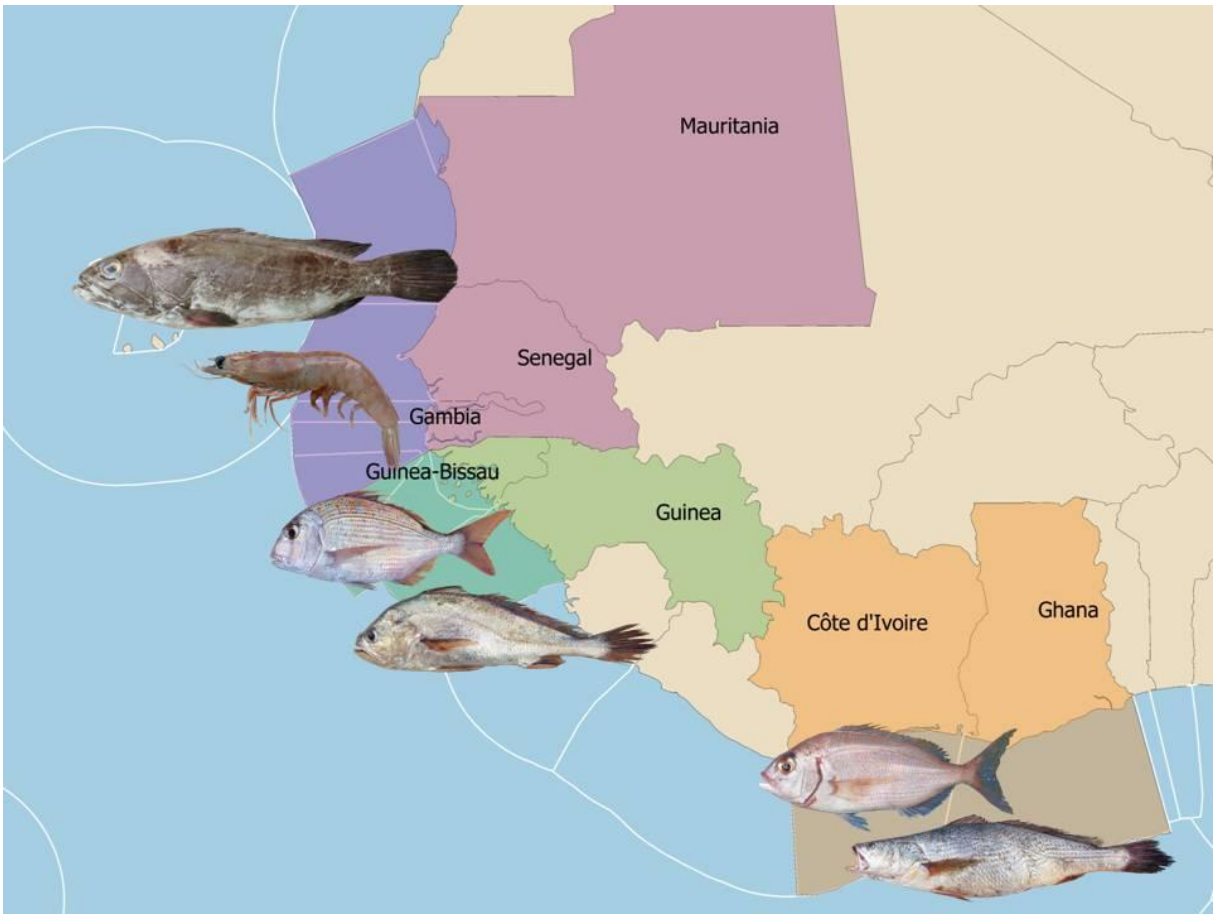
DEMERSTEM

PROTOCOLS FOR BIOLOGICAL SAMPLING



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A- GENERALITIES

A.1.- SPECIES AND SAMPLING AREA

Table 1.- Species by sampling place and country.

COUNTRY	SPECIES	SAMPLING PLACE
MAURITANIA	*Penaeus notialis* *Epinephelus aeneus*	Nouadhibou
		Nouakchott
SENEGAL-GAMBIE	*Penaeus notialis*	Saint Louis (Senegal River)- Port de Dakar
		Saloum -Casamance
		Banjul (Gambie)
	Epinephelus aeneus	Kayar
		Saloum –Casamance
		Banjul (Gambie)
GUINEA-BISSAU	*Penaeus notialis*	Cacheu
	Pagrus caeruleostictus	Bissau
		Cacine
	Pseudotolithus elongatus	Cacheu
		Cacine
GUINEA	*Pagrus caeruleostictus* *Pseudotolithus elongatus*	Kamsar, Katchek
		Conakry
CÔTE D'IVOIRE	*Pagellus bellottii* *Pseudotolithus senegalensis*	Abidjan
		San Pedro
GHANA	*Pagellus bellottii* *Pseudotolithus senegalensis*	Tema
		Takoradi

A.2.- TYPES OF SAMPLING : LENGTH FREQUENCIES AND BIOLOGICAL SAMPLING

Two types of sampling are carried out to obtain biological information of the target species: length frequency sampling and biological sampling. The two types of sampling are presented in Figure 1 and the main differences between both are explained in Table 2.

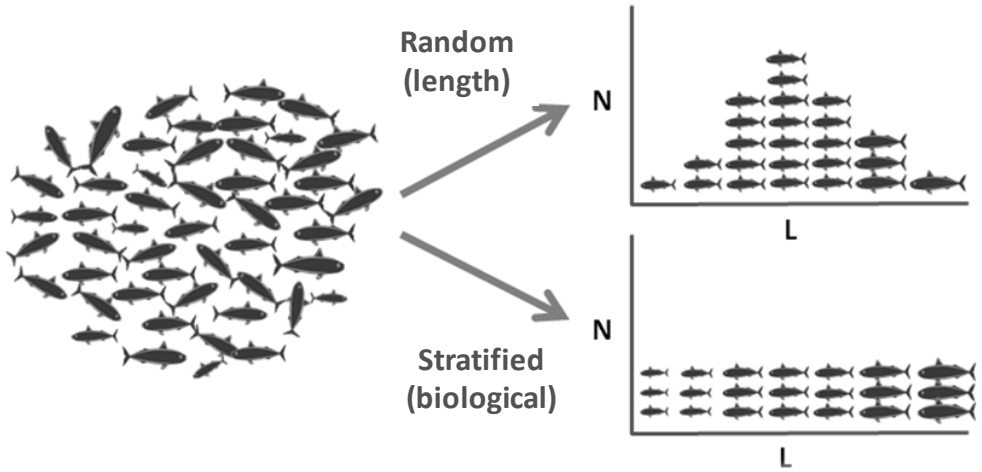


Figure 1.- Types of sampling (length and biological sampling).

Table 2.- Main differences between length frequency sampling and biological sampling.

SAMPLING	LENGTH FREQUENCY	BIOLOGICAL
Fishing activity dependent/independent:	Dependent. It is usually made together with landings sampling, completing it.	Independent.
Sampling place:	Artisanal fleet: ▪ Landing place. Industrial fleet: ▪ In the port (landings). ▪ On-board (observers).	Artisanal fleet: ▪ In the laboratory. Industrial fleet: ▪ In the laboratory. ▪ On-board (observers).
Type of sampling (see Figure 1):	Random sampling.	Stratified sampling (by length class).
Minimum number of individuals:	100 (aprox.) or more, if needed, until one (or several) modal lengths are observed in the catch distribution (Fig. 1).	The minimum established for each species. All catch sizes of a species should be equally represented in the sample.
Frequency:	Monthly or biweekly.	Monthly.
Form to use :	Length distribution.	Biological sampling.
Species and zones:	- The 2-3 species chosen by country. - The two sampling places established for each country.	

A.3.- COMPUTERIZING THE INFORMATION FROM DATA COLLECTION

The computerization of biological information from data collection must be carried out in 2 steps:

- Filling paper forms with pencil.
- Banking through the Android application Odk Collect (see Annex 10).

For morphometry, we recommend to take the photos at the same time as you enter the data on the ODK interface. Indeed, this app allows integrating photos, which will be associated with the individual data described in the form.

B- LENGTH-FREQUENCY SAMPLING

- **LANDING SAMPLINGS: 2 SPECIES PER ZONE, MONTHLY BASIS**
- **THIS IS INDEPENDENT OF THE BIOLOGICAL SAMPLING**
- **ONLY FOR THOSE COUNTRIES THAT HAVE NOT LENGTH SAMPLINGS ALREADY IMPLEMENTED (SEE TABLE 3)**

Objectives:

- **Growth studies** :
  - Length Frequency Analysis (LFA)
  - Growth parameters (Von Bertalanffy growth parameters from LFA)
  - Sexual variability
  - Geographical variability
  - Bathymetric variability (if depth information available. E.g.: from research surveys or on-board observers)

Methodology: measure a minimum of 50 individuals (fish) and 100 individuals (shrimps) in the landings place each month (minimal), following the methodology explained in Annex 2 (Length). These samples should be measured in the same landing place and therefore this length sampling, should be fast. Samples should be taken randomly and should be representative of the landings sizes.

IMPORTANT: These "length samples" (not bought) should be different than the "biological samples" (bought for the analysis in the laboratory).

FISH:

- With measuring boards (fish) or measuring tapes (large individuals).
- Minimum: **50** individuals.
- Measure: **Total Length** (TL).
- Measurements should be taken to the lower unit (lower cm or ½ cm):

  Measure to the lower cm: reading 12.7 cm record 12 cm
  Measure to the lower ½ cm: reading 12.7 cm record 12.5 cm

SHRIMPS:

- With calipers.
- Minimum: **100** individuals.
- Measure: Cephalotorax length or **Carapace length** (CarL).
- Measure should be taken to the lower unit (½ mm): e.g. 1: reading 22.3 mm record 22.0 mm; e.g. 2: reading 22.7 mm record 22.5 mm.

Table 3.- Length samples to be taken on landings (minimal: on a monthly basis).

COUNTRY	MAURITANIA		SENEGAL		GUINEA-BISSAU			GUINEA		CÔTE D'IVOIRE		GHANA	
SPECIES	P. not (SOP)	E. aen (GPW)	P. not (SOP)	E. aen (GPW)	P. not (SOP)	P. cae (BSC)	P. elo (PSE)	P. cae (BSC)	P. elo (PSE)	P. bell (PAR)	P. sen (PSS)	P. bell (PAR)	P. sen (PSS)
LENGTH Sampling	IMPLEMENT		IMPLEMENT	CONTINUE	CONTINUE	IMPLEMENT		CONTINUE		CONTINUE in Abidjan IMPLEMENT en San Pedro		IMPLEMENT	
SAMPLING PLACES	- Nouadhibou - Nouakchott		- St. Louis -Casamance	- St. Louis - Kayar - Mbour - Joal	Cacheu	- Bissau - Cacine	- Cacheu - Cacine	From 2017 In 10 landing places.		- Port d'Abidjan (weekly) -San Pedro (min. monthly)		- Tema - Takoradi	

C- BIOLOGICAL SAMPLING

C.1. SAMPLE SOURCES

The different sources for obtaining the monthly biological samples mainly are:

1. Option 1: Research surveys/Observers on-board fishing vessels.
2. Option 2: Samples in the landing site or at fish market.

If the country has the option of having surveys and/or observers on board fishing vessels, the Option 1 is recommended, as it is cheaper, with the advantage that samples obtained are geo-referenced. But if all the length classes required by species and area are not obtained by the option 1, it is important to buy the samples corresponding to those lacking lengths for completing the whole landed length range in all areas (Option 2) (see Figure 2).

Countries without surveys and/or observers on board should follow Option 2 (purchasing samples).

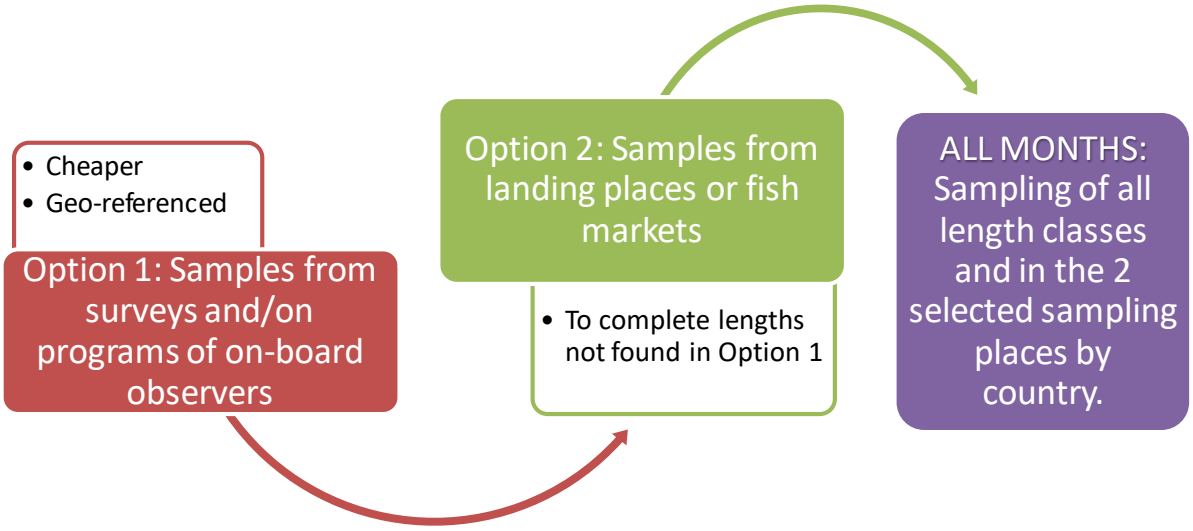


Figure 2.- Sources of the monthly samples of the species and places selected for biological sampling.

For large individuals of *E. aeneus*, which are very expensive, other solutions should be searched if the funds are limited to buy them (Option 2). In this case, we recommend sampling the individuals at the landing/sale place, without damaging them for their sale. Some sampling recommendations are made for this in this protocol.

C.1. 1. Sampling in surveys and/or observations on-board fishing vessels

Table 4 shows the research fishing surveys and/or on-board observer programs planned for 2019 and 2020, which can be used to obtain samples of the target species.

Table 4.- Surveys and programs of on-board observers in 2019 and 2020.

SURVEYS (2019-2020)		
COUNTRY	**2019 (month)**	**2020 (month)**
MAURITANIA	September	April and September
SENEGAL	_	?
GUINEA-BISSAU	November-December	November-December
GUINEA	_	September?
REGIONAL (F. NANSEN)	Ghana Côte d'Ivoire Guinea Guinea-Bissau	Mauritania? Senegal?
OBSERVERS		
COUNTRY	**Fleet**	**Year**
MAURITANIA	Spanish shrimpers (IEO)	2019
	Fish trawlers (IMROP)	2019-2020
SENEGAL	Shrimper and Fish trawlers	2019-2020
GUINEA-BISSAU	Spanish shrimpers (IEO)	2020
	Foreign industrial (PRAO)	2020
GUINEA	_	_
CÔTE D'IVOIRE	?	?
GHANA	Coastal trawlers (monthly)	2019-2020

Use these surveys and/or observer embarks to carry out samplings of the 6 SPECIES, if caught, always with **geo-referenced information**, at least for:

- Biological parameters (BIO)
- Genetics (GEN)
- Pictures for morphometry (MOR)

C.1.2. Samples at the landing place or at the fish market:

An initial calendar for biological sampling at the landing place or at the fish market was established in GT1-DEMERSTEM (Nouakchott, August 2019), with the aim of sampling a complete annual cycle, from September 2019 to August 2020.

However, in GT2-DEMERSTEM (Grand Bassam, February 2020), a review of the general and specific problems observed during the first five months of sampling was carried out, in order to clarify any doubts or possible errors. Due to several problems, the sampling schedule was restructured in certain case studies in order to have complete biological information over a complete annual cycle.

The frequency of biological sampling and collection of genetic samples and pictures for morphometry is the following:

- **Biological sampling - Monthly**
- **Morphometry & genetics – Biannual**

Tables 5 show the schedules for the biological sampling (BIO) and for the collection of pictures for morphometry (MOR) and samples for genetics (GEN), by case of study, species and country.

Tables 5.- Calendars for length-frequency sampling (T), biological sampling (BIO) and collection of pictures for morphometry (MOR) and samples for genetics (GEN), by case of study, species and country.

MAURITANIA – SENEGAL-GAMBIA

Epinephelus aeneus

PAYS	Zone/ Mois	Fév 20	Mar 20	Avr 20	Mai 20	Juin 20	Juill 20	Août 20	Sep 20	Oct 20	Nov 20	Déc 20	Jan 21
MAURITANIE	Noauadhibou	T BIO-MOR-GEN	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO-MOR-GEN	T BIO	T BIO
	Nouakchott	T BIO-MOR-GEN	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO-MOR-GEN	T BIO	T BIO
SENEGAL-GAMBIE	Kayar	T BIO-MOR-GEN	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO-MOR-GEN	T BIO	T BIO
	Saloum-Casamance	T BIO-MOR-GEN	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO-MOR-GEN	T BIO	T BIO
	Gambie	T BIO-MOR-GEN	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO-MOR-GEN	T BIO	T BIO

Penaeus notialis

PAYS	Zone/ Mois	Mar 20	Avr 20	Mai 20	Juin 20	Juill 20	Août 20	Sep 20	Oct 20	Nov 20	Déc 20	Jan 21	Fév 21
MAURITANIA	North	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO
	South	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO
SENEGAL-GAMBIE	Saint Louis	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO
	Saloum	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO
	Gambie	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO
GUINEA-BISSAU	Cacheu	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-GEN	T BIO	T BIO	T BIO	T BIO	T BIO

GUINEA-BISSAU – GUINEA

Pagrus caeruleostictus

PAYS	Zone/ Mois	Jan 20	Fév 20	Mar 20	Avr 20	Mai 20	Juin 20	Juill 20	Août 20	Sep 20	Oct 20	Nov 20	Déc 20	Jan 21
GUINEA-BISSAU	Bissau		T BIO-MOR-	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-	T BIO-MOR-	T BIO	T BIO	T BIO	T BIO	T BIO
	Cacine	T BIO-MOR-	T BIO-MOR-	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-	T BIO-MOR-	T BIO	T BIO	T BIO	T BIO	T BIO
GUINEA	Kamsar ou Katchek	T BIO-MOR-	T BIO-MOR-	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-	T BIO-MOR-	T BIO	T BIO	T BIO	T BIO	T BIO
	Conakry	T BIO-MOR-	T BIO-MOR-	T BIO	T BIO	T BIO	T BIO	T BIO-MOR-	T BIO-MOR-	T BIO	T BIO	T BIO	T BIO	T BIO

Pseudotolithus elongatus

PAYS	Zone/ Mois	Dec 19	Jan 20	Feb 20	Mar 20	Avr 20	Mai 20	Juin 20	Juill 20	Août 20	Sept 20	Oct 20	Nov 20
GUINEA-BISSAU	Cacheu	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR
	Cacine	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR
GUINEA	Kamsar ou Katchek	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR
	Conakry	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR

CÔTE D'IVOIRE – GHANA

Pagellus bellotii

PAYS	Zone/ Mois	Mar 20	Apr 20	May 20	Jun 20	Jul 20	Aug 20	Sep 20	Oct 20	Nov 20	Dec 20	Jan 21	Feb 21
CÔTE D'IVOIRE	Abidjan	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO
	San Pedro	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO
GHANA	Tema	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO
	Takoradi	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO

Pseudotolithus senegalensis

PAYS	Zone/ Mois	Mar 20	Apr 20	May 20	Jun 20	Jul 20	Aug 20	Sep 20	Oct 20	Nov 20	Dec 20	Jan 21	Feb 21
CÔTE D'IVOIRE	Abidjan	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO
	San Pedro	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO
GHANA	Tema	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO
	Takoradi	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO-MOR	T BIO	T BIO	T BIO	T BIO

C.2. BIOLOGICAL SAMPLING: METHOD

- **IN SURVEYS:** THE 6 TARGET SPECIES
- **LANDING SAMPLINGS:** THE TWO SELECTED SPECIES 2 SPECIES PER ZONE, MONTHLY BASIS

Objectives:

Obtain information on:

- Reproduction:
  - Sex-ratio
  - Gonadosomatic index (GSI) by month
  - Proportion of maturity stage by month
  - Spawning period
  - Spawning area
  - Maturity ogive and Length at first maturity (L_{50})
- Weight:
  - Length- weight relationship
  - Condition factor

For all, analysis of:

- Sexual variability
- Geographical variability
- Bathymetric variability (if depth information available. E.g.: from surveys or observers)

Parameters/samples to collect:

1. Pictures of the individuals (**BIANNUAL**)
2. External parasites (in body surface)
3. Length (TL or CarL, depending on the species) (mm)
4. Fresh (total) weight (g)
5. Sex
6. Maturity
7. Gonad weight (g)
8. Internal parasites
9. Gutted (eviscerated) weight (g)
10. External parasites (in gills)
11. Sample for genetics (**BIANNUAL**)
12. Collection and storage of otoliths

Methodology

1) Collect samples from one or more sources that can be complementary (surveys, observers, factories, purchases) of every species in each sampling place, covering all the complete length range of catches. This should follow a stratified sampling scheme: a specified number of individuals by the length class specified for each species in Annex 1, as a minimum.

