SPRFMO
South Pacific Regional Fisheries Management Organisation

7th MEETING OF THE SCIENTIFIC COMMITTEE

La Havana, Cuba, 7 to 12 October 2019

SC7-SQ10

Protocol for the collection of Jumbo flying squid muscle tissue for molecular analysis used by the Instituto del Mar del Peru

Peru

PO Box 3797, Wellington 6140, New Zealand
P: +64 4 499 9889 – F: +64 4 473 9579 – E: secretariat@sprfmo.int
www.sprfmo.int

IMARPE

South Pacific Regional Fisheries Management Organisation
2nd Squid Workshop and 7th Meeting of the Scientific Committee
La Havana, Cuba, 5–12 October 2019

PROTOCOL FOR THE COLLECTION OF JUMBO FLYING SQUID *Dosidicus gigas* MUSCLE TISSUE FOR MOLECULAR ANALYSIS USED BY THE INSTITUTO DEL MAR DEL PERU (IMARPE)

by

Giovanna Sotil*, Deivis Cueva, Marcos Espinel and Luis Mariátegui
Instituto del Mar del Peru (IMARPE)
Esquina Gamarra y Gerneral Valle s/n, Callao, Perú

* E-mail: gsotil@imarpe.gob.pe

2019

SUMMARY

Several molecular techniques from the traditional to the recent NGS (Next Generation Sequencing) are possible to be used in population genetic analysis, for the evaluation of different levels of variability throughout a species genome. The choice of a particular molecular marker depends not only on the resolution of the genetic variability but also on the sampling design such as periodicity, quality and type of tissue and DNA available, DNA integrity, etc. A critical aspect to consider is the tissues collection and their preservation to guarantee the good quality of DNA avoiding degradation over the time, and allowing its use for long field studies. Therefore, the use of lab protocols is important to ensure that different methods are performed with safe procedures.

In this sense, the following text provides a detailed description of the methodology that IMARPE is considering for the sampling of biological material of the jumbo flying squid *Dosidicus gigas*, which is being used for the population genetic analysis. Details are given for catch, preparation of the work area, sample collection and its conservation, along a scheme of collection sites in representative areas of the Peruvian jurisdictional waters. Finally, it is important to consider a standardize protocol for the evaluation of the jumbo flying squid, in order to compare data along spatial and temporal of the species' world distribution.

TABLE OF CONTENT

1. INTRODUCTION AND OBJECTIVE

The instructions that follow have been prepared considering the proposed "Sampling protocol of jumbo flying squid in the Southeast Pacific for genetic study" developed during the 6th meeting of the SPRFMO Scientific Committee (ref.: Annex 9 of the SPRFMO SC6-Report). Which has been modified, supplemented and expanded based on the experience, needs and requirements of the Instituto del Mar del Peru (IMARPE).

This paper is a contribution to the discussions to be held during the 2nd Squid Workshop and 7th Meeting of the Scientific Committee, La Havana, Cuba, 5–12 October 2019, in partial fulfillment of the task dealing with squid connectivity and the development of standardized approaches for the collection and analyses of genetic samples considered in the Expanded Scientific Committee Multi-Annual Work Plan (2019) of the Squid Working Group of the SPRFMO Scientific Committee (ref.: SPRFMO document SC7-Doc05).

These instructions establish the guidelines to be followed for the collection and storage of *Dosidicus gigas* muscle tissues, to be used in the evaluation of the specie's genetic variability, along its latitudinal and longitudinal distribution in the Peruvian jurisdictional waters. These studies will be complemented with the respective biological and biometric analyzes, in order to determine the population genetic structure, stocks identity and their association with phenotypic parameters.

2. SCOPE

The procedure is applied for the collection of samples of the muscle tissue of *Dosidicus gigas* captured on board IMARPE's research vessels by the Functional Area of Research on Marine Invertebrates and Macroalgae (AFIIMM), also responsible for the respective fisheries biology studies within of the General Directorate for Demersal and Coastal Resources Research (DGIRDL) of IMARPE. The Laboratory of Molecular Genetics of IMARPE's General Directorate for Aquaculture (DGIA) is responsible for establishing the guidelines for the collection of the sample, its storage and the molecular analysis. The general area covered and the main sampling subareas are shown in Figure 1.

3. DESCRIPTION OF THE COLLECTION AND SAMPLING

3.1. MATERIAL

- 2 fine-point tweezers.
- Stainless steel scalpel (scalpel blades).
- Scissors.
- Nitrile or latex gloves.
- Ethanol 96%.
- Paper towels.
- Box with 1.5 ml labeled microtubes containing ethanol 96%.
- Dissection tray.
- F05-B/IMP form for the biological data collection of cephalopods (Figure 2).

