

Evaluating Sampling Logistics in the North Arabian Sea for Implementation of Close-Kin Mark-Recapture for Indian Ocean Yellowfin Tuna

Report of the Pakistan pilot sampling project: implementation, methodology, results and operational lessons

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Purpose of this document. This paper reports how the Pakistan yellowfin tuna close-kin mark-recapture (CKMR) pilot sampling was carried out, what was collected, and what was learned about feasibility. It is not a stock assessment and presents no kinship or abundance estimates. Field sampling is complete; laboratory evaluation of DNA quality and cross-contamination for the first 100 fish is pending at CSIRO.

Executive Summary

WWF-Pakistan carried out a pilot project to test whether sampling of Indian Ocean yellowfin tuna (*Thunnus albacares*) for close-kin mark-recapture can be sustained from commercial landings in the northern Arabian Sea. The project was implemented with technical guidance from CSIRO and institutional support from the Marine Fisheries Department (MFD), Government of Pakistan. It responds to the staged approach to yellowfin CKMR set out in IOTC scientific work, which calls for sampling logistics, traceability, tissue preservation and contamination control to be tested before any large multi-year programme.

Sampling took place at Karachi Fish Harbour from 3 September 2025 to 20 May 2026. A five-member team sampled 1,898 yellowfin tuna on 96 sampling days in 37 consecutive weeks, an average of 51 fish per week. This is 3.6 times the original pilot target of 522 fish and 93.3% of the expanded operational target of 55 fish per week (2,035 fish). Fork length ranged from 35.5 to 96.5 cm (mean 53.7 cm, median 51.5 cm).

Measured against the size strata of the CKMR design study, 702 fish (37.0%) were <50 cm, 78 (4.1%) were >75 cm, and 1,118 (58.9%) were 50-75 cm. The 780 fish inside the two design strata equal 6.1% of the illustrative annual Region 1 allocation of 12,750 fish. The 1,118 intermediate fish can be used as juveniles only if otolith ageing shows they belong to the required young cohorts.

For every fish, five dorsal-muscle replicates were taken before the body cavity was opened: one in RNAlater (vial A) and four in 99% ethanol (vials B-E), in individually labelled 2 mL vials. Sex was recorded for 1,880 fish (1,156 males and 724 females), otoliths were collected from 1,879, and gonads and stomachs were examined in 1,880. The RNAlater replicate from the first 100 fish was sent to CSIRO in November 2025 for assessment of DNA quality and cross-contamination; results are expected to be presented during WPTT28. All other tissue is held frozen in Pakistan.

The pilot met and exceeded its sampling objective and shows that sustained, high-volume, port-based CKMR sampling is operationally feasible at Karachi Fish Harbour. It has established a trained team, standard operating procedures and a working sampling system, so Pakistan is in a position to continue sampling as a node in a coordinated Region 1 CKMR sampling network. The pilot also identified what is needed before scale-up: direct ageing of the 50-75 cm group, a deliberate >75 cm component, recording of vessel and trip for every fish, confirmation of DNA quality and contamination control from the CSIRO assessment, and a common protocol for all Region 1 sampling nodes. Standard operating procedures (SOPs) for sampling and for frozen storage were developed and applied in the pilot, and will be adopted for continued sampling. An outline for a multi-country, multi-year second phase is given in Section 9.

Key results

Indicator	Result
Original pilot target	522 fish
Expanded operational target	55 fish per week (2,035 over 37 weeks)
Total fish sampled	1,898
Sampling days / weeks	96 days / 37 weeks
Mean sampling rate	51 fish per week; 19.8 fish per sampling day
Fork-length range	35.5-96.5 cm
<50 cm / 50-75 cm / >75 cm	702 / 1,118 / 78
Fish within formal design strata	780 (6.1% of illustrative annual Region 1 allocation)
Otoliths / gonads / stomachs	1,879 / 1,880 / 1,880
Tissue sent to CSIRO	First 100 fish; assessment pending

1. Background and Rationale

Indian Ocean yellowfin tuna supports major industrial and artisanal fisheries across the basin. The IOTC stock assessment integrates catch, size-frequency, tagging and catch-per-unit-effort data. The 2024 assessment revised the estimated stock status to not overfished and not subject to overfishing, with a probability of 89%. The Scientific Committee nevertheless notes important uncertainties in the standardized catch-per-unit-effort index that drives the result and in the catches reported by some fisheries (IOTC-SC28, 2025).

Close-kin mark-recapture offers an independent source of information. Parent-offspring and half-sibling pairs identified genetically among sampled fish can be used to estimate absolute adult abundance, mortality and connectivity when combined with age, sex, time and location of sampling (Bravington, Skaug & Anderson, 2016). The method has been applied to southern bluefin tuna (Bravington, Grewe & Davies, 2016).

The design study for Indian Ocean yellowfin (Hillary et al., 2022) evaluated alternative sample sizes and age compositions. Under its assumptions, about 25,000-30,000 samples per year were expected to give informative estimates, and a five-year programme at around 30,000 samples per year (150,000 in total) gave useful precision. Performance was best with about 70% potential offspring and 30% adults. For planning, fish were divided into potential offspring (<50 cm fork length) and potential parents (>75 cm). Assuming even sampling across regions, the illustrative annual allocation for Region 1 was 10,500 fish <50 cm and 2,250 fish >75 cm, a total of 12,750. These are planning strata, not determinations of maturity. A staged approach beginning with pilot sampling was subsequently proposed (Williams et al., 2023). The IOTC programme of work for tropical tunas now includes a plan for a staged yellowfin CKMR project, and specifically a pilot project “to evaluate the logistics and feasibility of sampling, and levels of cross contamination of DNA” (IOTC-WPTT27, 2025; IOTC-SC28, 2025). The Pakistan pilot addresses that item.

