SPRFMO
South Pacific Regional Fisheries Management Organisation

11th MEETING OF THE SCIENTIFIC COMMITTEE

11 to 16 September 2023, Panama City, Panama

SC11 – JM08

Instructions for Sampling JM for population study

Republic of Peru

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INSTRUCTIONS FOR COLLECTING SAMPLES OF JACK MACKEREL *Trachurus murphy*i FOR POPULATION STUDY WITH A MULTIDISCIPLINARY APPROACH

by

Instituto del Mar del Perú (IMARPE)

This report contains information on the Jack mackerel stock and fishery in Peruvian jurisdictional waters that, we reiterate, the delegation of Peru, in use of its discretionary powers, voluntarily provides for the purpose of information and support to the scientific research work within the Scientific Committee of the SPRFMO. In doing so, while referring to Article 5 of the Convention on the Conservation and Management of High Seas Fishery Resources in the South Pacific Ocean and reiterating that Peru has not given the express consent contemplated in Article 20 (4) (a) (iii) of the Convention, Peru reaffirms that the decisions and conservation and management measures adopted by the SPRFMO Commission are not applicable within Peruvian jurisdictional waters.

2023

SUMMARY

The population structure and connectivity of Jack mackerel (JM) throughout its distribution range are important aspects to take into account in its stock assessment and management. Due to its complexity and limited understanding (given its wide distribution, uncertain migration patterns, different spawning and fishing areas, in addition to varying responses to the influence of environmental changes), JM population structure and connectivity should be evaluated along the whole species range following the same criteria, where sampling design plays a very important role for ensuring a subsequent holistic analysis. In this sense, a JM sampling protocol is proposed for the study of JM population structure and connectivity, focused on the population analysis with a multidisciplinary approach. Thus, guidelines for collecting, storing specimens and processing biological samples for the multidisciplinary study are proposed, including a temporal and spatial sampling criteria, different types of sampling (on board vessels and in ports, terminals and fishing factories), and conditions for their transport to laboratories. The subsequent processing of the specimens for their population study is also covered, including sampling protocols for studies of: reproductive biology, parasites, genetics, trophic ecology, and age and growth. All these steps are also presented in a schematic workflow to facilitate their easier implementation in laboratories of decentralized sites, as well as headquarters. Finally, we consider that information in this document may be a useful contribution to the Scientific Committee and the JM Connectivity Task Group, for the development of a regional sampling design and protocol.

1. INTRODUCTION AND OBJECTIVE

The understanding of the population structure and variability of Jack mackerel (JM), for the identification of stocks or population units and possible connectivity, are important for the JM assessment and management. During the SC9 held in 2021, some proposals were presented (SC9-JM04, SC9-JM08), and it was mention that an interpretation of JM population structure is necessary, with a multidisciplinary (e.g. morphometry, parasites, trophic ecology, among others, as well as genetics) and spatio-temporal approach (SC9-JM08). Thus, the JM Connectivity Task Group was established to develop a research plan for characterizing the population structure and variability of JM on a spatio-temporal scale, in order to disentangle its complex population structure and determine the existence of stocks or population units and their level of connectivity. Indeed, this requires a long-term multidisciplinary study, with a well-stratified sampling design and considering spatial and temporal variability. Therefore, as part of the ToR of the Task group, the following was mentioned: *"agree on protocols for collecting and processing samples and propose methods for analysis in each of the prioritised lines of research. Agree on the proper operational spatio-temporal scale for the sampling plan"* (SC10-Final report).

In this sense, the objective of this document is to present a set of instructions developed by IMARPE (Instituto del Mar del Perú), to establish guidelines and standardize the sampling of whole specimens of *T. murphyi* in Peruvian vessels and land sites, for carrying out a multidisciplinary study of the important aspects of its biology, fishery and population characteristics. This includes reproductive conditions, spawning areas, trophic ecology, parasitology, genetics, age and growth, general distribution, fishing areas, population structure, stock identity and connectivity. It is proposed and expected that these guidelines be considered, and adapted as appropriate, in the development of a more regional protocol.

