

Working Document 1

Protocol data collection WKMSSPDF 2012 (Workshop on Maturity Staging of Sole, Plaice, Dab and Flounder)

Version 2.0, April 2010

Contents

Protocol Data collection WKMSSPDF2.....	Error! Bookmark not defined.
1 Background.....	3
2 Data collection.....	5
2.1 Pictures.....	5
2.1.1 Technical details.....	5
2.1.2 Additional information.....	7
2.2 Histological samples	8
2.2.1 Technical details.....	8
2.2.2 Additional information.....	8
2.3 Details on fish sampled.....	10
3 Workshop and deadlines.....	11
References.....	11
Annex 1.....	12
Annex 2.....	13
Annex 3.....	13
Annex 4.....	14

1 Background

Sexual maturity stage is an important biological parameter used to estimate maturity ogives, spawning stock biomass, definition of spawning season and monitoring of changes in the spawning cycle. Maturity stage data are collected by various institutes using different macroscopic maturity scales or criteria for the same flatfish species. The variety of criteria may lead to bias in fisheries stock assessments or other biological analysis. In order to check for coherency of the criteria in the maturity scales and consistency of experts estimating maturity stages, a workshop will be held in 2012 on the sexual maturity staging of Northeastern Atlantic sole, plaice, dab and flounder.

During the workshop both pictures and fresh gonads will be examined to check for coherency between the participants. To check the relevance of the criteria used in the maturity scales histological samples will be taken from the gonads to identify the true maturity stage.

Both technicians and scientists working on the sexual maturity staging of sole, plaice, dab and flounder are invited to participate in the workshop.

This workshop is proposed by the ICES Workshop on Sexual Maturity Staging of Sole, Plaice, Dab and Flounder (WKMSSPDF) in 2010. Outcomes from this Workshop will be of interest to all ICES Working and Study Groups related to sole, plaice, dab and flounder, namely WGNSSK, WGBFAS, WGSSDS and WGNSDS, as well as to survey groups like the IBTSWG and WGBEAM. There is also a direct link with the EU DCR.

Proposed Term of References (TORs):

- a) Report on the use of the 2010 proposed common scale;
- b) Check the description of the characteristics of the stages of the 2010 scale;
- c) Calibrate staging of sole, plaice, dab and flounder using fresh fish, following the pattern of trial-discussion-retrial;
- d) Calibrate staging of sole, plaice, dab and flounder using photographs, following the pattern of trial-discussion-retrial;
- e) Validate macroscopic maturity determination with histological analysis.

The expectation of TOR a) has the goal of measuring the usefulness of the new 2010 maturity scales.

TOR b) to validate the criteria and descriptions to classify maturity stages of the new 2010 scales.

TOR c and d) calibrate maturity staging between the different laboratories.

TOR e) validate with histological analysis the macroscopic maturity stage, mainly the resting stages that are incorrectly classified as immature.

It is recommended that the Workshop be organised in January 2012. Participating institutes will be able to test the new scale and collect samples during 2010 and 2011.

2 Data collection

For the exchange and the workshop four different data sources will be used:

1. Pictures (digitally available)
2. Histological samples
3. Details on fish sampled
4. Fresh material (available at workshop)

In the following sections the protocol for data collection for pictures and histological samples and the details on the fish sampled is described.

2.1 Pictures

2.1.1 Technical details

To be able to compare all information, it is necessary to collect data conform a standard protocol.

The bullet points describe the essential information which has to be visible on the picture.

Guidelines for the pictures to be taken (section 8.2 in ICES, 2010).

When staging from pictures, it is necessary to standardise the way the pictures are taken. There have to be stringent procedures even down to equipment and/or settings used.

- Pictures have to be taken on fresh fish.
- Add at least sampling time, area, unique sampling number, fish length and species in the picture.
- Take care that the samples should be clean/tidy, preferable without intestines.
- Take at least six pictures:
 1. Dorsal side: overview of the fish on a measuring board, with the gonads visible in the fish. The ability to look at the whole fish with the gonad intact is vital to get the ratio of gonad to body length.

