ICES Special Request Advice
Northeast Atlantic ecoregion
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EU Request on the International Manual of Procedures (IMP) to be used in the NAFO Regulatory Area to guide the collection of samples from fisheries products for genetic analysis

Advice summary

ICES concludes that the International Manual of Procedures (IMP) will strengthen the ability to monitor and control for compliance with species labelling. ICES notes that the manual complies with the Common Fisheries Policy Control Regulation (EC) No 1224/2009, and is valuable in support of routine applications of genetic methods in fisheries management. ICES further concludes that the genetic techniques advocated in the IMP allow for unambiguous species identification.

ICES recommends that the reference to the maximum number of days from collection of a sample to its delivery to laboratory should be removed from the IMP as such a time limit is unnecessary when samples are adequately handled and preserved, as detailed in the IMP. ICES further advises that the manual would benefit from critical revision and editing to ensure unambiguity and clarity of the provided instructions for sample collection and preservation, data collection, and handling procedures as well as documentation of these steps. Additionally, the inclusion of schematic illustrations would help users to follow the instructions during sampling, sample processing, and transport to the laboratory.

ICES further recommends several practical steps for quality assurance and effective implementation of the IMP, such as: i) the training of inspectors for sample collection and storage, ii) the production of a one-page, weatherproof sampling protocol, and iii) the operational exchange of experiences between relevant authorities. ICES also endorses a pilot period to both test protocols and overall implementation of the manual.

Request

DGMARE Special Request to ICES:

Notwithstanding the already long process for the conclusion of the IMP, DGMARE, in the purview of further strengthen the quality of the IMP and in particular with the objective of further enlarge the peer reviewing process of the document, seeks for ICES review and for scientific advice about the IMP in specific on:

1. *The approach for collection of genetic material for sampling;*
2. *The sample material collection, preservation and transferring to laboratory;*
3. *Adequacy of the genetic technique advocate in the IMP provided that it should produce unequivocal evidence about species identification/misidentification; notably for presentation in court should that be the case.*

Moreover, as regarding point 1 of the present request, and as a result of the on-going EFCA consultation, DGMARE (D4) it was flagged potential implementation issue related with the number of days for the transferring of the genetic sample. The current version of the IMP foresees a maximum of 5 days between the sample collection up to its delivering in the laboratory. According to EFCA, this is deemed to be a very short period. EFCA has informed that at least a period of 7 days should be sought.

Therefore, in answering to point 1 of the current request, ICES is requested to advice on the relevant preservation method to ensure the integrity of the genetic samples for an extended period of at least 7 days of sampling delivery a proper laboratory facility.

Elaboration on the advice

Adequacy of the proposed IMP approach for collection of genetic material from sampling of fish for species identification

ICES notes that the proposed sampling design is based on previous knowledge of the probability of detecting mislabelled specimens in the target geographical area. The case studies presented in the IMP use catch data from previous years to define this probability. ICES recommends that the manual should be amended with information on: i) the degree of reliability of the catch data provided by fishers in relation to the correct identification of the involved species and ii) the

underlying assumption of the sampling design that "*the identities of the species being investigated are distributed normally or nearly so*" by presenting a few examples of which species and/or in which cases the assumption is applicable. ICES notes that this might be an important consideration in the context of legal investigations.

ICES recommends amending the guidelines by providing a very clear and robust proceedure for selection of the boxes for sampling. The amendments should include an infographic to illustrate this procedure.

ICES recommends amending the guidelines with detailed instructions on how to choose the fish or product to sample from a box by providing a robust, standardized, and well-documented sampling procedure. The requested amendments to the guidelines should consider that: i) the fish (product) may be frozen and ii) sampling can be performed both on board a vessel and at the port (on landed fish). The amendments should include an infographic to illustrate the procedure when selecting the boxes to be sampled.

ICES endorses the pilot period to test protocols and overall implementation of the manual in order to both verify the technical suitability of the approach and to estimate the probability values required for the statistical approach underpinning the sampling method. Consideration should be given to updating the manual based on the pilot test before starting routine applications.

Fully following the protocol is essentially important for assuring quality of the process. This is challenging under harsh weather conditions. Therefore, ICES recommends the training of inspectors in charge of sampling and sample processing prior to the work and suggests the production of one-page, weatherproof sample collection protocol to aid samplers for reference, supplemented by an infographic to improve clarity.

Preservation method for maintaining sample integrity for an extended period

Once collected, samples should be preserved in conditions that ensure their integrity over an extended period of time. Both methods suggested in the protocol (i.e. ethanol 96% and RNA*later*) are suitable preservatives for samples collected for genetic analyses. In both cases, it is crucial that at all times: i) the liquid fully covers the sample and ii) the ratio of sample to preservative is at least 1:5. Tissue samples should not be larger than 0.5 cm^3 (roughly of the size of the nail clip), which implies that the tissue sample from which the three subsamples should be collected should not be larger than 1.5 cm^3. Additionally, while storage of preserved samples at room temperature is acceptable for up to one month, for longer-term storage the preserved samples should be maintained at −20°C. RNA*later* allows easier sample transportation (as it is not flammable and thus does not require special transportation permits and conditions). ICES recommends updating the protocol accordingly.

ICES recommends removing from the IMP the reference to the maximum number of days from collection of a sample to its delivering to a laboratory as this is not required if samples are adequately handled and preserved.

ICES also recommends to amend the manual by stating that:

- the sample should be taken immedately after the fish has been caught, or the fish should be frozen for future sampling. Alternatively, the fish can be refrigerated (at 4°C) for one day prior to sampling;
- the preservative of equal volume should be replaced after 24 hours for long-term storage of samples at −20°C;
- ethanol to be used must be analytical or molecular grade.

