

Advanced Approaches to Studying the Population Diversity of Marine Fishes: New Opportunities for Fisheries Control and Management¹

D. A. Zelenina^a, Ja. Th. Martinsohn^b, R. Ogden^c, A. A. Volkov^a, I. A. Zelenina^d, and G. R. Carvalho^e

^aRussian Federal Institute for Fisheries and Oceanography, Moscow, 107140 Russia

^bEuropean Commission, Joint Research Centre, Institute for the Protection and Security of the Citizen, Maritime Affairs Unit G.04, TP051, 21027 Ispra (VA), Italy

^cTRACE Wildlife Forensics Network, Royal Zoological Society of Scotland, Edinburgh EH12 6TS, UK

^dMoscow State University, Biological Faculty, Moscow, 119899 Russia

^eBangor University, Bangor, UK, LL57 2UW

e-mail: dzel67@mail.ru

e-mail: jann.martinsohn@jrc.ec.europa.eu

e-mail: rob.ogden@tracenet.org

e-mail: g.r.carvalho@bangor.ac.uk

Received June 30, 2011

Abstract—Recent conceptual and technological advances now enable fisheries geneticists to detect and monitor the dynamics and distribution of marine fish populations more effectively than ever before. Information on the extent of genetically-based divergence among populations, so-called “population diversity”, is crucial in the quest to manage exploited living resources sustainably since it endows evolutionary potential in the face of environmental change. The generally limited dialogue between scientists, fisheries managers and policy makers, however, continues to constrain integration of population genetic data into tangible policy applications. Largely drawing on the approach and outputs from a European research project, FishPopTrace, we provide an example how the uncovering of marine fish population diversity enables players from genetics, forensics, management and the policy realm to generate a framework tackling key policy-led questions relating to illegal fishing and traceability.

DOI: 10.1134/S102279541120179

We focus on the use of single-nucleotide polymorphisms (SNPs) in European populations of cod, herring, hake and common sole, and explore how forensics together with a range of analytical approaches, and combined with improved communication of research results to stakeholders, can be used to secure sufficiently robust, tractable and targeted data for effective engagement between science and policy. The essentially binary nature of SNPs, together with generally elevated signals of population discrimination by SNPs under selection, allowed assignment of fish to populations from more areas and with higher certainty than previously possible, reaching standards suitable for use in a court of law. We argue that the use of such tools in enforcement and deterrence, together with the greater integration of population genetic principles and methods into fisheries management, provide tractable elements in the arsenal of tools to achieve sustainable exploitation and conservation of depleted marine fish stocks.

THE NATURE AND SIGNIFICANCE OF POPULATION DIVERSITY IN EXPLOITED FISHES

Although it is now well established that population diversity, that is, a measure of the number of distinct groups, or population units, recognizable by biological differentiation, is a key resource endorsed by the UN Convention on Biological Diversity, there continues within the world of fisheries biology and management a reluctance to actively manage stocks at a sub-specific level. The notion that species should be managed at a level below the species-level is long-standing and can be traced back to the turn of the last century, when F. Heincke and J. Hjort established the local self-sustaining population as opposed to the typological species as the preferred unit of study for fisheries management [1, 2]. Nevertheless, for various historical, political and socio-economic reasons [3], major international fisheries agencies collate and monitor fisheries resources according to geographic coordinates (e.g. ICES, FAO fisheries divisions), and it is at this level that policies and harvesting restrictions are

¹ The article was translated by the authors.

often imposed. Here, we focus on why the population or stock unit is important to delimit in fisheries management, and then discuss aspects of methodological developments and applications in the context of tractable tools for policy formulation and enforcement. Such considerations coincide with recent shifts in previously enduring paradigms in fisheries genetics [4].

The classical notion of marine fishes occupying “open” environments, with extensive gene flow and infinite population size, has shifted. Although such cases of wide-scale genetic homogeneity appear to exist [5, 6], there has been an escalation of instances where genetic structuring is evident, even across small spatial scales [7–9]. Although there is almost certainly a publication bias for studies detecting such differentiation, these examples show that extensive population structure in apparently homogenous species does indeed exist. There has also been corresponding evidence of adaptive variation at relatively small scales, even in species with high dispersal potential (reviewed in [4]). Our knowledge of the biology of marine organisms and their interplay with the physical and chemical environment in the oceans is less advanced compared to terrestrial species. The marine realm poses a number of very obvious challenges for research in population biology. Few marine organisms can be visually monitored, and therefore inferences on standard biological reference points such as population size, migration and individual behavior have to rely almost entirely on indirect measures. Numerous new analytical methods have been developed, significantly improving geographical resolution and statistical power, while enhancing speed of detection and reducing cost. Groundbreaking new tools have been developed such as “otolith microchemistry”, where the chemical composition of a fish otolith can be matched with composition of seawater to provide data on the geographical origin of individual fish [10]. Likewise, recent revolutions in molecular biology have facilitated development of very powerful tools for genetic analysis of population structure in marine fishes, with the majority of data on population structure originating in the last decade.

Whereas classical fisheries approaches typically focused on factors driving short-term demographic changes in populations (‘quantitative’ change), genetic approaches examine the extent to which changes in the composition of populations (‘qualitative’ change) influence both short-term alterations in phenotypic traits and longer-term response to natural and anthropogenic perturbations [11]. Although the notion that population dynamics and genetic structure are inextricably linked was emphasized long ago [12], traditional fisheries biology makes scant reference to genetic factors in fish population ecology, as evidenced by recent texts in the area [13, 14]. Yet, as is clear from the recent boom in publications in the field [15], the incorporation of such ‘qualitative’ thinking into fish and fisheries biology has generated new insights into

the temporal and spatial scale of change in fish populations and communities [16, 17], culminating in a considerable shift in how we view both the marine environment and some of its key inhabitants.

SUSTAINABILITY AND THE NEED FOR A POPULATION-LEVEL APPROACH

Three primary drivers highlight the significance of population diversity in exploited species. 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 [18]. It is therefore at the level of populations that genetic and ecological diversity should be described for conservation measures, which necessitates discrimination between populations in relation to their distribution and abundance across regional waters. To preserve the evolutionary legacy and future evolutionary-potential of a given marine fish species, it is important to secure viable populations covering the full geographical and environmental range [19]. The existence of biologically differentiated populations, so-called ‘biocomplexity’ [20], even in marine pelagic fishes [21, 22], has been credited with a major role in conferring resilience and in buffering overall productivity of fish population complexes [23]. Thus, a key aim of sustainable fisheries management is to identify the spatial and temporal scale of population structuring, and to devise tools to monitor its dynamics and contribution to overall fisheries production. Even apparently small genetic differences among populations of marine fishes at presumably neutral genetic markers could translate into important adaptive variation distributed among populations [16].