Label containing information on:

- Date
- Fish number
- Measuring board on the background
- Species (SOL, PLE, DAB, FLE)
- 2. Dorsal side: detail of picture 1, zoomed in on the gonads. Show the pressure characteristic on the picture to see if fish is running.

Label containing:

- Fish number
 - Species (SOL, PLE, DAB, FLE)
3. Ventral side: overview of the fish on a measuring board, with the gonads visible in the fish. The ability to look at the whole fish with the gonad intact is vital to get the ratio of gonad to body length.

Label containing information on:

- Date
 - Fish number
 - Measuring board on the background
 - Species (SOL, PLE, DAB, FLE)
4. Ventral side: detail of picture 3, zoomed in on the gonads. Show the pressure characteristic on the picture to see if fish is running.

Label containing:

- Fish number
 - Species (SOL, PLE, DAB, FLE)
5. Picture of gonads outside the fish, placed on a measuring board, allowing to view the gonad in more detail, blood vessels etc.

Label containing:

- Fish number
 - Species (SOL, PLE, DAB, FLE)
6. Picture of longitudinally cut gonad.

Label containing:

- Fish number
- Species (SOL, PLE, DAB, FLE)

Example: Annex 1 shows the do's and don'ts for the pictures.

Filenames:

Files have to be stored as *.jpg format, in a resolution as high as possible.

Filenames have to be composed as countryyear_species_fishnumber_number:

- Country codes as used in ICES databases
- Year in 4 digits
- Species codes SOL for sole, PLE for European plaice, DAB for dab, FLE for flounder
- Fishnumber: unique number for the fish in the year
- Number: referring to number 1 to 6 in the above protocol

Example:

Dutch plaice, fish number 142, dorsal overview: NED2008_ple_142_1.jpg

Dutch plaice, fish number 142, ventral detail: NED2008_ple_142_4.jpg

Belgian sole, fish number 101, ventral overview: BEL2008_sol_101_3.jpg

Belgian sole, fish number 101, gonad outside fish: BEL2008_sol_101_5.jpg

For each species, 2 fish per species per 10 cm class per sex per month should be collected. If it is not possible to collect data per month, please collect 5 fish per species per 10 cm class per sex per quarter.

Cm classes: 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, etc.

2.1.2 Additional information

In an *.xls file. The template is similar to the template used for WKMSSPDF2010 and will be available on the new sharepoint.

Variable	Units	Digits	Remarks
Country			ICES country code
Species			SOL (sole), PLE (European plaice), DAB (dab), FLE (flounder)
Year		4 digits	
Month			
Day			
Time		GMT	
Latitude	decimal	2	like 53.95
Longitude	decimal	2	like 3.75; W from 0°: -3.75
sample_number			
fish number			
picture name/histological_sample_number			
length	cm	1	total length
weight	g	0	total weight
gender			m=male, f=female
maturity scale			WKMSSPDF2010

maturity	as defined in the common maturity scales proposed by WKMSSPDF2010 (see ICES, 2010)		
maturity_stager	initials of the person staging the maturity		
gonad_weight	g	2	

2.2 Histological samples

2.2.1 Technical details

For histological samples one whole lobe of the gonad has to be put in 3.6% buffered formaldehyde solution (see Annex 2 for recipe). This should be done as soon as possible after catching the fish, within a maximum of 24 hours. Preferably the samples should be collected throughout the year. The protocol is as following:

1. weigh the fish (g)
2. measure the fish (to the mm below)
3. open the fish carefully, not to damage the gonads
4. stage the fish using your institute's maturity scale
5. take out the gonads
6. weigh the gonads (both lobes) (g, 2 digits)
7. put one whole lobe of the gonads and store in a jar with 3.6% formaldehyde, take care the gonad is completely covered in the fluid
8. put a note in the jar containing year, month, day, country, sample number, species, fish number

For each species, 2 fish per species per 10 cm class per sex per quarter should be collected.

