

Validating the origin of capture of fish using isotopes, lipids, trace elements and genetics (MMO1167 MMO1191 MMO1192 MMO1193 MMO1194)

Aim

The Marine Management Organisation's (MMO) sought an assessment of methods for confirming where fish sold ashore were originally caught. Four technique classes were identified as promising by the MMO including stable isotopes, lipids, trace elements and genetics. This collection of projects aimed to explore applicable techniques to trace the origin / location of capture of seafood landed in the UK, to understand the current levels of technological readiness, progress them to operational level and to consider synergies amongst techniques.

Introduction and methodology

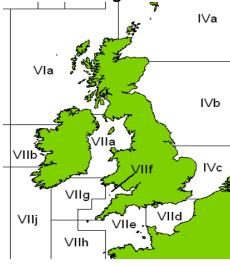
To successfully manage fishing pressure and fish stocks, it is necessary to know where fish are caught. Commercial fisheries catches must be identified to an ICES area (International Council for the Exploration of the Sea) for example ICES Area IVc, the Southern North Sea (Figure 1). In addition to restrictions in how much fish can be caught in each area, different ICES areas often having different catching rules and regulations. MMO therefore sought to increase confidence in, and understanding of, analytical methods that can verify where fish were caught to ICES Area or better resolution to supplement existing methods.

Objectives were to:

- identify potential methodologies to determine where a fish has been caught within the techniques of stable isotopes, lipids, trace elements and genetics
- evaluate the proposed methodologies to determine practical applicability in current form including; costs and benefits, ease of use and spatial resolution of catch allocation
- conduct work to increase Technological Readiness Level (TRL) of methodologies
- identify the program of work, including estimated costs, that is required to progress the technique to operational deployment

Five work packages contribute to this project, each employing a different methodology depending on the analytical technique and the existing knowledge base for the technique's application to defining the origin of fish:





- Stable Isotopes (MMO1192) conducted a i) a desk review of existing literature and ii) new data collection on anglerfish and sole.
- Lipids (MMO1193) undertook a proof of concept laboratory study testing whether Manilla clam samples from the Mediterranean and English Channel could be differentiated. The study used both non-targeted (any differences among resolved compounds) and targeted (fatty acid composition) examination of clam lipid biochemical make up.



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- Trace elements (MMO1194) was based on desk review of literature to explore the basis for the technique and its current technological readiness level.
- Genetics (MMO1191 MMO1167) encompassed two work packages, i) a desk review of the TRL of established techniques against seven major commercial species, and ii) new research assessing genetics in anglerfish and the potential to distinguishing seven different sea areas.

Results

Each technique was assessed against a TRL as defined by the <u>European Research Council</u>, the higher the number, the more advanced and operationally applicable; TRL 1 is "Basic principles observed" up to TRL 9 which is "Actual system proven in operational environment (commercially available)".

The following was found for each technique class:

- <u>Stable Isotopes</u> Readiness varies among species depending on data availability. Readiness in cod and herring were well advanced (TRL 7) and able to distinguish location to a sub-rectangle resolution. Plaice, dab, gurnard, anglerfish and haddock were estimated to all be at TRL 5 with potential to resolve large regional differences.
- <u>Lipids</u> The exploratory work conducted indicate lipid biochemical profiles can differentiate clams between the English Channel and the Adriatic. Differentiation was established using both non-targeted and targeted approaches. The potential for this technique was confirmed but requires more extensive spatial analysis. This method is currently at TRL 4 - technology validated in laboratory.
- <u>Trace elements</u> While trace element compositions of fish tissues have been routinely reported from UK waters (especially in relation to pollution and environmental toxicology), there are currently no substantial, consistent datasets available to predict spatial variations in elemental concentrations at a local or regional level. Overall TRL therefore lies between 3 and 5.
- <u>Genetics</u> is at an advanced stage of readiness but applicability is species dependant. For cod, hake and sole methods need little further work (TRL 9, TRL 8 and TRL 8 respectively) and tools can be implemented in the operational environment given resourcing. For plaice, genetics have been shown to work in a certain geographic context, but it is unclear whether they can be effective in other area of interest (TRL 4). For king scallops, technology has been shown to work (TRL 4). New analysis for anglerfish suggested individuals could be assigned to their capture origins (by comparing a large number of markers (1,977)). To make analysis quicker and cheaper a screening methodology was created that identified 166 outlier markers that differentiated areas although the resolution remained highest with the full dataset. Work moved anglerfish from TRL1 (a theoretical idea) to TRL6 (Technology demonstrated in relevant environment).



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Conclusions and recommendations

Four different techniques were proposed as potential methodologies to identify or validate the spatial origin of fish. Some techniques were advanced and could be operationalised now, especially genetics and in specific cases stable isotopes.

