

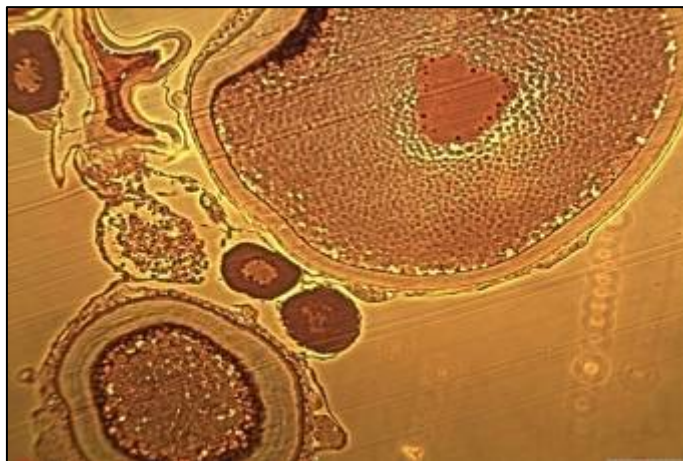
# **Apendiks / Appendix**

**Manual to determine gonadal maturity of North Atlantic cod (*Gadus morhua*) inshore in West Greenland. Macroscopic and histological**



# Manual to determine gonadal maturity of North Atlantic cod (*Gadus morhua*) inshore in West Greenland

## Macroscopic and histological



# ***Introduction***

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This manual is developed based on data collected in the Nuuk fjord system from December 2007 to November 2008 in collaboration with Greenland Institute of Natural Resources (Haidarz *et al.*, in prep.). It is meant as a field- and laboratory manual for determination of the maturity stages of Atlantic cod (*Gadus morhua*) in Greenland, primarily the inshore population.

## ***Structure of the manual***

The manual describes ten maturity stages (Tomkiewicz *et al.*, 2003) for Atlantic male and female cod respectively. For each particular stage is a description of the macroscopic- and histological characteristics along with illustrations that display the specific features of the stages. Each page of the manual show only one maturity stage of either male or female cod. *Note that the macroscopic and histological pictures from a given stage not necessarily represents the same fish.* The combination of both macroscopic- and histological descriptions and illustrations for each stage gives a better understanding of the developmental characteristics for the particular stages. The macroscopic descriptions of the ten maturity stages are based on the maturity manual by Tomkiewicz *et al.* (2002). The histological descriptions of the ten stages of female cod are based on Tomkiewicz *et al.* (2003). The workshop report from ICES (2008) and Morrison (1990) are used for both macroscopic and histological identifications for both male and female cod.

## ***Informations in the manual***

There is great variation within the stages, and the illustrations are therefore only meant as guidelines representing the general pattern. In addition the transition between the stages is gradual and it can be very difficult to differentiate two successive stages. To help the identification process some supplemental facts are added based on the study by Haidarz *et al.* (in prep.). The most likely period in which the particular stage can be seen; the fish length ( $L_T$ ), the clean fish weight ( $W_{cl}$ ) and the range of gonad weight ( $W_{GO}$ ). As an additional help ranges of gonadosomatic index ( $GSI$ ), describing the relative relation between  $W_{GO}$  and  $W_{cl}$ , from Tomkiewicz *et al.* (2002 and 2003) is added. For histological determination of the female gonadal maturity stage the most likely range of oocyte diameter ( $d$ ) and ratio of nucleus to oocyte diameter ( $n:o$ ) are added where relevant (Tomkiewicz *et al.*, 2003).

## ***Observations in the field***

When sampling in the field it is helpful for later histological validation of the maturity stage if as many details about the fish as possible are noted. In addition to total length, total weight, clean weight, gonad weight and liver weight, also the gonad length can be helpful. Most importantly note if the semen is running freely as the fish is pulled out of the water. The latter observation is very useful when vacillating between males in stage V and stage VI, which can be very difficult to distinguish both macroscopically and histologically; only stage VI has freely running semen.

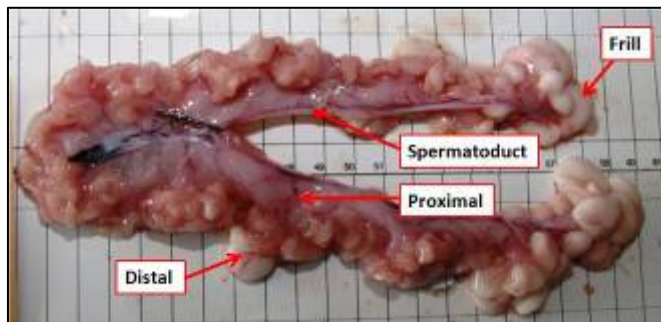
# ***Introduction***

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## ***Histological sampling***

When taking samples from the testes for histological analysis it is very important to cut the testes transversely and keep the orientation of the sample. Due to a spatial heterogeneity in the development of the tissue, the least developed germ cells are mainly concentrated in the distal part of the testis, while the most advanced germ cells are concentrated mainly in the proximal part (Morrison, 1990; and ICES, 2008). The combination of information from both the distal and the proximal part gives a stronger basis for a more precise identification of the maturity stage. The distal part (from the mesorchium) is the “frill side” and the proximal part is near the spermatoduct (Morrison, 1990) (Figure A).

When determining the reproductive stage for females histological, it is the most developed oocytes that are used as stage indicators (ICES, 2008).



**Figure A** Male reproductive organ

## ***References***

- ICES (2008). Report of the Workshop on Sexual Maturity Staging of Cod, Whiting, Haddock and Saithe (WKMSCWHS), 13–16 November 2007, Copenhagen, Denmark. ICES CM 2007/ACFM:33. 62 pp.
- Haidarz, K., Thomsen, S. S., Jensen, K. T. & Retzel, A. (in prep.) Maturity Determination and Spawning Characteristics for North Atlantic Cod (*Gadus morhua*) in the Nuuk Fjord System. MSc-thesis, Department of Biological Sciences, Aarhus University.
- Morrison, C. M. (1990). Histology of the Atlantic cod, *Gadus Morhua*; an atlas. Part three. Reproductive tract. *Canadian Special Publications of Fisheries and Aquatic Sciences* **100**.
- Tomkiewicz, J., Tybjerg, L., Holm, N., Hansen, A., Broberg, C., & Hansen, E. (2002). Manual to determine gonadal maturity of Baltic cod. DFUrapport. 116 - 02, Charlottenlund: Danish Institute for Fisheries Research. 49 pp.
- Tomkiewicz, J., Tybjerg, L. & Jespersen, A. (2003). Micro - and macroscopic characteristics to stage gonadal maturation of female Baltic cod. *Journal of Fish Biology* **62**, 253–275.

## Macroscopic characteristics

The ovaries are located posterior in the body cavity. They are very small and can appear translucent and glassy with thin walls. Blood vessels are almost not visible or at least very thin. Stage I can be seen all year round.  $L_T$ : 28 – 58 cm;  $W_{CL}$ : 200 – 1710 g;  $W_{GO}$ : < 12 g;  $GSI$  < 1.0.



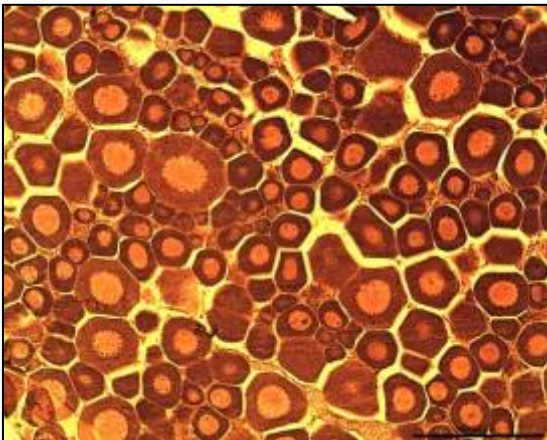
**Fish data:** Id: 3;  $L_T$ : 44 cm; Age: 4;  $W_{CL}$ : 718 g;  $W_{GO}$ : 4 g;  $GSI$ : 0.6; Date: March 11.

