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Working Document 1

Protocol data collection WKMSTB 2012 (Workshop on Sexual Maturity Staging of Turbot and Brill)

Version 2.0, April 2010

Contents

1 Background 50

2 Data collection 52

2.1 Pictures 52

2.1.1 Technical details 52

2.1.2 Additional information 54

2.2 Histological samples 55

2.2.1 Technical details 55

2.2.2 Additional information 55

2.3 Details on fish sampled 56

3 Workshop and deadlines 58

References 58

Appendix 1 59

Appendix 2 60

Appendix 3 61

Appendix 4 63

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 turbot and brill, namely WGNEW, 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) Agree on a common maturity scale for turbot (*Psetta maxima*) and brill (*Scophthalmus rhombus*) across laboratories comprising a comparison of existing scales and standardization of maturity determination criteria;

b) Calibrate staging of turbot and brill using fresh fish, following the pattern of trial-discussion-retrial;

c) Calibrate staging of turbot and brill using photographs, following the pattern of trial-discussion-retrial;

d) Validate macroscopic maturity determination with histological analysis;

e) Establish correspondence between old and new scales to convert time series;

f) Propose optimal sampling strategy to estimate accurate maturity ogives;

g) Address the generic ToRs adopted for maturity staging workshops (see 'PGCCDBS Guidelines for Workshops on Maturity Staging').

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 (TUR, BLL)

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 (TUR, BLL)

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 (TUR, BLL)

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 (TUR, BLL)

5. Picture of gonads outside the fish, placed on a measuring board, al-lowing to view the gonad in more detail, blood vessels etc.

   Label containing:

   - Fish number
   - Species (TUR, BLL)

6. Picture of longitudinally cut gonad.

   Label containing:

   - Fish number
   - Species (TUR, BLL)

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 TUR for turbot, BLL for brill
- Fishnumber: unique number for the fish in the year
- Number: referring to number 1 to 6 in the above protocol

Example:

Dutch turbot, fish number 142, dorsal overview:	NED2010_tur_142_1.jpg
Dutch turbot, fish number 142, ventral detail:	NED2010_tur_142_4.jpg
Belgian brill, fish number 101, ventral overview:	BEL2010_bll_101_3.jpg
Belgian brill, fish number 101, gonad outside fish:	BEL2010_bll_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			TUR (turbot), BLL (brill)
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			Walsh, IBTS, etc.

maturity			own maturity scale value
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 Appendix 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			TUR (turbot), BLL (brill)
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			Walsh, IBTS, etc.
maturity			own maturity scale value
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

From 5-9 March 2012 a workshop will be held in IJmuiden, Netherlands. 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 turbot and brill 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.

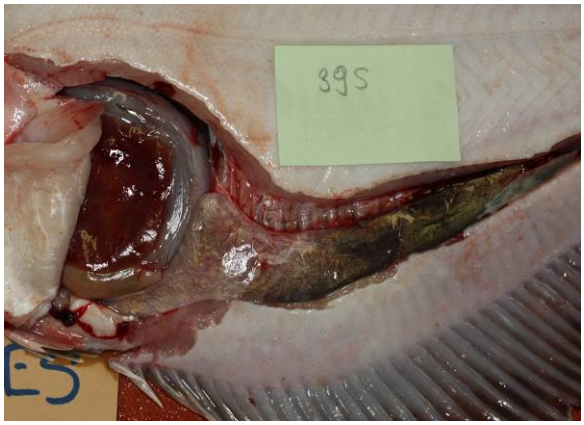
References

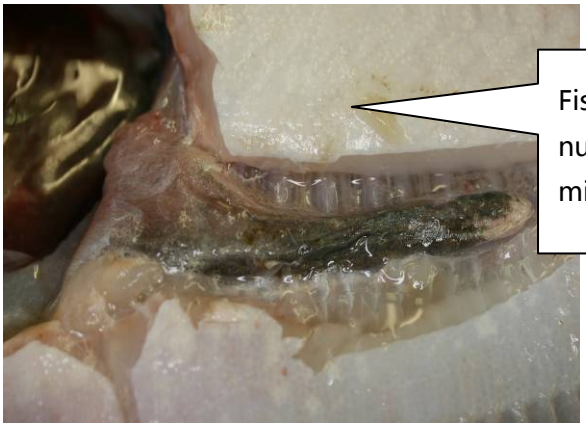
ICES. 2010. Report of the Workshop on Sexual Maturity Staging of sole, plaice, dab and flounder (WKMSSPDF), 22-26 February 2010, Ijmuiden, The Netherlands. ICES CM 2010/ACOM:50

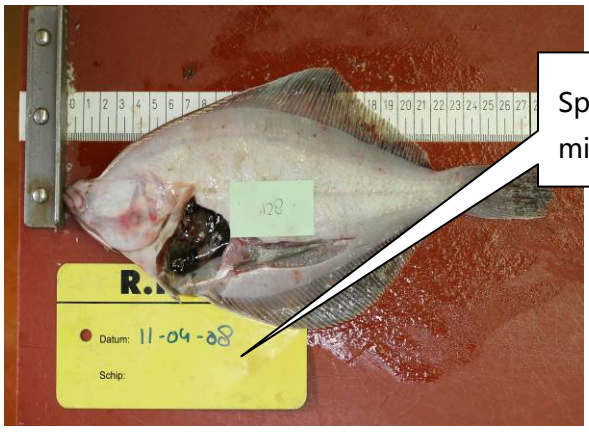
Appendix 1

Examples of pictures

DO **DON' T**







Appendix 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 $Na_2HPO_4.2H_2O$

Add together and stir until dissolved completely

39.9 g $NaH_2PO_4.H_2O$

Add the $NaH_2PO_4.H_2O$ to the solution and stir until dissolved completely

9030 ml purified water

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

Appendix 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 tem-
1	90% ethanol	2 hours	Room tempera-
2	Pour out the liquid and add fresh 90%	1 hour	Room tempera-
3	90% ethanol + Technovit 7100 (1:1 ratio) prepared by diluting Technovit 7100 (from used in steps 4).	2 hours or overnight	Store cool (+5°C) after the orbital
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 in the freezer.	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.

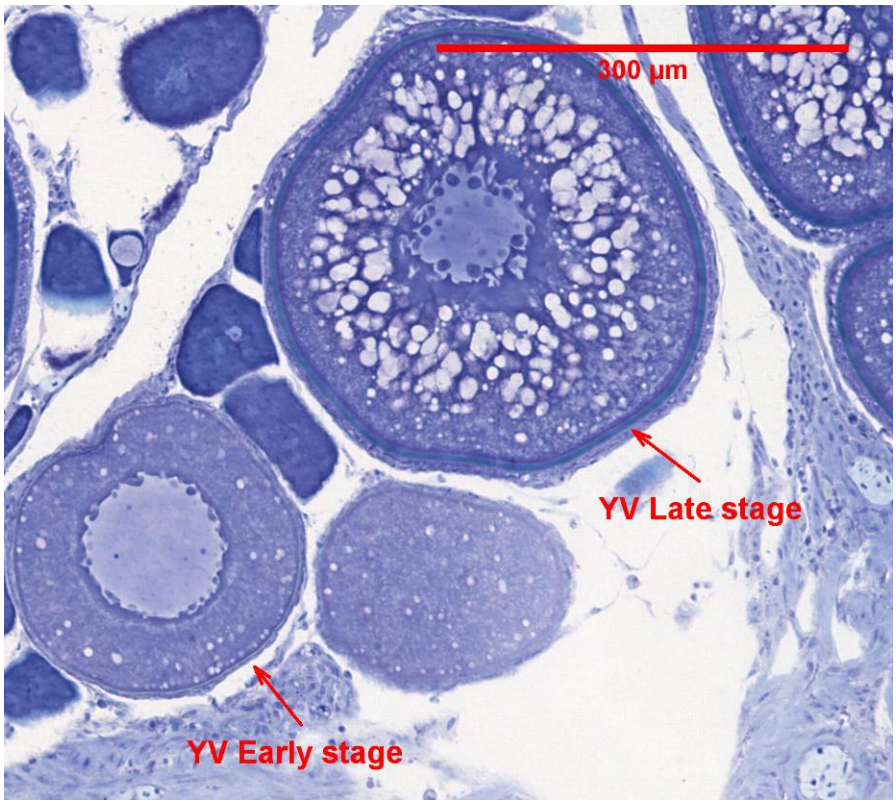
For individual slides: Cover the section with a few drops of 2 % Toluidine blue and pour the excess back in the bottle and rinse the section with hot (60°C) tap water for 20 seconds. Dry on a 60°C hot plate. Cover the section with a cover slip using two drops of mountex.

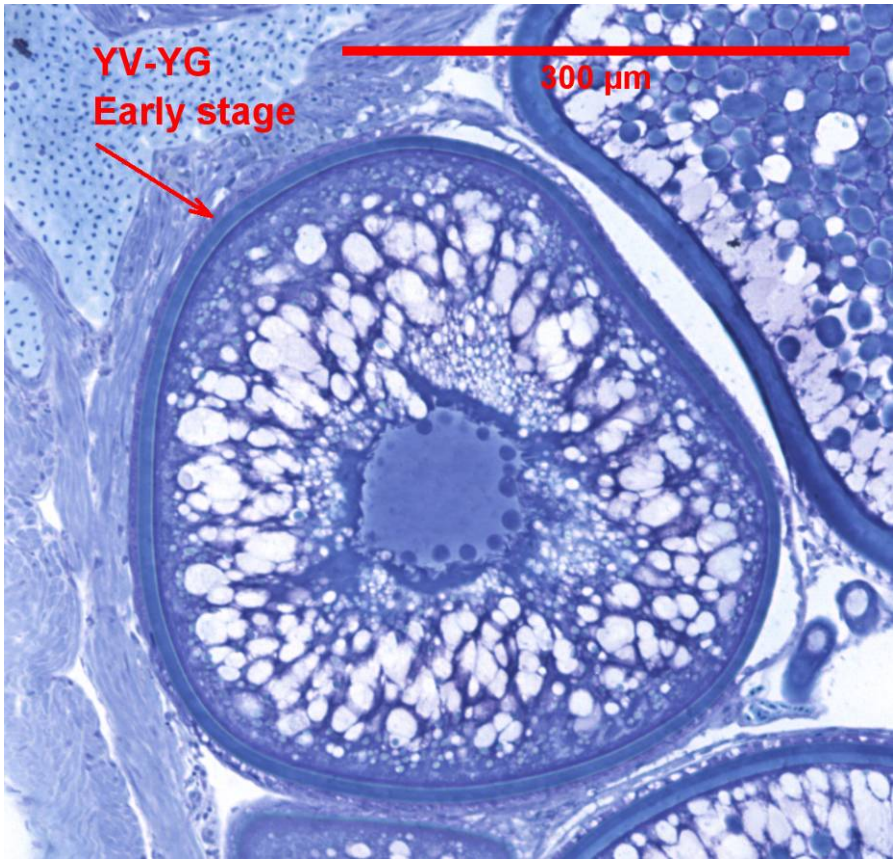
Appendix 4

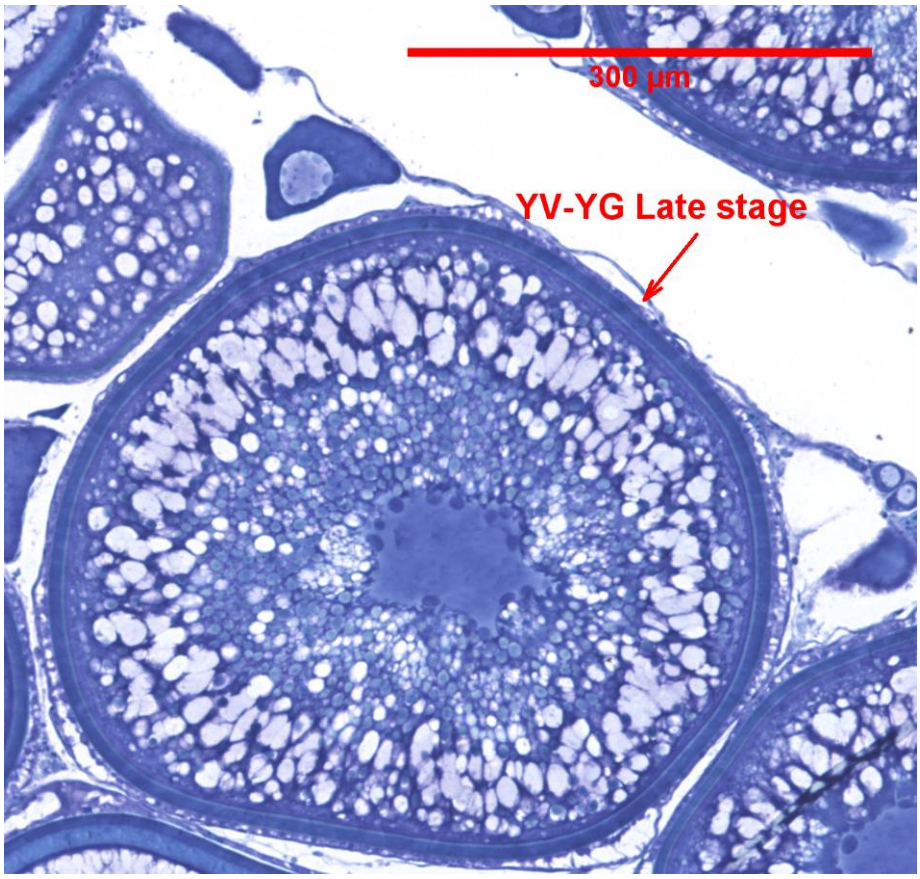
Maturity staging using histological sections

