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HSBI DNA SAMPLING GUIDE

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Voluntary HSBI Regional Guides

TOOLS FOR HIGH SEAS BOARDING AND INSPECTIONS

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HSBI DNA Sampling Guide

Document History

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PURPOSE STATEMENT

1. This document provides guidance to Authorised inspectors conducting tissue sampling and subsequent DNA sequencing and analysis (hereafter, broadly referred to as “DNA sampling”) as part of WCPFC High Seas Boarding and Inspections (HSBI¹). For CCMs wishing to use DNA sampling for HSBI, the development of this Guide also intends to:
 - support the establishment of a robust DNA sampling process by CCMs at the CCM’s level to verify species identification of individual specimens in support of HSBI
 - assist CCMs in ensuring that DNA data are credible and admissible using appropriate methods and procedures throughout the entire DNA sampling process
 - support the establishment of minimum practices at the CCM’s level which are necessary to ensure that DNA sampling produce accurate, precise analytical findings, and findings are conveyed in an unbiased, objective manner

¹ HSBI, refers to boarding, inspection, and related activities on the high seas within the Convention Area conducted pursuant to CMM 2006-08 Western and Central Pacific Fisheries Commission Boarding and Inspection Procedures or any successor CMM.

- provide guidance to CCMs on tools that can be used for gathering and preserving tissue samples during HSBI and the minimum standards for DNA sequencing and analysis.
2. This Guide sets out the minimum standards in the application of DNA sampling during a HSBI and the post analysis process, which includes:
 - tissue sampling
 - tissue sample handling, preservation, sealing and storage
 - tissue sample transfer/shipping
 - DNA extraction, sequencing, and analysis in accordance with accredited procedures
 - transmission of DNA results
 - DNA sequence, extracted DNA, and tissue sample retention and accessibility for flag CCM testing.
 3. The application of this Guide will be voluntary and apply to authorised HSBI activities within the WCPFC convention area.
 4. This guide can be modified in response to new information, technical innovations, and perspectives. It is expected that this guide will continue to evolve as the field develops.

Application of DNA sampling in WCPFC HSBI activities.

5. The aim of HSBI is to check whether a vessel is operating in compliance with the WCPFC Convention and all applicable WCPFC Conservation and Management Measure (CMM) obligations.
6. Inspectors conducting HSBI activities can detect and confirm species on board at the time of inspection. Sometimes, a visual inspection of morphological characteristics may be all that is needed to obtain a species identification.
7. Genetics offers a powerful tool to complement the work of Authorised inspectors conducting HSBI activities as it allows conclusive species identification.
8. Genetic analysis can be a useful method for species identification when species identity cannot be determined on a purely morphological basis, for example if the morphological characteristics are unfamiliar to the inspector, similar, or absent.
9. Genetic analysis through DNA sequencing of fish for identification can support investigations to verify a vessel's reported catch, through providing additional proof and the ability to confirm the identity of the species in question. Examples include, to determine between:
 - Pacific and Southern bluefin tunas
 - small-sized bigeye and yellowfin tunas, and
 - different bycatch species that are prohibited for retention.
10. DNA sequencing results can be used to corroborate other forms of evidence such as vessel logbooks and photographs taken by the Authorised inspectors. This can be used to support risk assessments to prioritise the vessel for further investigation and prosecution as determined by the flag CCM.

11. The use of DNA sampling during HSBI can assist the flag CCM with assessing compliance with vessel licensing and reporting obligations, including to:
 - confirm species identification
 - verify that only species which a vessel is authorised to catch are being retained and declared
 - verify catch reporting and catch log data
 - verify if protected species are being retained.

HSBI DNA SAMPLING Minimum Standards

Methods of tissue sampling (not limited to)

Laboratory-based analysis		Field-based analysis
Muscle Biopsy	Fin Biopsy	Other
DNA biopsy sampling involves taking a tissue sample from a single fish.	Tissue sample is collected from a single fish through cutting off a section of the fin.	Rapid field-based DNA test