## Histological characteristics

In this stage all the oocytes are in the perinuclear (PN) stage with a large central nucleus that stains a lighter colour than the rest of the oocyte. The nucleus has a ring of peripheral nucleoli. The ovary wall is thin and the ovigerous folds are easy to distinguish.  $d$ : < 80  $\mu$ m.

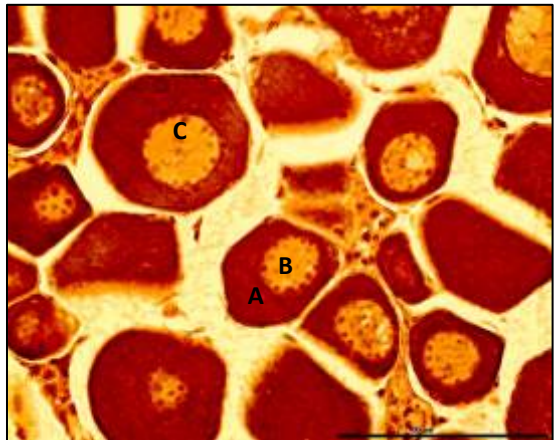
NOTE: Pictures are from a stage II female, but the extractions represent how a stage I would appear.

Magnification x10 bar 250  $\mu$ m



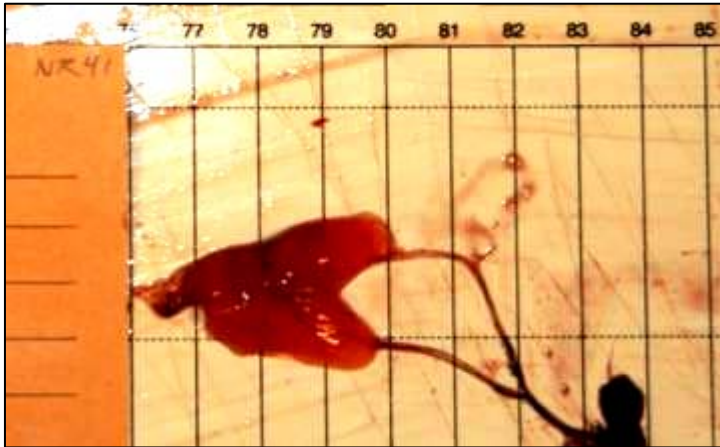
A: PN; B: Nucleus; C: Nucleolus

Magnification x40 bar 100  $\mu$ m



## Macroscopic characteristics

The ovaries are small and located posterior in the body cavity. They can appear translucent and glassy as in stage I but they can also appear somewhat blurred due to oocytes in CNR stage. They are soft and flatten on a solid sheet. Blood vessels are almost not visible or at least very thin. Stage II can be seen all year round.  $L_T$ : 34 – 69 cm;  $W_{CL}$ : 340 – 2700 g;  $W_{GO}$ : < 12 g;  $GSI$  < 1.5.

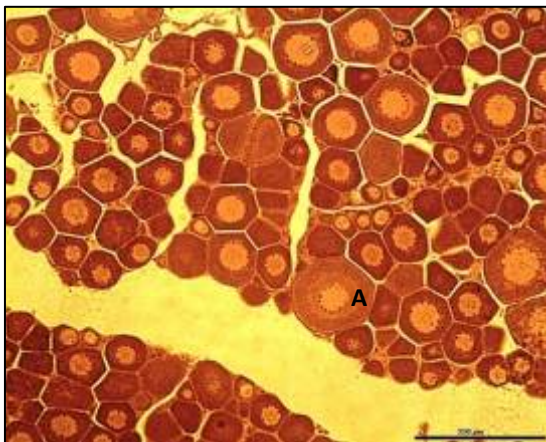


**Fish data:** Id: 41;  $L_T$ : 39 cm; Age: 4;  $W_{CL}$ : 510 g;  $W_{GO}$ : 2.8 g;  $GSI$ : 0.5; Date: February 5.

## Histological characteristics

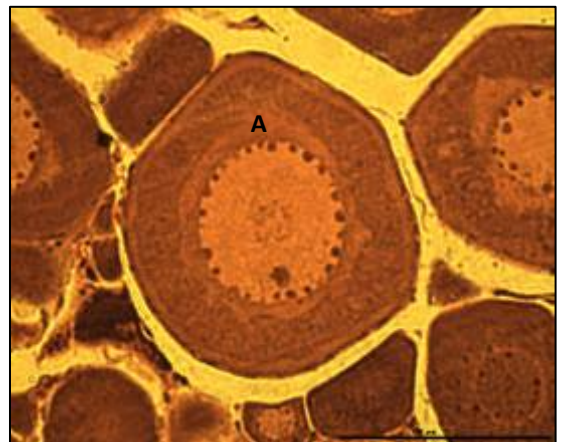
Some of the oocytes have grown in size and a light staining vasculated ring has emerged around the nucleus, this is called the circumnuclear ring (CNR) stage of the oocyte.  $d$ : 90-160  $\mu$ m.

Magnification x10 bar 250  $\mu$ m



A: CNR

Magnification x40 bar 100  $\mu$ m



## Macroscopic characteristics

The ovaries are still located posterior in the body cavity and are somewhat larger than stage I and II. They appear opaque with a creamy orange colour. They are firm and keep form on solid sheet. Blood vessels are becoming prominent. Stage III is most likely to be seen in the prespawning period from December – April, before that it is likely to be a stage IX and after it is likely to be stage VIII or IX.  $L_T$ : > 41 cm;  $W_{Cl}$  > 400 g;  $W_{GO}$ : 12 – 159 g;  $GSI$ : 1.0 – 7.5.



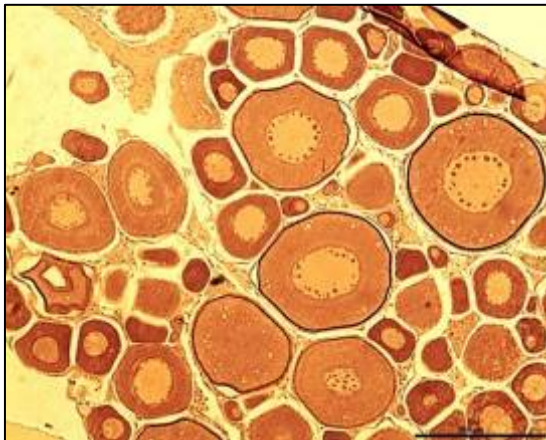
**Fish data:** Id: 35;  $L_T$ : 48 cm; Age: 4  $W_{Cl}$ : 890 g;  $W_{GO}$ : 15.7 g;  $GSI$ : 1.8; Date: February 5.

## Histological characteristics

The characteristic feature of this stage is the formation of cortical alveoli (CA) – transparent vesicles in the periphery of the cytoplasm. The CNR have disappeared in these CA oocytes. Yolk droplets appear in the periphery of the cell near the cell membrane and later between the CA, which indicates the beginning of vitellogenesis. The nucleoli become detached.  $d$ : 170-290  $\mu$ m;  $n:o$  > 0.4.