Therefore, this document is intended to be a contribution to the SC and its JM Connectivity Task Group, for the development of a regional sampling protocol for the study of JM population(s) and connectivity over its whole distribution range.

2. SCOPE

The procedure is applied for the collection, temporary storage and processing of samples of whole specimens of *T. murphyi* caught in Peruvian jurisdictional waters and the SPRFMO Convention area, either on board of IMARPE´s research vessels, on board of Peruvian commercial fishing vessels (industrial, small-scale, or artisanal), as well as in ports, terminals and fishing factories. In addition, it applies to the collection and processing of samples by professionals and technicians from coastal laboratories, decentralized headquarters and the headquarters of IMARPE, for population and connectivity studies.

The IMARPE units responsible for the design and implementation of this established guidelines for obtaining, storing specimens and processing biological samples for the multidisciplinary study are: Dirección General de Investigaciones de Recursos Pelágicos (Área Funcional de Recursos Transzonales y Altamente Migratorios, Área Funcional de

Investigaciones en Recursos Neríticos Pelágicos (including Laboratorio de Biología Reproductiva), Dirección General de Investigaciones de Recursos Demersales y Litorales (Laboratorio de Edad y Crecimiento and Laboratorio de Ecología Trófica), and Dirección General de Investigaciones en Acuicultura (Laboratorio de Genética Molecular and Laboratorio de Patobiología Acuática).

3. DESCRIPTION OF THE COLLECTION AND SAMPLING

3.1. Sampling criteria

- Obtain whole JM specimens of different sizes, taking into account a temporal and spatial criterion, as follows:

a. Temporal:

Make collections with a seasonal periodicity (considering an interval of 1 to 2 months per sampling group, covering the 4 seasons of the year).
Therefore, there will be 4 seasonal samples per sub-area per year.

b. Spatial:

Sampling is carried out with two spatial stratification criteria in the Peruvian jurisdictional waters: under a latitudinal (north, central and south regions) and longitudinal (distance from the coast) stratification.
Therefore, 9 sub-areas are considered for sampling groups per season, being a total of 36 sampling groups collected by year.

b.1. Within Peruvian jurisdictional waters

Collect along the latitudinal distribution of the resource, as follows:

(i) North: (Fig. 1, site 1) from Paita (- 4°S) to Huarmey (- 10°S),
(ii) Center: (Fig. 1, site 2) from Huarmey (- 10°S) to San Juan (- 15°30' S),
(iii) South: (Fig. 1, site 3) from Atico (- 16°S) to Morro Sama (- 18°S).

Collect along the longitudinal distribution of the resource (distance to the coast):

(i) near the coast (Fig. 1, sites a), between 0 and 60 mn,
(ii) intermediate, between 61 and 120 mn,
(iii) near the 200 mn limit (Fig. 1, sites c), between 121 and 200 mn distance from the coast.

b.2. Within the Convention area:

Collect in the north, central and south areas beyond the Peruvian jurisdictional waters (Fig 1, sites 9 to 11), in the north, central and south areas beyond the Chilean jurisdictional waters (Fig. 1, sites 12 to 14); and in areas in the mid Pacific Ocean (Fig 1, site 15).

