FISH and FISHERIES

FISH and FISHERIES, 2008, **9**, 462–472

Fisheries forensics: the use of DNA tools for improving compliance, traceability and enforcement in the fishing industry

Rob Ogden

School of Biological Sciences, Bangor University, Bangor, LL57 2UW, UK; and Wildlife DNA Unit, Tepnel Research Products & Services, Appleton Place, Livingston, EH54 7EZ, UK

Abstract

There is an increasing requirement for traceability of fish and fish products, both for consumer protection and for regulatory enforcement, in particular with respect to illegal, unreported and unregulated (IUU) fishing. IUU fishing is a serious global problem and one of the main impediments to the achievement of sustainable world fisheries. Genetic markers are used for the identification of species and populations in fisheries biology and can potentially also be used to support investigations and deter IUU fishing and food fraud. This review examines the development and application of non-human forensic genetic techniques for the purposes of species identification, population assignment and supply chain traceability in world fisheries. Key issues in the transfer from academic research to validated forensic methods are presented highlighting differences in how data are produced and interpreted. The rapid increase in genomic data production and subsequent future potential for greater spatial resolution of fish stocks is discussed alongside the biological and practical limitations of using DNA markers for fisheries enforcement.

Keywords Fish identification, food authentication, genetic markers, population assignment, validation, wildlife crime

Correspondence:
Rob Ogden, Tepnel Research Products & Services, Appleton Place, Livingston, EH54 7EZ, UK
Tel.: +44 1506 424290
Fax: +44 1506 424280
E-mail: rogden@tepnelscientific.com

Received 31 May 2008
Accepted 29 Aug 2008

Introduction **462**
Wildlife DNA forensics **463**
Validation – from research to forensic science 464
Genetic identification for fisheries enforcement **464**
Species identification 464
Fish stock and geographic origin identification 466
Supply chain traceability 468
The outlook for genetic tools in fisheries traceability **469**
Acknowledgements **470**
References **470**

Introduction

Fisheries management ideally aims to balance the optimization of yield with the sustainability of natural resources. Within this broad objective, the science of fisheries biology has traditionally focused on developing our understanding of the environmental and biological parameters that influence

© 2008 The Author
Journal compilation © 2008 Blackwell Publishing Ltd

population dynamics and stock structure, in order to determine current or future stock sizes and ultimately contribute to setting catch quotas. With over 60% of global marine stocks now known to be over-exploited or at their maximum sustainable limit (FAO Fisheries and Aquaculture Department 2007a), it is also necessary to ensure the implementation of these scientific findings through regulation and control of the fishing industry. Illegal, unreported and unregulated (IUU) fishing is estimated to be worth between $10bn and $23bn per year and poses a real threat to the sustainable management of regional fisheries, not only through direct depletion of stocks, but also by undermining the competitiveness of legal fishing efforts, driving the whole industry towards non-compliance. A great deal of progress has been made in regulating commercial fisheries through monitoring, control and surveillance (MCS) measures (FAO Fisheries and Aquaculture Department 2007b). 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. Since the early 1990s the opportunity to combine outputs from fisheries genetics research with applied regulation mechanisms has been recognized (Bartlett and Davidson 1991, 1992), leading to the development of a new set of tools for fisheries enforcement. This review aims to highlight recent progress and future potential in the production and application of forensic DNA methods for the investigation and prosecution of IUU fishing and illegal trade.

Regulation of the fisheries industry faces multiple challenges, but these can largely be grouped into two main categories. IUU fishing describes fish caught in contravention to regulations concerning the species, geographic origin or age of the fish, the amount of fish caught, the timing and duration of fishing effort and the equipment used to catch fish. Regional fishery management organizations monitor many of these issues through the deployment of on-board fisheries officers and the use of remote sensing technology to track vessel movements. Nevertheless, current MCS systems have limitations and it is not always possible to identify or produce evidence of IUU fishing. The second major challenge is to identify false product labelling that is used either to launder IUU fish into the legitimate marketplace or simply to defraud the consumer in order to obtain a higher sale price (Jacquet and Pauly 2008). While deceiving the public may appear less serious, it can represent health risks to consumers through substitution of species such as escolar (*Gempylidae*; mislabelled as sea bass, FSA – Food Standards Agency UK 2003) and pufferfish (*Fugu* spp.; mislabelled as monkfish, US FDA – United States Food and Drug Administration 2007), both known to cause serious illness. Mislabelling is also of growing concern to certification schemes that rely on credible species identification to support consumer demand for 'sustainable' products. False labelling, even of legally caught fish, destroys confidence in systems designed to reduce IUU fishing.

To address these regulatory issues, methods for identifying fish and fish products are needed, capable of application to routine audits within the industry and as enforcement tools for prosecuting serious illegal activities. The key requirement of any identification system is to be able answer three questions regarding a sample: What species is it? Where was it caught? Can it be traced to samples above and below it in the food supply chain? Each of these questions can be approached using molecular markers and genetic databases developed for fisheries research. However, in order to convert this research into a set of applied tools, the developmental and analytical processes involved require validation to internationally recognized forensic standards. This paper sets out to examine the steps involved in transferring fisheries genetic research into forensic genetic tools and provides a perspective on current and future applications of DNA identification methods to fisheries traceability.