Pakistan lies in the northern Arabian Sea within Region 1. Its tuna gillnet fishery lands a broad size range of yellowfin, including small and intermediate fish that are less common in some industrial fisheries. The project was therefore designed as an operational pilot, to test whether large numbers of individually traceable tissue samples, with linked biological data, can be collected week after week from commercial landings in Pakistan and handled to a standard suitable for CKMR. An earlier update was provided to WPTT27 (WWF-Pakistan, 2025).

2. Objectives and Delivery

The goal was to support the management of Indian Ocean yellowfin tuna by evaluating sampling logistics in the North Arabian Sea for CKMR. The four objectives were to:

1. develop standard operating procedures for collection, storage and transport of yellowfin biological samples;
2. establish a network of trained samplers;
3. sample 522 fish as a contribution to the Region 1 design allocation; and
4. examine DNA contamination among samples and adjust methods if needed.

Delivery against each objective is summarized in Table 1.

Table 1. Objectives, planned outputs and delivery.

Objective	Planned output	Delivered	Status
SOPs for collection, storage and transport	SOP suitable for CKMR sampling	SOP developed from the tissue sampling data recording and handling of the tissue samples applied throughout the pilot; to be adopted for continued sampling	Completed
Network of samplers	Trained samplers and coordination	Five-member team trained and operating at Karachi; MFD, fishers and CSIRO engaged. No sampling outside Karachi and no regional network yet	Partly achieved (national node established)
Sample 522 fish	522 fish in six size classes	1,898 fish sampled; 780 within the formal <50 cm and >75 cm strata	Exceeded
Examine DNA contamination	CSIRO assessment of about 50 fish	Tissue from the first 100 fish sent to CSIRO, November 2025	Shipment completed; results pending
Report to IOTC	Updates to working parties	Information paper to WPTT27; this report to WPTT28	Completed

3. Implementation

3.1 Partners and roles

WWF-Pakistan led sampling, laboratory coordination, data management and reporting. CSIRO advised on study design, size-class targets and molecular quality requirements. MFD provided access to its biological laboratory at Karachi Fish Harbour. WWF-Italy provided programme coordination.

Three consultative meetings were held before sampling with the Director General of MFD, technical officers and representatives of the tuna gillnet fishing community. The IOTC Secretariat issued a letter of support on 20 May 2025, and MFD issued a Letter of Intent on 16 June 2025 confirming laboratory access.

3.2 Preparation

Preparation covered stakeholder engagement, recruitment and training of the team, procurement of vials, preservatives and consumables, preparation of forms and the database, and arrangement of laboratory space and frozen storage.

3.3 Evolution of the sampling target

The initial matrix was 522 fish in six 10-cm size classes (Annex 1). Following discussion with CSIRO, and recognizing that CKMR needs large juvenile samples, the target was raised to 55 fish per week weighted toward the smallest class (Table 2). Over the 37 weeks sampled this corresponds to 2,035 fish. Once the original 522 fish had been reached, the partners agreed to continue with yellowfin rather than add other species, and the project was extended by three months to 30 June 2026 so that sampling could cover the whole gillnet season.

Table 2. Expanded weekly target.

Fork-length class	Weekly target	Share	37-week total
40-<50 cm	40	72.7%	1,480
50-<60 cm	5	9.1%	185
60-<70 cm	5	9.1%	185
70-<80 cm	2	3.6%	74
80-<90 cm	2	3.6%	74
90-<100 cm	1	1.8%	37
Total	55	100%	2,035

3.4 Planned and implemented

Implementation differed from the original plan in duration, sampling site, sampling frequency, number of fish and the size of the quality-control subset (Table 3).

Table 3. Differences between the original plan and implementation.

Element	Planned	Implemented
Duration	12 months to 31 March 2026	Extended to 30 June 2026; sampling 3 September 2025 - 20 May 2026
Ports	Karachi Fish Harbour and Gwadar	Karachi Fish Harbour only. Gwadar was dropped so that the pilot could focus on Karachi Fish Harbour, where the tuna landings take place
Frequency	Every week	96 sampling days; about 2.6 days per week, every week
Number of fish	522	1,898
Capture information	Fishing area from fisher interviews and logbooks	Not recorded: fish were sampled from the mixed catch in the landing area at Karachi Fish Harbour, not from an identified vessel or lot
Quality-control subset to CSIRO	About 50 fish	First 100 fish
Laboratory	MFD biological laboratory	MFD biological laboratory

4. Sampling Methodology

This section describes the method used in the pilot. The workflow is summarized in Figure 2 and a step-by-step standard operating procedure (SOP) was developed for the pilot and followed throughout. The developed SOPs will be adopted for continued sampling, with revision as needed once the CSIRO results are available. The sampling SOP is based for species identification, measurement, genetic tissue, gonads and otoliths.

4.1 Site and fishery

Fish were sampled at Karachi Fish Harbour from commercial landings, mainly of tuna gillnet vessels operating in the Pakistan EEZ and adjacent northern Arabian Sea, within Region 1 of the yellowfin assessment and CKMR design study (Figure 1). Vessel, trip and capture position were not recorded. The site is therefore a landing location, not a verified capture location.

