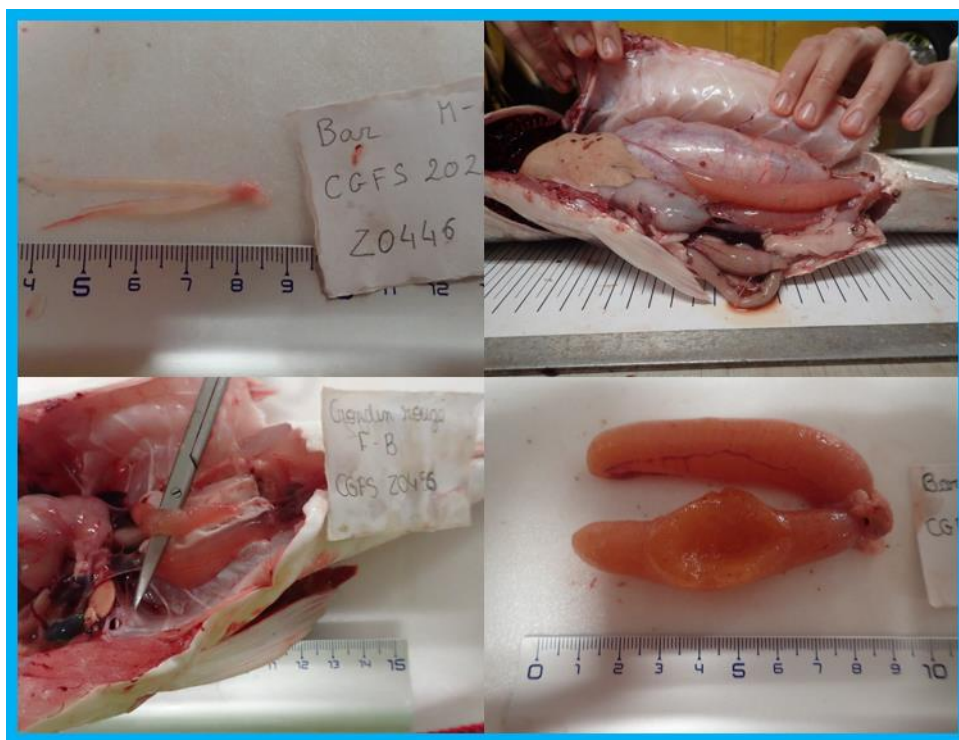


Fish gonads' photography protocol



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FISH GONADS' PHOTO PROTOCOL

The maturity phases used here is based on the **ICES WKASMSF scale**.

It is recommended to take several pictures at different angles at each step of the photographing process to ensure at least one exploitable photo.

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

Immature individuals :	Individuals with milt or roe:
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's 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 the gonads (for less glint, dry the gonads with paper towel)
- If possible, clean out blood, stomach contents and parasites
- Take photos of **both gonads in the abdominal cavity with the other organs around**
- Remove the other organs with a scalpel
- Take photos of **both gonads in the abdominal cavity without the other organs around**
- Remove both gonads from the fish with a scalpel without damaging or separating 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**

		
1 - Gonads inside the abdominal cavity with organs + ID tag	2 - Gonads inside the abdominal cavity without organs	3 - Gonads outside the abdominal cavity

1 – Context

Many scientific campaigns to assess abundances and fish stock distributions are being carried out in European coastal waters to measure the ecological and economic impacts of the fishery resource exploitation. In the context of annual scientific campaigns, maturity data acquisition is carried out for species of commercial interests. This data collection is partially undertaken during scientific prospecting missions and relies mostly on a visual estimation of sexual maturity (Coppin et al., 2019; Le Roy et al., 2020). A multitude of maturity scales and protocols have been established for maturity staging, leading to an overabundance of scales and staging methods that differ from country to institutions to species. 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 maturity scale has been created with the idea of being a universal scale, regardless of the species or sex of the individual, while respecting the species' biology and reproductive cycle (WKASMSF) (ICES, 2012, 2018, 2020). It is divided in 6 phases, with 3 of these categories with 2 possible subphases to take into consideration for batch spawners and species with long spawning cycles. Moreover, a visual estimation of the sexual maturity through the observation of the gonads with the naked eye is the simplest method. This universal scale is, therefore, the most used scale by IFREMER (Coppin et al., 2019; Le Roy et al., 2020).