Recent global estimates based on the analysis of 1519 FAO world fisheries over the last 50 years reveals that 366 fisheries’ collapses have occurred, representing nearly one fishery in four. More locally in European waters it is estimated that over 88% of stocks are overexploited. In combination with the high estimated levels of IUU activity (e.g. cod in North Sea 2009: reported landings 34000 t vs. actual removal of 91000 t), there is a major ongoing challenge to attaining sustainable yields, especially within the context of burgeoning climate change. There is thus an escalating need to develop an integrated and holistic approach to management of natural fish resources. In addition to population delimitation, and associated biological information on dynamics and distribution, there is an associated need in relation to enforcement of regulations to enhance the ability to trace fish and fish products. There is a corresponding need in relation to governance to integrate such data and traceability tools within a forensic framework to enable traceability within the context of enforcement and conservation policy. This in turn will facilitate implementation of effort limitation and regional allocation of quotas based on the relative abun-

dance of respective stocks, not only in wild populations, but also through enforcement based on fish products. Traceability tools can also play a species and population level, especially in the face of frequent mislabelling, fraud and to sustain “ecolabelling” schemes of stocks certified in various ways. Recent studies generated from the EU-funded Framework 7 project, “FishPopTrace” (<http://fishpoptrace.jrc.ec.europa.eu/>), have generated tools within the context of the Common Fisheries Policy (CFP), aimed at promoting sustainability through conservation of genetic resources, as well as in the protection of consumer interests. Incorporation of population diversity into management instruments and policies will further underpin an ecosystem-based approach to fisheries through recovery of declining stocks and associated resilience in feeding interactions. Conservation of fish stocks has a positive effect not only on the economics and sustainability of the industry, but also on long-term sustainability of biodiversity.

SINGLE-NUCLEOTIDE POLYMORPHISMS (SNPs) AND THEIR USE IN DETECTING POPULATION DIVERSITY

Genetic markers are heritable, discrete and stable. Their use in fisheries science dates back to the 1950s, and encompasses a wide scope of applications [24]. Generally, the analysis of genetic markers can be separated into DNA-analysis, revealing DNA sequence variation, and protein analysis, that reveals differences in amino acids. For practical purposes, samples of fish (or products) have to be collected, followed by DNA or protein extraction from tissue, nowadays a routine process facilitated by commercial kits. Protein analysis, such as isoelectric focusing (IEF) and related techniques [25], or assays based on antibodies binding to proteins with high specificity [26], can provide high species resolution and are still applied for fish food authenticity control.

Here we will focus on those DNA markers, most recently applied to analysis of population diversity and traceability in marine fishes; single nucleotide polymorphisms, or SNPs. Nuclear SNPs are sites in the genome with single base changes in a DNA sequence. SNPs are very abundant and widespread in most genomes, often every 200–500 bp. Individual SNP loci are less informative than microsatellites, but the ease of ascertainment due to their high abundance can generate equal or higher statistical power with higher quality data and better genomic coverage, making SNPs the best candidate for genetic markers in ecology, evolution and conservation. The application of SNPs to population genetics is not without some problems, including so-called “ascertainment bias”—the selection of loci based on an unrepresentative sample of individuals. For example if SNPs have been developed from a few individuals (small ascertainment depth), SNPs with high heterozygosities are preferentially found, providing a false impression of

overall genomic polymorphism. Likewise, if SNPs are developed from a biased sample of individuals (e.g. not covering the full range of populations), comparative analysis with respect to population-specific indices of variability can be biased. However, in the context of mixed stock analysis (MSA) for example, ascertainment bias is not expected to create problems. Population-biased ascertainment could result in marginally lower power for MSA in populations not included in the ascertainment sample; however, the high number of markers employed would most likely compensate for this. Microsatellite-based MSA, though the dominant approach, is beginning to be replaced by SNPs and individual assignment in routine studies of population-based management in Pacific salmon, primarily to assure reproducibility and transferability of data. Recent findings [27, 28] indicate that those SNP markers under selection typically exhibit high levels of divergence, making them especially valuable for disclosing subtle structuring and traceability studies. However, it is important to recognise that markers influenced by selection are less useful for estimating the extent of demographic forces such as levels of gene flow, most reliably inferred from neutral microsatellite or SNP markers.

Compared to other genetic markers, where routine genotyping and transfer of protocols between laboratories proves difficult, the information retrieved from SNPs is categorical (presence or absence of alleles, rather than their molecular size (bp)), and data can be standardized across laboratories for forensic applications [29]. Genotyping of the same specimens by microsatellites and SNPs markers performed in two different laboratories revealed obvious advantage of SNPs although the methods of analysis differed [30]. The rapid progress of DNA analysis technologies will have significant effects on the development of population analysis and traceability tools. High-throughput sequencing has declined dramatically in cost, while speed and quality of analysis has increased by orders of magnitude, allowing high throughput analysis of individuals. This is well illustrated by human genome sequencing, which can currently be accomplished in a few weeks at costs below US\$5000, compared to more than a year and more than US\$100 million in 2007 [31]. New sequencing technologies provide major new opportunities for genetic fish population analysis and consequently also for traceability in the fisheries sector as shown by FishPopTrace (<https://fishpoptrace.jrc.ec.europa.eu>) where in a few weeks over 100 million bases of sequence data have been generated for European hake (*Merluccius merluccius*), Atlantic herring (*Clupea harengus*) and common sole (*Solea solea*).

FISHPOPTRACE—IN A FEW WORDS ABOUT THE PROJECT

During the FishPopTrace project four marine fish species were investigated: Atlantic cod (*Gadus morhua* L.),

Atlantic herring (*Clupea harengus* L.), European hake (*Merluccius merluccius* L.) and common sole (*Solea solea* L.). The choice of target species was based on three main criteria related to conservation status, traceability issues and their life-styles. All the selected species are economically important, relatively widespread on a European scale, known to exhibit population structure and belong to EC priority species for enforcement and/or conservation. All of them are vulnerable to a varying degree of overfishing. These species represent four different life-styles ranging from a small pelagic herring and coastal flatfish sole to benthopelagic cod and demersal deep-sea hake. Two of them, cod and herring, have a more northern distribution than sole and hake. Such variety in life-styles and habitats allows us to project the results of our study to a wide range of marine fish.