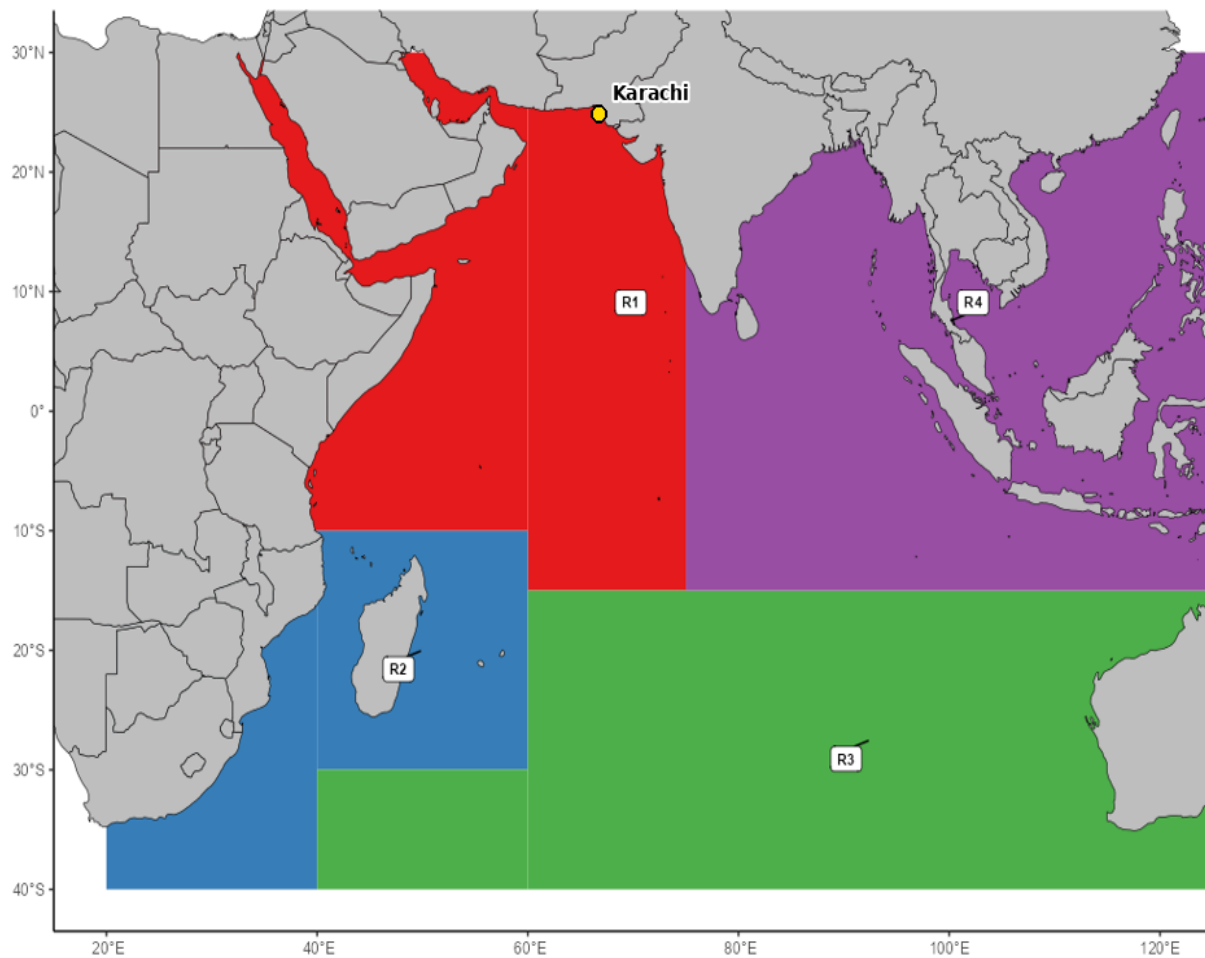


Figure 1. Study area the four regions used for the Indian Ocean yellowfin tuna assessment and the CKMR design study, with Region 1 (R1) in red and Karachi marked (after Fu et al., 2021; Williams et al., 2023).

4.2 Team and training

A five-member team was formed and completed seven days of training before sampling. Laboratory safety and biosafety training was delivered by the Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, from 4 to 8 August 2025. It combined lectures with supervised laboratory practice and covered biosafety levels and controls, putting on and removing protective equipment, hand hygiene, safe disposal of sharps, handling and sterilization of knives and other tools, organization of the workstation, opening and closing vials with one hand so that the cap and rim are not touched, preparation of the sampling kit, and safe handling, preservation and transport of biological and chemical material. A training manual was compiled for later reference. This was followed by hands-on training in species identification, measurement, tissue collection, contamination control, preservation, dissection, otolith extraction, photography, labelling and data recording, delivered by the project team.

4.3 Selection and transport

Fresh yellowfin were selected from each day's landings according to the target matrix. When too few fish of 40-50 cm were available, the next-smallest class landed that day was taken. Fish were taken from the mixed catch in the landing area, without reference to vessel or lot, and were chosen only on freshness and the required size classes. Selection was therefore directed by size and was not random. The species of every fish was confirmed before sampling. Fish were identified as yellowfin tuna from the external characters given in the protocol (pectoral fin, body markings, tail notch, body shape, eye size and head length), and identity was checked again from the liver during dissection: in yellowfin the liver is smooth

with the right lobe longer and thinner, whereas bigeye tuna has a striated liver with three rounded lobes of similar size. Fish were packed in ice in insulated boxes, taken to the laboratory and processed as soon as practicable.

4.4 Identification and traceability

Each fish received a unique Sample_ID before any tissue was removed, made up of the species code and a running number (for example YFT1898). Each tissue vial carries the Sample_ID with a replicate letter in front (AYFT1898 for the RNAlater vial; BYFT1898 to EYFT1898 for the ethanol vials). The same Sample_ID links the measurement record, the five tissue vials, sex and maturity, gonad, stomach and otolith records, and photographs.

4.5 Measurements

Straight fork length was measured from the tip of the upper jaw to the fork of the tail and recorded to 0.1 cm without rounding. Whole weight was recorded in kilograms. Curved fork length, body height and girth were recorded for some fish. Straight fork length is the basis for all size classes in this report. Implausible values were re-measured before dissection.

4.6 Genetic tissue

Tissue was taken from the dorsal muscle before the body cavity was opened. A wedge of muscle was cut out, and tissue was taken from its freshly exposed inner face to avoid skin, slime and blood from other fish. Five replicates were taken per fish. Each was a slice about 1.5-2.5 cm wide and no more than 5 mm thick, placed in a separately labelled sterile 2 mL vial with about 1.5 mL of preservative: one in RNAlater (vial A) and four in 99% ethanol (vials B to E). This gave roughly one part tissue to three parts preservative in each vial. The tissue was fully immersed, and the vials were closed immediately.

