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Protocol for biological and biometric sampling of Jumbo Flying Squid in use in Peru

Peru

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IMARPE

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PROTOCOL FOR BIOLOGICAL AND BIOMETRIC SAMPLING OF JUMBO FLYING SQUID *Dosidicus gigas* (D'ORBIGNYI) IN USE IN PERU

PROTOCOLO PARA MUESTREO BIOLÓGICO Y BIOMÉTRICO DE CALAMAR GIGANTE *Dosidicus gigas* (D'ORBIGNYI) EN USO EN EL PERÚ

by/por

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SUMMARY

The methodological procedures of the biological and biometric sampling of *Dosidicus gigas* adopted and in use by IMARPE are described. The materials to be used and how to use them are indicated. The types of sampling for obtaining biometric data and for obtaining biological data, and the way of carrying them out at the landing sites and/or on-board are described, indicating for each case the required size of the sample and the frequency of sampling. The main parts of the external and internal anatomy of *D. gigas*, the different phases of sampling and the scales of sexual maturity to be used for the observation and macroscopic determination of the stages of gonadal maturity of females and males are described.

KEYWORDS: *Dosidicus gigas*, biological – biometric sampling

RESUMEN

Se describen los procedimientos metodológicos del muestreo biológico y biométrico de *Dosidicus gigas* adoptados y en uso por IMARPE. Se indican los materiales a emplear y el cómo uarlos. Se describen los tipos de muestreo y la forma de llevarlos a cabo en los lugares de desembarque y/o a bordo, tanto para obtener datos biométricos como para obtener datos biológicos, indicándose para cada caso el tamaño de la muetra y la frecuencia del muestreo requeridos. Se describen las partes principales de la anatomía externa e internal del *D. gigas*, las distintas fases del muestreo y las escalas de madurez sexual a emplear para observación y determinación macroscópica de los estadios de madurez gonadal de hembras y machos.

PALABRAS CLAVE: *Dosidicus gigas*, muestreo biológico – biométrico

TABLE OF CONTENT

1. INTRODUCTION

This document is an extract of the Protocol for biological and biometric sampling of cephalopods (TAFUR *et al*. 2016) in use by the Peruvian Institute of Marine Research (IMARPE, Instituto del Mar del Perú), regarding the biometric and biological sampling of jumbo flying squid. It describes the standardized methodological procedures for the biometric and biological sampling of *Dosidicus gigas* that have been developed and agreed upon by IMARPE researchers as a main result of an *ad-hoc* Workshop for the Standardization of Protocols for Biological and Biometric Sampling of Marine Invertebrates, held at IMARPE, in May 2015.

The increased exploitation of fish resources and the depletion of some of the main stocks that were formerly part of the industrial fisheries caused the attention of the fishing vessels to be diverted to the so-called "unconventional marine resources", which include numerous species of cephalopods (JEREB & ROPER 2010).

Cephalopods are short-lived and fast-growing species that are heavily influenced by environmental variability (RODHOUSE *et al*. 2014). Squid represent the vast majority of the catches, mainly the catches of jumbo flying squid worldwide and in Peru (Fig. 1) and after a decline in 2009, their catches have been represented by jumbo flying squid (*Dosidicus gigas*) in the eastern Pacific and by the Argentine shortfin squid (*Illex argentinus*) in the southwest Atlantic. Since 2008, catches of cuttlefish and octopus have remained relatively stable at approximately 300,000 and 350,000 tons, respectively, although this represents a decrease for cuttlefish and an increase for octopus compared to previous years (FAO 2016).

Demand and consumption of cephalopods (cuttlefish, squid, and octopus) have slightly increased in recent years. Spain, Italy, and Japan remain the main consumers and importers of these species. Thailand, Spain, China, Argentina, and Peru were the largest