EVIDENTIARY PROCEDURES for DNA Sampling

12. The general principles and procedures for DNA sampling in fisheries monitoring and investigations:
 - a) Documenting and recording tissue sampling*
13. Tissue sampling should be documented using a recording device, including photographs and videos.
14. Tissue sampling should be conducted with witnesses' present (Authorised inspectors, master, crew, boarding party) and prioritise that witnesses from the fishing vessel are present. Authorised inspectors should ideally work in pairs.
15. Authorised inspectors should record in the HSBI report, including but not limited to the following information:
 - date of the inspection
 - vessel name
 - vessel location (coordinates at time of boarding)
 - tissue sampling information:
 - sample identification number
 - location of fish sampled (e.g. blast freezer, hold #)
 - description (processed state of fish)
 - comments, including the reason for or background of the tissue sampling
 - the master of the vessel must be provided with an interim copy of the report which includes details of any tissue sampling. The master must also be given to opportunity to include any objection or comment to be included in the final report.
- b) Collection and preservation of tissue samples*
16. Authorised inspectors should:
 - photograph and record tissue sampling (see chain-of-custody, below)

- take tissue samples, to the extent practicable, from the commercially least valuable part of the fish, such as the tail.
- collect, label, preserve and seal each tissue sample separately, at the sampling site.
- label each tissue sample with the following minimum details on the sample labels:
 - Date
 - Unique sample reference number
 - Vessel name
 - Collector name
- affix the waterproof sample labels directly to the sample vials or collection bags.
- keep the tissue samples in a cool, dark environment, preferably a freezer when available.

c) Preventing cross-contamination of tissue samples

17. Protective measures are necessary to prevent cross-contamination of samples. The following should be used for each individual sample:

- Using new, washed or unopened sampling tools
- Wear single-use disposable gloves.

d) Maintaining a chain of custody for tissue samples between the sample site and the testing laboratory

18. From the beginning to the end of the DNA sampling process, it is crucial to be able to demonstrate every single step undertaken to ensure traceability and continuity of the sample. The integrity of tissue samples and, later, extracted DNA samples, must be maintained as they pass from one person to another.

19. The 'chain of custody' is a continuous record of the life of the sample from the moment it was sampled to the moment it is analysed. Every step must be recorded and verified to ensure the sample is not tampered with, changed or lost. It is the Authorised inspector's responsibility to ensure chain of custody of the tissue sample by ensuring:

- the tissue samples are stored in a sealed bag or envelope.
- the chain of custody record is maintained.
- the tissue samples are sent to an accredited laboratory for testing as outlined in the CCM's DNA sampling procedures.

e) Transmission of DNA sampling results to the flag CCM

20. Authorised inspectors should note in the full inspection report, that DNA sampling occurred and the results will be provided to the flag CCM in due course.

21. Timing of DNA analysis and results will vary depending on circumstances, such as:

- time for tissue sample to return to port
- time to facilitate arrangements to deliver the tissue sample to an accredited laboratory
- time to deliver the tissue sample to an accredited laboratory
- time for extraction, sequencing, and analysis of the DNA by an accredited laboratory.

22. Once the DNA sampling results are received by the relevant authority of the inspection vessel, they should be provided to the flag CCM within 5 business days.

CCM DNA sampling and analysis procedures

23. CCMs wishing to use DNA testing for HSBI should share their DNA Sampling Procedures with the Secretariat for posting on the HSBI page on the WCPFC website.
24. The CCM's DNA Sampling Procedures should include:
- DNA sampling method for HSBI activities
 - DNA sampling procedures for HSBI activities
 - Chain of custody form
 - Details of testing Laboratory and credentials and recognised standards, these could include:
 - ISO 17025 / 9001 – *this accreditation supports laboratories in maintaining complex processes of testing and calibration to the highest standards and demonstrates to external clients that the laboratory outputs are valid and reliable.*
 - Quality Management Systems (QMS)
 - Society for Wildlife Forensic Science (SWFS) Standards and Guidelines for Wildlife Forensic Analysis – *the minimum standards and additional guidelines for wildlife forensic analysts in the sub discipline of DNA*
 - Genetic reference database – *used for species assignment for WCPFC catch and compliance*
 - Sample retention and accessibility – tissue and DNA extracts should be retained, for up to [2 years] to allow for future testing by the flag CCM, if requested.

Accessibility of DNA Sampling and Multi-language information

25. To assist the DNA sampling process during HSBI activities, it would be beneficial for the CCM's DNA sampling procedures to be translated into languages that are in use on fishing vessels and/or as pictographs to bridge any language barriers.
26. The following supporting documentation should be considered for translation into flag CCM languages by the Authorized inspector CCMs:
- HSBI multi-language cards
 - DNA sampling procedures provided online.
 - DNA sampling procedures given/shown to master of vessel prior to DNA sampling by HSBI Authorised inspectors.
27. In addition, flag CCMs should also consider providing information about DNA sampling procedures that may be used during HSBI Inspections to their fishing vessels in a language(s) used by their vessels.

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