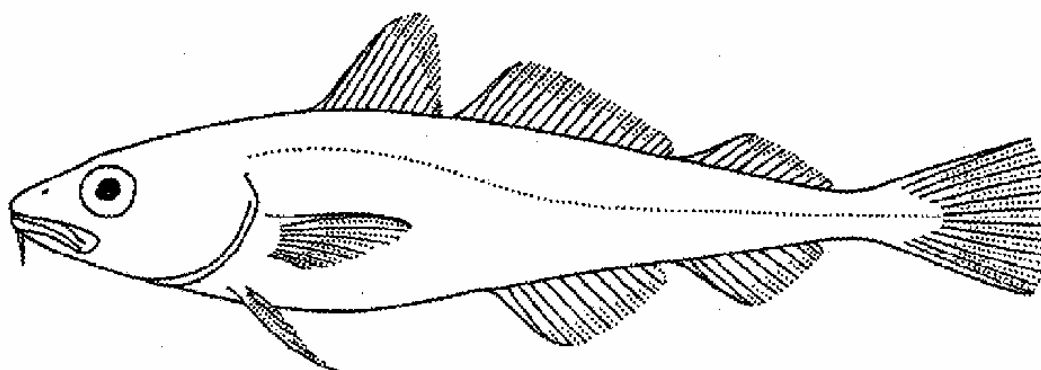


Manual to determine gonadal maturity of North Sea cod (*Gadus morhua* L)



R. H. Bucholtz, J. Tomkiewicz, F. Vitale,
J. Dalskov, I. Wilhelms, A. Sell, B. Bland,
I. Gibb, G. Power

DRAFT

Preface

This preliminary manual has been developed by participants of the ICES Workshop on Maturity Staging of Cod, Whiting, Haddock and Saithe (WKMSCWHS) conducted at DTU Aqua, Charlottenlund, Denmark 13-16 November 2007. The structure of the draft manual is based upon a 6-stage maturity scale proposed at the workshop and described in report of the workshop (ICES WKMSCWHS report 2007). Specimens illustrating the different maturity stages were sampled in cooperation between the participating countries during the IBTS 1Q and IBTS 3Q 2008.

The workshop participants and their respective institutes have all contributed to the development of this manual:

Tatjana Baranova	Latvia
Merete Fonn	Norway
Iain Gibb	UK, Scotland
Susanne Hansen	Denmark
Inger Hornum (instructor)	Denmark
Richard Humphreys	UK, England
Harald J. Larsen	Norway
Peter McCorrison	UK
Bart Martens	Belgium
Kerstin Schuhmann	Germany
Ivo Sics	Latvia
Rajlie Sjöberg	Sweden
Lisbet Solbakken	Norway
Yves Verin	France
Sally Warne	UK, England
Ken Coull	UK, Scotland

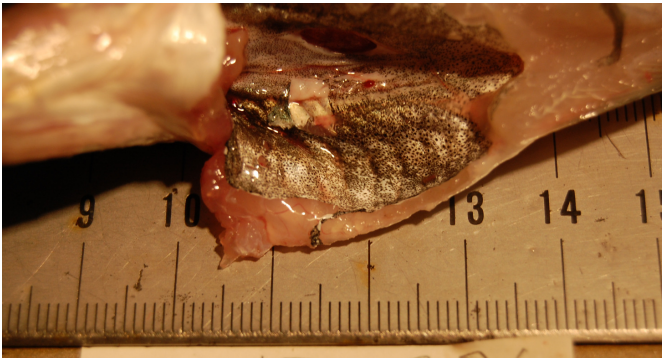
The following participants have contributed in the selection of the specimens applied in this manual and by providing suggestions for the description of each of the stages:

Francesca Vitale	Sweden
Ingo Wilhelms	Germany
Anne Sell	Germany
Barbara Bland	Sweden
Iain Gibb	UK, Scotland

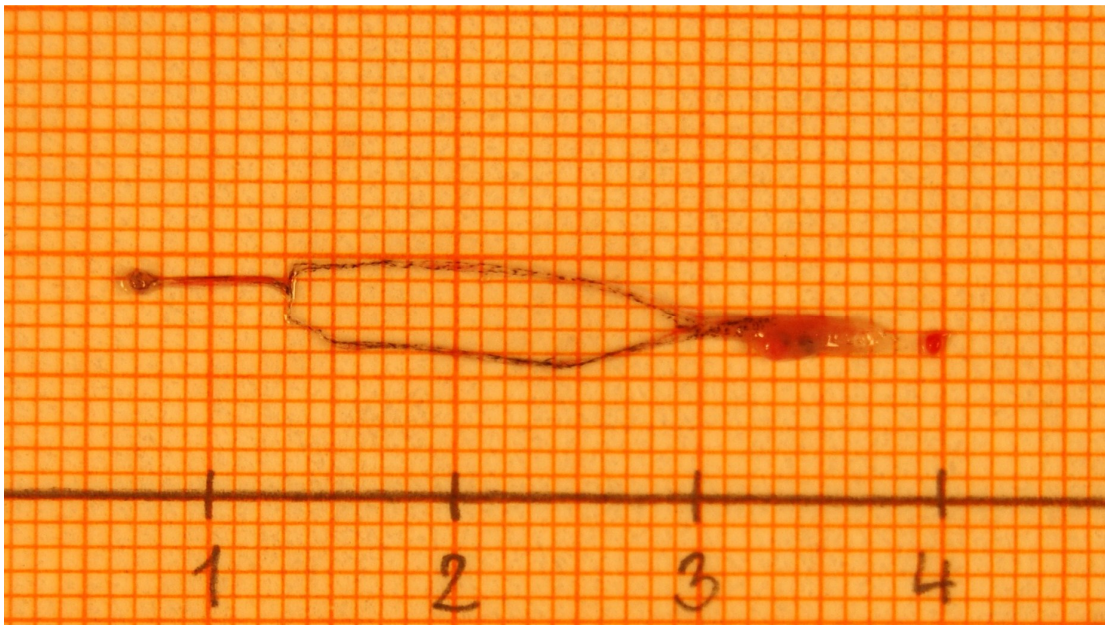
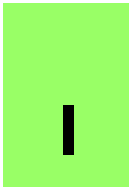
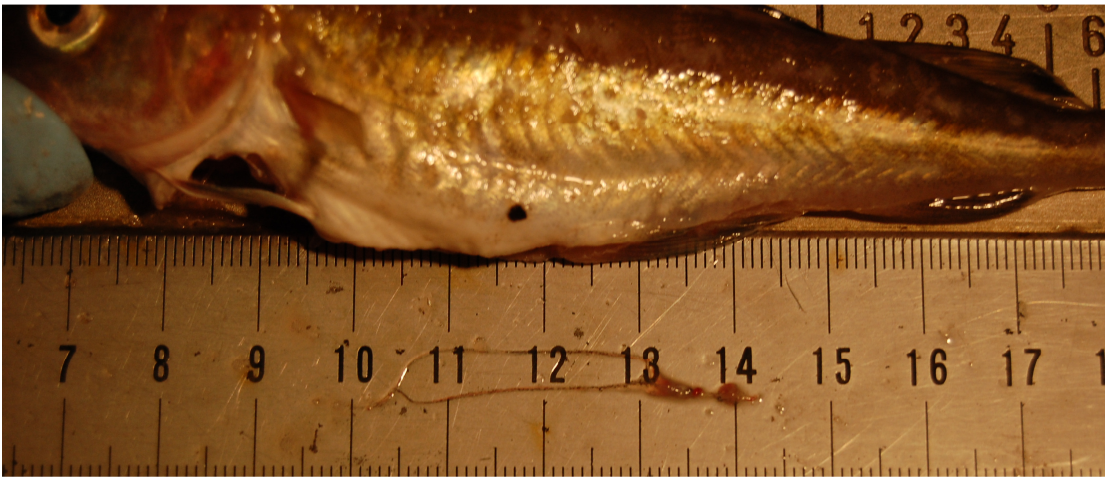
The editors:

Rikke H. Bucholtz	Denmark
Jonna Tomkiewicz	Denmark
Gavin Power	Ireland
Jørgen Dalskov	Denmark

I. Juvenile/immature (early)



Stage I (early):
Ovaries tiny paired organs posterior in body cavity close to bladder. Surface smooth. Glassy transparent to orange-reddish translucent.



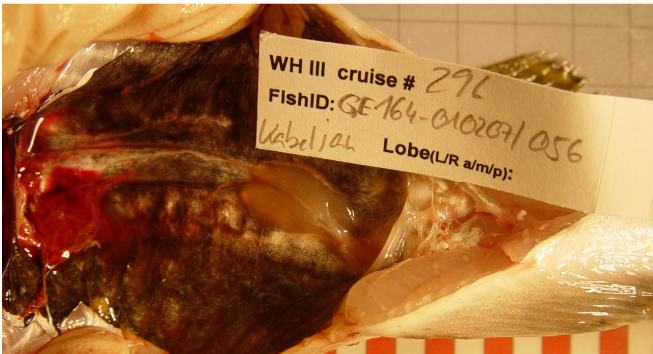
Specimen data

L_T: 15 cm
M_B: 34 g

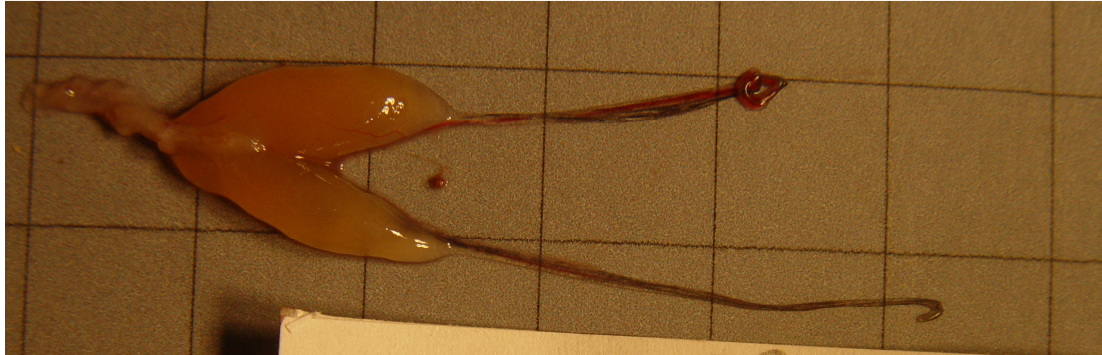
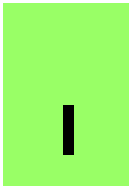
M_G: < 0.1 g
GSI: ~ 0

M: August 2007
ID: 071025/51

I. Juvenile/immature (early)



Stage I (early):
(continued)



Specimen data

L_T: 27 cm

M_B: 174 g

M_G: 0.5 g

GSI: 0.3

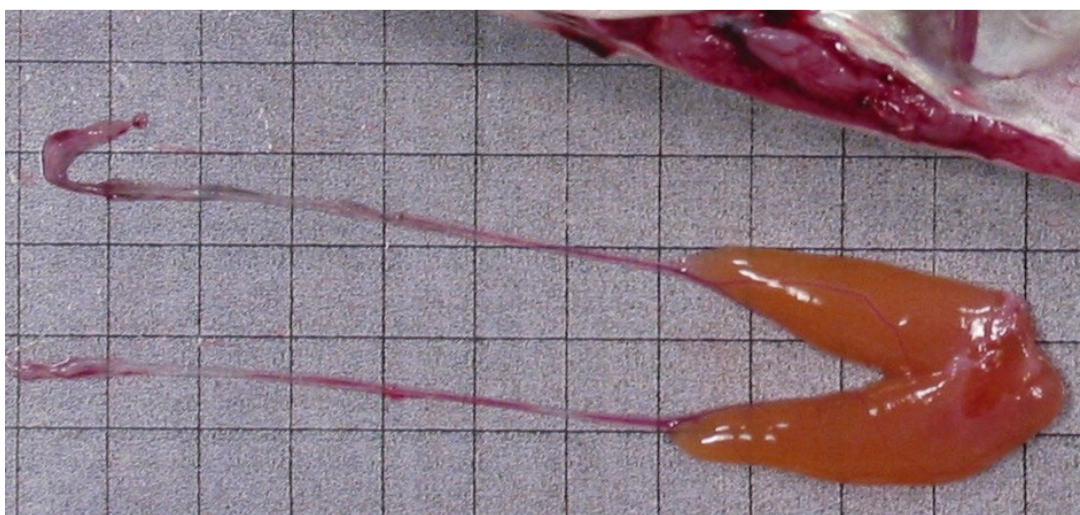
M: February 2007

ID: 070718/176

I. Juvenile/immature (preparation)



Stage I (preparation):
Ovaries small, but easily distinguishable posterior in body cavity, soft with smooth surface, blurred translucent, reddish-orange.