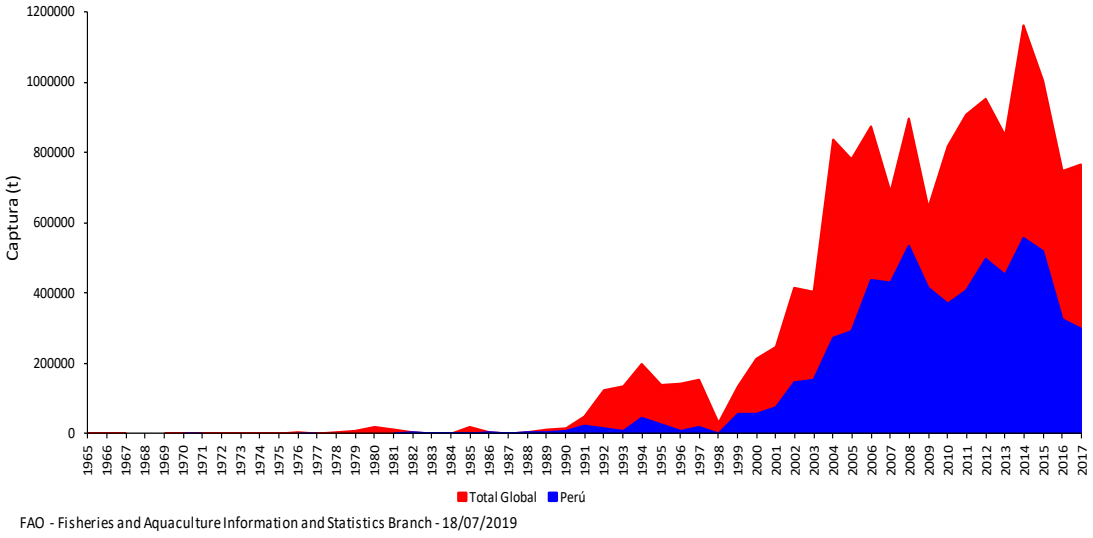


Figure. 1.- Total landings of jumbo flying squid *Dosidicus gigas* worldwide and in Peru. (Source: FAO 2019)

exporters of squid and cuttlefish, while Morocco, Mauritania, and China were the main exporters of octopus (FAO 2016).

Catches of *Dosidicus gigas* have increased, so between 2013 and 2014, it has had a positive variation of 37.1% (FAO 2017) and from 2014 to 2017, it showed a decrease of 34.3%.

The jumbo flying squid (*Dosidicus gigas*) (Fig. 2) is an endemic species of the eastern Pacific Ocean, distributed vertically between the surface and 1200 m and with a geographical range between 40°N (California, United States) and 47°S (southern Chile). In the Equatorial region, its range extends and narrows to the west, reaching 140°W. There is, therefore, a resident population in the Eastern Tropical Pacific (ETP), but the squid is not only distributed north and south of the ETP but also in important concentrations in the subtropical areas of both hemispheres.

In late 1999, an increase in the relative abundance (availability) of squid was observed, particularly in the northern zone (Talara, Paita, Sechura) and the southern zone of the country (Matarani), where a series of fisheries of national

Figure 2.- Jumbo flying squid *Dosidicus gigas*

importance are developed, such as the Peruvian hake and black anchoveta fisheries, and at the same time large concentrations of vinciguerria and euphausids are found. In the following years, the presence of squid in national fishing grounds increased and extended to the central zone (especially in El Niño years), becoming increasingly important in the fauna that accompanies the fisheries for mahi-mahi and other oceanic species (sharks and swordfish) developed in the oceanic zone. Since 2012, national landings of squid have been entirely artisanal (artisanal jigger fleet and occasionally by the purse-seiner fleet). The fishery for squid is the second most important in Peru, both in terms of catch volume and in terms of exported value.

This document is an extract of the Protocol for biological and biometric sampling of cephalopods (TAFUR *et al*. 2016) in use by the Peruvian Institute of Marine Research (IMARPE, Instituto del Mar del Perú), regarding the biometric and biological sampling of jumbo flying squid. It describes the standardized methodological procedures for the biometric and biological sampling of

Dosidicus gigas that have been developed and agreed upon by IMARPE researchers as a main result of an *ad-hoc* Workshop for the Standardization of Protocols for Biological and Biometric Sampling of Marine Invertebrates, held at IMARPE, in May 2015.

2. MATERIAL AND METHODS USED

The materials used in this process are listed below.