2) Bring samples to the laboratory. A sampling place should be appropriately prepared in the laboratory. This place must have electricity, water and good hygienic conditions. The worktable should be prepared with:

   - Sampling material (measuring boards or callipers, scales, dynamometers, dissection material, baskets, fish trays, etc.). See Annex 8, with list of sampling material.
   - The forms to be filled, depending on the species to be sampled (fish / shrimp).
   - Plasticized maturation key of the species.

Photo: Preparing a biological sampling. Bernal Vilela, FAO

3) Organize the samples, preparing the number of individuals needed by length class (Annex 1), and aligning them one after the other.

Photo: Organizing individuals of *P. notialis* by length class. Eva García Isarch. IEO

4) Prepare the forms, filling the headings with main fields: sample origin, with the most detail possible (port, fishing area, survey-trawl number), date, species name (scientific name and FAO code), sample weight, etc. See Annex 9.

It is important that the forms be filled with pencil, since the ink of the pens can be run or erased with water. It is important to write clearly.

5) Take **biological parameters** of each individual, ideally in the same order they are in the form: length, fresh (total) weight, sex, maturity stage, gonad weight and gutted (eviscerated) weight. See Annex 2 for procedures and Annex 3 for maturity stages.

   Ideally, the sampling should be carried out in teams of 2 people: one person sampling and the other writing down. In the case of on-board observers, who have to work alone, the use of recorders is recommended.

   FISH:

   - **Length:** the corresponding total length (TL) measurement is taken with a measuring board or a measuring tape and the exact measurement is given in mm. See Annex 2.
   - **Fresh (total) weight**: the entire individual is weighed with the precision balance or with a hanging scale and the exact measurement is given in grams (with a decimal, in small species).
   - **Sex:** the animal is dissected, all the organs are removed to expose the gonad and determine if the specimen is male (code 1) or female (code 2). If the gonad is very small or it is damaged and sex cannot be determined with the naked eye, it should be stated as undetermined (code 3).
   - **Maturity:** once the visceral cavity was opened and gonads exposed for sex determination, the assignment of the maturity stages is carried out through the macroscopic observation of the gonadal characteristics that can be observed with the naked eye (*visu*). The degree of maturation will be determined, following the criteria of the 5 stages-key in Annex 3.
   - **Gonad weight**: Gonads (ovaries or testicles) will be removed and weighted on a precision scale. The weight should be recorded in grams and with a minimum of 1 decimal.
   - **Gutted (eviscerated) weight:** after completely removing the organs from the specimen, its weight (gutted) is taken, using the same instrument as with the total (live) weight (hanging scale or precision scale).

     For large individuals of *E. aeneus* that must be sampled at the landing / sale location, without damaging it for sale, we recommend removing the viscera and putting them in a bag. This must be weighed with a dynamometer. The gutted weight would be the total weight minus the weight of the viscera.
   - Any observations that are considered relevant in the "observations" field are noted.

SHRIMP:

- **Length:** the carapace (cephalotorax) length (CarL) is measured with calliper and the exact measurement is given in mm and with a decimal.
- **Fresh (total) weight**: the entire individual is weighted in the balance and the exact measurement is given in grams and with a decimal.
- **Sex:** the sex of the animal is determined by its external characteristics (males: presence of petasma; females: absence of petasma). See Annex 2.
- **Maturity:** the assignment of the maturity stages is carried out through the observation of external characteristics with the naked eye (*visu*), following a key of 4 stages for females and of 2 stages for males (see Annex 3).

  For **females**, we should record:
  - **Maturity**: by observing the gonad, using the 4-stages maturity key.
  - If it is fertilized, that is, if the spermatophore is observed on the thelycum. "Yes" or "No" should be noted.

  For **males**, we should record:
  - If the petasma is joined or no (write “yes” or “no”).
  - If sperm mass is observed in the coxas of the 5th pair of pleopods or thoracic appendages (write “yes” or “no”).
- Any observations that are considered relevant are noted in the "observations" field (e.g.: molting individuals).

Table 6.- Summary table of biological sampling.

PARAMETER	MEASURE	OBSERVATIONS
LENGTH	- Total length (LT) for fish (mm). - Carapace length (CarL) for shrimps (mm, with one decimal).	Measuring equipment: - Measuring board for fish - Measuring tape for large fish. - Callipers, for shrimps.
FRESH (TOTAL) WEIGHT	In grams, with one decimal if weighted with precision scale.	Weight of the whole individual. With hanging scale or electronic scale.
SEX	1: Male 2: Female 3: Indeterminate	Sexed by internal characters (fish) or external characters (shrimps). (Annex 2).
MATURITY	For males and females, following the established keys.	Always bring maturity keys of target species (Annex 3).
GONAD WEIGHT	In grams, with 1 decimal minimum.	With precision scale.
GUTTED (EVISCERATED) WEIGHT	In grams, with one decimal if weighted with precision scale.	Weight of the empty individual, without internal organs. With hanging scale or electronic scale.

It is recommended to align the sampled specimens in trays, following the order in which they are sampled. If possible, the number of individual (same as indicated in the form) should be indicated with a label. This is useful, in case any data need to be checked.

6) **External and internal parasites** should be searched, extracted and storage as explained in Annex 6.

7) Once that a number of individuals have been sampled, it should be checked that the minimum of 10 individuals per length class established in Annex 1 have been sampled. Otherwise, individuals with the missing lengths should be searched and sampled. It may happen that all length classes cannot be completed in one single sampling in one month. If this is the case, two steps must be followed:

    a. Complete the total number required with individuals of the closer length class (e.g.: if there are only 9 individuals in the length class 18-21 of PSE, complete the length class 22-25 with 11 individuals).

    b. Try to have a complete coverage of all the length classes on a quarterly basis: 30 individuals by length class. The classes that could not be sampled in one single month should be sampled in the quarter.

8) Last sampling stage is the collection and storage of otoliths, following procedures explained in Annex 5.

9) Forms review: the forms should be well reviewed before finishing the sampling. If an error is detected, it can be checked, having the individuals located in the trays, in the order that corresponds to their number in the form. The field of the header "sample weight" (in grams), which is obtained as the sum of the weights of all individuals, should be noted.

10) Cleaning: everything must be cleaned and collected, as well as the instruments cleaned thoroughly.

D- COLLETION OF TISSUE FOR GENETIC ANALYSIS

EVERY 6 MONTHS, AS INDICATED IN TABLE 1.

The frequency of collection of tissues for genetic analysis for each species, zone and country is indicated in Tables 5.

A sample of tissue from 50 individuals sampled for biology and morphometry should be collected every 6 months, following this procedure:

1.- Preparation of the material before sampling

Operator has to wear cleaned gloves.

Before sampling, prepare 2 mL tubes with screw cap (vials) with at least 1 mL of non-denatured Ethanol 96%. Each vial must be labelled with **Sample ID** according to the labelling code reported in Annex 4. Label the vials twice with pens containing water-resistant ink, write the same code on the cap and on the side of the vial. Cover the label on the side of the vial with Scotch tape to prevent label erasing due to probable ethanol escapes. In addition, include a waterproof paper inside the tube with the code written with pencil (as ethanol may dissolve the ink outside the vial).

2. - Sampling procedure

IMPORTANT: Samples can be taken from fresh or frozen specimens.

Samples cannot be obtained from specimens that have been exposed to or fixed in formalin.

1) Cut with surgical instruments a **1 cm^3** muscle sample from each individual in the **red** region indicated in Figure 3. Note that larger pieces are not needed and can result in bad DNA quality due to low ethanol/tissue ratio.

   Tissue should be removed from only the right side of the fish/shrimp. Do not damage the left side of the fish as this is the side used for morphometry.

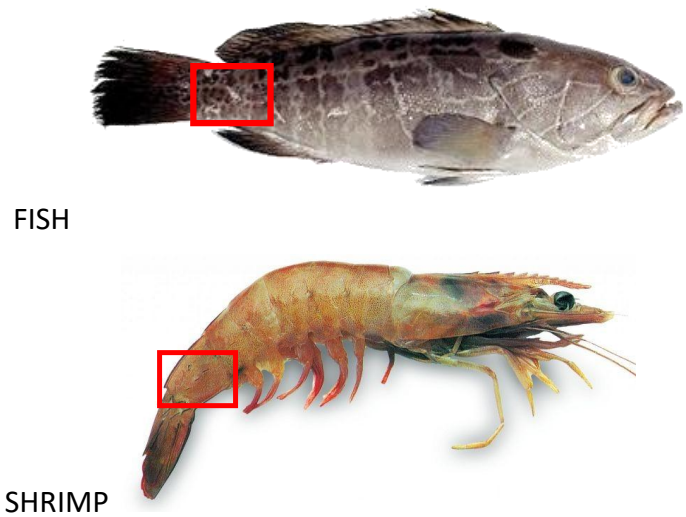


Figure 3.- Location of muscle samples for genetic in fish (top) and shrimp (bottom).

2) Put the tissue clip into the ID-labelled vial with ethanol 96%. Fill the tube with ethanol. Ensure the tissue volume is no more than 30% of the liquid volume and tightly close the cap (Figure 4).

Figure 4.- Example showing ratio of tissue/ethanol and sample size.

3) Clean surgical instruments for each sampled animal with water and commercial ethanol and dry it with a new scrip paper each time.

4) Store the vial containing the tissue at -20°C; if it is not possible, make sure that temperature does not exceed 4°C.

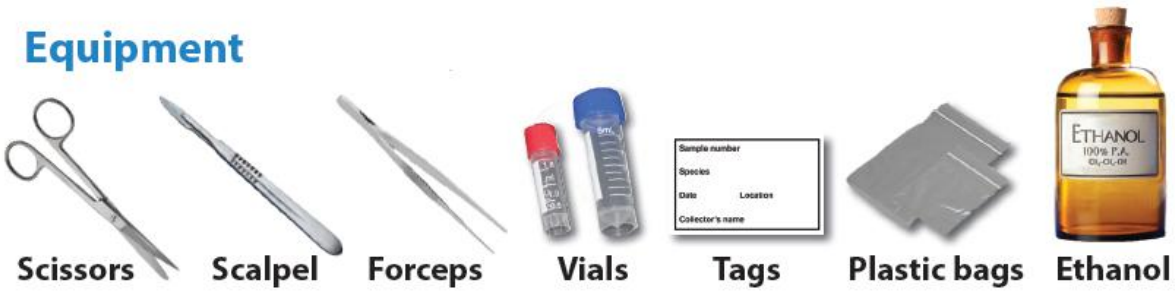
5) 4-5 days after sampling, carefully remove the ethanol from the microtube and replace it by new ethanol.

6) Samples should be sent for their analysis to IEO (Vigo): montse.perez@ieo.es

3.- Codification of samples and information to compile

All tubes must be coded twice (side and cap) using the same code as used for files of photos.

The codes of the individuals sampled for genetics, as stated in Annex 4, should be added to the forms of biological samplings.



(Source: FAO, 2016).

Make sure that the equipment (scissors, forceps, scalpels, etc.) be sterilized; do not handle tissue with bare hands so as not to contaminate it.

E- COLLETION OF PICTURES FOR MORPHOMETRY

EVERY 6 MONTHS, AS INDICATED IN TABLES 5.

Objective:

- Use morphometric techniques for stock identification. The historical development of stock identification methods has paralleled the advancement of morphometric techniques. Multivariate methods can be applied to quantify variation in growth and form among stocks. More recent advances have been facilitated by image processing techniques, more comprehensive and precise data collection, more efficient quantification of shape, and new analytical tools (Cadrin, 2000).
- Spatial variability, sexual and/or bathymetric variability (if depth information available) and /or ontogeny variability (e.g.: by length ranges).

Generalities:

Analysis of morphometry will be carried out by IEO using Truss Network methods (see Annex 7). This methodology is based on the analysis of a number of measures taken form pictures of each individual of the target species.

Frequency of pictures collection for morphometry for each species, zone and country is indicated in Tables 5.

Pictures from 50 individuals should be taken for morphometry every 6 months. Ideally, they will be taken during one month covering during the peak of spawning (Murta et al., 2008) and 6 month later, following the sampling scheme of Tables 5. The individuals should be the same sampled for biology and genetics, and pictures files should be named with the same codes used for genetics and biology (Annex 4).

The selected samples should represent the widest length range of the catch lengths (Annex 1) and this should be as much common as possible among the neighbour countries targeting the same species (case of study).

Summary procedure for photographing specimens for morphometry:

1. Place the fish straight on a **uniform background**, with all the fins visible and the pectoral fin extended backwards.
2. Find the **best position** of the fish to avoid shadows and reflections that can make difficult to find the landmarks.
3. Use the pins to make all the **landmarks visible**.
4. Check that no pin prevents the location of the landmarks.
5. Place the **measuring tape/ruler** on the top of the background.
6. Place the **3 cm scale** next to the pectoral fin.
7. Place the **code-label (sample ID)**.
8. Take the **picture**, using the grid option in order to align the individual in the photo.
9. **Check** the following before taking the photo: entire fish framed; no reflections or shadows; well focused: possible to read the numbers on the tape/ruler and on the code label.
10. **Name the picture file** with the code of the photographed individual (sample ID).

Procedure:

1) The fresh specimens (always before being gutted) have to be **located on** plain coloured or an artificial **background** contrasting the specimen's colour, on a surface where needles and pins can be nailed. The specimen should be positioned over the right side and photographed in left horizontal lateral position.
2) A **plastic ruler/measuring tape** must be positioned on the upper part of the background, together with other smaller **measuring scale** (3 cm) alongside the body of the specimen, as indicated in Figure 5. **Label the code** (sample ID) of the individual on a small label and put it on the corner to let the code appearing in the photo. The sequence of digital images (the file) must be codified the same way (see examples in Figure 5).
3) **Fish** should be **positioned** as follows:

- **In a straight line on its right side**: an imaginary line from the tip of the snout to the centre of the caudal fin (often parallel to the lateral line) is the most obvious axis that can be used. To achieve straight alignment, a ruler can be placed along the longitudinal axis of the fish.
- The **mouth** should be **slightly open** (but never completely open), pinning the tip of the lower lip, to better see the snout tip. Opercle should be fully closed and if flared, can be held down with a dissecting needle. Failure to secure the jaws and opercles in the standard position will result in changes to the shape of the head.
- Paired fins should be folded against the body, and the **unpaired fins** (e.g., dorsal and ventral) should be **fully erected** and spread and can be fixed in this position by using pins (see Figure 5). In the case of fish with two dorsal fins; distance between them must be visible in the image.

4) The **shape** of the body should be **fully visible** in a way that the landmarks could be easily located and marked in the image processing step to be later carried out in the lab by IEO (see Annex 7). These are:

Fish landmarks:

1. Anterior tip of snout on the upper jaw
2. Posterior tip of the upper jaw
3. Anterior insertion of 1st dorsal fin
4. Posterior insertion of 1st dorsal fin
5. Anterior insertion of 2nd dorsal fin
6. Posterior insertion of 2nd dorsal fin
7. Insertion of 1st dorsal caudal ray
8. Insertion of 1st ventral caudal ray
9. Posterior insertion of anal fin
10. Anterior insertion of anal fin
11. Anterior insertion of pelvic fin
12. Dorsal insertion pectoral fin (to be confirmed)
13. Origin of soft dorsal fin (in species with one dorsal fin, to be confirmed)

A total of 7 landmarks are used for the target fish species with 1 dorsal fin (E*. aeneus, P. caeruleostictus* and *P. bellottii*) and 8 landmarks for the target fish species with 2 dorsal fins (*P. elongatus* and *P. senegalensis*). 18-19 landmarks are used for *P. notialis*.

5) **Take a picture**, using a digital camera positioned in a horizontal tripod camera, if possible.

   - Appropriate lighting should be used. For that, we should search an area with **good lighting**, both natural and artificial.
   - The use of the flash will depend on the natural light conditions. Sometimes is convenient even with adequate lighting. In case of any doubt, we recommended to take a photo without flash and another with flash. In this way, we could verify that shadows or reflections are avoided, as they that could make difficult to locate the landmarks and therefore, make right measurements.
   - The specimen should be conveniently oriented in relation to the light source to **avoid shadows and reflections**. In case of taking pictures with natural light, direct exposure to sunlight should be avoided.
   - In order to keep the individual well aligned within the picture, we recommend activating the function "grid" that allows a **better framing** of the image. For a better framing, it is also very convenient to choose a panoramic-type option (16:9 or 19:9). Square-type options (3:3 or 1:1) should be avoided.
   - **Movements** while taking the picture should be **avoided**, in order to obtain clear and focused photographs. Thus, the camera must be held firmly with both hands and with the elbows attached to the body. If the camera has a stabilizer (OIS), it should always be used.