Several tools are available to understand the extent to which fish populations interbreed and to trace back the geographic origin of landed fish. They are divided into several groups namely artificial external tags; natural tags, including morphometric characteristics, chemical content and shape of the otoliths and the parasites composition; and various genetic markers. However as one of the goals of the project was to design the methodology suitable not only for population studies but for traceability purposes as well the choice of the main tool was based on its capacity to work good irrespective of the material analyzed whether a fresh/ethanol fixed fish tissue or a processed fillet (by the way the logo of FishPopTrace project (Fig. 1) reflects its main idea: “from ocean to fork”). Thus only genetic DNA-markers answered the desired conditions, and among the wide spectrum of them SNPs were chosen because of their reproducibility and capacity to be detected not only in genomic DNA of good quality but even in processed food were DNA may be digested to relatively short fragments.

On the other hand some phenotypic characteristics may be very useful as indicators of the fish origin though they are not available throughout the food supply chain. Since the beginning of the last century fisheries scientists have made regular collection of commercial fish to monitor abundance, growth and conditions. The otoliths collected for aging have frequently been archived and now they may be used for creation a historical baseline. Based on comparison of their shape and chemical composition one may trace population dynamics.

Besides generally acknowledged population tools the aim of the project was to find new traceability tools. The idea is that environmental differences, such as water temperature or the fish nutrients might influence specific features locally making it possible to trace the fish origin. Three different approaches—fatty acids profiles, gene expression and protein expression—were tested for their fitness for traceability needs. Statistical analysis of the fatty acids composition in the fish tissues revealed a clear differentiation of geographical popula-



Fig. 1. The FishPopTrace logo: “from ocean to fork”.

tions both within the Atlantic or the Mediterranean as well as between these two basins. To examine the gene expression, a DNA microarray for analysis of 14898 genes in the muscle of hake collected from six different areas from the North Atlantic to the Mediterranean was developed. We demonstrated that for certain areas (North Sea, Aegean Sea) it might be possible to trace back fish to their geographical origin measuring the expression of few marker genes. Protein expression analysis also allowed the identification of different hake populations.

FISHPOPTRACE—A CASE STUDY IN DETECTION OF POPULATION DIVERSITY

It is now possible (unpublished data; see FishPopTrace brochure available at: <http://fishpop-trace.jrc.ec.europa.eu/>) to assign using SNPs fish to populations from more areas and with higher certainty than previously possible, reaching standards which can be used in a court of law. Based on use of the most highly distinct genes among populations, “minimum assays with maximum power” with from 10–30 SNPs have been generated. These assays have been developed to target some of the most pertinent needs for traceability tools in European fisheries management. For example, fast, efficient and forensically robust tools to discriminate between cod from Canada, North Sea, Baltic Sea and Northeast Arctic populations have been developed, between North Sea and North Atlantic herring, between sole from the Irish Sea and Thames and between hake from the Mediterranean and Atlantic areas. The core approach was the development of a “SNP chip” for each of three species: sole, hake, and herring. These DNA-covered microchip-like devices enabled testing the identity of 1536 possible SNPs for each group of individuals from a specific population. Once all individual fish were characterised, the frequency of each SNP variant was determined, creating a potentially diagnostic pattern, or “population signature.” The FishPopTrace consortium investigated questions relevant to fisheries and to European consumers.

A common concern for the latter is the source of Atlantic cod, *Gadus morhua*. Fish from the Baltic are worth less because they tend to have lower quality flesh and higher levels of contaminants. The cod team used

its SNP chip to examine, without knowing the source, samples from both locations. By looking at 20 SNPs, the researchers correctly identified the origin of each individual fish. With just 10 SNPs, 96% of the unknown samples were still correctly identified. The SNP chip for sole (*Solea solea*) also performed well. This flatfish attracts the highest price of the four species and is severely over-fished in Europe. Only two of the twelve fishery areas within European waters are considered to be fished within safe biological limits. A key question is whether sole from the North Sea can be distinguished from populations in the Mediterranean, which are considered to be of higher quality. Just one SNP could reveal which sole was which with 96% accuracy.

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

Long-considered a sentinel of studies on marine fish population structure, the most challenging test case was perhaps Atlantic herring, (*Clupea harengus*). A geographically widespread and abundant species, with complex seasonal migratory behaviour, herring within European waters typically display only minor and sometimes transient genetic differences among populations. By applying the SNP chip to herring, however, it was possible to accurately distinguish many populations, including those in the northeast Atlantic and North Sea, a goal important to a joint EU—Norwegian fishery management plan. The flexibility of combining differing numbers of SNPs allowed the identification of some herring populations at smaller scales, even around the United Kingdom, where there is substantial misreporting of catches. Thus it has been possible by varying the numbers used on a SNP-chip, to assign individuals back to their source population across different geographic scales with high levels of certainty and reproducibility. Such outputs are especially significant since previous types of genetic markers either detect levels of population differences that are too low, or there are inherent difficulties in comparing data generated from different laboratories. The use of a marker system such as SNPs, which is essentially based on the presence or absence of large numbers of single genetic variants means that data can be compiled from sources in a much more reliable and high throughput way. The approach thereby enables the generation of baseline and ongoing additions for subsequent genetic monitoring of the distribution and

dynamics of fish populations or stocks. Moreover it is imperative that any such tools can be used in a legal context, necessitating forensic validation. This has been achieved for SNP markers within FishPopTrace across a range of policy-driven IUU scenarios.

TRACEABILITY AND FORENSIC APPLICATIONS

“Law without enforcement is just good advice” (A. Lincoln). This quote is as relevant to regulations governing the sustainable management and conservation of marine resources as any other type of legislation. The global level of Illegal, Unreported and Unregulated (IUU) fishing and supply chain fraud offer financial gains on a scale that attracts organized criminal groups as well as unscrupulous individuals; the tools required to tackle such activities and enforce fishing regulations need to be equally sophisticated. Over the past two decades, a great deal of progress has been made in regulating commercial fisheries through monitoring, control and surveillance (MCS) measures [32]. A range of technologies are utilized to identify infringements relating to individual vessels, but problems still exist in areas of catch identification and subsequent fraud throughout the food supply chain. Modern biological classification increasingly relies on genetic data to help define species boundaries and, as we have seen, is also of great importance in elucidating the structure of populations within species. It is therefore obvious to consider the use of DNA analysis to identify the origin of fish in order to support investigations into IUU fishing and mislabeling.

The application of scientific analysis to provide legal evidence is known as *forensic* investigation. DNA forensics has rapidly become the single most important forensic technique available for identifying human evidence and in recent years attention has turned to non-human, or wildlife DNA forensics [33]. The development of forensic techniques for genetic species identification of fish and fish products began twenty years ago [34, 35] and a number of prominent examples of its successful application to legal prosecution exist (see [36] for review). However, existing fisheries legislation often refers to fish stocks or geographic regions, rather than species, as the relevant units for law enforcement and this imposes the need for methods identifying the population of origin of landed fish. The production of genetic assays for the forensic identification of fish populations and hence the geographic origin of fish and fish products was a key objective of FishPopTrace.