In order to help with the identification of maturity phases, a tool was provided: the maturity identification guide (Garren & Le Roy, 2021). This guide based on the ICES 2018 maturity scale is a compilation of files with simple commentary and photos, describing the different aspects of males and females gonads for different species. 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. These summary sheets allow visual identification of maturity phases while on board. The creation of this guide stemmed from François GARREN and Didier LE ROY and their experience on data gathering during scientific campaigns and the use of ICES scales. Their goal was to provide a simple and reliable tool to help with maturity identification. It is for now, 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 convey a realistic representation of the gonads' visual aspects. Therefore, it has been requested to improve these sheets with simple commentary and new photos, for a more faithful representation of the reality out on the field. This request falls within the project for the harmonisation of maturity data acquisition methods for bony fish of commercial interest.

From this context, this protocol has been created with the purpose of acquiring as many photos as possible, for different types of maturity phases of each sex and species from French fisheries, and to set up colour chart to help with the visual colour identification of gonads. This protocol was established during the scientific campaigns CGFS, EVHOE and IBTS of 2021 and 2022, and ACCOBIOM in the Caribbean islands, and details the photography method used for fish gonads, as well as the collected data usage (Lazard et al., 2022; Le Roy et al., 2021). It is important to clarify that the photos are taken depending on the species available at every trawl hauls and the needs of the campaigns. Photos are taken for each species, each sex and each maturity phases.

2 - Protocol

2.1 Equipment list

The equipment listed below must be prepared before every fish gonads' photography. This list is not exhaustive but aims to standardize the content of the photos included in a database.

- **White background**, polystyrene plate type,
- **Camera** (no specific model) with charger,
- **Millimeter ruler**, preferably longer than the sample,
- **Identification paper** (ID tag),
- **Dissection equipment** (scissors, scalpel, scalpel blade, knife...),
- **Safety pins**,
- **Absorbent paper**.

During scientific campaigns, it is also important to think about keeping the photos that have been taken every day and saving them on a **hard disk** or an **SD card**.

2.2 Photography

In order to get the best gonad representation possible, with the best angle of view, it is recommended to take several pictures. For that, three different types of shots were taken:

- 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 of the cavity

Prior preparation of the camera and the picture area is strongly recommended, to take into account the area's lighting and avoid glints from overexposure. Photos must be taken with white or black background to contrast with the fish and its gonads that may be translucent. A ruler is used for scale, and it is recommended to indicate the individual's species, sex and maturity phase on a little piece of paper (ID tag) (**Figure 1 and 2**). The area and date of sampling may also be noted.

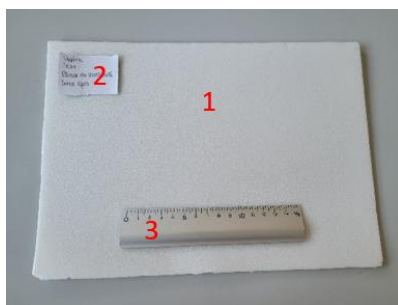


Figure 1. Fish gonads' photoshoot workbench: 1) White background, 2) ID tag, 3) ruler.

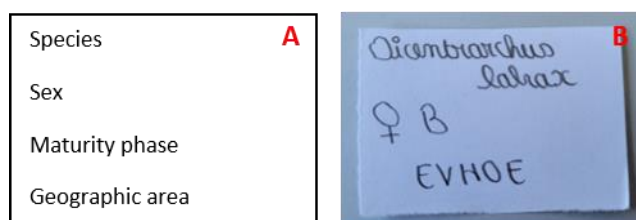


Figure 2. A) Main details to indicate in the ID tag; B) ID tag example.

Gonads are located inside the abdominal cavity, making it necessary to open it. The location within the cavity differs between round fish and flat fish. In both cases, it is recommended to start opening with small scissors 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 (**Figure 3**). The goal is to open the abdominal part and reveal the abdominal cavity and the different organs within. It is crucial to be cautious while opening at every step of the protocol in which gonads are involved, to avoid cutting through them or ripping them out.