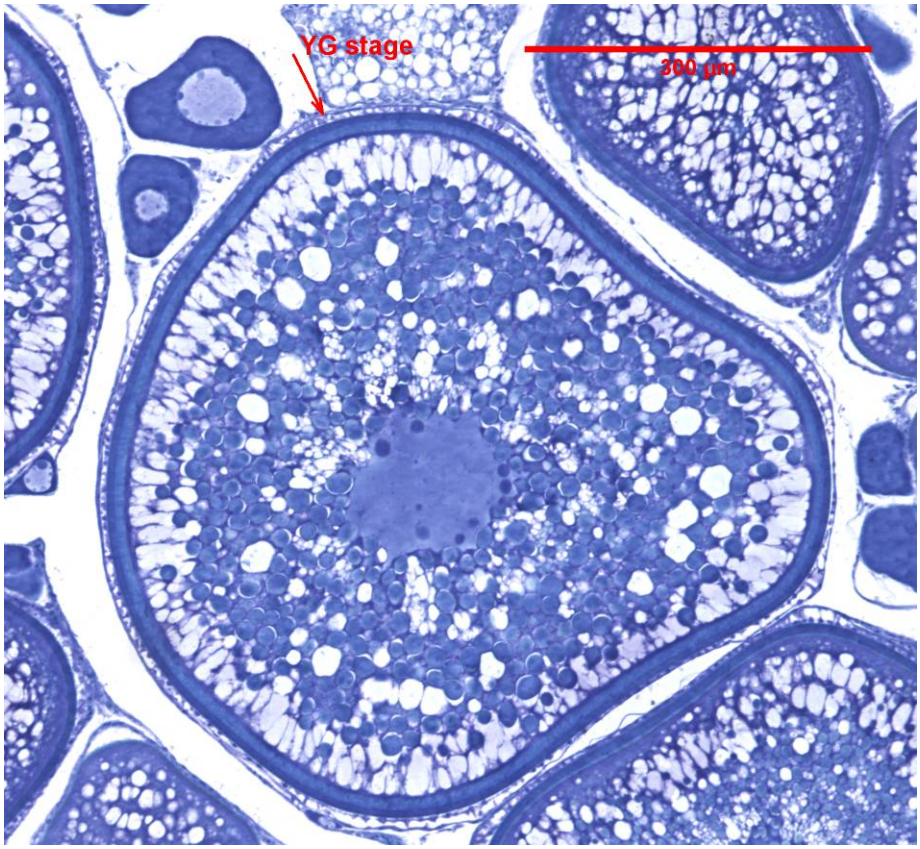
Maturity stage	Female histological appearance	Male histological appearance
Virgin	Well-spaced ovigerous folds orientated towards the center of the ovary; oogonia and primary oocytes at both the chromatin nucleolus and perinucleolus stage; oocyte size 10-60µ	
Developing virgin or resting	Few spaces between 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 sprindlelike cells constitute the follicle layer; oocyte size 150-400µ	
Later developing	Yolk granules becoming larger (yolk spherules) proliferate; oil droplets spread throughout the the cytoplasm, while at the end of the stage they coalesce and accumulate around the nuclear; zona radiate is present; granulose cells become cuboidal; oocyte size 400-600µ	

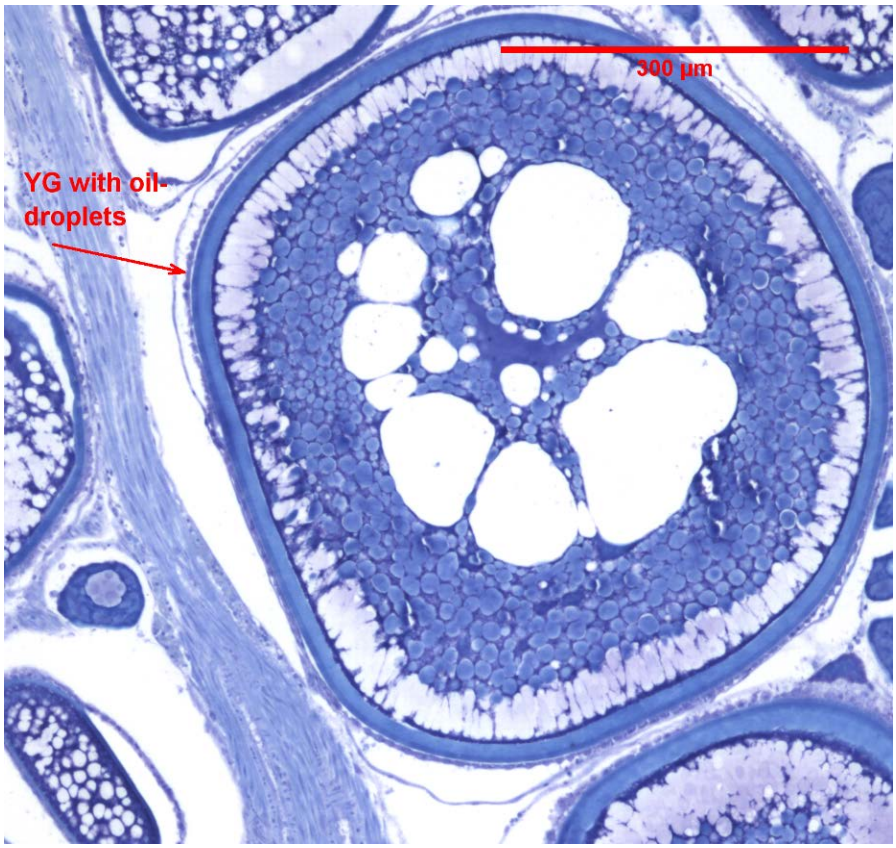
Maturity stage	Female histological appearance	Male histological appearance
Ripe/Running	Yolk spherules coalesce to globules or plates; large oil droplets follow the nucleus migration to the animal pole where the nucleus disperses its content into the cytoplasm; oocyte size 600-800µ By the nucleus dispersion rapid uptake of fluid (hydration) takes place; zona radiata loosing its striation and becoming very thin; oocyte size 700-1200µ	
Partly spent	Present post ovulatory follicles (POFs); oocyte in any developing 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	

