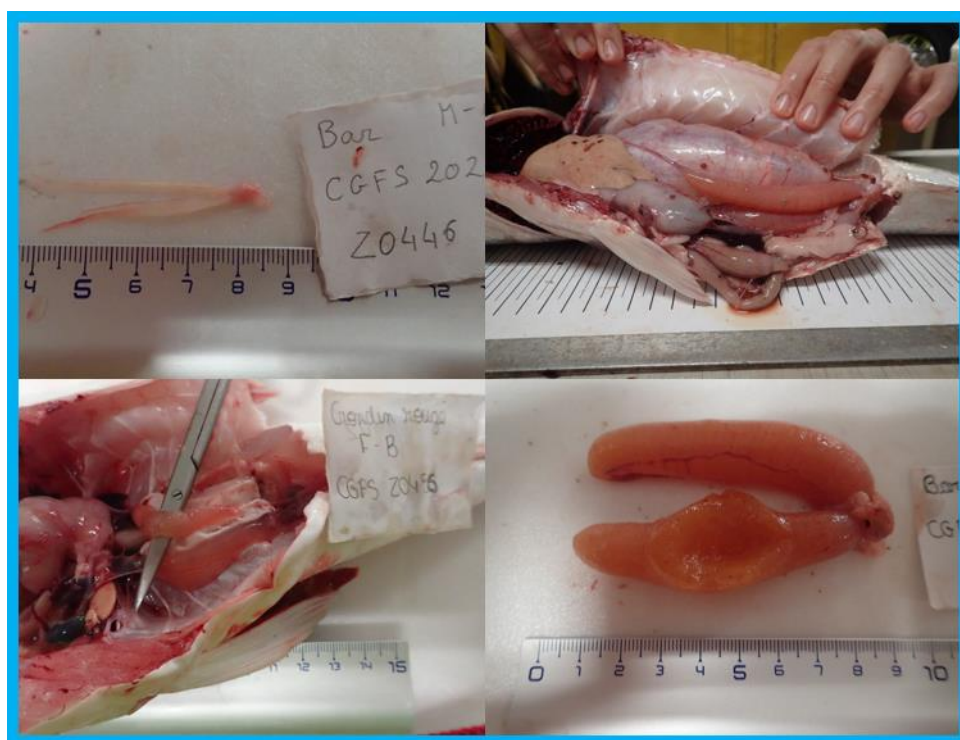


## Fish gonads photography protocol





## FISH'S GONADS PHOTOS PROTOCOL

The maturity phases of the gonads that are photographed is based on the **ICES universal scale**.




It is recommended to take several photos at different angles at each step of the photographing process in order to make sure to have at least one exploitable photo.

Each photo must be taken with a **graduated scale** and an **ID sheet** indicating the species, its sex and its maturity phase (+ geographical origin if necessary)

Immature individuals case :	Individuals with milt case:
Highlight the transparency of gonads with a metal object placed underneath 	Take a photo before opening the abdominal cavity 

### For all maturity phases :

- Open the fish abdominal cavity with a scalpel or a knife without damaging the gonads
- Properly clear out and separate the inner lining of the cavity, leaving the other organs around the gonads
- If needed, maintain the lining with pins
- Place the fish on a white background so as to have optimum lighting for gonads (- reflection possible)
- If needed, clean out blood, stomach contents and parasites
- Take photos of **both gonads into the abdominal cavity with the other organs around**
- Remove the other organs with a scalpel
- Take photos of **both gonads into the abdominal cavity without the other organs around**
- Remove both gonads from the fish with a scalpel without damaging them
- If possible, keep both gonads attached to each other, as found in the abdominal cavity
- Still on a white background, take photos of **both gonads outside the abdominal cavity**

		
<b>1</b> - Gonads inside the abdominal cavity with organs	<b>2</b> - Gonads inside the abdominal cavity without organs	<b>3</b> - Gonads outside the abdominal cavity

## 1 – Contextualisation

Many scientific campaigns to assess the abundance and the fish stock distribution are being carried out on European coasts in order to measure the ecological and economic impact of the fishery resource exploitation. It is in this context of annual scientific campaign that a maturity data acquisition is carried out for bony fish with commercial interest. This collection is undertaken partially during scientific prospecting missions and relies mostly on visual criteria (Coppin et al. 2019; Le Roy, Giraldo, et Coppin 2020). Maturity scales and protocols have been carried out in order to acquire as much data as possible. The scale established in 2012 by the International Council for the Exploration of the Sea (ICES) during a Workshop named « *Workshop for maturity staging chairs* » is one of them. This scale has been created with the idea of a universal scale, regardless of the species or sex of the individual and with the respect of the species biology and reproductive cycle (WKMATCH) (ICES 2012, 2018, 2020). It is divided in 4+2 phases: 4 main inevitable phases plus 2 optional phases. All of those phases represent the gonadic development phases and are common to every species of bony fish. Moreover, they are macroscopically observable and are the simplest to identify during campaigns. This universal scale is therefore, the most used scales by IFREMER (Coppin et al. 2019; Le Roy et al. 2020).