Magnification x10

bar 250  $\mu$ m



**A:** CA; **B:** Yolk

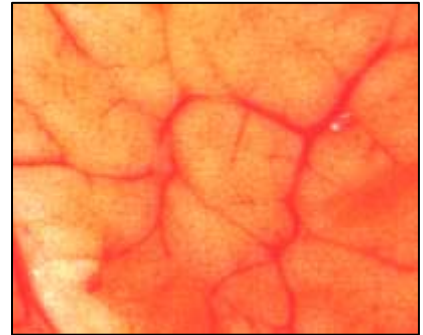
Magnification x20

bar 100  $\mu$ m



## Macroscopic characteristics

The ovaries extend to middle of body cavity. They appear opaque and blurred with a creamy yellowish colour. They are firm and keep form on solid sheet. Blood vessels are prominent. Vitellogenic oocytes are visible as small opaque granules. Stage IV is most likely to be seen in the prespawning period from December – May.  $L_T$ : > 41 cm;  $W_{CL}$ : > 400 g;  $W_{GO}$ : 12 – 328 g;  $GSI$ : 3.0 – 14.0.

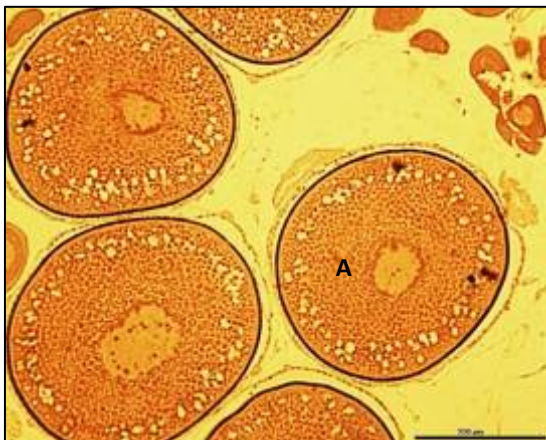


**Fish data:** Id: 20;  $L_T$ : 60 cm; Age: 5  $W_{CL}$ : 2047 g;  $W_{GO}$ : 174 g;  $GSI$ : 8.5; Date: March 26.

## Histological characteristics

Yolk droplets enlarge and fill most of cytoplasm which increases the oocyte size – The oocytes are in late vitellogenic stage. The nucleus becomes irregular shaped but is still located in the center of the oocyte.  $d$ : 300-530  $\mu\text{m}$ ;  $n:o$  < 0.4.

Magnification x10 bar 250  $\mu\text{m}$



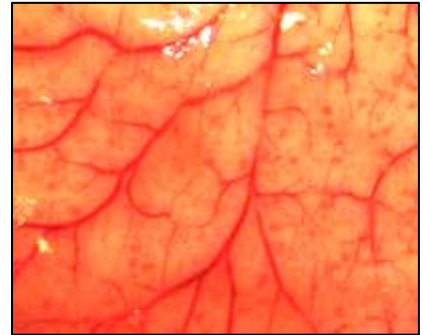
A: Yolk

Magnification x10 bar 250  $\mu\text{m}$



## Macroscopic characteristics

The ovaries are large and extend into the anterior part of the body cavity. They appear opaque with a creamy pink colour. They are soft with viscous fluid, which can be seen when the ovary is cut open. They contain single hydrated oocytes that appear glassy, which is the main indication of a spawning stage. Blood vessels are prominent. Stage V is most likely to be seen in the spawning period from late April and in May.  $L_T$ : > 41 cm;  $W_{CL}$  > 400 g;  $W_{GO}$ : 139 – 1180 g;  $GSI$ : 12.0 – 25.0.



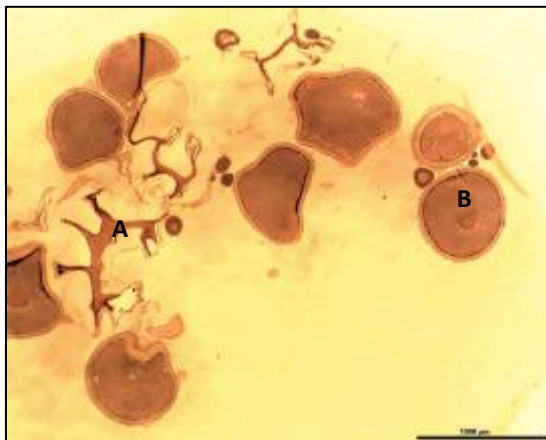
**Fish data:** Id: 15;  $L_T$ : 57 cm; Age: 5  $W_{CL}$ : 1480 g;  $W_{GO}$ : 174.4 g;  $GSI$ : 11.8; Date: April 30.

## Histological characteristics

Vitellogenic oocytes with yolk granules throughout the cytoplasm (VT3) dominate. The position of the nucleus becomes acentric as it migrates towards the micropyle, and eventually is no longer visible in hydrated oocytes (HYD). Hydrated oocytes contain a homogenous content of coalesced yolk and can have an irregular shape due to the histological processing. There might be single postovulatory follicles (POF) from hydrated oocytes which have been ovulated into the lumen of the oocyte.  $d$ : 500-970  $\mu$ m.

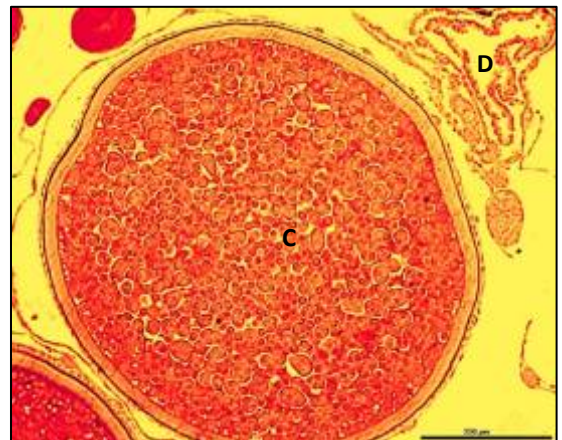
Magnification x2.5

bar 1000  $\mu$ m



Magnification x10

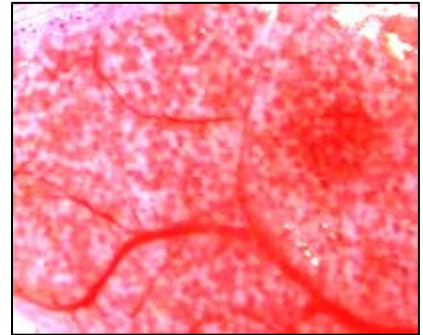
bar 250  $\mu$ m



**A:** HYD; **B:** migrating nucleus; **C:** Oocyte filled with yolk just before hydration; **D:** POF

## Macroscopic characteristics

The ovaries are distended and fill most of the body cavity. They appear granulated filled with hydrated glassy oocytes and vitellogenic opaque oocytes. They are very soft and lumen contains viscous fluid. Blood vessels are prominent. Stage VI is most likely to be seen in May and June.  $L_T$ : > 41 cm;  $W_{CL}$  > 400 g;  $W_{GO}$ : 163 – 2260 g;  $GSI$ : 15.0 – 60.0.