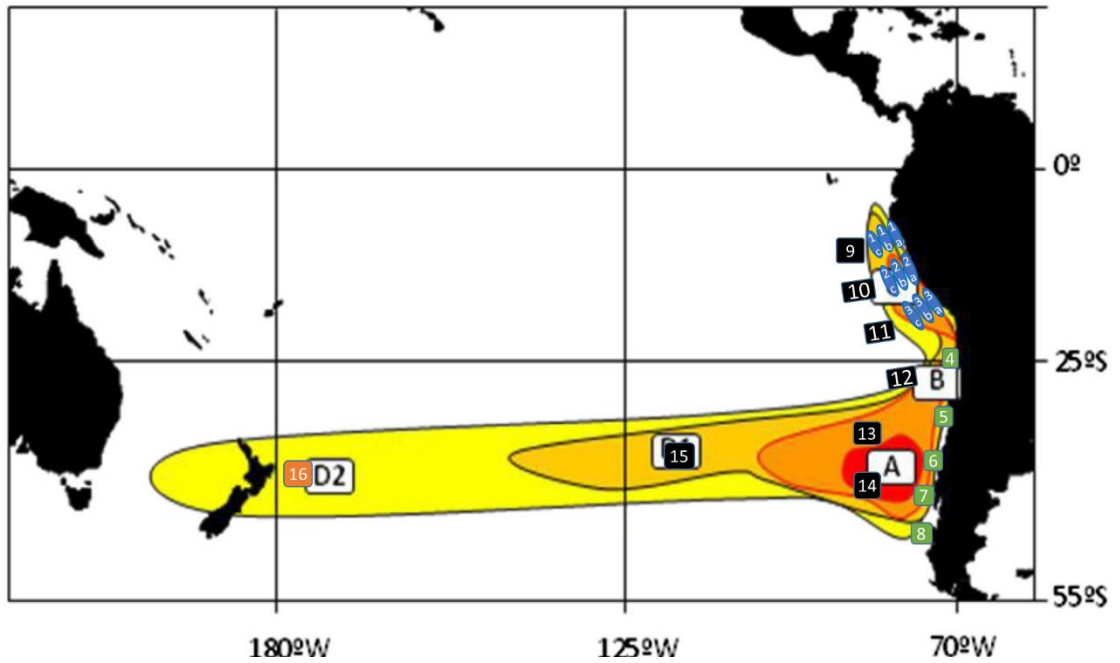

Figure 1. Proposed areas for collecting *T. murphyi* specimens in Peruvian jurisdictional waters (1 to 3, blue), and in Convention waters (9 to 15, black). Numbers in green and orange indicate areas in the Chilean and New Zealand jurisdictional waters, respectively. Based on a proposal in SC9-JM08 and modified from Gerlotto et al. (2012).

3.2. Sample collection

For obtaining samples with the spatial and temporal criteria indicated in 3.1 (from a seasonal sampling at different points of the geographical distribution of the resource), sampling on board IMARPE research vessels, on board commercial vessels, as well as at docks, terminals and fishing factories, should be done within 24 hours of capture.

3.2.1. On board vessels.

a. Collect whole specimens from:

(i) Onboard IMARPE research vessels

- Select specimens from one fishing set, corresponding for one of the sampling sub-area (indicated in 3.1), per season and per year.
- Select 60 whole specimens, including different lengths.
- Number of samples can be completed with the next set from the same sub-area, at near time from the previous set.
- If samples are obtained at all 9 sub-areas, then a total of 540 specimens will be collected in one season per year, in the Peruvian jurisdictional waters.
- Obtain data of abiotic parameters (*e.g.*, sea surface temperature, sea surface salinity, among others), as well as biotic parameters (*e.g.*, accompanying fauna, plankton, among others).

(ii) Onboard Peruvian commercial fishing vessels

- According to the expected capture zone, a previous communication with IMARPE is suggested for coordinating sampling sub-areas (of item 3.1).
- From one fishing set of one of the trips made by a vessel in a week, randomly select a maximum of 120 individuals per boat trip, in one sub-area per season of the year.
- The number of samples can be completed including specimens caught from the next set from the same sub-area, at near time previous set.

b. Place whole specimens in plastic bags.

c. Label each bag by placing a label (on canson paper written with pencil) with the following information:

CATCH DATA
Vessel name: _____
Date: _____
Catch area: _____
Set number: _____
Geographical position (Latitude and Longitude): _____
Sampler: _____

d. Close the labeled bag with the help of adhesive tape so that it is airtight.

e. Place the bag in an on-board freezer (0°C to below) to ensure its preservation. If a freezer is not available, keep the specimens on ice and try to deliver the samples for processing as soon as possible (approximately 1 day).

f. According to the landing zone, coordinate with the nearest IMARPE coastal laboratory for the reception of the sample and its subsequent processing.