4.7 Contamination control

CKMR relies on finding rare genetic matches, so transfer of DNA between fish or mislabeling can create false or uninterpretable results. Five controls were applied in the pilot. One fish was processed at a time, and only the vials of that fish were open. The sampler put on new gloves for every fish. Genetic tissue was taken before internal dissection. The skin was wiped with clean moistened tissue before cutting, and neither fingers nor the pipette tip touched the tissue, the mouth of the vial or the inside of the cap. Between fish, the instruments and cutting board were rinsed with water, wiped twice with clean paper tissue and washed with 99% ethanol.

The cleaning procedure follows the developed protocol. Its effectiveness will be confirmed by the CSIRO assessment of the first 100 fish.

4.8 Preservation and storage

Vials were kept cool, below 4 °C, for the first 24 hours for RNAlater samples, and then stored frozen at about -35 °C with backup power. The freezer was operated under a dedicated deep-freezer SOP covering access, daily checks, maintenance and emergency action. A separate inventory sheet links Sample_ID, vial, box and position. Repeated thawing was avoided.

4.9 Biological sampling

After the genetic vials were sealed and checked, the body cavity was opened.

- **Sex and maturity.** Sex was assigned from the gonads by eye. Gonads were measured, weighed, staged and photographed. Ovaries were recognized by their pinkish-orange color and circular cross-section with a central lumen, and testes by their firm, white-grey tissue and triangular cross-section. Maturity was staged on the macroscopic scale for yellowfin tuna in: 1 immature, 2 early maturing, 3 late maturing, 4 ripe, 5 spent. Where the stage could not be determined, stage 1 was recorded. All gonad photographs are kept as an inventory linked to the Sample_ID for later cross-checking. Staging was cross-checked by Mr Hamid Badar, a retired fisheries biologist of the Marine Fisheries Department.
- **Stomach.** Feeding status, stomach length and weight, prey identity, number, size, weight and digestion state were recorded.
- **Otoliths.** Both sagittal otoliths were removed by the “lifting the lid” method, cleaned of tissue and membrane, rinsed, air-dried and stored dry in a labelled vial.
- **Other tissues.** For 1,880 fish, liver, heart, gill, dorsal muscle and tail muscle were frozen for metal analysis at a later stage. These are not part of the CKMR dataset.

4.10 Data management

Data were entered into a database linking each fish to all samples and measurements, normally on the day of sampling. Fish counts were reconciled against Sample_IDs, and vials against records and freezer inventory.

4.11 Transfer to CSIRO

The RNAlater replicate (vial A) of the first 100 fish was shipped by courier to the CSIRO laboratory in November 2025, accompanied by the CSIRO import permit, and was received. It will be used to assess DNA quality, suitability for genotyping and cross-contamination. The four ethanol replicates of these fish were kept in Pakistan.

4.12 Size classifications used in this report

Two classifications are used: the 10-cm operational classes of the target matrix (40-<50 to 90-<100 cm, with fish <40 cm shown separately), and the design-study strata (<50 cm, 50-75 cm inclusive, >75 cm). Sampling dates are also grouped into early (September-October), middle (November-January) and late (February-May) parts of the season. These groupings describe sampling coverage only; they are not indices of catch rate or abundance.