Wildlife DNA forensics

The use of genetic analysis to identify non-human evidence began shortly after the first DNA fingerprints were applied to human forensic investigation (Gill *et al.* 1985). Individual DNA identification systems such as single- and multi-locus probes were transferred directly from advancing human techniques (Thommasen *et al.* 1989; Wetton and Parkin 1997), while in parallel, DNA started to be used for species identification of trace evidence based on molecular phylogenetic methods (Cronin *et al.* 1991). Non-human forensic genetics is now routinely applied to the investigation of serious human crimes such as murder (Menotti-Raymond *et al.* 1997), instances of animal persecution (Dawnay *et al.* 2008) and cases of illegal trade in products

© 2008 The Author

ranging from olive oil (Pasqualone *et al.* 2004) and ivory (Wasser *et al.* 2004) to timber (Deguilloux *et al.* 2002), rice (Steele *et al.* 2007) and traditional medicines (Hsieh *et al.* 2003; Wetton *et al.* 2004; Peppin *et al.* 2008). To address these issues, researchers now utilize multiple marker types including microsatellites, insertion/deletions and single nucleotide polymorphisms (SNPs) in combination with analytical methods such as nucleotide sequencing, fragment analysis and a diverse range of SNP genotyping approaches (Sobrino *et al.* 2005). Wildlife DNA forensics continues to draw on conservation genetics (Ogden *et al.* 2008) and molecular ecology (Baker 2008) for novel approaches to genetic identification, though in the transfer of these methods to forensic tools for law enforcement, it is necessary to look to human forensic genetics as the analytical standard for the field.

Validation – from research to forensic science

The wide array of molecular identification techniques offers huge potential for the monitoring and enforcement of fisheries regulations, but at the same time presents multiple challenges to those attempting to transfer research methods into applied tools. Perhaps the most pressing issue is the need to find an acceptable balance between the utilization of academic research to develop genetic identification methods, and the expectations and requirements of the forensic science community to present clear, unequivocal evidence produced using validated, robust techniques.

In any enforcement application, all processes of data production and interpretation may be subject to challenge. Sample collection and transfer, laboratory and statistical analyses, the accuracy of the reference database and the presentation of findings are all scrutinized in search of potential sources of doubt in the evidence. It is therefore essential that each stage of the method is assessed and validated prior to enforcement use and all applications are undertaken in a quality assured environment (reviewed by Ogden *et al.* 2008). A summary of criteria assessed during the validation of forensic genetic applications is presented in Table 1. While it is unlikely that non-human forensic geneticists will ever have sufficient resources to undertake the depth of validation studies produced in the human field, the limitations and assumptions involved in analysis must be recognized and where possible, quantified, prior to forensic application.

Genetic identification for fisheries enforcement

Species identification

Identifying the species to which a fish sample or product belongs is the first and sometimes only issue in fisheries enforcement investigations. While unprocessed fish can be identified by trained inspectors with the support of taxonomists, almost all fish products lose their defining morphological characters during the early stages of processing making them impossible to identify with traditional taxonomic approaches. Since the early 1990s, the use of DNA markers to identify fish species has provided an alternative identification method that is now widely employed in fisheries and food regulation. For example, at the US NOAA Marine Forensics Laboratory over 2500 evidence items were received for DNA identification between 2006 and 2007 (T. Knott, personal communication). Applications range from the investigation of illegally traded caviar (DeSalle and Birstein 1996) and shark fins (Abercrombie *et al.* 2005) through to the issues of consumer protection and food fraud (Marko *et al.* 2004). With the development of multi-species array-based techniques (Kochzius *et al.* 2008; Teletchea *et al.* 2008) and assays designed for highly processed fish products (Jerome *et al.* 2003), species identification is becoming routine. A great deal of work has been published in this area, with several review articles highlighting the range of techniques and applications currently in use for trade monitoring (Rasmussen and Morrissey 2008; Kochzius 2008; Gil 2007; Bossier 1999). The following section focuses on the methods suitable for generating forensic evidence for enforcement applications.

The development of species identification techniques has relied upon the availability of a number of molecular markers that typically display variation among rather than within species. The mitochondrial DNA cytochrome oxidase I (COI) and cytochrome b (cyt b) gene regions are most commonly targeted and have formed the basis of large reference databases (FishBOL and FishTrace, http://www.fishtrace.org) that enable samples to be identified through DNA sequence matching. DNA nucleotide sequencing has been validated for species

© 2008 The Author

Table 1 A summary of the key considerations in the transfer of genetic identification methods from research to forensic science. Forensic requirements (column 2) describe the criteria considered and supporting information necessary to validate each process. Validation scope (column 3) indicates the variables to be investigated and/or data to be produced within validation studies. Design of appropriate validation studies is technique, taxon and application specific; this table is provided as an introduction to this area and researchers considering forensic applications are advised to thoroughly assess their own specific requirements.

