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Validating the origin of capture of fish - Genetic methodologies (MMO1191)



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MMO1191 - Validating the origin of capture of fish - Genetic methodologies

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Executive Summary

In this project the available methods for determination of fish catch geographic origin were reviewed, based on state-of-the-art DNA technologies, for seven main types of commercial fish species of significant importance for landings in England and Wales. These are: Cod (*Gadus morhua*), Hake (*Merluccius merluccius*), Sole (*Solea solea*), Plaice (*Pleuronectes platessa*), Anglerfish (*Lophius piscatorius*), Rays or Skates (Elasmobranchii: Rajidae), King scallop (*Pecten maximus*).

Genomic Single Nucleotide Polymorphisms (SNPs) are found to be the tool of choice for assigning individuals to populations or geographic areas. All species in focus have been investigated with these methods, but with various degrees of intensity and success. In the case of ray/skate products methodologies were examined for species-level identification.

The level of implementation of available techniques were assessed against the Technology Readiness Levels (TRL) as defined by the [European Research Council](#) (2014) (Table 1): the higher the number meaning the more advanced and operationally applicable. It was found that for cod, hake and sole, the TRL is at an advanced stage and tools can be implemented in the operational environment. For plaice, tools have been shown to work in a certain geographic context, but it is unclear whether they can be effective in other area of interest. For king scallops and anglerfish, technology has been shown to work (scallops), and markers are being developed (anglerfish), but it remains to be demonstrated whether the areas of interest for landings will be robustly distinguishable.

Table 1 - Technology Readiness Levels

Level	Description
TRL 1	Basic principles observed
TRL 2	Technology concept formulated
TRL 3	Experimental proof of concept
TRL 4	Technology validated in lab
TRL 5	Technology validated in relevant environment (industrially relevant environment in the case of key enabling technologies)
TRL 6	Technology demonstrated in relevant environment (industrially relevant environment in the case of key enabling technologies)
TRL 7	System prototype demonstration in operational environment
TRL 8	System complete and qualified
TRL 9	Actual system proven in operational environment (competitive manufacturing in the case of key enabling technologies; or in space)

For ray/skate, established, validated DNA barcoding methods are fully operational, while a novel, rapid and portable tool, currently being developed for finfish species, could also be tailored and validated relatively swiftly.

Table 2 - Summary table of Technology Readiness Levels (TRL) of DNA tools available for the species under focus in this study

Species	Technique	TRL	TRL Description
Cod	Genomic SNPs	9	Actual system proven in operational environment
Hake	Genomic SNPs	8	System complete and qualified
Sole	Genomic SNPs	8	System complete and qualified
Plaice	Genomic SNPs	4	Technology validated in lab
Anglerfish	Genomic SNPs	3	Experimental proof of concept
Ray/Skate	DNA Barcoding	9	Actual system proven in operational environment
Ray/Skate	Universal qPCR* ('FASTFISH ID')	7	System prototype demonstration in operational environment
King scallop	Genomic SNPs	4	Technology validated in lab

* "qPCR" here stands for *quantitative real-time Polymerase Chain reaction*: a method that allows the visualisation, through a fluorescent probe, of the amount of target DNA amplified.

1. Introduction

As part of the Marine Management Organisation's (MMO) ongoing work looking at enhancing provenance and traceability, the MMO sought an assessment of methods for confirming where fish sold ashore were originally caught to potentially support and verify other systems for managing compliance and enforcement. This report concerns genetics, one of four technique classes previously identified as promising by the MMO, along with stable isotopes, trace elements and lipids.

Fishery products are characterised by remarkable biological diversity, not only in terms of number of species commercially harvested, but also in terms of complex spatial substructure among demographically separated stocks. Some of these biological units are more vulnerable to, and less resilient from, commercial harvesting; hence, management strategies should be devised to recognise this biocomplexity, and enforcement agencies should be enabled to monitor the robustness of enacted management actions.

Full transparency in the seafood supply chain depends on the development of processes that can trace a seafood product to a particular area inhabited by a given wild population. DNA 'markers' (i.e. short sequences of DNA that are sufficiently variable to distinguish animals belonging to different groups) have gradually established themselves among the most appropriate tools for ascertaining species identity in traded seafood, and to estimating probabilities of a product or specimen belonging to a certain stock or geographic area.