6) Write "Y" (Yes) in the corresponding column "Photo" of the biological sampling form (Annex 9) to indicate that the picture of that individual has been taken for morphometry.

7) To send the pictures:

   7.a) **<u>Classic procedure:</u>**

   - As images are translated from the camera to the computer and among software programs, a series of compressions and decompressions occurs. To avoid the degradation of the image quality, the image raw file format (RAW) or TIFF is desirable because no image compression occurs and the full spectrum of brightness levels is recorded by the camera. If pictures are taken with mobile phones, file format used will be JPEG. The minimum resolution of the camera should be 5 MP (images of 5 million pixels, with a resolution of 2560 x 1920).
   - Name the **image file** with the **ID-code** (as stated in Annex 4) of the photographed individual.
   - **One picture** (**maximum two**), should be chosen by specimen, those **with the greatest quality**. These pictures should be sent to IEO (eva.garcia@ieo.es), where morphometry analysis will be performed with an image software (as OTOLAB or ImageJ), following the methods explained in Annex 7. Some essays of the pictures will be made during the first sampling months, to ensure their quality in the correspondent sampling months.

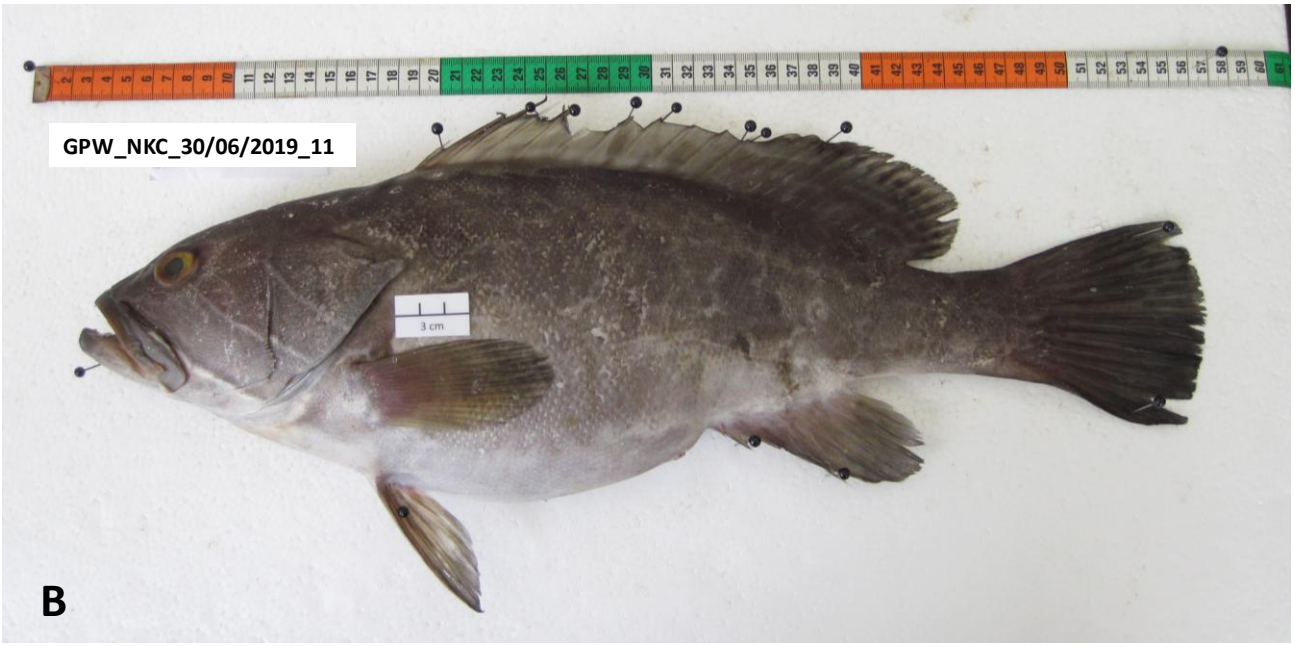
7-b) <u>Procedure with the Android application for entering data:</u>

If you enter your information in parallel on the electronic form (via the Android ODK collect application), the photo taken with the mobile or tablet is directly integrated into the form, for each individual:

Renaming photos is then unnecessary because each photo is associated with the individual described. When you take the photo, you can check its quality, accept it or not, and take another one. In the end, only a photo will be kept and transmitted so be sure to take the best.

If you use a digital camera to take the picture, you should rename the picture file. In this case, when entering data in the form Android, you should click on "Choose an image" rather than "Take a Photo". Then select the photo (a single photo) chosen for the individual.

A



B

Figure 5.- Pictures for morphometry: *Penaeus notialis* (A) and *Epinephelus aeneus* (B). José González Jiménez. IEO.

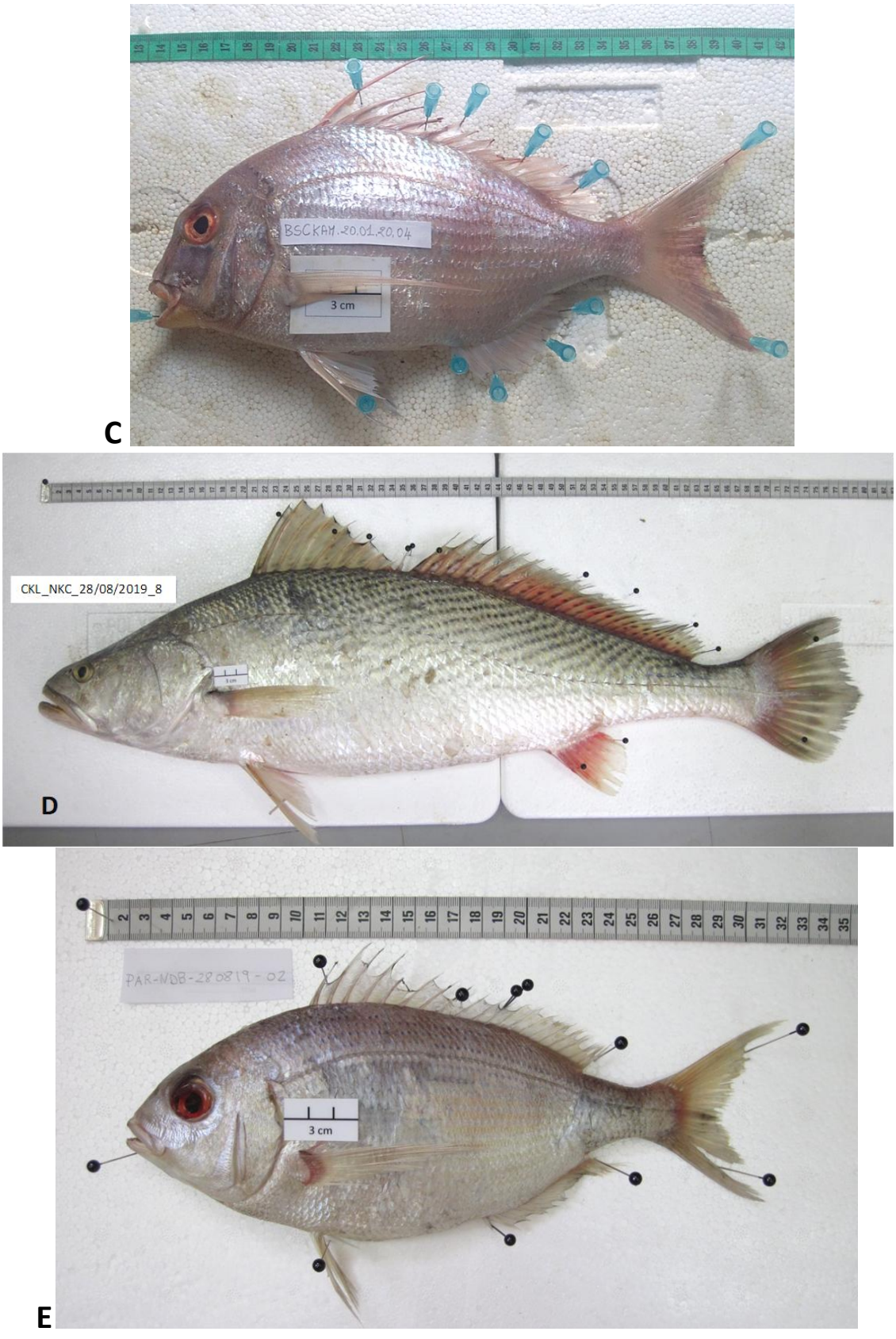
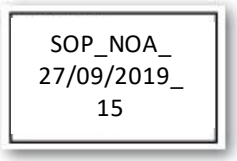


Figure 5 (cont.) - Pictures for morphometry: *Pagrus caeruleostictus* (C), *Pseudotolithus senegallus* (D) (valid for *P. typus et P. senegalensis)* and *Pagellus bellottii* (E). C: CRO, D and E: José González Jiménez. IEO.

Equipment

Digital camera

Ruler



Tags

Board

Source: FAO, 2016.

Pins

3 cm scale

*Use **pins** with small pinheads to avoid covering the landmarks with them.

F- ORDER STEPS TO FOLLOW IN MONTHLY AND SIX-MONTHLY SAMPLINGS

MONTHLY SAMPLINGS:

Day 1-2:

For each individual:

1. External parasites (in body surface)* (Ext PAR). Indicate "N" (no) if they are present but no stored, for any reason
2. Length (TL or CarL)
3. Fresh (total) weight (TW)
4. Sex
5. Maturity (Mat)
6. Gonad weight (GW)
7. Internal parasites* (int PAR)
8. Gutted (eviscerated) weight (Eviscer. W)
9. External parasites (in gills)* (Ext PAR gills)
10. Collection and storage of otoliths* (OTO)

*Always ensuring they are labelled with the same and correct codes (Annex 4). Indicate "N" (no) if they are present but no stored, for any reason.

SIX-MONTHLY SAMPLINGS:

Day 1-2:

13. Pictures of the individuals*, previously labelled in the order to be sampled (codes of Annex 4) (Photo)
14. External parasites (in body surface)* (Ext PAR)
15. Length (TL or CarL)
16. Fresh (total) weight (TW)
17. Sex
18. Maturity (Mat)
19. Gonad weight (GW)
20. Internal parasites* (Int PAR)
21. Gutted (eviscerated) weight (Eviscer.W)
22. External parasites (in gills)* (Ext PAR gills)
23. Sample for genetics*(GEN)
24. Collection and storage of otoliths* (OTO)

* Always ensuring they are labelled with the same and correct codes (Annex 4). Indicate "N" (no) if they are no taken/stored, for any reason.

Day 4-5:

- Carefully remove the ethanol from the microtubes with the genetic samples and replace them with new ethanol.

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ANNEX 1-

LENGTH CLASSES FOR BIOLOGICAL SAMPLING OF THE TARGET SPECIES

ANNEX 1- Number of individuals to sample by length class for each selected species and month/quarter

P. notialis **(SOP)**		
mm CarL	No. Ind (M)	No. Ind (Q)
<20	10	30
20-22	10	30
23-25	10	30
26-28	10	30
29-31	10	30
32-34	10	30
35-37	10	30
38-40	10	30
41-43	10	30
>43	10	30
Total	100	300

10 classes
3 mm/class

E. aeneus **(GPW)**		
cm LT	No. Ind (M)	No. Ind (Q)
<30	8	24
30-39	8	24
40-49	8	24
50-59	8	24
>59	8	24
TOTAL	40	120

5 classes
10 cm/class

P. caeruleostictus **(BSC)**		
cm LT	No. Ind (M)	No. Ind (Q)
<16	10	30
16-20	10	30
21-25	10	30
26-30	10	30
31-35	10	30
>35	10	30
TOTAL	60	180

6 classes
5 cm/class

P. elongatus **(PSE)**		
cm LT	No. Ind (M)	No. Ind (Q)
<14	10	30
14-17	10	30
18-21	10	30
22-25	10	30
26-29	10	30
30-33	10	30
>33	10	30
TOTAL	70	210

7 classes
4 cm/class

P. bellottii **(PAR)**		
cm LT	No. Ind (M)	No. Ind (Q)
<12	10	30
12-15	10	30
16-19	10	30
20-23	10	30
24-27	10	30
>27	10	30
TOTAL	60	180

6 classes
4 cm/class

P. senegalensis **(PSS)**		
cm LT	No. Ind (M)	No. Ind (Q)
<15	10	30
15-19	10	30
20-24	10	30
25-29	10	30
30-34	10	30
35-39	10	30
>39	10	30
TOTAL	70	210

7 classes
5 cm/class

M=Month
Q= Quarter

It may happen that all length classes cannot be completed in one single sampling in one month (red column). If this is the case, two steps must be followed:

a) Complete the total number required (100, 60 or 70, depending on the species) with individuals of the closer length class (e.g.: if there are only 9 individuals of the length class 18-21 of PSE, complete the length class 22-25 with 11 individuals).

b) Try to have all length sizes perfectly coverage on a quarterly basis: 30 individuals by length class (grey column). The classes that could not be sampled in one single month should be sampled in the quarter.

ANNEX 2-

COLLECTING BIOLOGICAL PARAMETERS (BIO)

ANNEX 2- COLLECTING BIOLOGICAL PARAMETERS

1. LENGTH

Procedure for measuring a **FISH** (from FAO, 2016):

1) Place the fish on the measuring board lying on its right side, snout to the left. Use a measuring tape if the fish is longer than the measuring board.
2) Gently press its snout against the headpiece.
3) Make sure the mouth is closed, and the body and tail are straightened along the mid–line.
4) Read the **total length** (tip of snout or jaw, the most anterior, to extreme end of tail in a straight line, Figure 1-A). **Avoid using the "pinched tail" length** (Figure 1-B).
5) The standard measurement units used are millimetres (mm).

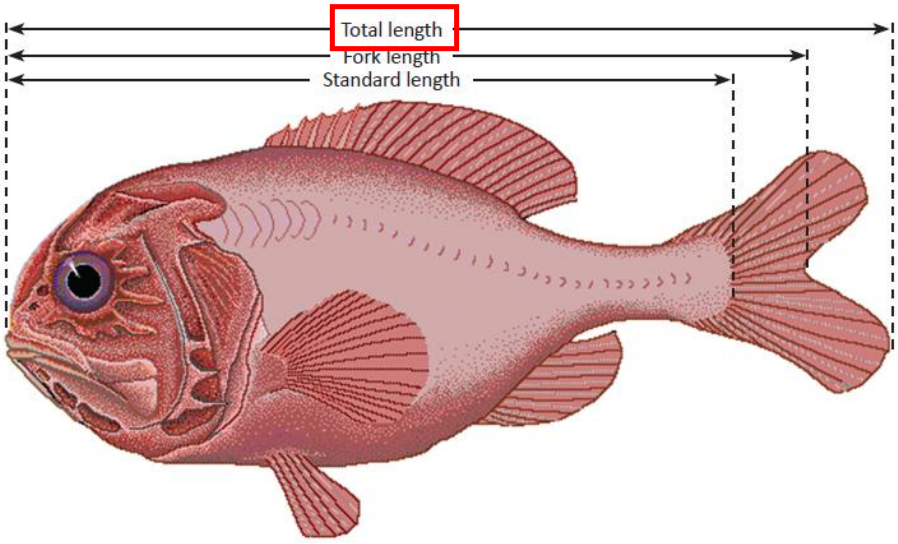


Figure 1-A. Fish measurements (FAO, 2016).

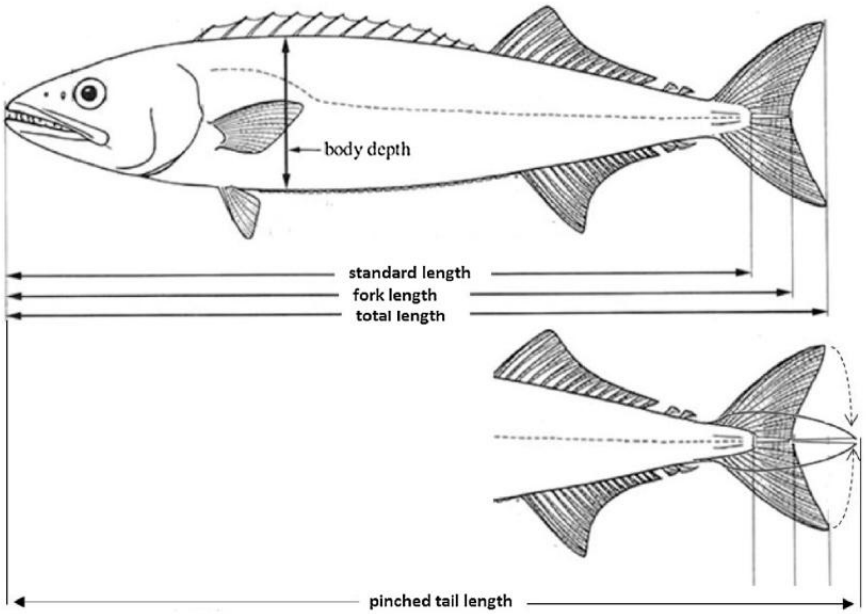


Figure 1-B.- Fish length measurement methods (Hansen et al., 2018).

The fish should be measured while it is fresh and wet. If the fish is in *rigor mortis* (stiffness after death) it should be flexed gently before it is measured.

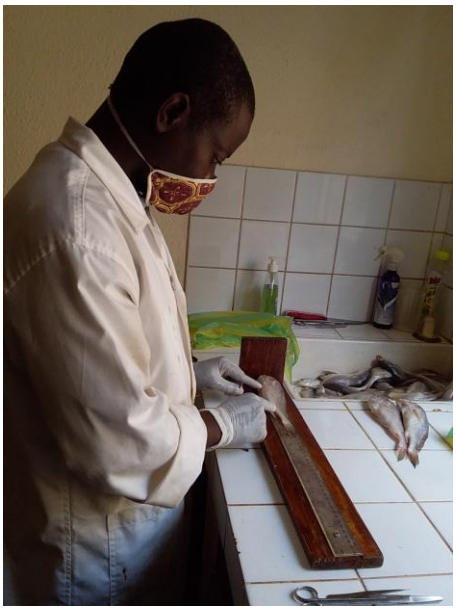


Photo: CRO.

Procedure for measuring a **SHRIMP** (*P. notialis*):

1) Take the shrimp with your left hand, with the eyes and rostrum to the left. Take the calliper with the right hand.
2) Fit the calliper between the base of the rostrum and the middle point of the back of the carapace.
3) Read the **carapace (cephalotorax) length** (CarL, length front to back of carapace, Figure 2).
4) The standard measurement units used are millimetres (mm), with on decimal. E.g.: 24.3 mm).