Specimen data

L_T: 47 cm

M_B: 945 g

M_G: 2.9 g

GSI: 0.3

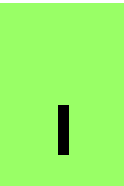
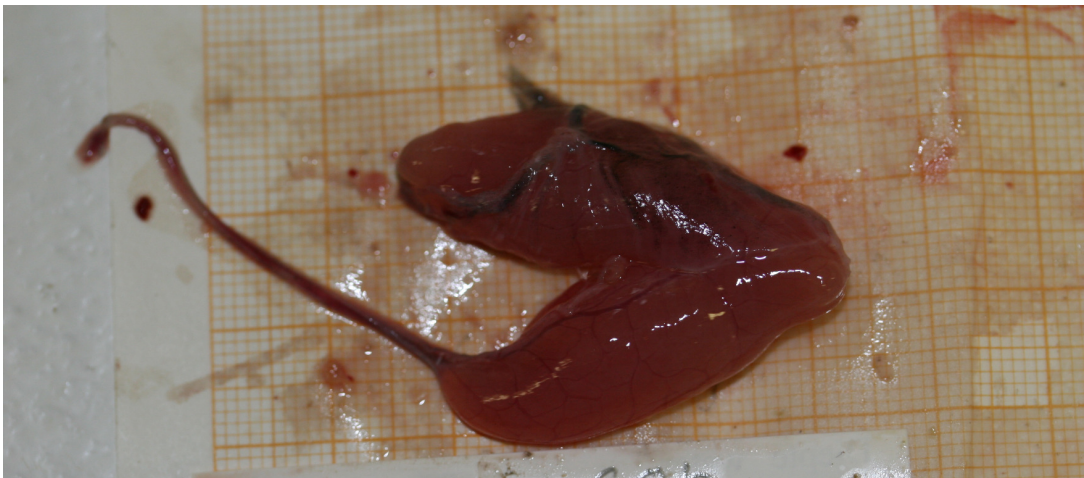
M: January 2007

ID: 070718/25

I. Juvenile/immature (preparation)



Stage I (preparation):
(continued)



<i>Specimen data</i>		
L _T : 54.5 cm	M _G : 4.4 g	M: February 2007
M _B : 1392 g	GSI: 0.3	ID: 070718/60

II. Maturing (early)



Stage II (early):

Ovaries still small and restricted to posterior body cavity. Structure more firm, swollen appearance with an uneven surface, opaque orange-red colouration with a greyish cast in repeat spawners. Oocytes become visible.



II

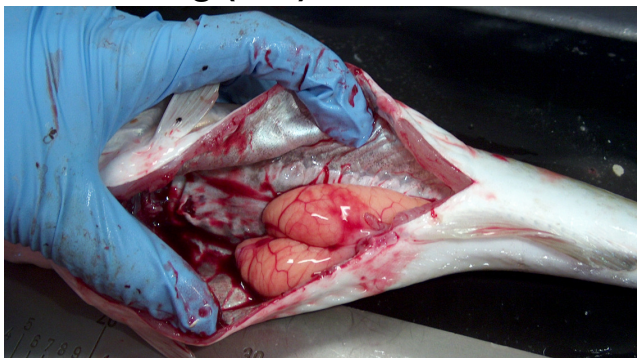
Specimen data

L_T: 39.5 cm
M_B: 502 g

M_G: 4.9 g
GSI: 1.1

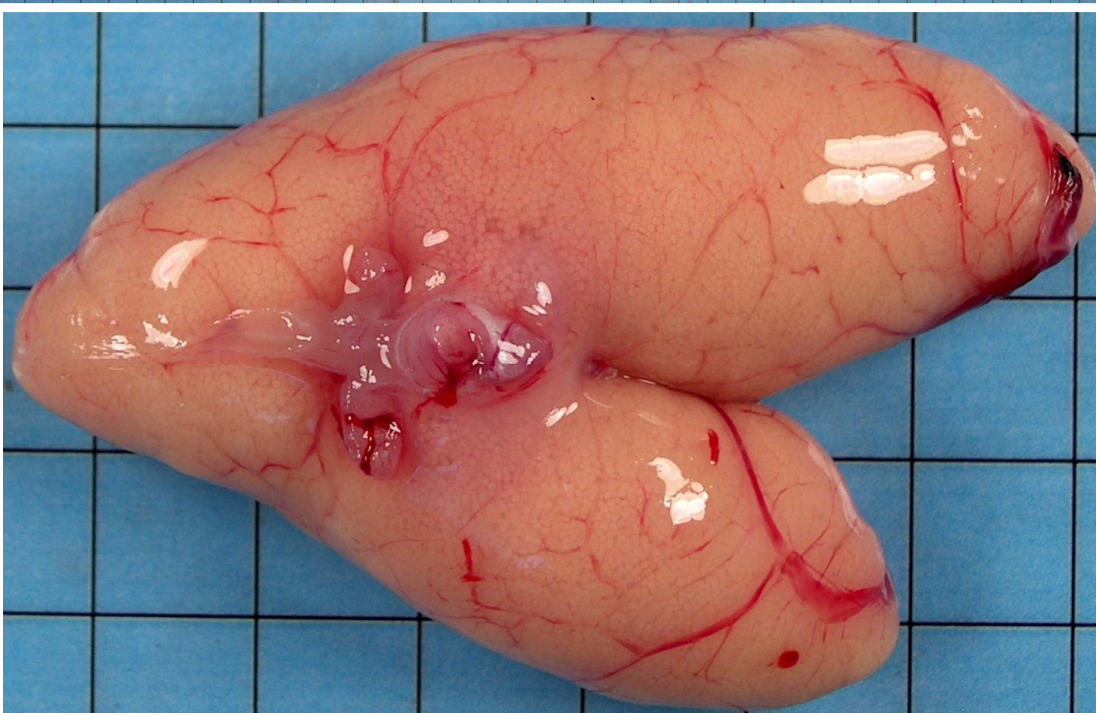
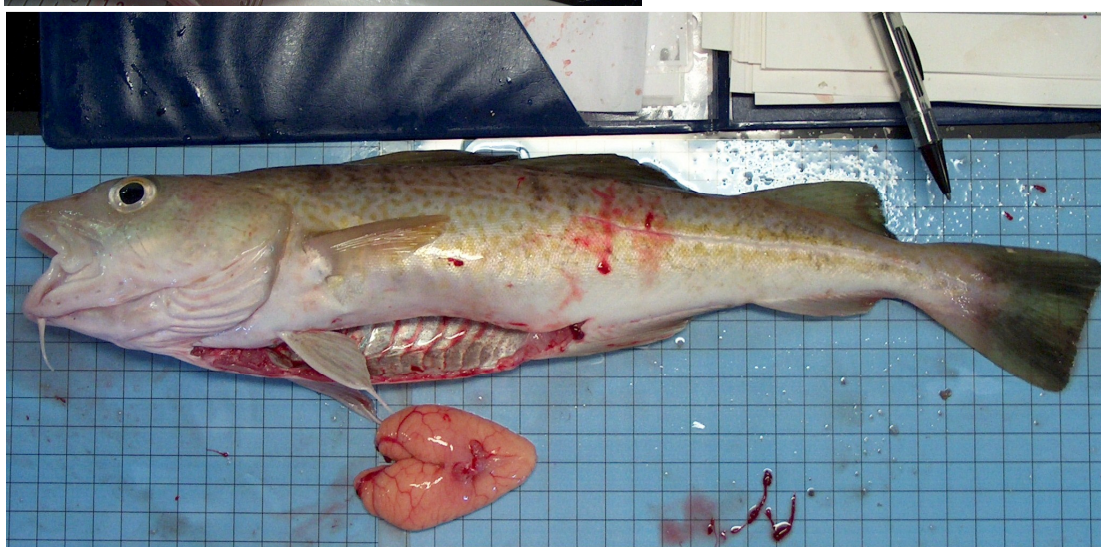
M: February 2007
ID: 070718/211

II. Maturing (late)



Stage II (late):

Ovaries occupy between half and 2/3 of the body cavity, plump and firm with prominent blood vessels, opaque, orange to creamy yellow. Oocytes clearly visible and densely packed.



Specimen data

L_T: 37 cm

M_B: 498 g

M_G: 22 g

GSI: 5.2

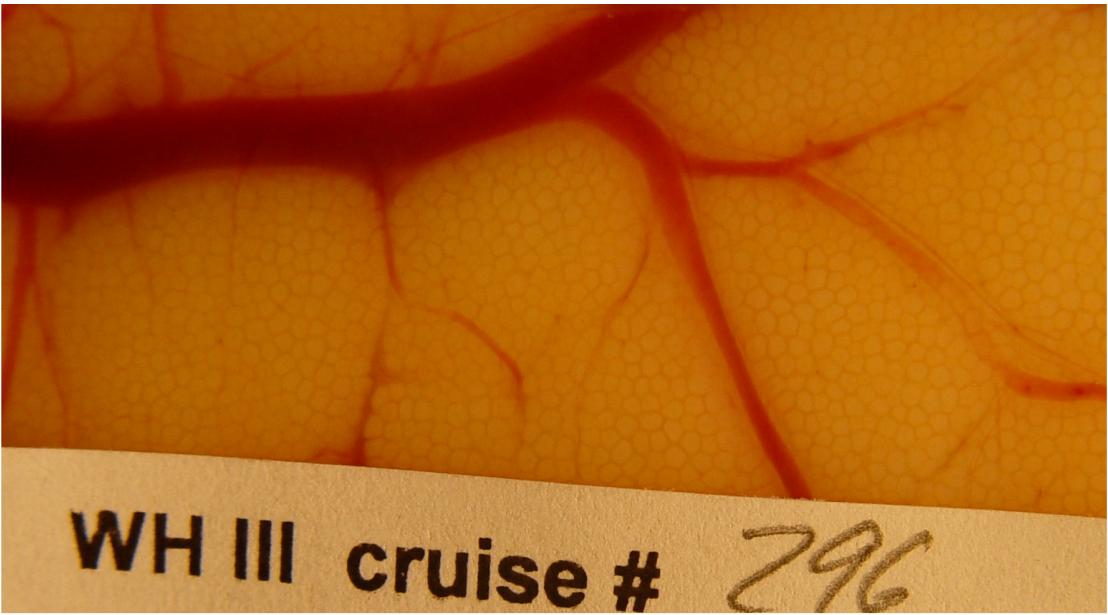
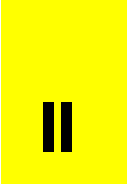
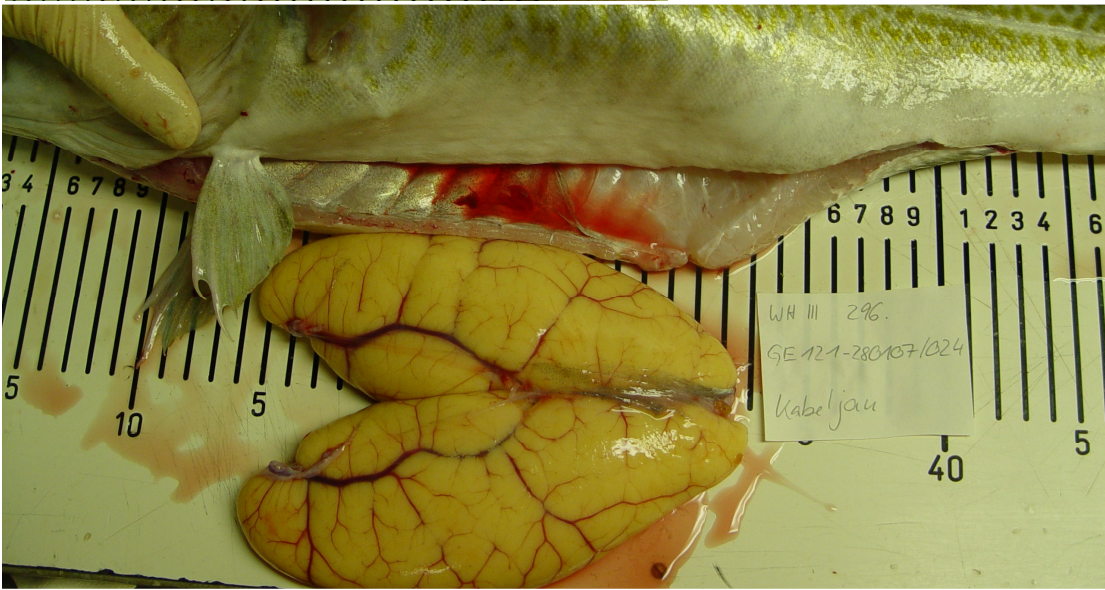
M: February 2007