3.2.2. Obtaining samples at ports, terminals and fishing factories

Note: Prior to obtaining samples of whole specimens, coordinate with the staff of the coastal and headquarter laboratories in charge of biological sampling.

a. Collect whole specimens

b. Perform sampling for each vessel that has completed a JM fishing trip, following the criteria in 3.1 above, ensuring that a diverse range of sizes or organisms are collected.

c. Conduct all activities in coordination with coastal laboratory staff. Randomly select approximately 100 individuals from each landing boat vessel.

d. Place whole specimens in a clean plastic bag (50-100 L approximately).

e. Label each bag by placing a label (on canson paper) as indicated in point 5.2.1.c. above.

f. Transport the samples to the nearest coastal laboratory for processing.

3.3. Processing of samples for population studies

Processing of JM samples should be done in the following order (3.3.1 to 3.3.9). The list of materials required are indicated in Annex 3.

3.3.1. Biological sampling (before evisceration)

NOTE: Biological sampling shall be done in two stages. The initial one before evisceration (section 3.3.1), and the other after evisceration (3.3.3).

a. Using the ichthyometer and precision balance, measure (cm) and weigh (grams) each complete specimen.
b. On the work table, arrange the specimens in increasing order of size and indicate each group that is discriminated by 1 cm of total length (maintain this order during all the activities indicated in 3.3). Record the information in the Biological Sampling Sheet Format, which is routinely used (Annex 1).

(Samples obtained or gathered in coastal laboratories and to be sent to headquarters, should be placed in adiabatic boxes for shipment)

3.3.2. Obtaining ectoparasites

a. Do not collect ectoparasites if the specimens are frozen.
b. Prior to collection, prepare a funnel with grade 4 filter paper, with a diameter of 125 mm, in a collection vessel (Erlenmeyer flask).
c. Select 10 JM specimens: the 5 smaller and 5 larger ones (10 specimens total).
d. After measuring and weighing each of the specimens (not eviscerated), place them on a clean plastic tray.
e. With the help of a rinse-bottle, rinse the body of the fish on both sides with 0.85% saline solution, using between 50 to 100 ml of solution. Perform this procedure by gently scraping the skin (from the head to the caudal fin) with a scalpel blade laterally, so as not to cause structural damage to the parasites collected.
f. In the same tray, wash the mouth of the specimen with the saline solution.
g. Filter the washing liquid by pouring it into the funnel with filter paper, and rinse the walls of the tray with the rinse-bottle to be sure to collect any remnants.
h. Place the filter (with the filtered solid material) in a 200 ml bottle containing approximately 150 ml of 70% ethanol.
i. Label the bottle by placing a label inside (on a canson paper written with pencil) and write the following information: species, collection area, date, specimen number, and indicate "SKIN and MOUTH" (parts of the specimen that have been filtered).
j. Label the outside of the bottle with a marker indicating: species name, serial number of the sample, origin of each filtrate: mouth and skin, and date. The sample obtained will be used for the identification of ectoparasites.
k. Before processing the next specimen, wash the tray and scalpel with plenty of water.
l. Send the vials to the Laboratorio de Patobiología Acuática, at IMARPE headquarters.

3.3.3 Eviscerated biological sampling

a. Extract gonads, stomachs and intestines from each specimen.
b. Place them in order in a tray.
c. Weigh the eviscerated specimens (in grams).
d. Weigh the gonads (in grams).
e. Catalog the gonads according to validated maturity scales.
f. Record the information indicated on the routinely used fish Biological Sampling Sheet format (Annex 1).
g. Continue sample collection as indicated below.

3.3.4 Gonads collection

Apply this procedure only to adult female specimens (greater than 26 cm total length):

a. Once the biological sampling is finished, take the complete gonad from each specimen, remove the ovaries and place them inside a plastic bag or plastic flasks containing 10% buffered formalin. Consider a size of bag or flask that will ensure the storage of the full gonad, keeping it completely covered with formalin, which is essential to ensure a good fixation.
b. Label the sample obtained, by placing a canson paper label inside the bag or bottle with the main data of the sample (written with pencil): species, date, area, vessel, capture and serial number of the sample.
c. Repeat the procedure for each adult female specimen.
d. In the case of hydrated gonads, remove them very carefully to avoid breaking them.
e. On the Biological Sampling Sheet format (Annex 1), indicate the samples collected by assigning an identification code.
f. Send the bags or flasks with the gonad samples to the nearest Laboratorio de Biología Reproductiva (Tumbes Coastal Laboratory, Huanchaco, Chimbote or IMARPE Headquarters) of IMARPE, for the respective histological analysis (study of the spawning fraction).