The research focus of FishPopTrace was to develop a series of markers and databases for selected fisheries that could be used to investigate and define population structure. In order to convert this research into a set of applied tools, the developmental and analytical processes involved in characterizing populations required validation within a forensic framework. In practical

Summary of validation studies performed to demonstrate the performance characteristics of the SNP genotyping assays for population assignment

Validation study	Purpose	Approach	Output
Sample type	To assess the range of samples from which DNA could be reliably recovered for subsequent analysis	Assess the recovery and PCR amplification of DNA using multiple methods on processed and preserved fish;	Matrix of suitable sample types for subsequent testing
SNP marker	To confirm the existence and inheritance pattern of the SNP markers	Genotyping and Sanger sequencing of candidates SNPs; familial genotyping	Confirmation of SNP markers in target species genome
Genotyping assay	To demonstrate accuracy and reproducibility	Sensitivity, specificity and reproducibility studies	Data on the optimum performance conditions of the assay
Reference data	To demonstrate the reliability and accuracy of data	Individual genotyping of selected SNPs and populations to confirm SNP chip results	Accurate population reference data for assignment of unknown samples
Assignment statistics	To evaluate the assignment method and statistical power of the assays	Examination of assignment performance across populations and SNP panels	Accurate, precise identification of population genetic origin

terms this meant going beyond the usual level of scientific rigour by designing and implementing a series of formal validation studies, each of which tested separate aspects of the genetic techniques developed in earlier stages of the project. This work included specific validation of sample type, SNP marker, reference data, genotyping assay and assignment statistics (table). The resulting data forms a body of evidence that supports the application of DNA SNP genotyping

for the categorical assignment of fish and fish products to their genetic population of origin. The validation studies were designed to cover a range of testing scenarios in order to demonstrate the performance of the assays across different possible enforcement applications and to address likely legal challenges. All of this information ultimately formed the basis of Standard Operating Procedures (SOPs), documents that set out exactly how to perform the new assignment methods

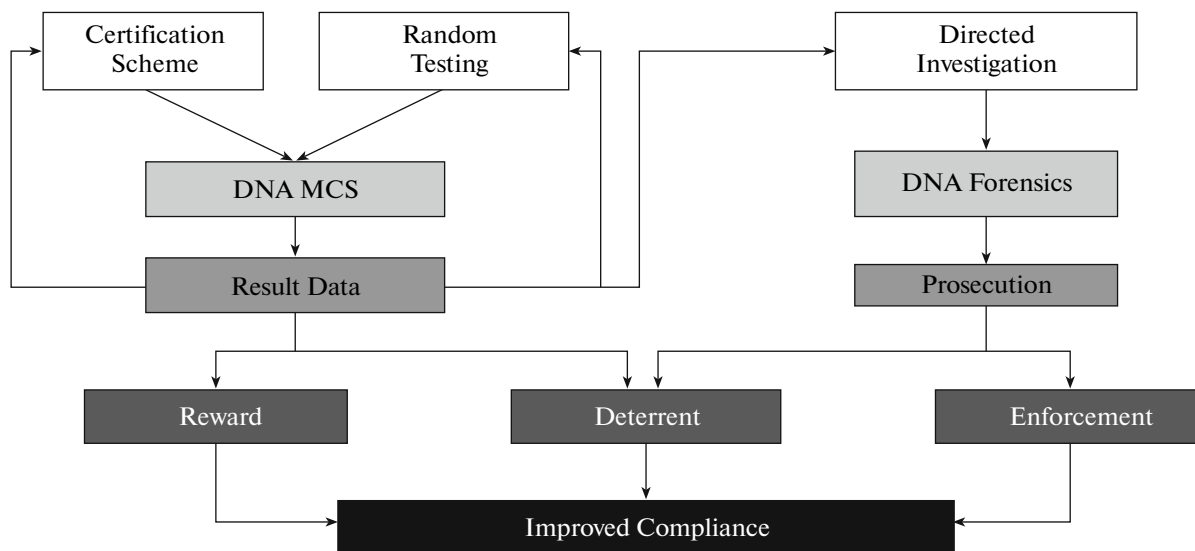


Fig. 2. Flow diagram depicting the impact of genetic identification methods on monitoring (DNA MCS) and enforcement (DNA forensics) to improve compliance with fishing regulations. Both types of application incorporate a deterrent effect to reduce IUU fishing; an essential element of any effective enforcement system. Reproduced from [37].

in each of the target species. The SOPs represent the delivery of applied tools developed by FishPopTrace, beyond the generation of novel research.

The scale of IUU fishing and subsequent illegal trade is unlikely to ever be controlled by enforcement alone. The overall aim of fisheries authorities is to maximize compliance with regulations and there are several strategies for achieving this. The basic system of DNA-based population assignment may be applied in the context of monitoring, certification schemes, or enforcement action and a combined approach is likely to be preferable (Fig. 2). It is important to note that traditional research techniques are only sufficiently robust for use in surveillance and monitoring; they should not be used to generate forensic evidence. The inclusion of a formal forensic validation process within FishPopTrace expands the ways in which genetic data can support fisheries management, enabling a credible threat of criminal prosecution. Although ultimately the use of DNA forensics in investigations is likely to be restricted by the resources available to fisheries enforcement agencies, the deterrent effect resulting from such applications is expected to play an important role in promoting responsible fishing and trade (Fig. 2).

FishPopTrace has demonstrated the technical feasibility of producing forensic traceability systems in four target species. From an applied perspective, this is only the beginning; there is now a massive opportunity to extend the range of origin assignment questions to other geographic regions or indeed to more species. The next steps are therefore to increase the number of assays available to address individual species \times region assignment issues and to develop capacity within fisheries inspectorates, industry and testing laboratories to be able to collect samples, access services and deliver results, respectively. To this end, one of the final activities of FishPopTrace was to identify and engage with stakeholders from across the international fisheries sector.

ENDOWING THE FISHPOPTRACE LEGACY: DISSEMINATION AND TECHNOLOGY TRANSFER

Bridging the gap between science and stakeholders in the fisheries sector. FishPopTrace started out as an academic research project to test the hypothesis that there is detectable genetic population stratification of the four marine fish species, cod, hake, herring and sole which could be applied, for example for traceability purposes. Keeping in mind that for a long time marine fish population stratification had been considered to be only faint at best and that the FishPopTrace consortium decided to use very advanced and, in a marine context, largely untested approaches to this end (otolith microchemistry and single nucleotide polymorphisms (SNPs)) this has been a highly ambitious goal. The project could have culminated in publishing

valuable results in scientific articles, and could have ended there.