ID: 070718/221

II. Maturing (late)

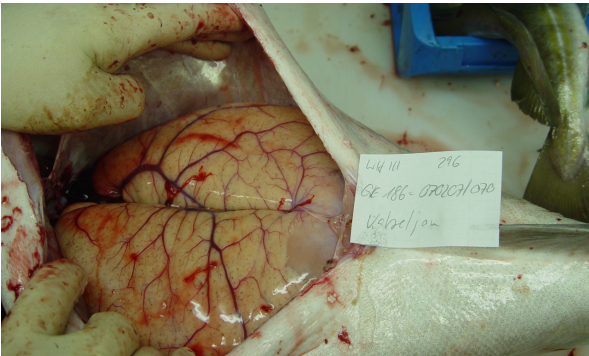


Stage II (late):
(continued)



Specimen data		
L _T : 63 cm	M _G : 498 g	M: January 2007
M _B : 3312 g	GSI: 20.4	ID: 070718/52

III. Spawning (initiation)



Stage III (initiation):
Ovaries extending into anterior body cavity; distended and soft; opaque, orange to creamy yellow. Single glassy, hydrated oocytes among abundant opaque, vitellogenic oocytes (round and larger than in II). Viscous fluid or hydrated eggs in lumen may occur.



Specimen data		
L _T : 83 cm	M _G : 1134 g	M: February 2007
M _B : 7375 g	GSI: 24.2	ID: 070718/178

III. Spawning (initiation)



Stage III (main period – no picture):
Ovaries fill most of body cavity; very distended and soft; appear granulated orange- to reddish-grey from mixture of opaque and glassy oocytes. Lumen containing viscous fluid in excess or hydrated eggs.



Specimen data

L_T: 72 cm
M_B: 5476 g

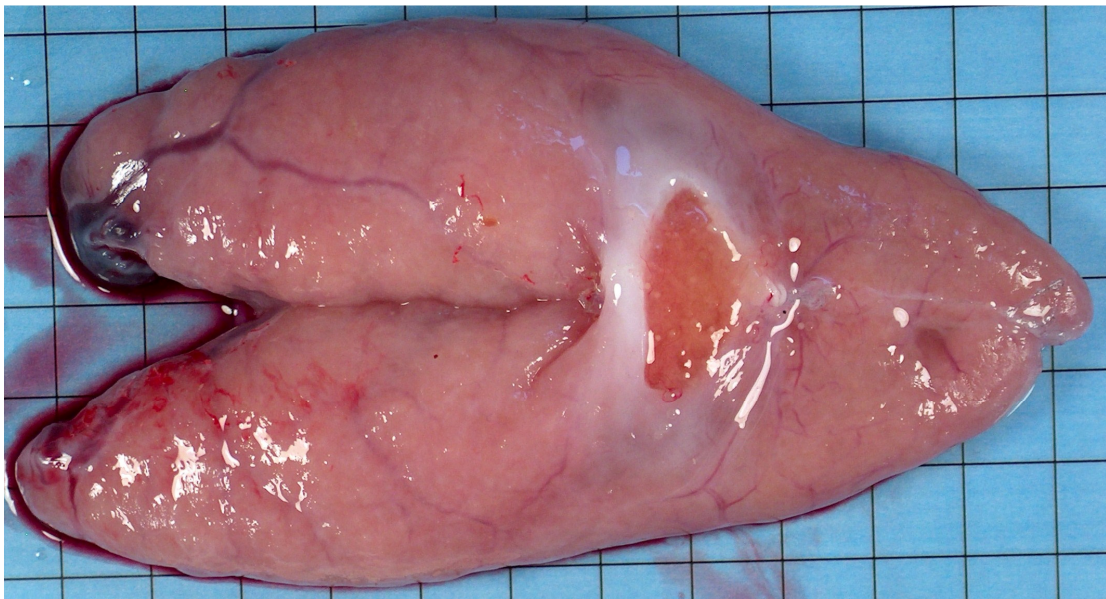
M_G: 896 g
GSI: 28.1

M: February 2007
ID: 070718/26

III. Spawning (cessation)



Stage III (cessation):
Ovaries shrunk to posterior body cavity; flabby with prominent blood vessels; opaque reddish-grey. Hydrated oocytes present; opaque oocytes few or absent. Lumen with excess fluid and frequently hydrated eggs.



Specimen data

L_T: 74 cm
M_B: 3668 g

M_G: 86 g
GSI: 2.7

M: February 2007
ID: 070718/218



III. Spawning (cessation)

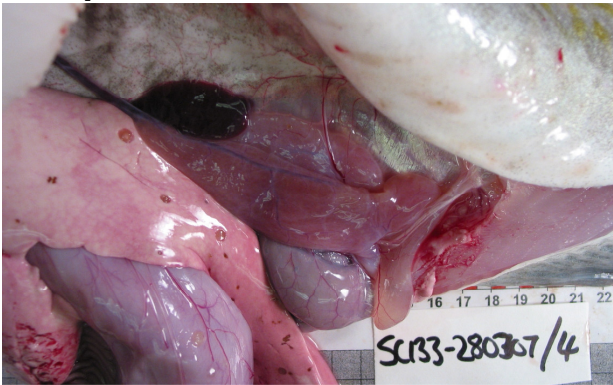


Stage III (cessation):
(continued)

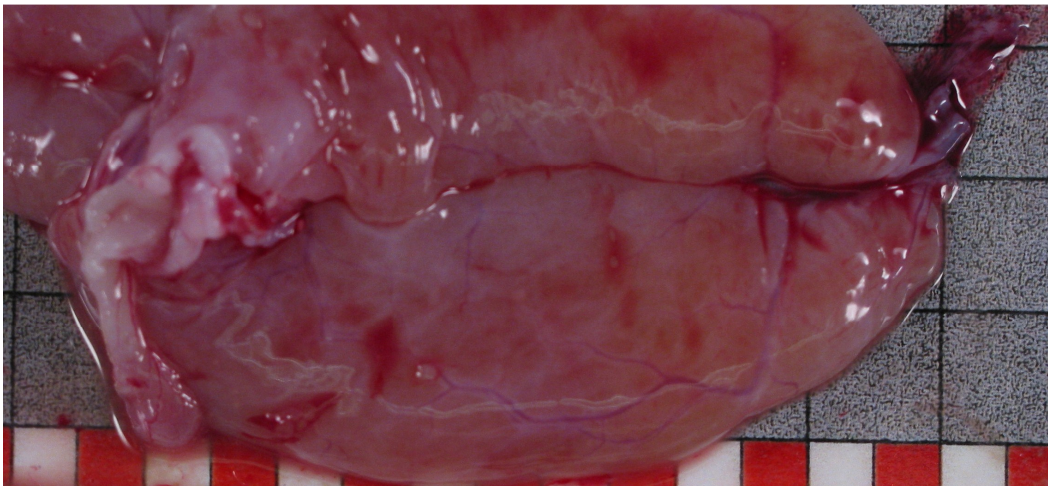
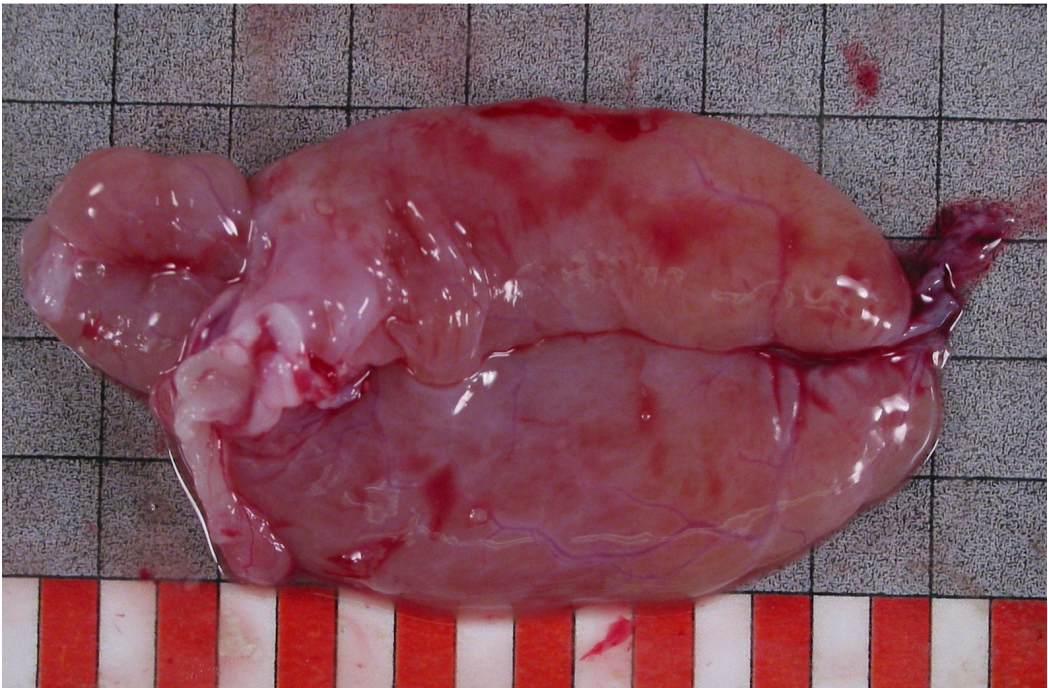


Specimen data		
L _T : 65 cm	M _G : 345 g	M: March 2007
M _B : 2686 g	GSI: 15.6	ID: 070718/24

IV. Spent

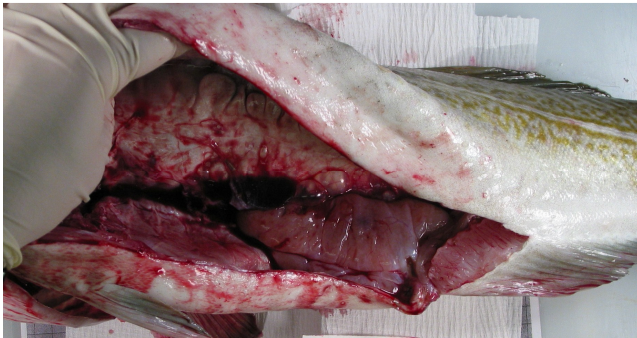


Stage IV:
Ovaries contracted; slack with grayish cast; rich in blood vessels; dim translucent reddish-grey. Vitellogenic oocytes absent; but single hydrated eggs or atretic oocytes (opaque irregular granules) may occur.