Nevertheless, wild organisms targeted by fisheries include hundreds of species globally with diverse ecological and life history traits, which will determine the degree of genetic divergence between biological units. This will in turn affect the ability of DNA markers to distinguish animals from putatively different populations.

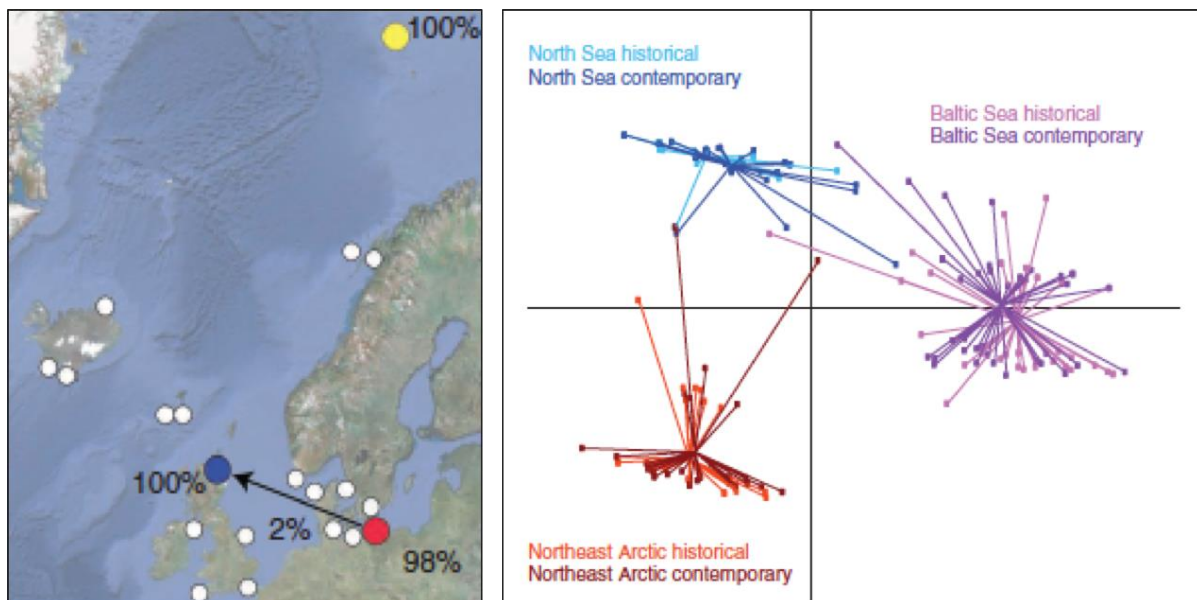
The present report reviews existing DNA-based tools for population / geographic area assignment in selected commercial fish species and evaluates the 'Technology Readiness Level' (TRL) of current methodologies, in view of providing advice to the MMO and other management authorities. The state-of-the-art for five finfish species (cod, hake, sole, plaice and anglerfish), one bivalve (king scallop), and a family of commercial elasmobranchs (rays/skates) was reviewed, by examining relevant scientific and grey literature, as well as consulting colleagues involved in research and development for these taxa.

Below the evaluation for the seven taxa is illustrated separately and reasoned advice is offered with ways forward for each one of them suggested.

2. Atlantic cod

Atlantic cod (*Gadus morhua*) is probably the most intensely studied marine fish species, and has also played a pivotal role in the development of genomic tools for both fundamental and applied purposes. Following from the [FishPopTrace project \(2011\)](#) which focused on cod, hake, sole and herring, Defra commissioned [Trace DNA Wildlife Forensics](#) to develop and validate Standard Operating Procedures (SOP) for the genetic traceability of at least some of these species ([Defra 2014](#)). The Defra SOP acted as a knowledge transfer from the primary findings presented in Nielsen et al 2012. Cod proved to be the species for which the most successful tools could be developed, the strongest genetic assignment with the lowest number of markers, for stocks of high relevance to UK landings, particularly in ICES areas IV.