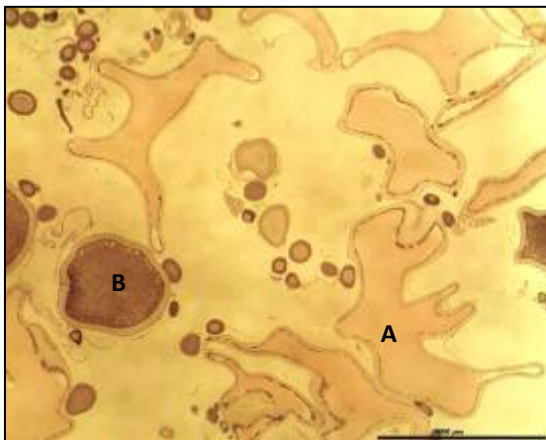


**Fish data:** Id: 2;  $L_T$ : 61 cm; Age: 5  $W_{CL}$ : 1880 g;  $W_{GO}$ : 214.5 g;  $GSI$ : 11.4; Date: May 25.

## Histological characteristics

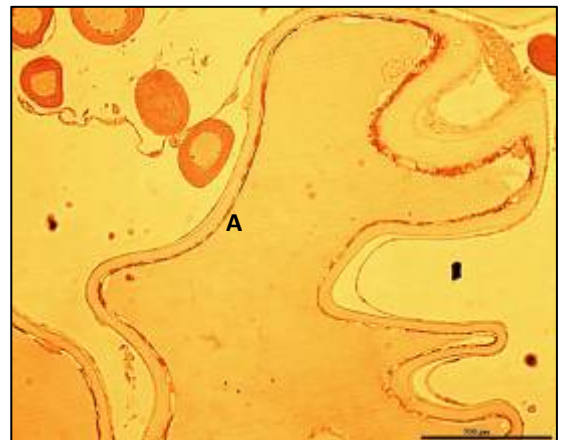
This stage contains the same developmental features of the oocytes as stage V (VT3, HYD and POF) but they are now all abundant.  $d$ : 510-1000  $\mu$ m.

Magnification x3.2 bar 1000  $\mu$ m



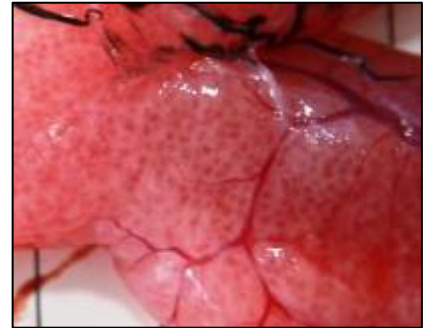
**A:** HYD; **B:** VT3

Magnification x10 bar 250  $\mu$ m



## Macroscopic characteristics

The ovaries have shrunk to middle and posterior part of body cavity. Hydrated glassy oocytes are still present and visible lying in viscous fluid. The ovaries are flabby with a grayish colour and walls are thickened. Blood vessels are prominent. Stage VII is most likely to be seen from May to July.  $L_T$ : > 41 cm;  $W_{CL}$  > 400 g;  $W_{GO}$ : 19 – 219 g;  $GSI$ : 3.0 – 8.0.

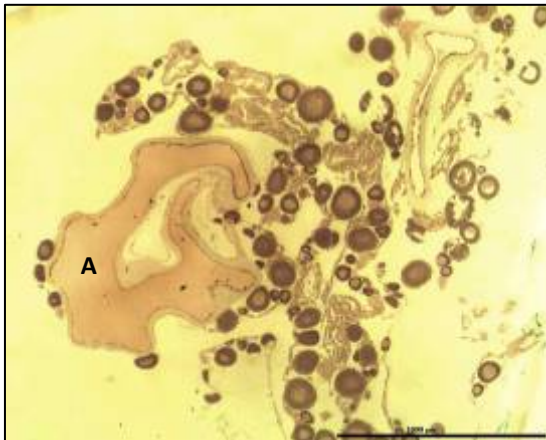


**Fish data:** Id: 9;  $L_T$ : 48 cm; Age: 4  $W_{CL}$ : 1060 g;  $W_{GO}$ : 32.3 g;  $GSI$ : 3.0; Date: May 22.

## Histological characteristics

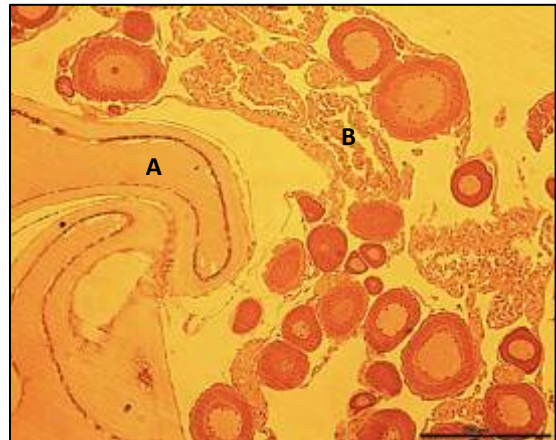
In this stage there are very few if any VT3 oocytes; the number of hydrated (HYD) oocytes are only moderate as most of them have been ovulated and as a logical consequence the presence of empty follicles (POF) are now eminent.  $d$ : 450-930  $\mu$ m.

Magnification x4 bar 1000  $\mu$ m



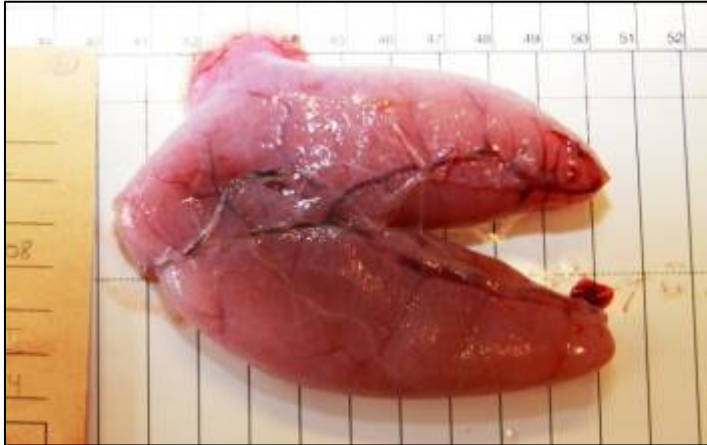
**A:** HYD; **B:** POF

Magnification x10 bar 250  $\mu$ m



## Macroscopic characteristics

Ovaries have contracted to posterior part of body cavity. Few hydrated glassy oocytes can still be visible but also atretic eggs that appears as opaque irregular granules. The walls are thickened and opaque often with a grayish colour. Blood vessels are prominent. Stage VIII is most likely to be seen from May.  $L_T$ : > 41 cm;  $W_{CL}$  > 400 g;  $W_{GO}$ : 6 – 306 g;  $GSI$ : 2.0 – 3.0 but with atresia it can be up to 10.

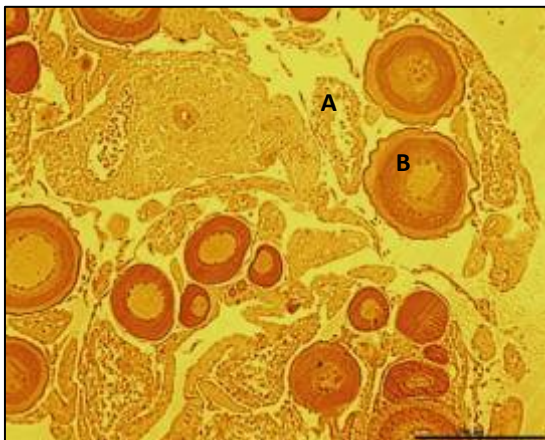


**Fish data:** Id: 5;  $L_T$ : 69 cm; Age: 6  $W_{CL}$ : 2540 g;  $W_{GO}$ : 72 g;  $GSI$ : 2.8; Date: May 25.

## Histological characteristics

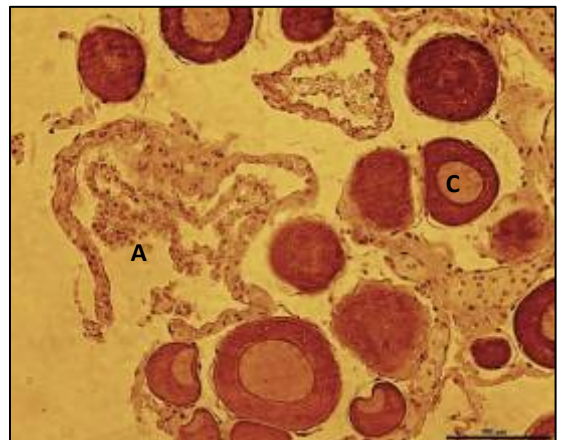
The postovulatory follicles are dominant but the presence of perinuclear and circumnuclear oocytes are increasing. Some of the vitellogenic oocytes that failed to mature completely are starting to reabsorb; this is called atresia.  $d$ : 450-930  $\mu$ m.