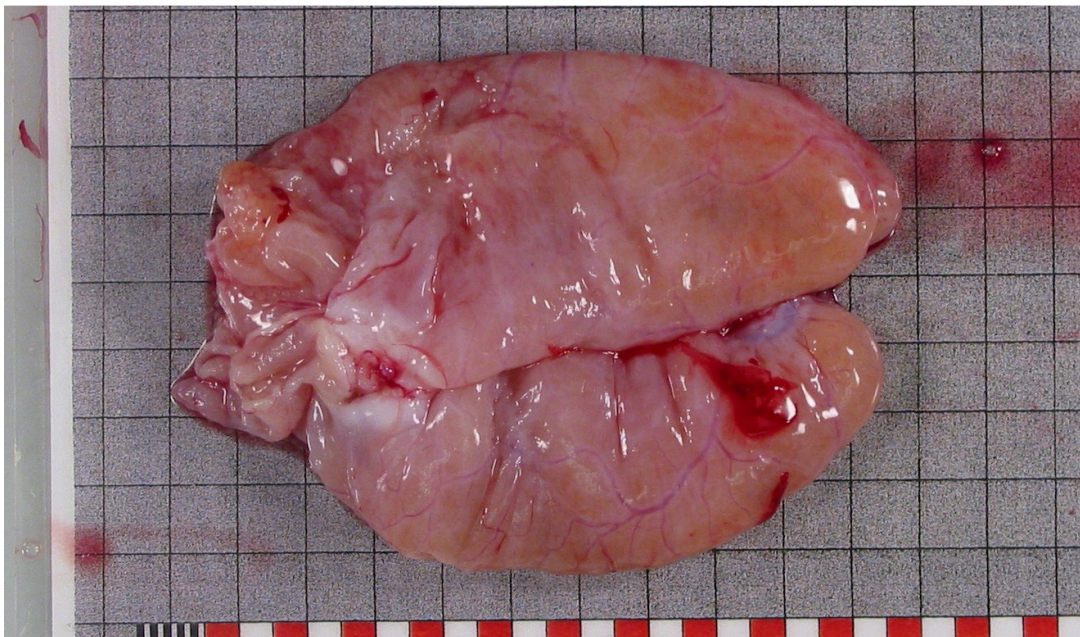


<i>Specimen data</i>		
L _T : 61 cm	M _G : 20 g	M: March 2007
M _B : 2436 g	GSI: 1	ID: 070718/21

IV. Spent



Stage IV:
(continued)



Specimen data

L_T: 76 cm

M_B: 4566 g

M_G: 82 g

GSI: 2.4

M: March 2007

ID: 070718/23

V. Skip of spawning (November-February?)

Stage V:

No suitable example of a stage V specimen was available from the samples. The following is a tentative description:

Ovaries relatively small as in stage I (preparation); soft with thickened walls; non-transparent; reddish-grey – often with a cast. Separation of stage II in early maturation and stage V is often hampered by the thickened walls and a greyish cast of females in stage V. The orange-like colouration, indicating yolk formation (stage II), is more easily distinguished if the tissue is cut open.

V. Resting (March-October?)

Stage V:

No suitable example of a stage V specimen was available from the samples. The following is a tentative description:

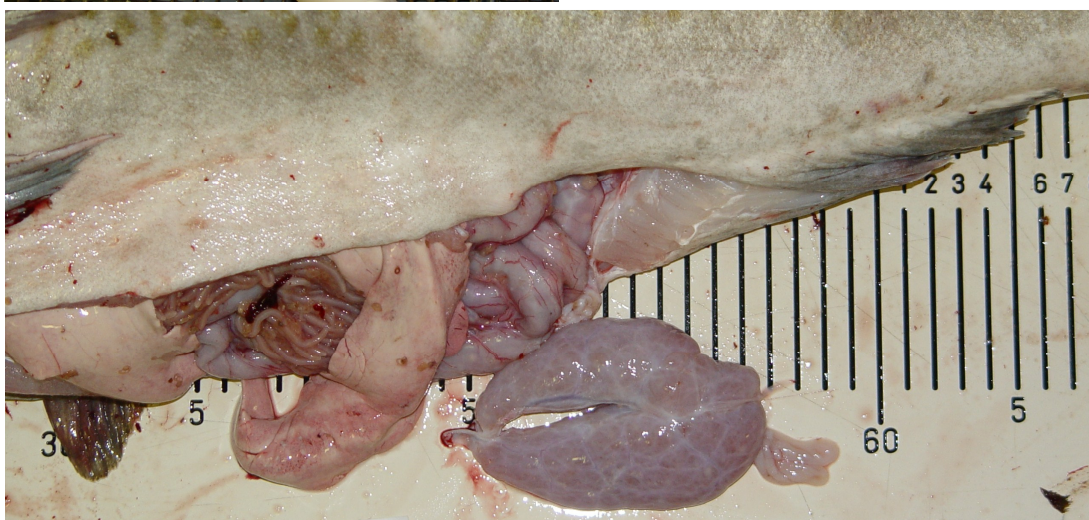
Ovaries small as in stage I (preparation) but with signs of previous spawning; e.g. greyish cast and somewhat uneven walls; blurred translucent, reddish-grey, but more granulated and opaque than in stage I (preparation). Separation of stage II in early maturation and stage V is often hampered by the thickened walls and a greyish cast of females in stage V. The orange shine indicating yolk formation

VI. Abnormal



Stage VI:

Stone roe. Ovary has a thick wall, grey-whitish cast and hard parts.



Specimen data

L_T: 99.5 cm
M_B: 8970 g

M_G: 92 g
GSI: 1.2

M: IBTS 3Q 2007
ID: 071025/86

VI. Abnormal

Stage VI:

Intersex: Macroscopically this specimen would be determined as a female, but the mixture of oocytes and spermatocytes are visible histologically.



Specimen data

L_T: 22 cm

M_B: 93 g

M_G: 1 g

GSI: 1.2

M: February 2007

ID: 071025/73

I. Juvenile/immature (preparation)



Stage I (early – no picture):
Testes emerge as a pair of thin strings along air bladder. Lobules tiny, glassy transparent to reddish translucent in larger specimens.



Specimen data

L_T: 28 cm
M_B: 186 g

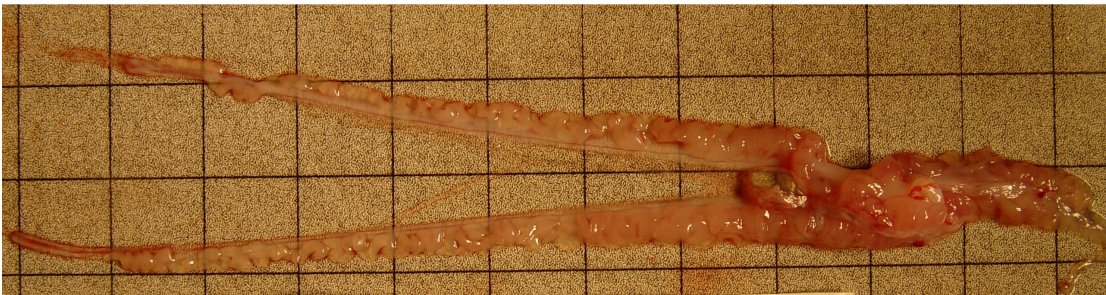
M_G: 0.1 g
GSI: 0.1

M: January 2007
ID: 070718/174

I. Juvenile/immature (preparation)

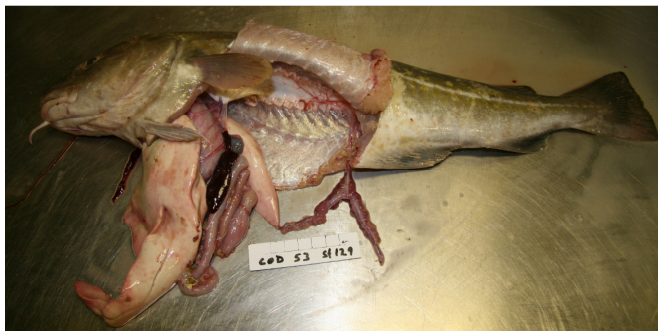


Stage I (preparation):
Testes small, but distinguishable along air bladder. Lobules small, blurred translucent reddish-white.



Specimen data		
L _T : 55 cm	M _G : 1.3 g	M: February 2007
M _B : 1790 g	GSI: 0.1	ID: 070718/171

II. Maturing (early)

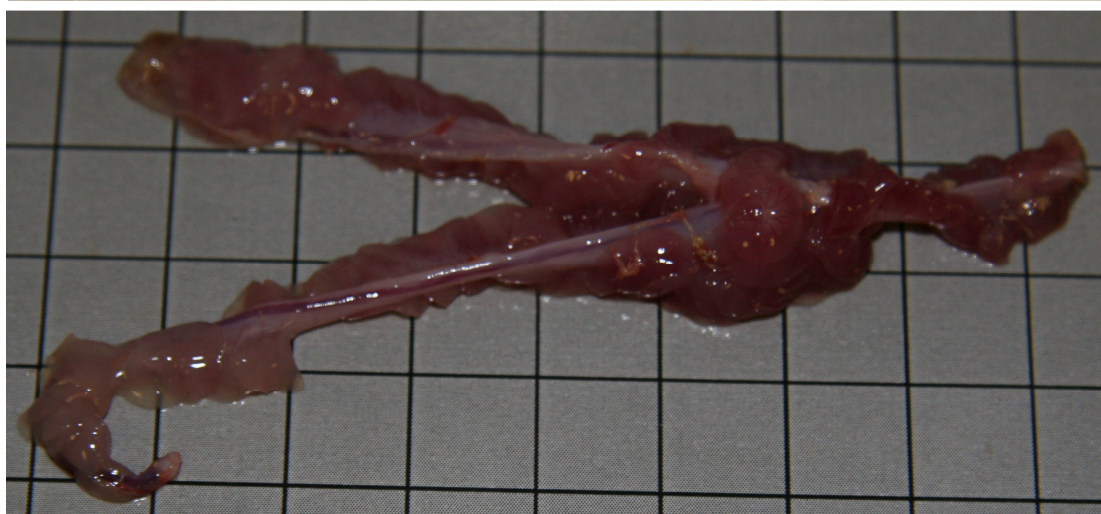


Stage II (early):

Testes still small, close to air bladder. Lobules plump and soft, rich in blood vessels, completely or partially opaque, reddish.



