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# Evidence-Based Monitoring: Molecular Tools for Fisheries Oversight

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# Evidence-Based Monitoring: Molecular Tools for Fisheries Oversight

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## Table of Contents

## 1. Glossary

**Assay** – A combination of genetic tools (e.g., primers, probes, enzymes) used to detect and analyse specific DNA regions or species of interest in a biological sample.

**ANSI/ASB** – American National Standards Institute / Academy Standards Board. These bodies develop and publish consensus-based standards, including those for forensic science.

**BLAST** – Basic Local Alignment Search Tool. A tool that compares unknown DNA sequences to known sequences in public databases (e.g., GenBank) or curated reference datasets to find regions of similarity.

**Chain of Custody (CoC)** – The documented and unbroken trail of sample handling and transfer from collection to analysis, essential for ensuring evidentiary integrity in forensic investigations.

**CKMR (Close-Kin Mark-Recapture)** – A genetic method used to estimate population size and demographic parameters by identifying close familial relationships (e.g., parent-offspring pairs) within a sample.

**DNA (Deoxyribonucleic Acid)** – The molecule that carries genetic instructions in all known living organisms and many viruses.

**Environmental DNA (eDNA)** – Genetic material released by organisms into their environment (e.g., water, sediment). In fisheries, eDNA can be used to detect presence of target, bycatch, or protected species on fishing grounds or vessels.

**ENFSI-APST** – European Network of Forensic Science Institutes – Animal, Plant and Soil Traces working group. Develops best practices for non-human forensic science in Europe.

**ISO/IEC 17025** – An international standard that specifies requirements for the competence of testing and calibration laboratories, widely used in forensic accreditation.

**Locus (plural: Loci)** – A fixed position on a chromosome where a particular gene or genetic marker is located. Multiple loci are used in species and population-level genetic comparisons.

**Oxford Nanopore Technologies (ONT)** – A portable, long-read DNA sequencing technology suitable for field-based applications. ONT allows sequencing of longer fragments, improving taxonomic resolution compared to short-read methods.

**Polymerase Chain Reaction (PCR)** – A widely used technique to amplify specific segments of DNA, enabling further genetic analysis.

**Primer** – A short strand of DNA that binds to a specific DNA region to initiate amplification during PCR.

**Reference Database** – A curated collection of DNA sequences from verified species used to identify unknown samples through sequence comparison.

**Single Nucleotide Polymorphisms (SNPs)** – Single base-pair variations in the genome. SNPs are useful for population genetics, stock structure analysis, and CKMR studies due to their abundance and resolution.

**Society for Wildlife Forensic Science (SWFS)** – An international organisation dedicated to the advancement of wildlife forensic science, including development of standards and professional training.

**Laboratory Information Management System (LIMS)** – Software used in laboratories to manage samples, metadata, workflows, and maintain chain of custody in compliance with regulatory and forensic standards.

**qPCR (Quantitative PCR)** – A PCR-based technique that quantifies DNA in real time and can be used for species-specific detection when targeting known sequences.

**Voucher Specimen** – A physical specimen (or photographic/morphological record) of a known species from which a reference DNA sequence is generated and archived in a curated collection or museum.

## 2. Executive summary

Regional Fisheries Management Organizations (RFMOs) play a critical role in the sustainable management and conservation of global fisheries. However, traditional monitoring methods are increasingly limited in their ability to detect species accurately, verify catch composition, and support enforcement in the face of modern challenges such as IUU fishing, bycatch, and opaque supply chains. As fisheries governance becomes more complex, there is growing interest in the use of molecular tools that offer high-confidence species identifications and support evidentiary requirements in monitoring, control, and surveillance (MCS).

This report explores the application of molecular monitoring tools ranging from DNA barcoding and metabarcoding to portable qPCR and LAMP assays for both field and laboratory-based use. These tools can provide species-level identification from a diverse array of samples (e.g., biopsies, brine tanks, swabs), and can enhance MCS workflows by supporting risk-based inspections, trade verification, and compliance decision-making.

To ensure reliability and defensibility, molecular monitoring must be underpinned by quality assurance frameworks and minimum standards. This report draws on established international wildlife forensic bodies (e.g., ANSI/ASB, SWFS, ENFSI-APST) to define minimum standards required for critical elements such as chain-of-custody protocols, method validation, analyst training, and the role of curated reference databases. It acknowledges that while ISO/IEC 17025 accreditation remains the gold standard for forensic laboratories, alternative forms of assessment such as the SWFS Assessment Program, offer practical pathways for laboratories operating in resource-limited settings to demonstrate technical credibility.

Through platform comparisons, case studies, and sampling scenarios, this report provides practical guidance and minimum standards for integrating molecular monitoring tools into RFMO frameworks. It also outlines opportunities for the Western and Central Pacific Fisheries Commission (WCPFC) to lead regional efforts by developing validated primer sets and reference databases tailored to commonly landed species, thereby improving data consistency and forensic robustness across member states.

# 3. Introduction

Fisheries Monitoring, Control, and Surveillance (MCS) is critical for ensuring compliance with management regulations, preventing Illegal, Unreported, and Unregulated (IUU) fishing, monitoring interactions with threatened and endangered species, and maintaining sustainable fish stocks. Regional Fisheries Management Organizations (RFMOs), particularly those that focus on highly migratory species such as tuna, oversee vast ocean areas and manage fisheries that contribute significantly to global food security, economies and livelihoods. However, these organisations face substantial hurdles in fulfilling their mandates, including complex governance structures, the influence of diverse geopolitical interests, and the challenges of consistently integrating scientific advice into management decisions.