Figure 1 - Genotyped baseline populations (left) and validated genetic assignment of recent and historical samples of cod (modified from Nielsen et al. 2012)



From a pool of more than 1200 Single Nucleotide Polymorphisms (SNPs) employed to characterise cod population structure in the North East Atlantic, smaller panels (between 10 and 48) of ‘super-SNPs’ (markers that can discriminate between certain geographical groups with high reliability) were designed, in order to increase the discriminating power with the lowest possible effort and cost.

The exercise involved comparisons between North Sea (ICES areas IV) vs Western Baltic (ICES areas III) and between North Sea vs North East Arctic (ICES areas V and II), and successful assignment could be achieved with as low as 10 SNP assays (investigative procedures). Baseline genotypes for several North East Atlantic stocks are available and stored with the [Joint Research Centre](#), which are likely to be reliable for decades, and can serve to validate further SNP assignment panels.

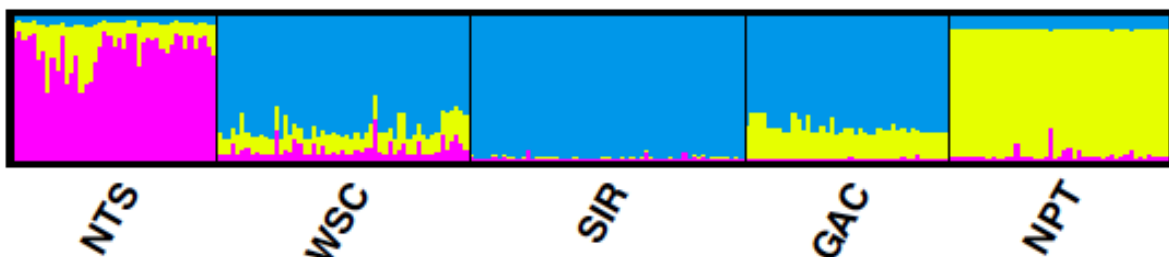
This method is ready to be immediately implemented and the tests can be carried out by technicians with biochemistry and molecular biology training in research institutes and approved United Kingdom Accreditation Service control laboratories.

3. Hake

Hake (*Merluccius merluccius*) was also targeted by [FishPopTrace project \(2011\)](#). Genetic analyses showed the existence of a strong genetic break between Atlantic and Mediterranean hake populations, which would only require a handful of markers to distinguish these two evolutionary units with 100% certainty.

More relevant to the UK context, hake showed significant spatial substructure also along the North West Atlantic shelf, from Northern Portugal to Western Scotland, and between the Western shelf and the North Sea (Figure 2).

Figure 2 - Assignment of individual hake genotypes collected from: the North Sea (NTS), Western Scotland (WSC), Celtic Sea (SIR), Galician Coast (GAC) and Northern Portugal (NPT).



Each thin bar represents an individual fish, and the colour of the bars represents the genetic assignment of an individual to any of three main population clusters identified in the area (modified from Milano et al. 2014). For instance, all fish from the Celtic Sea are strongly assigned to the “blue” stock, while most of the Galician fish – which are still assigned the blue stock – have a stronger genetic contribution of the “yellow” stock from Northern Portugal, presumably as a result of partial gene flow.

Building on these results, Defra obtained from Trace DNA Wildlife Forensics a validated tool for the distinction of hake samples collected in the North Sea or the Western shelf/slope. The currently existing procedure uses between 10 and 20 SNPs to distinguish North Sea from Western shelf hake with around 90-92% confidence. Given the substructure existing within the Western shelf, and the recent discovery of substructure also along the North Sea – Skagerrak – Kattegat cline (Westgaard et al. 2017) new studies are currently ongoing, to characterise stock boundaries with increased robustness.