Magnification x10 bar 250  $\mu$ m



**A:** POF; **B:** CNR; **C:** PN

Magnification x20 bar 100  $\mu$ m



# Stage IX – Regeneration: Resting / spawning omission

## Females

### Macroscopic characteristics

Ovaries are small and restricted to posterior part of body cavity. They can be semi translucent and look similar to immature ovaries in preperation but they can also bee opaque with a grayish colour. Blood vessels can be prominent and often more visible than in stage II. Stage IX can be seen in the prespawning- and spawning period (December-June) as skip-of-spawning; in the postspawning period as spent; or the rest of the year as resting.  $L_T > 41$  cm;  $W_{Cl} > 400$  g;  $W_{GO}: 2 - 87$  g;  $GSI: 1.0 - 3.0$ .

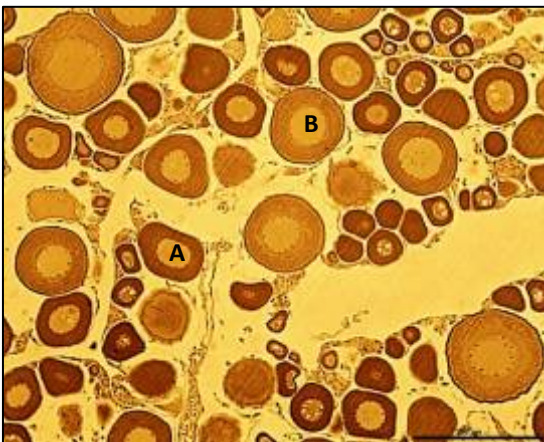


**Fish data:** Id: 9;  $L_T$ : 64 cm; Age: 5  $W_{Cl}$ : 2140 g;  $W_{GO}$ : 12.2 g;  $GSI$ : 0.6 g; Date: June 12.

### Histological characteristics

Resembles stage II a lot with mainly perinuclear and circumnuclear oocytes but in this stage there are also traces of previous spawning in form of atretic (AT) oocytes and maybe a few postovulatory follicles that have not yet been resorbed. The ovary wall is thickened  $d: 140-170$   $\mu$ m.

Magnification x10 bar 250  $\mu$ m



A: PN; B: CNR; C: AT

Magnification x20 bar 100  $\mu$ m



## **Stage X – Degeneration: Reduced fertility**

### **Females**

#### **Macroscopic characteristics**

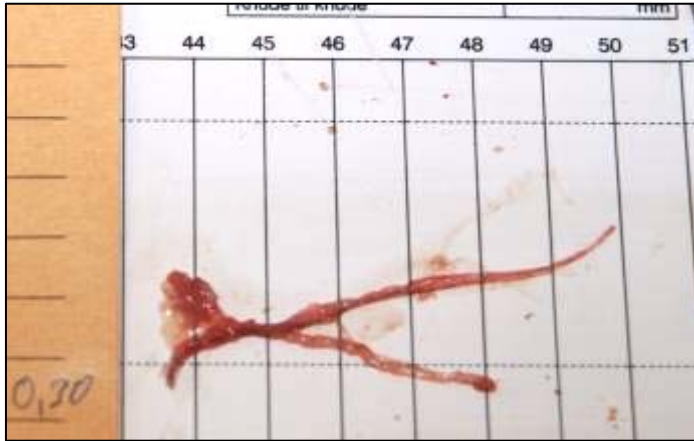
No data; but this stage is recognized by an abnormal appearance of the ovary e.g. hard brown parts of connective tissue. Stage X can be seen all year round.

#### **Histological characteristics**

An abnormal appearance of the oocytes; maybe lot of atretic eggs or hydrated oocytes which have not been ovulated but instead encapsulated in connective tissue. The abnormal features might only appear in certain areas of the ovary.

## Macroscopic characteristics

Testes appear as two very thin strings lying along the air bladder. The frills are also tiny and can be difficult to distinguish. There are no signs of previous spawning. Stage I can be seen all year round.  $L_T$ : 30 - 56 cm;  $W_{CL}$  200 - 1500 g;  $W_{GO}$ : < 3 g;  $GSI$  < 0.1.



**Fish data:** Id: 41;  $L_T$ : 34 cm; Age: 3  $W_{CL}$ : 300 g;  $W_{GO}$ : 0.2 g;  $GSI$ : 0.1; Date: February 21.

## Histological characteristics

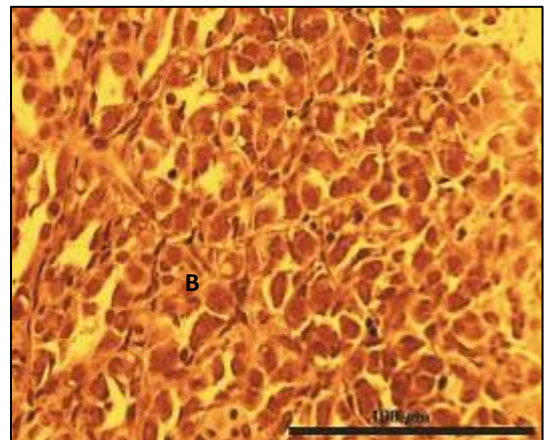
This stage is characterized by only having spermatogonia (SG) with a distinct nucleus. They are mainly located distally but some can also be found proximally lining tubules or efferent ducts. They are either found as single cells or lodged within small cysts. The walls of the testes are thin in this stage.

Magnification x10 bar 250  $\mu$ m



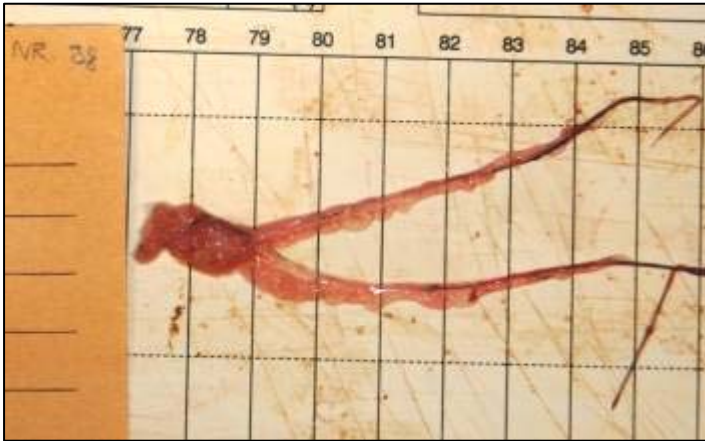
**A:** Efferent duct; **B:** SG; **C:** Frills

Magnification x20 bar 100  $\mu$ m



### Macroscopic characteristics

Testes are still very small and lying along air bladder. The frills are a little more prominent than in stage I. There are no signs of previous spawning. Stage II can be seen all year round.  $L_T$ : 34 – 52 cm;  $W_{CL}$  320 – 1280 g;  $W_{GO}$ : < 3 g;  $GSI$ : 0.1 – 0.5.



**Fish data:** Id: 38;  $L_T$ : 42 cm; Age: 4  $W_{CL}$ : 650 g;  $W_{GO}$ : 0.8 g;  $GSI$ : 0.1; Date: February 5.  
Note: This is a stage I but looks similar to a possible stage II.

### Histological characteristics

This stage is similar to stage I but some of the spermatogonia have divided into primary spermatocytes that are found in cysts.

Note: There are no histological pictures of this stage, but the germ cells present in this stage can be seen in stage III; next page.