Accurate and comprehensive monitoring of fisheries is important for sustainable management. This includes gathering reliable data on target catch, the incidental capture of non-target species, fishing effort, and adherence to conservation regulations and mandates. Bycatch, in particular, poses a significant threat to marine biodiversity, often impacting vulnerable, endangered, or protected species, and undermining the long-term health of marine ecosystems (Komorosoke & Lewison 2015). Traditional methods of at-sea monitoring, which heavily rely on human observers deployed on fishing vessels, face inherent limitations in terms of spatial and temporal coverage, cost-effectiveness, and potential biases, as well as safety concerns for observers (Clarke et al. 2013; Ruiz et al. 2015; Williams et al. 2016). These limitations necessitate the exploration and adoption of innovative monitoring technologies that can provide more comprehensive, accurate, and efficient data to support the work of RFMOs.

Molecular methods have emerged as powerful tools in fisheries science, offering a suite of techniques that can address many of the challenges associated with traditional monitoring approaches. These methods leverage the analysis of DNA to provide high sensitivity and accuracy in species identification for target and incidental catch, processed products, or from non-invasive environmental samples. Molecular techniques can also reveal population dynamics, life history traits, and intricate ecosystem interactions, all of which are important for effective fisheries management and conservation (Dudgeon et al 2012; Gilbey et al 2021). The rapid advancements in genetic markers and sequencing technologies over the past decade have further propelled the application of molecular methods in fisheries science, offering opportunities for enhanced monitoring.

## 3.1 Evidentiary use of DNA in Fisheries Enforcement

DNA-based tools are increasingly being adopted in fisheries management not only for monitoring but also as formal compliance and risk assessment instruments. In the context of the WCPFC, molecular monitoring can play a critical role in detecting misreporting, identifying bycatch of protected species, and verifying the legality of products in the seafood supply chain. Importantly, wildlife forensic testing has a well-established history of evidentiary use in compliance investigations and legal proceedings. Wildlife forensic DNA evidence can and has met the threshold for use in prosecutable cases when laboratories adhere to established minimum standards (see Iyengar et al 2014 for extensive list of cases).

Minimum standards include rigorous sample handling, validated analytical methods, transparent chain of custody, and defensible reporting procedures as outlined throughout this report (see section 7 *Minimum Standards and Best Practices for Molecular Monitoring*). While ISO/IEC 17025 accreditation is considered the gold standard for human forensic laboratories, it is not a prerequisite for evidentiary use in wildlife forensics. Recognising that accreditation may be financially or logistically out of reach for many wildlife and fisheries laboratories, the Society for Wildlife Forensic Science

(SWFS) has established alternative assessment pathways that evaluate laboratory quality systems against field-specific criteria. These programs, alongside resources such as standard operating procedures (SOPs) and training frameworks enable non-accredited laboratories to demonstrate robust forensic practices.

Several international bodies including the American Academy of Forensic Sciences (AAFS), the European Network of Forensic Science Institutes (ENFSI), and SWFS, acknowledge that high-quality, defensible forensic analysis can be delivered outside of formal accreditation, provided internal quality management systems (QMS) are in place and proficiency is demonstrated. This precedent supports a pragmatic and inclusive model for the WCPFC, enabling the region to build molecular monitoring capacity for compliance purposes without imposing unnecessary regulatory barriers, while still meeting the evidentiary standards required for enforcement.

# 4. Core Molecular Workflows for Fisheries Monitoring

## 4.1 Sampling Strategies for Molecular Monitoring

Molecular methods in fisheries monitoring are grounded in DNA analysis, enabling accurate identification of species from biological samples. Sampling commonly involves collecting tissue biopsies (e.g., muscle or fin) from landed carcasses during portside or at-sea inspections. These samples provide high-confidence data for species identification and can also support more advanced analyses such as population genetics or Close-Kin Mark-Recapture (CKMR), particularly when collected at scale and accompanied by detailed metadata (Punt et al., 2024).

In addition to tissue sampling, newer approaches target environmental DNA (eDNA); DNA left behind in water, ice, or on surfaces. This includes sampling from catch tanks, trawl nets, brine tanks, hoppers, or freezer holds (Maiello et al., 2022; Green et al., 2024). These methods can capture broad species diversity but require stringent field and laboratory protocols to minimise contamination risk.

## 4.2 Ensuring Chain of Custody and Sample Integrity

Accurate species identification using molecular tools relies not only on robust sampling and laboratory methods but also on the integrity of the chain of custody; the process of documenting the handling of samples from collection through to analysis and reporting. In fisheries monitoring and compliance contexts, maintaining a clear and verifiable chain of custody is critical to ensure the admissibility, credibility, and traceability of DNA evidence, particularly when used for enforcement or regulatory actions (SWFS Technical Working Group, 2018; Frankham et al 2025).

Chain of custody procedures must be transparent, tamper-proof, and consistently applied across jurisdictions. The following minimum standards are adapted from international forensic standards (ANSI/ASB Standard 019 (2019); Frankham et al 2025) and tailored for use in fisheries molecular monitoring.