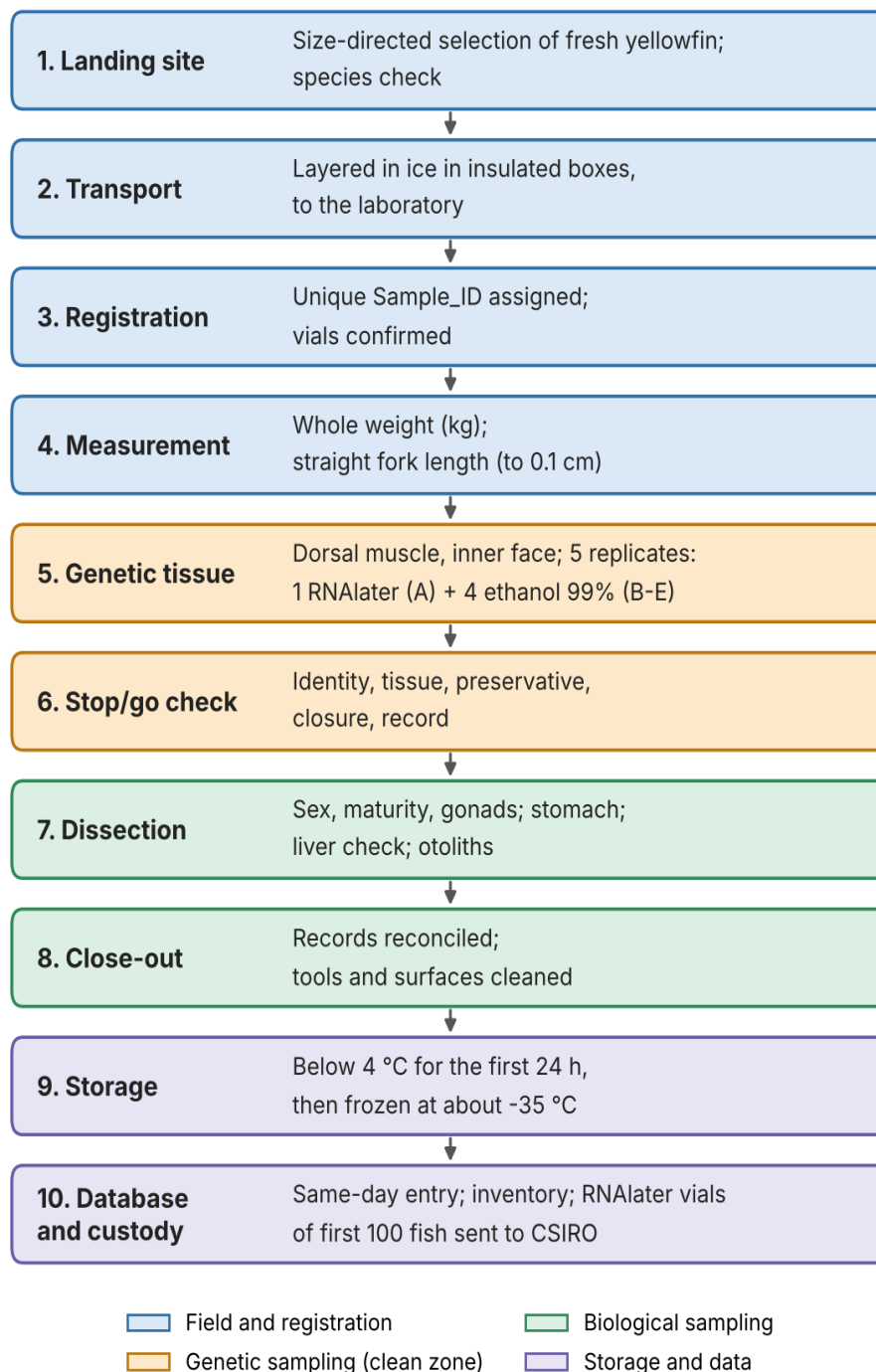


Figure 2. Workflow of the pilot, from selection at the landing site to storage and transfer.

5. Logistics, Quality Assurance and Deviations

5.1 Capacity

Five people sampled 1,898 fish in 96 days: 19.8 fish per sampling day, about 2.6 sampling days per week. Five replicates per fish gives an expected 9,490 tissue vials to label, preserve, store and track; a final vial-by-vial reconciliation of the archive, including the material sent to CSIRO, is still to be completed. Processing time per fish, staff time by task and consumables per fish were not recorded and cannot be

reported. They should be recorded in detail in the next phase, because cost per fish is the figure most needed to plan scale-up.

5.2 Laboratory continuity

The MFD laboratory was the processing facility. To cover public holidays, heavy rain in Karachi and other unforeseen conditions, WWF-Pakistan arranged back-up facilities at University of Karachi laboratories for tissue extraction and storage. The back-up facilities were not needed and were not used.

5.3 Deviations and gaps

No major failure occurred. Deviations and remarks were recorded for each fish in a comments column of the database. No leaking vials, freezer failures or suspected contamination events were reported.

The main gap is the absence of vessel, trip and capture positions. Without them, it is not possible to examine whether close relatives are clustered within trips, or to use location in analysis.

Molecular validation of the samples is still pending. The pilot demonstrates that sampling, archiving and transfer are feasible; it does not yet show that the archive is suitable for kinship analysis.

6. Results

6.1 Sampling delivered

In total 1,898 fish were sampled on 96 days in 37 weeks between 3 September 2025 and 20 May 2026 (Figure 3), 3.6 times the original target and 93.3% of the expanded target.

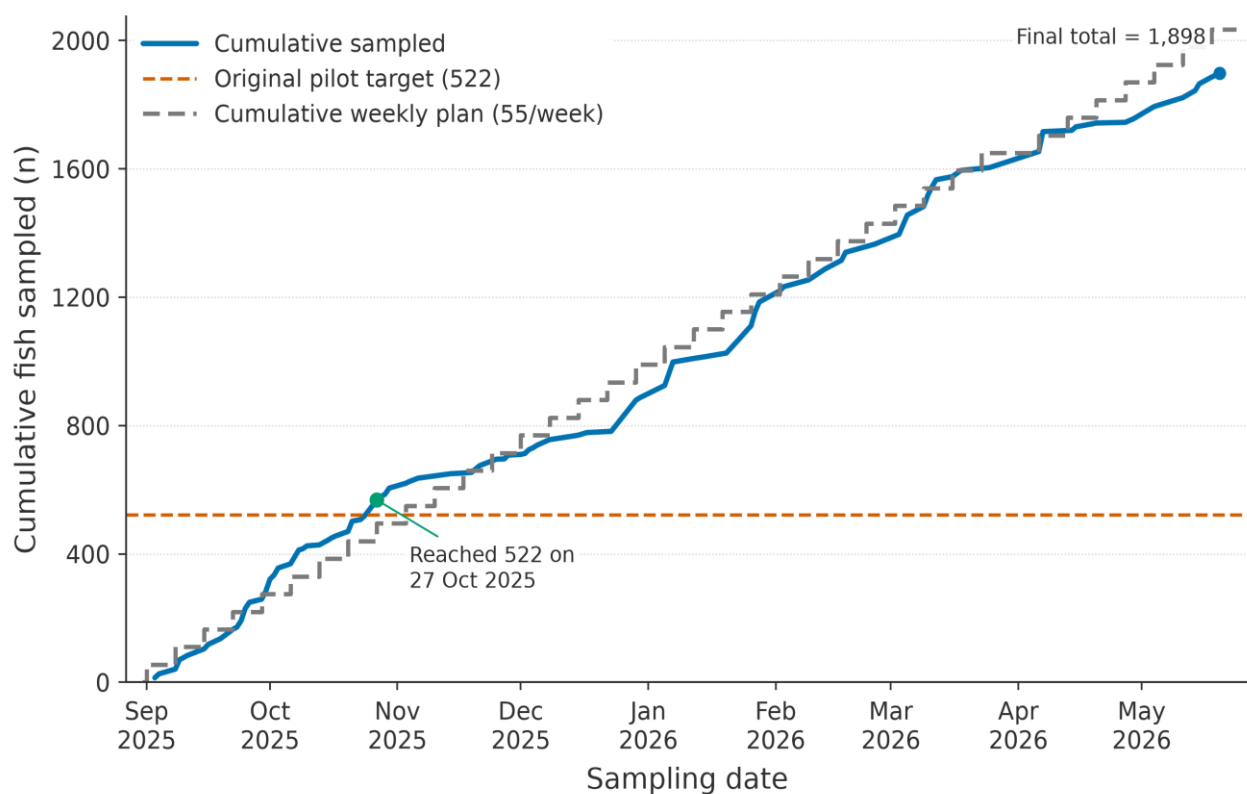


Figure 3. Cumulative number of yellowfin tuna sampled at Karachi Fish Harbour, September 2025 - May 2026.