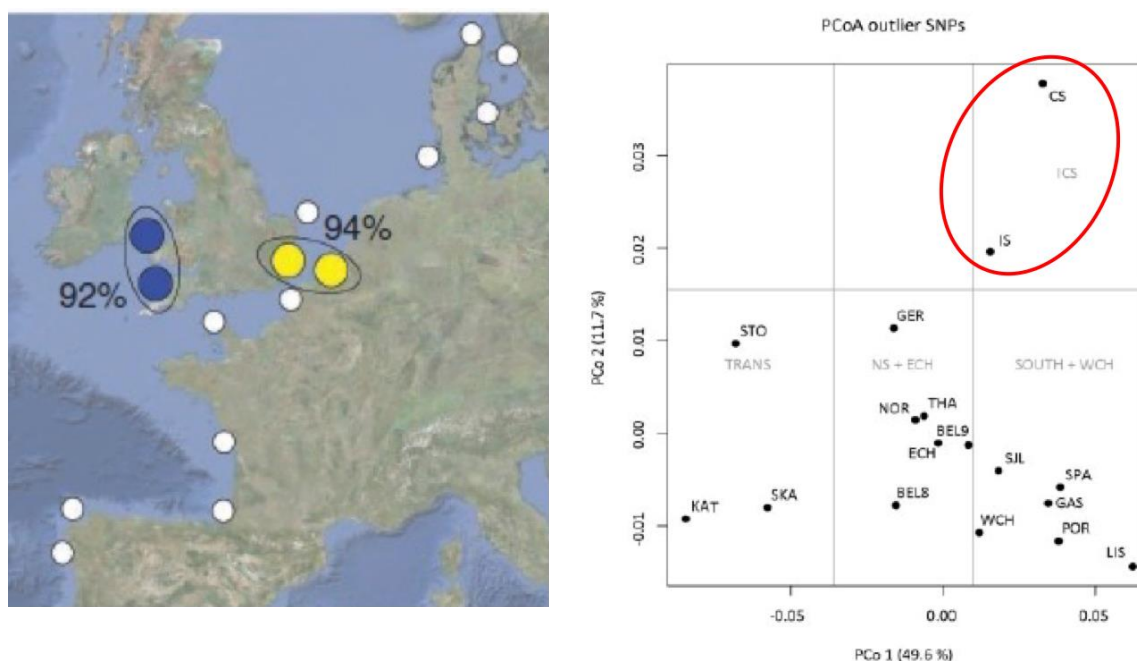
Nevertheless, for the purpose of distinguishing Atlantic from Mediterranean hake, and North Sea from Western shelf hake, the existing tools derived from FishPopTrace are adequate, and with minimal readjustment, they can be considered operational, at technology readiness level 8.

4. Dover sole

Dover sole (*Solea solea*) was another target species for [FishPopTrace project \(2011\)](#), and genetic analyses showed the existence of four distinguishable Atlantic populations: the Baltic Sea transition zone, the Bay of Biscay, the North Sea / Eastern English Channel and the Irish/Celtic Sea (Cuveliers et al. 2012, Diopere et al. 2017). The [Defra 2014 report](#) illustrates the efficacy of SNP panels (<48 markers) devised for the discrimination between the areas of greatest relevance to UK landings.

While fish from the Western English Channel (ICES areas VIIe-d) can be distinguished from the Irish/Celtic Sea (ICES areas VIIa-g) with >95% probability (Figure 3), the differences between these regions and the Bay of Biscay (ICES areas VIII) or the North Sea (ICES areas IV) are less stark, only allowing assignment with less than 90% confidence, which – albeit highly informative from a biological standpoint, may be too weak for enforcement purposes.

Figure 3 - Genotyped sole populations, highlighting areas of greater genetic distinctiveness (left, from Nielsen et al 2012) and genetic relationships among all studied areas (right, from Diopere et al 2017 – the red ellipse highlights the Irish/Celtic Sea samples).



Sole from the Mediterranean are also easily distinguishable from Atlantic contingents with high confidence, using just a handful of SNPs (though this distinction is likely not to be relevant to traceability of English landings). Furthermore, new genome-wide SNPs have been developed and analysed at the University of Leuven, Belgium (unpublished), which are likely to offer additional power for geographic assignment. Even without any new markers, the currently existing tools are complete and qualified to at least discriminate Irish/Celtic Sea catches from Western Channel ones; however, due to lack of application in the operational environment so far, technology readiness should be considered to be at level 8.

5. Plaice

For nearly two decades, plaice (*Pleuronectes platessa*) has been known to show negligible genetic structure across the North East Atlantic (Hoarau et al. 2002), with only the Icelandic stock being significantly distinct from the rest of the populations.