Process	Forensic requirements	Validation scope	Examples
Sample type	Demonstration of DNA recovery (quantity and quality) from different samples	Sample type Sample condition Storage condition	1
Sample collection & transfer	Documented, auditable chain of custody from sample source to laboratory	System audit trail – see QA	See QA
Laboratory analysis	Demonstration of reliable data production using established techniques, operating within tested analytical conditions. Evidence that all reasonable sources of potential analytical variation have been considered and accounted for understanding of method limitations	*Genetic markers:* PCR conditions Mode of inheritance, independence Species specificity *Lab platforms/techniques:* Precision, accuracy, reproducibility Inter-laboratory calibration	2,3,4,5 6,7
Statistical analysis	Quantifiable results Measurement of uncertainty Ability to evaluate multiple hypotheses Ability to account for biological parameters Evaluation (peer-review) of algorithms/approaches	Simulation across a defined range of parameter values Testing for concordance of simulations with empirical data	8,9,10,11,12,13
Reference data	Authenticated reference materials Documented sample sources Data quality control	Audit trail from specimens to data Control, calibration and reproducibility	*Species ID:* 14,15 *Population ID:* 16 *Data quality:* 17
Evidence presentation	Evaluation of prosecution and defence hypotheses, rather than null and alternative hypotheses	Validation not applicable. Refer to relevant national forensic guidelines	8,9 National guidelines
Quality assurance (QA)	Analytical process performed in a QA environment Production and use of standard operating procedures	External independent audit of all analytical and control systems	QA standards: ISO17025* GLP/GMP†

Notes: 1. Jerome *et al.* 2003; 2. SWGDAM (2003); 3. Branicki *et al.* 2003; 4. Dawnay *et al.* 2007; 5. Dawnay *et al.* 2008; 6. Lygo *et al.* 1994; 7. Koumi *et al.* 2004; 8. Evett and Weir 1998; 9. Balding 2005; 10. Manel *et al.* 2005; 11. Hauser *et al.* 2006; 12. Altschul *et al.* 1997; 13. Ratnasingham and Hebert 2007; 14. BOLD (http://www.barcodinglife.org); 15. FishTrace (http://www.fishtrace.org); 16. Seeb *et al.* 2007; 17. Parson *et al.* 2004.

*ISO17025 specifies the general requirements for competence to carry out tests under the International Organization for Standardization.

†Good laboratory practice/good management practice.

identification applications (Wilson *et al.* 1995) and is favoured in forensics as it provides the most comprehensive information, and therefore strongest evidence, of all single marker DNA analysis methods. When used in combination with universal primers, nucleotide sequencing enables fish to be identified without any prior knowledge of their species of origin (Kyle and Wilson 2007). However, when faced with samples containing multiple species, or DNA that has fragmented through degradation or

© 2008 The Author
Journal compilation © 2008 Blackwell Publishing Ltd,

processing, sequencing with universal primers is limited and alternative methods are required.

The differences observed among species at a genetic marker are largely due to single base pair changes in the DNA sequence, known as SNPs. SNPs can be directly utilized to identify species through the design of species-specific primers or probes, or through the use of restriction enzymes (PCR-RFLP). While the reduction of genetic information from an entire sequence to the characterization of several SNPs increases the potential for misidentification, it does allow single target species to be identified from complex mixtures. For example, Chapman *et al.* (2003) successfully employed species-specific primers to detect DNA originating from the great white shark, *Carcharodon carcharias* (Lamnidae). They designed a multiplex PCR assay that simultaneously targets two gene regions, the mitochondrial cyt b and nuclear ITS2 regions, returning positive results for great white shark samples from around the world, whilst excluding 68 non-target shark taxa. The inclusion of two genetic markers and subsequent validation against a large number of alternative species and species mixtures was central to making this technique suitable for subsequent enforcement work. SNP-based methods, including PCR-RFLP, are accepted within the forensic genetic community for fishing investigations (Withler *et al.* 2004), however, they are applied in the context of species detection, rather than species identification, with each assay having a defined scope that describes the total set of species that have been tested during development. The strength of the subsequent evidence therefore depends on the completeness of the species set and is reinforced by demonstrating the same result at multiple DNA markers.

The power to identify species offered by DNA sequencing and the ability to analyze mixed DNA through SNP approaches can be combined. By using species-specific PCR-primers it is possible to generate a single DNA sequence for the target species from a sample containing multiple species DNA. This sequence can then be identified to species level to categorically demonstrate its presence in the sample, even for fragments as small as 109 bp (Hajibabaei *et al.* 2006), and is therefore a popular method in wildlife DNA forensics (Peppin *et al.* 2008).

Fish stock and geographic origin identification

One of the most pressing issues in fisheries management is to understand the level of population structure within a species in any given geographical area. Knowledge of spatio-temporal population dynamics is essential to validate models of fish ecology and subsequently predict patterns of dispersal, migration, recruitment and population size (Hauser and Carvalho 2008; Waples *et al.* 2008). In addition to output management tools (catch limits, TACs, minimum landing sizes) there is a growing need to develop control through input management tools (capacity and effort limitation). Such a policy requires information on the relative dynamics and abundance of fish stocks from particular regions. The determination of fish population limits is also a necessary precursor to the development of tools to identify the geographic origin of samples. Genetic analysis has again been shown to offer methodological solutions and is forming the basis of many applications that are vitally important if regulations to safeguard regional stocks are to be enforced.