We considered this, however, to be unsatisfactory and set the goal to bridge the often prevailing gap between academic science and stakeholders like the fisheries management sector, control authorities and policy making institutions. To this end a technology transfer strategy has been developed and implemented throughout the project activity. The technology transfer approach was based on several components. These were primarily aimed at public awareness building, facilitation of data and results accessibility beyond the project funding period, and the provision of applications adapted to the needs of end-users.

The challenges: the academic island; data dispersal; non-accessibility of research results to end-users. Several challenges arise when a research project originating from the academic environment sets out to engage with a wider public. The academic realm, particularly when linked to fundamental research, is often perceived as being in an insular state: Generated data and results might be of value for the development of 'real-world' applications but seldom, or only over long time periods, reach beyond the publishing in scientific articles. There are a number of reasons for this, a very prevalent one being the dispersal and loss of data after research projects come to an end. Due to the very nature of research funding, researchers have to move on after the conclusion of projects, plan for new projects and look for new funding opportunities. This does not leave resources to create and maintain a proper data storage infrastructure, i.e. professionally curated databases. For the fish(eries) genetics field this has been identified as a considerable problem and impediment to scientific progress by the ICES Working Group on the Application of Genetics for Fisheries and Mariculture (WGAGFM), which suggested to establish a meta-database cataloguing molecular data in the field of fish and shellfish population genetics [38]. However such an endeavour requires financial support, the provision of long-term resources, and careful planning.

The challenges: solutions. FishPopTrace decided therefore to develop in the meantime its own database, compiling sampling and experimental data, largely accessible to the public through the FishPopTrace website (<http://fishpoptrace.jrc.ec.europa.eu>), and maintained beyond the project's research phase. This should also ensure that data can be integrated into other existing or planned marine data hubs such as the European Marine Observation and Data Network (EMODNET) [39], or that they can be accommodated in fisheries management data collection schemes such as the European Union data collection framework (DCF) [40]. Moreover, to increase coherence and awareness about research in the marine fish and fisheries field, information on other related past and ongoing research projects was assembled on the FishPopTrace website and a web-based meta-crawler

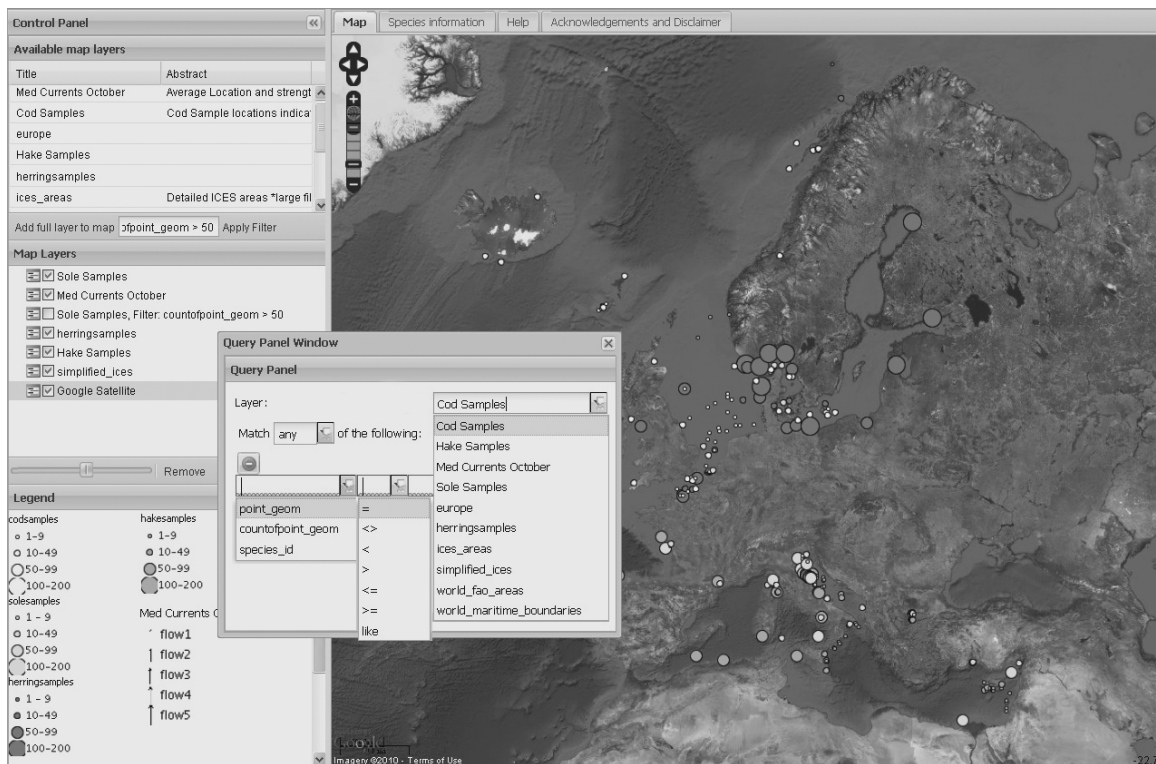


Fig. 3. Geo-visualisation platform (detail). The public web-based, GIS driven geo-brower is currently under development (<http://fishpoptrace.jrc.ec.europa.eu/data-access>). It enables the integration of population genetic data with oceanographic and environmental parameters (e.g. currents, salinity, sea surface temperature, primary production) in a geographic context. Here the sample locations of all the four FishPopTrace target species (cod, hake, sole and herring) are plotted. The opened query window allows to filter the locations according to sample size (courtesy of Eoin MacAoidh, JRC Ispra).

tool developed which provides access to data and results of such projects. The meta-crawler tool relies on web accessibility to databases of other projects, and while trying to identify suitable projects a clear lack of properly sustained project databases became apparent further emphasizing the danger of data loss and dispersal and the need for tackling this issue. However when reaching out to the non-scientific community the mere provision of data and results are insufficient. They need to be put in a context relevant for stakeholders and disseminated using a language that is commonly comprehensible. This is why FishPopTrace decided to develop a geo-visualisation platform. This public web-based, Geographic Information System (GIS) driven tool enables the integration of population genetic data with oceanographic and environmental parameters (e.g. currents, salinity, sea surface temperature, primary production) in a geographic context (Fig. 3). This can be exploited on one hand by scientists in seascape genetic approaches [41], on the other hand it can be used to address questions relevant to fisheries management such as the extent to which natural populations of marine fish (biological units) match stocks (the management units), and ultimately it can be used as a supportive fisheries management

decision finding tool. The FishPopTrace geo-visualisation tool is currently under development (<http://fishpoptrace.jrc.ec.europa.eu/map/geobrowser.html>).