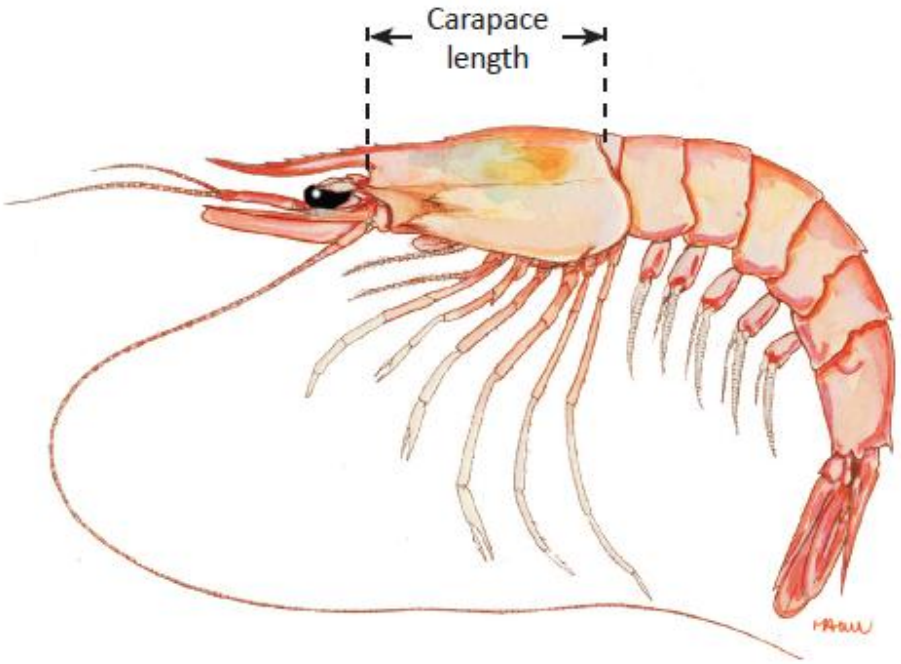


Figure 2. Carapace length (FAO, 2016).

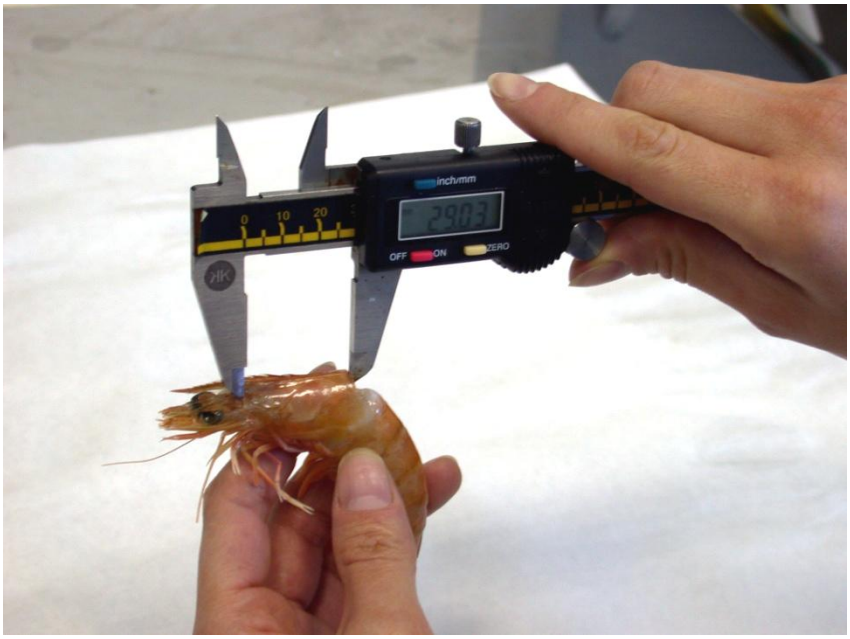


Photo: Eva García Isarch. IEO.C.O. Cádiz

Measuring equipment:

- **Measuring board**: for fish.

- **Calliper**: for shrimps

- **Measuring tape**: for fish with sizes exceeding the measuring boards. Attention should be paid not to follow the contour of the fish but to take a straight line measurement.

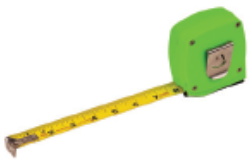


Table 1.- Summary table with procedures for measuring fish and shrimps.

GROUP	MEASURE	Abrev.	UNITY	EQUIPMENT
FISH	Total length	TL	mm	Measuring board or Measuring tape
SHRIMP	Carapace or Cephalotorax length	CarL	mm (with one decimal)	Calliper

2. FRESH WEIGHT

It is the weight of the whole individual, before being gutted. To weight each individual, precision electronic scales should be used whenever possible. Hanging scales could be used for large individuals with weights bigger than those of the electronic scale.

Each individual will be weighted, indicating fresh weigh in grams (exact, and with one decimal for shrimps).

Photo: CRO

<u>Weighting equipment</u> (FAO, 2016):

Hanging scale:

Often used by observers at sea.

Waterproof and covers a variety of weight ranges and levels of precision.

Electronic scale

Used in good working conditions such us a dry lab.

Not usually taken to the sea.

Table 2.- Summary table with procedures for measuring fish and shrimps.

GROUP	MEASURE	UNITY	EQUIPMENT
FISH	Fresh weight Eviscerated weight	Gram (g)	Electronic scale or hanging scale
SHRIMP	Fresh weight	Gram (g) (with one decimal)	Electronic scale

3. SEX

FISH- By internal characters:

For bony fish, without external characters that allow us to determine sex, we will proceed to dissect the specimens for the observation of the gonads. Normally, the visceral cavity must be opened, so that the gonads (ovaries in females and testicles in males) are exposed.

Procedure:

1) Ventrally open the fish by making a cut parallel to the spine forward from the anus.
2) Move stomach and intestines to the side.
3) The gonads can be located close to the spine below the intestines.
4) Determine the sex of the fish following Table 3.

Sex differentiation by visual examination may be difficult or impossible among small virgin individuals. In this case, they are classified as undetermined (code 3).

Among the specimens that have passed the virgin-immature stage, the differentiation between sexes can usually be made with the naked eye (*visu*) by observing the external characteristics of the gonads, following the generalities in Table 3 .

Table 3.- Basic differences in the appearance of male and female gonads of fish.

MALES (Code 1)	FEMALES (Code 2)
The testicles are flat, white and their ventral edges often have a wavy line.	The ovaries are tubular and granular.
Off-white, or grayish color.	Pink, reddish or orange colors.
Flattened shape, like knife edge.	Rounded or cylindrical shape, bag shape.

Hermaphroditism is found in some fish target species:

Epinephelus aeneus:

- Several studies have contributed to the belief that as most species of the Family Serranidae, *E. aeneus* is a **protogynous hermaphrodite** species (individuals are born as females, and then changes sex to males).
- However, in Senegalese waters, this species is found to be functionally **gonochoric** (i.e. the sexes are separate) (Ndiaye et al., 2013).

***Pagrus caeruleostictus*:**

This species is considered as:

- **Rudimentary hermaphrodite** species: only very young individuals have organs of both sexes, although they are still totally immature and therefore unable to produce gametes. When the animal grows, it develops one of the two sexes and keeps it throughout its life (Chakroun-Marzouk and Kartas, 1987, in Tunisian waters).

- Bonnet (1969) found **only two hermaphroditic** individuals of 35 and 46 cm length on the coasts of Mauritania and Senegal and could not specify what phenomenon it is, since males and females were found in a substantially equal number.
- However, other authors consider this species to be **gonochoric** in Mauritania (Navarro et al., 1943) and Ghana (Rijavec, 1973).
- In Egypt, recent studies suggest that *P. caeruleostictus* have extensive **hermaphroditism** with a proportion of **protogyny** or an extent of secondary **gonochoric** (Ismail et al., 2018).

Pagellus bellottii:

- In Senegalese water, *P. bellottii* is defined as **protogynous hermaphrodite** (Ndiaye, 2014).
- In contrast, the hermaphrodism character was not confirmed in Côte d'Ivoire (Kouame et al., 2018).

SHRIMP (P. notialis)- By external characters:

The sex of penaeid shrimps can be determined by looking at the abdominal region, close to the abdomen and walking legs.

Males:

- The male has a pairs of modified abdominal appendices or pleopods on the first abdominal segments (the petasma) that deliver sperm to the female's external receptacle (the thelycum) located between the bases of the fifth walking legs. The petasma and thelycum are located on the ventral surface.
- In mature individuals, the petasma is always joined. Only in immature individuals (still very small in size), the petasma is not joined and it is divided into 2 membranes, one in the right pleopod and one in the left.
- In addition, mature males are characterized by the presence of sperm mass, which accumulate in the coxas (bases) of the fifth pair of thoracic appendages or pereiopods. It is seen with the naked eye or by slightly pressing the coxas.

Females:

- Females lack petasma.
- They have thelycum, which is a modification of the ventral part of the cephalothorax at the height of the 3rd, 4th and 5th pair of pereiopods or thoracic appendages. This structure is where the male deposits his spermatophore.
- The females of *P. notialis* have "open thelycum", which means that the spermatophore is adhered externally, being able to know if the female has been fertilized (they have spermatophore).

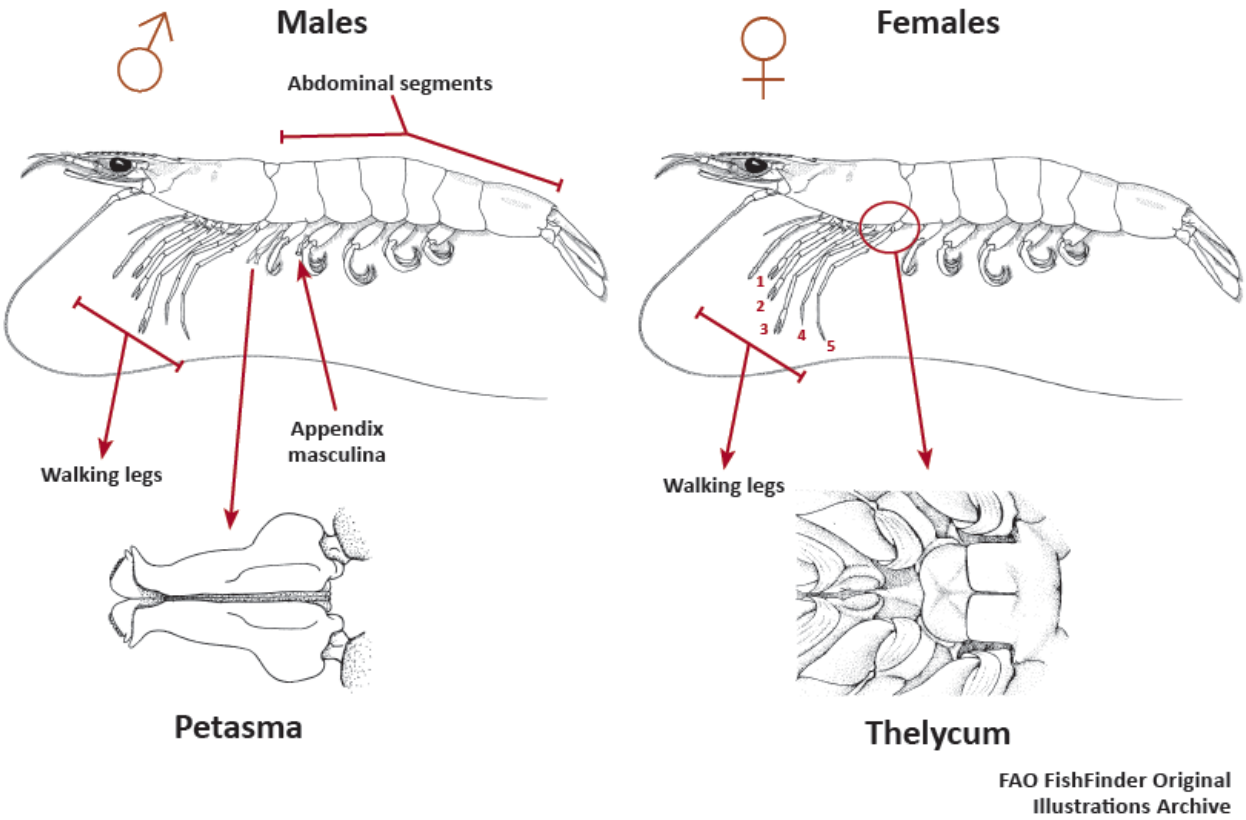


Figure 3.- Morphological differences between males and females of *Penaeus notialis*. (FAO Fishfinder Illustration).

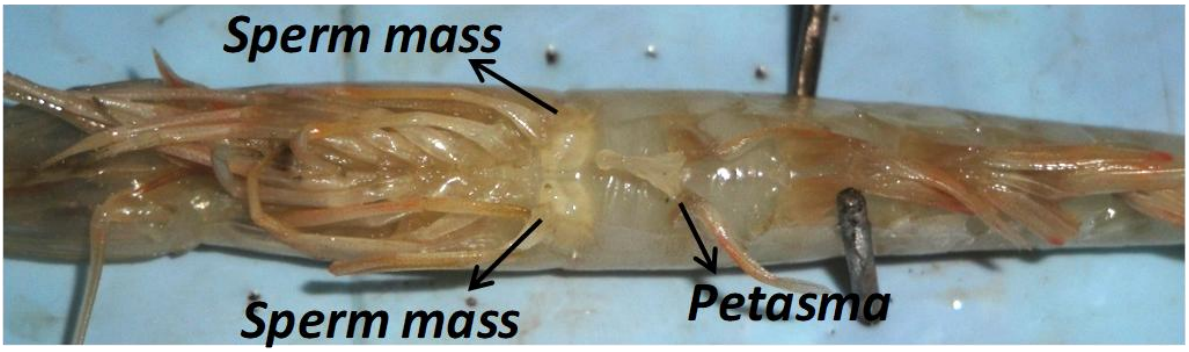
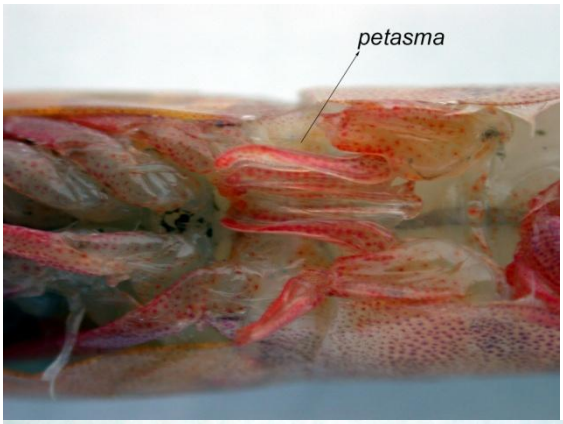
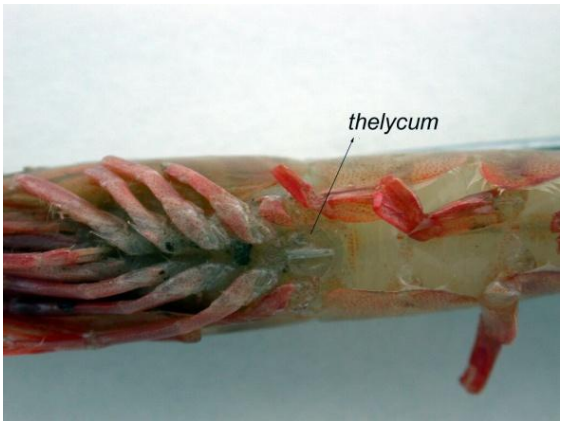


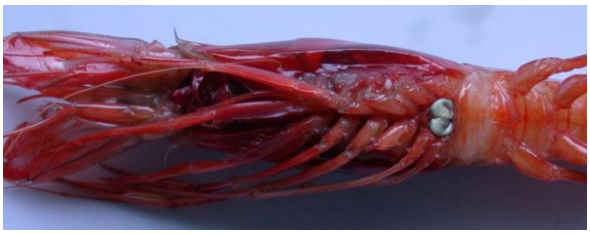
Photo- Male of *Penaeus notialis*. IEO. C.O. Cádiz

MALE



FEMALE





Fertilized *Aristeomorpha foliacea* female. Spermatophore adhered to the thelycum

Photos: Eva García Isarch. IEO. C.O. Cádiz

4- MATURITY STAGE

FISH

For fish, we will proceed to dissect the specimens for the observation of the gonads and determination of the maturity stage (see Annex 3). Once the visceral cavity was opened and gonads exposed for sex determination, the assignment of the maturity stages is carried out through the observation of characteristics of the gonad that can be observed with the naked eye (*visu*).

The degree of maturation will be determined, following the criteria of the 5 stages key in Annex 3. The maturation keys should be always plasticized for their use in the lab.

The maturation keys of the most important species or groups are found in Annex 3.

SHRIMP

The assignment of the maturity phases is carried out through the observation of external characteristics that can be observed with the naked eye (*visu*). See Annex 3. Females→ 4 stages . Males→ 2 stages.

5. GONAD WEIGHT

The gonad (ovary or testicle) will be removed, trying not to break it. Any remaining tissue that does not correspond to the gonad is removed. The gonad is located on the precision scale (previously tared). If it breaks, the pieces that make up the entire gonad will be added. The weight is recorded in the form, in grams and with a minimum of 1 decimal.

For shrimps: only the posterior lobes of the ovaries will be weighted. No weight of male gonads should be taken.

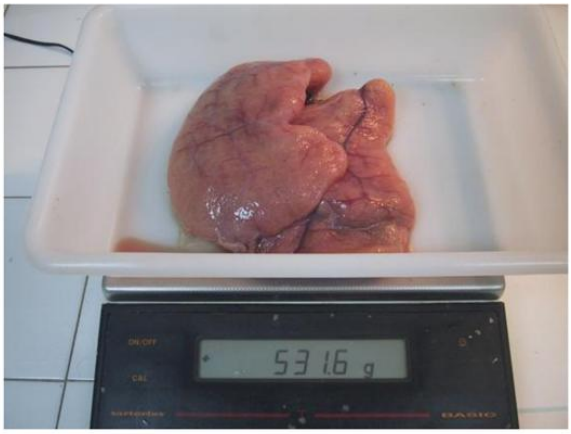


Photo: Fish gonad: Fernández et al., 2012.

Photo: Weight of posterior lobes of shrimp ovary. IEO. Cádiz.

5. GUTTED (EVISCERATED) WEIGHT

It is the weight of the animal, without the internal organs. The same instrument used for live weight (precision scales or dynamometer) will be used, indicating the weight in grams (with a decimal, if it is with precision balance) and writing it down in the corresponding forms.

