Système d'Informations Halieutiques

Action Paramètres biologiques

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Sample collection protocol for the extraction of female gonads in the megrim (*Lepidorhombus spp.*) for maturity staging through histology

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1.0	November 2019	Conception of the document
1.1	December 2019	Finalization of the document (pictures added)

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Context/Generalities/Objectives

This protocol was established during project MATO (MATurité Objective des poissons par l'histologie quantitative) for the evaluation of sexual maturity of exploited stock species, as well as to harmonize the sampling process of gonad extraction before the mounting between slide and slip of the gonad sections.

This document follows the work of different workshops that aimed to improve the compilation of data on sexual maturity. The WKMATCH (WorKshop for MATurity staging CHairs, 2012) defined a universal evaluation grid for sexual maturity staging of different species. During this workshop, two main recommendations were made, underlining the need to improve and complete the knowledge on sexual maturity, as well as to harmonize the practices used to determine these sexual phases.

This sampling protocol will be restricted to the female gonad of the megrim.

1. List of materials

- Dissection kit (scalpel, blades, surgical scissors, forceps)
- Dissection board
- Name tags for the tissue processing embedding cassettes (Annex1)
- Precision scale (>1 kg, precision 0.01 g)
- Petri dish (or small container of the same shape to hold the gonads)
- Pencil
- Permanent marker
- Tissue processing embedding cassettes
- Davidson solution
- Ethanol (70 %)
- Plastic laboratory bottles (2 L)
- Large plastic can (10 L)
- Refrigerator (4°C)
- Paper towels
- Duct tape
- Long forceps (35 cm)
- Latex gloves
- Measuring cylinder (1000 ml)

2. Sampling procedure on the field

2.1. Macroscopic parameters

This sampling procedure can be done by one operator, but it is preferable to have at least 3 readers for the visual estimation of the fish's sexual maturity.

The data to be collected on the fish are the following, and can be noted in the table from Annex 2 :

- **Fish_ID**
- **Fish code**
- **ICES division** (where the fish was caught)
- **Date** (that the fish was caught on)
- **Total length of the fish** (in cm, rounded down)
- **Ungutted weight** (in g, rounded down)
- **Otoliths_ID**
- **Visual maturity** (3 different readers if possible, using the WKMATCH scale)
- **Dorsal** and **Ventral gonad weight** (precision of 0.01g, rounded down)
- **Number of sections**

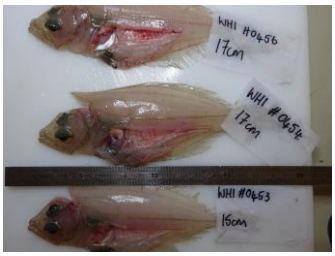
The **Fish_ID** is an identification number that will follow the fish for the entire study, and is composed of : Fish Code / Fished date / Sampling Zone / Total fish length / Ungutted fish weight / Sex / Sexual maturity.

For example, a female Lepidorhombus boscii caught on the 5th of November 2019 in the ICES division VIId , weighting 124 grams for 26 centimeters and estimated to be in the Ba maturity phase will be named : *LEPIBOS 051119 7D 26 124 F Ba*