II



Specimen data

L_T: 52 cm

M_B: 1683 g

M_G: 4.4 g

GSI: 0.3

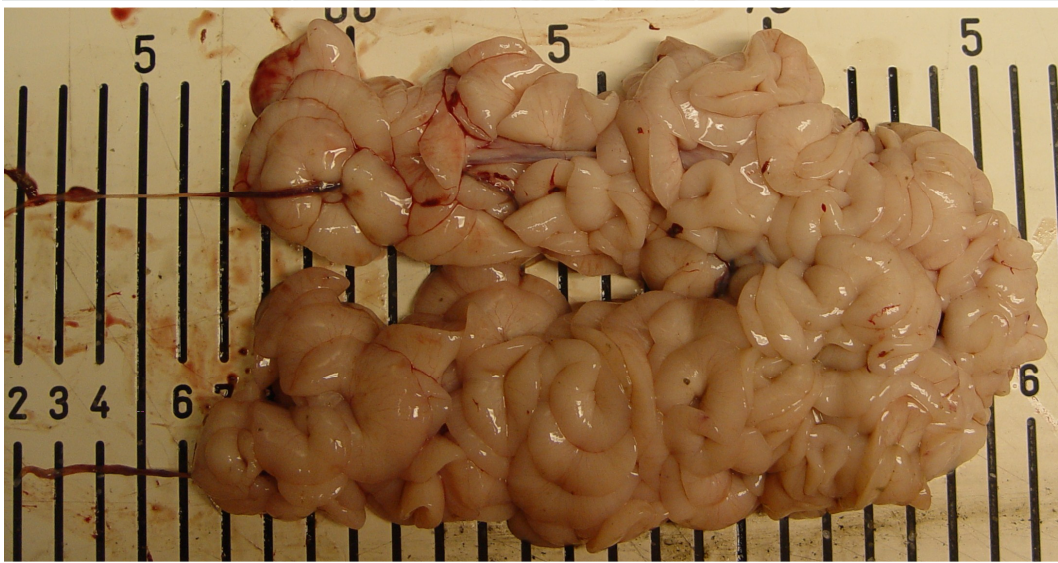
M: IBTS 3Q 2007

ID: 070718/109

II. Maturing (late)



Stage II (late):
Testes enlarged and prominent dorsal in body cavity; lobules plump and brittle; reddish-white. Empty transparent spermatoducts with prominent blood vessels; no sperm release.



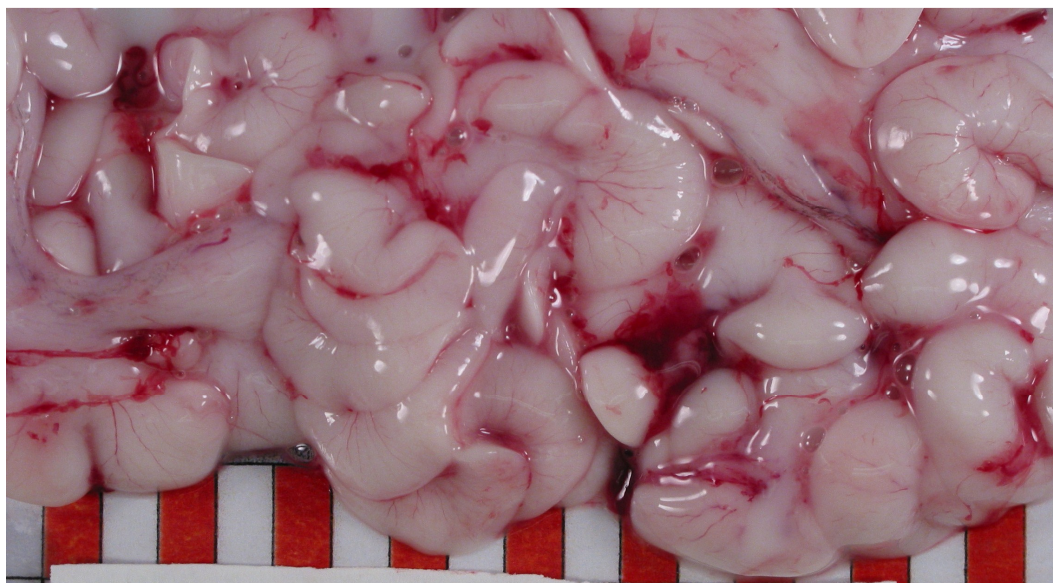
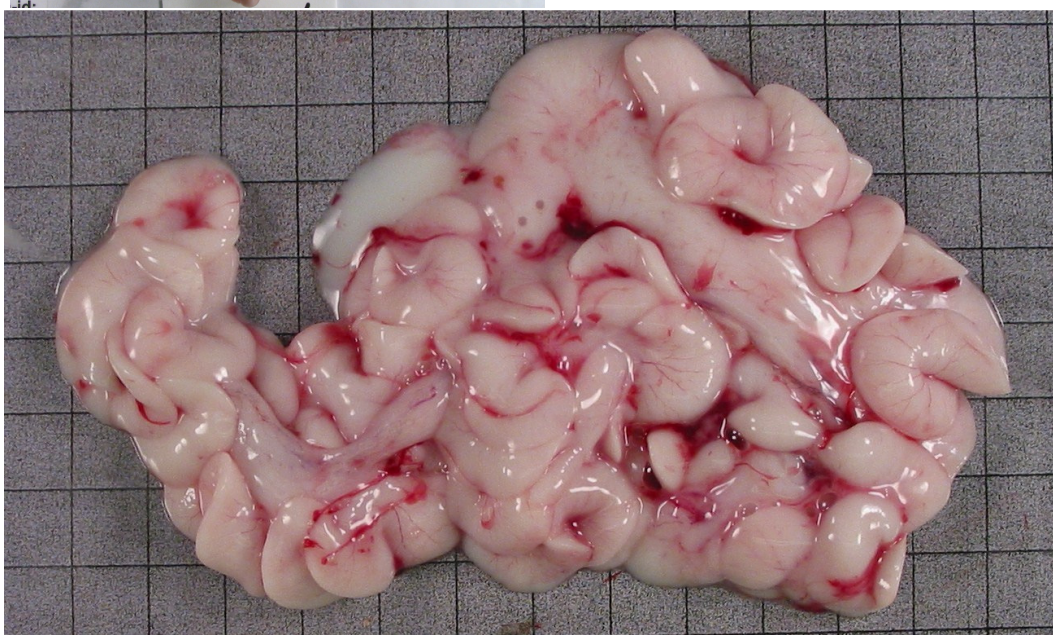
<i>Specimen data</i>		
L _T : 71 cm	M _G : 320.2 g	M: January 2007
M _B : 3940 g	GSI: 10.1	ID: 070718/175

III. Spawning (main period)



Stage III (initiation – **no picture**):

Testes extending into ventral part of body cavity. Lobules distended and brittle, opaque creamy-white. Spermatoducts filled with viscous semen and viscous droplet may be released from vent.



Specimen data

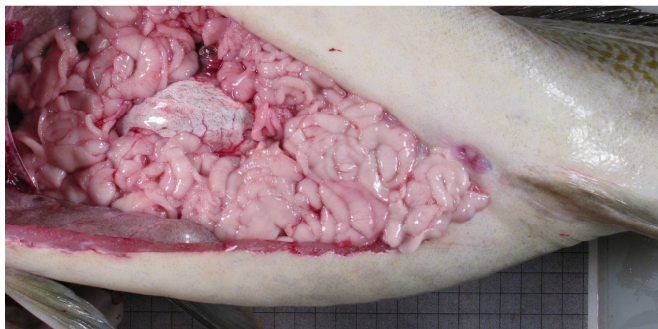
L_T: 44 cm
M_B: 890 g

M_G: 55 g
GSI: 7.3

M: March 2007
ID: 070718/142



III. Spawning (main period)



Stage III (main period):

Testes large and prominent in body cavity. Lobules still plump, but soft; completely opaque, whitish.

Spermatoducts filled with fluid, milky semen that easily flows from vent.



Specimen data

L_T: 77 cm

M_B: 5472 g

M_G: 609 g

GSI: 13

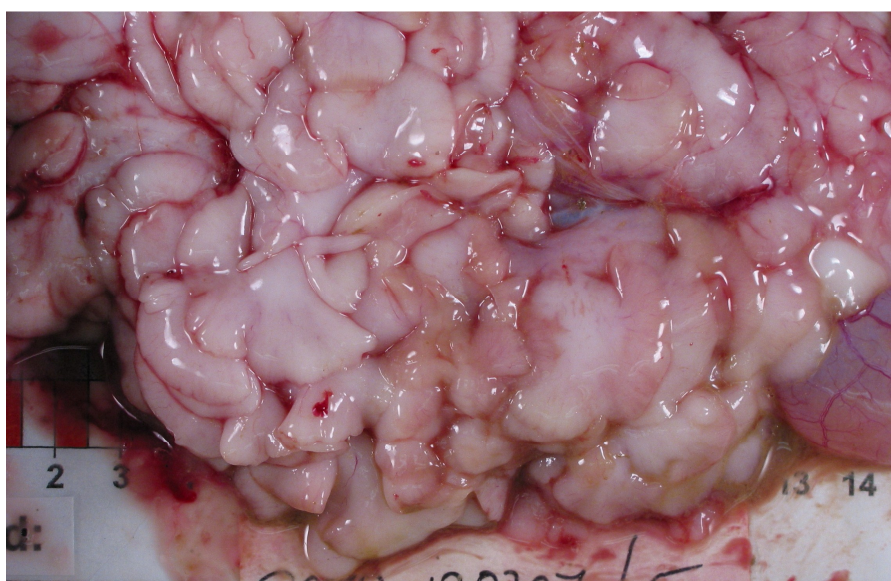
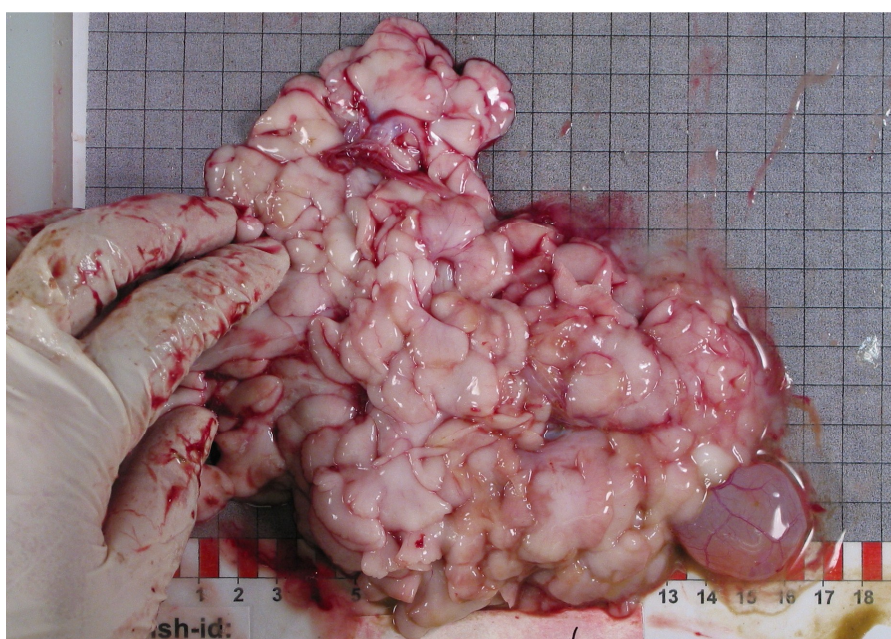
M: March 2007

ID: 070718/145

III. Spawning (cessation)



Stage III (cessation):
 Testes shrunk to dorsal part of body cavity; soft and flabby. Lobules almost empty, opaque, reddish-white. Spermatoducts still with fluid semen that easily flows from vent.