Equipment:

Ciseaux

Scalpel

Pince

Ciseaux à poisson

Couteau à dents

Marteau et ciseau
(pour l'extration des otolithes des grandes thiofs)

ANNEX 3-

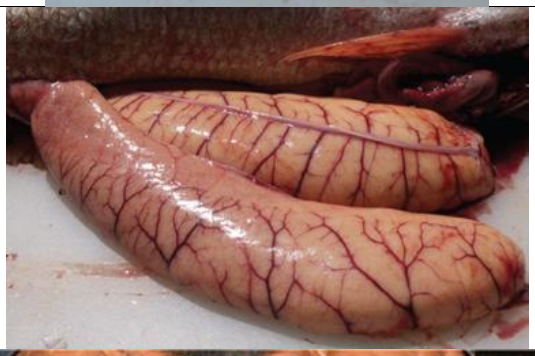
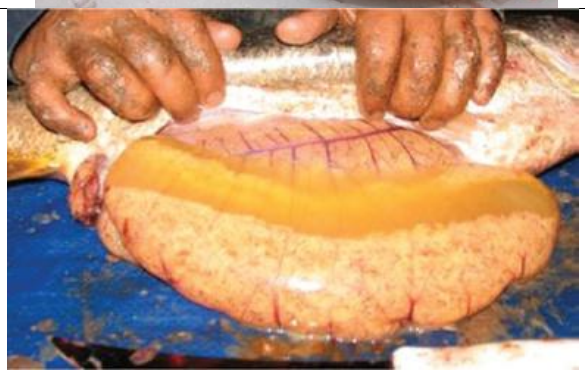
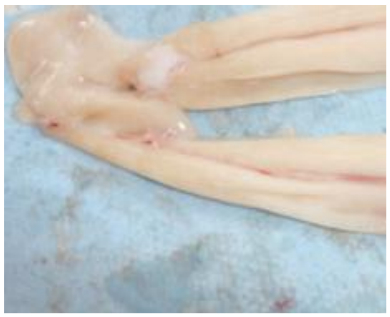
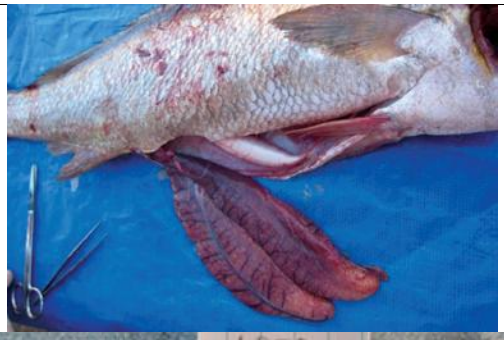
MATURITY KEYS

MATURITY KEY FOR DEMERSAL FISH

MATURITY KEY FOR DEMERSAL FISH (5-STAGE)

STAGE	GENERALITIES	FEMALE	MALE
1 INMATURE/ RESTING	Small, thin and transparent gonads. In virgin individuals it is difficult to distinguish sex (in these cases, sex=3, undetermined).	Ovary small, firm and translucent or pinkish/grey. No eggs visible to the naked eye. Minimal vascularisation.	Testis small, translucent, whitish, appear as thin strips lying close to the vertebral column.
2 MATURING	Small and filamentous gonads, with poorly visible blood supply.	Ovary more extended, about 1/2 length of body cavity. Firm consistency. Ovary opaque, rounded, pinkish or yellow-orange (depending on species), showing certain vascularisation. No eggs visible to the naked eye.	Testis extending for about 1/2 length of body cavity. Testis white, flat, convoluted, easily visible to the naked eye. No milt produced when pressed or cut.
3 NEARLY RIPE	Gonad about 2/3 length of body cavity.	Ovary large starting to swell the body cavity (2/3). Colour varies according to species (red, pink, orange), showing vascularisation. Granular appearance, but no transparent or translucent eggs visible.	Testis large (2/3). Whitish to creamy and convoluted. No milt produced when pressed or cut.
4 GRAVID (FEMALE) OR RIPE (MALE)	Bulky gonads that occupy between 2/3 to full length of the visceral cavity. Perfectly visible, abundant and branched blood irrigation.	Ovary large (2/3-total). Colour ranging from pink to reddish orange, with conspicuous superficial blood vessels. Firm consistency. Large transparent, ripe (translucent) eggs visible, sometimes running under pressure.	Testis large (2/3-total) and thick. Colour opalescent white. Sperm flows under abdominal pressure.
5 SPENT	Reduction of the size of the gonads, which present lax and empty aspects. Abundant irrigation and highly branched capillaries.	Ovary shrunken, flaccid, dark red colour (hemorrhagic). Soft consistency. It contains a few residual eggs and many small eggs.	Testis shrunk, flabby, dirty white in colour, with traces of bleeding (hemorrhagic). Sperm absent or residual.

SCIANIDAE (Family of *Pseudotolithus* spp.)

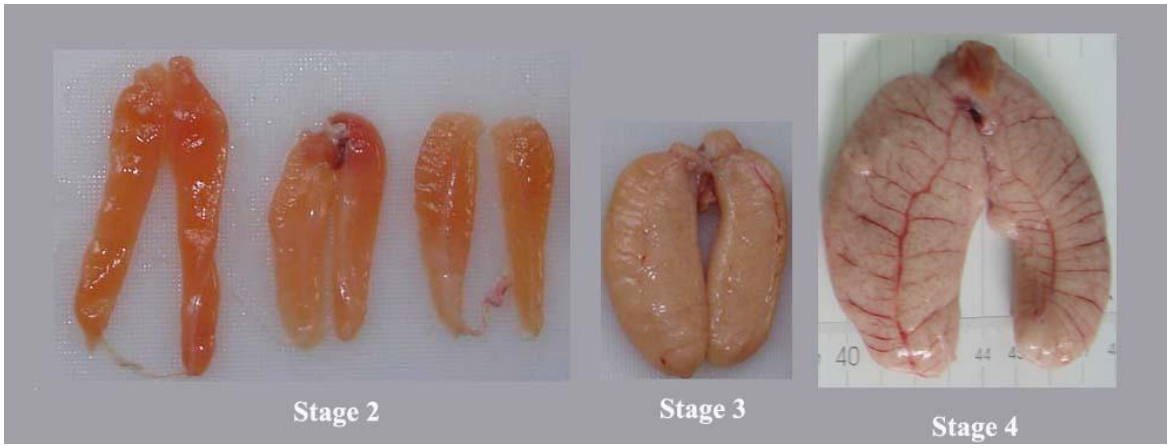
Stage	FEMALE	MALE
1-INMATURE		
2-MATURING		
3-NEARLY RIPE		
4- GRAVID (F) or RIPE (M)		
5- SPENT		
1- RESTING		

Fotos: Mascareñas I., G. Hinojosa, B. Erisman, O. Aburto-Oropeza. 2013. *Cynoscion othonopterus.*

MATURITY KEY FOR *Pagrus caeruleostictus* (5-STAGE)- Ismail et al., 2018

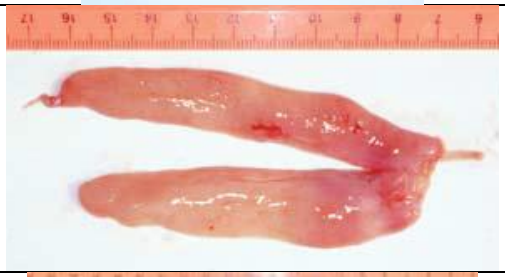
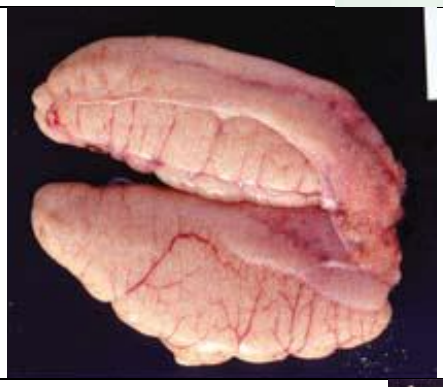
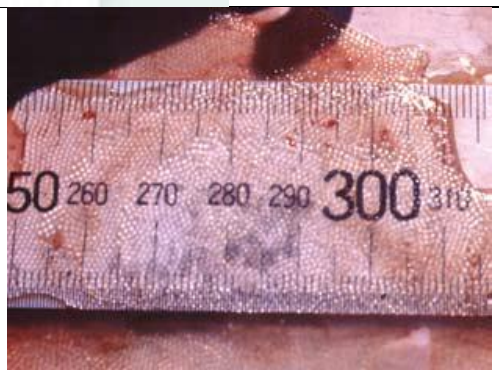
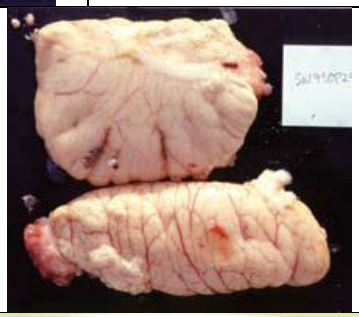
STAGE	FEMALE	MALE
1 INMATURE/ RESTING	Ovaries are very thin semitransparent tubes.	Testes are thin transparent but wider than ovaries.
2 MATURING	Ovaries are semi-transparent pale orange or yellowish wider tubes with no visible eggs.	Testes appear as white thinner tubes.
3 NEARLY RIPE	Ovaries are larger in size and orange in colour with visible oocytes as small granules.	Testes are larger, deeper with creamy-white colour.
4 RIPE AND RUNNING	Ovaries reach the maximum diameter with larger oocytes, deep orange colour with plentiful veins and arteries.	Testes are very large and fragile when handled and sperm can extrude if the testis break.
5 SPENT	Ovaries' size decreased, reddish in colour, and flaccid.	Testes are pinkish white in colour, shrunken in size.

Pagrus auratus- Female gonads (Stewart et al. 2010).

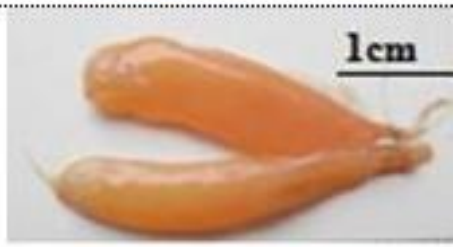


Pagrus auratus – Male gonads (Stewart et al. 2010).

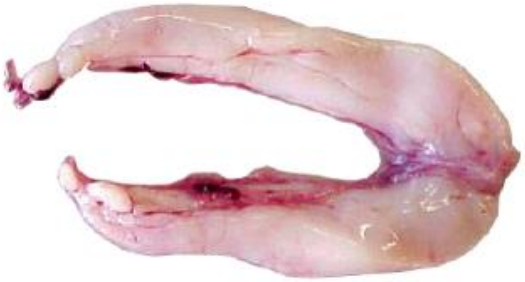
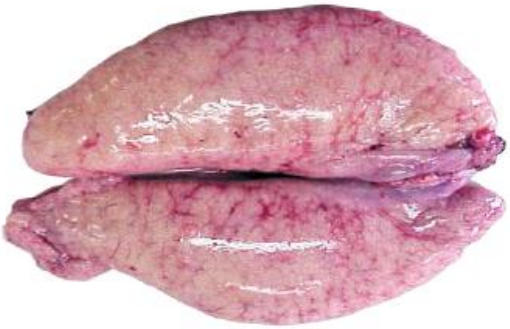
***Pagrus auratus*- Female gonads (Mackie et al., 2009)**

STAGE	FEMALE
1 INMATURE	
2 MATURING	
3 NEARLY RIPE	
4 GRAVID	 
5 SPENT	
1 RESTING	

Pagellus bellotii (pictures from Kouame et al., 2018)

STAGE	FEMALE	MALE
1 INMATURE OR RESTING		
2 MATURING		
3 NEARLY RIPE		
4 GRAVID (FEMALE) OR RIPE (MALE)		
5 SPENT		

OTHER SPARIDAE- ***Dentex gibbosus*** (Alves et al. 2011)

	FEMALE	MALE
1		
2		
3		
4		
5		
1		

MATURITY KEY FOR

Penaeus notialis

Penaeus notialis-FEMALES (4-stages key)[1]

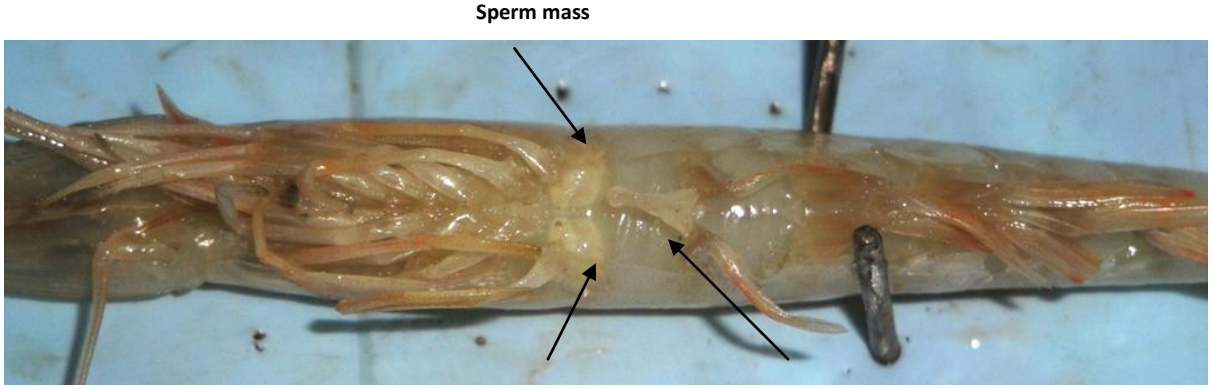
STAGE	DESCRIPTION	Gonad COLOUR	OBSERVATIONS	PHOTO
1-INMATURE	Ovaries thin, transparent and not visible to the naked eye. If dissected, two thin, transparent tubes (gonadal lobes) are attached to the dorsal part of the stomach, not extending to the abdomen.	Translucid	Only a thin black tube (full digestive) or transparent (empty digestive) visible in the abdominal part to the naked eye.	
2-DEVELOPING OR RESTING	Ovaries barely visible without dissection.	Off-white, pale yellow or cream-orange	Gonad visible through the cephalothorax and abdomen, although their abdominal lobes are quite thin. The difference with state III (in addition to thickness) is that in state II the gonad abdominal lobes are not observed in the last segments of the abdomen.	
3-MATURING	Ovaries clearly visible through the tegument, to the naked eye. Ovaries developed and turgid, with the cephalic and abdominal lobes occupying the entire distal portion. Grainy appearance.	Dark yellow, orange or light green	Gonad abdominal lobes visible until the end of the abdomen.	
4-MATURE	The ovaries are turgid and extend throughout the dorsal area. The posterior or abdominal lobes are well developed. The eggs are well visible.	Different shades of dark green	Gonads larger than in the previous state. Widening of the gonadal lobe, after passing through the first abdominal segment.	

[1] Key and pictures: IEO-C.O. Cádiz

Pictures of 4 maturity stages in dissected females- *Parapenaeus longirostris*

Penaeus notialis-MALES (2-stages key)[2]

STAGE	PETASMA	SPERM IN COXAS OF THE 5TH PAIR OF THORACIC LEGS
1. IMMATURE	Not joined	Absent
2. MATURE	Joined	Present



MATURE MALE

[2] Key and pictures: IEO-C.O. Cádiz

ANNEX 4-

LABEL CODES

FOR SAMPLES AND PICTURES

ANNEX 4- LABEL CODES USED FOR GENETIC/PARASITES SAMPLES AND PICTURES FOR MORPHOMETRY

The code for each individual consists of:

- FAO code for the name of the species.
- Three alpha code for the name of the port/sampling area or
- Three alpha code for the name of the survey, and number of haul.
- Date in the format day/month/year.
- Number of the specimen within each sampling (01-50).

	NAME	CODE
Species	*E. aeneus*	GPW
	P. notialis	SOP
	P. caeruleostictus	BSC
	P. elongatus	PSE
	P. senegalensis	PSS
	P. bellottii	PAR
Sampling places	Noauadhibou	NDB
	Nouakchott	NKC
	Saint Louis	SLO
	Saloum	SAL
	Casamance	CAS
	Gambia	GAM
	Kayar	KAY
	Cacheu	CCH
	Cacine	CAC
	Bissau	BIS
	Kamsar	KAM
	Katchek	KAT
	Conakry	CON
	Abidjan	ABJ
	San Pedro	SPE
	Cape 3	CPE
	Tema	TMA
	Takoradi	TKO
Surveys	Name of survey and number of haul	
Date	Day/Month/Year	

E.g.1) **GPW_NDB_15/02/20_03→** individual number 3 of *E. aeneus* from Noauadhibou (Mauritania) sampled on the 15/02/2020.

E.g.2) **SOP_CAS_03/08/20_12→** individual number 12 of *P. notialis* from Casamance (Senegal) sampled on the 03/08/2020.

E.g.3) **PSE_CCH_10/01/20_08→** individual number 8 of *P. elongatus* from Cacheu (Guinea-Bissau) sampled on the 10/01/2020.

E.g.4) **BSE_KAM_07/07/20_45→** individual number 45 of *P. caeruleostictus* from Kamsar (Guinea) sampled on the 07/07/2020.

E.g.5) **PSS_ABJ_12/10/19_36→** individual number 36 of *P. senegalensis* from Abidjan (Côte d'Ivoire) sampled on the 12/10/2019.

E.g.6) **PAR_TKO_12/12/19_23→** individual number 23 of *P. bellottii* from Takoradi (Ghana) sampled on the 12/12/2019.

E.g.7) **GPW_EAF58_15/02/20_05→** individual number 5 of *E. aeneus* from the trawl number 58 during the survey EAF Nansen sampled on the 15/02/2020

E.g.8) **SOP_OSM125_21/12/19_12→** individual number 12 de *P. notialis* from the trawl number 125 of observer (O) onboard shrimper trawlers (S) in Mauritania (M), sampled on the 21/12/2019

ANNEX 5-

COLLECTION AND STORAGE OF OTOLITHS (OTO)

ANNEX 5- COLLECTION AND STORAGE OF OTOLITHS

Otoliths are pairs of bone structures of calcium carbonate that act as a balancing mechanism. They are located in the skull, specifically, in the saccule of the inner ear, under the brain and behind the eyes, in separate cavities (ear vesicles) on both sides of the cranial cavity (Figure 1). The sagittal otoliths (the biggest) are those used for age determination of most fish.

Objectives:

In addition to determining the age of individuals, otoliths can be used for stocks identity studies, since their shape may vary depending on where the individual comes from. These differences are due to the different habitats and oceanographic characteristics.