## Macroscopic characteristics

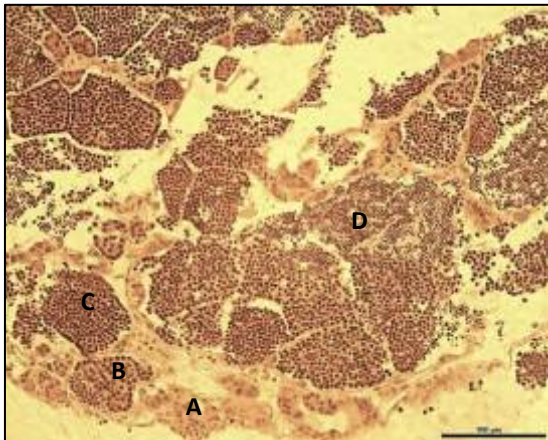
This stage is recognized by testes that are somewhat larger than stage II, due to multiple cell divisions (see histological characteristics below). They have easily distinguishable and soft frills rich in blood vessels. Spermatoducts are translucent and with no signs of semen. Stage III can primarily be seen in the prespawning period from December – April.  $L_T$ : > 36 cm;  $W_{CL}$  > 360 g;  $W_{GO}$ : 3 – 88 g;  $GSI$ : 0.5 – 6.0. Note: There is no picture of a testis in stage III. In comparison to stage IV (next page) the frills are not as distended.

## Histological characteristics

Almost all the cysts with spermatogonia (SG) have now divided into cysts with primary spermatocytes (SC1). Some of the cysts with SC1 have divided into cysts with secondary spermatocytes (SC2), and some of these have divided further into spermatides (ST). The cysts have now enlarged considerably due to the numerous cell divisions. There might also be a few cysts with nonmotile flagellated maturing spermatozoa lying with their heads together, facing the interstitial tissue between the tubules and the flagella pointing in the same direction (see histological pictures in next page).

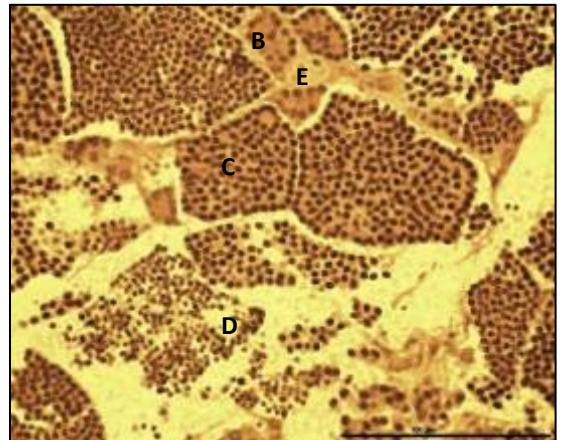
NOTE: : Pictures are from a stage IV male, but the extractions represent how a stage III would appear.

Magnification x20 bar 100  $\mu$ m



A: SG; B: SC1; C: SC2; D: ST; E: Interstitial tissue

Magnification x40 bar 100  $\mu$ m



## Macroscopic characteristics

Testes are enlarged and the frills are very prominent, opaque and whitish with many blood vessels. The Spermatoducts are also prominent but with no signs of semen. Stage IV can primarily be seen from December – May.  $L_T$ : > 36 cm;  $W_{CL}$  > 360 g;  $W_{GO}$ : 14 – 66 g;  $GSI$ : 1.0 – 18.0.

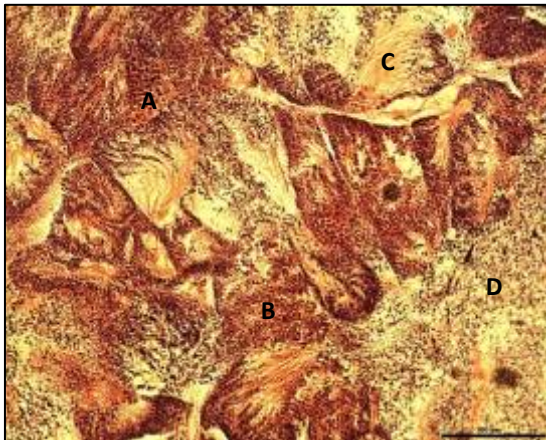


**Fish data:** Id: 7;  $L_T$ : 69 cm; Age: 7  $W_{CL}$ : 3130 g;  $W_{GO}$ : 635 g;  $GSI$ : 20.3; Date: February 21.

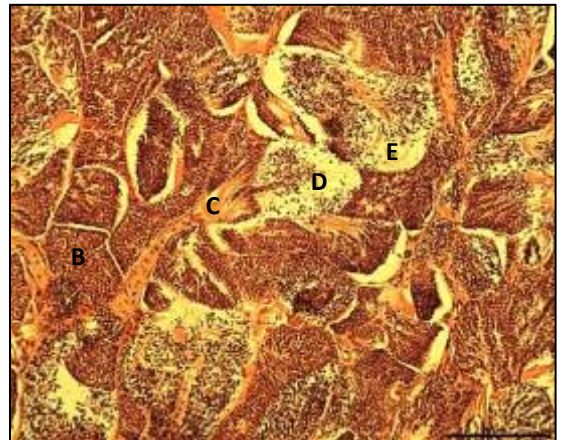
## Histological characteristics

In this stage all the cell types of spermatogenesis are present. The earlier stages; spermatogonia and spermatocytes (SC) predominate in the distal part of the testis and the later stages spermatides (ST) and spermatozoa (SZ) predominate in the proximal part. Some cysts with maturing SZ in the proximal part break down and form tubules with mature SZ. These tubules are lined with developing cysts of earlier developmental stages of cells. There might be few motile SZ in the large efferent ducts near the spermatoducts.

Magnification x20 bar 100  $\mu$ m



Magnification x20 bar 100  $\mu$ m



**A:** SC; **B:** ST; **C:** Maturing SZ; **D:** Mature SZ; **E:** Tubule from merged cysts

## Macroscopic characteristics

Testes are very large and fill most of body cavity. Frills are prominent and can be quite brittle; they are opaque with a creamy white appearance and with many blood vessels. The spermatoducts now contain semen appearing as a viscous opaque white fluid. Stage V can primarily be seen in the beginning of the spawning period from February – April.  $L_T$ : > 36 cm;  $W_{Cl}$  > 360 g;  $W_{GO}$ : 14 – 1200 g;  $GSI$ : 3.0 – 22.0.



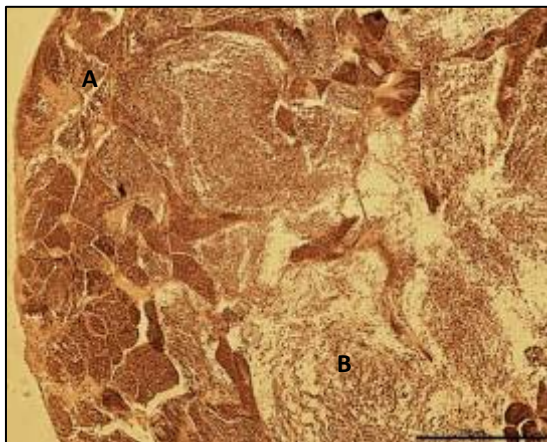
**Fish data:** Id: 38;  $L_T$ : 52 cm; Age: 4  $W_{Cl}$ : 1190 g;  $W_{GO}$ : 176 g;  $GSI$ : 14.8; Date: February 21.

## Histological characteristics

Proximally almost all the cysts have broken down and coalesced to form large tubules filled with mature and ripe spermatozoa (SZ). There are however still cysts of developing cell stages lining the tubules, mainly maturing SZ lying with their heads together and the flagella pointing in the same direction. At the far distal side all the early development stages; spermatogonia, spermatocytes, spermatids and also maturing SZ can be found.