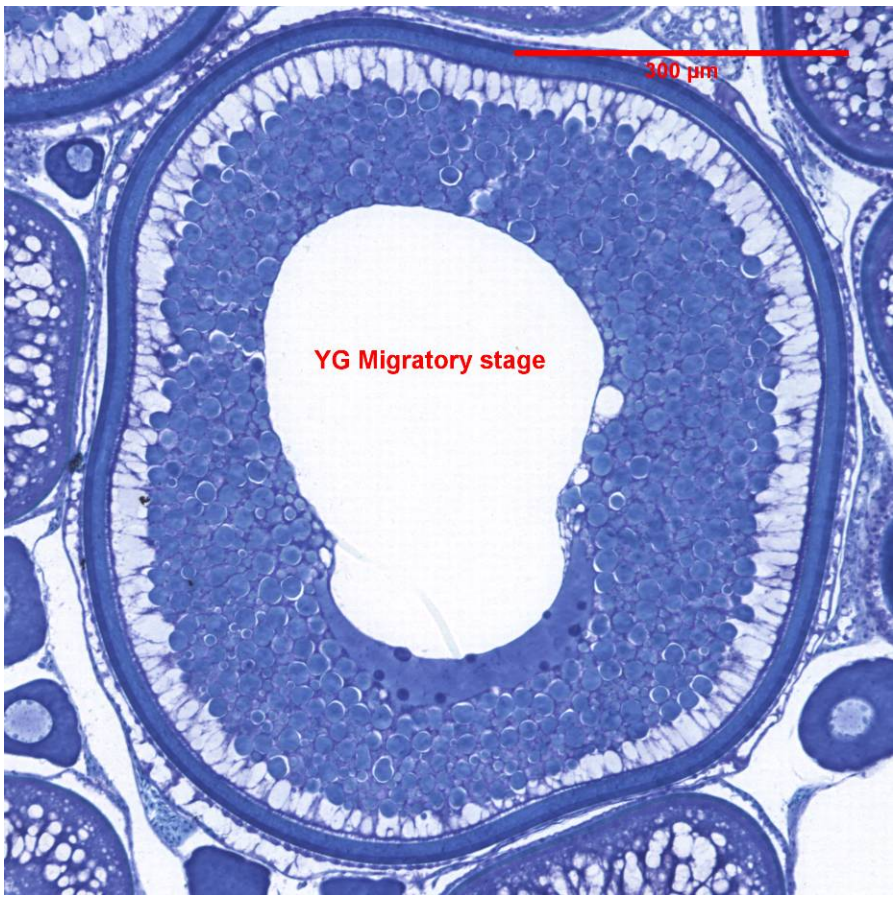

300 µm
YV Late stage
YV Early stage


YV-YG
Early stage
300 µm

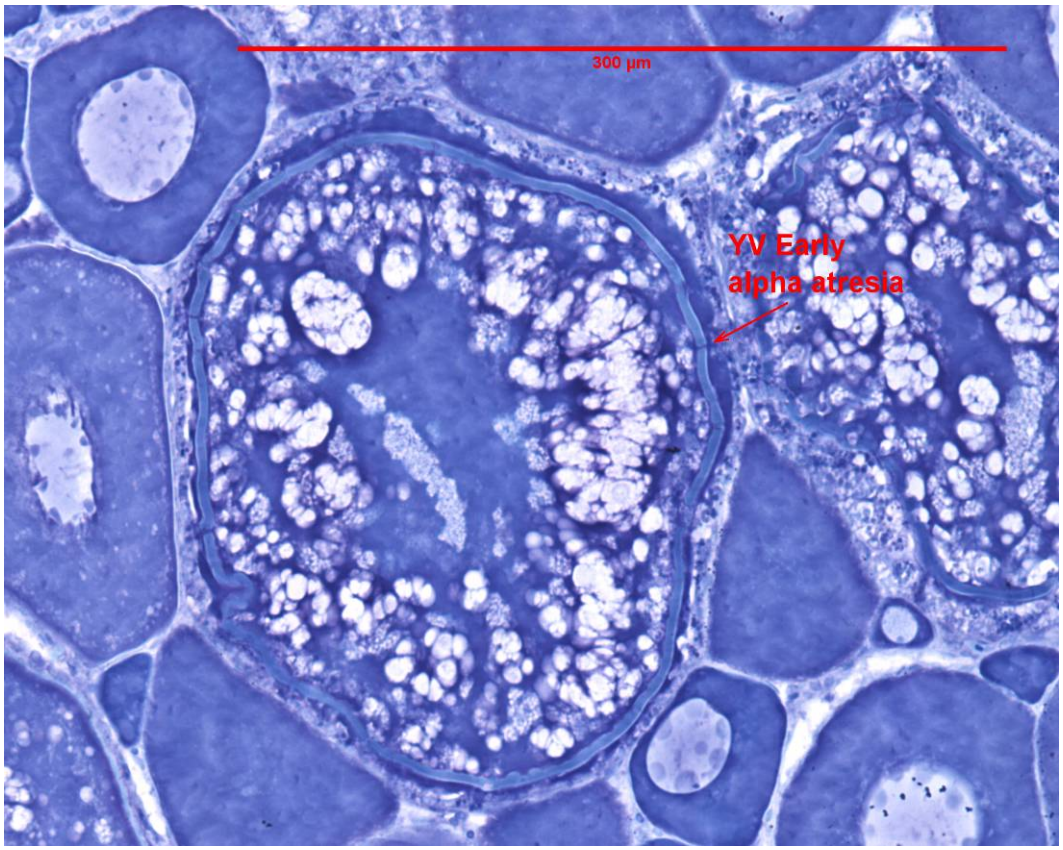

300 µm
YV-YG Late stage

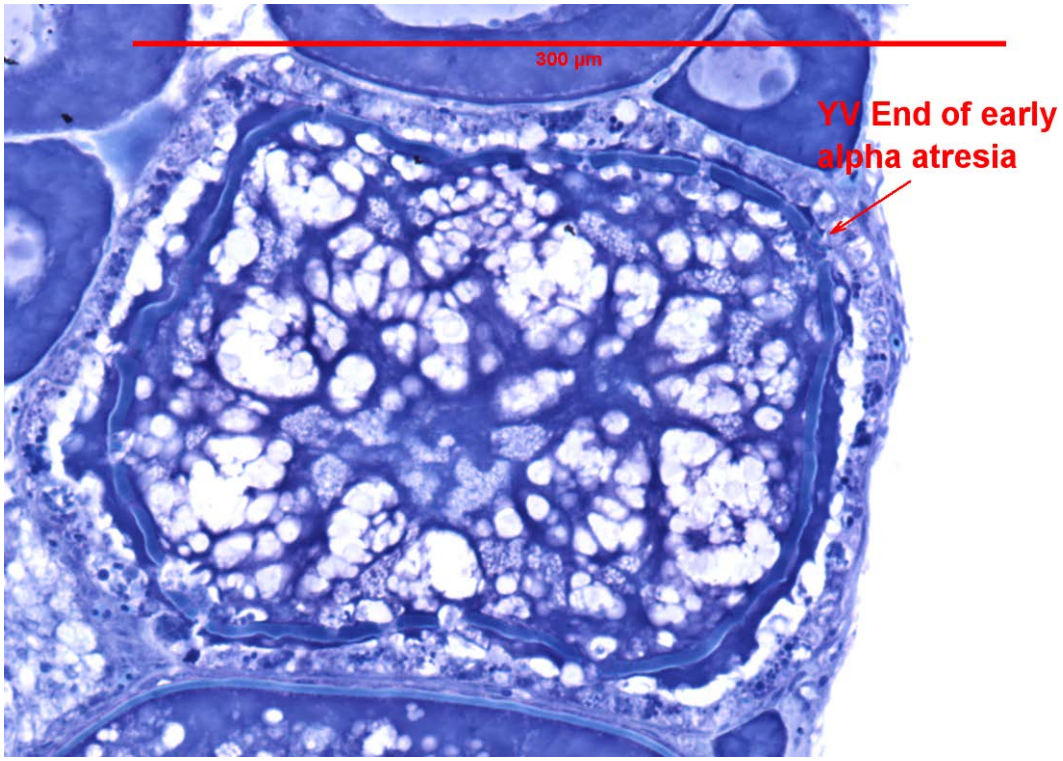

YG stage
300 µm

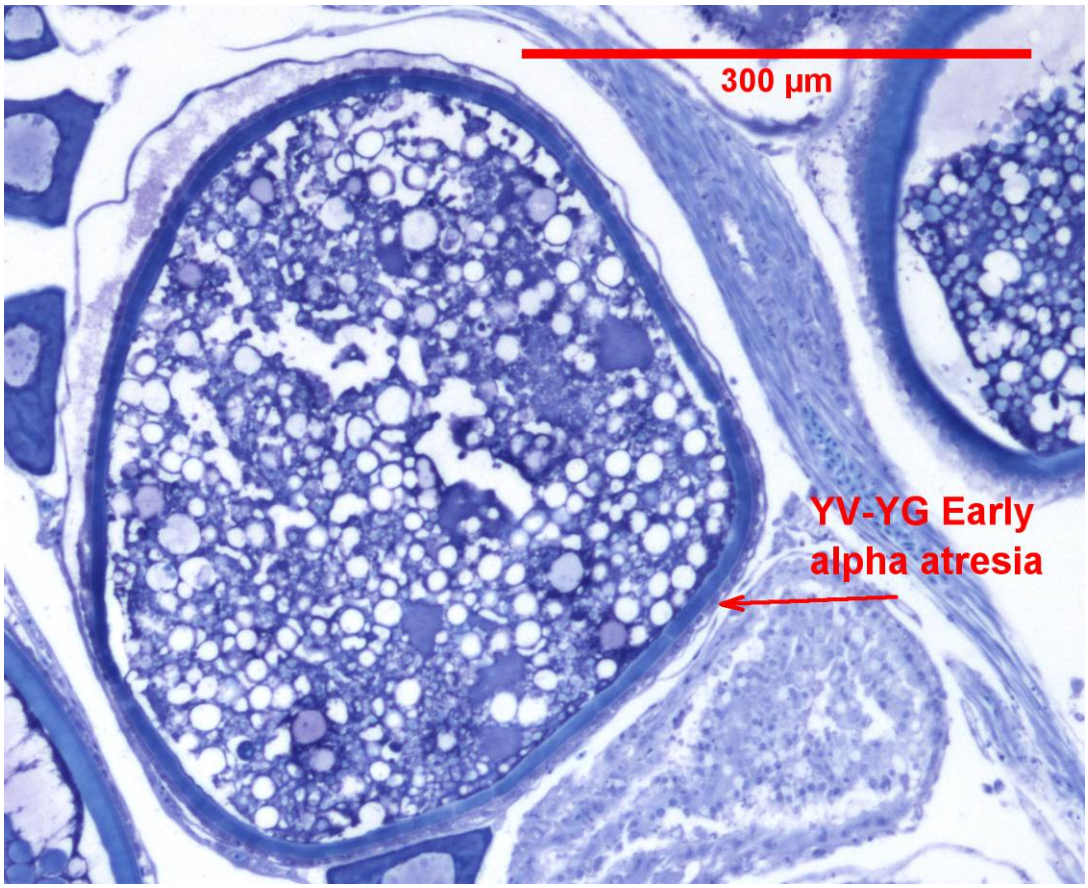

300 µm
YG with oil-droplets


300 µm
YG Migratory stage

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

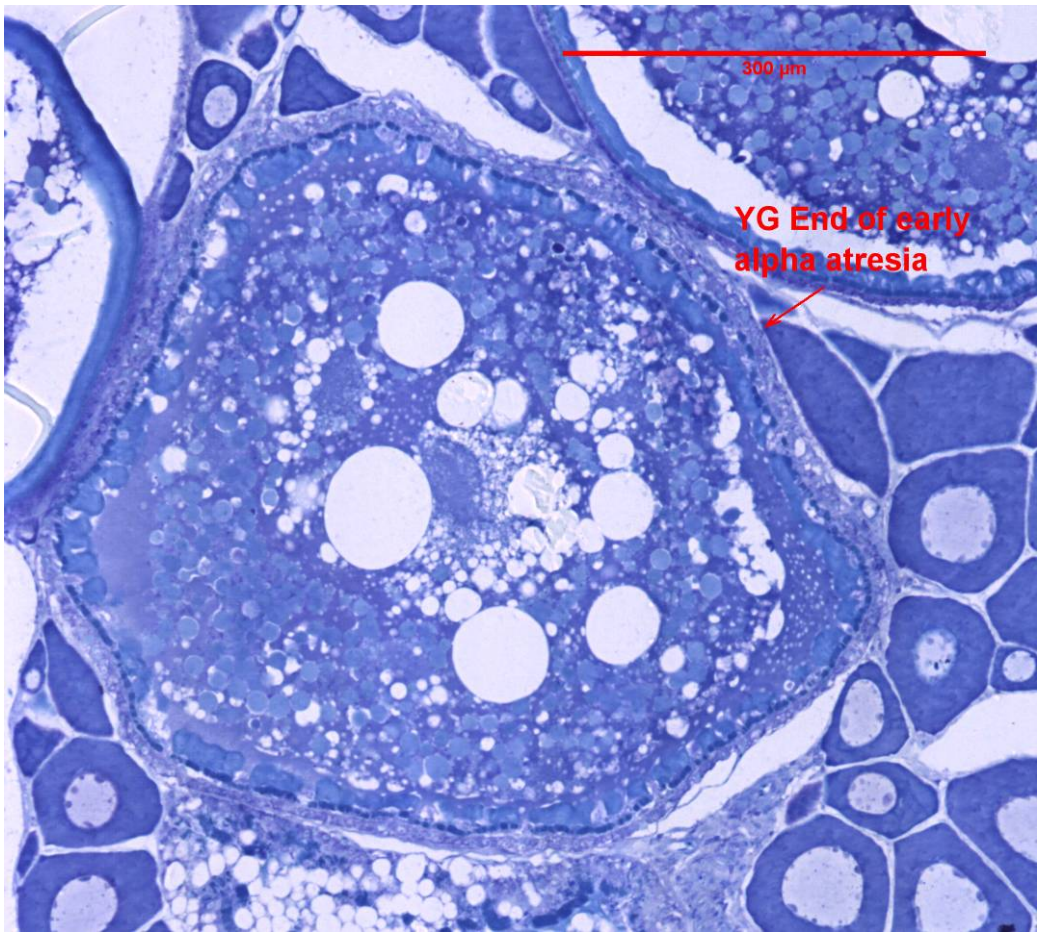





300 µm
YV-YG Early
alpha atresia


300 µm
YV-YG End of
early alpha atresia


300 µm
YG Early
alpha atresia


300 µm
YG End of early
alpha atresia

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Working Document 2: Reference Document Maturity Stages of Turbot and Brill

Contents

Reference Document Maturity Stages of Turbot (*Psetta maxima*) 73

Reference Document Maturity Stages of Brill (*Scophthalmus rhombus*) 91

Reference Document Maturity Stages of Turbot (*Psetta maxima*)

1 Introduction

This document contains the descriptions and reference pictures for the sexual maturity stages of turbot (*Psetta maxima*). The descriptions as well as the reference pictures were discussed and created by WKMSTB 2012.

It should be mentioned that reliable macroscopic maturity staging of fish can only be done in the period from two months before the spawning season until the end of spawning. Outside that period, histological samples should be taken to identify the maturity stage. The description of the stages focuses thus primarily on this period.

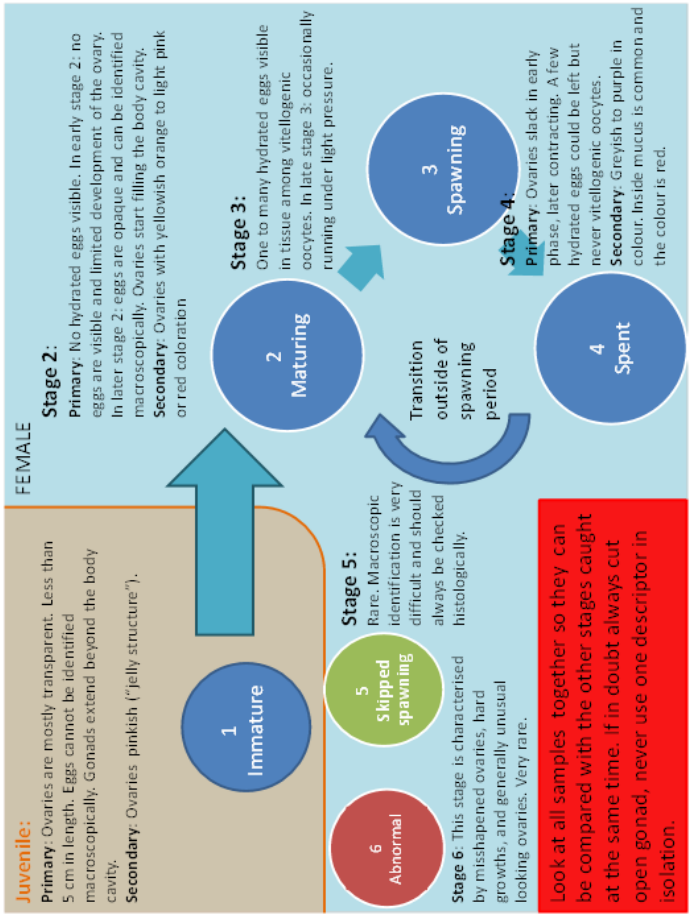
In general, sexual maturity development in fish is as follows: after the juvenile stage, of which the duration varies by species, fish will mature. In the maturation cycle, the gonads mature (stage 2), the fish will spawn (stage 3) and after that will be spent (stage 4). The next year, this cycle repeats itself.

