline ever since; the orange roughy, a species thought to live up to 150 years and only reach sexual maturity at 30 years or older, are now in significant decline due to overexploitation. Similarly, Patagonian toothfish, which can live for 50 years or more, are now targeted by a rapidly expanding, mainly unregulated fishery, and scientists fear that stocks will collapse. Despite the expanding fishery, and increasing interest from other industries, such as gold mining and oil companies, research is lagging considerably and there is limited available biological information about these species. Basic data on population structure, effective population sizes and connectivity is lacking. This ToR will summarize the available information about population genetics of deep-sea fish and identify research priorities and needs in relation to recent and future trends in deep-sea fisheries.

(Lead: Sarah Helyar and Jens Carlsson)

Resource requirements:	None required other than those provided by the host institute
Participants:	The Group is normally attended by some 15–25 members and guests.
Secretariat facilities:	None.
Financial:	None.
Linkages to advisory	ACOM

committees:	
Linkages to other committees or groups:	SIMWG , WGECO, WGMAFC, WGMASC
Linkages to other organizations:	Linkage with the EC Joint Research Centre at Ispra, Italy

Annex 4: Recommendations

Recommendation	For follow up by:
1. Pursuing the development and implementation of a web-based fish population genetic meta-database, under the responsibility of WGAGFM, within the remit of ICES and in collaboration with the European Commission, as proposed in the WGAGFM reports of 2007 and 2008	WGAGFM, ICES data centre
2. The meta-database should ultimately serve as a portal cataloguing relevant information on existing genetic data, primary and secondary reports on genetic research, and available biological samples for genetic analysis, indicating the repositories and contacts from which such data, samples and other relevant information can be obtained	WGAGFM, Fisheries geneticists
3. The Working Group or a subgroup thereof, reviews, by September 2009, the type of data to be included. If needed the currently incorporated data categories will be complemented and a comprehensive reference list of the selected data types be produced and presented at the WGAGFM meeting 2010	WGAGFM
4. A review be completed by April 2010 on the scope for including historical datasets that are not accessible by IT;	WGAGFM
5. A web-based crawler tool, originally developed for the FP7 project FishPopTrace (https://fishpoptrace.jrc.ec.europa.eu) by the EC Joint Research Centre, be put at the disposal of the WGAGFM to catalogue relevant electronically available genetic data and make this accessible via a web interface to end-users; furthermore, together with ICES and the European Commission it be explored whether, and under which conditions, for this purpose a special website dedicated to ICES-WGAGFM, and implementing the crawler tool, can be developed;	WGAGFM, SCICOM
6. The first projects used for Crawler development are the completed EU FishTrace project (www.fishtrace.org) and, if possible, the ongoing EU Salsea-Merge project (http://www.nasco.int/sas/salseamerge.htm)	WGAGFM
7. Possibilities should be explored to enhance and support the efforts underlying this ToR with respect to storing, managing and accessing relevant population genetic metadata, particularly where it is currently difficult to access through the web. If appropriate, and available, alternative resources and collaborations for database development and web-based tools that ensure accessibility to such data, should be considered;	WGAGFM, Fisheries geneticists
The following tentative deadlines for delivery and reporting on progress for this ToR: R3: Delivery September 2009; Report WGAGFM Meeting 2010; R4: Delivery November 2009; Report WGAGFM Meeting 2010; R5: Delivery of prototype Crawler foreseen April 2009; Implementation for WGAGFM after clarification of consent by the WGAGFM; Fishprace Consortium, ICES and the European Commission.	WGAGFM
8. The development and application of traceability tools that can be applied throughout the food supply chain ("ocean-to-fork"). Here, DNA-based methods are ideal as they support traceability ranging from whole organism, blood and tissue remains to processed product.	Fisheries geneticists
9. We further recommend integration of the DNA approach with other independent techniques, such as analyses of otolith microchemistry, fatty acid, stable isotopes, pigments, etc.	Fisheries geneticists
10. In view of recent technical advances, additional investment should be made in the development of appropriate tools to detect and monitor populations (or other identifiably significant subspecific units).	Fisheries geneticists