Specimen data

L_T: 90 cm

M_B: 6565 g

M_G: 206 g

GSI: 3.6

M: March 2007

ID: 070718/148

IV. Spent



Stage IV:

Testes contracted, close to air bladder; rich in blood vessels. Lobules empty, flabby, reddish potentially with a greyish cast.

Spermatoducts with signs if previous distension, often with visible remains of semen.



Specimen data

L_T: 72 cm

M_B: 3838 g

M_G: 2.9 g

GSI: 0.1

M: February 2007

ID: 070718/168

V. Skip of spawning (November-February?)

Stage V:

No suitable example of a stage V specimen was available from the samples. The following is a tentative description:

Testes relatively small but with slightly larger lobules than in stage I (preparation) and spermatoducts often with a greyish cast.

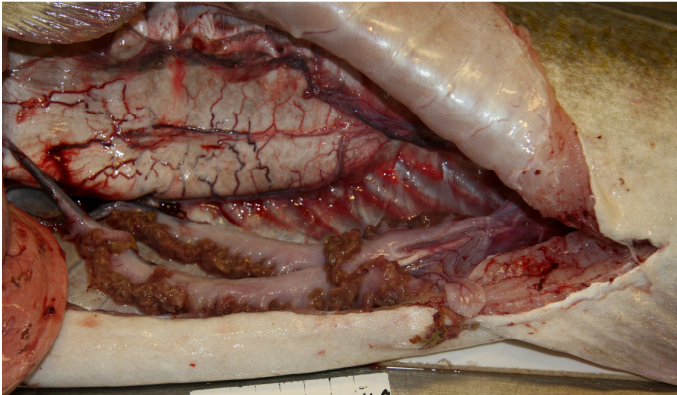
V. Resting (March-October?)

Stage V:

No suitable example of a stage V specimen was available from the samples. The following is a tentative description:

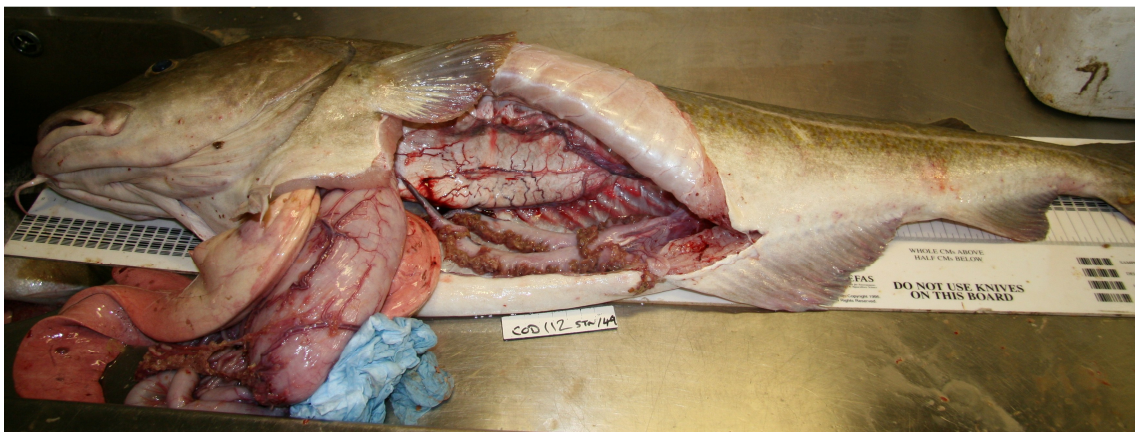
Testes small (as in stage I preparation) but with signs of previous spawning; e.g. lobules slightly larger lobules than in stage I (preparation); spermatoducts often with a greyish cast.

VI. Abnormal



Stage VI:

Testes with adipose tissue formation; affected parts undeveloped, hard and yellowish.



Specimen data

L_T: 99 cm

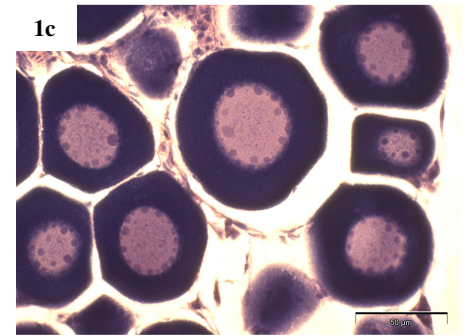
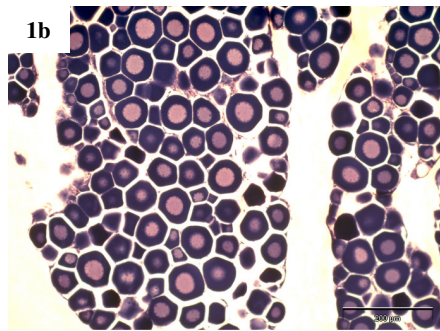
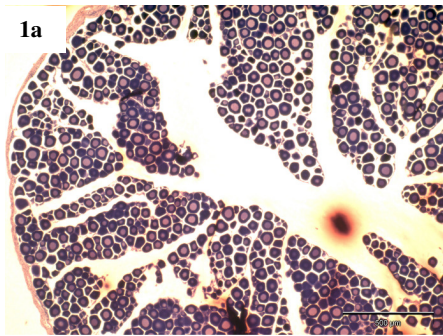
M_B: 10600 g

M_G: 62.2 g

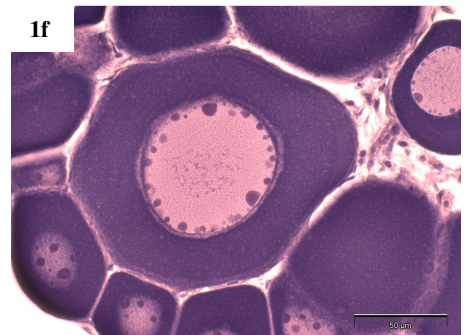
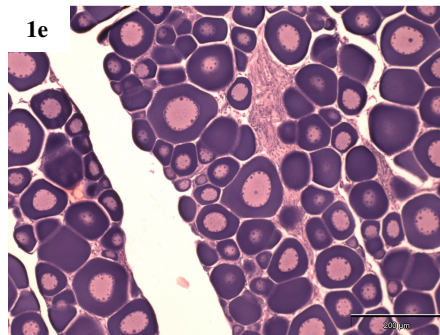
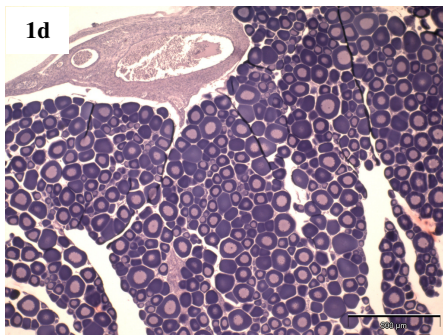
GSI: 0.7

M: IBTS 3Q

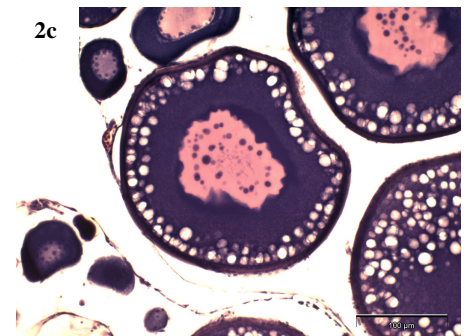
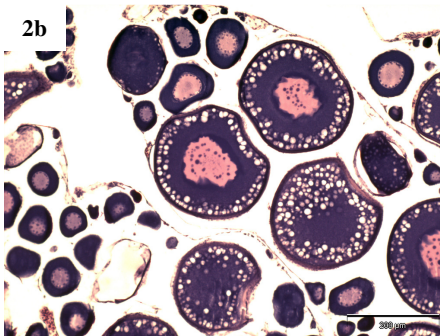
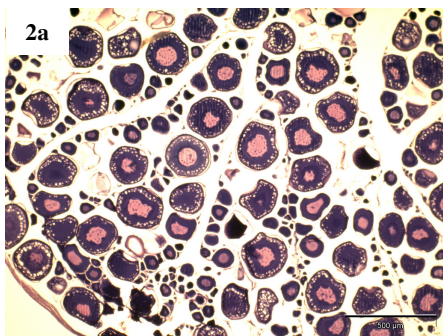
ID: 070718/111



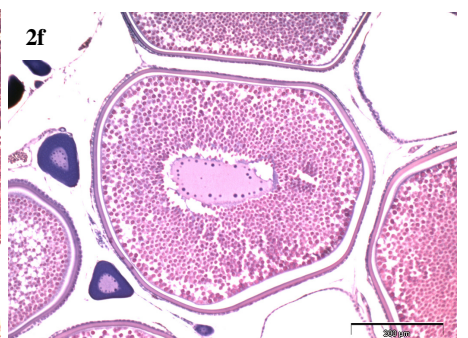
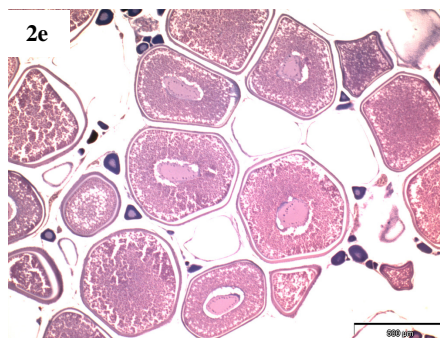
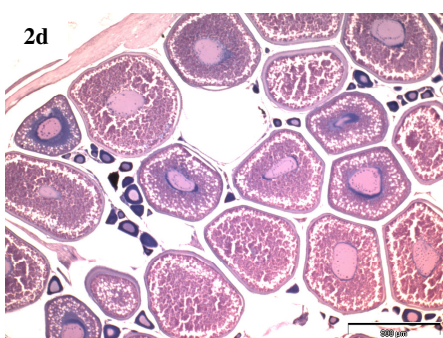
I. Juvenile/immature (early). Oocytes are small with dense stained cytoplasm and a central nucleus with few, large peripheral nucleoli around its edge, oögonia are always present at this premature stage although sometimes not visible (scale bars **1a.** 500 µm **1b.** 200 µm **1c.** 50 µm; specimen 070718/176).



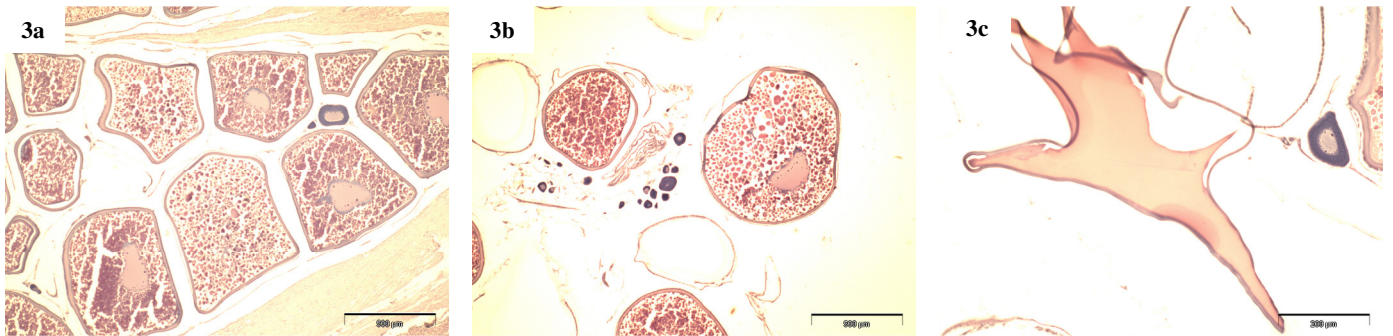
I. Juvenile/immature (preparation). A portion of the oocytes have started the primary growth (**1f**), characterized by a slight increase in size and by the presence of a light stained area around the nucleus, the so called circumnuclear ring. This ring shows that cytoplasmatic changes are occurring (scale bars **1d.** 500 µm **1e.** 200 µm **1f.** 50 µm; specimen 070718/25).