From a forensic genetic perspective, identifying the geographic origin of a sample is equivalent to identifying its biological population. The term 'population' has no single definition and its meaning can vary between ecological and evolutionary interpretations that place different emphasis on how individuals are grouped (Waples and Gaggiotti 2006). Although many management applications will focus on a demographic, ecological approach to population structuring, genetic identification techniques must relate to a population definition that focuses on levels of reproductive cohesion (Waples *et al.* 2008). This disparity has implications for fisheries enforcement. Genetic variation among populations reflects levels of gene exchange among spawning stocks and does not necessarily relate to feeding aggregations, which may be composed of multiple evolutionary populations (Carlsson *et al.* 2007). Consequently molecular markers may be used to trace an individual salmon to the specific river drainage where it spawned (Beacham *et al.* 2008), but possibly not to where it was caught. This is clearly important in an enforcement context, as it requires a link to be established between the geographic distribution of stocks subject to IUU fishing and their genetic source populations.

Methods for genetic population identification, or assignment, are well-developed in fisheries research and extensive information exists relating to differing levels of genetic variability, structure and gene flow for many species. However, the conversion of these methods into enforcement tools that seek to

© 2008 The Author

categorically define geographic origin is complicated by the potential mobility of individual fish and the consequent gene flow between regions. Although molecular markers offer great potential for defining populations and identifying the origin of fish (Nielsen *et al.* 2001), marker selection must carefully consider their individual power to discriminate candidate populations in the context of each specific investigation. For some species, such as the Patagonian toothfish (*Dissostichus eleginoides,* Nototheniidae), markers reveal clear phylogeographic structure that directly correlates with geographically distinct fisheries allowing unambiguous assignment of samples on the basis of divergent genetic populations (Shaw *et al.* 2004). However, for other species at different spatial scales, geographically defined fishery stocks have so far proved very difficult to distinguish using molecular markers (Carvalho and Hauser 1998; Mariani *et al.* 2005). Furthermore, for enforcement applications, there is the additional requirement for population sub-division to coincide with management boundaries (e.g. ICES zones) or international borders (e.g. transnational rivers). Non-alignment of political regions and biological distributions is common in fisheries and represents one of the principal limitations to the use of genetic identification in enforcement activities (Waples *et al.* 2008).

The choice of molecular marker is important to achieve the required level of population discrimination. Identifying highly divergent populations that have experienced very limited gene flow over a long period of evolutionary time may be achieved using mitochondrial DNA markers such as the control region (D-loop), or occasionally COI or cyt b. The control region may exhibit fixed haplotype differences among regions (Ikeda *et al.* 2003) enabling categorical population assignment, which is preferable in an enforcement context. For populations that have undergone more recent divergence or exhibit greater gene flow, mitochondrial DNA markers usually fail to provide sufficient resolution, and population assignment methods have instead traditionally focussed on nuclear microsatellite loci as neutral markers capable of reflecting regional reductions in gene flow. While microsatellites may occasionally exhibit private alleles in single populations, genetic differentiation is normally achieved on the basis of divergent allele frequencies, enabling a probabilistic assignment of individual samples to their putative source population.

Such approaches have been successfully applied to various species in fisheries research, such as cod, trout and salmon (Hansen *et al.* 2001). Progress in this area has been accompanied by the development of various statistical assignment methodologies (Manel *et al.* 2005) and software packages (Hauser *et al.* 2006) that enable questions of population number, degree of differentiation and power of assignment to be addressed. The ability to accurately assign an individual fish to its source population is a function of the level of underlying genetic structure, the quality of the comparative datasets (size, representation and data accuracy), the number and variability of the molecular markers and the relative genetic uniqueness of the individual sample. The potential for this body of research to spawn enforcement tools for geographic origin assignment has long been recognized (Ward 2000 and Primmer *et al.* 2000), however, with the notable exception of the Pacific NorthWest salmon fishery (Withler *et al.* 2004) there are relatively few examples of such technology transfer in IUU fishing investigations.

One issue often raised by research scientists during the conversion from population assignment techniques to forensic identification methods is the relative level of statistical certainty required by the research and legal communities. Research scientists are familiar with testing hypotheses against error rates of 1% (1 in 100), while human forensic geneticists routinely report odds of 1 in 1 billion. This may give the impression that enormously powerful marker panels are always required to provide forensic evidence. Probability thresholds of $T > 0.999$ and $T < 0.001$ have been suggested for population assignment and exclusion respectively, in non-human DNA forensics (Manel *et al.* 2002). While these figures would certainly provide compelling evidence, questions over the value of the test statistic required for forensic applications are not strictly relevant. No scientist would (or should!) feel comfortable translating a *P*-value of 0.001 into a definitive legal statement regarding the source of a sample, for three reasons: (i) there is room for uncertainty in the genetic data, (ii) there is no scope for allowing the incorporation of non-genetic (prior) information relating to the sample's origin and (iii) there is no scope to evaluate the evidence under the defence hypothesis. Instead, many forensic geneticists favour the formulation of a likelihood ratio that compares the probability of observing the evidence (sample profile) under the prosecution and defence hypotheses. This allows the scientist to account for

© 2008 The Author
Journal compilation © 2008 Blackwell Publishing Ltd,

uncertainty, circumstance and alternative claims, and importantly, does not place any quantitative restriction on how significant the result must be. A quantitative estimate of the relative likelihood of the evidence is produced; the larger the number, the more probable the evidence given the allegation. This framework is widely employed to present human and non-human individual DNA profile matches (Evett and Weir 1998; Dawnay *et al.* 2008) and ancestral race identification (Phillips *et al.* 2007) and is recommended here for cases involving fish population assignment.