Incidentally, if the condition of the fish is low, it might decide to skip a spawning season (stage 5), or the gonads of a fish do not develop in a normal way (stage 6).

Chapter 2 describes the sexual maturity stages of female fish, chapter 3 contains maturity scales for males.

It is important to realise that turbot in the Baltic Sea grows slower than in the North Sea. This means also that in the North Sea turbot is still juvenile at a larger size (up to 30-35 cm) than in the Baltic Sea (juveniles up to approx. 28 cm, male even smaller). The staging descriptions are based on turbot caught in the Baltic Sea and the North Sea, and on brill caught in the North Sea. The descriptions of the sexual maturity stages are identical to the stages for brill.

2 Female



2.1 Stage 1 – Juvenile

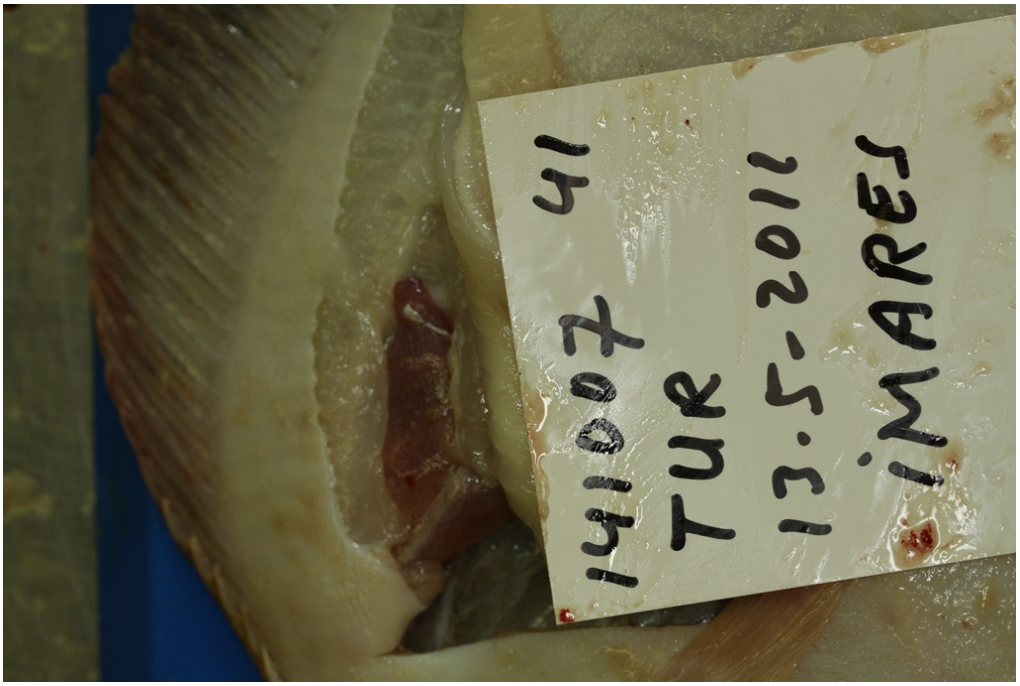
Primary:

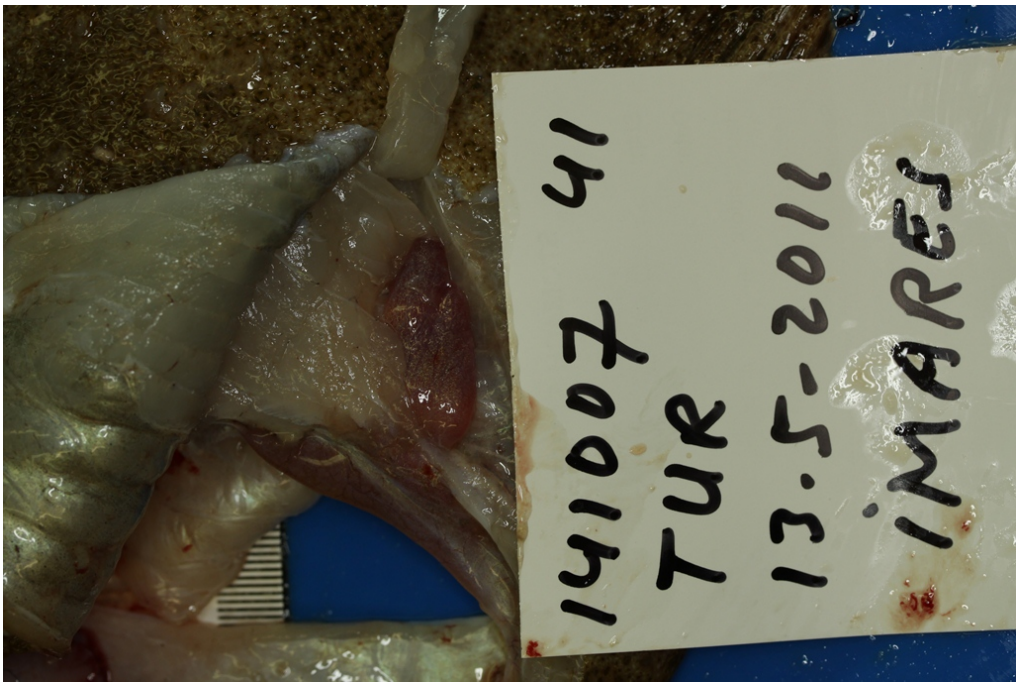
- Ovaries are mostly transparent
- Gonads extend beyond the body cavity, but are less than 5 cm in length
- Eggs cannot be identified macroscopically

Secondary:

- Ovaries pinkish ("jelly structure")
- Blood vessels hardly discernable

pictures: ovary in the fish NED2011_tur_141004_053_1.jpg, overview (upper) and in detail NED2011_tur_141004_053_6.jpg (lower)





pictures: ovary in the fish NED2011_tur_141007_041_4.jpg, white side (upper) and dark side, NED2011_tur_141007_041_2.jpg (lower)

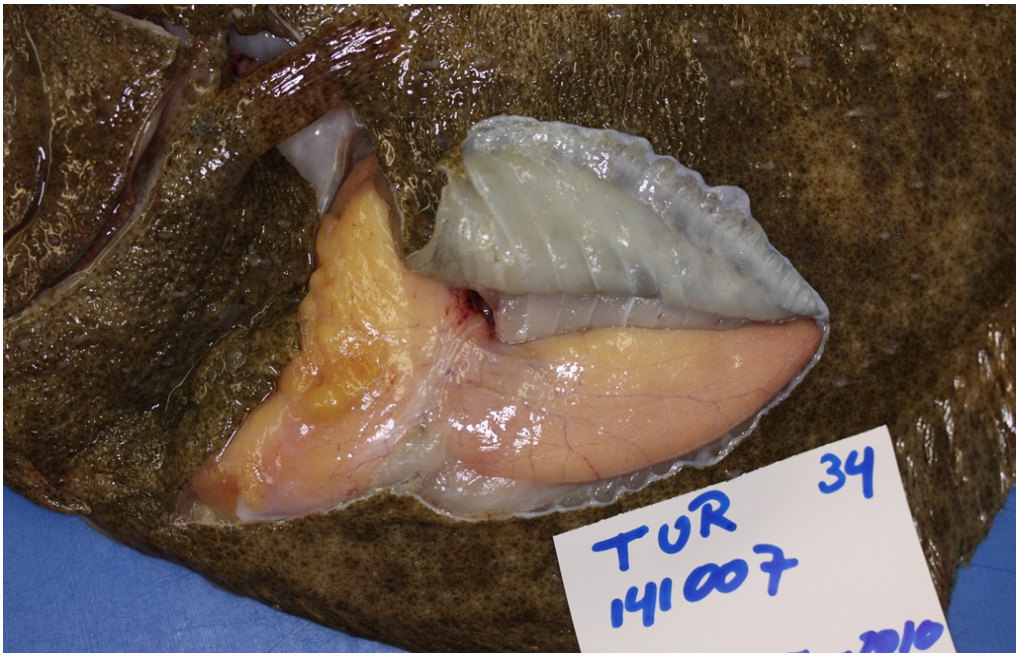
2.2 Stage 2 – Maturing

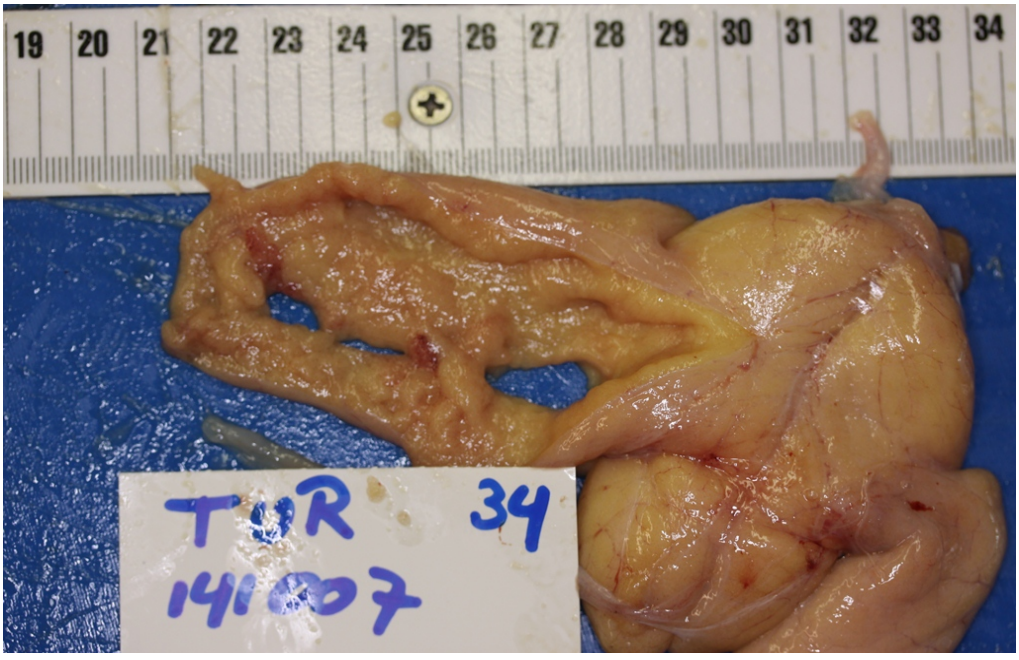
Primary:

- No hydrated eggs visible
- In early stage 2: no eggs are visible and limited development of the ovary
- In later stage 2: eggs are opaque and can be identified macroscopically
- Ovaries start filling the body cavity.

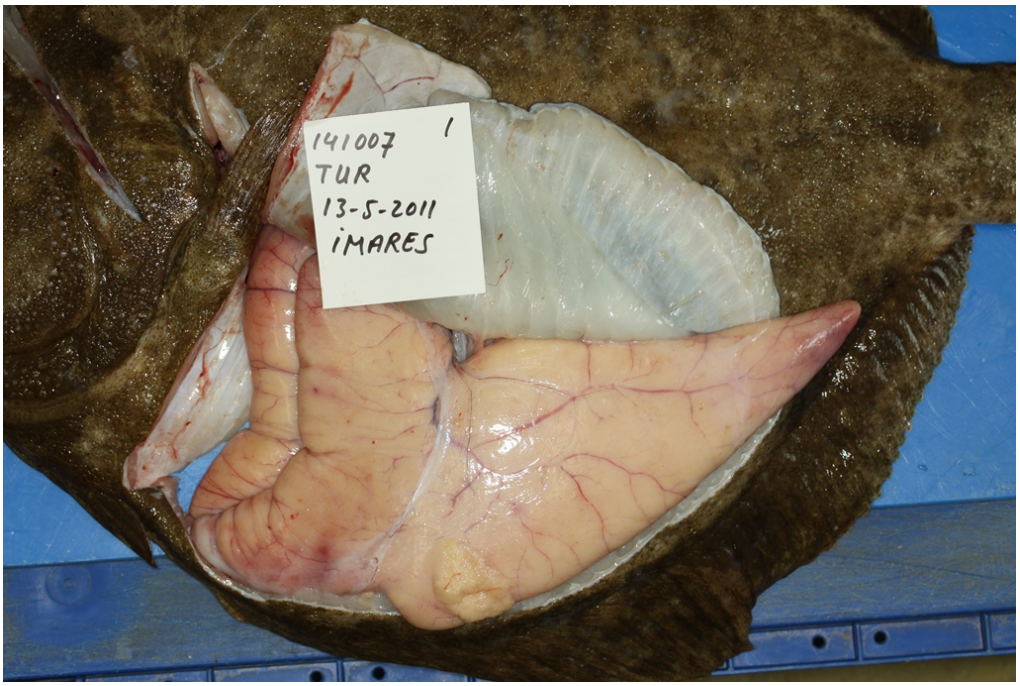
Secondary:

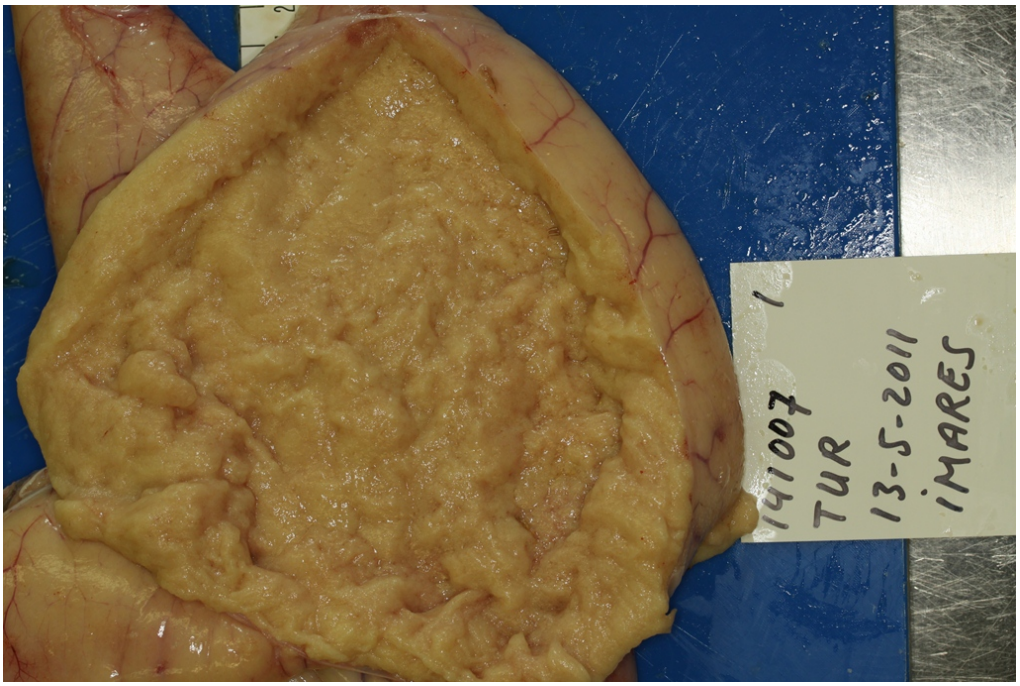
- Ovaries with yellowish orange to light pink or red coloration
- The eggs do not flow at light pressure
- Blood vessels larger and diversified





pictures: ovary in the fish NED2010_tur_141007_034_2.jpg (upper) and cut open outside the fish NED2010_tur_141007_034_6.jpg (lower)





pictures: ovary in the fish NED2011_tur_141007_001_2.jpg (upper) and cut open outside the fish NED2011_tur_141007_001_6.jpg (lower)

2.3 Stage 3 – Spawning

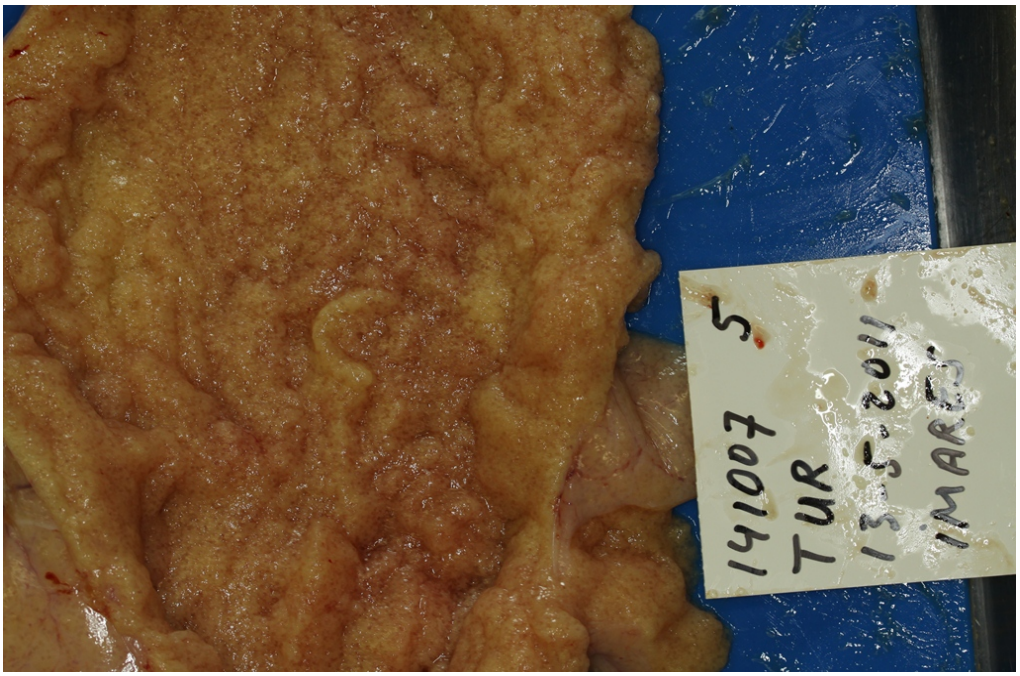
Primary:

- one to many hydrated eggs visible in tissue among vitellogenic oocytes
- in late stage 3: occasionally running under light pressure

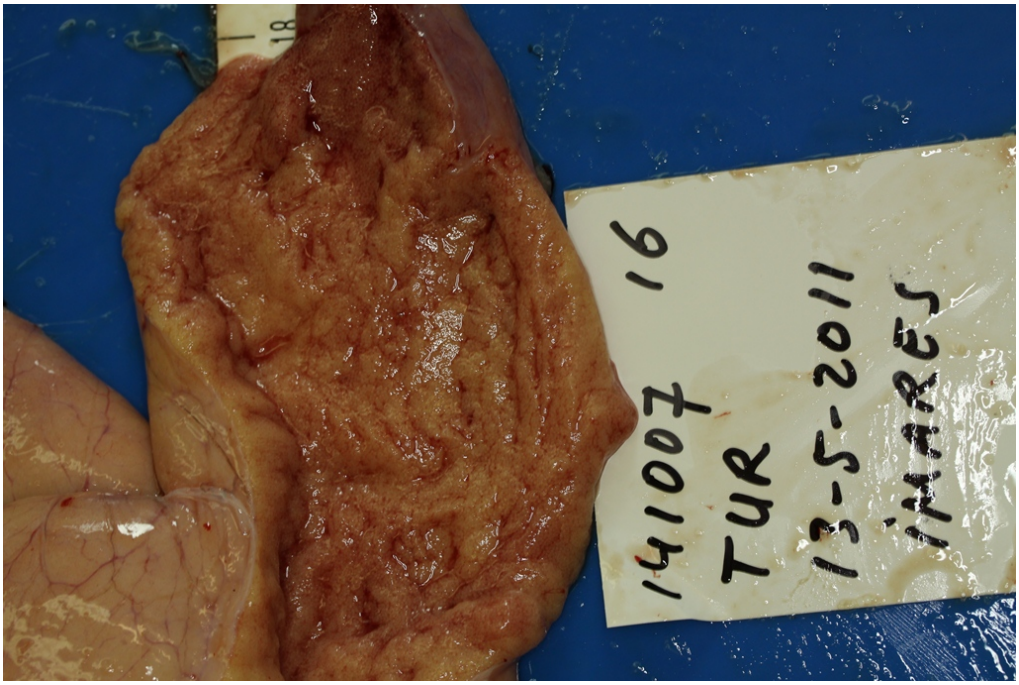
Secondary:

- ovaries do not change in size and volume compared to stage 2

NB: check both ovaries as eggs are small and hydrated eggs can easily be missed



pictures: ovary in the fish NED2011_tur_141007_005_2.jpg (upper) and cut open outside the fish NED2011_tur_141007_005_6.jpg (lower)



pictures: ovary in the fish NED2011_tur_141007_016_2.jpg (upper) and cut open outside the fish NED2011_tur_141007_016_6.jpg (lower)

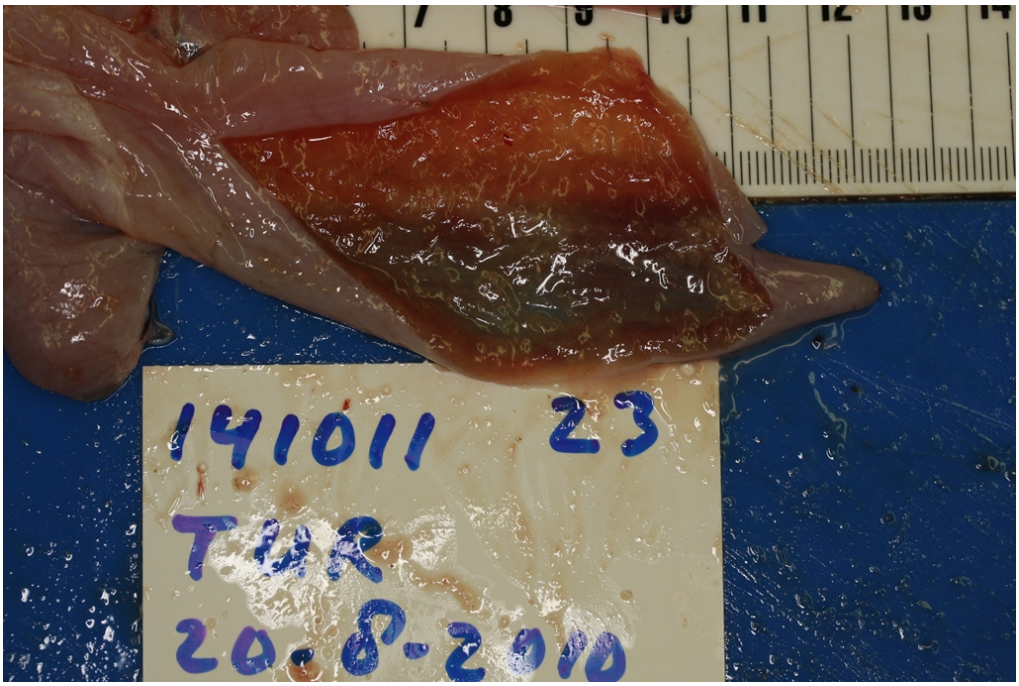
2.4 Stage 4 – Spent

Primary:

- ovaries slack in early phase, later contracting
- a few hydrated eggs could be left but never vitellogenic oocytes

Secondary:

- colour: greyish to purple
- inside mucus is common (colour: red)



pictures: ovary in the fish NED2010_tur_141011_023_5.jpg (upper) and cut open outside the fish NED2010_tur_141011_023_7.jpg (lower)

2.5 Stage 5 – Skipped spawning

Rare. Macroscopic identification is very difficult and should always be checked histologically.

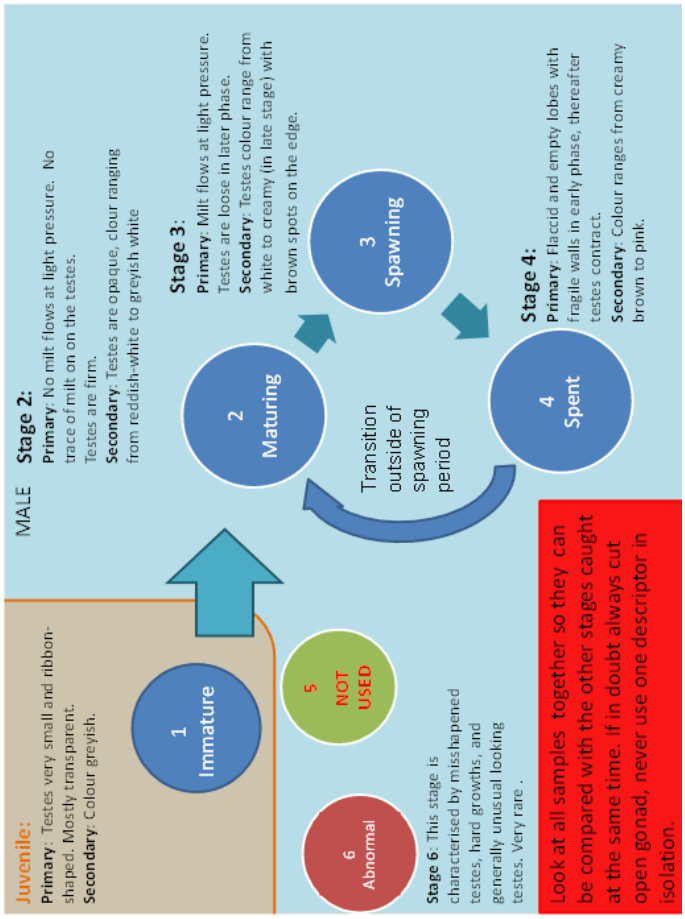
No pictures available.