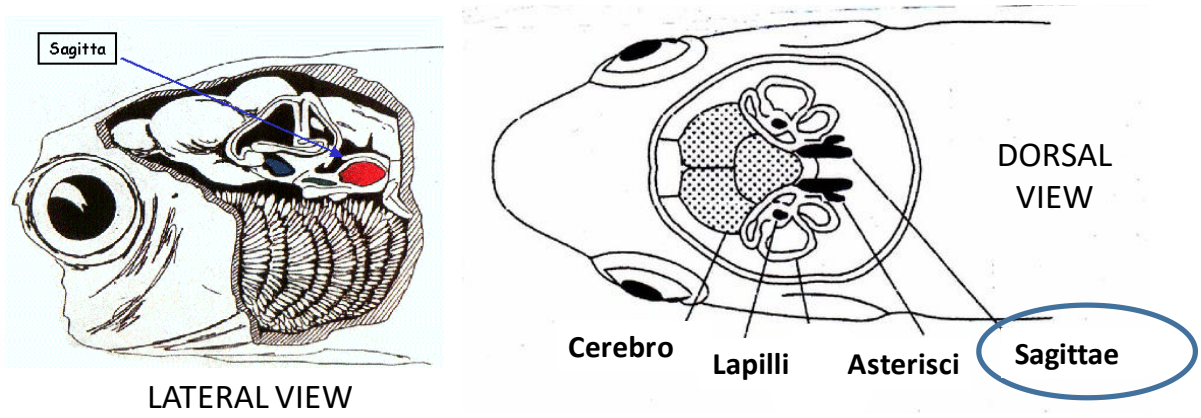


Figure 1. Location of sagittal otoliths. Lateral and dorsal views.

Procedure to collect otoliths:

The otoliths are located slightly behind and below the brain and are nestled within separate cavities, one on either side of the mid–line.

The slicing and cutting tools vary according to the cranium size and robustness, but in general consist of knives. Never use scalpel for the otoliths extraction. Use a serrated knife, which is much safer. A handsaw or even a hammer and a chisel might be needed for very big individuals (e.g.: *E. aeneus*).

1) In general, there are different ways to extract otoliths, which can be more or less effective depending on the species and size of the fish.

   a. *By an oblique section of the dorsal area of the neurocranium* (Figure 2)

   Hold the fish vertically, firmly placing the abdomen on the sampling table and cut down in the vertical plane. To make this cut, the head of the specimen must be firmly held by inserting the index finger and the thumb (left or right hand) in the eye sockets.

   The neurocranium, placed in front of the eyes, should be cut with an inclination of about 45^{o}. Once the skull is opened and the brain moved forward to the anterior part of the fish head, the two largest otoliths (sagittae) are easily detected and can be removed with stainless steel tweezers.

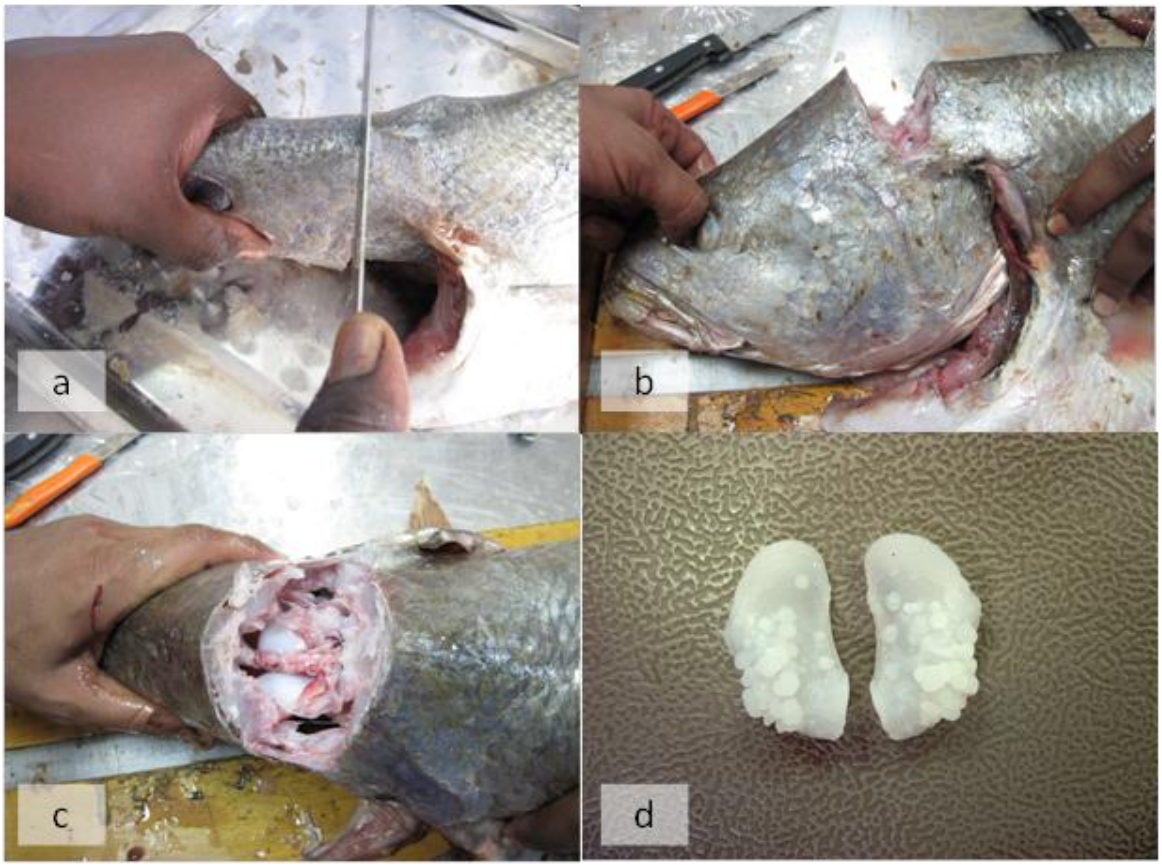


Figure 2.- Procedure for collecting otoliths of *Pseudotolithus* spp of large size, by an oblique cut of the dorsal area of the neurocranium. Photos: José González Jiménez. IEO.

b. B*y a longitudinal section of the ventral area of the otic (ear) capsules* (Figure 3)

Place the specimen with the abdomen upwards and make a cut section on the jugular region. Insert your hand into the gill chamber through the sectioned area, take the gills and pulled them up and forward to extract them and expose the ear capsules. These are found at the base of the neurocranium and constitute a large part of the roof of the gill chamber. They are easily recognizable by their characteristic bulbous shape.

Make a longitudinal cut, starting it at the posterior edge of the otic capsule and directing it forward.

2) Gentle probing with the forceps is often successful in locating unseen otoliths as they are hard and dense and 'feel' different from the bone/cartilage tissue.

3) Carefully remove the pair of otoliths using the forceps. The otoliths are brittle and will break if handled roughly.

4) Carefully and slightly clean the 'jelly' off the otoliths. If there is any tissue adhering to the otolith, clean each one by gently wiping it on a clean part of your glove on the back of your hand. Dry them carefully, putting them on blotting paper (or paper napkin), and place <u>both otoliths</u> in the labelled tube.

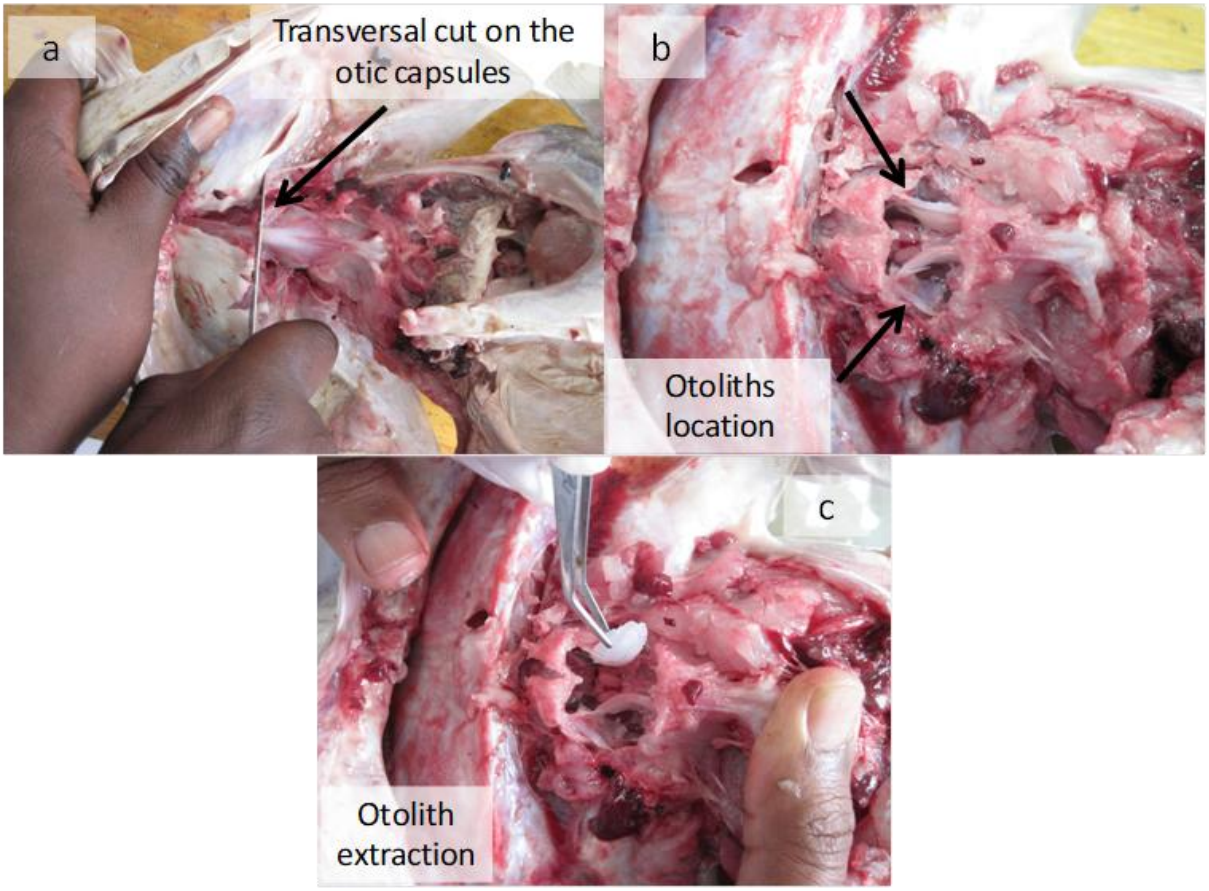


Figure 3.- Procedure for collecting otoliths of *Epinephelus aeneus*, by a longitudinal section of the ventral area of the otic capsules. Photos: José González Jiménez. IEO.

5) Store the tubes in boxes and send them to IEO (Cádiz), for the otoliths shape analysis. eva.garcia@ieo.es
6) For the **otoliths shape analysis** (in IEO):
   - One of the two pairs (right or left) will be always used for analysis.
   - The otolith will be located on a black background for catch image and digitalization.
   - Image software (OTOLAB or ImageJ) will be used for taking measures: area, perimeter, length, width, circularity, etc.
   - Different shape indices will be used: circularity, ratio between otolith perimeter and otolith area, ratio between otolith length and otolith width.

ANNEX 6-

COLLECTION AND STORAGE OF PARASITES (PAR)

ANNEX 6- COLLECTION AND STORAGE OF PARASITES

Objectives:

Parasites are used as tags for studies on stocks identity. The basic principles of using parasites as tags is that fish can become infected with a particular parasite only when they come within the endemic area of that parasite, the endemic area being the geographic region in which transmission of the parasite can take place. If infected fish are found outside the endemic area, we can infer that these fish had been within that area at some time in its past history.

Sampling procedure:

Samples should be examined as soon as possible after their caught, otherwise the samples must be stored in frozen (-30 °C) until their examination.

Parasitological examination should be made after the pictures for morphometry, during the months these should be taken.

1) **For external parasites (ectoparasites):** copepods and monogeneans.
   - Scan the fish macroscopically.
   - Remove gill arches and scan them during a standard time of 2 minutes.

   If found, first try to take a picture of the fish with the parasites. After, remove the parasites and wash them in saline solution, then in distilled water. Finally, store them in alcohol 96°, in 5 ml tubes, well labelled with the code of the individual.

2) **For internal parasites (endoparasites):** larval and adult nematodes:

   Look carefully to the **internal viscera cavity and surface of the internal organs** (gonads, stomach, intestine, etc) for the detection of nematodes (e.g.: *Anisakis* spp.) during a standard duration of 2 minutes observation.

   Use the following criteria to record the infection level in the forms for biological samplings (Annex 9) and to take samples of parasites of each fish:

NIVEAU D'INFECTION	Number of parasites seen in 5 minutes	Nombre de parasites à stocker
0 (No infection)	0	0
1 (Low infection)	1- 20	All
2 (Medium infection)	20-50	20
3 (High infection)	>50	20

   If found, first try to take a picture of the fish with the parasites. After, pick out larval and adult nematodes of all internal organs found in 2 minutes, removed them and wash them and in saline solution, then in distilled water; finally, store them in alcohol 96°.

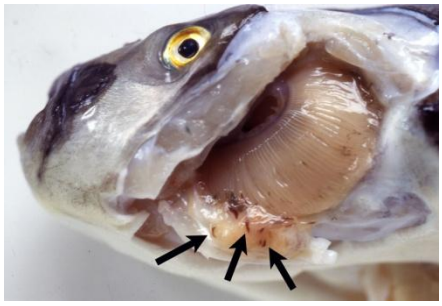
Use tubes of 5 ml with caps, including up to 20 worms per tube. Collect the number of worms indicated in the table. Close the cap also by using parafilm. If more than one tube is needed label them and include this information on the label (e.g. 1(3), 2(3) and 3(3) if three vials were needed). Label each tube of the collected nematodes as indicated and store them.

3) Finally, all the tubes will be stored in the same box, indicating, on its top, the host animal (e.g.: *P. elongatus, E. aeneus*) and the sampling location.
4) Indicate the samples for parasites taken in the forms used for biological sampling (Annex 9).
5) Samples will be sent to IEO (Cádiz) and analyzed, if possible. eva.garcia@ieo.es

ECTOPARASITES

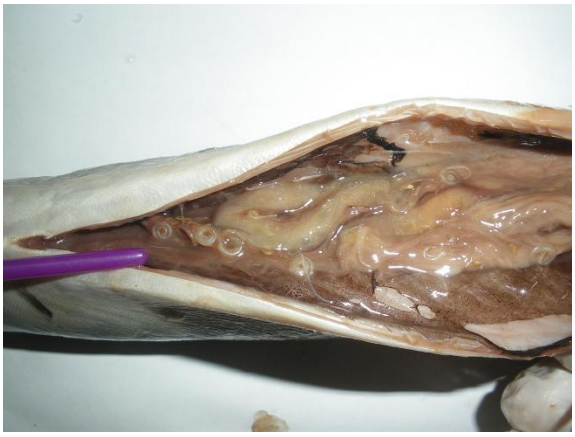
Isopoda

Copepoda



Monogeans

NEMATODES



Nematodes in internal cavity

Nematodes in fillets

ANNEX 7-

IMAGES ANALYSIS FOR MORPHOMETRY (MOR)- IEO

ANNEX 7- IMAGES ANALYSIS FOR MORPHOMETRY (IEO)

Objective: Study of the individual and otoliths shape, used for **stock identity.**

Procedure in laboratory (IEO):

The images of the 6 target species sent by the Institutions from coastal States will be analyzed in the IEO. Software for images analyses as OTOLAB or ImageJ might be used. They allow measuring the distances established for the Truss Network (TN).

Target species have been organized in 3 groups:

- **Group 1- Fish with 1 dorsal fin**: *E. aeneus, P. caeruleostictus* and *P. bellottii*

  They will have 7 landmarks and 14 distances will be measured.

- **Group 2**- **Fish with 2 dorsal fins**: *P. elongatus and P. senegalensis*.

  They will have 8 landmarks and 16 distances will be measured.

- **Group 3- Shrimp.** *P. notialis*

  They will have 18-19 landmarks and 38-43 distances will be measured.

GROUP 1- FISH WITH 1 DORSAL FIN

Epinephelus aeneus, Pagrus caeruleostictus and *Pagellus bellottii*

A total of 7 landmarks and 14 distances have been preliminary selected for Group 1-fish, although other distances might be further added, if needed. These are the following:

Group 1_ 7 Landmarks:

1. Anterior tip of the snout on the upper jaw
2. Anterior insertion of 1st dorsal fin
3. Insertion of 1st dorsal caudal ray
4. Insertion of 1st ventral caudal ray
5. Anterior insertion of anal fin
6. Anterior insertion of pelvic fin
7. Posterior tip of the upper jaw

Group 1_ 14 Distances:

D01. Anterior tip of the snout on the upper jaw – Anterior insertion of 1st dorsal fin (1→2)

D02. Anterior tip of the snout on the upper jaw – Anterior insertion of pelvic fin (1→6)

D03. Anterior tip of the snout on the upper jaw – End of the mouth (1→7)

D04. Posterior tip of the upper jaw – Anterior insertion of 1st dorsal fin (7→2)

D05. Posterior tip of the upper jaw - Anterior insertion of pelvic fin (7→6)

D06. Anterior insertion of 1st dorsal fin – Anterior insertion of pelvic fin (2→6)

D07. Anterior insertion of 1st dorsal fin – Anterior insertion of anal fin (2→5)

D08. Anterior insertion of 1st dorsal fin – Ventral insertion of caudal fin (2→4)

D09. Anterior insertion of 1st dorsal fin – Dorsal insertion of caudal fin (2→3)

D10. Anterior insertion of anal fin – Dorsal insertion of caudal fin (5→3)

D11. Anterior insertion of anal fin – Ventral insertion of caudal fin (5→4)

D12. Anterior insertion of pelvic fin – Anterior insertion of anal fin (6→5)

D13. Anterior insertion of pelvic fin – Ventral insertion of caudal fin (6→4)

D14. Insertion of 1st dorsal caudal ray – Ventral insertion of caudal fin (3→4)