Recently, a Danish multidisciplinary study (Ulrich et al. 2017) applied novel SNP markers to shed light on plaice spatial genetic patterns between the North Sea and the Western Baltic. Results showed significant differences between the two regions, and intermediate features along the transition zone (Figure 4 and Figure 5), which is consistent with patterns observed in other species across the same region, yet less stark.

Figure 4 - Map of plaice samples screened in Ulrich et al. (2017)

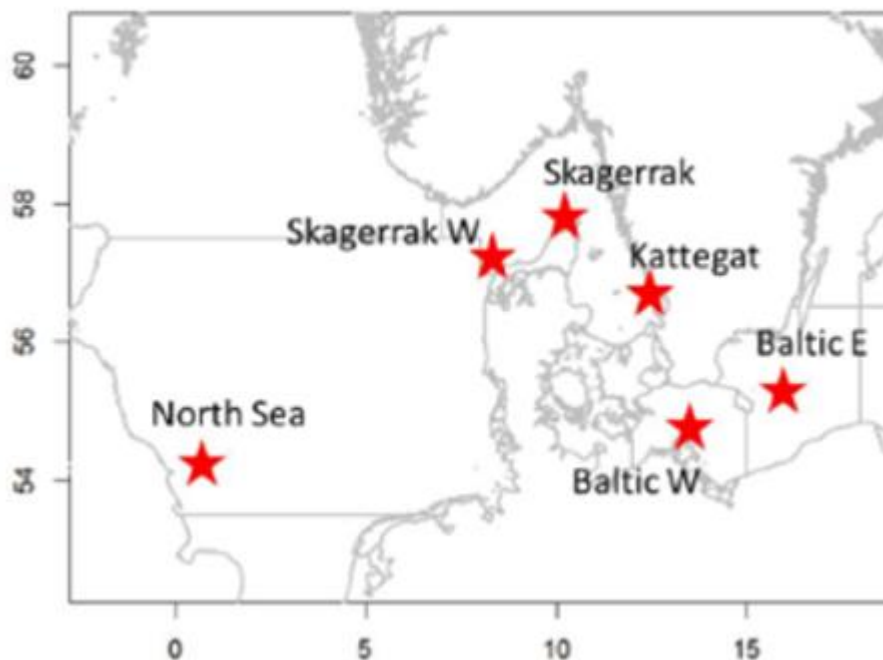
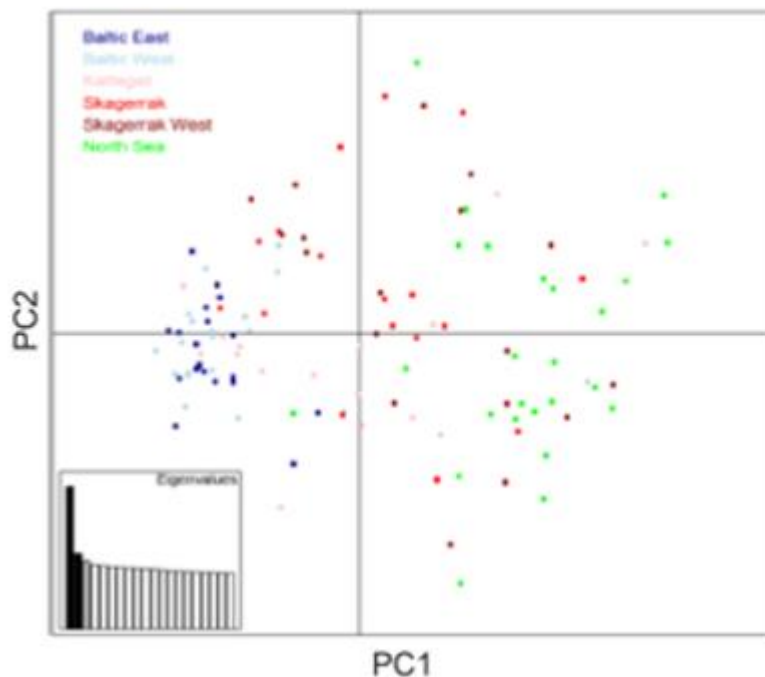


Figure 5 - Principal Component Analysis of 118 individual plaice genotypes screened at >5000 SNPs from Ulrich et al. (2017)



Strong distinction is observed between Baltic (blue shades) and North Sea (green) plaice, with intermediate variation found between Kattegat and Western Skagerrak (red shades) (Figure 5).