3.2. CATCH AND INFORMATION GATHERING

a. The capture of specimens for the collection of tissue is done in three general representative areas of the Peruvian jurisdictional waters (Figure 1), in the north (from

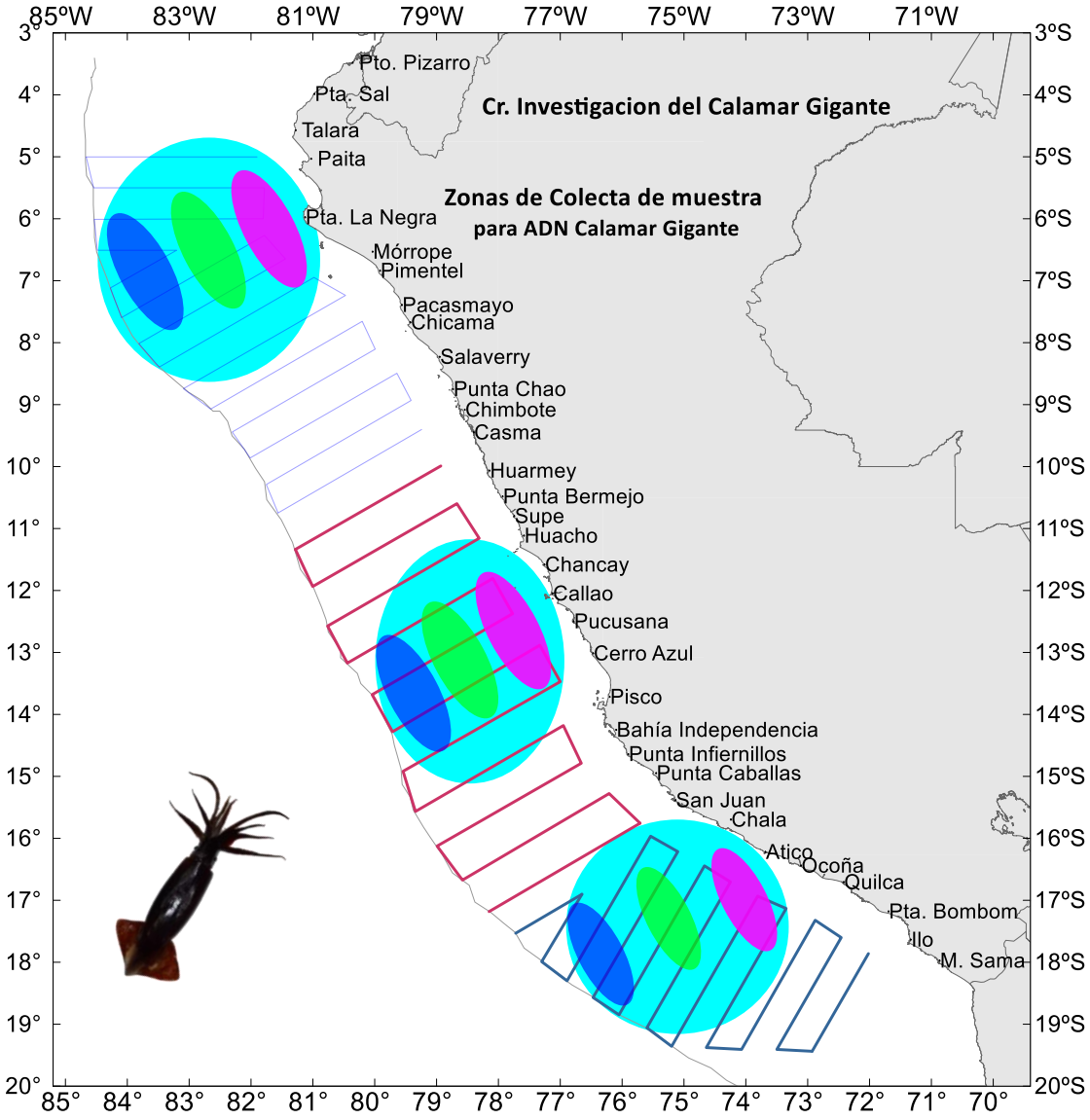


Figure 1.- Collection areas proposed for the population genetic analysis of the Jumbo flying squid *Dosidicus gigas*

Paita to Pimentel), center (from Huacho to Pisco) and south (from Chala to Ilo), according to the plan established in the Scientific Research Cruises of jumbo flying squid.

b. Collect approximately 180 specimens in each general area, of which 60 will be collected within 40 nm distance from the coast, another 60 specimens from the furthermost offshore 30-60 nm band covered by the research cruise and the remaining 60 specimens from the intermediate band covered by the cruise.
c. The capture of specimens is to be performed according to the procedure used for the biological and biometric sampling of cephalopods, established by the AFIIMM of IMARPE (Tafur *et al.*, 2016).