Cm classes: 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, etc.

Putting gonads into cassettes and in 70% ethanol.

In the lab the gonads will be embedded, sectioned and stained for later determination of the maturity stage. If your lab is able to create histological slides, see Annex 3 for the protocol on sectioning of the gonads and Annex 4 for the description and maturity staging. If your lab is not able to create histological slides, please contact: cindy.vandamme@wur.nl

2.2.2 Additional information

In the jar:

1. Year

2. Month
3. Day
4. Sample number
5. Country
6. Species
7. Fish number

In an *.xls file. The template is similar to the template used for WKMSSPDF2010 and will be available on the new sharepoint.

Variable	Units	Digits	Remarks
Country			ICES country code
Species			SOL (sole), PLE (European plaice), DAB (dab), FLE (flounder)
Year		4 digits	
Month			
Day			
Time		GMT	
Latitude	decimal	2	like 53.95
Longitude	decimal	2	like 3.75; W from 0°: -3.75
sample_number			
fish number			
picture name/histological_sample_number			
length	cm	1	total length
weight	g	0	total weight
gender			m=male, f=female
maturity scale			WKMSSPDF2010
maturity			as defined in the common maturity scales proposed by WKMSSPDF2010 (see ICES, 2010)
maturity_stager			initials of the person staging the maturity
gonad_weight	g	2	

2.3 Details on fish sampled

An Excel template is available to store all additional data in. The template is similar to the template used for WKMSSPDF2010 and will be available on the new sharepoint. The template consists of five worksheets: (1) readme, with all the variables and their units listed (2) sample information, (3) fish information, (4) maturity_fresh, (5) maturity_hist. If you do not do histological stageing, leave the worksheet blank. Always fill in the sample information and the fish information. Fill in the sheets completely.

If you send any pictures, please send the last version of the *.xls file with the pictures.

The file should be sent to: ingeborg.deboois@wur.nl

3 Workshop and deadlines

In 2011 an exchange of pictures will take place before the actual workshop. Experts will be asked to stage the fish from the screen and the results will be compared. Meanwhile, the histological data will be analysed. A comparison will be made between the maturity staged in the field and the histological maturity.

From 9-13 January 2012 a workshop will be held in Oostende, Belgium. The results of the exchange will be presented as well as the results on the comparison of histological slides and in situ staging. During the workshop, for sole, plaice, dab and flounder fresh material will be available for staging by experts.

Data can be uploaded to the sharepoint when operational. Please put your first data as soon as possible. Pictures can be put on the sharepoint, in Data in the folder with your country name. Please keep a version of the *.xls file with it.

We would like to receive the pictures as soon as possible, we prefer having some batches instead of receiving everything as one bunch. So please, update the picture list and the Excel file regularly.

References

ICES. 2010. Report of the Workshop on Sexual Maturity Staging of sole, plaice, dab and flounder (WKMSSPDF), 22-26 February 2010, Ijmuiden, The Netherlands. ICES CM 2010/ACOM:50

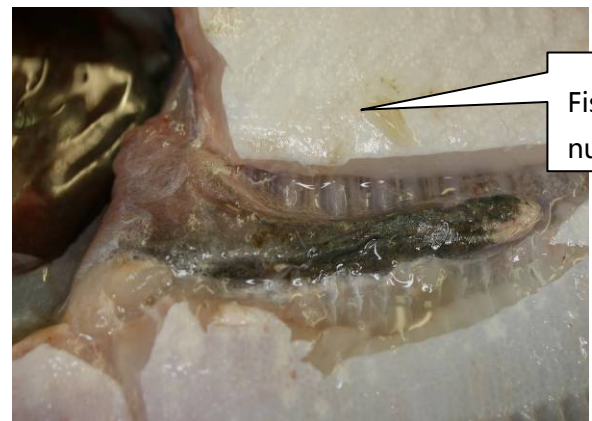
Annex 1

Examples of pictures

DO



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Annex 2

Recipe for 3.6% formaldehyde buffered with sodium phosphate. The recipe is similar to the one used for WKMSSPDF2010 and will be available on the new sharepoint.