Best Practice Protocols for Chain of Custody (CoC):

- **Unique Sample Identification**
  Each sample must be labelled with a unique ID (e.g., barcode or QR code), linked to metadata including date, location, collector name, vessel or facility, and sampling method. Labels should be waterproof and affixed directly to sample vials or collection bags.
- **Field Data Capture and Metadata Recording**
  A field datasheet (physical or digital) must accompany each sample, documenting collection

details, sample type (e.g., fin, water, swab), suspected species (if known), and the storage medium (e.g., ethanol, silica, dry ice).

- **Sealing and Storage**
  Samples should be stored in secure, tamper-evident containers (e.g., sealed bags or cryoboxes). Any removal, transfer, or opening of a sample must be logged and justified.
- **Transfer Documentation**
  A signed chain-of-custody form must be completed each time a sample changes hands or location, whether from vessel to port, port to lab, or between institutions. This form includes details of sender, receiver, date, purpose of transfer, and condition of sample.
- **Laboratory Processing Records**
  Laboratories should maintain logs of when and how samples were processed, including who accessed them and the methods used. Digital tracking systems (e.g., Laboratory Information Management System, LIMS) are recommended for high-throughput environments.
- **Sample Retention and Storage**
  Following analysis, remaining DNA extracts and tissues should be archived in a secure biobank for future verification, subject to permitting, time and ethical standards.

### 4.3 Commonly Used Molecular Techniques in Monitoring and Compliance

A range of molecular techniques are now available to support species identification in fisheries monitoring. These methods vary in their resolution, speed, and suitability for different sample types and monitoring contexts (see section 8 *Technical Considerations for Molecular Monitoring Tools* for detailed information).

- **DNA Barcoding** – Tests for an unknown species from a single source sample. e.g., a biopsy sample taken from tuna carcass in a freezer hold or sample taken from an unknown fillet.
- **DNA Metabarcoding** – Simultaneous identification of multiple species from a mixed sample (e.g., eDNA in brine water)
- **Quantitative PCR (qPCR)** – A cost effective method to test for a species of interest from a sample which can be single or mixed source. Requires species-specific primers to be developed and produces more rapid results as sequencing is not required.
- **High Resolution Melt (HRM)** – A post-PCR analysis method that distinguishes genetic variants based on differences in DNA melting curves. It can be used to screen for species-specific genetic signatures and detect closely related species.
- **Loop-Mediated Isothermal Amplification (LAMP)** – A rapid test for a species of interest from a single source sample. Designed to be used for rapid on-the-spot identification of species of interest. Primer/probe development requires significant resources and time.

### 4.4 Integrating Molecular Tools into Fisheries MCS Workflows

Molecular monitoring enhances traditional MCS by providing accurate, verifiable data on catch composition and species identity across the seafood supply chain. These tools can be used during inspections, at port, or further downstream at markets and processors.

A typical protocol may involve:

- **At-sea or portside inspections**, where muscle biopsies, fin-clips, scales or swabs are taken.
- **On-site rapid screening,** using portable qPCR or LAMP devices to detect key species.
- **Laboratory confirmation**, where required, using barcoding or metabarcoding methods with established reference databases.

When appropriately applied, molecular monitoring support efforts to strengthen fisheries reporting, verify catch composition, and enhance transparency and accountability in line with international management and trade requirements.

## 5. Sampling Opportunities Across the Seafood Supply Chain


Molecular monitoring methods can be deployed at multiple points throughout the seafood supply chain, offering flexibility in how, when, and where DNA samples are collected. The choice of sampling location depends on the monitoring objective, for example whether to verify species identity, detect illegal or unreported catch, track product movement, or assess compliance with trade regulations.

**Key sampling opportunities include:**

1. **At-Sea Inspections:** Onboard sampling during active fishing operations, including from gear (nets, hooks), live wells, or brine tanks.

2. **Portside Inspections:** Sampling of landed catch as it is offloaded from vessels, often involving biopsy, swab, or eDNA methods.

3. **Pre- and Post-Freight Handling:** Sampling frozen or processed products during container loading/unloading or cold storage checks.

4. **Fish Markets:** Verification of species identity in unpackaged, processed, or whole fish sold in domestic markets.

5. **Processing Facilities:** Collection before, during, or after processing, including sampling of by-products or product intermediates.

6. **Artisanal Fishery Landings:** Supporting data collection in small-scale fisheries, where conventional monitoring is often limited.

7. **Export Inspection Points:** Sampling at customs, freight hubs, or shipping points for species verification in export documentation.

8. **Retail Outlets:** Market surveillance at supermarkets, grocery stores, or wholesalers to verify species identity in packaged, fresh or frozen seafood products.

9. **Restaurant and Food Service:** Sampling of prepared seafood dishes in restaurants or catering services to identify seafood provenance, detect species substitutions and ensure menu accuracy.

## 6. Field-Deployable vs Laboratory-Based Molecular Methods

The integration of molecular tools into fisheries MCS spans a spectrum from rapid field-based screening to full laboratory-based forensic analysis. Field-deployable technologies such as LAMP assays, portable qPCR devices, and handheld sequencing platforms offer considerable operational advantages: they can be used in remote locations, produce results within a few hours and support immediate decision-making during inspections. However, these tools are primarily designed for screening or intelligence-gathering, rather than formal evidentiary purposes. Their portability often comes at the cost of reduced sensitivity, taxonomic resolution, and reproducibility. In many cases, these methods are still undergoing validation, with limited inter-laboratory comparison or benchmarking against reference standards.