The following conclusions can be drawn:

- Stable Isotopes The combined stable isotope compositions of carbon (C) nitrogen (N) and sulphur (S) shows great promise as a geographic indicator for marine food stuffs. C N and S isoscapes have been built for the entire UK shelf, and initial reference datasets are being compiled for major cod fishery locations across the North East Atlantic Area. Limitations of stable isotope based geographic assignment in general are the relatively small number of available isotope tracers (usually 2 or 3) and the potential for behavioural (i.e. non-location) influences on tissue isotopic compositions. The lack of reference datasets of the isotopic composition of known origin fish remains a barrier to wider use.
- Lipids There is promising evidence that very different fishing regions such as UK vs. Adriatic based clams can be differentiated based on their fatty acid profiles and / or their biochemical profile. Potential biomarkers (tentatively identified) have been discovered that can differentiate clams from the Adriatic Sea compared to the English Channel.
- Trace elements Trace element based spatial approaches have proven successful in elemental discrimination among spatially separated fish stocks. The suite of elements that can be used is limited by the level of physiological control of many elements in fishes, the relative homogeneity of marine waters, and the cost of assembling suitable reference samples. Spatial variation in bioavailable trace element compositions tends to occur at local, small spatial scales rather than broad regional scales, and in the case of certain elements largely reflects current and historic anthropogenic inputs to the marine environment. Trace elements would best be employed with other methods and could provide fine scale differentiation that cannot be obtained using any other natural tracer.
- Genetics The validated procedures for cod, hake, and to some extent sole, can be employed immediately, there has been significant investment already made by the UK government in previous years. Commercial accredited laboratories are available to provide this service following the Defra Standard Operating Procedures. This is likely to cost upwards of £20 a sample in a laboratory.

The following recommendations are made for next steps:

 Stable Isotopes - The TRL for stable isotope methods will be best advanced through the development and maintenance of reference datasets particularly assessing species-specific isotopic variation across key locations.



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- Lipids To advance the TRL two follow-on studies are recommended: work to confirm the tentatively identified markers and secondly a study looking at additional sea areas with greater numbers of samples.
- Trace elements The TRL for trace element methods will be best advanced through a combination of predictive spatial modelling and the development and maintenance of reference datasets.
- Genetics The availability of spatial data varies greatly by species, outside of cod, hake and sole the required baseline information needs to be generated and investigated as to whether the diagnostic SNP polymorphisms exist for the areas of interest. Improvements can also be made to the existing tools to further refine the analysis.

Combining techniques

Although all techniques were able to identify spatial differentiation was possible, further work is required to confirm if detectable differences align with ICES management areas. Spatial differences identified by each technique are driven by different process that are usually un-related, e.g. feeding patterns for stable isotopes, geology for trace elements and interchange in populations for genetics. MMO therefore expects it may be possible to improve spatial differentiation by combining the results of different techniques to improvement in traceability above that provided by any method in isolation. Use of multiple techniques can also address caveats in one or other techniques. For example, fish migrants and hybrids were identified by genetics and could incorrectly be assigned to a different population. Advanced techniques such as genetic assignment might best be applied in concert with other non-genetic methods, such as stable isotopic or trace element analyses or alongside other conventional enforcement intelligence. In the case of genetic analysis information from a second source may mean the number of genetic markers necessary to assign a fish to a population may be reduced. For some species the TRL is high across a number of techniques for example cod, making multi-class assessments viable for this species now and therefore increasing the evidential certainty of an origin assignment.

MMO comments

This series of reports has shown there are a number of techniques available to the MMO to validate the origin of capture of fish. The readiness levels of these techniques vary by species, where some have been more widely studied and others more likely to present differences based on a species behaviour including for example site attachment. Reports make recommendations to the MMO on how to implement techniques for marine enforcement officers and these can be considered. An appropriate and robust chain of evidence and analytical handling with be important factors to consider. Genetic material for example becomes degraded unless frozen or preserved. Preserved samples are less suitable for multi-technique analysis. Commercial laboratories are available now to run these techniques. Sample costs and processing time are declining year on year. Collectively theoretical underpinning, existing and continually expanding references databases and exiting available analytical facilities all increase the feasibility of this work.



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The legal basis for prosecution drawing on analytical techniques herein is also developing including two recent cases in Denmark using genetics. In 2003, a fisher declared 7,759 kg of cod as being harvested from the eastern Baltic Sea. Inspectors observed that the fish resembled North Sea cod in appearance and size and were associated with a large by-catch of pollack which is unusual for the Baltic Sea. DNA-analysis showed that the provenance probability of the cod allegedly from the eastern Baltic was one in 6 million and 1 in a 1,000 that the fish originated from the western Baltic. The case was judged in court in 2005 and the verdict, substantially influenced by the DNA evidence was delivered in early January 2006. The 7,759 kg of confiscated cod were worth 25,131 USD and a fine of 9,218 USD was imposed.

The second case took place in 2006, when two fishing vessels landed 922 tonnes of sprat from the Baltic Sea. The vessels were only allowed to harvest 400 tonnes each from the Baltic Sea, and the vessel owners invented a trip in the logbooks claiming the fish in excess was caught in the North Sea. Genetic testing showed that it was highly unlikely that the catch originated (partly) from the North Sea. Confronted with this evidence, combined with satellite tracking records, the fishers accepted a fine and the confiscation of the catch without going to court. Fines accounted for 24,055 USD and the confiscated catch accounted for 41,238 USD.

There is great potential in this area of work and the MMO will continue to have a keen interest in complementing the marine compliance work done currently with new and innovative ways of working.

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Further information

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