An Excel file is available to calculate the amounts needed of the different chemicals. When the sharepoint is not yet operational, the sheet can be asked for at cindy.vandamme@wur.nl.

3.6% buffered formaldehyde for histological samples

970 ml 37% formaldehyde

81.8 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$

Add together and stir until dissolved completely

39.9 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$

Add the $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ to the solution and stir until dissolved completely

9030 ml purified water

Add the purified water to make 10 liters of solution and stir

Annex 3

Embedding, sectioning and staining

Preparing resin blocks

Use the two 5 mm sections in the cassettes, following these steps :

Step	Infiltration solution	Duration	Process tempera-
1	90% ethanol	2 hours	Room temperature
2	Pour out the liquid and add fresh 90% ethanol	1 hour	Room temperature
3	90% ethanol + Technovit 7100 (1:1 ratio) prepared by diluting Technovit 7100 (from used in	2 hours or overnight	Store cool (+5°C) after
4	Replace the liquid with Technovit 7100 (from step 5)	2-3 days	Store cool (+5°C) after
5	Replace the liquid with freshly prepared Technovit 7100	1 day	Store cool (+5°C) after
6	Transfer the sections from the cassettes to the moulds. Store tissue with catalyzed resin in moulds	2-3 hours or overnight	-6°C
7	Polymerise by adding Technovit 7100: hardener (15:1) in the freezer	2 hours	-6°C

8	Leave overnight	overnight	Room temperature
9	Block up using Technovit 3040.	15 minutes	Room temperature

Store the blocks in a box containing 70% glycerol.

Disposal of waste resin (in the fume cupboard)

After step 3 the 1:1 resin mix should be put in an aluminium tray and left in the fume cupboard over a few days to allow the EMS to evaporate from the resin. Use about 1 g hardener to 100g resin to polymerise and wrap the block in a poly bag for disposal. Caution the reaction is exothermic and potentially hazardous if too much hardener is added.

Sectioning the blocks

Use a microtome to cut 5 µm sections and dry at 100°C.

Staining the sections

Recipe 2 % Toluidine blue

2 % Toluidine blue and 1 % Sodium tetraborat (Borax). The borax is dissolved in the distilled water and then the dye added under constant stirring. Filter the solution before use.

For individual slides: Cover the section with a few drops of 2 % Toluidine blue and pour the excess back in the bottle and rinse the section with hot (60°C) tap water for 20 seconds. Dry on a 60°C hot plate. Cover the section with a cover slip using two drops of mountex.