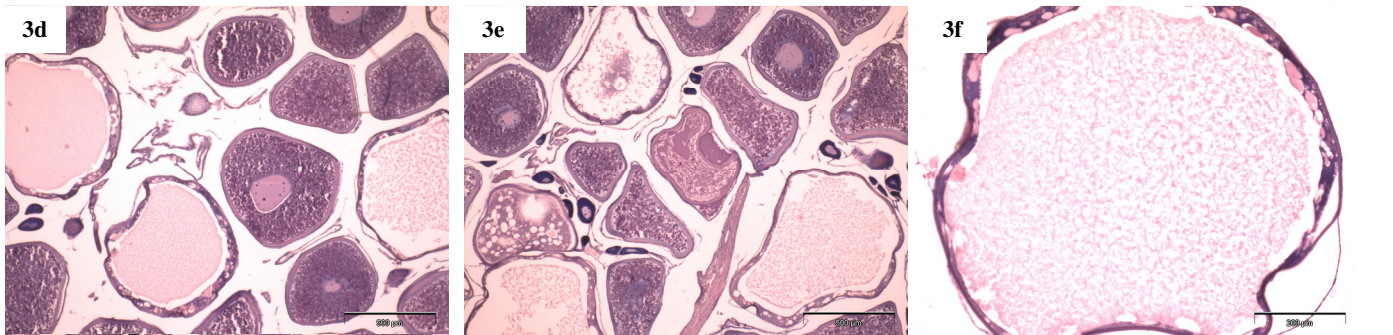
II. Maturing (early). The circumnuclear ring moves towards the outer part of the cell and gradually disintegrates, while spherical and transparent vesicles (cortical alveoli) appear in the superficial half of the cytoplasm, which is now fair stained. The maturation process is in progress, and the individual will normally develop within the current spawning season (scale bars **2a.** 500 µm **2b.** 200 µm **2c.** 100 µm; specimen 070718/202).



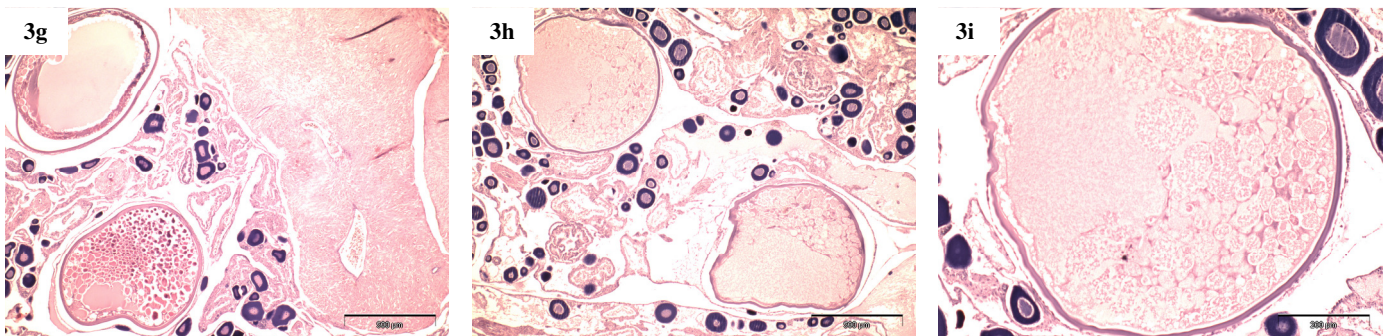
II. Maturing (late). Granules of yolk intensely stained, initially appear peripherally, but as they increase in number and size, they tend to spread out together with the cortical alveoli, throughout the cytoplasm. This process is termed vitellogenesis. The shape of the nucleus becomes irregular, but it is still centrally located (scale bars **2d.** 500 µm **2e.** 500 µm **2f.** 200 µm; specimen 070718/221).



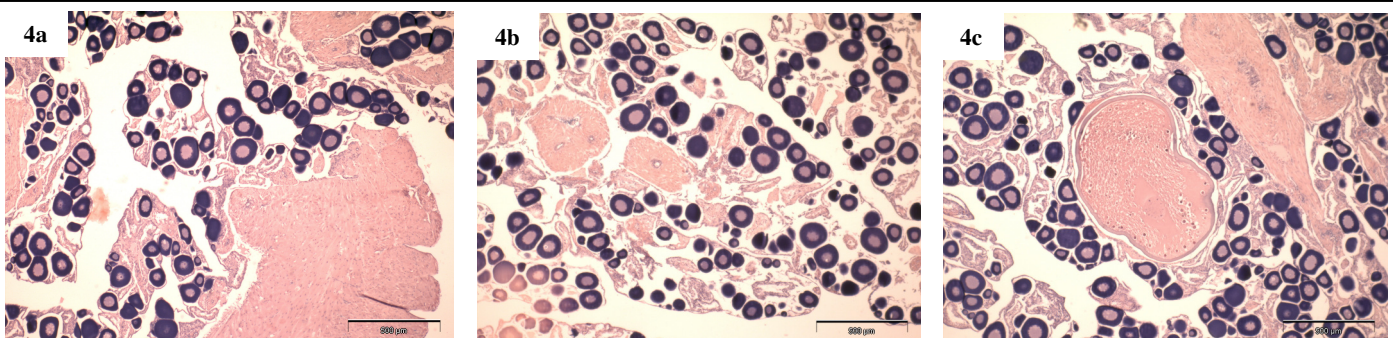
III. Spawning (initiation). The nucleus migrates to the periphery and breaks down, yolk granules coalesce forming large irregular spheres (3a, 3b), a water influx occurs and hydrated eggs (3c) with a completely homogeneous content are formed. At the ovulation, oocytes are released into the lumen, while the post-ovulatory follicles (3b) remain in the ovary (scale bars 3a. 500 µm 3b. 500 µm 3c. 200 µm, specimen 070718/178).



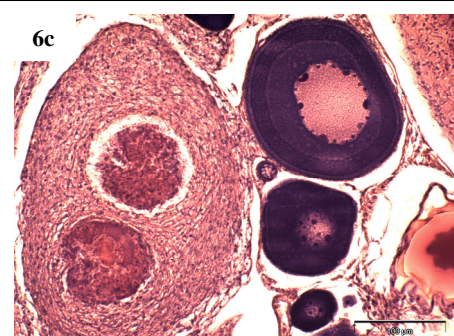
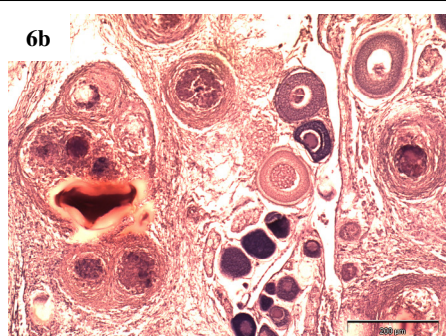
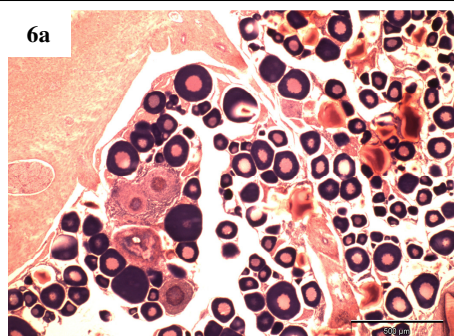
III. Spawning (main period). As the spawning proceeds the number of hydrated eggs and post-ovulatory follicles increase (scale bars 3d. 500 µm 3e. 500 µm 3f. 200 µm; specimen 070718/64).



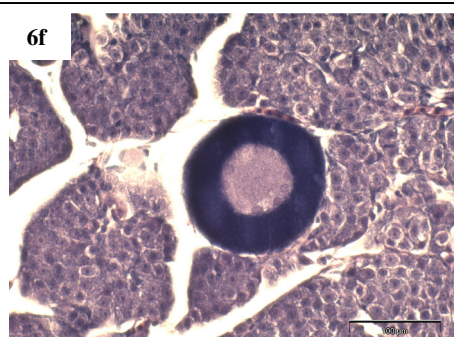
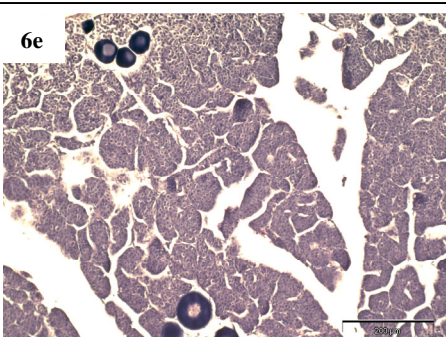
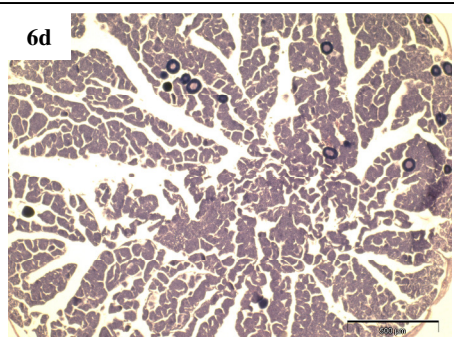
III. Spawning (cessation). At this late stage of spawning the hydrated eggs, post-ovulatory follicles and resting oocytes in the perinuclear and circumnuclear stages are dominant and the ovary wall (3g) becomes more thick and folded (scale bars 3g. 500 µm 3h. 500 µm 3i. 200 µm; specimen 070718/218)



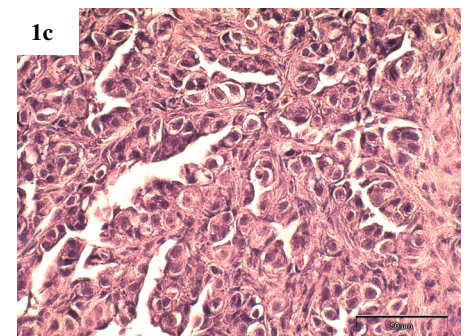
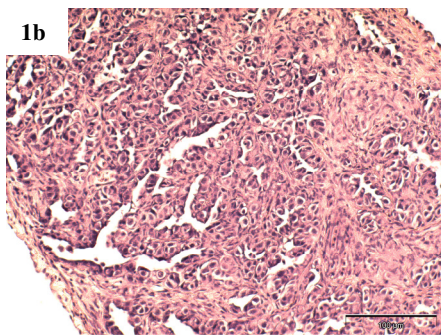
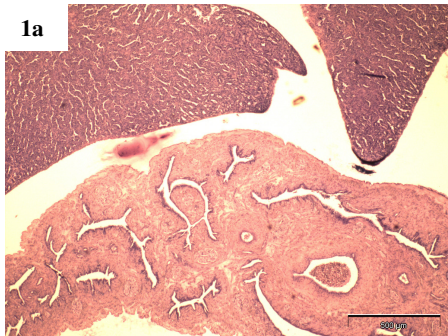
IV. Spent. At the end of the spawning season, the ovary enters the spent stage and post-ovulatory follicles, marking the occurred ovulation, are abundant among perinuclear or circumnuclear stage oocytes. The development of vitellogenic oocytes sometimes fail and they undergo a process called “atresia” (4c) consisting in an intra-ovarian resorption (scale bars 4a. 500 µm 4b. 500 µm 4c. 500 µm; specimen 070718/23)



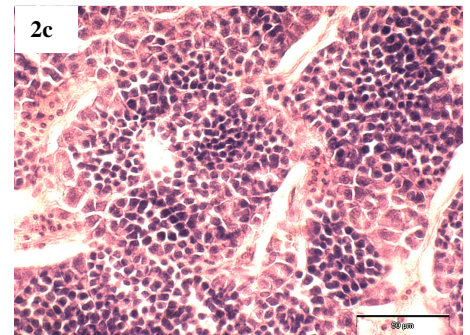
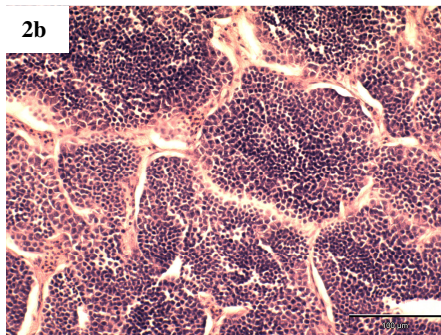
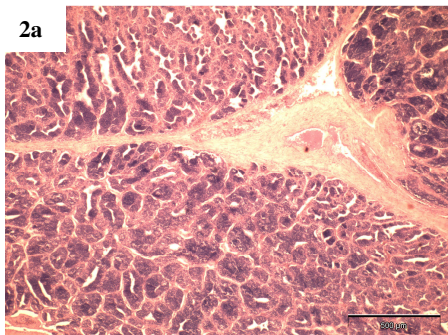
VI. Abnormal (stone roe). The reproductive tissue is transformed into connective tissue (scale bars **6a.** 500 µm **6b.** 200 µm **6c.** 100 µm, specimen 071025/86).