- Board
- Tape measure
- Electronic scale with a 0.01 g accuracy
- Dissecting Equipment: Scissors, watchmaker's tweezers, straight tweezers
- Steel knife
- Vernier calibrator or caliper square
- Plastic trays, Tupperware or airtight box
- Stationery: Pens, pencil, eraser, correction fluid
- Blotting-paper
- Plastic apron
- Cartons to carry otoliths
- Biometric Sampling Form
- Biological Sampling Form
- 70% alcohol
- 5 mL pill dispenser bottles
- Canson paper for labels

2.1. SAMPLING TYPE

The sampling type considered in cephalopods is carried out as follows:

- For obtaining biometric data, it will be a simple random sampling
- For obtaining biological data, it will be a stratified random sampling

2.2. COLLECTION OF THE SAMPLE AT THE DOCK OR LANDING SITE

- The technicians or professionals seconded for this purpose must wear an institutional identification or uniform dress of IMARPE, or of the institutional entity to which they belong, for easy access to the facilities of artisanal wharves or places of unloading.
- To purchase the sample directly from the fisherman, avoiding as far as possible the intervention of intermediaries (collectors), the sample will be representative of the total catch (structure by the size of the sample must be equal to the structure observed in the catch).
- To record important data such as gear and/or fishing method used, catch area (geo-referenced where possible), depth (fathoms and/or meters), species composition (kg), total catch (kg), and other relevant observations.
- To record the name and registration of the vessel, the total number of crew members, as well as the name of the skipper (if possible).

– The minimum sample size and frequency of biometric and biological sampling of jumbo flying squid are detailed in Tables 1 and 2 (YAMASHIRO et al. 2018).

Tabla 1.- Tamaño mínimo de muestra y frecuencia de muestreo biométrico del calamar gigante *Dosidicus gigas*, por lugar de muestreo

Table 1. Minimum sample size and biometric sampling rate of the jumbo flying squid *Dosidicus gigas*, per sampling location

Lugar de muestreo Sampling site	Tamaño de muestra Sample size	Frecuencia de muestreo Sampling frecuency
Desembarcadero/Laboratorio	120 ejemplares por embarcación	Semanal
Dock/Laboratory	120 specimens per vessel	Weekly
Embarcación artesanal	120 ejemplares por zona de pesca	Salida
Artisanal vessel	120 specimens per fishing area	Departure
Embarcación industrial	120 ejemplares por operación de pesca	Diaria
Industrial vessel	120 specimens per fishing operations	Daily
Barco de investigación	120 ejemplares por estación o lance	Diaria
Research vessel	120 specimens per station or set	Daily

Tabla 2.- Tamaño mínimo de muestra y frecuencia de muestreo biológico del calamar gigante *Dosidicus gigas*, por lugar de muestreo

Table 2. Minimum sample size and biological sampling frequency of jumbo flying squid, *Dosidicus gigas*, per sampling site

Plataforma de muestreo Sampling platform	Tamaño de muestra Sample size	Frecuencia de muestreo Sampling frecuency
A bordo de embarcación pesquera	10 ejemplares por sexo abarcando el rango de tallas de la captura, por zona de pesca	Diaria
On board fishing vessel	10 specimens by sex covering the size range of the catch, by fishing area	Daily
A bordo de barco de investigación	10 ejemplares por sexo e intervalo de talla por grado latitudinal	Diaria
On board research vessel	10 specimens by sex and size range by latitudinal degree	Daily

2.3. TRANSPORT AND PROCESSING OF THE SAMPLE

The sample must be transported to the laboratory in no more than 2 hours.

The sampling should be processed or carried out immediately upon arrival of the sample at the laboratory. If this is not possible, keep the sample refrigerated (at 0 to 4°C).

2.4. BIOMETRIC SAMPLING

- The weight of the sample should be recorded in grams before biometric and/or biological sampling.
- The dimension to be measured is the dorsal mantle length in mm with a tape measure, from the proximal edge of the mantle to the distal edge following the gladius in a straight line (ML).
- The frequencies by size of the specimens should be recorded with vertical bars, by forming groups of 5 (the 5TH bar must join transversally the previous four) in the biometric sampling form (IMARPE's sampling 'Format – 01'), including as well as the date, fishing area, weight of the catch (kg), weight of the sample (kg), name of the vessel, and names of the people who carried out the sample.

