



## Aim

This proof of principle study investigated a non-targeted metabolomics approach using Mass Spectrometry (MS) to evaluate if this is a potential approach to distinguish between line and hanging net caught mackerel.

## Introduction and methodology

As part of the Marine Management Organisation's (MMO) work looking at enhancing provenance and traceability an assessment of approaches for confirming by which method fish sold ashore were originally caught was sought. This will potentially support and verify other systems for managing compliance and enforcement. Certain fisheries regulations only apply to particular species and certain rules only apply to the specific type of fishing gear which was used to catch the fish.

Based on the premise that different fishing gears cause different stress on the fish this project attempted to replicate a non-targeted metabolomics approach used in Black et al. (2017) using Rapid Evaporative Ionisation MS to attempt to observe differences in the biochemical make up between line caught and net caught Atlantic mackerel (*Scomber scombrus*). Mackerel from Cornwall was sourced for the project both line caught and net caught which were frozen and sent to the laboratory for testing in the mass spectrometer.

## Results

From the positive mode data set approximately 4,500 potential compounds were detected from all of the samples analysed. The negative data set provided approximately 3,600 potential compounds giving a total of approximately 8,000 potential compounds to further interpret.

Analysis showed sixteen peaks (potential compounds) across both MS modes found to be significantly different between the sample sets. Seven of these compounds were tentatively identified in non-targeted analysis to allow further analysis. Working from the assumption that net caught fish will be under extra stress than line caught fish, the level of compounds were compared between the two sample groups, it was found for example, glutathione abundance was 30 times lower in the net caught sample set compared to the line caught set. This decrease was found to agree with Nakano et al (2014). Other compounds tentatively identified as significantly decreasing in the net caught sample set include erythrose-1-phosphate, phosphonopyruvate and the peptide phenylalanyl-gamma-glutamate. Some compounds were found to be the reverse in that they were increased in the net caught fish: epoxy docosahexaenoic acid (DHA), the vitamin C related compound ascorbate-2-sulfate and 2-hydroxy-4-(methylthio)butanoic acid which was 71 times higher.

In targeted analysis the sugar based molecules glucose, glyceric acid and levoglucosan all were found to have a significantly lower abundance in the net caught sample set which agrees with the findings in Wen et al. (2018). The non-proteinogenic amino acid ornithine and amino acid metabolite 3-aminoisobutyric acid likewise had significantly lower abundance in the net caught



sample set, however Wan et al, (2008) reported these as significantly increasing in a stress related sample set in their study.

## Conclusions and recommendations

In this controlled study where we had control of where the samples were sourced, how they were caught and then how they were subsequently stored / transported for analysis, there is promising evidence to show metabolomics can determine if a fish sample has been exposed to a stressful capture such as hanging net.

The use of both the full compound dataset and a number of key metabolite compounds have promising implications for the approach. A number of the tentatively identified compounds have previously been identified as stress related in previous literature.

In particular the ascorbate / glutathione metabolism changes alongside DHA (upregulated to possibly deal with tissue stress) potentially provides a promising set of metabolites that could be measured in a targeted fashion to determine the physical / abiotic stress that may be induced by net capture. It is likely that the observations described above would be applicable to the majority of fish species, however the biomarkers may change within species.

This approach could have serious limitations in a real world setting with limited control over the handling of fish to be tested. It is recommended that there be a follow-on study to test a much greater number of samples, with a range of locations, over a time period, looking at different species and comparing net and line with trawl net.

## MMO comments

This project has shown there is a potential new tool to assist with catch verification and traceability with regards to which fishing gear type was used to catch fish. The technology is currently at a Technological Readiness Level (TRL) of 4, there is significant work needed to address potential limitations in a real world application of this and increase the level to a complete system for an operational environment. This project will help guide MMO priority setting in technology and innovation planning.

## Further information

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