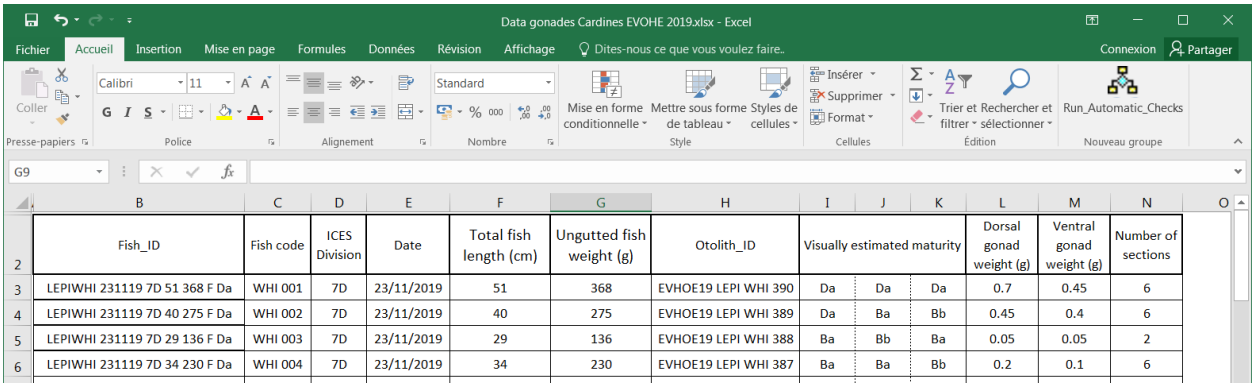
The **Fish Code** follows the species codification index (Rubin type) used during the EVOHE campaigns (Mahé & Poulard, 2005). This code is just to give the fish a smaller name during the data analysis.

For **Otolith_ID**, most sampling campaigns will not give an identification number to the fish, but they will give one to the otoliths when these are extracted. When this is the case, in order not to lose the information we have on the fish, it is very important to link this ID number to the fish and its gonads (*cf.* **section 2.2**).

Visual maturity should be estimated by 3 different operators. To minimize the chances of the readers influencing one another, they should each visually estimate the fish's sexual maturity without the presence of the other two. The sexual maturity should be estimated using the WKMATCH (ICES, 2012 ; 2018a) scale.



Number of sections is the number of sections cut within the gonad, and thus the number of gonad pieces put into embedding cassettes. If the entire gonad was extracted and put in one piece inside the embedding cassette, put a 1. If the gonad was cut into three sections, and thus put into three different cassettes, put a 3. If both gonads were sampled and cut into 3 sections, then you will have 3 sections for 2 gonads, meaning a total of 6 sections for one fish, so put a 6 in that column.



	B	C	D	E	F	G	H	I	J	K	L	M	N
2	Fish_ID	Fish code	ICES Division	Date	Total fish length (cm)	Ungutted fish weight (g)	Otolith_ID	Visually estimated maturity			Dorsal gonad weight (g)	Ventral gonad weight (g)	Number of sections
3	LEPIWHI 231119 7D 51 368 F Da	WHI 001	7D	23/11/2019	51	368	EVHOE19 LEPI WHI 390	Da	Da	Da	0.7	0.45	6
4	LEPIWHI 231119 7D 40 275 F Da	WHI 002	7D	23/11/2019	40	275	EVHOE19 LEPI WHI 389	Da	Ba	Bb	0.45	0.4	6
5	LEPIWHI 231119 7D 29 136 F Da	WHI 003	7D	23/11/2019	29	136	EVHOE19 LEPI WHI 388	Ba	Bb	Ba	0.05	0.05	2
6	LEPIWHI 231119 7D 34 230 F Da	WHI 004	7D	23/11/2019	34	230	EVHOE19 LEPI WHI 387	Ba	Ba	Bb	0.2	0.1	6

Figure 1 : Example of a filled in sampling sheet

2.2. Gonad extraction

Once the macroscopic parameters have been collected, place the megrim facing up. The shape of the gonad can be seen through transparency, to separate males from females. For this protocol, **only female gonads will be sampled**.

Figure 2 : Photographe of a female Lepidorhombus boscii (left) and a female Lepidorhombus whiffiagonis (right)

Position the fish so to have the ovary close to you and **make a cut, about 1 cm under the lateral line, from behind the operculum (gill cover) all the way to the caudal fin**. This will allow the skin to be pulled back and uncover the ovary without damaging it. Cutting the skin of the fish will use the blade of the scalpel very fast. Don't hesitate to change the blade regularly (every 3 to 4 fish). Moreover, a second blade can be used just for the sectioning of the gonads. This will ensure that a clean cut is made and minimize the risks of ripping the gonad with a dulled blade.