6.2 Size composition

The number of fish sampled in each operational size class is compared with the target in Table 4.

Table 4. Fish sampled by operational size class.

Fork-length class	Target	Sampled	Share of sample	Sampled / target
<40 cm	-	3	0.2%	-
40-<50 cm	1,480	699	36.8%	47.2%
50-<60 cm	185	897	47.3%	484.9%
60-<70 cm	185	183	9.6%	98.9%
70-<80 cm	74	66	3.5%	89.2%
80-<90 cm	74	34	1.8%	45.9%
90-<100 cm	37	16	0.8%	43.2%
Total	2,035	1,898	100%	93.3%

Fish of 40-<50 cm were not available in the numbers planned, so samplers took the next-smallest fish and the 50-<60 cm class became the largest (Figure 4). Because selection was directed by size, this distribution does not represent the size composition of the catch.

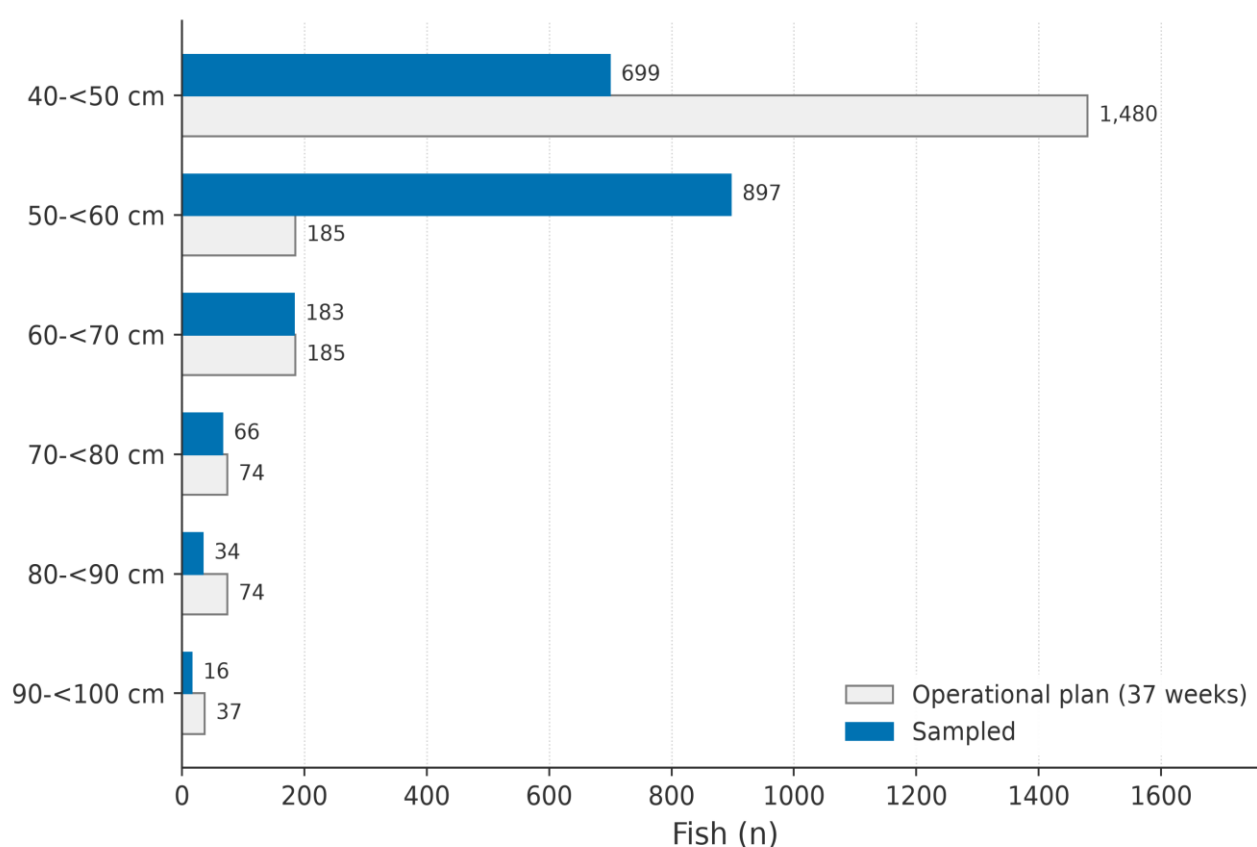


Figure 4. Planned and achieved numbers of yellowfin tuna by fork-length class.

6.3 Comparison with the CKMR design strata

Table 5 sets the pilot sample against the illustrative annual allocation for Region 1.

Table 5. Pilot sample against the illustrative annual Region 1 allocation.

Design stratum	Illustrative annual allocation	Pilot	% of allocation
<50 cm (potential offspring)	10,500	702	6.7%
>75 cm (potential parents)	2,250	78	3.5%
Both strata	12,750	780	6.1%
50-75 cm (outside the strata)	-	1,118	-

The 1,118 fish of 50-75 cm fall between the two strata and cannot at present be counted toward either. If ageing confirmed that all of them belong to the required juvenile cohorts, the juvenile set would rise to 1,820 fish (17.3% of the juvenile allocation); the realized figure will depend on the ageing results. For reference, all 1,898 fish are equal in number to 14.9% of the 12,750 total.

6.4 Coverage through the season

Sampling effort and fish size in each part of the season are given in Table 6.

Table 6. Sampling by part of the season.