In addition to the need for a panel of validated markers, the construction of a likelihood ratio requires sufficient population genetic data to allow the evidence to be evaluated according to the hypotheses of both the prosecution and defence. This usually means that allele frequency estimates are needed from the source population claimed by the defendant as well as that alleged by the prosecution. Accordingly, it is the limited availability of datasets representing multiple populations that often governs the scope of enforcement tools. However, as any forensic analysis is undertaken within the context of a specific investigation, there are restrictions on selection of source populations that remove the need for complete species datasets. When evaluating the likelihood of the genetic evidence, the forensic scientist is also obliged to consider the effect of circumstantial information such as vessel limitations or remote sensing data that influence the number of alternative source populations that need to be considered. This is important as it may prevent the defendant from deliberately claiming that the source population was one from which no comparable allele frequency data is available.

Despite this, the level of resource required to generate population genetic data for each species and geographic region subject to fisheries management is vast. While future methods are likely to facilitate a much broader enforcement approach, it is currently not feasible to address every IUU fishing issue involving geographic origin using population assignment methods. The following section examines possible alternative approaches to utilize genetic variability for product traceability through the food supply chain, from fish to fork.

Supply chain traceability

While the direct detection of IUU fishing is not always possible, enforcement activities can still proceed through investigations that target the point at which IUU fish are laundered into the legitimate food supply chain. Supply chains are increasingly regulated through the use of documented audit trails that trace goods from production to point of sale in order to assure the origin and quality of products. Supply chain verification is usually achieved through the use of batch-specific certificates or barcodes detailing the content of a shipment; however, where sufficient drivers exist, such as public health or endangered species conservation, molecular markers are also used to authenticate trade. For example, in the UK, concern over BSE infection in cattle has led to beef products being identified with individual DNA profiles (Meghen *et al.* 1998), while in SE Asia, timber exports are being validated using a DNA certification scheme (Lawson 2007) to prevent the laundering of illegally logged wood. Targeting investigations at the fish supply chain offers an alternative approach to fisheries regulation and a number of molecular-based detection methods are already being applied.

Aside from the need to authenticate the species being traded (discussed earlier), traceability at any point in the food supply chain is restricted to demonstrating product receipt from one stage up in the chain and product provision to one stage down in the chain. This logic removes the requirement for molecular genetic markers to identify samples, instead they can be employed to exclude samples. Exclusion is simpler than identification as it does not require the same level of comparative population data or statistical interpretation. By screening a batch of fish at any point in the supply chain, subsequent adulteration can be detected by resampling and discovery of genetic material that was not present in the original batch. Dense supply chain sampling allows identification of the exact point at which illegal fish entered the chain. From a practical fisheries perspective, such methods would not use DNA markers for tracing individual fish, but instead use markers that characterize the genetic variation found within approved fisheries. For example, microsatellite panels currently used to identify farmed salmon broodstock from different suppliers could also be used to demonstrate changes in the genetic composition of a batch of fish as it is infiltrated with IUU products along the supply chain. The scale of sampling required would then be restricted to that typically used to provide a comprehensive estimate of allele frequencies in a population genetic study. In addition to enabling enforcement authorities to

© 2008 The Author

investigate IUU activity, genetic tracing also provides a method that can be employed by the fisheries industry or independent certification schemes to demonstrate the source of specific products.

The application of molecular markers for supply chain authentication is being instigated by the Marine Stewardship Council (A. Jackson, personal communication) as part of the ongoing TASC project (Traceability and Assurance in the Supply Chain), while in Russia, genetic traceability is already being employed to verify the farmed-origin of caviar (S. Rastorguev, personal communication). The financial and logistical resources required to develop such supply-chain traceability systems may support their widespread future use across many different fisheries of concern to conservation and enforcement authorities.

The outlook for genetic tools in fisheries traceability

The potential use for genetic identification in fisheries traceability was one of the earliest recognized applications of non-human DNA forensics. For almost two decades, this field has developed through the horizontal transfer of techniques from both human forensic science and molecular fisheries research. Our ability to generate genetic data is now increasing at a phenomenal rate, potentially removing many of the barriers currently faced by applied geneticists. In this emerging era, what can the fishing industry expect from molecular genetic tools and which limitations are likely to remain?

The continued development of global DNA databases containing authenticated reference sequences should soon enable the genetic identification of every fish species. Such resources also enable the development of assays for the detection of specific taxa and this provides the capacity to produce tools for authenticating almost all fish products, including mixed species and processed goods. With advances in genotyping technology, routine genetic monitoring will become increasingly cost effective. Single SNP assays are already commercially available at below \$1/€1 per sample and as uptake of genetic methods increases, costs will fall (Hauser and Seeb 2008). However, it should be noted that the cost of analysing forensic evidence samples is primarily driven by quality assurance procedures, not technology costs.