2.5. BIOLOGICAL SAMPLING

- The mantle length (ML) of each specimen is measured with a tape measure to the lower centimeter and weighed on a balance of 50 kg capacity and 1 g approximation. A 50 kg 'Roman' scale can be used for on-board sampling.
- The dissection of the specimens placed in ventral position is carried out and the degree of gonadal maturity is determined by using the scale proposed by PEREA *et al*. (2018).
- In the case of females, the length of the nidamental gland (mm) is measured and the reproductive structures are separated: nidamental glands, oviducts, oviductal gland, and the ovary. In males, the spermatophoric complex (sac and organ) and the testicle are separated, whose length is measured in mm. The reproductive structures of males and females are weighed on a 0.01 g approximation precision scale.
- The digestive gland is separated and weighed.
- The degree of stomach fullness is determined according to an empirical scale of 4 stages (0=empty, 1=half full, 2=full, and 3=completely full), the stomach is separated for dissection and qualitative analysis of food items, which are classified into fish (F), crustaceans (C), squids (Sq), others (O), and red liquid (RL). In the samples, the stomachs are collected for analysis in the IMARPE's Laboratory of Trophic Ecology, according to the established protocol.
- In females, the presence or absence of spermatophores in the oral veil is observed and recorded.

– The statoliths are collected, those who are found in the nuchal region (under the siphon), by using a scalpel blade to make a transversal cut, and with the help of a watchmaker's tweezer, the pair of statoliths are extracted, cleaned, and place in cartons (the label will be the serial number or length and sex).

– The mantle is weighed.

3. ILLUSTRATED ASPECTS OF THE ANATOMY AND SAMPLING

The main aspects of the morphology of the jumbo flying squid and of the way in which the biological and biometric sampling work is conducted on board by IMARPE staff are described below through the use of illustrations, schematic designs and photographs. These sections include graphical descriptions of the external and internal anatomy of the jumbo flying squid (section 3.1., Figures 3, 4, 5, 6 and 7), as well as of the various stages of the sampling on board (section 3.2., Figures 8a to 8l) and of the gonadal maturity scales used for females (section 3.3.) and males (section 3.4.).

3.1. ANATOMY OF THE JUMBO FLYING SQUID

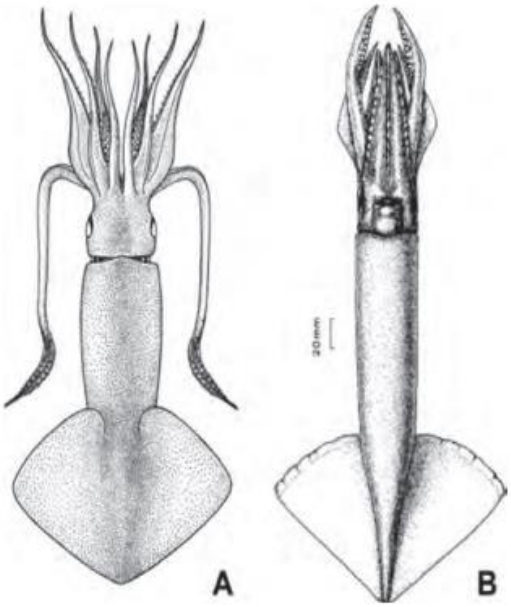


Figura 3.-Calamar gigante *Dosidicus gigas*. A vista dorsal (Tomado de ROPER *et al.* 1984 en MARKAIDA 2001). B vista ventral (Tomado de WORMUTH 1976 en MARKAIDA 2001)

Figure 3. Jumbo flying squid *Dosidicus gigas*. A dorsal view (Taken from ROPER *et al.* 1984 in MARKAIDA 2001). B ventral view (Taken from WORMUTH 1976 in MARKAIDA 2001)

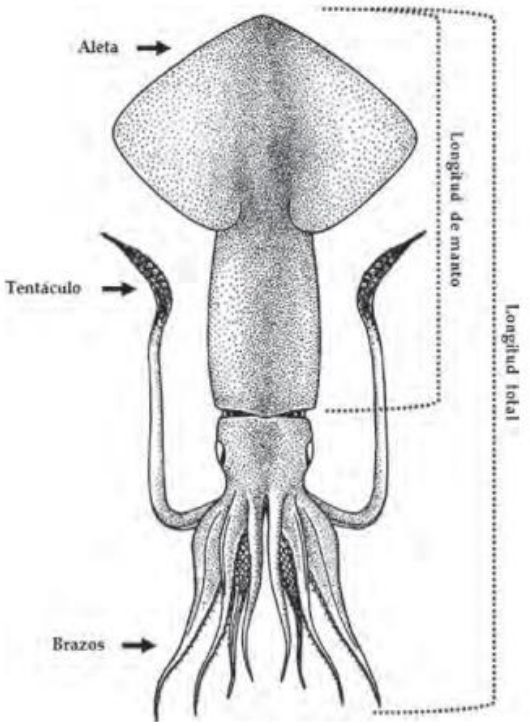


Figura 4.- Vista dorsal de *Dosidicus gigas* (modificada de ROPER *et al.* 1984)