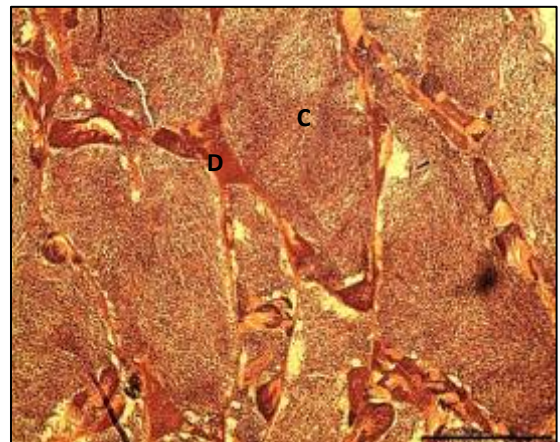
Magnification x10

bar 250  $\mu$ m



Magnification x10

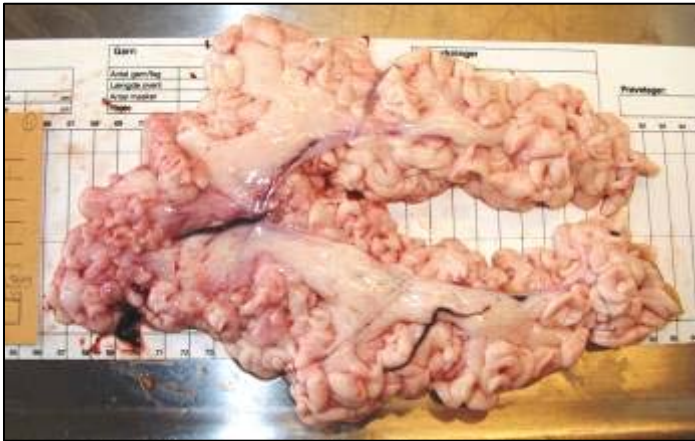
bar 250  $\mu$ m



**A:** Distal side with maturing germ cells; **B:** Ripe SZ; **C:** Large tubules with mature SZ; **D:** Tubule walls lined by developing germ cells

## Macroscopic characteristics

Testes are still very large and fill most of body cavity. Frills are prominent and brittle; they are opaque and creamy white. The spermatoducts contain semen as a thin fluid that appear whitish opaque but can also be more translucent. Stage VI can primarily be seen from April – June.  $L_T$ : > 36 cm;  $W_{Cl}$  > 360 g;  $W_{GO}$ : 14 – 619 g;  $GSI$ : 3.0 – 25.0.

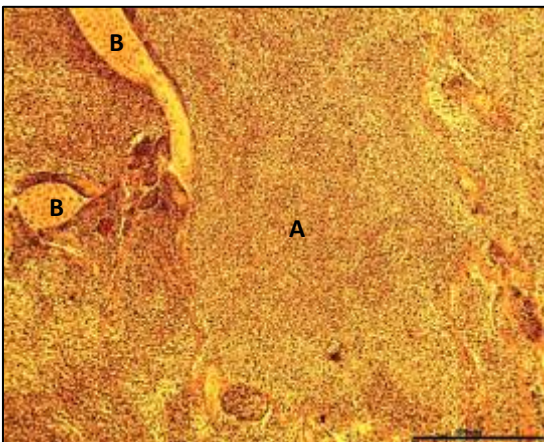


**Fish data:** Id: 11;  $L_T$ : 81 cm; Age: 8  $W_{Cl}$ : 4960 g;  $W_{GO}$ : 367.7 g;  $GSI$ : 7.4; Date: May 8.

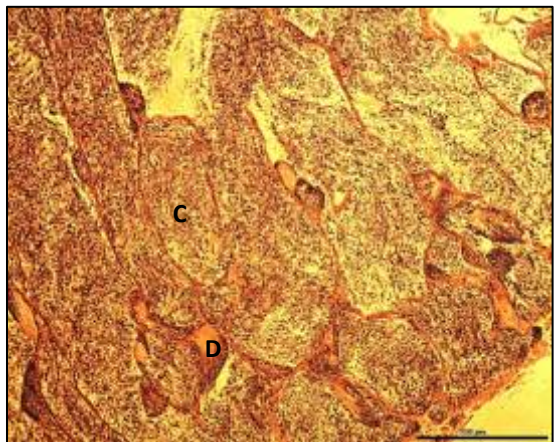
## Histological characteristics

Tubules and efferent ducts are filled with spermatozoa (SZ) and in the proximal part of the testes the sperm ducts and efferent ducts contain ripe SZ which lies disorderly. In the proximal part there are no cysts of maturing SZ where the heads and the flagella are aligned (difference between stage V and VI). In the far distal part there might however still be some maturing SZ like in stage V and developing germ cells can still be found.

Magnification x10 bar 250  $\mu$ m



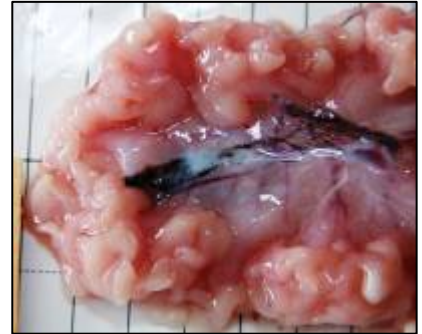
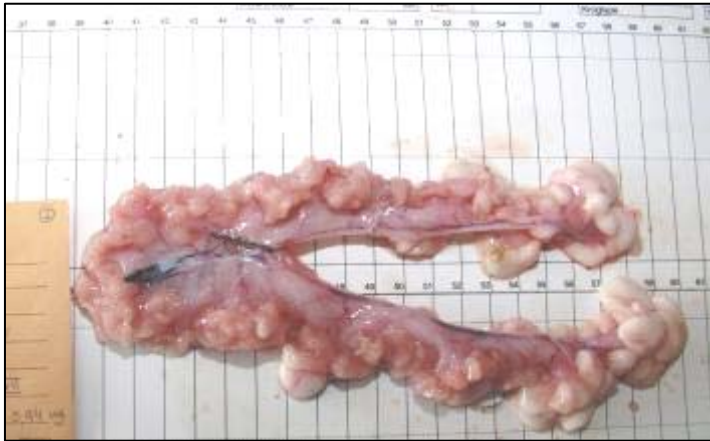
Magnification x10 bar 250  $\mu$ m



**A:** Ripe SZ; **B:** Blood vessels; **C:** Tubule with mature SZ; **D:** Cyst of maturing SZ lining tubule wall

## Macroscopic characteristics

Testes have shrunk considerably and frills have become flabby and empty; they are still opaque and creamy white with prominent blood vessels. The spermatoducts still contain semen as a thin semi translucent fluid. Stage VII can primarily be seen towards the end of the spawning period from May and throughout the postspawning period.  $L_T$ : > 36 cm;  $W_{CL}$  > 360 g;  $W_{GO}$ : 4 – 244 g;  $GSI$ : 0.5 – 4.0.