Adequacy of procedures for sample material collection, preservation, and transfer to laboratory

Although the protocol advocates the use of samples of fin clip or muscle tissue, samples from other tissue (such as gill) can also be taken. ICES advises updating the protocol accordingly.

Detailed recording of sampling procedure – including sample collection, preservation, and transfer to laboratory – needs to be ensured. Currently, several of the relevant statements in the manual are insufficient to assure sample quality and to ensure maintenance of integrity of collected samples over longer periods of time.

ICES recommends the following revisions to the manual:

- Establishing a mechanism to link each sample to the metadata of the catch it came from;
- Revising the sample collection protocol to ensure unambiguity of instructions and steps in the sample collection, preservation, and transportation process as well as documentation of these steps;
- Specifying detailed actions to prevent and detect cross-contaminations and recording errors during the process.

Adequacy of the genetic technique advocated in the IMP, provided that it should produce unequivocal evidence about species identification/misidentification

The IMP presents the most common DNA-based methods for species identification, focusing on Sanger sequencing and quantitative PCR (qPCR). ICES notes that the suggested methods are suitable for unambiguous species identification. ICES recommends replacing the term 'Sanger sequencing' with 'DNA sequencing' to accomodate developments in novel sequencing technologies.

ICES endorses that unambiguous taxonomic identification of samples without any prior information should be done using sequencing.

ICES recommends that the protocol clearly specify that the approach for species identification should be designed on a species- and case-specific basis. It should include issues such as interspecific hybridization and levels of genetic variability for the chosen genetic marker.

Basis of the advice

This advice on the IMP for genetic sampling has been issued in response to a request from the European Commission, and it specifically addresses the procedures for sampling, storing, and transporting tissue samples from fish and fish products from fisheries in the Northwest Atlantic Fisheries Organization (NAFO) area. Thus, the advice is based on the topics and approaches covered in the IMP, with the aim of securing representative sampling of the fishery.

It should be noted that, as a sampling strategy, the IMP only considers random sampling. ICES considers that there are situations where a more targeted sampling approach could be appropriate. ICES advice is based on the methodologial approach used in the protocol.

ICES does not provide advice on the use of the IMP's techniques and procedures in a court of law as it is outside our purview and the scope of this advice. That said, ICES does find that, with the feedback that ICES provides on the IMP, the sampling procedures and genetic techniques described represent the best practices on the matter.

The current ICES advice is based on obtaining the best quality DNA samples for downstream species identification analyses. It should be noted that: i) DNA degradation (the genome breaking down into small pieces) starts when the fish dies and ii) determining the exact times after which and conditions under which the DNA is no longer valid for genetic analyses is not possible. Therefore the advice is based on ensuring the best conditions for delaying DNA degradation, which are: i) collecting the sample as close as possible to the fish's death, ii) keeping the dead fish at the coolest temperature possible, and iii) storing the sample using appropriate preservative conditions (Rodriguez-Ezpeleta *et al*., 2013).

Additional information

The IMP is not the first case of a guide being provided on the collection of samples from fisheries products for genetic analysis, but it is the first time that DNA techniques will be used on a large scale in European waters (and beyond) to monitor for compliance with species labelling rules. In addition to the Portuguese manual of procedures (DGRM, 2015), which was the basis for the elaboration of the IMP, another relevant example is the test project carried out by the Danish AgriFish Agency, which explored the feasibility of fishery inspectors undertaking *in situ* tissue sampling for DNA analysis (Martinsohn *et al*., 2019). With respect to genetic sampling strategies, the Office of Law Enforcement and Marine Forensics Laboratory of the US National Oceanic and Atmospheric Administration (NOAA) supported the legal investigation into a

large-scale false labelling scheme of catfish imported into the United States of America and intentionally mislabelled as higher-value fish species (Martinsohn *et al*., 2019).

Genetic methods to trace escaped farmed salmon back to their farms of origin have been developed and implemented by the regulatory authorities in Norway for over a decade (Glover *et al*., 2008; Glover, 2010). These methods have also been adapted and implemented to successfully trace rainbow trout (Glover, 2008) and Atlantic cod (Glover *et al*., 2010, 2011) escapees.

DNA barcoding represents the highest level of direct information on genetic differences between species and provides the necessary baseline for designing and validating assays and protocols for qPCR. The latter method can still be tested and validated to forensic standards with unequivocal species identification and may be more cost-efficient for analysing higher numbers of samples (Zhang *et al*., 2013).

New methods are under development (e.g. onsite high throughput sequencing and paper strip based reactions) which require further work before routine applications (Pomerantz *et al*., 2018). These should be considered when updating the protocol in the future.

The protocol advises using Genbank as the reference database for taxonomic assignment. However, it has been documented that Genbank contains errors (Li *et al*., 2018). There are more reliable databases available, such as BOLD, but these are less comprehensive. Therefore, prior verification of available reference databases is desirable to ensure reference data are available and there is a sufficient taxonomic resolution.

Concerning uncertainties on taxonomic assignment, the protocol mentions that when 100% similarity is not obtained, a result should be confirmed with a phylogenetic tree. It should be noted that there are situations when even a 100% similarity or closest relative in a phylogenetic tree to a sequence in the database does not mean accurate taxonomic assignment. This can occur when there is mitochondrial introgression from one species to another, e.g. about 2% of Atlantic bluefin tuna contain albacore mitochondrial DNA (Diaz-Arce *et al*., 2016), or when the fragment sequenced does not contain enough variation to distinguish between closely related species (Tahir and Akhtar, 2015). For this reason, assays developed for species identification should be specific to each case study, considering information available in the literature and performing any additional research required.

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