Traceability within species will be largely governed by our ability to identify sufficiently powerful molecular markers to differentiate target populations. Population genetic research for many fisheries has not even begun and a great deal of research is required to understand what exists, before identification methods can be developed. In extensively researched species, the priority is to increase the level of spatio-temporal population resolution. This may be achieved through the addition of more neutral genetic markers, but for many species there are likely to be limits to such assignment approaches due to levels of migration between populations (Waples 1998). The gradual movement away from microsatellites toward SNP markers offers the possibility to remove some of these barriers by targeting variation in genes under selection, as well as traditional neutral markers. Single gene markers, for example the *Pan I* locus, have demonstrated variation across populations (Westgaard and Fevolden 2007; Pampoulie *et al.* 2008) and such approaches have led to the development of forensic identification methods based on a very low number of highly informative markers (Schwenke *et al.* 2006). The advent of genome sequencing and subsequent SNP discovery in commercially important species (Van Tassell *et al.* 2008), should increase the availability of non-neutral molecular markers capable of assigning fish to geographic origin (Luikart *et al.* 2003).

Molecular markers, like any other technology, do not offer a complete solution to fisheries regulation and are best used in combination with complimentary approaches. Current MCS methods for vessel monitoring provide a wealth of information that can help to refine evidence produced from genetic techniques. For the analysis of whole fish, otolith microchemistry and morphological data can be used to examine the life history of an individual animal, indicate its geographical movements and region of capture (Campana and Thorrold 2001; Swan *et al.* 2006). A current European initiative, FishPopTrace (https://maritimeaffairs.jrc.ec.europa.eu/web/fishpoptrace/) is focussing on such an integrated approach for four commercially exploited fish species: cod (*Gadus morhua*, Gadidae), hake (*Merluccius merluccius*, Gadidae), herring (*Clupea harengus*, Clupeidae) and sole (*Solea solea*, Soleidae). Using a combination of array-based SNP screens to select informative population genetic markers and otolith data relating to ecological and life history population structuring, this project aims to develop a series of validated marker systems for tracing geographic origin for application to fisheries enforcement.

The need for accurate product labelling to generate consumer confidence in any initiative designed to

© 2008 The Author
Journal compilation © 2008 Blackwell Publishing Ltd,

promote good fisheries management is obvious. The use of genetic techniques in supply chain traceability is in its infancy, but offers huge potential for the authentication of both farmed and sustainably-caught fish. Importantly, it provides the opportunity for industry self-regulation which should potentially allow finite enforcement resources to be more effectively targeted at IUU fishing and trade. The scale and importance of the global fishing industry requires technological solutions that have the ability to support law enforcement where necessary, but primarily to encourage compliance with increasingly stringent management policies.

Acknowledgements

The author would like to thank L. Hauser, G. Carvalho, J. Martinsohn and two anonymous referees for their comments that helped improved this paper.

References

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

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J.H., Zhang, Z., Miller, W. and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389–3402.

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

Balding, D.J. (2005) *Weight of Evidence for DNA Profiles*. John Wiley & Sons Inc., London.

Bartlett, S.E. and Davidson, W.S. (1991) Identification of *Thunnus* tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome b genes. *Journal of Fisheries and Aquatic Science* **48**, 309–317.

Bartlett, S.E. and Davidson, W.S. (1992) FINS (forensically informative nucleotide sequencing): a procedure for identifying the animal origin of biological specimens. *Biotechniques* **12**, 408–411.

Beacham, T.D., Wetklo, M., Wallace, C. *et al.* (2008) The application of microsatellites for stock identification of Yukon River Chinook. *The North American Journal of Fisheries Management* **28**, 283–295.

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

Branicki, W., Kupiec, T. and Pawlowski, R. (2003) Validation of cytochrome b sequence analysis as a method of species identification. *Journal of Forensic Sciences* **48**, 83–87.

Campana, S.E. and Thorrold, S.R. (2001) Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Canadian Journal of Fisheries Aquatic Sciences* **58**, 30–38.

Carlsson, J., McDowell, J.R., Carlsson, J.E. and Graves, J.E. (2007) Genetic identity of YOY bluefin tuna from the eastern and western Atlantic spawning areas. *Journal of Heredity* **98**, 23–28.

Carvalho, G.R. and Hauser, L. (1998) Advances in the molecular analysis of fish population structure. *Italian Journal of Zoology* **65** (Suppl.), 21–33.

Chapman, D.D., Abercrombie, D.L., Douady, C.J., Pikitch, E.K., Stanhope, M.J. and Shivji, M.S. (2003) A streamlined, bi-organelle, multiplex PCR approach to species identification: Application to global conservation and trade monitoring of the great white shark, *Carcharodon carcharias*. *Conservation Genetics* **4**, 415–425.

Cronin, M.A., Palmisciano, D.A., Vyse, E.R. and Cameron, D.G. (1991) Mitochondrial-DNA in wildlife forensic-science – species identification of tissues. *Wildlife Society Bulletin* **19**, 94–105.

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

Dawnay, N., Ogden, R., Thorpe, R.S., Pope, L.C., Dawson, D.A. and McEwing, R. (2008) A forensic STR profiling system for the Eurasian badger: a framework for developing profiling systems for wildlife species. *Forensic Science International: Genetics* **2**, 47–53.

Deguilloux, M.-F., Pemonge, M.H. and Petit, R.J. (2002) Novel perspectives in wood certification and forensics: dry wood as a source of chloroplast, mitochondrial and nuclear DNA. *Proceedings of the Royal Society of London, B*, **269**, 1039–1046.

DeSalle, R. and Birstein, V.J. (1996) PCR identification of black caviar. *Nature* **381**, 197–198.

Evett, I.W. and Weir, B.S. (1998) *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists*. Sinauer Associates Inc., Sunderland, Massachusetts.