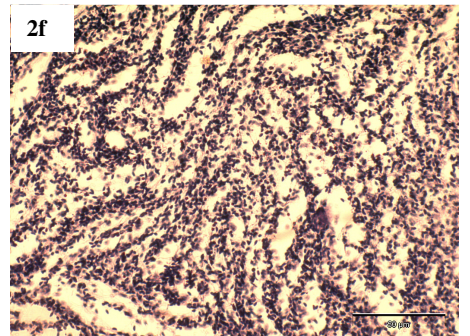
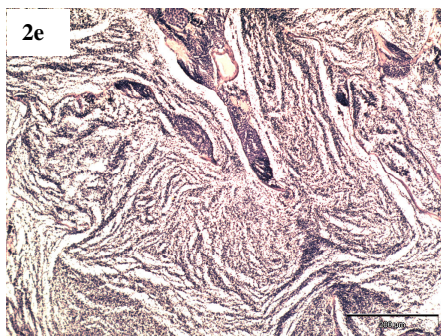
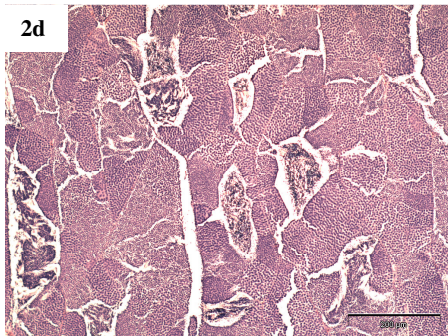
VI. Abnormal (intersex). A few perinuclear oocytes are visible among abundant spermatogonia (scale bars **6d.** 500 µm **6e.** 200 µm **6f.** 100 µm, specimen 070718/73).



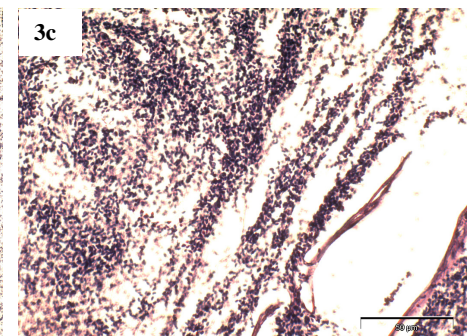
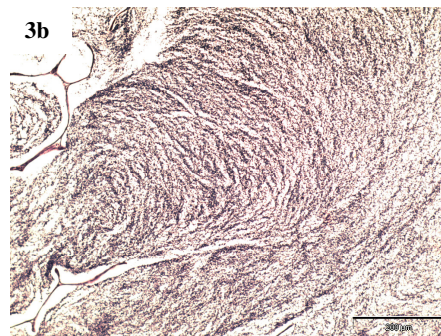
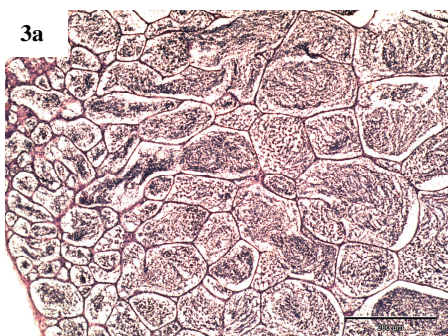
I. Juvenile/immature (preparation). 'Germ cells' or spermatogonia are characteristic of this stage; most are located distally. Singular germ cells lodge within a cyst and divide mitotically into groups of germ cells, which again divide into primary spermatocytes once the immature tissue is in preparation (scale bars **1a.** 500 µm **1b.** 100 µm **1c.** 50 µm; specimen 070718/171).



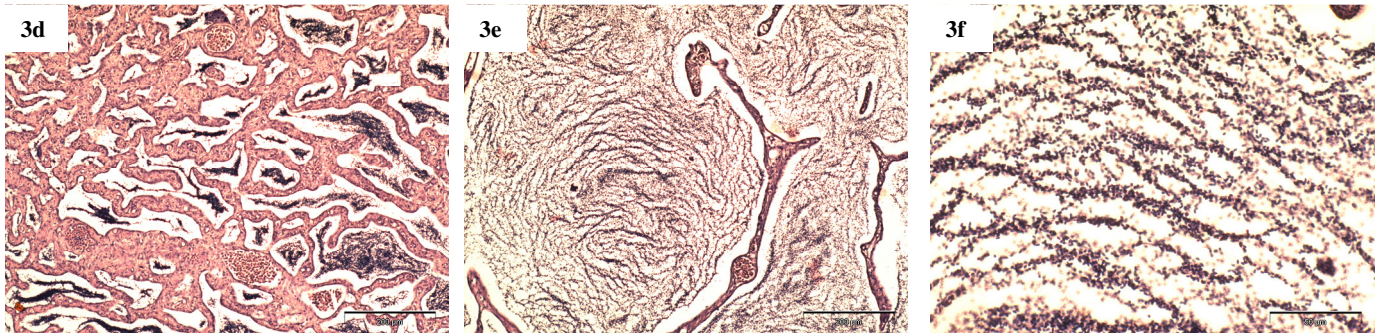
II. Maturing (early). The primary spermatocytes stain more deeply than spermatogonia or germ cells and divide into secondary spermatocytes, the nucleus of which appear more mottled. A further two divisions produce haploid spermatids with elliptical shaped nucleus. The spermatids develop flagella and become flagellate spermatozoa (scale bars **1d.** 500 µm **1b.** 100 µm **1c.** 50 µm; specimen 071025/109).



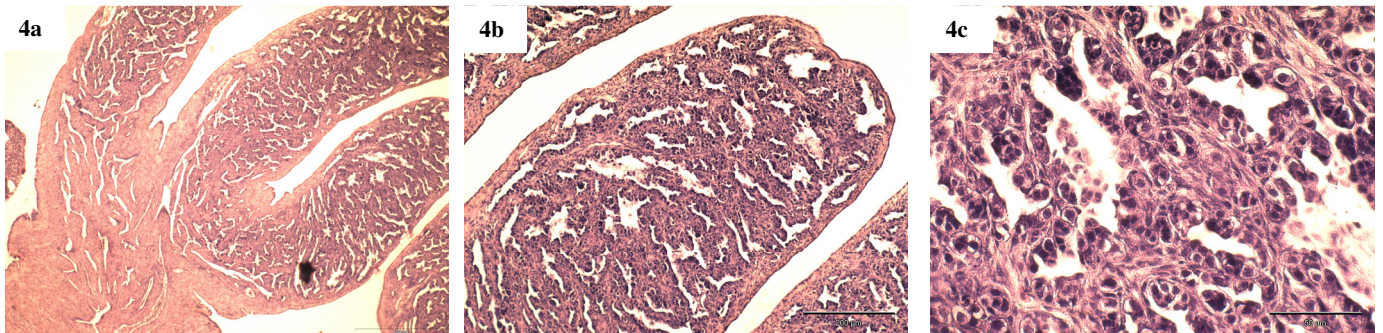
II. Maturing (late). In late maturing tissue the numbers of spermatids and flagellate spermatozoa increases rapidly, but no motile sperm are present in the sperm duct. Flagellate spermatozoa are present in higher numbers in the more rapidly developing proximal tissue (scale bars **2d.** 200 µm - proximal **2e.** 200 µm - distal **2f.** 50 µm - distal; specimen 070718/175).



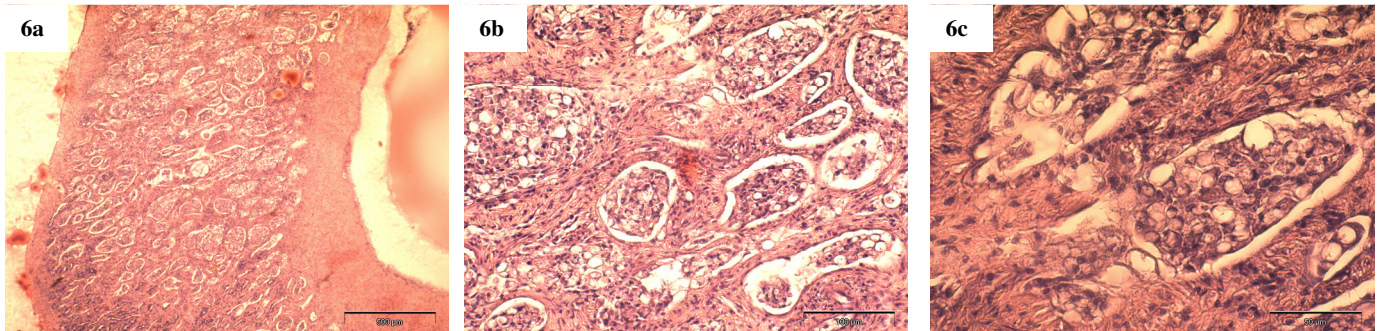
III. Spawning (main period). The number of spermatozoa increases rapidly. Cyst and lobule walls disappear and long tubules are formed proximally. Mature spermatozoa become aligned. Migrating germ cells are only visible at the extreme distal edges. The sperm duct and proximal efferent duct system contains ripe spermatozoa (scale bars **3a.** 200 µm-proximal **3b.** 200 µm-distal **3c.** 50 µm-distal; specimen 070718/142).



III. Spawning (cessation). The interlobular walls increase in thickness especially in the distal part of the lobules, while the proximal part is still full with ripe spermatozoa (scale bars **3d.** 200 μ m-distal **3e.** 200 μ m-proximal **3f.** 50 μ m-proximal; specimen 070718/148).



IV. Spent. A great reduction of sperm as well as an increase in thickness in the interlobular walls and the stroma of the testis. Remaining germ cells and thick septa of connective tissue can be seen in the distal end. Atretic spermatozoa are present in the collapsing efferent ducts, proximal tubules and in the sperm duct (scale bars **4a.** 500 μ m **4b.** 200 μ m **4c.** 50 μ m; specimen 070718/168).



VI. Abnormal. Abnormal testicular tissue contains histological irregularities in the developmental process of spermatogenesis. Mass atresia of maturing or ripe structures may be visible in some or all tissue regions as in this case (scale bars **6a.** 500 μ m **6b.** 100 μ m **6c.** 50 μ m; specimen 070718/111).