Period	Fish	Sampling days	Weeks	Mean FL (cm)	Median FL (interquartile range), cm
Early (Sep-Oct)	605	34	9	52.1	50.0 (47.0-54.0)
Middle (Nov-Jan)	580	32	13	55.1	53.0 (49.5-57.1)
Late (Feb-May)	713	30	15	54.0	52.0 (48.0-58.0)
Total	1,898	96	37	53.7	51.5

The three periods contributed 31.9%, 30.6% and 37.6% of the sample. Sampling was sustained throughout the season.

6.5 Linked biological samples

Almost every fish has a complete set of linked records (Table 7).

Table 7. Completeness of records.

Component	Fish	Completeness
Genetic tissue (five replicates)	1,898	100%
Fork length and weight	1,898	100%
Sex recorded	1,880	99.1%
Gonads examined	1,880	99.1%
Gonads photographed	1,874	98.7%
Otoliths collected	1,879	99.0%
Stomachs examined	1,880	99.1%

Of the 1,880 fish examined, 1,156 (61.5%) were male and 724 (38.5%) were female, a ratio of 1.6 males per female. No fish was recorded as indeterminate; the 18 fish without a record were not examined. Most fish were immature: 1,449 (77.1%) were at stage 1 and 370 (19.7%) at stage 2, while 61 fish (3.2%) were at stages 3 or 4 (Table 8). The stage 1 total includes fish whose stage could not be determined. This is consistent with a sample dominated by fish below 60 cm. Sex and stage were assigned by eye and have not been validated by histology, so the sex of the smallest immature fish in particular should be treated with caution.

Table 8. Macroscopic maturity stage by sex (1 = immature; 2 = early maturing; 3 = late maturing; 4 = ripe). No fish was recorded at stage 5 (spent).

Sex	Stage 1	Stage 2	Stage 3	Stage 4	Total
Male	945	175	31	5	1,156
Female	504	195	25	0	724
Total	1,449	370	56	5	1,880
Share	77.1%	19.7%	3.0%	0.3%	100%

Table 9 shows how each type of data collected can be used.

Table 9. Use of the data collected.

Data	Use
Genetic tissue, sampling date	Required for kin identification and CKMR modelling
Fork length, otoliths	Required to assign age and birth year; otoliths are essential for the 50-75 cm group
Sex, maturity, gonad measurements	Supports CKMR interpretation; informs reproductive biology
Weight, other body measurements	Length-weight relationships
Stomach contents, other tissues	Not used in CKMR; available for diet and other studies

6.6 Archive status

The RNAlater replicate of 100 fish is at CSIRO with the ethanol replicates retained in Pakistan; tissue from the other 1,798 fish is in frozen storage in Pakistan pending decisions on custody, transfer and genotyping.

7. Lessons

Throughput. One trained team of five can deliver about 1,900 fully documented fish in a season at one landing site. Access to fish was not the constraint; the constraints were the availability of the desired sizes and the lack of supporting information needed for analysis.

Small juveniles are limited at Karachi. The 40-<50 cm class reached 47.2% of its target despite being the first priority every day. Planning should not assume that a juvenile allocation defined as <50 cm can be met from one landing site.

Age, not length, should decide the use of 50-75 cm fish. These fish are 58.9% of the collection. A stratified otolith-ageing study across 40-75 cm, covering the whole season, would show how many belong to usable cohorts.

Potential parents need their own target. Only 78 fish >75 cm were sampled because the matrix gave them a small allocation. This says nothing about the availability of large yellowfin in the fishery.

Vessel and trip must be recorded at the landing. This requires arrangements with boat owners or auction agents, since fish are currently selected from the mixed catch in the landing area.

Contamination control should be confirmed by the genetics laboratory. Feedback from the first 100 fish should be obtained before large-scale sampling, and the SOP revised if needed.

Regional standardization. A multi-country programme needs common identifiers, measurement methods, tissue type, preservation, source information, contamination controls, custody procedures and quality controls. Laboratory-critical details should be set centrally, not adapted by each node.

8. Limitations

- One landing site and mainly one gear; the sample does not represent the Region 1 fishery or population.
- Selection was directed by size. The length distribution describes the collection, not the catch.
- Vessel, trip and capture position were not recorded.
- DNA quality and cross-contamination have not yet been evaluated; results for the first 100 fish are pending.
- 1,118 fish (58.9%) fall outside the two design strata pending ageing; only 78 potential parents were sampled.
- Sex and maturity were assessed by eye without histological validation.
- A single season cannot provide the multi-cohort comparisons that CKMR requires.

9. Outline for a Second Phase

The pilot was designed to inform a multi-year, multi-country programme for Region 1. The elements in Table 10 are proposed for discussion; numbers are to be set with CSIRO and the participating CPCs.

Table 10. Outline of the second phase.

Element	Proposal
Duration	At least three, preferably five, consecutive fishing seasons, consistent with the design study
Pakistan node	Continue at Karachi Fish Harbour at about 50-55 fish per week, with a fixed weekly quota for >75 cm fish
Other nodes	Landing sites in four other Region 1 CPCs, each trained on the common SOP and forming, with Pakistan, a network of sampling nodes run by participating countries and organizations
Targets	An allocation for each node within the Region 1 total, based on measured availability by size, and revised after ageing results
Protocol	The SOPs developed in the pilot adopted as the starting point for a common Region 1 SOP, finalized with the genetics laboratory
Ageing	Stratified otolith ageing of the 2025-26 collection in year 1, then routine ageing of a subsample each season
Genotyping	Staged: quality check of each node's first samples before routine collection; arrangements for the existing 1,798-fish archive
Data	Common minimum data fields and a shared database with agreed access rules
Coordination	A technical coordination group reporting annually to WPTT
Costing	Per-fish cost to be measured and used to budget each node

The immediate priorities before a second phase begins are: (1) complete the CSIRO assessment of the first 100 fish; (2) age a stratified subsample of the 1,879 otoliths; and (3) revise the SOP in the light of the CSIRO results.