3.3. PREPARATION OF THE WORK AREA

a. Clean the area and materials (tray, scissors, scalpel and tweezers) with 96% ethanol, and dry with paper towel, make sure there is no paper or other residues remain between the grooves of the material.
b. Put on gloves, wash your hands with alcohol and dry with paper towel.

F 05 - B / IMP

INSTITUTO DEL MAR DEL PERÚ
MUESTREO BIOLÓGICO DE CEFALÓPODOS

MÉTODO DE CAPTURA	OPERACIÓN	OBSERVADOR	PÁGINA N°

ESPECIE

FECHA	POSICIÓN		PROFUNDIDAD DE PESCA m.	TSM	EMBARCACIÓN	ÁREA PESCA	OBSERVACIONES
	LATITUD	LONGITUD					
	S	W					

N°	LM mm	PESO TOTAL g	PESO MANTO g	SEXO ♂♀	COPULACIÓN S N	PESO ÓRGANOS REPRODUCTORES Y GLÁNDULAS ANEXAS			L.G. NID L. TEST. D.G.OVID mm	MADUREZ	LLENURA ESTÓMAGO	PESO CONTEN. ESTOMAC. g	PESO G. DIG. g	DESCRIPCIÓN CONTENIDO ESTOMACAL	OBSERVACIONES
						TESTÍCULO OVARIO g	COMPLEJO ESPT. OVIDUCTO Y GLAND. g	SACO ESPT. GL. NID. g							
1										1 2 3 4 5	0 1 2 3				
2										1 2 3 4 5	0 1 2 3				
3										1 2 3 4 5	0 1 2 3				
4										1 2 3 4 5	0 1 2 3				
5										1 2 3 4 5	0 1 2 3				
6										1 2 3 4 5	0 1 2 3				
7										1 2 3 4 5	0 1 2 3				
8										1 2 3 4 5	0 1 2 3				
9										1 2 3 4 5	0 1 2 3				
0										1 2 3 4 5	0 1 2 3				
1										1 2 3 4 5	0 1 2 3				
2										1 2 3 4 5	0 1 2 3				
3										1 2 3 4 5	0 1 2 3				
4										1 2 3 4 5	0 1 2 3				
5										1 2 3 4 5	0 1 2 3				
6										1 2 3 4 5	0 1 2 3				
7										1 2 3 4 5	0 1 2 3				
8										1 2 3 4 5	0 1 2 3				
9										1 2 3 4 5	0 1 2 3				
0										1 2 3 4 5	0 1 2 3				

RESPONSABLE DEL MUESTREO:

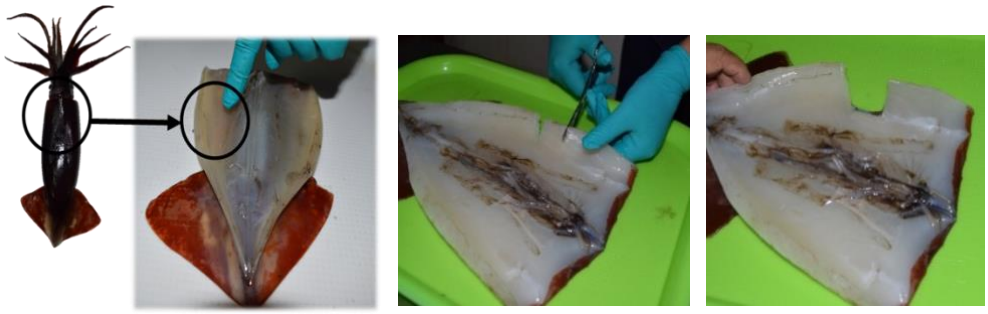
Figure 2.- Datasheet for the collection of biological data of cephalopods

c. Make sure you have at hand the F05-B/IMP form (Figure 2) and the microtubes box for tissue collection.