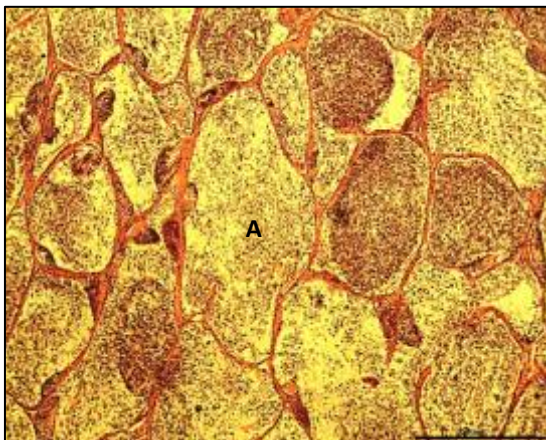


**Fish data:** Id: 2;  $L_T$ : 87 cm; Age: 8  $W_{CL}$ : 5940 g;  $W_{GO}$ : 102.2 g;  $GSI$ : 1.7; Date: May 22.

## Histological characteristics

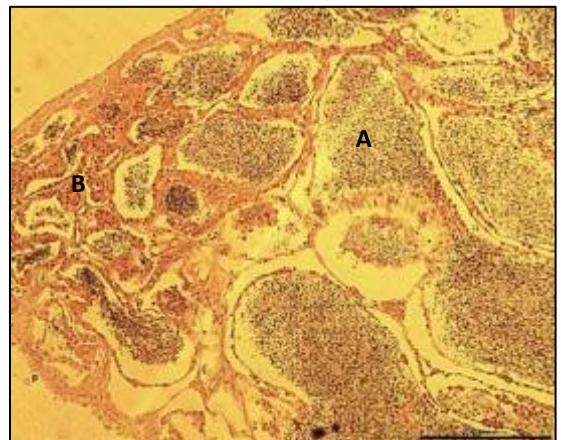
The density of spermatozoa (SZ) decrease but they are still ripe especially in the proximal ducts. The walls of the interlobular tissue thicken primarily in the distal part, and blood vessels become more apparent. Distal more spermatogonia (SG) emerge and there can be cysts of spermatides and spermatozoa which have not developed.

Magnification x10 bar 250  $\mu$ m



**A:** Ripe SZ; **B:** Cysts of SG

Magnification x10 bar 250  $\mu$ m



## Macroscopic characteristics

Testes are again small and lobules and spermatoducts are flabby and empty but with signs of previous distension and there can be remnants of semen. The testes are rich in blood vessels and appear reddish and the walls are somewhat thickened. Stage VIII can primarily be seen towards the end of the spawning period from May and throughout the postspawning period.  $L_T$ : > 36 cm;  $W_{CL}$  > 360 g;  $W_{GO}$ : 1 – 35 g;  $GSI$ : < 1.5.

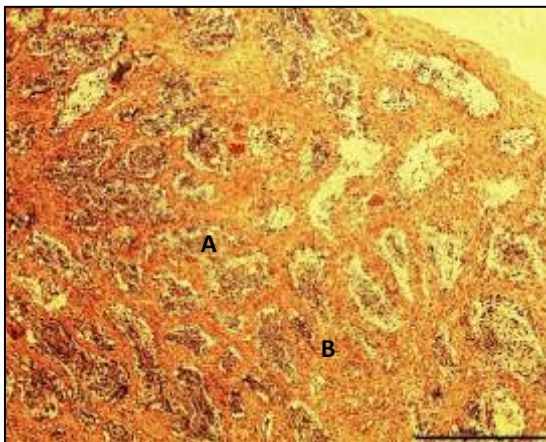


**Fish data:** Id: 15;  $L_T$ : 47 cm; Age 4  $W_{CL}$ : 900 g;  $W_{GO}$ : 6.6 g;  $GSI$ : 0.7; Date: May 8.

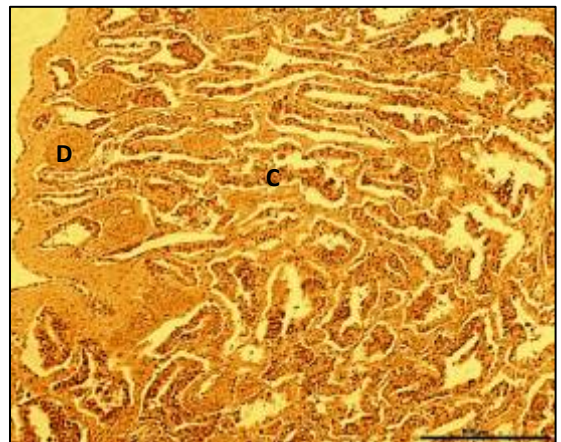
## Histological characteristics

A resorption of unspawned spermatozoa (SZ) and thickening of the interlobular walls compared with stage VII. There can still be a few ripe spermatozoa but also atretic spermatozoa with no flagella can be seen in the collapsing efferent ducts. Spermatogonia (SG) can be prominent especially in the distal parts and lining collapsed tubules.

Magnification x10 bar 250  $\mu$ m



Magnification x10 bar 250  $\mu$ m



**A:** Collapsed tubules with unspawned SZ; **B:** Thickened interstitial tissue and SG; **C:** Cysts of SG lining interstitial tissue; **D:** Blood vessel

# Stage IX – Regeneration: Resting / spawning omission Males

## Macroscopic characteristics

Testes are small and can appear similar to stage II but frills and spermatoducts are more distended with signs of previous spawning. They are rich in blood vessels and appear reddish with thickened walls. Stage IX can be seen during the prespawning- and spawning period (February – June) as skip-of-spawning; in the postspawning period as spent; or the rest of the year as resting.  $L_T$ : > 36 cm;  $W_{CL}$  > 360 g;  $W_{GO}$  < 26 g;  $GSI$ : < 1.5.



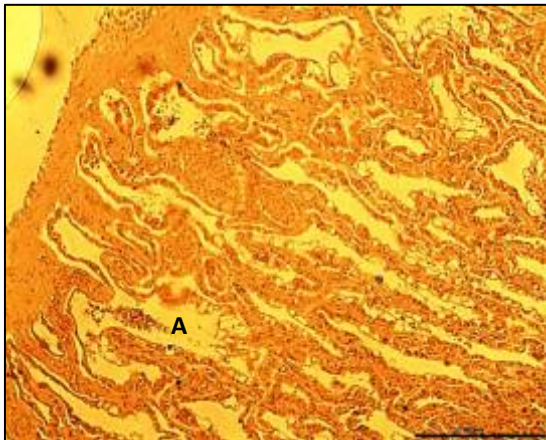
**Fish data:** Id: 13;  $L_T$ : 44 cm; Age: 4  $W_{CL}$ : 820 g;  $W_{GO}$ : 5.8 g;  $GSI$ : 0.7; Date: May 22.

## Histological characteristics

Proximal there can still be few spermatozoa (SZ) left but they are degenerating and some may be atretic. Distal this stage may resemble stage II with many spermatogonia (SG) in cysts or separately, and cysts of spermatocytes can also occur. The walls however are thicker than stage II and blood vessels are prominent.

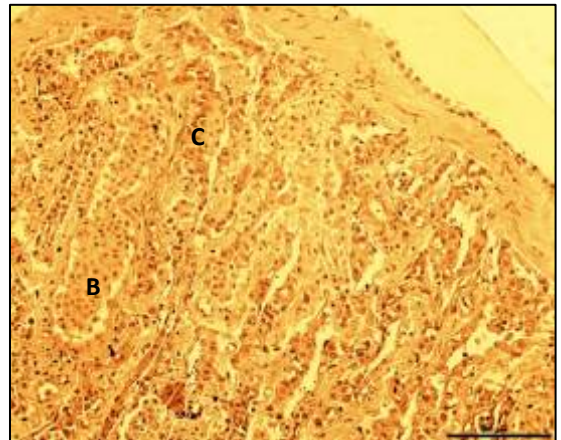
Magnification x10

bar 250  $\mu$ m



Magnification x20

bar 100  $\mu$ m



**A:** Collapsed ducts with remnants of SZ; and SG lining the interstitial tissue; **B:** Blood vessel; **C:** SG

### **Macroscopic characteristics**

No data; but this stage is recognized by an abnormal appearance of the testis e.g. a dark yellowish colour of the frills. Stage X can be seen all year round.

### **Histological characteristics**

An abnormal appearance of the some of the germ cells and some of the tissue, but it might be in only certain areas of the testis.