As outlined above, illegal fishing activities and fraud along the supply chain are a major concern in the fisheries sector and pose a challenge to control and enforcement measures. Traceability, albeit indispensable to fisheries control, is currently mostly based on labeling rules and certification schemes, and powerful tools to independently test the truthfulness of the information provided are lacking. To this end FishPopTrace put particular focus on origin assignment, to strengthen the ability of control and enforcement authorities to independently test the geographical origin of fish and fish products. Also here considerable effort needs to be put in properly communicating research and resulting applications to stakeholders. A crucial component and asset with respect to availability to control and enforcement authorities and courts of law is the forensic framework developed by FishPopTrace (see above). In particular, carefully designed inter-laboratory validations and the development of Standard Operating Procedures guarantee that the protocols designed for origin assignment can be

adopted by control and enforcement authorities and will be accepted before courts of law.

On costs and benefits. Interestingly especially DNA-based technologies have already found widespread use in fisheries control and enforcement throughout the world as shown by numerous examples [42]. While the general trend is that the costs for DNA analysis decrease swiftly, which is paralleled by a constant increase in analytical power [43] its value for control and enforcement purposes might not yet be taken for granted by stakeholders in the fisheries sector.

For this reason FishPopTrace decided to assess the value of DNA-based analytical applications for fisheries control and enforcement objectively by performing a Cost Benefit Analysis (CBA). A CBA weighs costs and benefits of procedures or actions in monetary terms and can thereby rationalise and facilitate the decision finding process on their implementation. To obtain data on the use of DNA-base analysis, 73 institutions (control/enforcement and customs laboratories, national fishery ministries) in 26 countries have been contacted. At the writing of this article the CBA is being evaluated and the results will soon be published.

Towards the integration into a policy framework (FishPopTrace as an example for a successful dialogue between science and the policy realm). One of the prime achievements of FishPopTrace, with respect to 'communicating science to stakeholders' was its engagement with the European Union Common Fisheries Policy (CFP). As the activities of each fishing fleet affect the opportunities of other fleets, the 27 EU member countries have decided to manage their fisheries in collaboration, through the CFP. This policy brings together a range of measures aimed at achieving a healthy and sustainable European fishing industry [44]. Scientific advice is anchored in the CFP. For example the International Council for the Exploration of the Sea (ICES) and STECF, which is the European Commission's Scientific, Technical and Economic Committee for Fisheries, are consulted at regular intervals on matters pertaining to the conservation and management of living aquatic resources, including biological, economic, environmental, social and technical considerations. However, fisheries genetics, even though being fundamental to the understanding and conservation of the living resource fish, are not yet taken into account at all on EU-level.

FishPopTrace took advantage of two occasions to draw the attention to the potential of genetics and genomics for fisheries management on the EU policy level. In 2008 it responded with an opinion to the public consultation launched by the European Commission on a reform of the CFP control scheme (http://ec.europa.eu/fisheries/partners/consultations/control/contributions/index_en.htm).

We regard the dialogue that materialized between the policy and scientific realm as a very positive example for a successful communication between different stakeholders. For instance the impact assessment by

the European Commission that later accompanied the Commission proposal for a new regulation establishing a community control system for ensuring compliance with the rules of the Common Fisheries Policy, FishPopTrace has been explicitly mentioned [45, 46]. Moreover in the final new control regulation, article 13 'New technologies' refers explicitly to "traceability tools such as genetic analysis" having a potential to improve compliance with the rules, even though that implies by no means any obligation for the EU member states to use genetic analysis [47]. In 2009, the European Commission in its Green paper on a reform of the CFP [48] outlined the challenges facing Europe's fisheries and launched a public debate on the way EU fisheries are managed (http://ec.europa.eu/fisheries/reform/consultation/index_en.htm). Also to this consultation the FishPopTrace consortium answered with the document "*Integrating Genetic, genomic, and Chemistry Tools into an improved Management Scheme under the Common Fisheries Policy Remit.*" Both consultation contributions can also be downloaded from the FishPopTrace website.

Into the future. Preserving and enhancing result and application availability beyond the term of a projects natural life. As mentioned, a major impediment for the transfer of research results into applications, here for fisheries management, arises from the relative short life of scientific project consortia. Both the communication of scientific applications to and uptake by stakeholders require time and ideally a unrelenting dissemination effort. We believe that by building a properly curated database which is connected to a public web-interface and linked to a geo-visualisation platform, FishPopTrace has created an opportunity for the continued engagement with stakeholders beyond the project funding period. This approach will help to disseminate the opportunities both for fisheries management in general and control and enforcement in particular that have emerged through FishPopTrace activities. For example the origin assignment tools and the established forensic framework can provide a precious asset for control and enforcement authorities, but their uptake will depend on the proper future engagement with the end-users. That requires on one hand effortless access to the tools and on the other hand continued availability of FishPopTrace consortium members for information and support. We made sure that both requirements are met, and hope that these efforts will help to finally integrate genetics properly into fisheries management, including control and enforcement.

CONCLUSION

Although our consideration of molecular markers and population diversity in the context of fisheries management has been selective, we have aimed to emphasize the breadth and practical utility of their application, with some specific illustration. As pointed

out elsewhere [4], the application of population genetic markers in fisheries management falls broadly into two types: their use as “tags” (e.g. traceability markers) to identify individuals and populations, such as in population assignment and traceability of individuals, and the rather more prosaic, but fundamental role that population diversity (genetic resources) plays in the resilience and recovery of exploited populations. Coincident with the availability of markers such as single nucleotide polymorphisms that enhance the targeting of genes under selection, as well as exploring linkages between ecological trait and genetic variation, has been the burgeoning of genomic technologies. The generation of large DNA sequence data bases and expressed sequence tags (ESTs) provides the basis for studies on gene expression, as well as facilitating a search for candidate genes [17, 49], both of which afford novel ways for examining adaptive variation in the wild. In addition to classical approaches for detecting local adaptation [50], innovative methods based on parentage analysis that enable empirical estimates of fitness variation in wild fish [51, 52], common garden experiments [53, 54], and the application of landscape genetics to examine covariance in spatial patterns of environmental and genetic variation [8, 55, 56] have further advanced our insights into the dynamics of adaptation in wild fish.