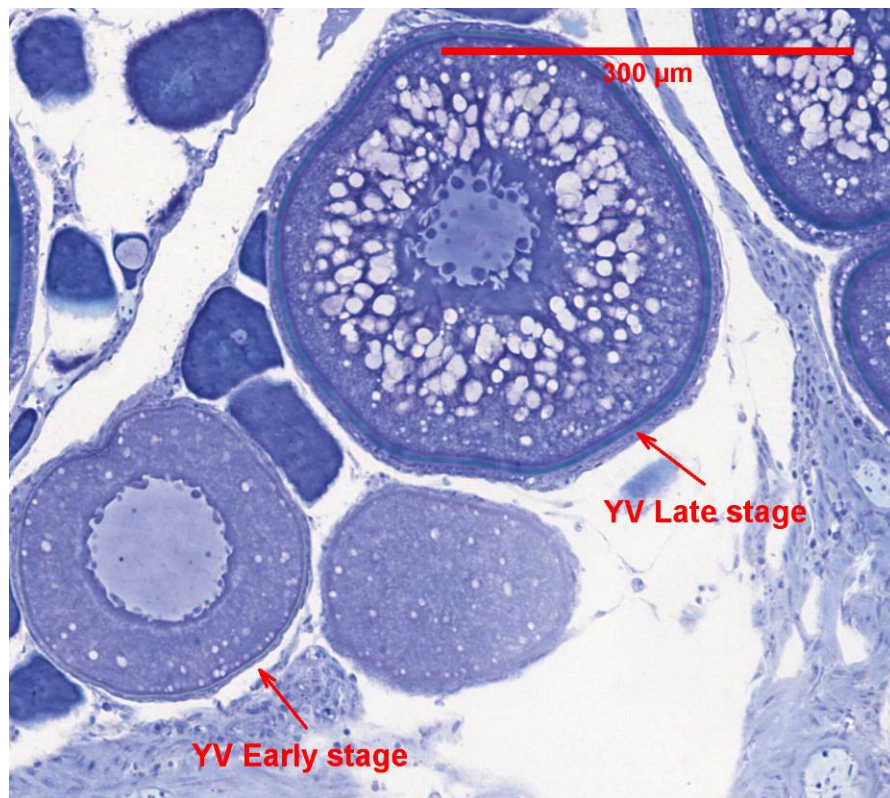
Annex 4

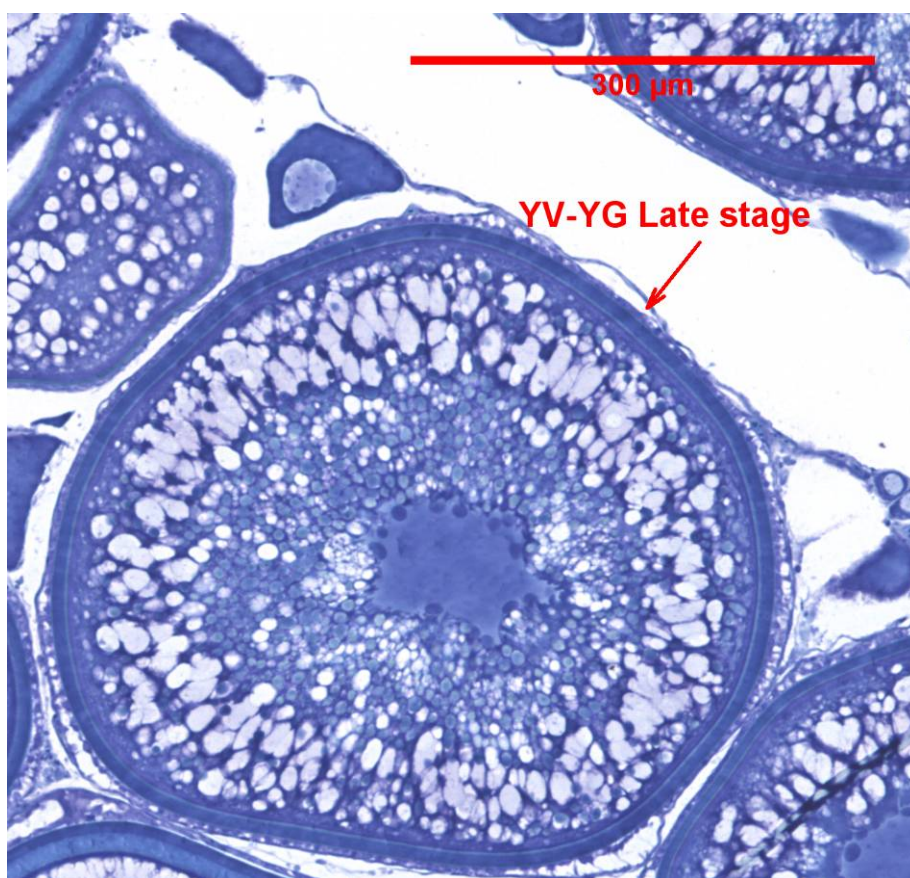