2.6 Stage 6 – Abnormal

This stage is characterised by mis-shapened ovaries, hard growths, and generally unusual looking ovaries. For example: stony gonads, both sexes present in the gonad, etc. Very rare.

No pictures available.

3 Male


MALE
Juvenile:
Primary: Testes very small and ribbon-shaped. Mostly transparent.
Secondary: Colour greyish.
1 Immature
Stage 2:
Primary: No milt flows at light pressure. No trace of milt on the testes. Testes are firm.
Secondary: Testes are opaque, clour ranging from reddish-white to greyish white
2 Maturing
Stage 3:
Primary: Milt flows at light pressure. Testes are loose in later phase.
Secondary: Testes colour range from white to creamy (in late stage) with brown spots on the edge.
3 Spawning
Stage 4:
Primary: Flaccid and empty lobes with fragile walls in early phase, thereafter testes contract.
Secondary: Colour ranges from creamy brown to pink.
4 Spent
Transition outside of spawning period
5 NOT USED
6 Abnormal
Stage 6: This stage is characterised by misshapened testes, hard growths, and generally unusual looking testes. Very rare .
Look at all samples together so they can be compared with the other stages caught at the same time. If in doubt always cut open gonad, never use one descriptor in isolation.

3.1 Stage 1 – Juvenile

Primary:

- testes very small and ribbon-shaped
- mostly transparent

Secondary:

- colour: greyish
- blood vessels hardly discernable.

No pictures available.

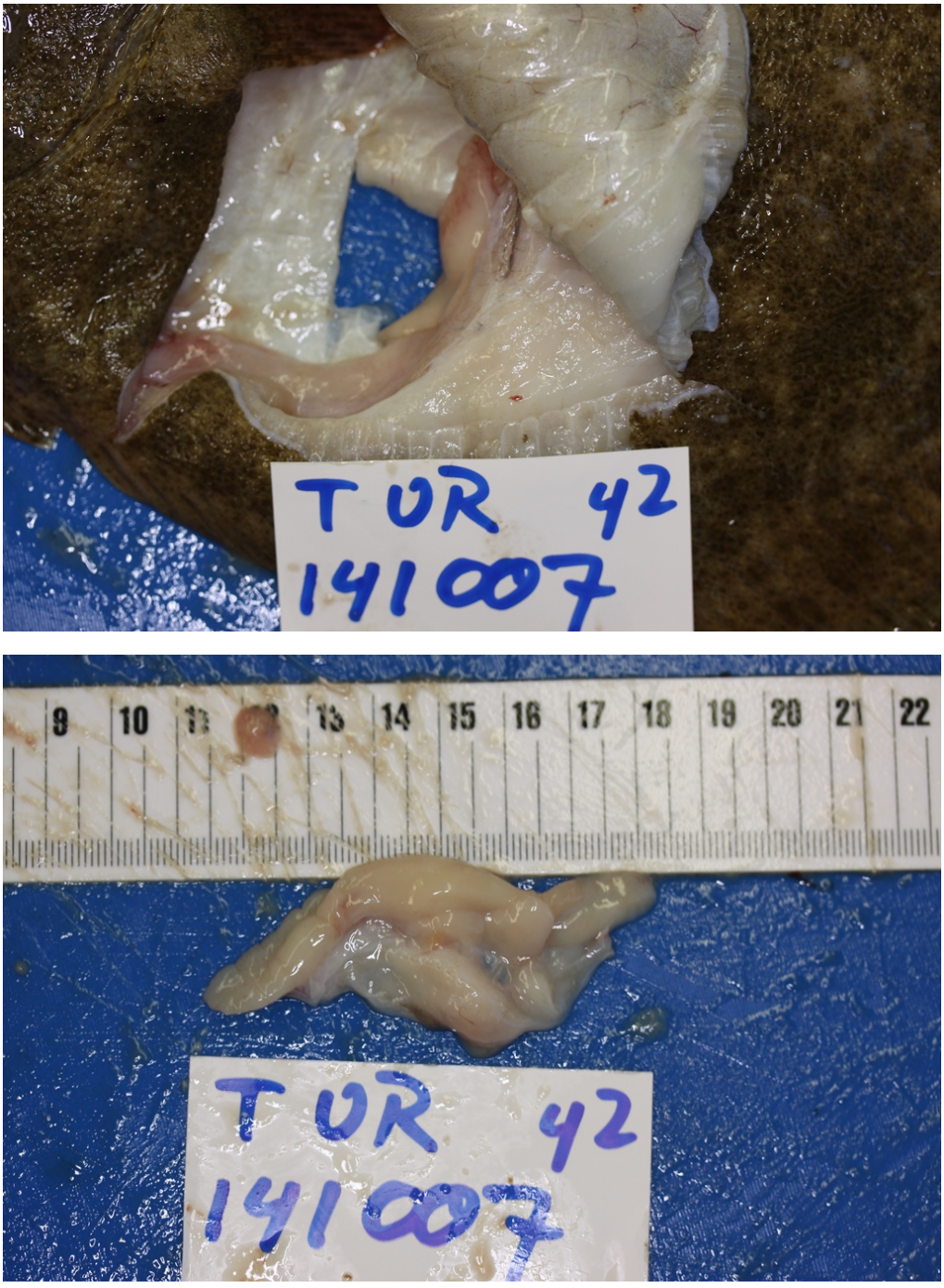
3.2 Stage 2 – Maturing

Primary:

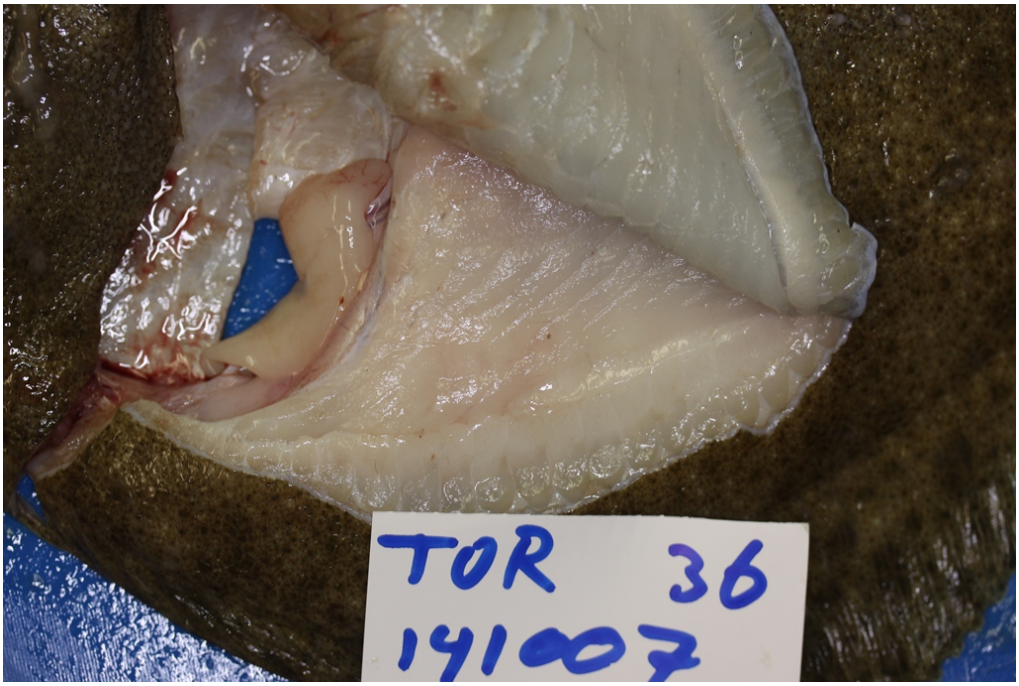
- no milt flows at light pressure
- no trace of milt on the testes
- testes are firm

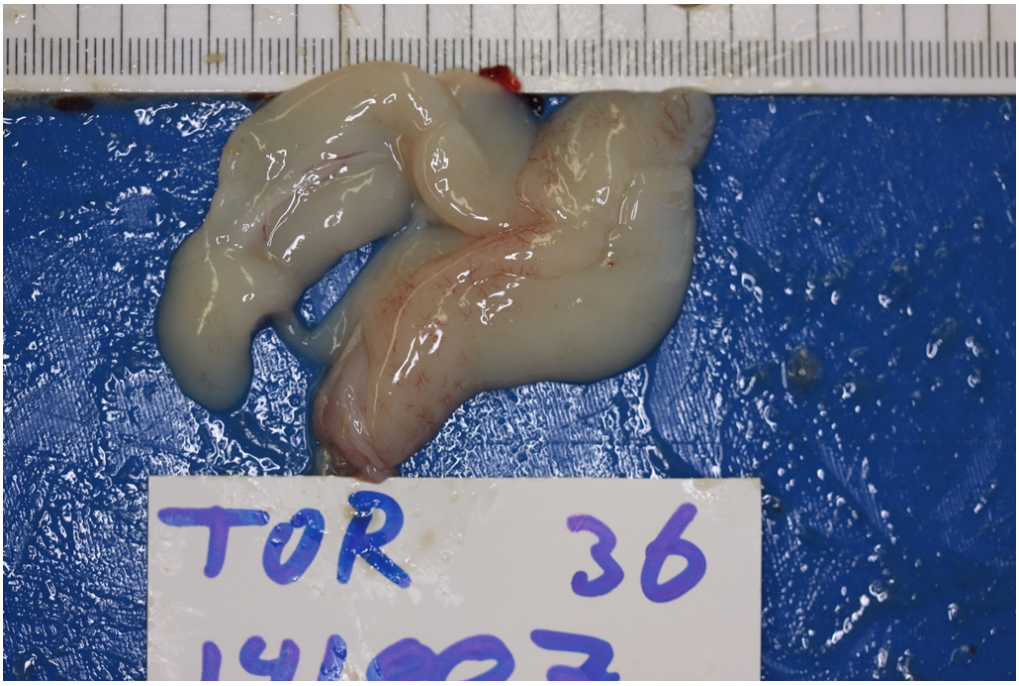
Secondary:

- testes are opaque
- colour: ranging from reddish-white to greyish white
- spermatoducts are transparent and empty
- blood vessels can be present