Figure 3 : Photographe of a female Lepidorhombus whiffiagonis with the dorsal ovary uncovered

Both ovaries must be extracted. The **dorsal gonad (coded D)** can be extracted when the megrim is facing upwards. The **ventral gonad (coded V)** can be extracted when the megrim is facing downwards.

Figure 4 : Photographe of a female Lepidorhombus boscii with the ventral ovary uncovered

Once the ovary is uncovered, the sexual maturity phase of the fish can be visually estimated by three different operators. After this, the ovary is removed and weighted with a precision scale (in g., with a precision of 0.01g.). To not smear the scale, the gonad can be put in a petri dish. Do not forget to **wipe the petri dish between each use**, with a paper towel, and to **tare the scale with the empty petri dish on it before weighing the next gonad**.

Figure 5 : Lepidorhombus boscii ventral ovary in a petri dish on a scale, being weighted

Once the gonads have been weighted, they must be placed inside tissue processing embedding cassettes. There can only be **one** gonad, or gonad section, **per cassette**.

- **If the ovary length is smaller than 3 cm** : place the ovary flat down into a tissue processing embedding cassette, with a name tag. On this name tag should figure the Fish_ID followed by V if this is the ventral gonad, or by D if this is the dorsal gonad. *Example : LEPIBOS 051119 7D 26 124 F Ba D*

- **If the ovary length is longer than 3 cm** : cut a section of 1 cm in the anterior part of the ovary, a section of 1 cm in the median part of the ovary, and a section of 1 cm in the anterior part of the ovary. Once you have the 3*1 cm sections cut out from your ovary, place each section into separate cassettes with their corresponding nametags. The name tags are the Fish_ID followed by the letters V (for the ventral gonad) or D (for the dorsal gonad), and the number : 1 (for the anterior section), 2 (for the median section) or 3 (for the posterior section). *Example : LEPIBOS 051119 7D 26 124 F Ba V3*

Figure 6 : From left to right, cutting process for ovaries of 3cm or more. Each section is placed into its own cassette with its unique identification tag

Once each sampling has been put into individual cassettes, place them into a plastic laboratory bottle (2L). One bottle can hold around 70 cassettes. Topping the bottle with the cassettes is not advised since the cassettes must then be submerged in a Davidson solution (Annex 3).

Davidson being a dangerous solution, all handling of this solution must be done under a fume hood, while wearing gloves. Once the cassettes are in the bottle, under a fume hood use a measuring cylinder to pour Davisdon into the bottle until the cassettes are completely submerged. Try not to go over the 1.8L mark, so as to avoid spillage when handling the bottle. Close the bottle, identify it (date, project name, species) and stock it at a temperature of 4°C for 24 to 48 hours.

After 24 to 48 hours, bring the bottle back under the fume hood and, using gloves and long forceps, remove the cassettes from the Davidson solution and submerge them into a new plastic bottle containing ethanol (70%). Inside the alcohol, the samples can be stocked several weeks if needed.

During this transfer from Davison to ethanol, it is strongly advised to trim the edges of the sectioned ovaries, so as to get a straight rim. Use color coded cassettes so as to know which ones contain sectioned samples.

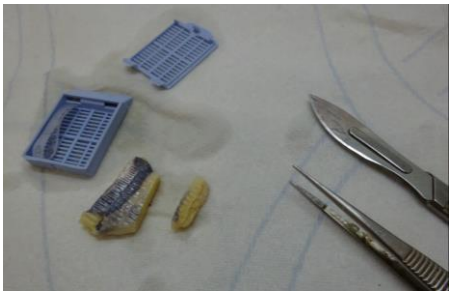
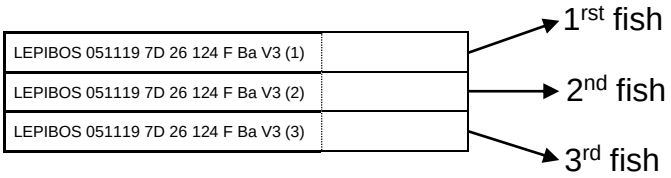