By contrast, laboratory-based methods benefit from controlled environments, trained personnel, and the ability to implement internationally recognised forensic standards. Accredited laboratories or those working to best practice, for example, following the guidance in ANSI/ASB Standards 048, 111, and 180, and SWFS recommendations can deliver results suitable for use in legal or compliance proceedings. These standards prescribe validated workflows, stringent quality control, and defensible documentation, including chain of custody protocols and taxonomic assignment criteria. While field tools are not expected to meet the same level of validation, they can serve as powerful frontline tools to guide inspections and flag samples for further testing under forensic conditions.

A pragmatic, tiered approach where field diagnostics are used for screening and risk prioritisation, and laboratory methods provide confirmatory testing, ensures that molecular monitoring is both operationally feasible and scientifically robust.

## 7. Minimum Standards and Best Practices for Molecular Monitoring

As molecular technologies become integral to fisheries monitoring and enforcement, they must be implemented within a recognised framework of minimum standards and best practices. The field of wildlife forensics provides a well-established foundation for such frameworks, ensuring that molecular evidence is generated and interpreted with scientific rigour, traceability, and legal defensibility. This section draws on international wildlife forensic standards to guide minimum standards for molecular testing in the WCPFC.

## 7.1 Laboratory Operations and Quality Assurance

Forensic wildlife laboratories must maintain documented Standard Operating Procedures (SOPs), contamination-controlled environments, and rigorous documentation from sample receipt through reporting (ANSI/ASB Std 019). Laboratories are expected to implement clean workflows with spatial separation of pre- and post-PCR areas, use personal protective equipment and negative controls, and maintain calibration and maintenance logs for equipment (SWFS Technical Working Group, 2018). These practices help safeguard data integrity and minimize the risk of contamination and are critical considerations when conducting species identification from trace or degraded DNA.

Accreditation under ISO/IEC 17025 remains the gold standard for forensic laboratories, but it is not always feasible, particularly for smaller or emerging facilities. In such cases, the ANSI/ASB standards (e.g., Std 019) and the Society for Wildlife Forensic Science (SWFS) (Frankham et al 2025) guidelines provide robust, community-accepted alternatives. These frameworks offer structured pathways for implementing quality assurance, chain of custody procedures, and defensible reporting practices, particularly in laboratories not operating within traditional criminal justice systems.

## 7.2 Analyst Competency and Training

DNA analysts must undergo documented training in molecular techniques, quality control, and taxonomic interpretation. In the context of mitochondrial DNA (mtDNA), training must include sequence alignment, detection of ambiguous bases or stop codons, and use of reference databases for taxonomic assignment (ANSI/ASB Std 111). Competency assessments should be repeated every two years and cover both theoretical and practical components. The SWFS guidelines also stress the need for continued professional development, particularly for staff involved in developing or interpreting novel molecular methods (SWFS Technical Working Group, 2018; Frankham et al 2025).

## 7.3 Chain of Custody and Sample Tracking

Maintaining sample integrity from field to lab is fundamental to defensible molecular monitoring. Chain of custody must be documented in detail, recording sample ID, collector, location, date, preservation method, and every transfer point (ANSI/ASB Std 019). Storage conditions must be recorded, and all packaging should be tamper-evident. SWFS recommends that metadata accompany all field collections and that samples be labelled using unique, non-repeating identifiers to avoid confusion during processing (SWFS Technical Working Group, 2018).

In fisheries settings, where inspections may occur at sea or across jurisdictions, this level of documentation is crucial to ensuring that evidence or monitoring data is admissible in regulatory or legal settings.

## 7.4 Method Selection and Validation

A foundational principle of molecular monitoring is “fit-for-purpose” tool development. Methods selected for field or laboratory use must undergo rigorous validation under realistic operating conditions. This includes defined assessments of specificity (the method’s ability to distinguish closely related species), sensitivity (the minimum DNA concentration required for reliable detection), repeatability (consistency of results within a single laboratory or operator), and reproducibility (consistency across different laboratories or users). In addition, precision and accuracy must be evaluated to determine how reliably the method produces correct identifications.

Validation should follow minimum standards as outlined in ANSI/ASB Standard 048, which includes: (1) clear documentation of the method’s intended purpose and scope, (2) specification of required reagents, instrumentation, and workflow steps, and (3) implementation of a quality assurance framework using positive/negative controls, calibrators, and reproducible documentation protocols.

For example, a qPCR assay designed to detect a specific bycatch species must be tested against closely related species to confirm it does not cross-amplify, and the assay must return accurate results at low DNA concentrations. Similarly, DNA metabarcoding pipelines should be validated using mock communities and replicate analyses to assess species detection rates and false positive occurrences. The SWFS guidelines further emphasize that any method used in an enforcement context must be repeatable, transparent, and fully documented. Laboratories must retain raw data and analysis files to allow independent verification and legal scrutiny (SWFS Technical Working Group, 2018).