Figure 4. Dorsal view of *Dosidicus gigas* (modified from ROPER *et al.* 1984)

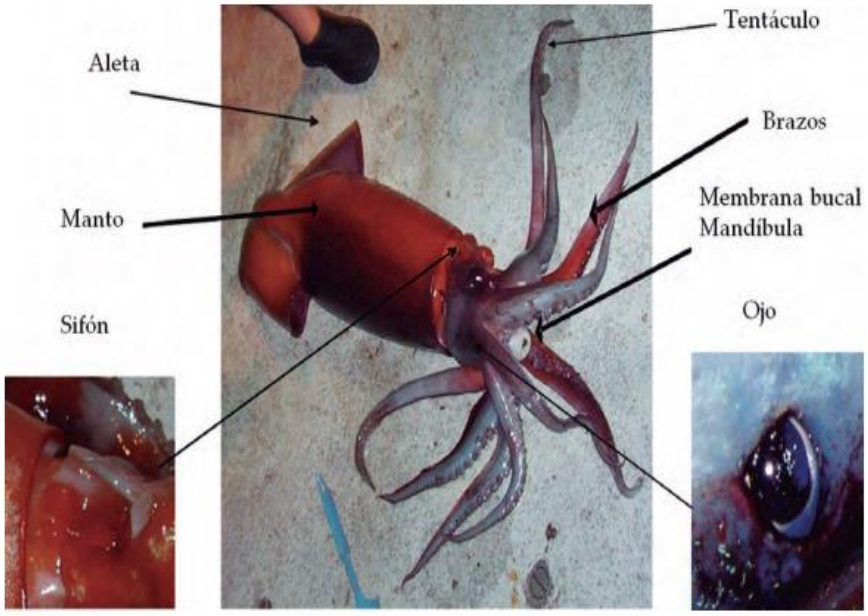


Figura 5.- Vista ventral de *Dosidicus gigas*

Figure 5. Ventral view of *Dosidicus gigas*

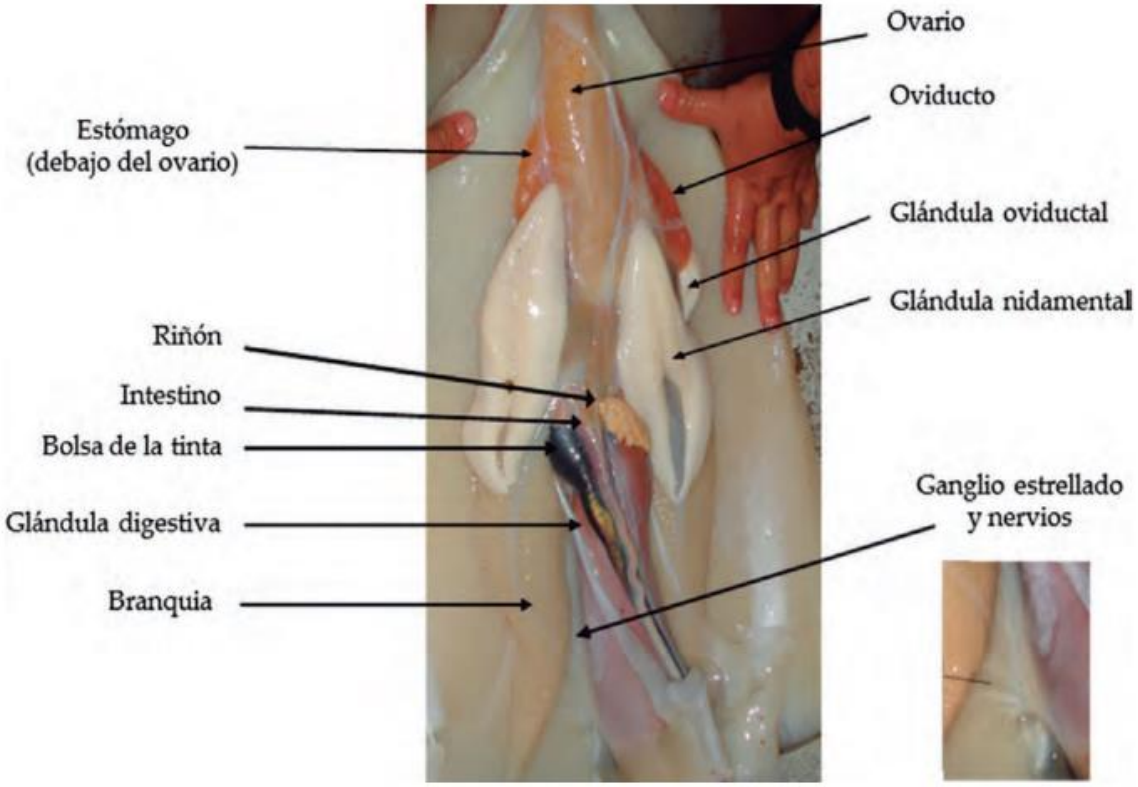


Figura 6.- Anatomía interna de *Dosidicus gigas* - Hembra

Figure 6. – Internal anatomy of *Dosidicus gigas* - Female

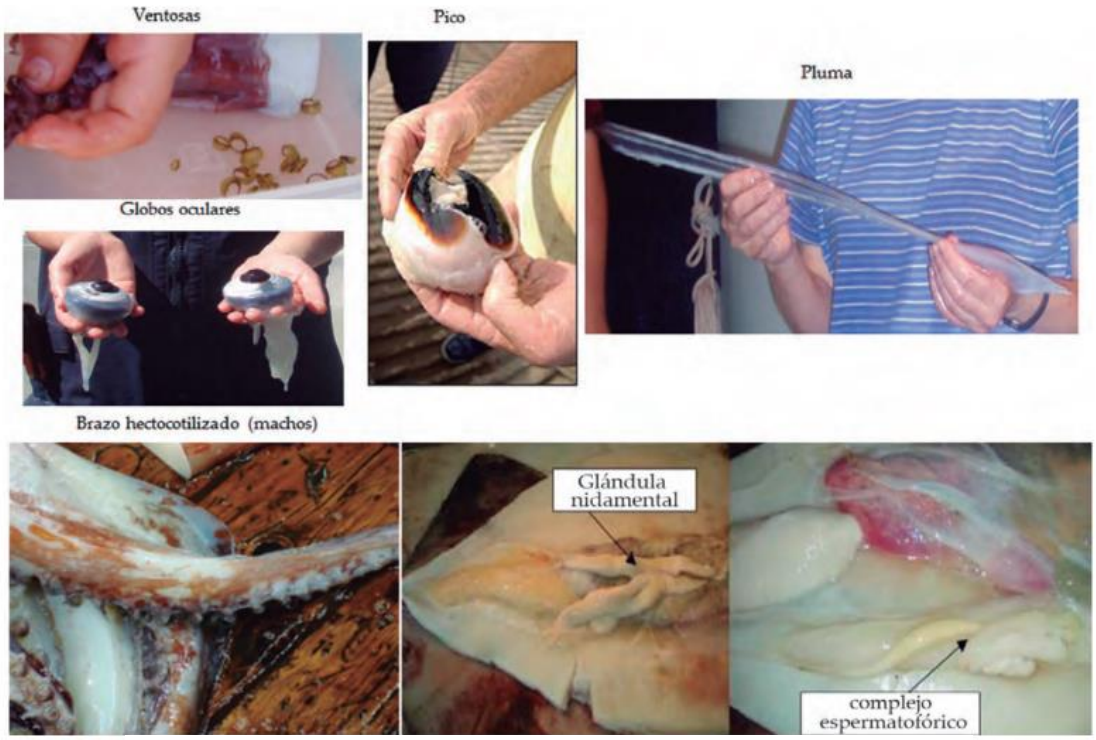


Figura 7.- Algunas estructuras externas e internas de *Dosidicus gigas*

Figure 7. – Some external and internal structures of *Dosidicus gigas*

3.2. STAGES OF THE SAMPLING ON BOARD

Figure 8.- Various stages of sampling on board scientific vessels: a) Catch of jumbo flying squid *Dosidicus gigas* with hand line with jigs on board the R/V José Olaya Balandra; and, b) Jumbo flying squids aligned deck for sampling; (…continues)