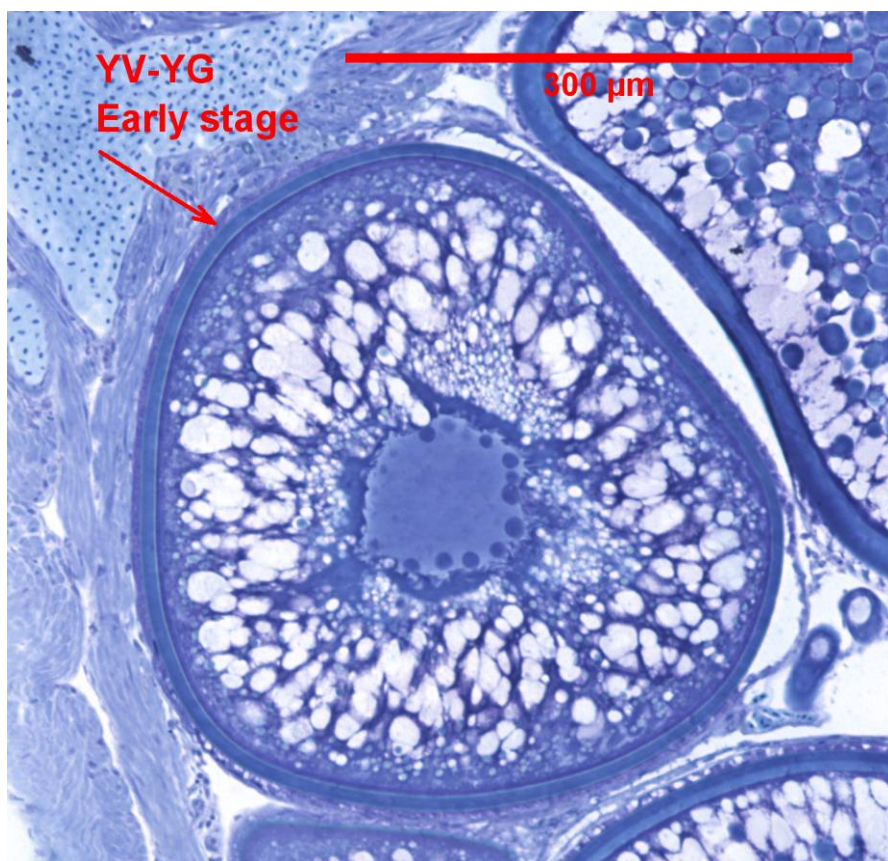
Maturity staging using histological sections