3.3.5 Stomachs collection

a. Select the first 3 specimens for each size, and collect the stomachs (empty or full).
b. Place the stomachs in order in a clean tray.
c. Consecutively, from the first to the last, tie with a single wick thread each stomach through the esophageal section.
d. Tie the label with the data before the first stomach to identify the beginning of the series collected.
e. Place the collected stomachs in a bag with 96% ethanol.
f. Send the samples to the Laboratorio de Ecología Trófica at IMARPE Headquarters.

3.3.6 Muscle tissue collection for isotope and genetic analysis

a. For this activity, always use disinfected gloves, tweezers and scalpel blades (washing the material with ethanol and drying with paper towel).
b. Obtain muscle tissue from the 15 smallest and 15 largest specimens (total of 30 specimens). For this purpose, make a cut of the dorsal part close to the head, of approximately 2 cm^3.
c. Place the tissue in a 15 ml falcon tube or 1.5 ml microtube, containing 96% alcohol (10 ml if using falcon tubes or 1 ml if using microtubes), and close it tightly.
d. Verify that the tissue is completely submerged in the alcohol (keep approximately 3 times the volume of the alcohol with respect to the tissue).
e. Label the container with the tissue on the outside, indicating the sample code (according to the specimen indicated on the Biological Sampling Sheet form, Annex 1).
f. Place the tubes in a cryobox or hermetic plastic bag, and label indicating the area and date of collection.
g. Before taking the next sample, clean the dissection material using a paper towel and 96% alcohol, verifying that there are no muscle tissue remnants.
h. Keep the samples refrigerated (ideally between 0 and 10 °C), if possible.
i. On the biological sampling form, indicate with a "G" the specimens collected.
j. Send the collected tubes to the Laboratorio de Genética Molecular at IMARPE headquarters.

3.2.7 Collection of endoparasites and ectoparasites in gills

From the 10 specimens selected for ectoparasites (5 smaller and 5 larger):

a. Always wear gloves to perform this activity.
b. Place one specimen in a clean plastic tray.
c. Remove the gills of the specimen and place them in a container (plastic bottle) containing 200 ml of 70% alcohol. This will be used later for the study of ectoparasites in the gills of the specimens.
d. Place a label (canson paper and written with pencil) inside the flask, stating the above-mentioned data.
e. Close and label the bottle (as indicated for ectoparasites, 3.3.2).
f. Before processing the next specimen, previously wash the tray with abundant water and clean the gloves.
g. After collecting otoliths from these 10 specimens, place each eviscerated specimen and its internal organs in separate bags, accompanied by a label stating the above-mentioned data. Keep them frozen for later examination and identification of endoparasites.
h. Send the flasks and frozen specimens to the Laboratorio de Patobiología Acuática at IMARPE headquarters.

3.2.8 Collection of otoliths

a. From the specimens sorted by size during biological sampling, select 10 individuals from each size.

b. Using tweezers or the finger, break the isthmus of the fish.
c. Remove the gill bundle and clean the area of tissue remnants (such as blood, muscle, etc.) until the spine and neurocranium (otic capsule) are visible.
d. Break the spine at the base of the skull, transversely, using scissors.
e. Open the braincase carefully with the tweezers. Be careful when exerting force so as not to break the otoliths located inside the braincase. Once the braincase is open, the otoliths will be clearly visible.
f. Remove the pair of otoliths, clean them of tissue debris using tweezers, and dry them.
g. Store the otoliths in an "otolith cardboard" container and label them appropriately (Fig. 2) with the following information: common name, place of origin or cruise name, date, size, sex (0 females and 1 male), carton number (if more than one carton), catch (cruise).
h. At the end, cover each carton with tape to prevent loss of otoliths.
i. Send the collected otoliths to the Laboratorio de Edad y Crecimiento at the headquarters of IMARPE.