It is only through the judicious identification and monitoring of population diversity, especially those features determined genetically, that it becomes possible to develop strategies to maximize and conserve genetic resources for adaption to environmental change. Indeed, there has been an identifiable shift in the nature of marine fisheries genetics in the past decade—from predominantly descriptive studies that provide the spatial and temporal framework of patterns of genetic variability in the wild, to increasingly mechanistic approaches that explore empirically some of the biological (e.g. life history, population demography and connectivity) and environmental factors (e.g. oceanic hydrography, environmental stress, harvesting) shaping such patterns. Importantly, such efforts not only inform our understanding of the distribution and dynamics of species, but provide also a range of tools and conceptual platform for the design and implementation of management and conservation strategies. The key to future developments will continue to depend upon the integration of principles and practice, especially between scientists and practitioners in fisheries biology and management. A key message to embrace is acknowledgement and promotion of the ethos that fundamental features of a species biology (recruitment, migration and mortality) depend upon the nature, pace and extent of interactions between the genotypic composition of populations and their environment.

ACKNOWLEDGMENTS

FishPopTrace was funded by the European Community’s Seventh Framework Programme under grant agreement no. KBBE-212399 in the area of “Food, Agriculture and Fisheries, and Biotechnology”.

REFERENCES

1. Sinclair, M., *Marine Populations: An Essay on Population Regulation and Speciation*, Seattle: Univ. Washington Press, 1988.
2. Carvalho, G.R. and Hauser, L., Molecular Genetics and the Stock Concept in Fisheries, *Special Issue of Reviews in Fish and Fisheries Biology*, Carvalho, G.R. and Pitcher, T.J., Eds., 1994, vol. 4, pp. 351–373.
3. Hammer, C. and Zimmermann, C., The Role of Stock Identification in Formulating Fishery Management Advice, in *Stock Identification Methods: Applications in Fishery Science*, Cadrin, S.X., Friedland, K.D., and Waldman, J.R., Eds., Elsevier, 2005, pp. 631–658.
4. Hauser, L. and Carvalho, G.R., Paradigm Shifts in Marine Fisheries Genetics: Ugly Hypotheses Slain by Beautiful Facts, *Fish Fisheries*, 2008, vol. 9, no. 4, pp. 333–362.
5. Mariani, S., Hutchinson, W.F., Hatfield, E.M.C., et al., North Sea Herring Population Structure Revealed by Microsatellite Analysis, *Mar. Ecol. Progress Ser.*, 2005, vol. 303, p. 245.
6. Cassista, M.C. and Hart, M.W., Spatial and Temporal Genetic Homogeneity in the Arctic Surfclam (*Macrotromeris polynyma*), *Mar. Biol.*, 2007, vol. 152, pp. 569–579.
7. Ruzzante, D.E., Wroblewski, J.S., Taggart, C.T., et al., Bay-Scale Population Structure in Coastal Atlantic Cod in Labrador and Newfoundland, Canada, *J. Fish Biol.*, 2000, vol. 56, pp. 431–447.
8. Jorgensen, H.B.H., Hansen, M.M., Bekkevold, D., et al., Marine Landscapes and Population Genetic Structure of Herring (*Clupea harengus* L.) in the Baltic Sea, *Mol. Ecol.*, 2005, vol. 14, pp. 3219–3234.
9. Knutsen, H., Jorde, P.E., Andre, C., and Stenseth, N.C., Fine-Scaled Geographical Population Structuring in a Highly Mobile Marine Species: The Atlantic Cod, *Mol. Ecol.*, 2003, vol. 12, pp. 385–394.
10. Campana, S.E., Otolith Elemental Composition as a Natural Marker of Fish Stocks, in *Stock Identification Methods: Applications in Fishery Science*, Cadrin, S.X., Friedland, K.D., and Waldman, J.R., Eds., Elsevier, 2005, pp. 227–246.
11. Frankham, R., Stress and Adaptation in Conservation Genetics, *J. Evol. Biol.*, 2005, vol. 18, pp. 750–755.
12. Elton, C.S., Periodic Fluctuations in the Numbers of Animals: Their Causes and Effects, *J. Exp. Biol.*, 1924, vol. 2, pp. 119–163.
13. Jennings, S., Kaiser, M.J., and Reynolds, J.D., *Marine Fisheries Ecology*, Malden: Blackwell, 2001.
14. Hallerman, E.M., *Population Genetics: Principles and Applications for Fisheries Scientists*, Bethesda: American Fisheries Society, 2003.