Although this study is based on thousands of SNPs, it did not develop and validate a specific procedure for stock traceability; furthermore, the samples analysed did not include important areas that are relevant to UK landings, so there currently is no information on the patterns or levels of genetic distinctiveness among plaice populations of interest to UK catches. Although genomic SNPs have shown potential to serve as location markers in other demersal species, there is no way to tell whether they will prove effective in the case of plaice, until a systematic study is carried out for this purpose.

Technology readiness of genetic traceability in plaice populations must presently be considered at level 4.

6. Anglerfish

The white anglerfish (*Lophius piscatorius*) is managed as three separate stocks in the NE Atlantic, organised along a north-south direction, which does not correspond to actual biological boundaries.

Although studies have attempted to clarify the patterns of population structure in this species, very little information is available from genetic methods. The only exceptions are the study by Blanco et al. (2008) and an unpublished PhD thesis (O'Sullivan et al. 2005), which respectively used eight and nine microsatellites, to study samples spanning from Iceland and Norway (O'Sullivan et al. 2005) to Northern Spain and Portugal (Blanco et al. 2008). Neither study detected significant spatial differentiation, with the exception of a possible isolation of a population in the Northern Bay of Biscay (Blanco et al. 2008). Yet no robust marker set exists to assist with monitoring catch origin.

Two projects ([EU-funded GECKA Project](#)) and one supported by the MMO (MMO1167) are currently developing genomic markers in order to resolve the population structure of anglerfish in NE Atlantic, and it is reasonable to expect that these genome-wide studies could detect enough spatial variance to develop the tools required for traceability. However, at this stage, technology readiness can only be deemed to be at level 3.

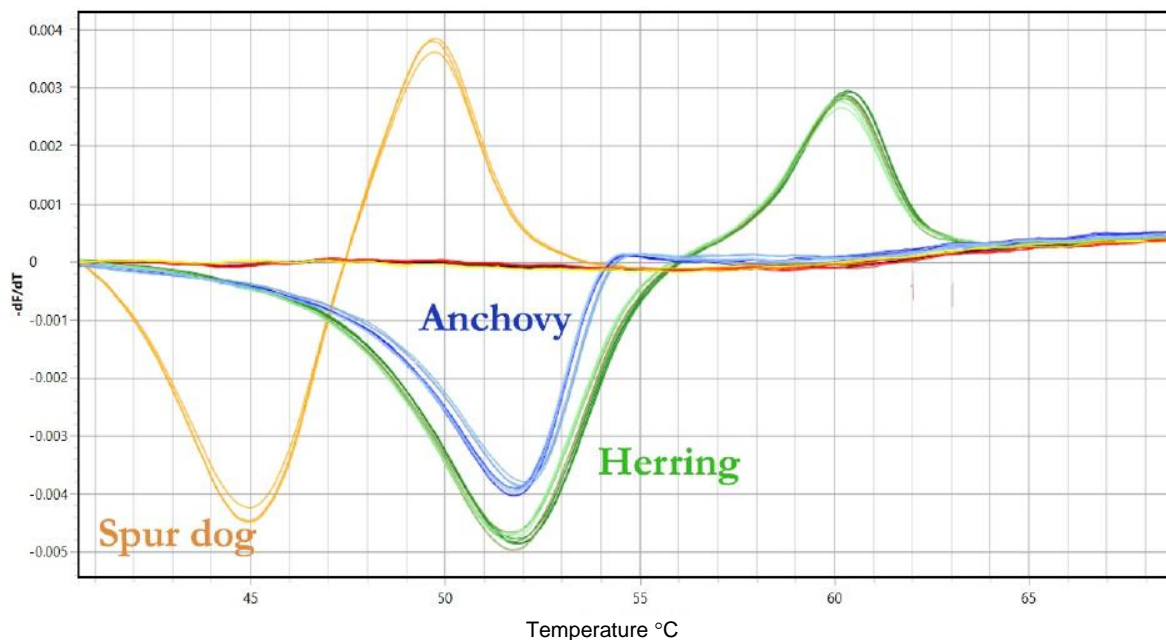
7. Ray/Skate – Species Determination

The primary issue around the landing and trade of batoid elasmobranchs commonly known as ‘ray’ or ‘skate’ (Family Rajidae) pertains to the identification of species, rather than the area of origin of specific contingents within a given species. Up until recently, landings only reported just “ray”, disregarding variation in population status among species.