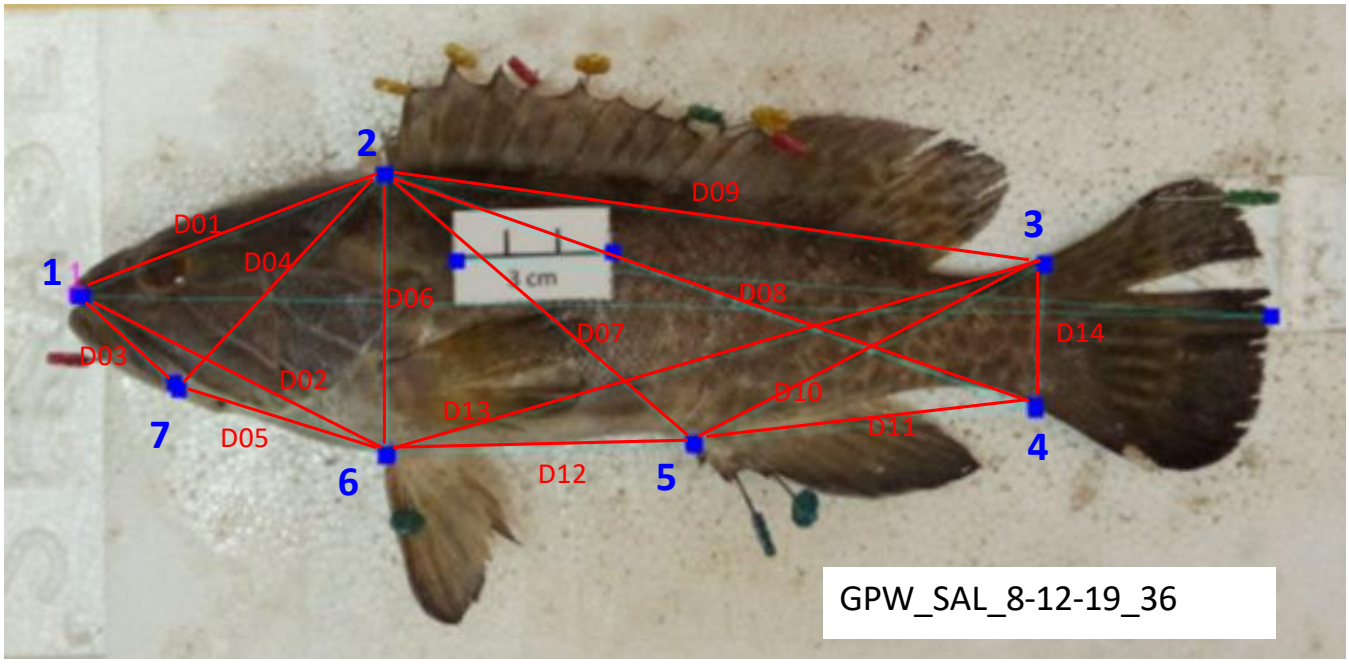


Figure 1.- Number and position of landmarks (in blue) and distances (D, in red) used for truss network. Example of Group 1: *Epinephelus aeneus* (GPW). Photo: CRODT

GROUP 2- FISH WITH 2 DORSAL FINS

Pseudotolithus elongatus and *Pseudotolithus senegalensis*

A total of 8 landmarks and 16 distances have been preliminary selected for Group 2-fish, although other distances might be further added, if needed. These are the following:

Group 2_8 Landmarks:

1. Anterior tip of the snout on the upper jaw
2. Anterior insertion of 1st dorsal fin
3. Anterior insertion of 2nd dorsal fin
4. Insertion of 1st dorsal caudal ray
5. Insertion of 1st ventral caudal ray
6. Anterior insertion of anal fin
7. Anterior insertion of pelvic fin
8. Posterior tip of the upper jaw

Group 2_16 Distances:

D01. Anterior tip of the snout on the upper jaw – Anterior insertion of 1st dorsal fin (1→2)

D02. Anterior tip of the snout on the upper jaw – Anterior insertion of pelvic fin (1→7)

D03. Anterior tip of the snout on the upper jaw – Posterior tip of the upper jaw (1→8)

D04. Posterior tip of the upper jaw – Anterior insertion of 1st dorsal fin (8→2)

D05. Posterior tip of the upper jaw – Anterior insertion of pelvic fin (8→7)

D06. Anterior insertion of 1st dorsal fin – Anterior insertion of pelvic fin (2→7)

D07. Anterior insertion of 1st dorsal fin – Anterior insertion of 2nd dorsal fin (2→3)

D08. Anterior insertion of 1st dorsal fin – Anterior insertion of anal fin (2→6)

D09. Anterior insertion of 2nd dorsal fin – Anterior insertion of anal fin (3→6)

D10. Anterior insertion of 2nd dorsal fin – Dorsal insertion of caudal fin (3→4)

D11. Anterior insertion of 2nd dorsal fin – Ventral insertion of caudal fin (3→5)

D12. Anterior insertion of anal fin – Dorsal insertion of caudal fin (6→4)

D13. Anterior insertion of anal fin – Ventral insertion of caudal fin (6→5)

D14. Anterior insertion of pelvic fin – Anterior insertion of 2nd dorsal fin (7→3)

D15. Anterior insertion of pelvic fin – Anterior insertion of anal fin (7→6)

D16. Dorsal insertion of caudal fin – Ventral insertion of caudal fin (4→5)

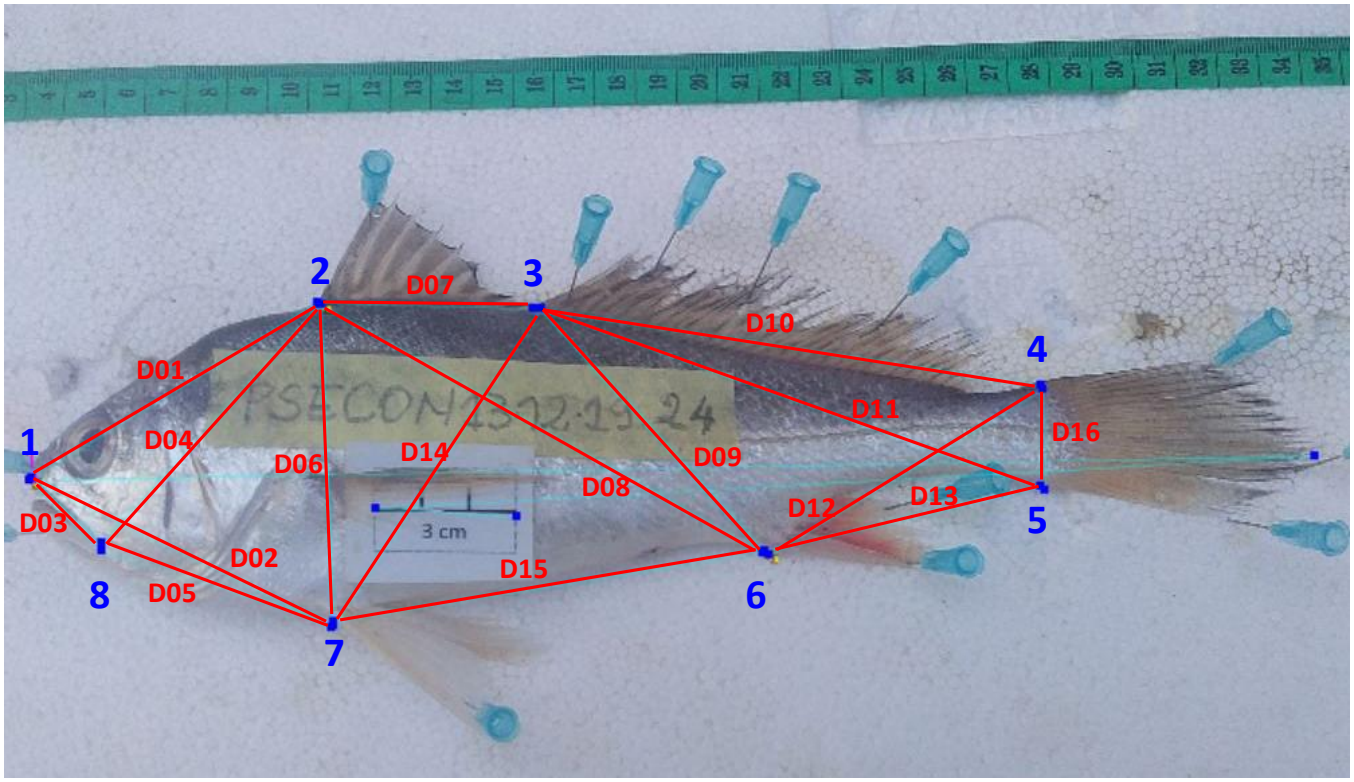


Figure 2.- Number and position of landmarks (in blue) and distances (D, in red) used for truss network. Example of Group 2: *Pseudotolithus elongatus (PSE).* Photo: CRO

GROUP 3 - SHRIMPS

Penaeus notialis

A total of 18-19 landmarks and 40-43 distances have been preliminary selected for shrimps, although other distances might be further added, if needed. These are the following:

Group 3_18-19 Landmarks:

1. Base of the rostrum
2. The first upper teeth of the rostrum
3. The upper of the first segment base
4. The upper of the second segment base
5. The upper of the third segment base
6. The upper of the fourth segment base
7. The upper of the fifth segment base
8. The upper of the sixth segment base
9. The upper of the final segment base
10. Caudal base
11. End of the sixth segment bottom

12. End of the fifth segment bottom
13. End of the fourth segment bottom
14. End of the third segment bottom
15. End of the second segment bottom
16. End of the first segment bottom
17. The base of the first walk leg
18. The base of the antenna
19. The base of carapace

Groupe 3_ 38-43 distances.

Truss distance				
D01. 1→2	D11. 4→5	D21. 6→14	D31. 9→10	D41. 17→18
D02. 1→18	D12. 4→15	D22. 6→15	D32. 9→11	D42. 17→19
D03. 1→19	D13. 4→16	D23. 7→8	D33. 9→12	D43. 18→19
D04. 2→3	D14. 4→17	D24. 7→12	D34. 10→11	
D05. 2→17	D15. 5→6	D25. 7→13	D35. 11→12	
D06. 2→19	D16. 5→14	D26. 7→14	D36. 12→13	
D07. 3→4	D17. 5→15	D27. 8→9	D37. 13→14	
D08. 3→16	D18. 5→16	D28. 8→11	D38. 14→15	
D09. 3→17	D19. 6→7	D29. 8→12	D39. 15→16	
D10. 3→19	D20. 6→13	D30. 8→13	D40. 16→17	

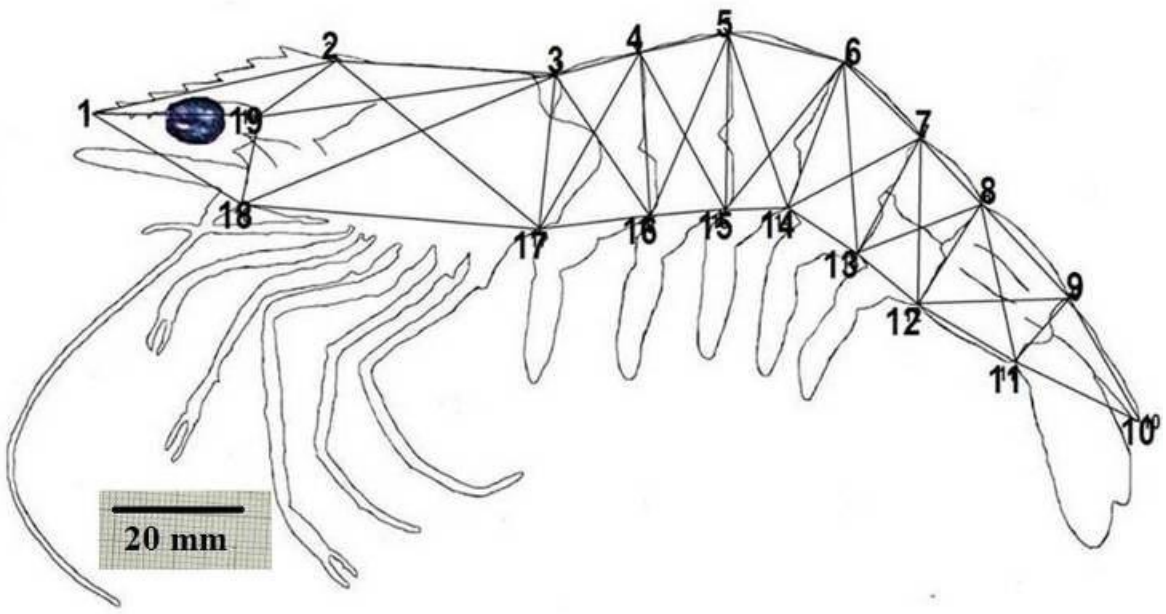


Figure 3.- Number and position of landmarks used for truss network in the banana shrimp (*Penaeus merguiensis*). Source: Marini et al., 2017. Example for Group 3: *Penaeus notialis*.

ANNEX 8-

LIST OF MATERIAL

ANNEX 8- LIST OF EQUIPMENT

MATERIAL	MINIMUM BY SAMPLING PLACE	SAMPLING
Icthyiometer or measuring board	1	BIO-LENGTH
Measuring tape	1	BIO-LENGTH
Calliper	1	BIO-LENGTH
Hangig scale or electronic scale (30kg)	1	BIO-LENGTH
Precision scale 0.1 g	1	BIO
Plastic trays 5 l and 10 l	10	BIO
Plastic apron	2	BIO-LENGTH
Latex gloves		BIO-LENGTH
Knife (small, medium, big sized)	1 x 3	BIO
Scalpels	3	BIO
Scissors (small, medium, big sized)	1 x 3	BIO
Tweezers (small, medium, big sized)	1 x 3	BIO
Dissecting needles	3	BIO
5 ml tubes with screw caps	2400	PAR, OTO
2 ml tubes with screw caps	200	GEN
Undenatured ethyl alcohol 96% (10 l)	1	GEN, PAR
Petri dishes		BIO, OTO, PAR
Digital camera	1	MOR
Polyurethane panels (or similar)	2	MOR
Coloured board-photo background	2	MOR
Dissecting pins and needles (box)	1	MOR
Plastic tags	70	BIO, MOR, GEN, PAR
Permanent markers different thickness	10	BIO, MOR, GEN, PAR
Pencils, sharpeners, erasers		BIO-LENGTH
Sampling forms		BIO-LENGTH
Clipboard Folder	2	BIO-LENGTH
Plasticised maturity keys	2	BIO
Blotting paper		BIO-LENGTH
Tracer paper for labels		GEN, PAR
Roll of paper towels		BIO-LENGTH
Corrosion inhibitor		BIO-LENGTH
Plastic bags		BIO-LENGTH

BIO= Biological sampling; LENGTH= length sampling; GEN= sampling for genetics; MOR= sampling for morphometry; OTO= collecting otoliths; PAR= collecting parasites.

1. Roll of paper towels
2. Measuring board or icthyometer
3. Corrosion inhibitor
4. Hanging scale
5. Precision scale
6. Dissection tools
7. Knifes
8. Dish
9. Latex gloves
10. Pencil
11. Sampling form
12. Plastic tray 5 l

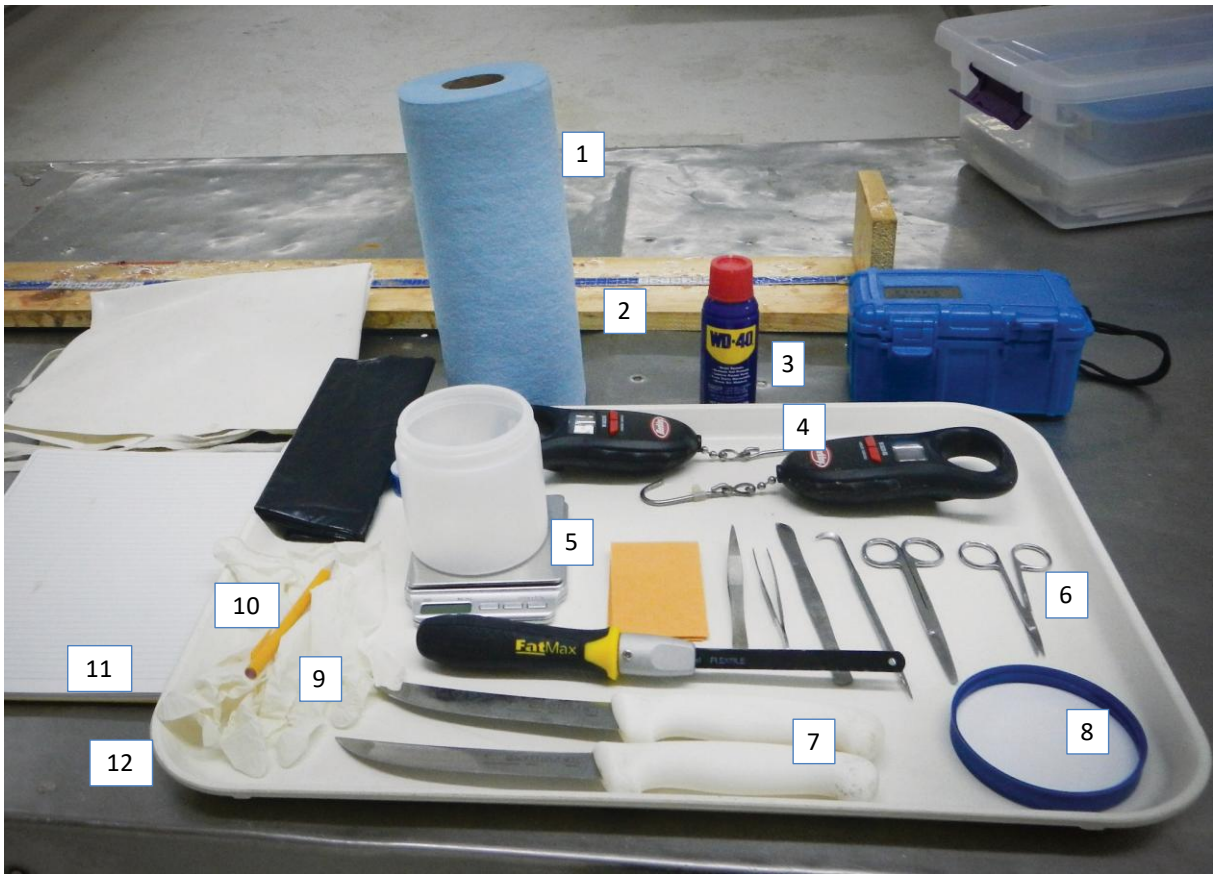


Photo: Mascareñas et al., 2013.