FAO Fisheries and Aquaculture Department (2007a) *The State of World Fisheries and Aquaculture 2006*. Food and Agriculture Organization of the United Nations, Rome.

FAO Fisheries and Aquaculture Department (2007b) Combating Illegal, Unreported and Unregulated Fishing Through Monitoring Control and Surveillance, Port State Measures and Other Means. In: *Meeting Document COFI/2007/7*, Food and Agriculture Organization of the United Nations, Rome.

FSA – Food Standards Agency UK (2003) *Mislabelling of Escolar Fish Species as Sea Bass and Potential Health Issues Associated With Consumption*. Published on 20/08/03 at: http://www.food.gov.uk/enforcement/alerts/2003/aug/escolarfhw (downloaded on 19/08/08).

© 2008 The Author

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

Gill, P., Jeffreys, A.J. and Werrett, D.J. (1985) Forensic applications of DNA 'fingerprints'. *Nature* **318**, 577–579.

Hajibabaei, M., Smith, M.A., Janzen, D.H., Rodriguez, J.J., Whitfield, J.B. and Hebert, P.D.N. (2006) A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology Notes* **6**, 959–964.

Hansen, M.M., Kenchington, E. and Nielsen, E.E. (2001) Assigning individual fish to populations using microsatellite DNA markers. *Fish and Fisheries* **2**, 93–112.

Hauser, L., Seamons, T.R., Dauer, M., Naish, K.A. and Quinn, T.P. (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**, 3157–3173.

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

Hauser, L. and Seeb, J.E. (2008) Advances in molecular technology and their impact on fisheries genetics. *Fish and Fisheries* **9**, 473–486.

Hsieh, H.M., Huang, L.H., Tsai, L.C., Kuo, Y.C., Meng, H.H., Linacre, A. and Lee, J.C.I. (2003) Species identification of rhinoceros horns using the cytochrome b gene. *Forensic Science International* **136**, 1–11.

Ikeda, M., Nunokawa, M. and Taniguchi, N. (2003) Lack of mitochondrial gene flow between populations of the endangered amphidromous fish, *Plecoglossus altivelis ryukyuensis,* inhabiting Amami-oshima Island. *Fisheries Science* **69**, 1162–1168.

Jacquet, J.L. and Pauly, D. (2008) Trade secrets: renaming and mislabeling of seafood. *Marine Policy*, **32**(3), 309–318.

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

Kochzius, M. (2008) Trends in fisheries genetics. In: *The Future of Fisheries in North America* (Proceedings of the 50th Anniversary Symposium of the American Institute of Fishery Research Biologists, Seattle, February 13–15th 2007). R. Beamiash and B. Rothschild, eds. Springer, Seattle, (in press).

Kochzius, M., Nölte, M., Weber, H. *et al.* (2008) DNA microarrays for identifying fishes. *Marine Biotechnology* **10**, 207–217.

Koumi, P., Green, H.E., Hartley, S. *et al.* (2004) Evaluation and validation of the ABI 3700, ABI 3100, and the MegaBACE 1000 capillary array electrophoresis instruments for use with short tandem repeat microsatellite typing in a forensic environment. *Electrophoresis* **25**, 2227–2241.

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

Lawson, S. (2007) *Certisource DNA Legality Assurance System – Independent Assessment*. Available at: http://www.certisource.net/data+downloads/02_Independent_Certisource_Report_Summary_09-2007.pdf (last accessed on 26/05/08).

Luikart, G.H., England, P., Tallmon, D.A., Jordan, S. and Taberlet, P. (2003) The power and promise of population genomics: from genotyping to genome-typing. *Nature Reviews Genetics* **4**, 981–994.

Lygo, J.E., Johnson, P.E., Holdaway, D.J. *et al.* (1994) The validation of short tandem repeat (STR) loci for use in forensic casework. *International Journal of Legal Medicine* **107**, 77–89.

Manel, S., Berthier, P. and Luikart, G. (2002) Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. *Conservation Biology* **16**, 650–659.

Manel, S., Gaggiotti, O.E. and Waples, R.S. (2005) Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology and Evolution* **20**, 136–142.

Mariani, S., Hutchinson, W.F., Hatfield, E.M.C. *et al.* (2005) North sea herring population structure: isolation by distance and age-class effect. *Marine Ecological Progress Series* **303**, 245–257.

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

Meghen, C.N., Scott, C.S., Bradley, D.G., MacHugh, D.E., Loftus, R.T. and Cunningham, E.P. (1998) DNA based traceability techniques for the meat industry. *Animal Genetics* **29** (Suppl. 1), 48–49.

Menotti-Raymond, M.A., David, V.A. and O'Brien, S.J. (1997) Pet cat hair implicates murder suspect. *Nature* **386**, 774.

Nielsen, E.E., Hansen, M.H., Schmidt, C., Meldrup, D. and Grønkjær, P. (2001) Population of origin of Atlantic cod. *Nature* **413**, 272.

Ogden, R., Dawnay, N. and McEwing, R. (2008) Wildlife DNA forensics – bridging the gap between conservation genetics and law enforcement. *Endangered Species Research* (in press) doi: 10.3354/esr00144.

Pampoulie, C., Steingrund, P., Stefánsson, M.O. and Daníelsdóttir, A.K. (2008) Genetic divergence among East Icelandic and Faroese populations of Atlantic cod provides evidence for historical imprints at neutral and non-neutral markers. *ICES Journal of Marine Science: Journal du Conseil* **65**, 65–71.