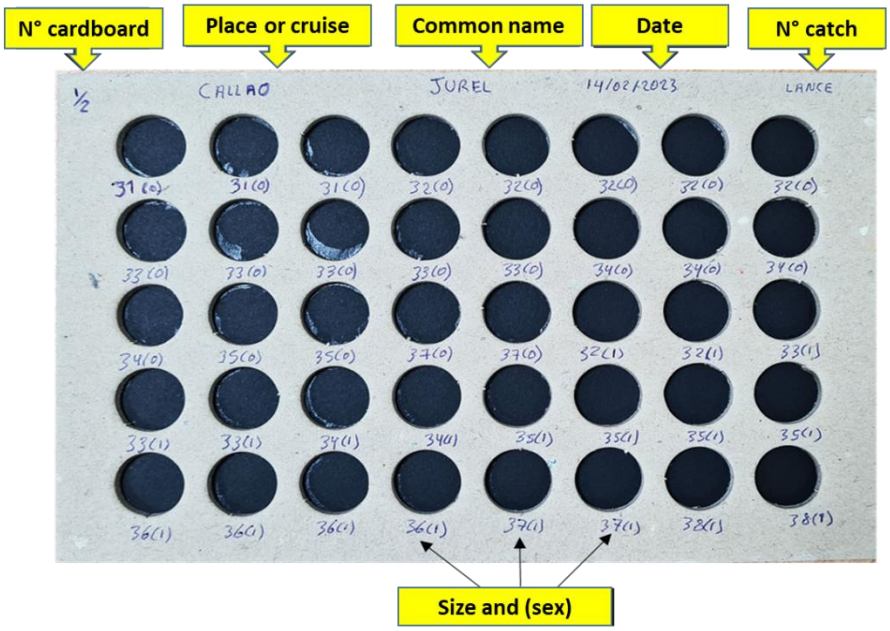

Figure 2. Label of JM otolith cardboards

ANNEXES

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F 03-B/IMP

ESPECIE	
MUESTRA N°	
CRUCERO	
NOMBRE: BUQUE/ LANCHA	

PUERTO				
LONGITUD		FECHA	DÍA	MES AÑO
TOTAL		DE		
HORQUI.		A		

N° PARA COMPUTO

N°	LONG. (cm)	PESO TOTAL (g)	PESO EVISCE. (g)	SEXO	MADU	LONG. GONADA (mm)	PESO GONADA (g)	OTOLITOS N° SERIE	G	EDAD	AREA	N°	LONG. (cm)	PESO TOTAL (g)	PESO EVISCE. (g)	SEXO	MADU	LONG. GONADA (mm)	PESO GONADA (g)	OTOLITOS N° SERIE	G	EDAD	AREA
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2												2											
3												3											
4												4											
5												5											
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RESPONSABLE		

HEMBRAS	0
MACHOS	1

L	LANCE N°	
TIPO DE PESQUERÍA	INDUSTRIAL	1
	CONSUMO	2

OBSERVACIONES

ANNEX 1. Biological Sampling Sheet Format

ANNEX 2. Workflow for the collection of jack mackerel samples.

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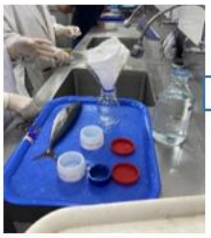
3.3.1. Biological sampling (before evisceration)

- Measure and weigh each specimen. Complete the Biological Sampling Form.
- Before eviscerating the specimens, go to point 3.3.2.


3.3.2. Obtaining ectopararasites

- Select 10 specimens (the 5 smallest and 5 largest).
- Place filter paper in funnel.
- Place a specimen in the tray and rinse it with 0.85% saline solution, gently scraping the skin with a scalpel.
- Rinse the mouth of the specimen.