10. Recommendations for WPTT28

The WPTT is invited to:

1. **NOTE** that the pilot sampled 1,898 yellowfin tuna over 37 consecutive weeks at Karachi Fish Harbour, showing that sustained port-based CKMR sampling is feasible in the northern Arabian Sea, and that molecular validation of the samples is pending.
2. **NOTE** that 59% of the fish were 50-75 cm, between the design strata, and **ADVISE** whether fish in this range confirmed by direct ageing to belong to the required cohorts may be included in the juvenile sample, and on a suitable ageing subsample design.
3. **NOTE** that standard operating procedures for CKMR sampling and frozen storage of yellowfin tuna were developed and applied in the pilot and will be adopted for continued sampling, and **ADVISE** on the minimum data fields and quality controls to be standardized across Region 1 sampling nodes.
4. **CONSIDER** the outline for a multi-year, multi-country second phase in Region 1, including arrangements for custody, genotyping and data access for the existing Pakistan archive.

11. Conclusions

The Pakistan pilot set up and ran a port-based sampling system for yellowfin tuna CKMR for a full gillnet season. It sampled 1,898 fish on 96 days in 37 consecutive weeks, 3.6 times its original target, with five tissue replicates per fish and linked otolith, reproductive and stomach records for about 99% of fish.

Of these, 702 fish were <50 cm and 78 were >75 cm, so 780 fish fall within the published design strata. The value of the 1,118 fish of 50-75 cm depends on ageing, for which the otoliths are in hand.

The pilot demonstrates logistical feasibility. It does not yet demonstrate molecular suitability, which awaits the CSIRO results. With ageing, a dedicated component of large fish, vessel and trip recording, confirmed contamination control and sampling over several years, Karachi Fish Harbour can serve as a sampling node in a coordinated Region 1 CKMR programme, and the procedures developed can be shared with other nodes.

Acknowledgements

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References

- Bravington, M.V., Grewe, P.M. & Davies, C.R. 2016. Absolute abundance of southern bluefin tuna estimated by close-kin mark-recapture. *Nature Communications* 7: 13162.
- Bravington, M.V., Skaug, H.J. & Anderson, E.C. 2016. Close-kin mark-recapture. *Statistical Science* 31(2): 259-274.
- Fu, D., Urtizberea Ijurco, A., Cardinale, M., Methot, R., Hoyle, S. & Merino, G. 2021. Preliminary Indian Ocean yellowfin tuna stock assessment 1950-2020 (Stock Synthesis). IOTC-2021-WPTT23-12.
- Hillary, R.M., Tremblay-Boyer, L., Williams, A.J., Hill, N.J. & Preece, A.L. 2022. Indian Ocean yellowfin tuna close-kin mark-recapture design study. CSIRO, Australia. IOTC-2022-WPM13-12.

IOTC-SC28. 2025. Report of the 28th Session of the IOTC Scientific Committee. China, 1-5 December 2025. IOTC-2025-SC28-R[E], Rev.1: 269 pp.

IOTC-WPTT27. 2025. Report of the 27th Session of the IOTC Working Party on Tropical Tunas. Seychelles, 21-25 October 2025. IOTC-2025-WPTT27-R[E]: 75 pp.

Williams, A.J., Tremblay-Boyer, L., Hillary, R.M. & Preece, A.L. 2023. A close-kin mark-recapture pilot study for Indian Ocean yellowfin tuna. IOTC-2023-SC26-11.

WWF-Pakistan. 2025. Pilot sampling to support CKMR for Indian Ocean yellowfin tuna in the north Arabian Sea (Pakistan). IOTC-2025-WPTT27(DP)-INF01.

Annex 1. Target Matrices and Sample Inventory

A1.1 Original target matrix (522 fish)

The original matrix used broad descriptive labels for its size classes. In this report the design cut-offs (<50 cm and >75 cm) are applied to the measured length of each fish, so the 70-80 cm class is not assigned as a whole to the adult stratum.

Class label	Fork length (cm)	Target	Share
Juvenile-focused	40-50	367	70.3%
Subadult	50-60	50	9.6%
Subadult	60-70	50	9.6%
Larger fish	70-80	20	3.8%
Larger fish	80-90	20	3.8%
Larger fish	90-100	15	2.9%
Total		522	100%

A1.2 Expanded target matrix and achievement

Fork-length class	Weekly target	37-week total	Sampled	Difference
<40 cm	-	-	3	+3
40-<50 cm	40	1,480	699	-781
50-<60 cm	5	185	897	+712
60-<70 cm	5	185	183	-2
70-<80 cm	2	74	66	-8
80-<90 cm	2	74	34	-40
90-<100 cm	1	37	16	-21
Total	55	2,035	1,898	-137

A1.3 Status against the CKMR design strata

Category	Fish	Interpretation
<50 cm	702	Within the potential-offspring stratum
50-75 cm	1,118	Between strata; use depends on ageing
>75 cm	78	Within the potential-parent stratum
Within strata	780	6.1% of the illustrative annual Region 1 allocation (12,750)
All fish	1,898	

A1.4 Archive inventory

Component	Status
Fish sampled	1,898
Tissue vials	Five per fish (vial A in RNAlater; vials B-E in ethanol); 9,490 expected; vial-level reconciliation to be completed
Sent to CSIRO	RNAlater replicate (vial A) of the first 100 fish (November 2025); ethanol replicates retained in Pakistan
CSIRO assessment	DNA quality and cross-contamination; pending
Held in Pakistan only	1,798 fish, frozen at about -35 °C
Otoliths	1,879 fish
Sex and maturity records	1,880 fish (1,156 males, 724 females)
Gonad records	1,880 fish (1,874 photographed)
Stomach records	1,880 fish
Other frozen tissues (liver, heart, gill, dorsal and tail muscle)	1,880 fish, for later metal analysis