3.4. COLLECTION OF THE SAMPLE

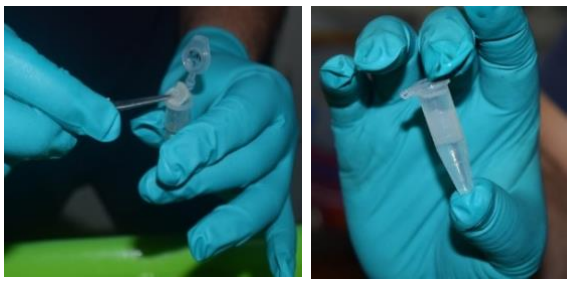
See detailed scheme in Figure 3 and proceed as follows

a. Place a specimen on top of a clean tray.
b. Take the biometric and biological information of the specimen, and fill out the F05-B/IMP form (Figure 2).
c. Open the specimen with the scissors and remove the viscera.
d. With the scissors, cut the inner part of the mantle to obtain a muscle portion of 4 cm x 4 cm, approximately. Consider the area to be cut one third away from the upper edge of the organism, close to the head. Avoid contamination of the sample with possible remains of visceral tissue.
e. Place the muscle portion on top of the clean tray and with a scalpel subsample the middle area of the tissue obtained in 3.4.d. above, cutting out approximately 0.5 cm^3 of muscle.
f. Rinse tissue with ethanol.
g. With a clean tweezer, place the extracted piece of tissue in a microtube containing 1 mL of 96% ethanol. Be sure to close well the microtube.



Place specimen on top of a clean tray. Using scissors, cut a 4x4cm square portion the mantle muscle, at 1/3 distance of fromthe upper edge.

Subsample a 0.5 cm^3 tissue from the middle area of the previously sampled, and rinse with ethanol.



With clean tweezers, place tissue in a microtube containing ethanol 96%. Verify tissue is completely submerge in ethanol.

Verify microtube´s label and place tube inside the corresponding box. Store in refrigerator.

Figure 3.- Schematic description of the main step for collecting a sample of the muscle tissue of *Dosidicus gigas* for molecular analisis

h. Verify that the tissue is completely submerged in the ethanol, maintaining a volume of ethanol approximately 3 times larger than the volume of tissue.
i. Check the microtube label and place it in the corresponding box.
j. Keep the microtubes in refrigeration until they arrive to the Molecular Genetics Laboratory of IMARPE.
k. Clean the collection material used prior to the next tissue sample.

3.5. CONSERVATION OF THE SAMPLE

a. The AFIIMM staff delivers to the staff of the Laboratory of Molecular Genetics the samples and the data (in digital format) as collected with form F05-B/IMP.
b. Once in the laboratory, place each sample in a new and sterile 2 mL screw cap cryovial, containing 1 mL of 96% ethanol, maintaining the ratio of 3:1 (ethanol volume relative to tissue volume).
c. Assign a new code to each sample, according to what is established in the Laboratory of Molecular Genetics. Example of sample code: **DgN 0518-11**; where letters and numbers refer to:
   - Two first letters related the scientific name of the species collected (e.g. Dg = *Dosidicus gigas*).
   - A capital letter indicating the collection area (e.g.: N = North, C = center, S = South).
   - Cruise date: indicating the month and year (e.g., 0518 = May 2018).
   - The number of the specimen sampled (example 11 = eleventh sample collected during the cruise).
d. Place sample tubes in cryovial holder boxes, labeled as follows:
   - First line: write which tissue is stored
   - Second line: indicate "POP Dgi #" - box number.
   - Third line: write month and year (separated by a hyphen) in which samples were collected. If they correspond to two-month collection, they will be separated by a "/".
e. Store boxes in the laboratory's tissue bank, at 4 °C, and record the information in the digital database, considering:
   - Box name
   - Cruise
   - Collection date
   - Collection area
   - Location of the box (in the refrigerator)
   - Sample's interval numbers
f. Data provided by AFIIMM and the new code assigned to each sample are also included in the laboratory database.
g. Annually, verify the preservation state of samples (e.g. volume of ethanol, tissue, labels), and each three years change ethanol to all samples.
h. Samples can remain well preserved for 10 years or more, provided the aforementioned considerations are adhered to.

4. REFERENCES

Steinke D. & Hanner R. (2011) The FISH-BOL collaborators' protocol. Mitochondrial DNA, 22: sup1:10-14. DOI: 10.3109/19401736.2010.536538.

Tafur R, Mariátegui L, Condori W & Buitrón B (2016). Protocolo para muestreo biológico y biométrico de cefalópodos Informe Instituto del Mar del Perú 43(4): 375-401.