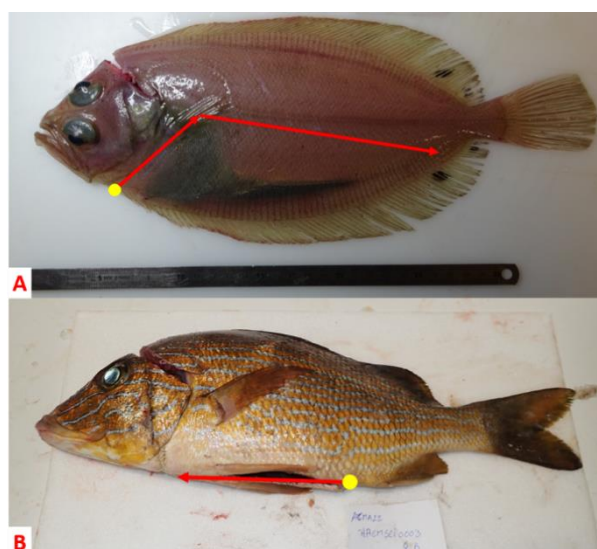


Figure 3. Abdominal cavity opening section for A) flat fish and B) round fish (red arrows) starting from the uro-genital opening (yellow circle).

In order to take the two first types of photos, it is important to wide open the cavity, clearing off the extra flesh to the sides and pinning them backwards to give a clear sight of the gonads, with the best lighting (**Figure 6a and b**). To do that, it is possible to use pins and cut out the extra flesh with the scalpel to clear off the sides as much as possible (**Figure 4 and 5**).

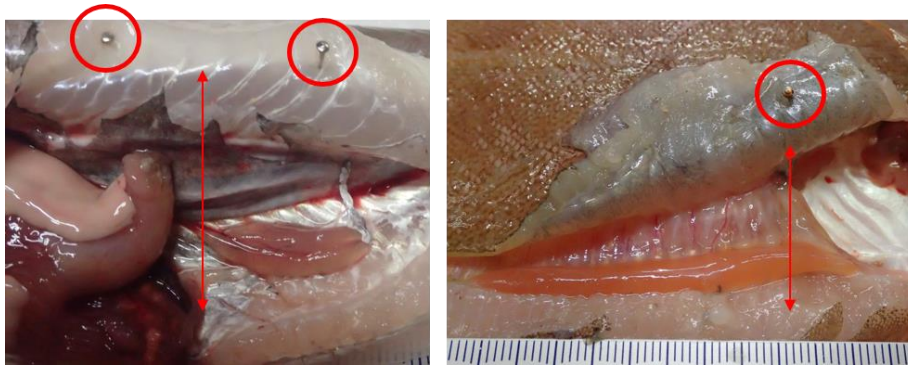


Figure 4. Examples of fish's abdominal cavity open with safety pins (red circles).



Figure 5. Possible additional notches (red areas) to help the opening of the fish's' abdominal cavity.

For the second type of photo, the other organs are either pushed aside or cut out from the cavity, depending on the species or the size of the gonads (**Figure 6b**). It is also recommended to remove the lining that attaches the gonads to the inner cavity and other organs. Finally, blood or stomach contents that could be in the cavity must be cleaned out. The photo can be taken when the gonads are cleared from the other organs but still within the abdominal cavity.

For the final picture, the gonads must be taken out of the cavity (**Figure 6c**). For that, the lining attaching the gonads to the inner cavity 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.

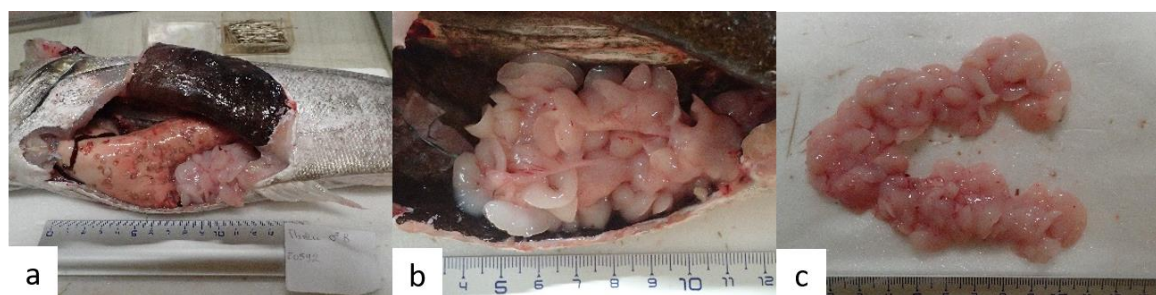


Figure 6. The 3 types of photography: a) gonads inside the fish with other organs; b) gonads inside the fish without organs; c) gonads outside the fish

When taking the picture of an A individual, it can be interesting to highlight the transparency of the gonad by putting the tip of a knife blade, or any other coloured equipment, underneath it (**Figure 7a**). In the same way, if it is a phase C individual (spawning), it can be useful to show the

“fluent” criteria (**Figure 7b**). For female gonads, cutting open the gonad is also a good way to show on the photos that there are non-hydrated or hydrated eggs (**Figure 7c**).