In order to help the identification of maturity phases, a tool was created and provided on board : the maturity identification guide (ifremer 2021). This guide is based on the ICES maturity scale and is composed of files with simple commentary and photos of the different aspect of the gonads for males and females. A total of 13 species/family of bony fish are represented: anchovy, sea bass, monkfish, sea bream, *Gadidae sp.*, red gurnard, herring, mackerel, hake, flat fish, red mullet, sprat and sardine. The files allows IFREMER scientists to have a tool to help visual identification of maturity phases. The guide was created from the initiative of François GARREN and Didier LE ROY mostly with their experience and the ICES scale. Their goals was to provide a simple and reliable tool to help the maturity identification. It is however the only tool available, and shows some imperfections. In fact, many phases either do not have photos to represent them or are of low quality, which does not represent all the gonads visual aspects. Therefore, it has been requested to improve the files with simple commentary and new photos to represent the reality as much as possible. This request falls within the project of harmonisation of maturity data acquisition methods for bony fish with commercial interest.

It is in this context that this protocol has been created, with the purpose to acquire as many photos as possible, with different types of maturity phases for each sex of species from French fisheries and to create a visual aspect colour chart for gonads to complete the files. This

protocol was established during the scientific campaign CGFS, EVHOE and IBTS of 2021 and 2022, and corresponds to the photography method of fish gonads, as well as the collected data use. It is important to clarify that the photos are taken depending on the availabilities and the needs of fish at every trawl hauls. Photos are taken for each species, each sex and each maturity phases.

## 2 - Protocol

### 2.1 Photography

In order to get the best gonads representation possible and to be able to choose the best view, it is recommended to take several images of them. In order to do that, it was decided to take three different types of shots:

- Photos of the gonads in the cavity with the other organs
- Photos of the gonads in the cavity without the other organs
- Photos of the gonads outside the cavity

The prior preparation of the camera is wildly recommended. It is a way to adapt to the brightness and to prevent reflects. Photos must be taken with white or black background to contrast with the fish and its gonads that may be translucent. The scale is done with ruler, and it is recommended to indicate the individual species, sex and maturity phase on a little piece of paper.

Gonads are located inside the abdominal cavity. It is then necessary to open it. The location of the cavity differs between round fish and flat fish. In both cases, it is recommended to start opening with a knife or a scalpel through the uro-genital opening. This opening is located on the ventral part of fish, which is underneath rounded fish and on the left/right side of flat fish. The goal is to open that abdominal part to reveal the abdominal cavity and the different organs that are in there. It is crucial to be cautious while opening, but also for every part of the protocol in which gonads are involved, in order to avoid cut through them or rip them out.

In order to take the two first types of photos, it is important to wildly open the cavity, to clear off the sides and maintain them to distinguish as much as possible the gonads and have the best lightening. To do that, it is possible to get help with pins and to do extra cuts with the scalpel to clear off the sides as much as possible (**Figure 1a**).

For the second type of photos, the other organs are either pushed aside or cut off from the cavity, depending on the species or the size of the gonads. It is also recommended to remove lining that attaches the gonads to the sides and the other organs. Finally, blood or stomach contents that could be in the cavity must be cleaned out. The photo can be taken when gonads differ from the other organs and the sides (**Figure 1b**).

Then, gonads must be taken out of the cavity. In order to do that, the lining must be completely removed with a knife or a scalpel. It is recommended to keep both gonads attached to each other, as originally found in the cavity (**Figure 1c**).