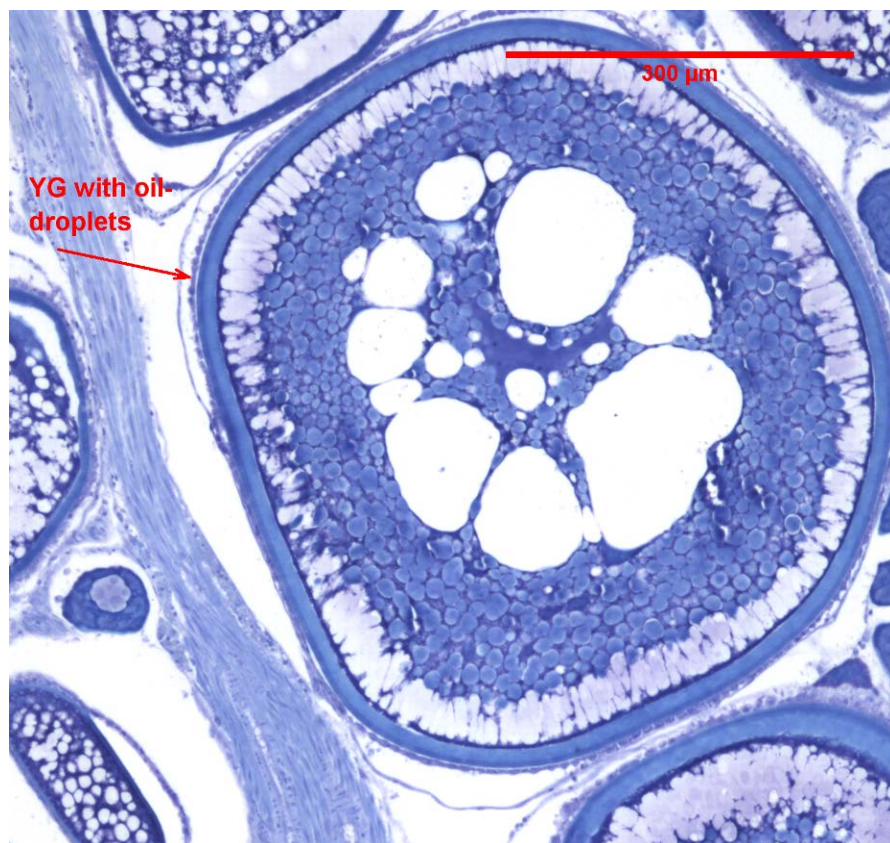
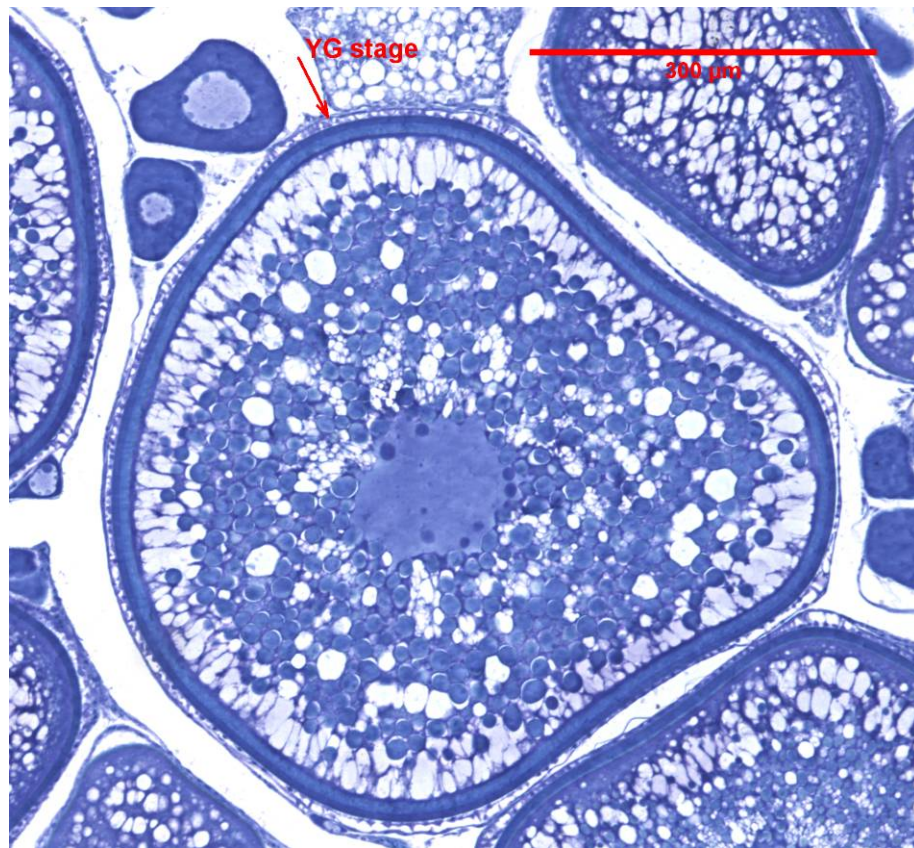
Maturity stage	Female histological appearance	Male histological appearance
Virgin	Well-spaced ovigerous folds orientated towards the center of the ovary; oogonia and primary oocytes at both the chromatin nucleolus and peri-	