Recommendation	For follow up by:
11. The establishment of a statistically rigorous sampling scheme, allowing assessment of spatio-temporal variation is needed	Fisheries geneticists
12. The application of marker information, such as Single Nucleotide Polymorphism data, that is fully transferable across analyses and laboratories.	Fisheries geneticists
13. Sample and data repositories are built and a statistically rigorous framework is set up.	Fisheries geneticists
14. Technical and statistical tools and procedures are fully validated to internationally recognized forensic standards.	Fisheries geneticists
15. To engage with programmes at the global level such as the DNA barcoding enterprise coordinated by the Consortium for the Barcode of Life (Fish-Bol).	Fisheries geneticists
16. Traceability systems are developed that recognize units that may carry both a geographic signature and those that are also biologically identifiable.	Fisheries geneticists, SCICOM
17. The application of traceability tools should be extended to include methods for conservation of marine genetic resources and ecosystem-based management	Fisheries geneticists, SCICOM
18. An appropriate strategy to promote the uptake of traceability tools by international agencies through focussing on a few methods with the highest discriminatory power, greatest reproducibility, simplest validation and most flexibility with respect to the type of tissue and degree of processing.	WGAGFM, Fisheries geneticists
19. The development of the above mentioned technologies should be accompanied by a sound technology transfer strategy, engaging relevant stakeholders, such as managers, consumers, wholesalers, enforcement authorities and policy-makers.	WGAGFM, Fisheries geneticists, SCICOM
20. Promote and actively encourage research at national and international (e.g. EU) levels into the identification and optimization of molecular genetic marker suites that can resolve population structuring in other species under the ICES remit	WGAGFM, SCICOM
21. Encourage the development of integrated, transnational molecular genetic databases on marine species under the ICES remit, using the SALSEA-Merge genetic database as a prototype/model	SCICOM, ICES data centre
22. Make provision to host and curate trans-national genetic databases on marine species covered by its remit	SCICOM, ICES data centre
23. Support and promote extension of the SALSEA-merge database for European Atlantic salmon stocks to encompass stocks in the Western Atlantic	WGAGFM, SCICOM
24. Support endeavours to extend work on the use of genetic markers to advance understanding of the marine ecology of Atlantic salmon beyond the life of the existing EU SALSEA-Merge project	WGAGFM
25. Review the potential of use molecular genetic markers in other marine species under ICES remit for monitoring spatial and temporal movements of individuals populations and stocks to advance understanding of their marine ecology	WGAGFM
26. Given the evidence of broad scale population structure in Atlantic cod between regions (e.g. Baltic Sea vs. North Sea, northeast Arctic cod vs. Norwegian coastal cod), further studies investigating micro-geographical structure within regions should be encouraged.	WGAGFM, SCICOM
27. The development of a standardized framework for the integration of genetic data across research groups to allow a more robust definition of management units in cod should be promoted.	WGAGFM, Fisheries geneticists
28. Given the ongoing development of genomic resources in cod, genetic markers subject to selection should be developed to be used in association with neutral markers to resolve finer scale population structure and provide new insights in relation to management units.	Fisheries geneticists
29. Cross disciplinary approaches combining genetics with life-history characters, physiology, ecology and modelling should be	Fisheries geneticists, Fisheries biologists

Recommendation	For follow up by:
encouraged to improve our understanding of population structure in cod.	
30. Where current management units contradict identified genetic populations, management should be reviewed to assure conservation of intraspecific biodiversity	SCICOM, Fisheries managers
31. Include specific provisions within their new science plan to deal with the issues associated with impacts of population maladaptation (leading to a loss of genetic fitness, productivity) on fish recruitment processes and species distributions caused by environmental changes, such as those induced by climate shifts	SCICOM
32. Encourage the EU to put out a call under FP7 for research projects to explore the impact of population maladaptation on fish recruitment processes and species distributions caused by environmental changes, such as those induced by climate shifts, to help develop overall predictive models of effects on ecosystem and population productivity	SCICOM
33. Support the routine collection of data/ material that will allow for monitoring of genetic changes over time under the Data Collection Regulation	SCICOM
34. Increase awareness of genetic issues related to environmental change in other international forums such as EFARO, etc	SCICOM
35. Host a Special theme session on genetic processes underlying population responses to climate change	SCICOM