ANNEX 9-

SAMPLING FORMS

LENGTH DISTRIBUTION 1 cm -FISH (SURVEYS)

SURVEY		**HAUL**		**DATE**	

Species				
FAO Code				
Total Weight (g)				
Sample Weight (g)				
Initial Length				
Final Length				

0			0			0			0		
1			1			1			1		
2			2			2			2		
3			3			3			3		
4			4			4			4		
5			5			5			5		
6			6			6			6		
7			7			7			7		
8			8			8			8		
9			9			9			9		
0			0			0			0		
1			1			1			1		
2			2			2			2		
3			3			3			3		
4			4			4			4		
5			5			5			5		
6			6			6			6		
7			7			7			7		
8			8			8			8		
9			9			9			9		
0			0			0			0		
1			1			1			1		
2			2			2			2		
3			3			3			3		
4			4			4			4		
5			5			5			5		
6			6			6			6		
7			7			7			7		
8			8			8			8		
9			9			9			9		
0			0			0			0		
1			1			1			1		
2			2			2			2		
3			3			3			3		
4			4			4			4		
5			5			5			5		
6			6			6			6		
7			7			7			7		
8			8			8			8		
9			9			9			9		
0			0			0			0		
1			1			1			1		
2			2			2			2		
3			3			3			3		
4			4			4			4		
5			5			5			5		
6			6			6			6		
7			7			7			7		
8			8			8			8		
9			9			9			9		

LENGTH DISTRIBUTION 1 cm- FISH (LANDINGS)

LANDING SITE ______ **DATE** ______

Species				
FAO Code				
Total Weight (g)				
Sample Weight (g)				
Initial Length				
Final Length				

0			**0**			**0**			**0**		
1			**1**			**1**			**1**		
2			**2**			**2**			**2**		
3			**3**			**3**			**3**		
4			**4**			**4**			**4**		
5			**5**			**5**			**5**		
6			**6**			**6**			**6**		
7			**7**			**7**			**7**		
8			**8**			**8**			**8**		
9			**9**			**9**			**9**		
0			**0**			**0**			**0**		
1			**1**			**1**			**1**		
2			**2**			**2**			**2**		
3			**3**			**3**			**3**		
4			**4**			**4**			**4**		
5			**5**			**5**			**5**		
6			**6**			**6**			**6**		
7			**7**			**7**			**7**		
8			**8**			**8**			**8**		
9			**9**			**9**			**9**		
0			**0**			**0**			**0**		
1			**1**			**1**			**1**		
2			**2**			**2**			**2**		
3			**3**			**3**			**3**		
4			**4**			**4**			**4**		
5			**5**			**5**			**5**		
6			**6**			**6**			**6**		
7			**7**			**7**			**7**		
8			**8**			**8**			**8**		
9			**9**			**9**			**9**		
0			**0**			**0**			**0**		
1			**1**			**1**			**1**		
2			**2**			**2**			**2**		
3			**3**			**3**			**3**		
4			**4**			**4**			**4**		
5			**5**			**5**			**5**		
6			**6**			**6**			**6**		
7			**7**			**7**			**7**		
8			**8**			**8**			**8**		
9			**9**			**9**			**9**		
0			**0**			**0**			**0**		
1			**1**			**1**			**1**		
2			**2**			**2**			**2**		
3			**3**			**3**			**3**		
4			**4**			**4**			**4**		
5			**5**			**5**			**5**		
6			**6**			**6**			**6**		
7			**7**			**7**			**7**		
8			**8**			**8**			**8**		
9			**9**			**9**			**9**		

LENGTH DISTRIBUTION BY SEX AND 1/2 mm- *Penaeus notialis* **SURVEYS**

SURVEY : ..... **DATE: .....**

HAUL: ..... **SPECIES: .....*Penaeus notialis* .....** **CODE: .....SOP.....**

Total Weight: ..... g

Sample Weight: .....g

Total NUMBER:.....

MALES WEIGHT: ..... No.....	FEMALES WEIGHT: ..... No.....	INDETERMINED WEIGHT: ..... No.....
Inicial length:	Inicial length:	Inicial length:
Final length:	Final length:	Final length:

	MALE			FEMALE			INDETERMINED	
0			0			0		
0.5			0.5			0.5		
1			1			1		
1.5			1.5			1.5		
2			2			2		
2.5			2.5			2.5		
3			3			3		
3.5			3.5			3.5		
4			4			4		
4.5			4.5			4.5		
5			5			5		
5.5			5.5			5.5		
6			6			6		
6.5			6.5			6.5		
7			7			7		
7.5			7.5			7.5		
8			8			8		
8.5			8.5			8.5		
9			9			9		
9.5			9.5			9.5		
0			0			0		
0.5			0.5			0.5		
1			1			1		
1.5			1.5			1.5		
2			2			2		
2.5			2.5			2.5		
3			3			3		
3.5			3.5			3.5		
4			4			4		
4.5			4.5			4.5		
5			5			5		
5.5			5.5			5.5		
6			6			6		
6.5			6.5			6.5		
7			7			7		
7.5			7.5			7.5		
8			8			8		
8.5			8.5			8.5		
9			9			9		
9.5			9.5			9.5		
0			0			0		
0.5			0.5			0.5		
1			1			1		
1.5			1.5			1.5		
2			2			2		
2.5			2.5			2.5		
3			3			3		
3.5			3.5			3.5		
4			4			4		
4.5			4.5			4.5		
5			5			5		
6			6			6		
6.5			6.5			6.5		
7			7			7		
7.5			7.5			7.5		
8			8			8		
8.5			8.5			8.5		
9			9			9		
9.5			9.5			9.5		
0			0			0		

LENGTH DISTRIBUTION BY SEX AND 1/2 mm- *Penaeus notialis* **LANDINGS**

LANDING PLACE: ..... **DATE: .....**

SPECIES: .....*Penaeus notialis* ..... **CODE: .....SOP.....**

Total Weight: ..... g

Sample Weight: .....g

Total NUMBER:.....

MALES WEIGHT: ..... No.....
Inicial length:
Final length:

FEMALES WEIGHT: ..... No.....
Inicial length:
Final length:

INDETERM.WEIGHT: ..... No.....
Inicial length:
Final length:

	MALE			FEMALE			INDET.	
0			0			0		
0.5			0.5			0.5		
1			1			1		
1.5			1.5			1.5		
2			2			2		
2.5			2.5			2.5		
3			3			3		
3.5			3.5			3.5		
4			4			4		
4.5			4.5			4.5		
5			5			5		
5.5			5.5			5.5		
6			6			6		
6.5			6.5			6.5		
7			7			7		
7.5			7.5			7.5		
8			8			8		
8.5			8.5			8.5		
9			9			9		
9.5			9.5			9.5		
0			0			0		
0.5			0.5			0.5		
1			1			1		
1.5			1.5			1.5		
2			2			2		
2.5			2.5			2.5		
3			3			3		
3.5			3.5			3.5		
4			4			4		
4.5			4.5			4.5		
5			5			5		
5.5			5.5			5.5		
6			6			6		
6.5			6.5			6.5		
7			7			7		
7.5			7.5			7.5		
8			8			8		
8.5			8.5			8.5		
9			9			9		
9.5			9.5			9.5		
0			0			0		
0.5			0.5			0.5		
1			1			1		
1.5			1.5			1.5		
2			2			2		
2.5			2.5			2.5		
3			3			3		
3.5			3.5			3.5		
4			4			4		
4.5			4.5			4.5		
5			5			5		
6			6			6		
6.5			6.5			6.5		
7			7			7		
7.5			7.5			7.5		
8			8			8		
8.5			8.5			8.5		
9			9			9		
9.5			9.5			9.5		
0			0			0		

FISH- BIOLOGICAL SAMPLING **SURVEYS**

SURVEY: ..... **HAUL: .....** **DATE: .....** **SPECIES: .....** **FAO CODE: .....**

TOTAL WEIGHT (g): ..... **SAMPLE WEIGHT (g): .....**

N	CODE	Photo (Y-N)	Ext PAR (0-3)	TL (mm)	TW (g)	SEX	MAT (1-5)	Gonad W (g)	Int PAR (0-3)	Eviscer. W (g)	Ext PAR-gills (0-3)	GEN (Y/N)	OTO (Y/N)	OBSERVATIONS
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														
19														
20														
21														
22														
23														
24														
25														
26														
27														
28														
29														
30														
31														
32														
33														
34														
35														

FISH- BIOLOGICAL SAMPLING **LANDINGS**

LANDING PLACE: …… **DATE:** ..... **SPECIES:** ..... **FAO CODE:** ……

TOTAL WEIGHT (g): ..... **SAMPLE WEIGHT (g):** .....

N	CODE	Photo (Y-N)	Ext PAR (0-3)	TL (mm)	TW (g)	SEX	MAT (1-5)	Gonad W (g)	Int PAR (0-3)	Eviscer. W (g)	Ext PAR-gills (0-3)	GEN (Y/N)	OTO (Y/N)	OBSERVATIONS
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														
19														
20														
21														
22														
23														
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BIOLOGICAL SAMPLING ***Penaeus notialis*** **SURVEYS**

SURVEY: ..... **HAUL:** ..... **DATE:** ..... **SPECIES:** *Penaeus notialis*

TOTAL WEIGHT (g): ..... **SAMPLE WEIGHT (g):** ..... **FAO COD:** ....SOP

						FEMALE			MALE			
						W=			W=			
N	CODE	Photo (Y-N)	CarL (mm)	TW (g)	SEX	MAT (1-4)	Gonad W (g)	Fecund. (Y/N)	Joint Petasma (Y/N)	Sperm mass- coxas 5th pleip. (Y/N)	GEN (Y/N)	OBSERVATIONS
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BIOLOGICAL SAMPLING ***Penaeus notialis*** **LANDINGS**

LANDING PLACE: …… **DATE:** …… **SPECIES:** *Penaeus notialis*

TOTAL WEIGHT (g): …… **SAMPLE WEIGHT (g):** …… **FAO COD:** …. SOP

						FEMALE			MALE			
						W=			W=			
N	CODE	Photo (Y-N)	CarL (mm)	TW (g)	SEX	MAT (1-4)	Gonad W (g)	Fecund. (Y/N)	Joint Petasma (Y/N)	Sperm mass- coxas 5th pleip. (Y/N)	GEN (Y/N)	OBSERVATIONS
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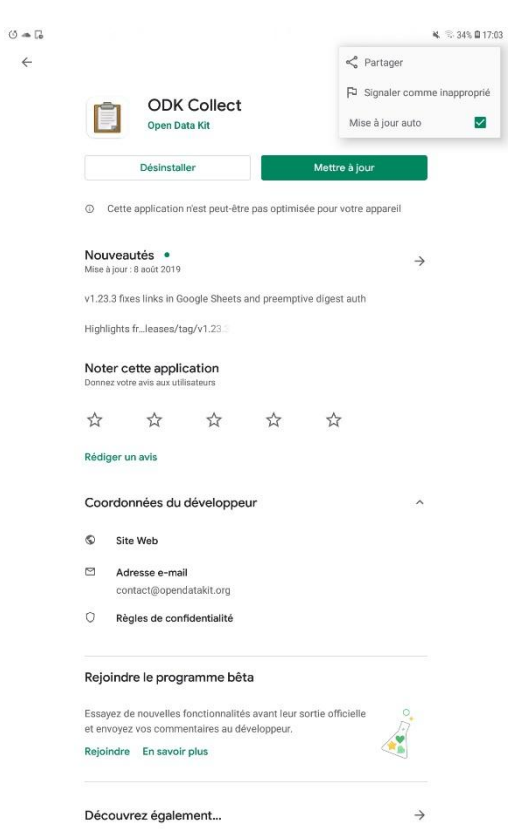
ANNEX 10-

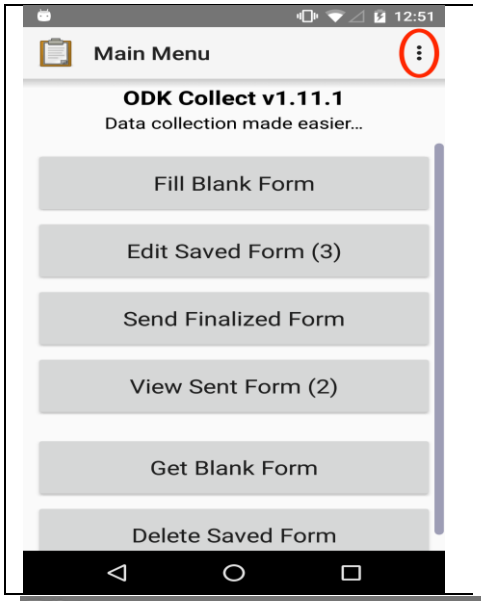
ANDROID ODK COLLECT INPUT APPLICATION

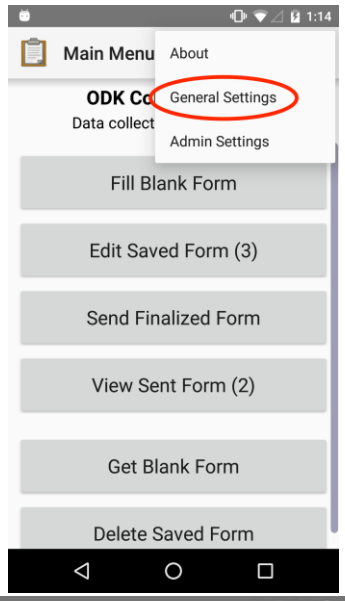
Jérôme Guitton

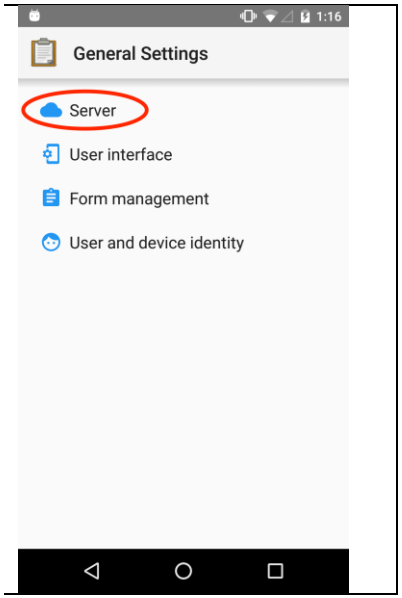
Agrocampus Ouest

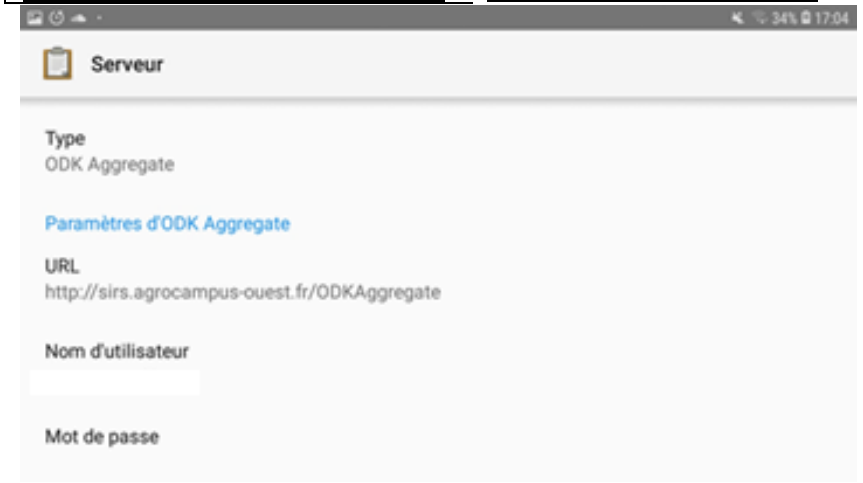
- The app is to be used with an Android tablet or phone. The ODKcollect app must be installed from the Play Store.
- Once installed, the application must be configured. In particular, we must indicate the server where to get the form templates and where the forms entered will be filed (http://sirs.agrocampus-ouest.fr/ODKAggregate/)



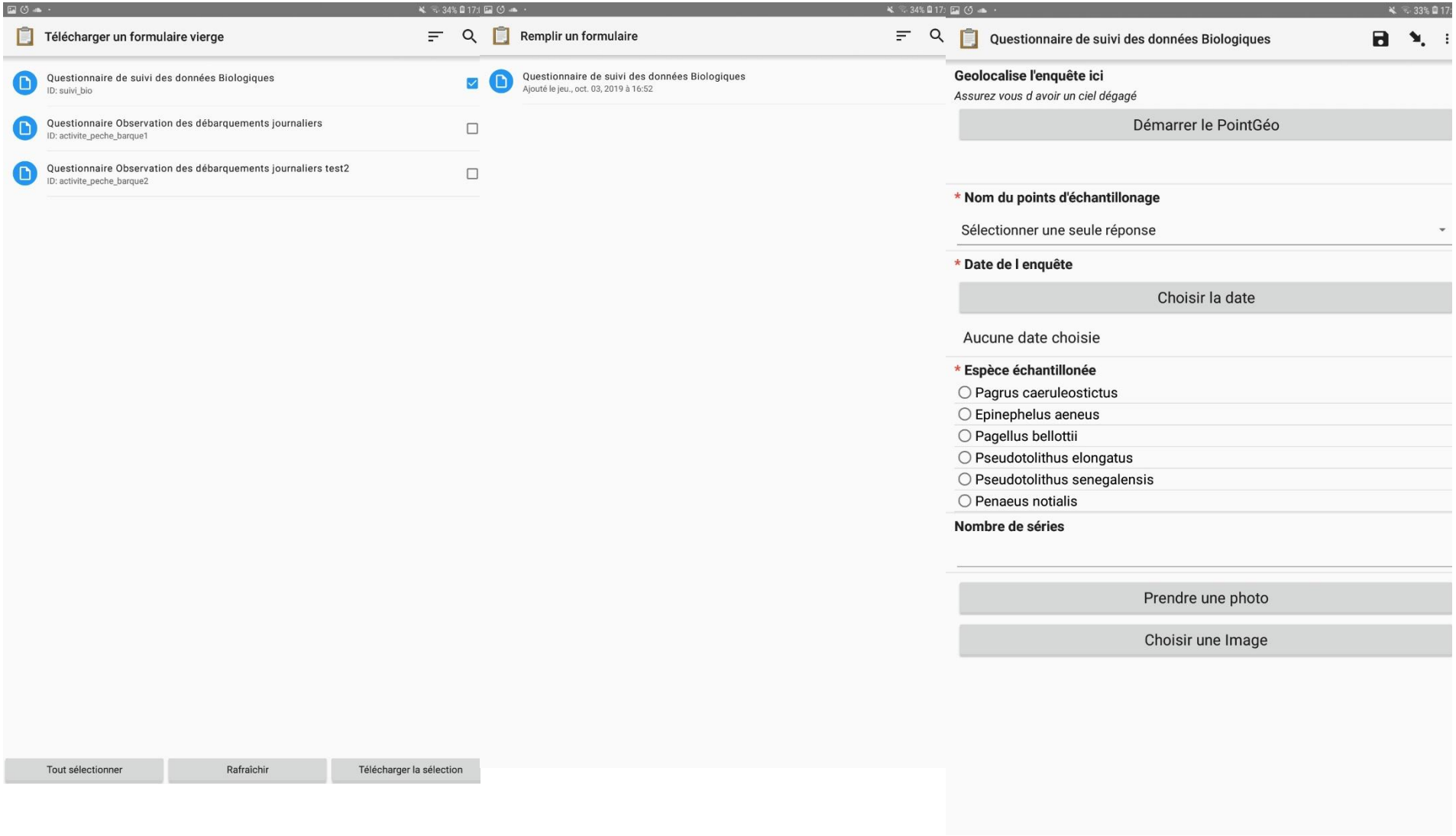








- Once configured, we should download the form **(Get Blank form)**:



- Finally, we can fill it out and send it to the server (**send Finalized form**).

Demonstration videos have been made available to users.