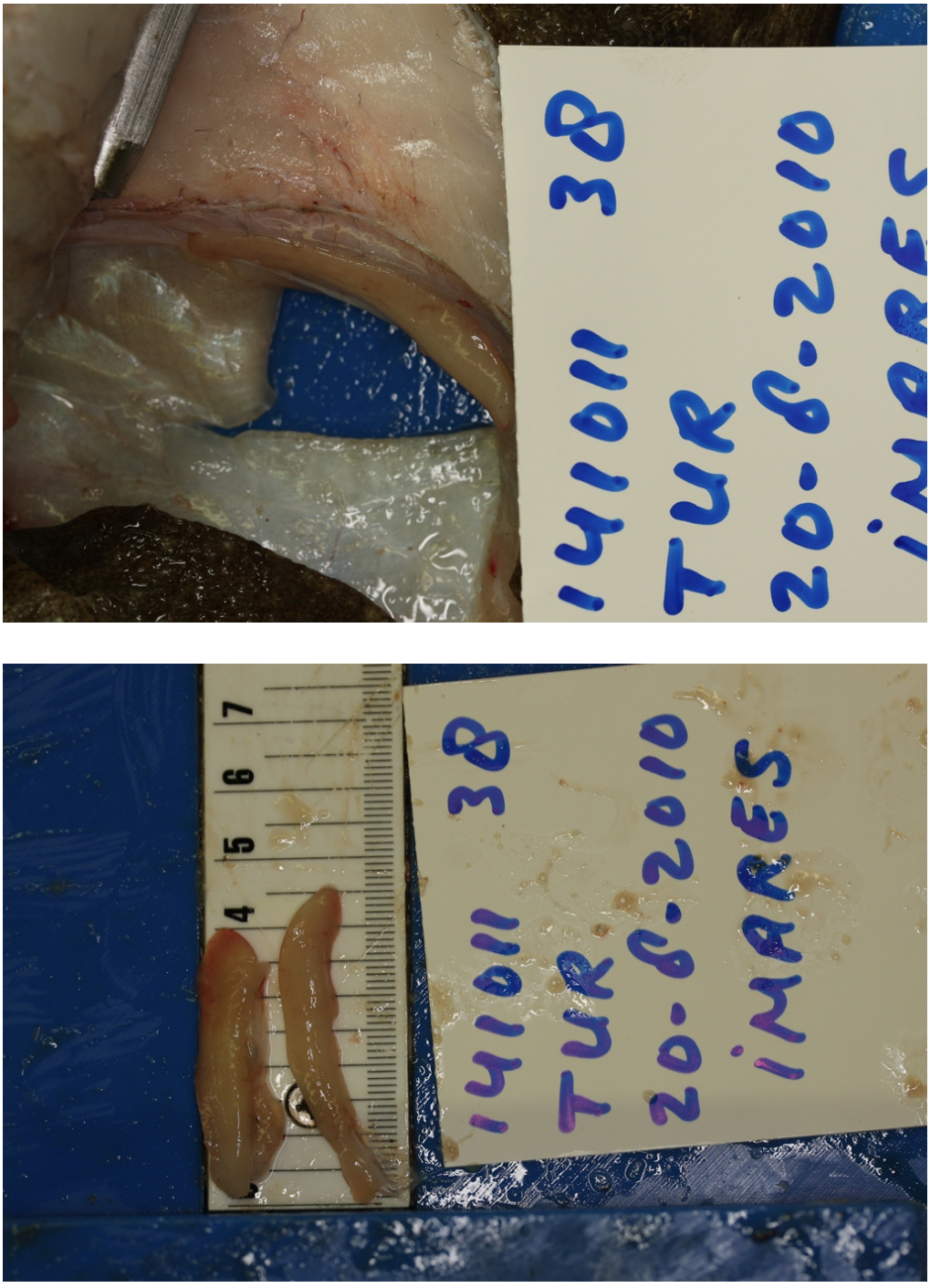


pictures: testis in the fish NED2010_tur_141007_042_2.jpg (upper) and outside the fish NED2010_tur_141007_042_5.jpg (lower)





pictures: testis in the fish NED2010_tur_141007_036_2.jpg (upper) and outside the fish NED2010_tur_141007_036_5.jpg (lower)



pictures: testis in the fish NED2010_tur_141011_038_2.jpg (upper) and outside the fish NED2010_tur_141011_038_5.jpg (lower)

3.3 Stage 3 – Spawning

Primary:

- milt flows at light pressure
- testes are loose in later phase.

Secondary:

- colour: ranging from white to creamy (in late stage) with brown spots on the edge
- spermatoducts are well developed and filled with milt

No pictures available.

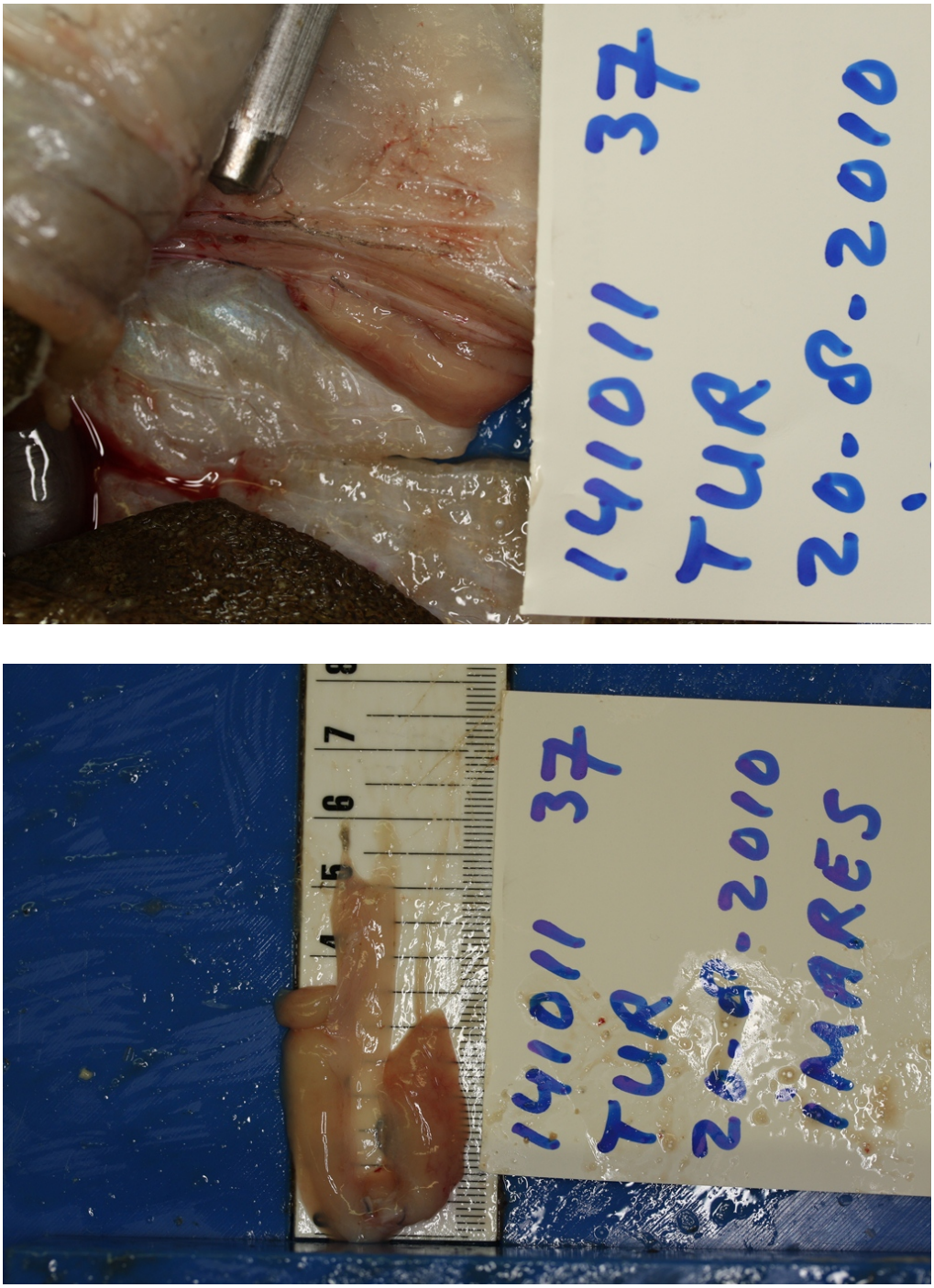
3.4 Stage 4 – Spent

Primary:

- flaccid and empty lobes with fragile walls in early phase
- in a later phase testes contract.

Secondary:

- colour: creamy brown to pink.
- Milt residue can be present



pictures: testis in the fish NED2010_tur_141011_037_2.jpg (upper) and outside the fish NED2010_tur_141011_037_5.jpg (lower)

3.5 Stage 5 – Skipped spawning

This stage is not used for male fish.

3.6 Stage 6 – Abnormal

This stage is characterised by mis-shapened testes, hard growths, and generally unusual looking testes. Very rare.

No pictures available.

Reference Document Maturity Stages of Brill (*Scophthalmus rhombus*)

1 Introduction

This document contains the descriptions and reference pictures for the sexual maturity stages of brill (*Scopthalmus rhombus*). The descriptions as well as the reference pictures were discussed and created by WKMSTB 2012.

It should be mentioned that reliable macroscopic maturity staging of fish can only be done in the period from two months before the spawning season until the end of spawning. Outside that period, histological samples should be taken to identify the maturity stage. The description of the stages focuses thus primarily on this period.

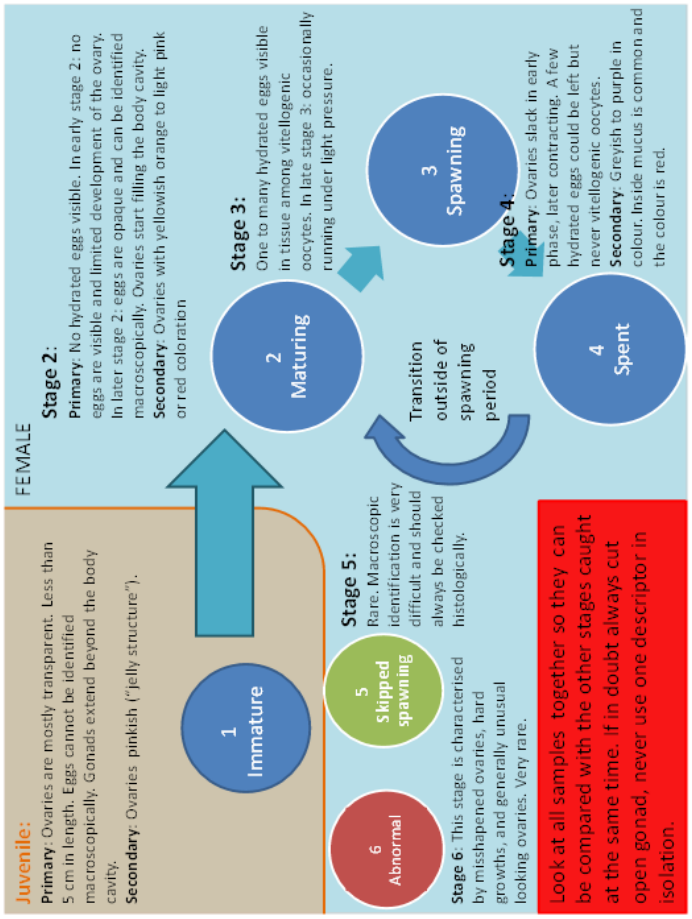
In general, sexual maturity development in fish is as follows: after the juvenile stage, of which the duration varies by species, fish will mature. In the maturation cycle, the gonads mature (stage 2), the fish will spawn (stage 3) and after that will be spent (stage 4). The next year, this cycle repeats itself.

Incidentally, if the condition of the fish is low, it might decide to skip a spawning season (stage 5), or the gonads of a fish do not develop in a normal way (stage 6).

Chapter 2 describes the sexual maturity stages of female fish, chapter 3 contains maturity scales for males.

It is important to realise that turbot in the Baltic Sea grows slower than in the North Sea. This means also that in the North Sea turbot is still juvenile at a larger size (up to 30-35 cm) than in the Baltic Sea (juveniles up to approx. 28 cm, male even smaller). Although catches of brill are not so frequent in the Baltic, it is assumed this also applies to brill.
The staging descriptions are based on brill caught in the North Sea. The descriptions of the sexual maturity stages are identical to the stages for turbot.

2 Female


FEMALE
Juvenile:
Primary: Ovaries are mostly transparent. Less than 5 cm in length. Eggs cannot be identified macroscopically. Gonads extend beyond the body cavity.
Secondary: Ovaries pinkish ("jelly structure").
1 Immature
Stage 2:
Primary: No hydrated eggs visible. In early stage 2: no eggs are visible and limited development of the ovary. In later stage 2: eggs are opaque and can be identified macroscopically. Ovaries start filling the body cavity.
Secondary: Ovaries with yellowish orange to light pink or red coloration
2 Maturing
Stage 3:
One to many hydrated eggs visible in tissue among vitellogenic oocytes. In late stage 3: occasionally running under light pressure.
3 Spawning
Stage 4:
Primary: Ovaries slack in early phase, later contracting. A few hydrated eggs could be left but never vitellogenic oocytes.
Secondary: Greyish to purple in colour. Inside mucus is common and the colour is red.
4 Spent
Transition outside of spawning period
5 Skipped spawning
Stage 5:
Rare. Macroscopic identification is very difficult and should always be checked histologically.
6 Abnormal
Stage 6: This stage is characterised by misshapened ovaries, hard growths, and generally unusual looking ovaries. Very rare.
Look at all samples together so they can be compared with the other stages caught at the same time. If in doubt always cut open gonad, never use one descriptor in isolation.