Figure 7. Different types of particular case photography: a) with knife blade; b) individuals with milt; c) open gonads

2.3 Data usage

A database with photos taken for this protocol, as well as an Excel file gathering all the details about those photos, have been established in order to complete and improve the maturity identification tool for fish of commercial interest. In the database, photos are sorted and categorized in folders depending on the species, the sex and the maturity phase of the individuals. Moreover, species are identify by their scientific names. Here is the path to each photos in the database :

Photo_MATURITE > Species scientific name > Sex > Maturity phase

Example : Photo_MATURITE > Dicentrarchus labrax > M > A

The photos in the database must be in “.jpg” format. No specific dimension is required.

This database can be found in the website xxx.

All the information regarding the individuals in the photos of the database are gathered in the Excel file “photo_mat” in the “ALL” sheet (**Figure 8**). Here are the details listed 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) ;
- **“sppeng”** : Vernacular name of the species or species group;
- **“Species”** : Scientific name of the species or species group;
- **“Sex”** : Sex of the fish;
- **“phase ID”** : Maturity phase (WKASMSF)
- **“Link”** : Link to the photo;
- **“spplatTRUE”** : Scientific name of the species;

- “**sppengTRUE**” : Vernacular name of the species;
- “**Date**” : Date the photo was sorted;
- “**Campaign**” : Campaign during which the photo was taken;
- “**Area**” : Geographical area (ICES or not) that correspond to the campaign;
- “**Commentary**” : Commentary about the photo.

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 and are associated to specific species (**Table 1**). Therefore, the real scientific and vernacular name of the species are indicated in the “sppengTRUE” and “spplatTRUE” columns.

Table 1. List of species group with their corresponding species name for species from European coastal waters

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 other flat fish
<i>Gadidae sp.</i>	EVERY gadidae

For each photo, all the details must be indicated, except for the link to the photo, which is automatically generated with the following formula (n = line number) (**Figure 8**):

=LIEN_HYPERTEXTE(« **(Your path to the database)** \Photo_MATURITE\« &Hn& »\« &En& »\« &Fn& »\« &An& »..JPG »)

Diagram illustrating the required data fields for each photo report in the Excel file, with labels pointing to specific columns:

- ID photo**: Points to column A (Name)
- Type**: Points to column B (Type)
- Sex**: Points to column E (Sex)
- Maturity phase**: Points to column F (Maturity phase)
- Link**: Points to column G (Link) - Note: The link in the table is highlighted in red.
- Geographical area**: Points to column L (ICES area)
- Scientific name**: Points to column D (Species2)
- Vernacular name**: Points to column C (sppeng)
- TRUE Species**: Points to column F (Maturity phase)
- Date**: Points to column J (Date)
- Campaign**: Points to column K (Campaign)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
	Name	Type	sppeng	Species2	Sex	Maturity phase	Link	spplatTRL	spp	Date	Campaign	ICES area	Color	Comm
1	P5250141	INT	Honeycomb co	Acanthostrac	F	A	C:\Users\ale\Acanthostrac Honey			13/06/2022	ACCOBIOM	Arc Antillais		
2	P5250142	INT	Honeycomb co	Acanthostrac	F	A	C:\Users\ale\Acanthostrac Honey			13/06/2022	ACCOBIOM	Arc Antillais		
3	P5250145	EXT	Honeycomb co	Acanthostrac	F	A	C:\Users\ale\Acanthostrac Honey			13/06/2022	ACCOBIOM	Arc Antillais		
4	P5250146	EXT	Honeycomb co	Acanthostrac	F	A	C:\Users\ale\Acanthostrac Honey			13/06/2022	ACCOBIOM	Arc Antillais		
5	P5250147	EXT	Honeycomb co	Acanthostrac	F	A	C:\Users\ale\Acanthostrac Honey			13/06/2022	ACCOBIOM	Arc Antillais		
6	P5250148	EXT	Honeycomb co	Acanthostrac	F	A	C:\Users\ale\Acanthostrac Honey			13/06/2022	ACCOBIOM	Arc Antillais		
7	P5250108	INT	Honeycomb co	Acanthostrac	F	C	C:\Users\ale\Acanthostrac Honey			13/06/2022	ACCOBIOM	Arc Antillais		

Figure 8. Details for each photo that must be reported (except the link in red) in the Excel file.

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