## 7.5 Reference Databases and Taxonomic Evaluation

Genetic identifications rely heavily on public databases such as GenBank, Barcode of Life Database (BOLD), and MitoFish, which provide broad access to reference sequences for species assignment. However, these databases also carry risks, particularly for taxonomic groups like tunas and sharks, where high volumes of entries often include mislabelled species or inconsistent annotations, leading to potential misidentification despite data abundance (ANSI/ASB Std 180).

To improve the reliability of forensic identifications, ANSI/ASB Standard 180 sets out a series of minimum requirements for evaluating and reporting sequence-based taxonomic assignments:

- Exclude low-quality reference sequences, including those from environmental samples, synthetic constructs, and batch uploads (e.g., sequences tagged as "WGS," "library," "NGS," or lacking metadata).
- Assess sequence integrity, ensuring no ambiguous bases (Ns) or premature stop codons are present in coding regions.
- Prioritise sequences derived from vouchered specimens, published in peer-reviewed studies, or included in curated datasets such as RefSeq.
- Report the top sequence match with: accession number, gene region, percent identity (ideally ≥ 99%), query coverage (≥ 98%), alignment length, and E-value (near zero).
- Ensure the match does not have equally strong alignment to multiple species, if it does, report at genus or family level.
- For problematic taxa such as *Thunnus*, lower identity scores (e.g., ≥96%) may be acceptable only if the assay has been validated (as per ANSI/ASB primer validation std 036), and when the next closest species is ≥2% different in identity.
- Document all search parameters used, including alignment tool version, database version/date, and scoring criteria.

These requirements help ensure that identifications are transparent, defensible, and supported by reliable reference data. When followed, they strengthen the scientific and legal validity of molecular evidence in monitoring and enforcement applications.

## 7.6 Reporting and Interpretation of Results

Clear, transparent reporting is a critical component of forensic science. ANSI/ASB Standard 029 sets out minimum requirements for reporting in wildlife forensic cases, including a clear description of the sample type and condition, methods used (including primer sets, reagents, amplification protocols, and sequencing platforms), loci targeted, and the reference databases consulted. The report must also document the analytical results, including match metrics (e.g., percent identity, alignment length, query coverage), and a clear statement of the interpretation.

Importantly, reports must specify the level of taxonomic certainty (e.g., species, genus, or family level), justified with reference to the quality and completeness of database entries and the distinctiveness of the genetic marker used. Ambiguities must be disclosed—for example, when interspecific and intraspecific variation overlap, when multiple species produce near-identical matches, or when key diagnostic regions are unavailable. These considerations are especially relevant for mixed-source

samples (e.g., brine tanks, surface swabs), where species-level resolution may be constrained by both biological and technical limitations (ANSI/ASB Std 180).

Reports should be written in plain language where possible, avoid overstating the certainty of conclusions, and include disclaimers if analytical limitations exist. Laboratories must also retain all supporting documentation—such as chromatograms, alignments, and raw sequence files—to support transparency and enable independent review or legal scrutiny.

## 8. Technical Considerations for Molecular Monitoring Tools

This section provides a detailed overview of the technical components that underpin molecular monitoring efforts in fisheries MCS, including recommended genetic primers, sequencing technologies, and the role of reference databases in taxonomic identification. These elements are critical to ensuring reliability, reproducibility, and defensible outcomes in molecular surveillance and enforcement contexts.

## 8.1 Recommended Primer Sets for Molecular Identification of Common WCPFC Species

The following table presents recommended primer sets for molecular identification of fish and shark species commonly encountered within the WCPFC region. Primer selection is based on demonstrated reproducibility, relevance to key target and bycatch taxa, and the availability of supporting reference sequences in public databases such as GenBank.

| Taxon Group | Method | Primer Names | Gene Region | Reference | Comments |
|---|---|---|---|---|---|
| **Teleost Fish** | | | | | |
| | Barcoding (excluding tuna) | FishF1<br>FishR1<br>FishF2<br>FishR2 | COI | Ward et al (2005) | Recommended for all fish species (excluding tuna) |
| | Metabarcoding (excluding tuna) | Short-read: Fish16sF/D 16S2R-Degenerate | 16S | Berry et al (2017), Deagle et al (2007), | Suitable for billfish (genus-level) |
| | | Long-read: Fish_12S_fw1-ONT<br>Fish_16S_rv1-ONT | 16S+12S | Dorenspleet et al (2025) | Long primers can more accurately identify species than short primers. |
| | Metabarcoding (Tunas) | ToCR3_F<br><br>ToCR1_R | D-Loop | Yoshitake et al (2021)<br><br>Green et al (*In prep*) | Enables identification of 4 species of tuna using ONT sequencing (*T. alunga, T. albacares, T. maccoyii, T. orientalis).* NB-Big-eye tuna (*T. obesus*) unable to be confidently detected |
| **Elasmobranch (shark / ray)** | | | | | |
| | Barcoding | ILEM / ASNM | ND2 | Naylor et al (2012) | ND2 gene region is the most robust at identifying chondrichthyes species. |
| | Metabarcoding | FishF1-degenerate<br>FishF2-degenerate | COI | Original: Ward et al (2005), Degenerate: West et al (2020) | Poor species level resolution because primers select a small gene region. Recommended to be used for genus level identification. |
| | | MiFish-E-F<br>MiFish-E-R | 12S | Miya et al (2015) | Poor species level resolution because primers select a small gene region. Recommended to be used for genus level identification. |
| | qPCR | Many primer pairs - see reference | COI | Cardeñosa et al (2017) | Considered a pre-screening tool to rapidly and cost effectively screen for some CITES listed species. Some false positives identified in initial validation study, therefore if positive detection sample must be sent away for secondary barcoding. |

## 8.2 DNA Sequencing Technologies for Laboratory and Field Applications

A range of sequencing and DNA analysis platforms are now available to support molecular monitoring workflows, from high-throughput laboratory systems to compact, field-deployable devices. Below is an overview of key technologies relevant to fisheries MCS applications, including their functionality, strengths, and limitations.