Parson, W., Brandstatter, A., Alonso, A. *et al.* (2004) The EDNAP mitochondrial DNA population database (EMPOP) collaborative exercises: organisation, results and perspectives. *Forensic Science International* **139**, 215–226.

© 2008 The Author
Journal compilation © 2008 Blackwell Publishing Ltd,

Pasqualone, A., Montemurro, C., Caponio, F. and Blanco, A. (2004) Identification of virgin olive oil from different cultivars by analysis of DNA microsatellites. *Journal of Agriculture and Food Chemistry* **52**, 1068–1071.

Peppin, L., McEwing, R., Carvalho, G.R. and Ogden, R. (2008) A DNA-based approach for the forensic identification of Asiatic Black Bear (Ursus thibetanus) in a traditional Asian medicine. *Journal of Forensic Sciences* Published Online: Aug 25 2008 12:19pm, doi: 10.1111/j.1556-4029.2008.00857.x

Phillips, C., Salas, A., Sánchez, J.J. *et al.* (2007) Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs. *Forensic Science International: Genetics* **1**, 273–280.

Primmer, C.R., Koskinen, M.T. and Piironen, J. (2000) The one that did not get away: individual assignment using microsatellite data detects a case of fishing competition fraud. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**, 1699–1704.

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

Ratnasingham, S. and Hebert, P.D.N. (2007) Bold: the barcode of life data system (http://www.barcodinglife.org). *Molecular Ecology Notes* **7**, 355–364.

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

Seeb, L.W., Antonovich, A., Banks, A.A. *et al.* (2007) Development of a standardized DNA database for Chinook salmon. *Fisheries* **32**, 540–552.

Shaw, P.W., Arkhipkin, A.J. and Al-Khairulla, H. (2004) Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep-water troughs as barriers to genetic exchange. *Molecular Ecology* **13**, 3293–3303.

Sobrino, B., Brion, M. and Carracedo, A. (2005) SNPs in forensic genetics: a review on SNP typing methodologies. *Forensic Science International* **154**, 181–194.

Steele, K.A., Ogden, R., McEwing, R., Briggs, H. and Gorham, J. (2007) InDel markers distinguish Basmatis from other fragrant rice varieties. *Field Crops Research* **105**, 81–87.

Swan, S.C., Geffen, A.J., Morales-Nin, B., Gordon, J.D.M., Shimmield, T., Sawyer, T. and Massuti, E. (2006) Otolith chemistry: an aid to stock separation of *Helicolenus dactylopterus* (bluemouth) and *Merluccius merluccius* (European hake) in the Northeast Atlantic and Mediterranean. *ICES Journal of Marine Science* **63**, 504–513.

SWGDAM (2003) *Revised Validation Guidelines*. Available at http://www.cstl.nist.gov/div831/strbase/validation/SWGDAM_Validation.doc (accessed on 15 January 2008).

Teletchea, F., Bernillon, J., Duffraisse, M., Laudet, V. and Hänni, C. (2008) Molecular identification of vertebrate species by oligonucleotide microarray in food and forensic samples. *Journal of Applied Ecology* **45**, 967–975.

Thommasen, H.V., Thomson, M.J., Shutler, G.G. and Kirby, L.T. (1989) Development of DNA fingerprints for use in wildlife forensic science. *Wildlife Society Bulletin* **17**, 321–326.

US FDA – United States Food and Drug Administration (2007) *FDA Warning on Mislabeled Monkfish – Fish Believed to be Puffer Fish; Contains Deadly Toxin*. Available at: http://www.fda.gov/bbs/topics/NEWS/2007/NEW01639.html (last accessed on 19/08/08).

Van Tassell, C.P., Smith, T.P.L., Matukumalli, L.K. *et al.* (2008) SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. *Nature Methods* **5**, 247–252.

Waples, R.S. (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89**, 438–450.

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

Ward, R.D. (2000) Genetics in fisheries management. *Hydrobiologia* **420**, 191–201.

Wasser, S.K., Shedlock, A.M., Comstock, K., Ostrander, E.A., Mutayoba, B. and Stephens, M. (2004) Assigning African elephant DNA to geographic region of origin: applications to the ivory trade. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 14847–14852.

Westgaard, J.-I. and 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.

Wetton, J.H. and Parkin, D.T. (1997) A suite of falcon single-locus minisatellite probes: a powerful alternative to DNA fingerprinting. *Molecular Ecology* **6**, 119–128.

Wetton, J.H., Tsang, C.F.S., Roney, C.A. and Spriggs, A.C. (2004) An extremely sensitive species-specific ARMs PCR test for the presence of tiger bone DNA. *Forensic Science International* **140**, 139–145.

Wilson, M.R., Dizinno, J.A., Polanskey, D., Replogle, J. and Budowle, B. (1995) Validation of mitochondrial-DNA sequencing for forensic casework analysis. *International Journal of Legal Medicine* **108**, 68–74.

Withler, R.E., Candy, J.R., Beacham, T.D. and Miller, K.M. (2004) Forensic DNA analysis of Pacific salmonid samples for species and stock identification. *Environmental Biology of Fishes* **69**, 275–285.

© 2008 The Author