15. Leis, J.M., van Herwerden, L., and Patterson, H.M., Estimating Connectivity in Marine Fish: What Works Best?, *Oceanogr. Mar. Biol.*, 2010, vol. 49, pp. 193–234.
16. Conover, D.O., Clarke, L.M., Munch, S.B., and Wagner, G.N., Spatial and Temporal Scales of Adaptive Divergence in Marine Fishes and the Implications for Conservation, *J. Fish Biol.*, 2006, vol. 69, pp. 21–47.
17. Larsen, P.F., Nielsen, E.E., Williams, T.D., et al., Adaptive Differences in Gene Expression in European Flounder (*Platichthys flesus*), *Mol. Ecol.*, 2007, vol. 16, pp. 4674–4683.
18. Bonin, A., Nicole, F., Pompanon, F., et al., Population Adaptive Index: A New Method to Help Measure Intraspecific Genetic Diversity and Prioritize Populations for Conservation, *Conservation Biol.*, 2007, vol. 21, pp. 697–708.
19. Nelson, K. and Soule, M., Genetical Conservation of Exploited Fishes, *Population Genetics and Fishery Management*, Ryman, N., and Utter, F.M., Eds., Seattle: Washington Univ. Press, 1987, pp. 345–368.
20. Michener, W.K., Baerwald, T.J., Firth, P., et al., Defining and Unraveling Biocomplexity, *BioScience*, 2001, vol. 51, pp. 1018–1023.
21. Mitchell, D.M., Biocomplexity and Metapopulation Dynamics of Pacific Herring (*Clupea pallasii*) in Puget Sound, *Master's Thesis*, Washington, DC: University of Washington, 2006.
22. Ruzzante, D.E., Mariani, S., Bekkevold, D., et al., Biocomplexity in a Highly Migratory Pelagic Marine Fish, Atlantic Herring, *Proc. R. Soc. London, Ser. B: Biol. Sci.*, 2006, vol. 273, pp. 1459–1464.
23. Hilborn, R., Quinn, T.P., Schindler, D.E., and Rogers, D.E., Biocomplexity and Fisheries Sustainability, *Proc. Natl. Acad. Sci. USA*, 2003, vol. 100, pp. 6564–6568.
24. Kochzius, M., Trends in Fishery Genetics, *The Future of Fisheries Science in North America*, Beamish, R.J. and Rothschild, B.J., Eds., Springer-Verlag, London, 2008, 1st Ed., pp. 451–491.
25. Rehbein, H., Identification of Fish Species by Protein- and DNA-Analysis, *Authenticity of Species in Meat and Seafood Products*, Perez-Martin, R. and Soletto, C.G., Eds., Association “International Congress on Authenticity of Species in Meat and Seafood Products”, 2003.
26. Taylor, W.J., Patel, N.P., and Jones, J.L., Antibody-Based Methods for Assessing Seafood Authenticity, *Food Agric. Immunol.*, 1994, vol. 6, no. 3, pp. 305–314.
27. Nielsen, E., Hemmer-Hansen, J., Poulsen, N., et al., Genomic Signatures of Local Directional Selection in a High Gene Flow Marine Organism, the Atlantic Cod (*Gadus morhua*), *BMC Evol. Biol.*, 2009, vol. 9, p. 276.
28. Stokstad, E., To Fight Illegal Fishing, DNA Gets Local, *Science*, 2010, vol. 330, pp. 1468–1469.
29. Sobrino, O.B., Briouan, M., and Carracedo, A., SNPs in Forensic Genetics: A Review on SNP Typing Methodologies, *Forensic Sci. Int.*, 2005, vol. 154, pp. 181–194.
30. Zelenina, D.A., Khrustaleva, A.M., Volkov, A.A., et al., A Case Study of Two Genetic Markers for Inter-Laboratory Collaboration: SNPs Provide Transportability without Standardization, *NPAFC Doc.*, 2005, no. 913, p. 14.
31. Metzker, M.L., Sequencing Technologies—the Next Generation, *Nat. Rev. Genet.*, 2010, vol. 11, pp. 31–46.
32. FAO Fisheries and Aquaculture Department: *Combating Illegal, Unreported and Unregulated Fishing through Monitoring Control and Surveillance, Port State Measures and Other Means. Meeting Document COFI/2007/7*, Rome: Food and Agriculture Organization of the United Nations, 2007.
33. Ogden, R., Dawnay, N., and McEwing, R., Wildlife DNA Forensics—Bridging the Gap between Conservation Genetics and Law Enforcement, *Endangered Species Res.*, 2009, vol. 9, pp. 179–195.
34. Bartlett, S.E. and Davidson, W.S., Identification of *Thunnus* Tuna Species by the Polymerase Chain Reaction and Direct Sequence Analysis of Their Mitochondrial Cytochrome b Genes, *J. Fisheries Aquatic Sci.*, 1991, vol. 48, pp. 309–317.
35. Bartlett, S.E. and Davidson, W.S., FINS (Forensically Informative Nucleotide Sequencing): A Procedure for Identifying the Animal Origin of Biological Specimens, *BioTechniques*, 1992, vol. 12, pp. 408–411.
36. Ogden, R., Fisheries Forensics: The Use of DNA Tools for Improving Compliance, Traceability and Enforcement in the Fishing Industry, *Fish Fisheries*, 2008, vol. 9, pp. 462–472.
37. Martinsohn, J.T. and Ogden, R., FishPopTrace—Developing SNP-Based Population Genetic Assignment Methods to Investigate Illegal Fishing, *Forensic Sci. Int.: Genet. Suppl. Ser.*, 2009, vol. 2, no. 1, pp. 294–296.
38. Verspoor, E., Zanzi, A., Gilbey, J., and Martinsohn, J.T., Pursuing the Establishment of a Meta-Database Cataloguing Molecular Data in the Field of Fish and Shellfish Population Genetics, *ICES WGAGFM Report*, 2010. ICES CM 2010. SSGHIE:12, pp. 4–17.
39. *European Commission: Building a European Marine Knowledge Infrastructure: Roadmap for a European Marine Observation and Data Network* (Commission Staff Working Document), Brussels: 07.04.2009. SEC(2009) 499 Final. pp. 1–69.
40. European Council Regulation (EC) No 199/2008 of 25 February 2008 Concerning the Establishment of a Community Framework for the Collection, Management and Use of Data in the Fisheries Sector and Support for Scientific Advice Regarding the Common Fisheries Policy, *Official J. Eur. Union*, L 60, pp. 1–12.
41. Galindo, H.M., Olson, D.B., and Palumbi, S.R., Seascape Genetics: A Coupled Oceanographic-Genetic Model Predicts Population Structure of Caribbean Corals, *Curr. Biol.*, 2006, vol. 16, pp. 1622–1626.
42. Martinsohn, J.T., Deterring Illegal Activities in the Fisheries Sector: Genetics, Genomics, Chemistry and Forensics to Fight IUU Fishing and in Support of Fish Product Traceability, *JRC Reference Reports: Scientific and Technical Research Series*, Luxembourg: European Commission, 2011.
43. Anonymous, Human Genome at Ten: The Sequence Explosion, *Nature*, 2010, vol. 464, pp. 670–671.
44. Hemmer-Hansen, J., Nielsen, E., Meldrup, D., and Mittelholzer, C., Identification of Single Nucleotide Polymorphisms in Candidate Genes for Growth and Reproduction in a Nonmodel Organism, the Atlantic

- Cod, *Gadus morhua*, *Mol. Ecol. Resour.*, 2011, vol. 11, suppl. 1, pp. 71–80.
45. Carvalho, G.R., Evolutionary Aspects of Fish Distributions: Genetic Variability and Adaptation, *J. Fish. Biol.*, 1993, vol. 43, suppl. A, pp. 53–73.
46. Rowe, S., Hutchings, J.A., Skjaeraasen, J.E., and Bezanson, L., Morphological and Behavioral Correlates of Reproductive Success in Atlantic Cod *Gadus morhua*, *Mar. Ecol. Proc. Ser.*, 2008, vol. 354, pp. 257–265.
47. Neff, B.D., Genetic Paternity Analysis and Breeding Success in Bluegill Sunfish (*Lepomis macrochirus*), *J. Hered.*, 2001, vol. 92, pp. 111–119.
48. Hutchings, J.A., Swain, D.P., Rowe, S., et al., Genetic Variation in Life-History Reaction Norms in a Marine Fish, *Proc. R. Soc. London, Ser. B*, 2007, vol. 274, pp. 1693–1699.
49. Marciel, J., Swain, D.P., and Hutchings, J.A., Genetic and Environmental Components of Phenotypic Variation in Body Shape among Populations of Atlantic Cod (*Gadus morhua* L.), *Biol. J. Linnean Soc.*, 2006, vol. 88, pp. 351–365.
50. Bekkevold, D., Andre, C., Dahlgren, T.G., et al., Environmental Correlates of Population Differentiation in Atlantic Herring, *Evolution*, 2005, vol. 59, pp. 2656–2668.
51. Hemmer-Hansen, J., Nielsen, E.E., Frydenberg, J., and Loeschcke, V., Adaptive Divergence in a High Gene Flow Environment: Hsc70 Variation in the European Flounder (*Platichthys flesus* L.), *Heredity*, 2007, vol. 99, pp. 592–600.