### *a. MinION (Oxford Nanopore Technologies)*

The MinION is a portable, real-time DNA/RNA sequencing device developed by Oxford Nanopore Technologies. It performs long-read sequencing by detecting changes in electrical current as DNA strands pass through nanopores. It does not require PCR amplification, making it compatible with degraded or complex samples such as eDNA.

Cost per sample: Moderate, USD $1500 per run, able to sequence 96 samples at a time

Reliability: Good for presence detection and identification of closely related species, sequencing accuracy improving but still lower than short-read illumina platforms.

Results in: 1-2 days

| Pros | Cons |
|---|---|
| • Field-deployable; fits in the palm of your hand | • Requires technical expertise for library prep and data analysis |
| • Generates long reads, enabling better species resolution (e.g., full D-loop or ND2 regions) | • Higher error rates than Illumina (though improving) |
| • Capable of near real-time analysis | • Expensive consumables and equipment for high-throughput runs |
| • Can detect multiple species in mixed samples without needing species-specific primers | • Sensitive to environmental conditions (temperature, humidity) |

### *b. Illumina Sequencing (e.g., MiSeq, NextSeq)*


Illumina's sequencing-by-synthesis technology is the gold standard for high-throughput, short-read DNA sequencing. Widely used in barcoding and metabarcoding studies, it enables simultaneous sequencing of thousands of samples.

Cost per sample: Moderate-High, USD $16,000 per run (NovaSeq), high-throughput capacity able to sequence 384 at a time

Reliability: Excellent base accuracy (>99.9%) and reproducibility; historically the standard for bulk barcoding and metabarcoding, however lower resolution species identification.

Results in: 1-2 months

| Pros | Cons |
|---|---|
| • High accuracy and sequencing depth<br>• Well-established protocols and workflows<br>• Supported by a wide range of reference databases<br>• Suitable for large sample batches | • Requires well-equipped laboratories<br>• Short fragments make species identification difficult<br>• Not suitable for real-time or in-field testing<br>• Inflexible for low-throughput or urgent tasks<br>• Turnaround times can be slow due to batching |

### *c. Biomeme Franklin™ qPCR Platform*


Biomeme is a handheld qPCR device designed for rapid, field-based DNA testing. It uses pre-loaded assay cartridges and smartphone connectivity to deliver species identification within hours.

Cost per sample: Moderate, USD $300 per run, up to 9 samples at a time

Reliability: High specificity and sensitivity when assays are properly validated; robust in field conditions

Results in: 2-3 hours

| Pros | Cons |
|---|---|
| • Portable and user-friendly; requires minimal training | • Limited number of assays per run |
| • Produces fast, quantitative results (e.g., species detection or CITES pre-screening) | • Only suitable for known targets with pre-developed assays |
| • Suitable for enforcement or compliance officers | • Requires cold storage for some reagents |
| • Assay kits can be stored and used in rugged conditions | • Not a sequencing platform—only confirms presence/absence of known species |

### *d. MicBio Platform (qPCR and HRM)*

MicBio is a portable diagnostic platform combining quantitative PCR with High Resolution Melt (HRM) analysis. HRM allows for the detection of closely related species based on melting curve profiles.

Cost per sample: Low, USD $200 per run, up to 48 samples per run

Reliability: Good under controlled conditions; requires consistent melt curve interpretation

Results in: 5 hours

| Pros | Cons |
|---|---|
| • Field-ready device suitable for species identification and variant detection | • Limited resolution compared to full sequencing |
| • HRM allows detection of fine-scale genetic differences | • HRM requires prior knowledge of sequence variation |
| • Cost-effective and fast turnaround | • Potential for false positives with closely related species |
| • Suitable for mixed or degraded DNA sources | • Validation required for use across multiple species groups |

*e. Nucleic Acid Barcoding Tool (NABIT), WildTech DNA Platforms*


NABIT and WildTech DNA are low-cost, rugged genetic testing platforms developed to assist with field-based biodiversity monitoring. They use simplified DNA extraction and amplification workflows, often integrated with mobile devices or custom hardware.

Cost per sample: Low, USD $2 per test, 1 sample per test.

Reliability: Promising but variable; dependent on assay development and pilot testing. No assays currently available for WCPFC species of interest.

Results in: 10-30 minutes

| Pros | Cons |
|---|---|
| • Extremely portable and low cost; designed for frontline conservation | • Still in early stages of commercial deployment |
| • Compatible with field DNA extraction methods | • Lower throughput and less precision than lab-based systems |
| • Can be used by non-experts | • Currently limited to specific use cases or pilot programs |
| • Good for community-based or artisanal fisheries monitoring | • May require integration with external labs for sequencing or confirmation |

### 8.3 Reference Databases for Accurate Species Identification

Molecular monitoring methods such as DNA barcoding and metabarcoding rely on the comparison of unknown DNA sequences to reference databases to assign species-level identifications. The accuracy of this identification step depends on the quality, coverage, and curation of the reference sequences available.