- Filter the washing liquid, rinsing the tray with saline solution.




- Remove the filter and place it in a bottle with 200 ml of alcohol 70%. Put the label inside.
- Label the walls of the bottle.

3.3.3 Eviscerated biological sampling

- Remove the gonads and stomach. Weigh eviscerated specimens and gonads.
- Catalog the gonads according to a validated maturity scale.

3.3.4 Gonads Collection

- Apply only to adult female specimens (>26 cm). Remove whole ovaries and place them in a plastic bag or flask with 10% buffered formalin. Include a label.

3.3.5 Stomachs collection

- Select the first 3 specimens per size, and collect the stomachs. Tie with wick thread each of the stomachs through the esophageal section, consecutively from the first to the last one. Tie the label. Place them in a bag with 96° alcohol.

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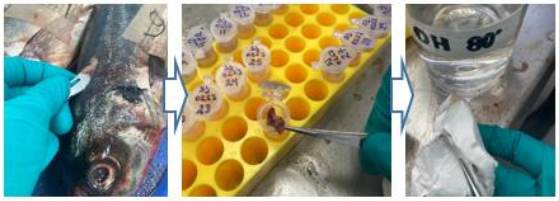
3.3.6 Muscle tissue collection for isotope and genetic analysis

- Take samples from the 15 smallest and 15 largest specimens.
- With forceps and scalpel (wearing gloves), cut 2 cm^3 of muscle from the dorsal part near the head. Place in a tube with ethanol. Label.
- Clean the collection material before the next specimen.



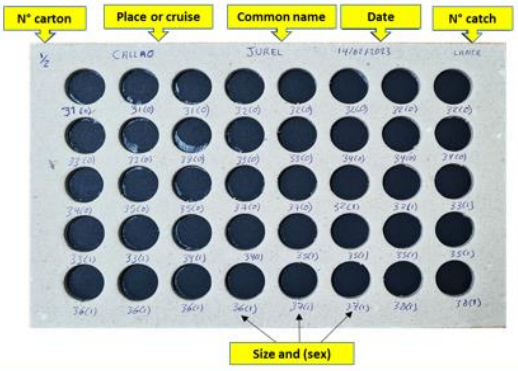
- Indicate with a "G" on the sampling sheet the specimens collected.

3.2.7 Collection of endo and ectoparasites in gills

- From the 10 specimens selected for ectoparasites, using gloves, remove the gills and place them in a bottle with 200 ml of 70% alcohol. Label.
- Wash the collection material with abundant water before the next specimen is taken.
- After removing the otoliths, place the eviscerated fish and internal organs (remaining from the stomach collection) in bags, label and freeze.

3.2.8 Collection of otoliths

- From the specimens sorted by size during biological sampling, select 10 individuals for each size.
- Extract the otoliths very carefully, using tweezers, clean them from tissue debris using tweezers, and dry them.
- Store the otoliths in a container "otolith cardboard" and label them appropriately (Fig. 2).
- At the end, cover each carton with adhesive tape to prevent loss of otoliths.



Annex 3. List of Materials

Biological material

- Whole specimens of Jack mackerel

Sample collection

- Plastic trays
- 50 and 100 L plastic bags for sample storage.
- 50 L adiabatic boxes
- Otolithic cardboards
- Adhesive tape
- Funnel
- Biological sampling form
- 1 L plastic bottles
- 200 ml plastic bottles
- Latex or nitrile gloves
- Scalpel handle and blades
- Ichthyometer
- Pencil
- Wick
- Absorbent cloths
- Canson paper
- Grade 4 filter paper with a diameter of 125 mm
- Paper towel
- Tweezers
- Rinse bottle
- Marker
- Dissecting scissors
- 15 ml falcon tubes or 1.5 ml micro tubes

Equipment

- Balance

Reagents

- 10% buffered formaldehyde
- Alcohol (ethanol) 96%.
- Alcohol (ethanol) 70%.
- Saline solution 0.85% (dilute 8.5 g of NaCl in 1 L of distilled water and shake until no salt granules are visible).