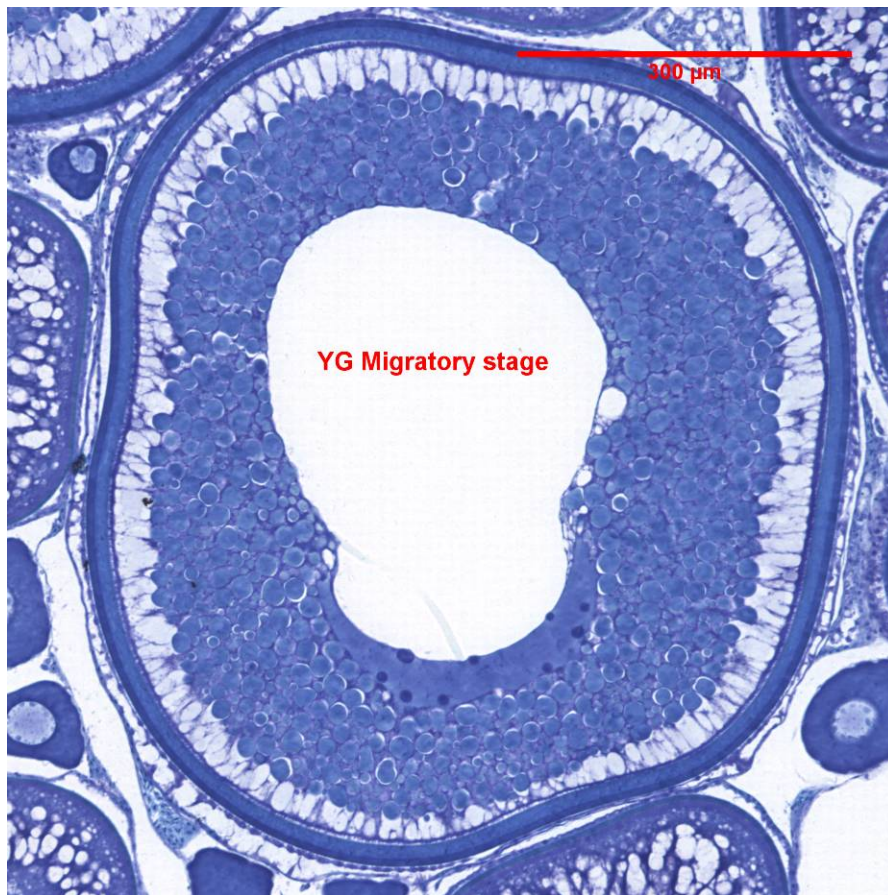
	nucleolus stage; oocyte size 10-60μ	
Developing virgin or resting	Few spaces between ovigerous folds; few oogonia, the majority of primary oocytes at the perinucleolus stage; oocyte size 20-150μ	
Early developing	Oocytes with cytoplasmic vacuoles (lipid droplets); yolk granules first appear in the cytoplasmic periphery while subsequently spread internally; elongated spindlelike cells constitute the follicle layer; oocyte size 150-400μ	
Later developing	Yolk granules becoming larger (yolk spherules) proliferate; oil droplets spread throughout the cytoplasm, while at the end of the stage they coalesce and accumulate around the nucleus; zona radiata is present; granulose cells become cuboidal; oocyte size 400-600μ	
Ripe/Running	Yolk spherules coalesce to globules or plates; large oil droplets follow the nucleus migration to the animal pole where the nucleus disperses its content into the cytoplasm; oocyte size 600-800μ By the nucleus dispersion rapid uptake of fluid (hydration) takes place; zona radiata losing its striation and becoming very thin; oocyte size 700-1200μ	
Partly spent	Present post ovulatory follicles (POFs); oocyte in any de-	

	veloping stage including that of ripe; possible oocytes in alpha or subsequent atresia stage	
Spent	Possible POFs; yolked oocytes of which 50% or more are in alpha stage atresia or no yolked oocytes but atretic follicles (beta or later stage atresia) and primary oocytes	









Pictures of the 3 different stages in early alpha atretic oocytes stained with toluidine blue.