Several reference databases are commonly used in fisheries applications:

- **GenBank (NCBI):** The most widely used and comprehensive repository of genetic sequences. However, it is open-access and user-submitted, with minimal taxonomic validation, leading to frequent misidentifications—particularly for closely related species like tunas.
- **RefSeq (NCBI):** A curated subset of GenBank with high-quality, non-redundant reference sequences. While more reliable, it covers fewer species, especially for non-model marine taxa.
- **BOLD (Barcode of Life Data System):** Designed specifically for DNA barcoding, with an emphasis on the COI gene region. It includes specimen metadata, geographic information, and links to voucher specimens when available.
- **EMBL-EBI & DDBJ:** European and Japanese nucleotide archives, respectively. These are part of the International Nucleotide Sequence Database Collaboration (INSDC) and largely mirror GenBank content.
- **MitoFish:** A specialised database of complete mitochondrial genomes and gene annotations for fish. It is particularly useful for studies requiring mitochondrial markers (e.g., D-loop, ND2), and has growing utility for long-read metabarcoding applications.

While GenBank remains the most accessible and widely used resource, its lack of curation poses significant risks for species misidentification. This is particularly problematic in fisheries monitoring, where enforcement decisions may depend on accurate and defensible species assignments. For example, multiple *Thunnus* sequences in GenBank are incorrectly labelled or inconsistently annotated, leading to ambiguous matches in BLAST searches and incorrect identifications.

In contrast, RefSeq and BOLD offer higher confidence due to curated content, though their species coverage is narrower and often lacks region-specific fisheries species. MitoFish, while still developing, provides valuable mitochondrial genome resources that complement these databases, particularly for designing long-read assays and resolving species in complex or degraded samples.

**Recommendation**

To improve the reliability and consistency of molecular monitoring in the Western and Central Pacific, there would be substantial value in establishing a WCPFC-endorsed reference database. This could include:

- Vouchered specimens with verified taxonomic ID
- High-quality photographic documentation
- Multiple gene regions (e.g., COI, 12S, ND2, D-loop, whole genomes)
- Metadata including location, collector, and date
- Open-access availability for MCS activities for WCPFC members

A regionally curated database would reduce misidentification risks, support consistent enforcement across jurisdictions, and underpin the credibility of molecular evidence in compliance and trade settings for WCPFC purposes.

# 9. Case Studies

## Case Study 1: High Seas Boarding Inspection – Biopsy Sampling for Species Identification


**Scenario Description:**

During a routine high seas boarding inspection (HSBI) in the Western and Central Pacific, fisheries officers board a longline vessel to verify compliance with reporting and species retention regulations. Genetic sampling can be initiated to confirm the species identity of large tuna carcasses stored in the freezer hold.

**Sampling Strategy:**

Small muscle or fin biopsy samples (~1 cm²) are collected from selected carcasses in the freezer hold. Samples are placed in pre-labelled vials containing ethanol or silica desiccant, with all metadata recorded, including suspected species, GPS location, vessel name, collector, and date. Each sample was logged on a tamper-evident chain-of-custody form compliant with forensic best practices (SWFS Technical Working Group 2018; ANSI/ASB 019, 2019).

**Rapid At-Sea or Portside Testing:**

Where immediate decisions are required (e.g., for potential release, seizure, or prioritised inspection), species-specific assays qPCR or barcoding can be used directly onboard or at port.

**Platforms**: minION (ONT), Biomeme Franklin or MicBio qPCR

**Assays Used**: COI or 16S-12S barcoding, qPCR tests for key species

**Timeframe**: 3 hours to 1 day from sample to result

**Limitations**: qPCR limited to known target species; dependent on validation and field training; currently under development for broader species panels.

**Compliance Value**: Useful for initial screening or triage to inform enforcement action

**Laboratory-Based Testing:**

All collected samples can be subsequently sent to a laboratory for confirmatory analysis using standardised barcoding protocols.

**Method**: COI, 16S-12S barcoding or ND2 sequencing, depending on taxonomic resolution required

**Databases Used**: BOLD, GenBank (with quality-filtering protocols)

**Validation**: Aligned with ANSI/ASB Standards 048 (2019) and 111 (2020) for forensic wildlife DNA testing

**Turnaround**: ~2–4 weeks

**Outcome**: High-confidence species identifications; resolution of misidentification issues common in visually similar tunas and sharks