Classic DNA barcoding is a very robust tool for the purpose of ray species identification, and its performance in the context of the UK seafood market was demonstrated by Griffiths et al. (2013), who showed a 100% assay success rate and a 100% species identification. This approach is fully operational and could be employed at any time to control specimen authenticity at any point of the supply chain. The protocol follows a Standard Operating Procedure produced by the LABELFISH project for Defra in 2015 ([available upon request](#)).

More recently, as part of the [SEATRACES](#) project a new fish species identification method is being developed, based on real-time quantitative polymerase chain reaction (qPCR), which can generate unique fluorescent signatures for a range of species for which reference fluorescent spectra are available. Although primarily aimed at commercial teleosts, initial tests suggest that the procedure can be applicable also to elasmobranchs: Figure 6 shows the fluorescent signature of spurdog (*Squalus acanthias*), compared to herring (*Clupea harengus*) and anchovy (*Engraulis encrasicolus*). This method does not require sequencing, which means that it is significantly cheaper, faster and portable (results can be generated in a few hours, on site).

Figure 6 - Fluorescent spectra obtained using the FASTFISH I.D. method for three species of fish collected during a pelagic survey.



Spurdog is clearly distinguishable (yellow trace) from anchovy and herring (in blue and green, respectively). This method is two steps away from being fully operational: it will require i) the provision of tissue samples from all the main ray species existing

in UK fishing grounds, so as to generate reference spectra for each one of them, ii) the validation of the assay using 'blind samples'.

Assuming availability of samples, this could be carried out and completed within a year.

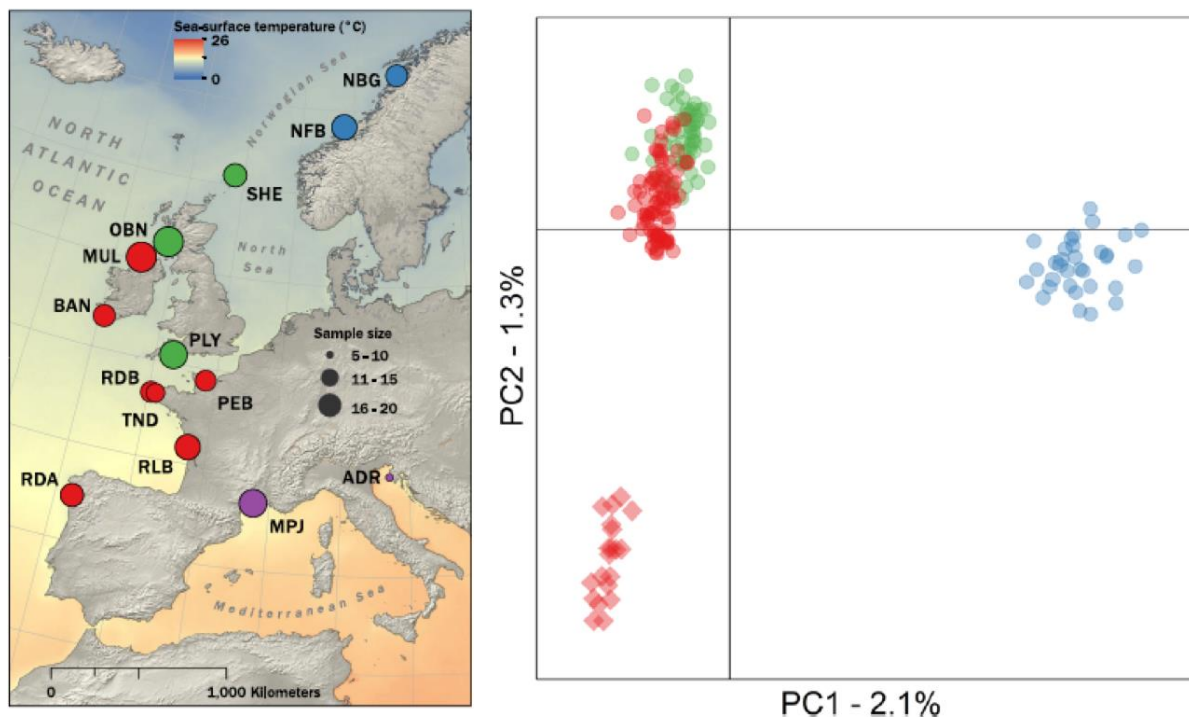
Summary	
DNA barcoding (lab-based)	TRL 9
'FASTFISH ID [®] ' (portable qPCR)	TRL 7