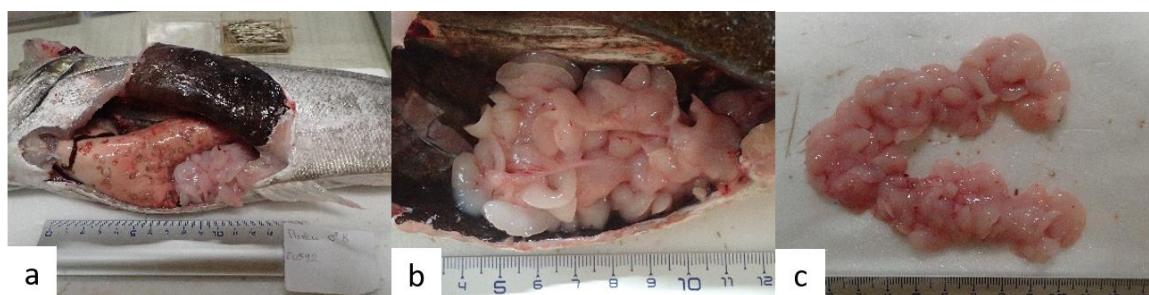


Figure 1. Different types of photography: a) gonads inside the fish with other organs; b) gonads inside the fish without organs; c) gonads outside the fish

In the case of the maturity phase corresponding to the immature phase A, it can be interesting to bring out to light the transparency of the gonad by putting a part of a knife blade or any other equipment underneath it (**Figure 2a**). In the same way, if it is a phase C male, it can be useful to bring out to light the “fluent” criteria (**Figure 2b**). Creating an opening on female gonads is also a way to show on the photos that there is non-hydrated or hydrated eggs (**Figure 2c**).



Figure 2. Different types of particular case photography: a) with knife blade; b) individuals with milt; c) open gonads

## 2.2 Data use

### 2.2.1 Database

A database with photos taken throughout this protocol has been established in order to complete and improve the maturity identification tool for fish with commercial interest. In the database, photos are sorted and categorized by files depending on the species, the sex and the maturity phase of the individuals. Moreover, species names are indicated in scientific names.

All the information about photos in the database are gathered in the Excel file “photo\_mat” in the “TOUT” sheet. Here are the information to list in the table for each photo:

- « Name » : Name of the photo ;
- « Type » : Type of the photo (INT = inside without organs, INT ORG = inside with organs, EXT = outside, EXT OUV = outside and open, FLUANT = fluent) ;
- « CommonName » : Vernacular name of the species or species group;
- « Species » : Scientific name of the species or species group ;

- « Phase » : Maturity phase ;
- « Campaign » : Campaign during which the photo was taken ;
- « Link » : Link to the photo ;
- « Commentary » : Commentary about the photo ;
- « Species2 » : Vernacular name of the species ;
- « CommonName2 » : Scientific name of the species ;
- « Date » : Date the photo was sorted.

It is important to note that for the “Species” data, few species are categorized by group. Those groups correspond to those used with the maturity identification guide. Here is the list of the different groups with their corresponding species:

Table 1. List of species group with their corresponding species

Group	Corresponding species
<i>Solea solea</i>	Sole
<i>Scophthlamus sp.</i>	Brill
	Turbot
<i>Lophius sp.</i>	Anglefish ( <i>L. piscatorius</i> )
	Anglefish ( <i>L. budegassa</i> )
<i>Flat fish sp.</i>	EVERY left flat fish
<i>Gadidae sp.</i>	EVERY gadidae

### 2.2.2 Maturity identification guide setup

The maturity phase identification guide corresponds to a two-sided sheet for each sex of each species of fish with commercial interest. At the top of the sheet is indicated vernacular and scientific names of species, and a table with the sex covered as well as photos of fish and their first maturity size. Then, for each maturity phase, simple commentaries are indicated as well as 2 photos of gonads. The guide is created with the RStudio software in html format.

The first maturity size is collected from the maturity data of the IFREMER fishery resources database established with the ICES scale. It corresponds to the range between quartiles of all the individuals in A and B phases with a size included between the minimum size from phase B and the maximum size from phase A. Data is then included in sheet 4 of the Excel file “matu\_scale”. Commentaries are also present in this file (sheet 1: French version; sheet 2: English version).

For the photography present in the guide, all the database photos are sorted according to their quality and their accuracy on the different gonads visual aspects. The selected photos are

then gathered in an Excel file "photo\_mat" sheet 2 and 3. These sheets are organised the same way as the "TOUT" one but has "NA" lines added for every species, sex and maturity phases that correspond to a picture that indicates if it is the case that there is no gonads photo. It also has a "Fiche" column added that corresponds to the information if the photo must be used or not for the guide (« yes », « no » or « na »).