Figure 7 : Section of an ovary that was cut at the edge to make a straight rim

2.3. Nametag format

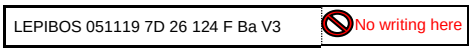
For the **nametags inside the cassettes** (Annex 1), if the Fish_ID is not available when the gonads are extracted, use the Otolith_ID followed by the letters V (for the ventral gonad) or D (for the dorsal gonad). If sections were made, add the number : 1 (for the anterior section), 2 (for the median section) or 3 (for the posterior section).

The identification (Fish_ID or Otolith_ID) on each nametag must be unique. If ever a Fish_ID or Otolith_ID are identical for 2 different fish, or more, add (#) at the end of the nametag.



The tags must **not be more than 5 mm high**, so as to able to close the cassette when the tag is laid upright.

Leave an **unwritten section of at least 1 cm** at the end of the tag. That part of the nametag will be imbedded into the paraffin, making any written information on that part unreadable.



Use “normal” paper. Any other kind of paper (like plastic coated paper) will deteriorate when put in the alcohol solutions.

If the nametags are written by hand on the field, **use a pencil** (grease pencil if possible). Any writings in marker, ballpoint pen, felt pen or any other kind of pen, will be erased when put into the ethanol. The ink used in printing machines are alcohol resistant, meaning that the nametags can be printed beforehand.

2.4. Sampling plan

This sampling plan was set up without any reliable data and is thus very empirical. It was based on previous works done on the sole (Solea *solea*) and the plaice (*Pleuronectes platessa*).

For each species, extract both gonads of :

- 5 females with a total length of 20 cm or lower
- 10 females with a total length of]20 cm; 25 cm]
- 5 females with a total length of]25cm;30 cm]
- 5 females with a total length of more than 30 cm

References

ICES, 2018a. **Report of the Workshop for Advancing Sexual Maturity Staging in Fish (WKASMSF)** (No. 38). International Council for the Exploration of the Sea, Copenhagen, Denmark. ICES CM/EOSG: 38. 75 pp.

ICES, 2018b. **Working Group on Biological Parameters (WGBIOP)** (Workshop). International Council for the Exploration of the Sea, Ghent, Belgium. ICES CM 2018/EOSG:07. 186pp.

ICES, 2012. **Report of the Workshop for maturity staging chairs (WKMATCH)** (Workshop). International Council for the Exploration of the Sea, Split, Croatia. ICES CM 2012/ACOM:58. 57 pp.

Mahe Jean-Claude, Poulard Jean-Charles (2005). **Manuel des protocoles de campagne halieutique. Campagnes EVHOE (EValuation des ressources Halieutiques de l'Ouest Europe)**. DSTH/LBP/05-xxx. https://archimer.ifremer.fr/doc/00036/14707/

Annex 1 : Name tag format to put inside the tissue processing embedding cassettes

Annex 2 : Table to fill for the data collection process

Fish_ID	Fish code	ICES Division	Date	Total fish length (cm)	Ungutted fish weight (g)	Otolith_ID	Visually estimated maturity			Dorsal gonad weight (g)	Ventral gonad weight (g)	Number of sections

Annex 3 : Protocol for the Davidson solution for tissu fixation

Davidson being a dangerous solution, all manipulations of these products must be done under a fume hood, while wearing gloves.

Using the measuring cylinder, add the different compounds in the following order:

- 400 mL of glycerol (1 volume)
- 800 mL of formol at 37% (2 volumes)
- 1200 mL of ethanol 95% (3 volumes)
- 1200 mL of filtered sea water or water from the tap (3 volumes)

This solution must be kept at a temperature of 4°C. Before use, add 360 mL (1 volume) of acetic acid 10%.