8. King scallop

Scallops represent the largest shellfish landings in the UK, and are only second to Norway lobster (*Nephrops norvegicus*) in terms of value; yet, surprisingly, there currently exists scant knowledge of population structure of these stocks, especially for the king scallop (*Pecten maximus*).

The most recent and geographically exhaustive study compared North East Atlantic samples, from Northern Spain to North Western Norway, using a panel of >80,000 SNPs (Vendrami et al. 2019), which detected strong separation between Norwegian sites and all the other collections from the Western European shelf (Figure 7).

Figure 7 - Geographical extent of king scallop samples genetically screened by Vendrami et al. (2019) (left), and bi-dimensional scaling of genetic differentiation among Atlantic samples (right). Blue refers to Norwegian samples, green to Shetland and Britain samples, red to Ireland and South Western Europe. In the right panel, samples from “MUL” are represented as diamonds. The purple “MPJ” sample refers to a Mediterranean collection of the sister species, *Pecten jacobaeus*.



The levels of differentiation between these two groups are substantial, and despite the Shetland population being situated somewhere in between these two regions, its genetic constitution appears similar to other British collections (Fig. 6, right panel). Unfortunately no samples were screened from along the North Sea coast. A strong genetic distinction was also observed in the Northern Irish population from Mulroy Bay (Fig. 6, red diamond data points in the right panel), but this collection was from an aquaculture facility that stocked scallop from locally sourced seed, which means that the sample had undergone an artificial bottleneck.

Although this study fails to record significant substructure among Northern Scotland, the Irish Sea, the Celtic Sea, the English Channel, the Bay of Biscay and North Western Spain, no specific analysis was carried out to investigate which of the outlying SNPs could be high-graded to separate some of these areas more robustly. Since a tailored validation study should be conducted, at this stage, technology readiness must be considered to be only at around level 4.

9. Conclusion and recommendations

The validated SNP assays (procedures) for cod, hake, and to some extent sole, should be employed, given the investment already made by the government in previous years. Commercial accredited laboratories are available to provide this service following the Defra SOPs. This is likely to cost upwards of £20 a sample in a laboratory.

In a subsequent phase, the existing tools could be further refined to improve through-put: currently, separate individual reactions must be carried out for each assay SNP and each individual, but several of these could be multiplexed, using different dyes, which could be simultaneously 'read' by qPCR systems. Further academic work in improving efficiency here could save time and money in the testing which would currently cost between £5 and £10 a sample to process.

The amount of genomic resources available for cod – and soon for hake – should offer the possibility for additional, greater resolution assays, aimed at tracing catches from additional areas (e.g. Celtic Sea, West of Scotland, etc.) or discriminate between units within the North East Arctic (e.g. distinguishing Icelandic from Barents Sea cod).

For anglerfish and plaice, the only way forward is to generate the required baseline information and verify whether diagnostic SNP polymorphisms exist for the areas of interest. The same applies for king scallop, with the slight advantage that for some regions there are some recently generated genomic resources, which could be mined for potentially useful SNP markers. It should be noted, however, that cooperation would be required with, and among, the research teams that produced SNP data sets, in order to effectively and efficiently assess their usefulness. To obtain more samples from more locations of interest for these species it could potentially be up to 2 years of work costing up to £30,000, a more thorough study involving multiple seasons to test for variability patterns could be up to £70,000.

For ray species identification the operational tools are simple and already available. The opportunity for the validation of a rapid, portable and cheaper method should, however be considered, as it would considerably streamline the procedure and facilitate tool intake. A current test in a laboratory would likely be around £10 a sample with a turnaround of around a week whereas some available portable devices could process a sample for around £3 with a turnaround sample of around 5 hours.

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