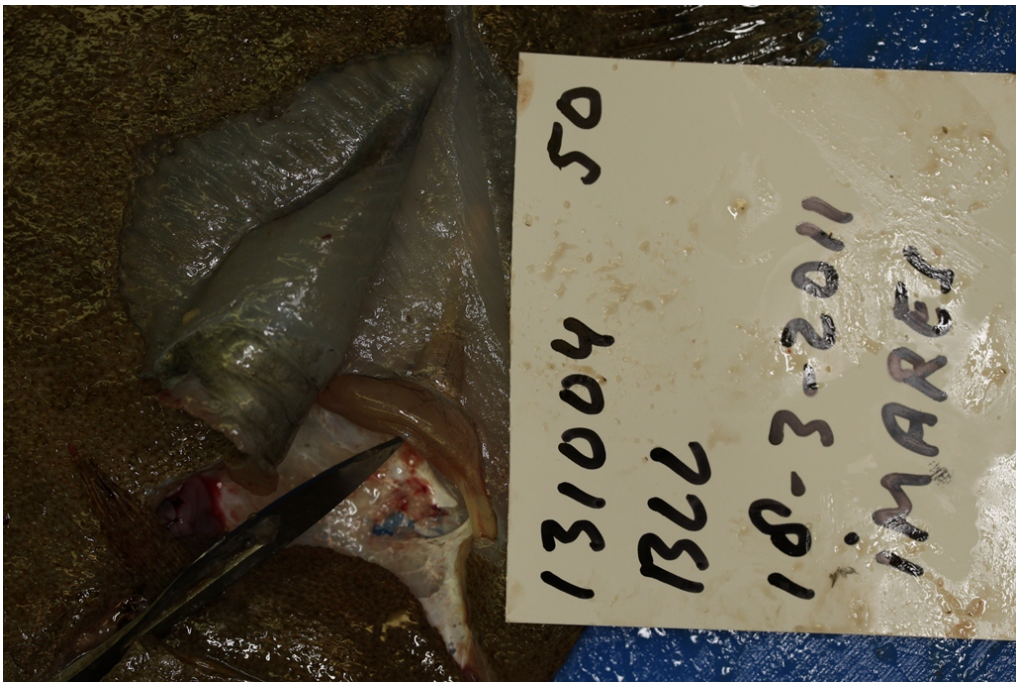
2.1 Stage 1 – Juvenile

Primary:

- Ovaries are mostly transparent
- Gonads extend beyond the body cavity, but are less than 5 cm in length
- Eggs cannot be identified macroscopically

Secondary:

- Ovaries pinkish ("jelly structure")
- Blood vessels hardly discernable



pictures: ovary in the fish NED2011_bll_131004_050_1.jpg, overview (upper) and in detail NED2011_bll_131004_050_2.jpg (lower)

2.2 Stage 2 - Maturing

Primary:

- No hydrated eggs visible
- In early stage 2: no eggs are visible and limited development of the ovary
- In later stage 2: eggs are opaque and can be identified macroscopically
- Ovaries start filling the body cavity.

Secondary:

- Ovaries with yellowish orange to light pink or red coloration
- The eggs do not flow at light pressure
- Blood vessels larger and diversified

pictures: ovary in the fish NED2010_bll_131015_006_1.jpg (upper left), detail NED2010_bll_131015_006_2.jpg (lower left), outside the fish (upper right) NED2010_bll_131015_006_5.jpg , and cut open NED2010_bll_131015_006_6.jpg (lower right).

pictures: detail of ovary in the fish NED2010_bll_131015_004_2.jpg (upper), overview NED2010_bll_131015_004_1.jpg (lower left) , and outside the fish NED2010_bll_131015_004_2.jpg (lower right).

2.3 Stage 3 - Spawning

Primary:

- one to many hydrated eggs visible in tissue among vitellogenic oocytes
- in late stage 3: occasionally running under light pressure

Secondary:

- ovaries do not change in size and volume compared to stage 2

NB: check both ovaries as eggs are small and hydrated eggs can easily be missed

pictures: detail of ovary in the fish NED2011_bll_131007_023_3.jpg (upper), overview NED2011_bll_131007_023_4.jpg (lower left) , and outside the fish NED2011_bll_131007_023_5.jpg (lower right).

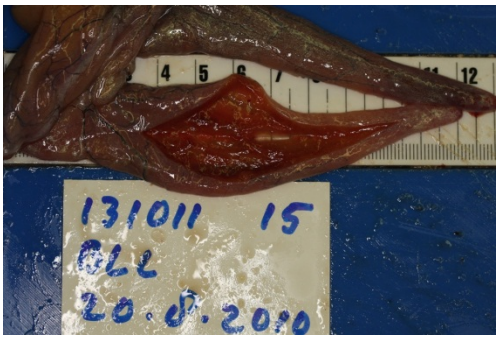
2.4 Stage 4 – Spent

Primary:

- ovaries slack in early phase, later contracting
- a few hydrated eggs could be left but never vitellogenic oocytes

Secondary:

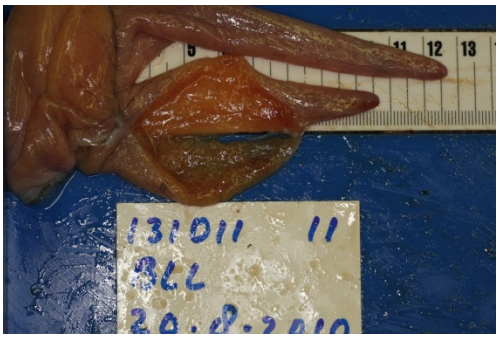
- colour: greyish to purple
- inside mucus is common (colour: red)



pictures: detail of ovary in the fish NED2010_bll_131011_015_2.jpg (upper), overview NED2010_bll_131011_015_1.jpg (lower left) , and cut open outside the fish NED2010_bll_131011_015_6.jpg (lower right).

2.5 Stage 5 – Skipped spawning

Rare. Macroscopic identification is very difficult and should always be checked histologically.



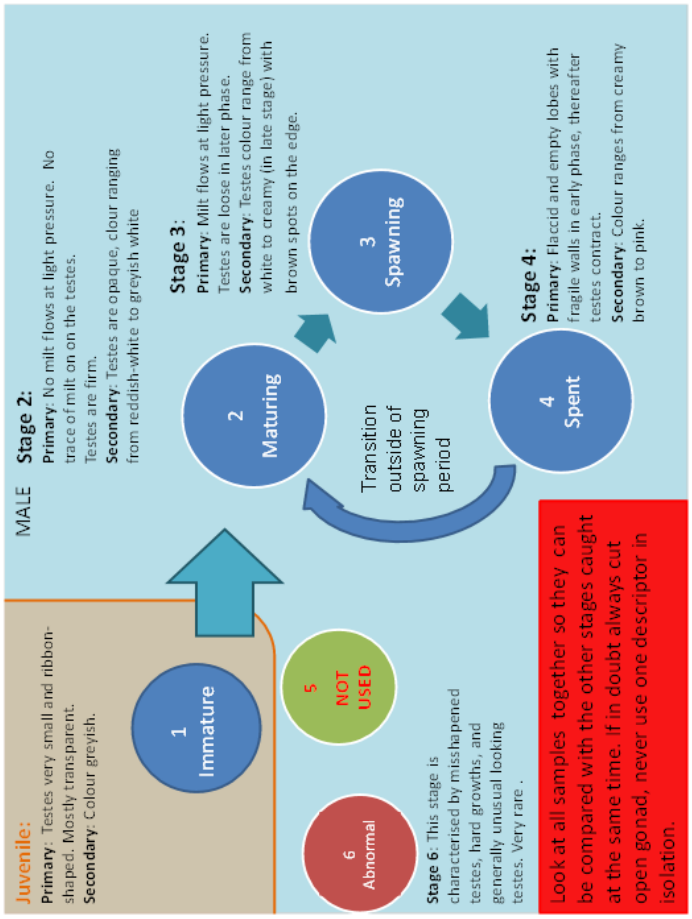
pictures: detail of ovary in the fish NED2010_bll_131011_011_2.jpg (upper), overview NED2010_bll_131011_011_1.jpg (lower left) , and cut open outside the fish NED2010_bll_131011_011_6.jpg (lower right).

2.6 Stage 6 – Abnormal

This stage is characterised by mis-shapened ovaries, hard growths, and generally unusual looking ovaries. For example: stony gonads, both sexes present in the gonad, etc. Very rare.

No pictures available.

3 Male


MALE
Juvenile:
Primary: Testes very small and ribbon-shaped. Mostly transparent.
Secondary: Colour greyish.
1 Immature
Stage 2:
Primary: No milt flows at light pressure. No trace of milt on the testes. Testes are firm.
Secondary: Testes are opaque, clour ranging from reddish-white to greyish white
2 Maturing
Stage 3:
Primary: Milt flows at light pressure. Testes are loose in later phase.
Secondary: Testes colour range from white to creamy (in late stage) with brown spots on the edge.
3 Spawning
Stage 4:
Primary: Flaccid and empty lobes with fragile walls in early phase, thereafter testes contract.
Secondary: Colour ranges from creamy brown to pink.
4 Spent
Transition outside of spawning period
5 NOT USED
6 Abnormal
Stage 6: This stage is characterised by misshapened testes, hard growths, and generally unusual looking testes. Very rare .
Look at all samples together so they can be compared with the other stages caught at the same time. If in doubt always cut open gonad, never use one descriptor in isolation.

3.1 Stage 1 – Juvenile

Primary:

- testes very small and ribbon-shaped
- mostly transparent

Secondary:

- colour: greyish
- blood vessels hardly discernable.

No pictures available.

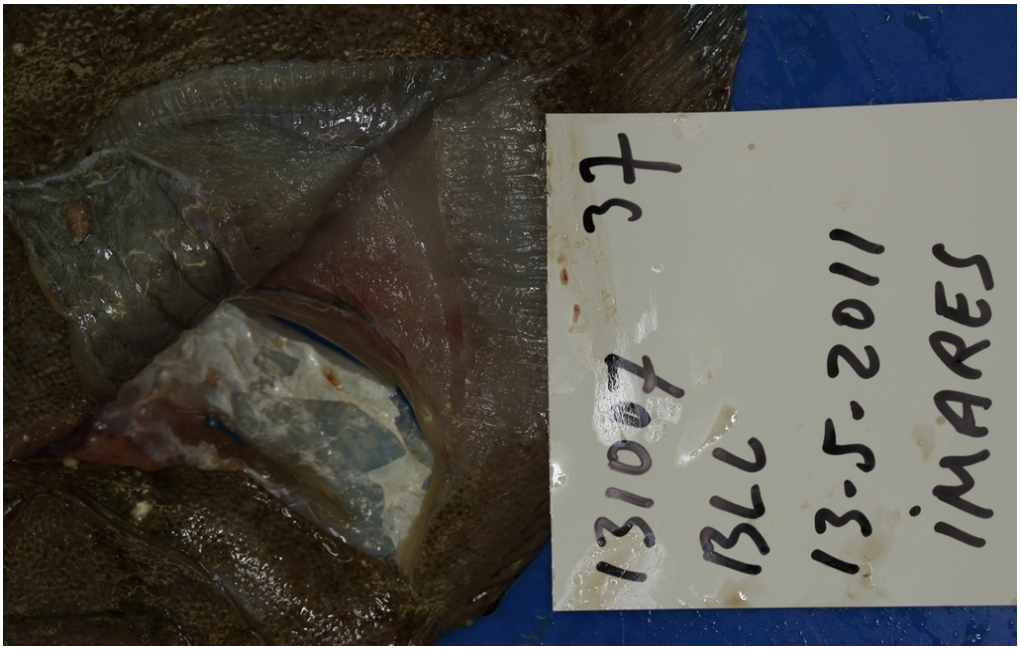
3.2 Stage 2 – Maturing

Primary:

- no milt flows at light pressure
- no trace of milt on the testes
- testes are firm

Secondary:

- testes are opaque
- colour: ranging from reddish-white to greyish white
- spermatoducts are transparent and empty
- blood vessels can be present



pictures: overview of testis in the fish NED2011_bll_131007_037_1.jpg (upper) and detail NED2011_bll_131007_037_2.jpg (lower)

3.3 Stage 3 – Spawning

Primary:

- milt flows at light pressure
- testes are loose in later phase.

Secondary:

- colour: ranging from white to creamy (in late stage) with brown spots on the edge
- spermatoducts are well developed and filled with milt

No pictures available.

3.4 Stage 4 – Spent

Primary:

- flaccid and empty lobes with fragile walls in early phase
- in a later phase testes contract.

Secondary:

- colour: creamy brown to pink.
- Milt residue can be present

No pictures available.

3.5 Stage 5 – Skipped spawning

This stage is not used for male fish.

3.6 Stage 6 – Abnormal

This stage is characterised by mis-shapened testes, hard growths, and generally unusual looking testes. Very rare.

No pictures available.