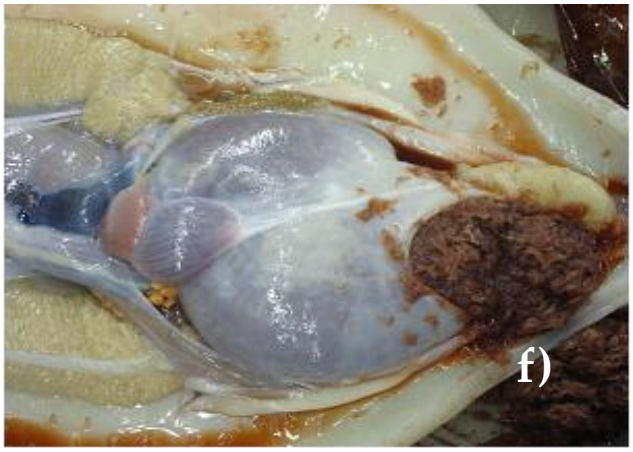
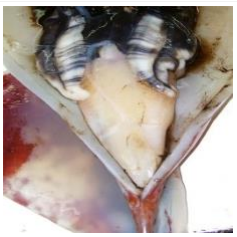


Figure 8 (cont.) .- Various stages of sampling on board scientific vessels: c) Measurement of the mantle length (ML); d) Dissection of a large squid during biological sampling; e) Female oral veil with evidence of copulation; f) Stomach with traces of euphausids; and, g) Dissection of very large specimens; (…continues)

Figure 8 (cont.).- Various stages of sampling on board scientific vessels: h) Dissection of a very large specimens; i) Tray with internal male structures; j) Small squids; k) Dissection of a small specimens; and, l) Gladius from a very large specimen

3.3. GONADAL MATURITY SCALE FOR FEMALES OF JUMBO FLYING SQUID

The description and macroscopic view of the four stages of gonadal maturity of females of jumbo flying squid (*Dosidicus gigas*) as described by PEREA *et al*. (2018) follows.

Stages	Description	Photos
I Immature	The ovary is shaped like a pyriform sac, which is elongated, not granular and clear in appearance; being translucent in the initial phase of this stage. The wall of the ovary is very thin.	
II Maturing	Ovary looks grainy, opaque, creamy or slightly yellowish.	
III Mature	Larger and turgid, more piriform in appearance, yellowish-cream to amber in color, due to the presence of a large number of mature oocytes. The wall of the ovary is thin. Oviduct of great size for being full of advanced mature oocytes. The nidamental gland is developed and turgid. It presents immature, maturing, and mature oocytes.	
IV Spawning	Less turgid, granular, with lots of cream-colored and amber oocytes, with a predominance of the latter, giving an orange hue to the ovary. The wall of the ovary is very thin. Flaccid nidamental gland. The oviducts may be compact and large because they are full of advanced mature oocytes or somewhat flaccid due to their evacuation. In addition, in the external part of the oviductal gland some advanced mature oocytes are observed, a sign of spawning in the squid. Immature, maturing, and mature oocytes are observed. In the oviducts, these are full of oocytes at maximum maturity and ovulated. In the ovary, at microscopic level, the presence of post-ovulatory follicles is observed.	

3.4. GONADAL MATURITY SCALE FOR MALES OF JUMBO FLYING SQUID

The description and macroscopic view of the three stages of gonadal maturity of males of jumbo flying squid (*Dosidicus gigas*) as described by PEREA *et al*. (2018) follows.

Stages	Description	Photos
I **Immature**	Small, flaccid, elongated, white-colored and translucent testicle.	
II **In Maturation**	A larger, somewhat consistent, whitish to creamy, but not translucent testicle. Whitish spermatophore sack.	
III **Mature**	A testicle of a more developed size with respect to the previous stages, turgid, white-colored and milky in appearance. Spermatophore sac is full of spermatophores; free spermatophores are observed in the abdominal cavity.	

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