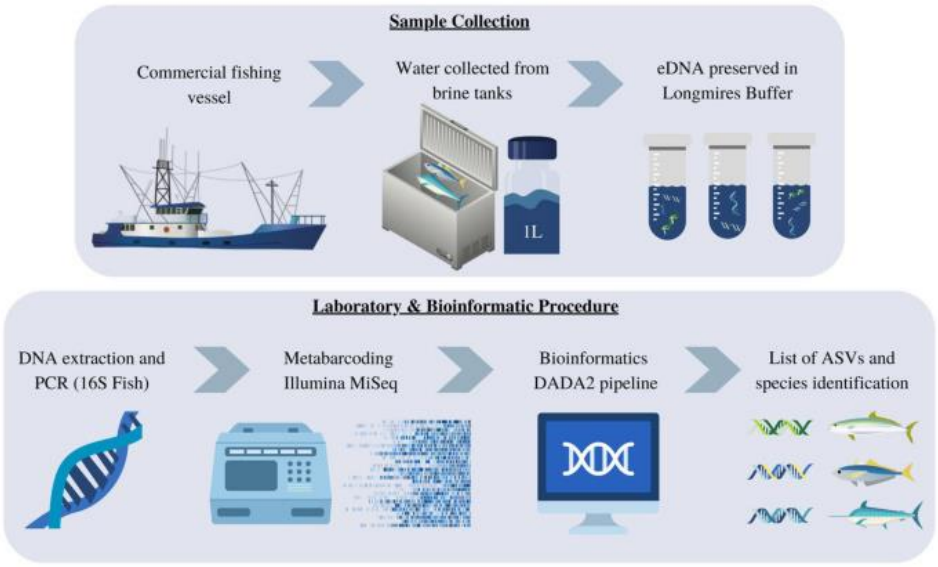
**Compliance and Evidentiary Value:**

Results obtained from both field-based and laboratory workflows were used to support potential compliance action, including species quota enforcement, bycatch violation investigation, and CMM adherence. Because chain of custody and lab validation followed forensic guidelines, the data were considered legally defensible under wildlife forensic standards (SWFS Technical Working Group 2018; ANSI/ASB 019 & 048, 2019).

**Limitations & Development needs:**

**Sampling:** Requires training in sterile technique and forensic sample handling
**Field Tools:** Validation of qPCR / LAMP assays for full species panels remains a development need
**Laboratories:** Need for WCPFC-endorsed reference databases to reduce identification ambiguity
**Capacity**: Integration into MCS workflows requires coordination between enforcement, scientific, and lab partners

## Case Study 2: Estimating Catch Composition via Brine Tank Meltwater Sampling




**Scenario Description:**

On many longline and purse seine vessels, landed catch is stored in chilled brine tanks to maintain freshness during transit. These tanks contain meltwater or concentrated brine that becomes mixed with tissue particles, blood, and other cellular material, resulting in the accumulation of environmental DNA (eDNA) from the stored fish. This eDNA offers a unique, non-invasive method to assess catch composition. Molecular monitoring of brine tank water enables efficient screening for species presence and can support the detection of unreported, misreported, or high-risk taxa, including no-take or restricted species.

**Sampling Strategy:**

Between 250–1000 mL of brine tank water is collected using a sterile container. A subsample of 30 mL is then decanted into 5–8 pre-labelled 50 mL tubes, each pre-filled with 15 mL of Longmire's buffer to preserve environmental DNA (eDNA) during transport and storage. All associated metadata including suspected species, GPS coordinates, vessel name, date, and collector are recorded at the time of sampling. Each sample is logged using a chain-of-custody form in accordance with wildlife forensic best practices (SWFS Technical Working Group 2018; ANSI/ASB 019, 2019).

**Rapid At-Sea or Portside Testing:**

Where immediate decisions are required (e.g., for potential release, seizure, or prioritised inspection), species-specific assays qPCR or metabarcoding can be used directly onboard or at port.

**Platforms**: minION (ONT), Biomeme Franklin or MicBio qPCR

**Assays Used**: 16S-12S metabarcoding (billfish), D-loop (tuna), qPCR tests for some CITES shark species available

**Timeframe**: 1 day from sample to result

**Limitations**: further validation of eDNA metabarcoding required, qPCR limited to known target species

**Compliance Value**: Useful for initial screening or triage to inform enforcement action

**Laboratory-Based Testing:**

All collected samples can be subsequently sent to a laboratory for confirmatory analysis using standardised barcoding protocols.

**Assays Used**: 16S-12S metabarcoding (billfish) or D-loop (tuna), qPCR tests for some CITES shark species available

**Databases Used**: BOLD, GenBank (with quality-filtering protocols)

**Validation**: Aligned with ANSI/ASB Standards 048 (2019) and 111 (2020) for forensic wildlife DNA testing

**Turnaround**: ~2–4 weeks

**Outcome**: High-confidence species identifications; resolution of misidentification issues common in visually similar tunas and sharks

**Compliance and Evidentiary Value:**

Brine tank eDNA provides a non-invasive tool to audit catch composition and detect unreported or high-risk species. When collected under chain-of-custody and analysed with validated metabarcoding methods, results can support compliance checks and risk assessments. However, as this is a relatively novel application, it is currently best suited for guiding inspections or identifying vessels for further scrutiny, rather than being used as standalone evidence in legal proceedings. With further validation of NGS methods under forensic standards (e.g., ANSI/ASB 048, 029), its use for formal compliance applications is expected to grow.

**Limitations & Development needs:**

**Sampling:** Sample collection is operationally simple, but maintaining robust chain-of-custody procedures is essential to ensure evidentiary integrity.
**Field Tools:** Further validation of metabarcoding assays is needed, including establishing detection thresholds and cut-off criteria for confident species assignments across full panels.
**Laboratories:** Development of WCPFC-endorsed reference databases is essential to reduce taxonomic ambiguity and improve consistency in species identification.
**Capacity:** Continued development of long-read sequencing and species-specific qPCR panels will enhance resolution and support future compliance-grade applications.

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